TREATMENT OF PSYCHOPATHOLOGICAL AND NEUROCOGNITIVE DISORDERS IN GENETIC SYNDROMES: IN NEED OF MULTIDISCIPLINARY PHENOTYPING AND TREATMENT DESIGN

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Editorial: Treatment of psychopathological and neurocognitive disorders in genetic syndromes: In need of multidisciplinary phenotyping and treatment design

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genetic neurodevelopmental disorder, contextual neuropsychology, perspective taking, psychopathological phenotype, etiology based assesment, interdisciplinary treatment

Editorial on the Research Topic
Treatment of psychopathological and neurocognitive disorders in genetic syndromes: In need of multidisciplinary phenotyping and treatment design

Introduction

Similar to the figures in a coloring book, which are set at the moment of printing, the framework for a new person is set the moment a human egg is fertilized. The genes that we are born with contribute to our vulnerability for a wide range of possible phenotypes, but which of these phenotypes will subsequently develop, is greatly influenced by contextual factors from the physical world and social environments we live in, and our lifestyles (e.g., nutrition and exercise).

When looking at neurodevelopmental disorders with known genetic underpinnings, understanding the interaction between genes and context is vital for identification and support strategies. A fruitful way to achieve this would be through involvement of multiple disciplines, both in their additive capacities and in their ability to proceed

from shared well-informed theoretical frameworks and clinical practice approaches. The inclusion of somatic, neuronal, cognitive and behavioral aspects, of rare genetic syndromes, and acquiring a more detailed understanding of altered brain development, new and fundamental insights can be gained, which can guide diagnosis and treatment decisions. In addition, a tailored approach to assessment and monitoring is necessary to understand how the contextual factors influence the (neuro)development of the individual patient under investigation. In the present Research Topic, nine studies have been brought together that illustrate ways to better assess the challenges that come with genetic neurodevelopmental disorders, to ameliorate symptoms, and in general to improve quality of life, not only of these individuals but also of their family members and other caregivers.

Personalized healthcare

The phenotype of individuals with genetic neurodevelopmental disorders can differ considerably, as a consequence of their individual gene x environment developmental history. For example, the social abilities of caregivers have a substantial impact on the social development of a child. Because of this, personalized healthcare is important. Landlust et al. adopted a multidisciplinary method of assessment which takes the full developmental context into account and therefore allows more meaningful interpretations and predictions of behavior. Also, the Mobile-Health project by Heunis et al. aims to enhance personalized healthcare by using a self-report checklists to document the individual needs of patients and provide a toolkit with recommendations for support strategies.

Early interventions

Since the context in which a child grows up can largely influence the development of the cognitive, social and behavioral symptoms, earlier interventions may have better developmental outcomes than later ones. Garg et al. found worse sleep behavior in infants with Neurofibromatosis type 1 compared to infants without a family history of neurodevelopmental difficulties. Interventions to promote sleep hygiene may therefore be an interesting early treatment option.

Personalized guidance on social behavior and social cognitive training may be another way in which early interventions can improve developmental outcomes and improve the quality of life for the affected individuals and their social network of family members and caretakers. Bouw et al. saw children between the age of 4–8 with Sex Chromosome Trisomies improve their facial emotion recognition skills drastically by using a neurocognitive training program. Their ability to identify standard facial communication of emotions improved to the extent that it was indistinguishable from non-disabled controls after completing the training. Social training can, however, also be beneficial to individuals with a genetic disorder of older ages. Dykens et al. developed an intervention to ameliorate social skill deficits in people aged 14–33 with Prader-Willi Syndrome. This intervention was seen to improve social cognition, motivation and communication skills of the affected persons and was linked to their reports having more friendships and reduced loneliness.

Early interventions may also have wider benefits, extending beyond the patient to their wider social network of family members and caretakers. Bos-Roubos et al. found a high prevalence of traumatic events and greater vulnerability to Posttraumatic Stress Disorder in family members of individuals with Prader-Willi Syndrome (PWS). The number and complexity of symptoms in individuals with PWS as well as the level of trauma symptom severity of their family members was positively associated with patient age. Thus, early interventions like those of Dykens et al. may not only improve the quality of life of the patients but also hold promise for the wider family.

Biology in context

Even though genetic neurodevelopmental disorders often present with a highly variable phenotype (i.e., symptoms can differ significantly within genetic syndromes), comparing symptoms *between* syndromes may reveal information about their neurobiological mechanisms. For example, Lubbers et al. compared profiles of autism symptoms between individuals with different genetic syndromes and found similarities between sets of syndromes. These similarities may reflect similarities in the underlying neurobiology and provide a frame of reference, which can guide both research and treatment decisions. Similarly, Alfieri et al. found evidence that the differences in adaptive functioning on the socialization domain in children with Duplication 7 Syndrome or Williams-Beuren Syndrome may arise from general differences between the syndromes, including levels of cognitive function, a finding which provides insight into the developmental mechanisms of symptoms.

Understanding developmental mechanisms also implies the possibility of influencing them and can hence be important for designing new types of treatment. In recent years, studies have found that the composition of the gut microbiome is different in children with Autism Spectrum Disorder (ASD) compared to typically developing children. Whether these microbiome differences contribute to the behavioral symptoms remains to be understood. However, Lu et al. reviewed the literature on interventions to rebalance the gut microbiome and found reason to believe that probiotic treatment and microbiota transfer therapy may improve different behavioral symptoms of ASD.

As we have seen, genes and body, cognition and behavior as well as the environmental context are all interconnected and interact. The multiple disciplinary approach that was

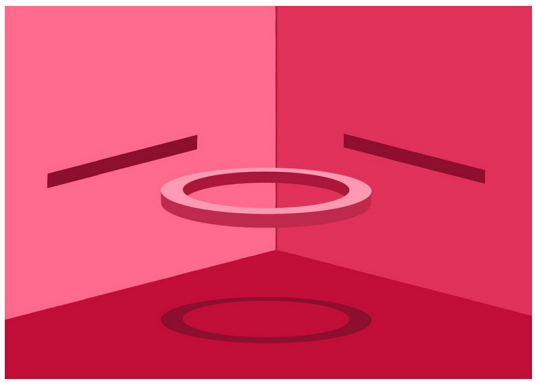

FIGURE 1
Different perspectives are needed: one eye cannot discern the ring.

used in and across these studies is therefore vital for improved understanding of the phenotypic presentations and mechanisms of genetic neurodevelopmental disorders. Contextual parameters should furthermore also be taken into account by means of tailored assessments. Early interventions, like social trainings, can influence the course of development and therefore improve the quality of life of patients and their relatives and caregivers. Interpreting genetic disorders within the context of individual development, can be highly valuable for efforts to provide efficient and effective healthcare.

Embodied embedded cognition pointing the way?

In summary, the present Research Topic may teach us that the combined etiology-inspired and context-sensitive approach employed in an inter- and transdisciplinary manner is essential for understanding both the neurobiological and developmental mechanisms involved. As metaphorically depicted in Figure 1, this approach is probably beneficial for providing the optimal scope and perspectives for devising innovative treatment interventions.

Author contributions

JE and CE: conceptualization and literature review and visualization. JE, CE, and EW: writing—original draft preparation. KW, LDe, MV, LV, and TK: writing—review and editing for important content. JE, EW, and TK: supervision. All authors have read and agreed to the final version of the manuscript.

Conflict of interest

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Early Preventive Intervention for Young Children With Sex Chromosome Trisomies (XXX, XXY, XYY): Supporting Social Cognitive Development Using a Neurocognitive Training Program Targeting Facial Emotion Understanding

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Background: Sex Chromosome Trisomies (SCTs; XXX, XXY, XYY) are genetic conditions that are associated with increased risk for neurodevelopmental problems and psychopathology. There is a great need for early preventive intervention programs to optimize outcome, especially considering the increase in prenatal diagnoses due to recent advances in non-invasive prenatal screening. This study is the first to evaluate efficacy of a neurocognitive training in children with SCT. As social behavioral problems have been identified as among the key areas of vulnerability, it was targeted at improving a core aspect of social cognition, the understanding of social cues from facial expressions.

Methods: Participants were 24 children with SCT and 18 typically developing children, aged 4–8 years old. Children with SCT were assigned to a training ($n = 13$) or waiting list (no-training) group ($n = 11$). Children in the training group completed a neurocognitive training program (The Transporters), aimed to increase understanding of facial emotions. Participants were tested before and after the training on facial emotion recognition and Theory of Mind abilities (NEPSY-II), and on social orienting (eyetracking paradigm). The SCT no-training group and typically developing control group were also assessed twice with the same time interval without any training. Feasibility of the training was evaluated with the Social Validity Questionnaire filled out by the parents and by children's ratings on a Visual Analog Scale.

Results: The SCT training group improved significantly more than the SCT no-training and TD no-training group on facial emotion recognition (large effect size; $\eta_p^2 = 0.28$), performing comparable to typical controls after completing the training program. There were no training effects on ToM abilities and social orienting. Both children and parents expressed satisfaction with the feasibility of the training.

Conclusions: The significant improvement in facial emotion recognition, with large effect sizes, suggests that there are opportunities for positively supporting the development

of social cognition in children with an extra X- or Y-chromosome, already at a very young age. This evidence based support is of great importance given the need for preventive and early training programs in children with SCT, aimed to minimize neurodevelopmental impact.

Keywords: neurocognitive training, facial emotion understanding, sex chromosome trisomies, Klinefelter syndrome, triple X

INTRODUCTION

Between 1:650 and 1:1000 children are born with a Sex Chromosome Trisomy [SCT; (1)]. SCT is characterized by an extra X- or Y-chromosome compared to the typical karyotype of 46, XX in girls and 46, XY in boys. Intellectual functioning is typically within normal limits, although somewhat lower on average, and SCT is related to a profile of specific cognitive vulnerabilities, for example in areas of executive functioning, language and social cognition [see for reviews: (2, 3)]. Children and adolescents with SCT also show higher percentages of clinical diagnoses of neurodevelopmental disorders, such as Attention Deficit/Hyperactivity Disorder (ADHD) and Autism Spectrum Disorder [ASD; (4–6)].

As SCT is a condition that are associated with increased risk for neurocognitive vulnerabilities and related neurobehavioral problems, these genetic conditions may serve as naturalistic "at risk" models of neurodevelopment. More specifically, the presence of an additional X- or Y-chromosome is known to convergently impact the maturation of brain functions and networks involved in social adaptive cognitive and behavioral development, often referred to as the "social brain" (7, 8). Therefore, the use of specific genetic conditions as models of more common behavioral and cognitive developmental disorders can reveal insights into early neurodevelopmental pathways that contribute to neurodevelopmental and -behavioral dysfunction in children. Therefore, research of the impact of genetic conditions such as SCT on development and potential effective interventions supporting development will help to elucidate the linkages among genetic, neurocognitive and neurobehavioral development.

Due to recent advances in non-invasive prenatal testing technology [i.e., the introduction of the NIPT; (9, 10)], it is possible to identify SCT as early as prenatally, resulting in increasing diagnoses of SCT. Give this rise in prenatal diagnoses of SCT, there is not only the opportunity to prospectively investigate early development, but also the opportunity and urgent need to study whether early preventive interventions may possibly reduce risk for difficulties in adaptive functioning and psychopathology later in life (11). However, to date, there has been no research evaluating the potential effects of early and preventive neurocognitive training in SCT. The present study aims at providing in this.

In defining the targets for early intervention in SCT, a key area may present the social domain, considering that social adaptation is among the key domains of vulnerability in SCT (3, 6, 10). Underlying cognitive mechanisms that may drive the risk of these social behavioral difficulties are social cognitive functions, referring to the mental processes that are used to perceive and process social cues, stimuli and environments, and underpin social adaptive functioning (12). With respect to SCT, recent reviews identify social cognition as among the key areas of difficulty from school age on (3, 6). Although outcomes are variable, reported vulnerable social cognitive abilities include reading social signals from social gaze directions, facial emotion understanding, face processing (accuracy and reaction time) and Theory of Mind, referring to the attribution of mental states, intentions and emotions to others (13). Calculated effect sizes indicated high to very high clinical significance. Interestingly, specific age dynamics during early development of social cognitive functions in young children with SCT were recently found (14), often described as the "growing into deficit" phenomenon (15), the effect that development is increasingly deviating compared to typical developing peers when children become older. Early intervening with children who are "at risk" for adverse development, but do not yet exhibit full expression of the syndrome, may provide the best advantages from intervention. By implementing intervention early in life, the course of social development may be shaped, preventing for adverse long-term outcomes (16). Given the difficulties in underlying social cognitive mechanisms in SCT that serve as building blocks for social adaptive functioning (12), it is important to study whether it is possible to support the development of social neurocognitive functions early in development by early intervention trials.

Training of emotion perception and understanding appears to be an important component of effective social cognitive interventions (17). In addition, emotion recognition develops already early in life (18), is proven to be vulnerable across the life-span of individuals with SCT, and therefore an important target to preventively support social cognitive development early in life of individuals with SCT. In typical early social cognitive development, the ability to recognize facial expressions correctly and to respond to them appropriately is vital for successful everyday social interaction, and a prerequisite for showing social adaptive behavior, responsive of social feedback that follows social interactions. This ability to recognize facial emotions, in turn, depends on basic social orientation, the spontaneous visual orienting of attention to naturally occurring and meaningful aspects of social interactions (i.e., eyes and faces), which is already present in the first 6 weeks of postnatal life (19); for a review on eye tracking studies, see (20). Later developing and higher order social cognitive skills as for example Theory of Mind likely depend on this very early propensity to orient

attention to social important information and to recognize facial emotions (21). Attributing and understanding mental states such as beliefs, desires and intentions of others and oneself with the ability to share these during social interactions (i.e., Theory of Mind) continue to develop throughout childhood, based on maturation of complex neural networks and high-order cognitive processes (22). We evaluated the efficacy of an emotion recognition training program in SCT on key areas of typically social cognitive development during early childhood that were found to be vulnerable in young children with SCT, i.e., on measures of emotion recognition, Theory of Mind and social orientation (14).

Most of the currently available training programs targeted to enhance emotion recognition are computer-based. These computer-based neurocognitive training programs provide the opportunity to teach emotion recognition in a controlled and structured environment with little social demands. Motivation and interest are usually easily to maintain, and materials are low-budget and therefore easy accessible (23, 24).

The efficacy of a home-based computer-based emotion recognition training in young children with SCT in the current study was evaluated by comparison of measures of emotion recognition, Theory of Mind and social orienting before and after the training. Training effects in young children with SCT were compared with two groups that did not attend the training program i.e., a waiting list group with SCT, and a group of typically developing children. Also, we studied feasibility and implementation of the training program in the SCT training group, based on self-report of the parents, and the children. Because studies on the efficacy of neurocognitive training that target to support early social cognitive development in SCT are lacking, this study is unique and may provide important implications for clinical care and future research aimed at improving evidence-based care for children with SCT in order to support optimal neurodevelopmental outcome.

MATERIALS AND METHODS

Recruitment

The present study is part of a larger ongoing longitudinal study (the TRIXY Early Childhood Study–Leiden, The Netherlands), which includes children with SCT and nonclinical controls. The TRIXY Early Childhood Study aims to identify neurodevelopmental risk in young children with an extra X or Y chromosome. Recruitment and assessment of the current study took place as part of this larger study, at the Trisomy of the X and Y chromosomes (TRIXY) Expert Center at Leiden University (LUBEC) in Leiden, The Netherlands. Children in the SCT group were recruited in cooperation with clinical genetics departments in the Dutch speaking parts of Western Europe.

Typically developing control children were recruited from the western part of The Netherlands, and approached with information brochures about the study. All participants were Dutch speaking, had normal or corrected-to-normal vision, and did not have a history of traumatic brain injury. The diagnosis of SCT was defined by trisomy in at least 80% of the cells, which was confirmed by standard karyotyping. For ethical reasons, children in the typically developing group were not subjected to genetic screening. As the prevalence of SCT is ∼1 in 1,000, the risk of having one or more children with SCT in the typically developing group was considered minimal and acceptable.

TABLE 1 | Background information of participant.

	SCT training group	SCT no-training group	TD control group
Age	5.86 (1.28)	6.33 (1.27)	5.87 (1.15)
Gender	5 boys, 8 girls	8 boys, 3 girls	9 boys, 9 girls
Parental education	5.58 (1.08)	6.10 (0.83)	5.58 (1.41)
Karyotype	8 XXX, 4 XXY, 1 XYY	3 XXX, 5 XXY, 3 XYY	n.a.
Recruitment strategy*	A:6, B:4, C:3	A:8, B:1, C:2	n.a.

SCT, Sex Chromosome Trisomy; TD, Typically Developing.
**A = Prospective follow-up, B = Information seeking parents, C = Clinically referred cases.*

Participants

A group of 25 children with SCT (range 4–8 years old) was included in this study. Children with SCT were assigned to a training group (SCT training group; n =14) or waiting list no-training group (SCT no-training group; n =11). See **Table 1** for background information of the participants. Likelihood Ratio tests were performed to investigate the ratios of age, gender and karyotype distribution across the study groups. Mean age across the three study groups did not differ between the three groups [$F_{(2, 39)} = 0.58$, $p = 0.566$]. Also, gender distribution did not differ between the three study groups [$\lambda(2) = 2.96$, $p = 0.227$]. There was no difference in distribution of karyotypes between the SCT training and SCT no-training group [$\lambda(2) = 3.50$, $p = 0.187$]. One girl with 47, XXX in the SCT training group dropped out of the study during the second week, as she was not motivated to continue watching the training episodes any longer.

For the SCT group, recruitment strategy was assessed, and three subgroups were identified: (1) "active prospective follow-up", which included families who were actively followed after prenatal diagnosis (58.3% of the SCT group), (22) "Information seeking parents", which included families who were actively looking for more information about SCT without having specific concerns about the behavior of their child (20.8% of the SCT group), and (3) "Clinically referred cases", which included families seeking professional help based on specific concerns about their child's development (20.8% of the SCT group). The distribution of recruitment strategy did not differ between the SCT training and SCT no-training group [$\lambda(2) = 2.25$, $p = 0.325$]. One out of nine boys with 47, XXY had received testosterone treatment (11%). Testosterone treatment was performed at the age of 1 year, 3 years before the start of the current intervention study.

Parental education of the primary caregiver was assessed, according to the criteria of Hollingshead (25). Scores of this scale include: 0 (no formal education), one (less than seventh grade), two (junior high school), three (partial high school), four (high school graduate), five (partial college

or specialized training), six (standard college/university graduation), and seven (graduate/professional training). Eighty-one percentage of all parents indicated that their child has a second caregiver. If two parents were available, level of education was averaged over both parents. No differences in parental education distribution between the three study groups were found [$\lambda(16) = 15.36, p = 0.498$].

Design of the Study

The current study had a repeated measures within-subject design. All participants were assessed twice: at baseline and 4 weeks later (follow-up). At baseline global level of cognitive functioning was measured, as well as receptive and expressive language skills. At both baseline and follow-up children facial emotion recognition, Theory of Mind, and social orienting was assessed. Between the baseline and follow-up assessment, children in the SCT training group participated in the emotion training program. Children in the SCT no-training group and the typical control group completed the baseline and follow-up assessment, but did not participate in the training program between baseline and follow-up, nor in other forms of early intervention as part of regular care. See **Figure 1** for an overview of the study design.

Emotion Training Program: The Transporters

The Transporters is a narrated and animated DVD series and was originally developed to teach young children between the age of three and eight about emotions, their causes and consequences, and their corresponding facial expressions [Changing Media Development, www.thetransporters.com; (26)]. The series consists of fifteen 5-min episodes, portraying key emotions including basic emotions and nine more complex emotions: happy, sad, angry, afraid, disgusted, surprised, excited, tired, unfriendly, kind, sorry, proud, jealous, joking and ashamed. The narrated stories are built around eight characteristics who are vehicles (e.g., trams, cars, railway) with real-life faces of actors showing the emotions. The emotion is presented in the context of the series plot, in which the emotions are labeled, facial expressions are highlighted, and the context of the emotional experience is provided within social interactions between the toy vehicles. The assumption behind The Transporters is that through repetitive watching of entertaining episodes children might enhance facial emotion recognition and understanding skills [see for an extensive description of The Transporters: (27)]. The Transporters has been proven to be successful in improving emotion understanding abilities in young children with ASD (27, 28), although mixed results were found with respect to efficacy of The Transporters in young children with ASD with a lower range of cognitive ability (29, 30). See **Figure 2** for screenshots of the first episode.

Children in the SCT training group watched the Dutch version of The Transporters in their home setting (De Ambelt, The Netherlands; resources.autismcentreofexcellence.org); they watched three episodes a day supervised by the parent, for 5 days a week and 4 weeks long (see **Figure 1**). The episodes were repeated in the same order every single week, in order to achieve repetitive watching of the episodes. Parents were provided with a detailed manual that consists of operating instructions, and a daily diary with a general introduction to the separate episodes, and exercises and questions to discuss with their child after watching the episodes. These exercises and questions were aimed to broaden the child's understanding of the emotional concepts as presented in the episodes, and to facilitate consolidation of learned skills. Examples of exercises and questions are: "Who is kind to you when you are in a bad mood? What does this person do? How does that make you feel? What do you do when you see that your mother/father/brother/sister/friend is sad or worried?" During the training period, the parent had a weekly call with the researcher to discuss and find solutions for any practical bottlenecks.

Instruments

Background Measures: Global Level of Cognitive and Language Development

At baseline, global level of cognitive and language development was assessed in all children. Four subtest of the WPPSI were used to estimate global level of intelligence [Block Design, Matrix Reasoning, Vocabulary, and Similarities; (31)]. Total IQ estimates were calculated based on this short form version of the WPPSI-III (32). The Peabody Picture and Vocabulary Test [PPVT; (33)] was used to measure receptive language level. To measure expressive language skills, the Clinical Evaluation of Language Fundamentals-Preschool, 2nd edition [CELF-Preschool; (34)] was administrated.

Facial Emotion Recognition

The Affect Recognition subtest of the NEPSY-II neuropsychological test battery (35) was used to assess children's ability to discriminate among common facial emotions from photographs of children, and was administrated at baseline and follow-up. The task has been normed with typically developing children aged 3–16 years old. During the task, participants are required to match faces of different children who show the same emotional expressions (happy, sad, angry, disgust, fear and neutral). The participant indicates if two expressions are the same or different, determines which two faces have similar expressions, or identifies two children with expressions that match a third child's face. The total raw score range is between 1 and 35, with higher scores reflecting a better ability to recognize facial expressions.

Theory of Mind

The ToM subtest of the NEPSY-II neuropsychological test battery (35) was used to assess children's understanding of mental states and other people's perspectives at baseline and post-training. The ToM subtest consists of two different subtasks: verbal tasks and contextual tasks. In the verbal tasks, the questions are based on verbal scenarios with (six items) or without (11 items) support of pictures. They measure the understanding of (false) beliefs, intentions, other's thoughts, ideas and comprehension of figurative language. Two items aim to measure the child's verbal and gestural imitation abilities, as imitation abilities are thought to be a basic ability for ToM skills. The child is asked to answer the tasks verbally, with the exception of an imitation question

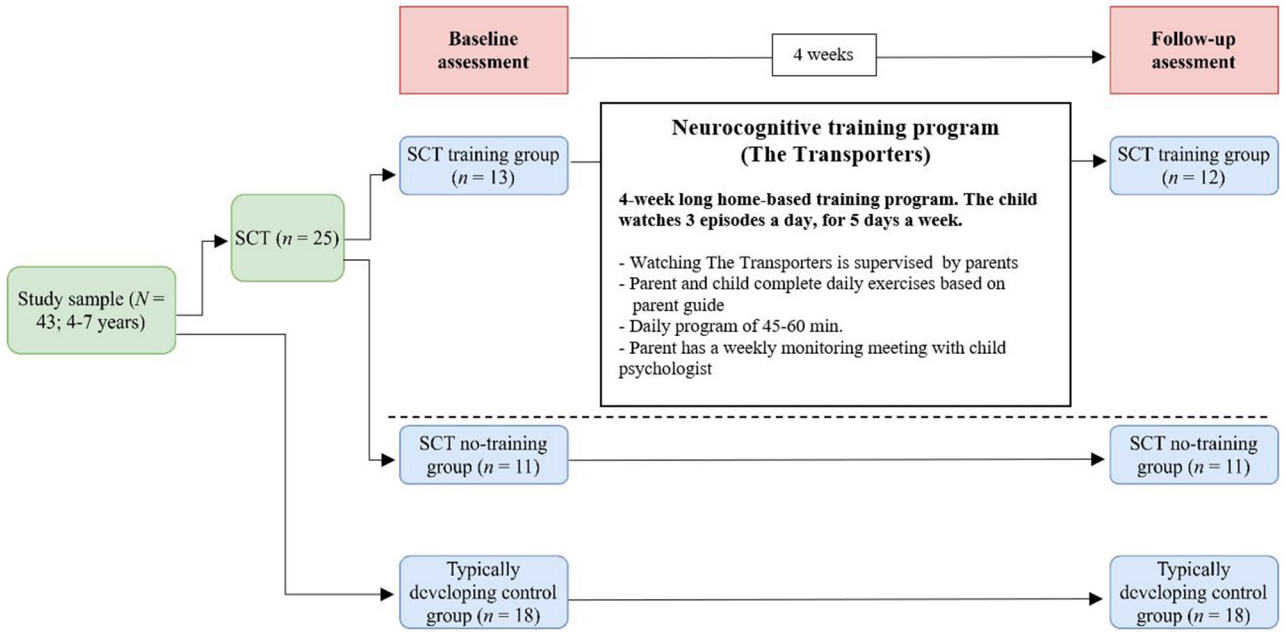


FIGURE 1 | Flow diagram of the study design with the SCT training group, the SCT no-training group and a typically developing control group. SCT, Sex Chromosome Trisomie.

FIGURE 2 | Screenshot of an episode from The Transporters training program: character William showing the emotion "happy".

where the child is asked to imitate gestures or words. In all of the items the child can answer very briefly; one word is often sufficient for a correct answer, and in two of the questions it is also possible to answer by pointing. The contextual tasks of the ToM subtest aim to measure the child's ability to relate affects to a broader social context. In these items the child is shown drawings with children in social contexts. In each drawing there is a target girl whose face is not shown. The child is asked to select one of four photographs of the same girl's face with different emotions selecting the emotion of the girl in the drawing. The child can answer by pointing. The total score range is between 1 and 28 (sum score of the 15 verbal tasks and six contextual tasks), with higher scores reflecting better ToM skills.

Social Orienting

Eye gaze fixations toward key sources of social information (eyes, faces) were measured during a Social Orienting Paradigm (see for a detailed description of the paradigm: 14), at both baseline and follow-up. The 30 s during video showed a social plot, in which social cues are reciprocally exchanged between a child and an adult. To prevent interference with language abilities, language used in the clip was not the same as the language of the participants (i.e., Italian vs. Dutch). In a group of non-clinical young children aged 3–7 years, this eyetracking paradigm was found to be related to real-life social behaviors, and independent of age, IQ, or gender (36). See **Figure 3** for a screenshot of the video clip.

Eyetracking Equipment and Procedures

Gaze data within specific areas of interest (AOIs) was collected using the Tobii X2-60 eyetracker (Tobii Technology AB, Danderyd, Sweden), which records the X and Y coordinates of the child's eye position at 60 Hz by using corneal reflection techniques. The computer with eyetracker was placed on a table adapted to the height of the seat, and the child was seated in a car seat at 65 cm viewing distance. A 5-point calibration procedure

FIGURE 3 | Screenshot of the video clip in the Social Orienting Paradigm.

FIGURE 4 | Visual Analog Scale used during the training program.

was used, with successful calibration defined as a maximum calibration error of one degree for individual calibration points (i.e., < 1 cm at a distance of 65 cm from the eyetracker). After the calibration procedure, the child was instructed to watch the movie clips and pictures on the computer. The paradigm started with an attention grabber (e.g., a moving picture of an animal, shown on a black background and accompanied by a sound) to direct the attention of the child to the screen.

Gaze data was processed using Tobii Studio (version 3.2.1), using the Tobii Identification by Velocity Threshold (I-VT) fixation filter. A fixation was registered if the velocity threshold for an eye movement exceeded 30°/s, and therefore controls for validity of the raw eyetracking data making sure only valid data were used (37). The "Dynamic AOI" tool was used to draw AOIs, drawn with a one centimeter margin, to ensure that the AOIs were sufficiently large outside the defining contours to reliably capture the gaze fixation (38). Dynamic AOIs were grouped into the following categories: eyes, faces and the whole screen. In order to evaluate the amount of nonvalid eye tracking data, the total visit duration toward the whole screen was calculated, divided by the duration of the clip, multiplied by 100, reflecting the percentage of valid data collected during each of the eye tracking tests. Proportions fixation duration were calculated by taking the total fixation duration within the AOI, divided by the total visit duration toward the whole screen of the individual child, multiplied by 100, reflecting the percentage of time children were attending to an AOI.

Feasibility of the Emotion Training Program

Social Validity Parents

The Social Validity Questionnaire [SVQ; (39)] was filled in by the primary caregiver of the children in the SCT training group at follow-up, and assessed the parent's perception of: (a) how easy the training was to incorporate into daily life; (b) how easy the training was to learn; and (c) whether the training was effective for the child and family. The SVQ consist of 15 items, and were rated on a five point Likert scale ranging from one "Totally disagree" to five "Totally agree". Examples of items are: "This training was easy to incorporate into my family life", "This training was not complicated to learn" and "I noticed meaningful increases in my child's social interaction with the people in his environment".

Perception of Children About the Training

Children in the SCT training group reported how much they liked the training episodes in a daily diary. Their perception about the training was rated on a Visual Analog Scale (VAS) at a daily basis on an interval from 0 to 100 (see **Figure 4**). According to Shields et al. (40), only responses that were properly marked on the VAS lines (the mark must be a single vertical line that is no more than 1 mm away from the VAS line) and responses that were marked along the entire length of the VAS line (as opposed to using just the end-points and/or the middle of the VAS line) were used in the analysis. A total score was computed for each child by taking the sum of the registrations divided by the number of registrations (with a maximum of 20 registrations). Furthermore, how much children liked the training was calculated for every single week.

Study Procedures

Assessments ate baseline and follow-up took place in a quiet room at the university or at home. Administration of the WPPSI, PPVT, CELF and NEPSY was performed on a table by trained child psychologists. The eyetracking procedure took place after the neurocognitive tests administration. The laptop with the eyetracker was placed in a small tent to standardize the testing environment, and to control for light conditions. The child was seated in a car seat in front of the eyetracker. The examiner was seated beside the child (directing Tobii Studio with a remote keyboard), and started the calibration procedure. Parents were allowed to stay in the room (out of sight) and were asked not to communicate with their child during the procedure. The Social Validity Questionnaire was filled in by the primary caregiver of the child.

Ethical Approval and Informed Consent

This study was approved by the Ethical Committee of Leiden University Medical Center, The Netherlands. Signed informed consent was obtained from the parents of all participating children, according to the declaration of Helsinki.

Data Analyses

Data were analyzed using the Statistical Package for the Social Sciences (SPSS), version 25. Baseline difference between study groups on background measures (global cognitive and language level) were analyzed with ANOVAs. In order to analyze the training effects, Repeated Measures MANOVAs were used with

TABLE 2 | Means (SD's) and group differences on cognitive and language functioning.

	SCT training	SCT no-training	TD control	*p*-value	Effect size (η_p^2)	Group differences
	***n* = 13**	***n* = 11**	***n* = 18**			
Global cognitive functioning standard score; WPPSI-III	92.77 (10.68)	91.09 (14.98)	107.78 (13.20)	0.002	0.28	A, B < C
Receptive verbal ability standard score; PPVT-III	103.00 (11.10)	97.82 (12.33)	111.39 (10.55)	0.009	0.22	B < C
Expressive verbal ability scaled score; CELF	9.31 (2.66)	8.36 (2.87)	10.33 (1.85)	0.108	0.11	

SCT, Sex Chromosome Trisomies; TD, typically developing; A, SCT training group; B, SCT no-training group; C, typical developing control group.

Time (baseline, follow-up) as within variable and Group (SCT training, SCT no-training, typically developing) as between variable. The interaction effect (Time x Group) was used to evaluate overall training effects. *Post-hoc* paired sample *t*-tests were carried out to analyze change in social cognitive abilities within the three study groups (SCT training, SCT no-training, typically developing). Repeated Measures MANCOVAs were carried out to test training effects, while covarying for cognitive and language abilities. Change in reported perception of children about the training was analyzed with a RM ANOVA. Level of significance was set at $p < 0.05$, two-tailed. Effect sizes were calculated with Cohen's *d* or partial η^2 when applicable.

RESULTS

Background Measures

Mean scores on cognitive background measures at baseline (global cognitive level, receptive and expressive verbal ability) are presented in **Table 2**. The three study groups do not differ on expressive verbal ability [$F_{(2, 39)} = 2.35$, $p = 0.108$]. However, the groups significantly differ in global cognitive functioning [$F_{(2, 39)} = 7.64$, $p = 0.002$], indicating lower functioning in the SCT groups (training and no-training), compared to the TD group. Both SCT groups perform similar on global cognitive functioning. Also, a significant difference on receptive verbal ability is found [$F_{(2, 39)} = 5.39, p = 0.009$], indicating lower ability in the SCT no-training group, compared to the TD group. No difference between the SCT training group and the no-training group was found for receptive verbal ability. Because of these differences between the SCT groups and the TD group, global cognitive functioning and receptive verbal ability are added as covariates in the analyses.

Training Effect: Facial Emotion Recognition

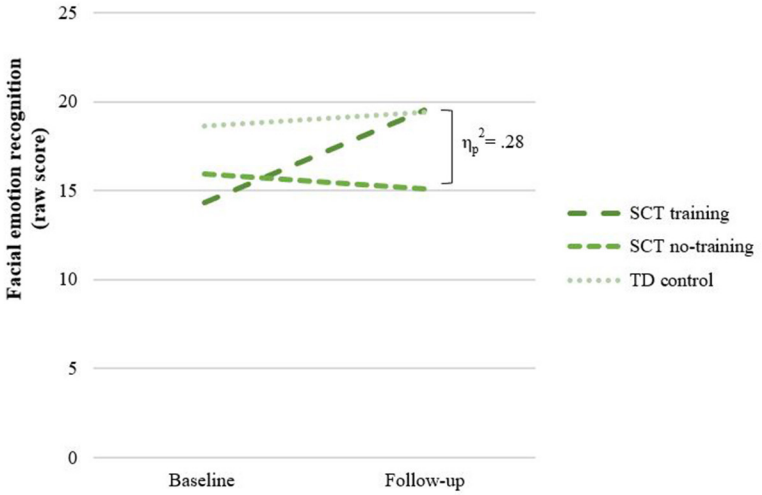
First, to evaluate the overall effect of the training a RM MANOVA is conducted with Time (baseline, follow-up) as within variable and Group (SCT training, SCT no training, typically developing) as between variable. The analysis yield a significant interaction effect for Time x Group [$F_{(2, 39)} = 7.50$, $p = 0.002$, $\eta_p^2 = 0.28$), with a large effect size. This significant interaction effect on emotion recognition skills remains, even when global intelligence and receptive language skills are added as covariates [$F_{(2, 37)} = 6.65$, $p = 0.003$, $\eta_p^2 = 0.26$]. Next, *post-hoc* paired sample *t*-tests are used to analyze the effect of Time within the three study groups. In the SCT training group, a significant change in emotion recognition abilities is found [$t_{(12)} = -3.72$, $p = 0.003$]. In the SCT no-training group, no significant change in emotion recognition is found [$t_{(10)} = 0.88, p = 0.401$], neither in the typically developing group [$t_{(17)} = -0.88, p = 0.393$]. These findings indicate a significant change in emotion recognition abilities in the SCT training group, that is not present in the SCT no-training group or typically developing control group. After completing the training, the SCT training group ($M = 19.54$, $SD = 4.72$) scores comparable to their typically developing peers [$M = 19.39$, $SD = 4.98$; $t_{(29)} = 0.84$, $p = 0.933$]. In terms of standard deviations, children in the SCT training group score 1.11 *SD* higher as compared to their average baseline score. See **Figure 5** for an illustration of the interaction effect on facial emotion recognition.

Training Effect: Theory of Mind

First, to evaluate the overall training effect on Theory of Mind, a RM MANOVA is conducted. No significant interaction effect is found for Time x Group [$F_{(2, 39)} = 0.31, p = 0.738$] indicating no training effect on Theory of Mind. These findings do not change when global intelligence and receptive language skills are added as covariates [$F_{(2, 37)} = 0.73, p = 0.488$]. See **Figure 6**.

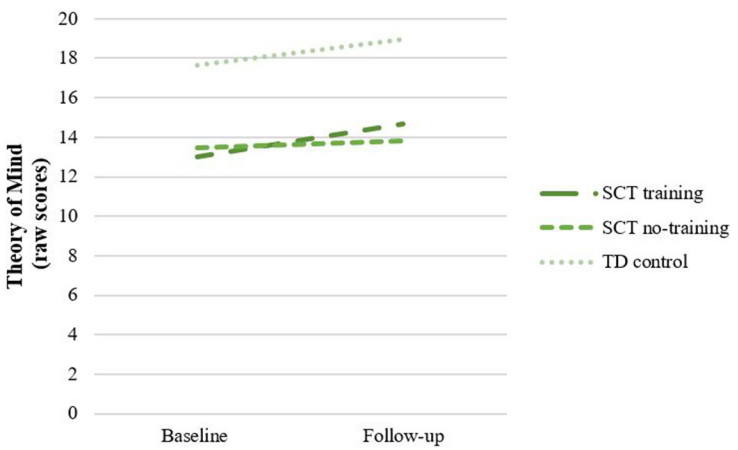
Training Effect: Social Orienting to Eyes and Faces (Eyetracking Paradigm)

Attention to the screen. The Social Orienting Paradigm was successfully completed by 42 children at baseline, and 41 children at follow-up (one boy with 47, XXY in the SCT no-training group was not able to complete the task at follow-up). At baseline, the total proportion valid on-screen fixation duration is 95.5%, indicating sufficiently high attention to the screen. Attention to the screen do not significantly differ between the



Emotion recognition	*M (SD)* baseline	*M (SD)* follow-up	Δ-value	*p*-value	Cohen's *d*
SCT training	14.31 (5.17)	19.54 (4.72)	5.23	.003	1.06
SCT no-training	15.91 (6.72)	15.09 (7.13)	-0.82	.401	0.12
TD control	18.61 (4.89)	19.39 (4.98)	0.78	.393	0.16

FIGURE 5 | Training effect on facial emotion recognition: mean scores before (Baseline) and after completing the training (Follow-up). SCT, Sex Chromosome Trisomies; TD, typically developing.



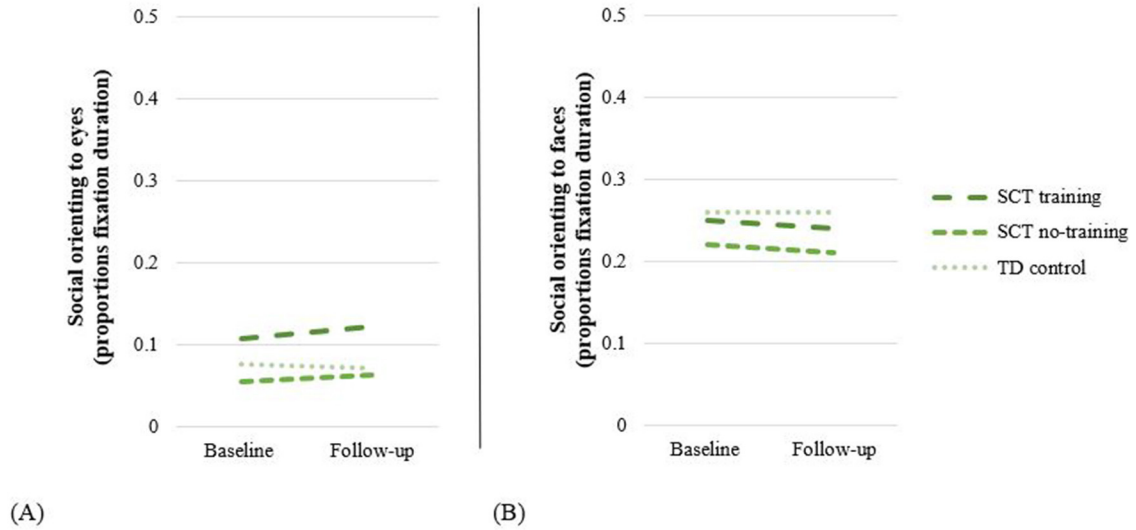
Theory of Mind	*M (SD)* baseline	*M (SD)* follow-up	Δ-value	*p*-value	Cohen's *d*
SCT training	12.31 (5.38)	13.85 (6.40)	1.54	.114	0.26
SCT no-training	13.45 (6.46)	13.82 (5.76)	0.37	.826	0.06
TD control	17.67 (4.24)	18.94 (5.01)	1.27	.097	0.27

FIGURE 6 | Theory of mind (total scores) before (Baseline) and after completing the training (Follow-up). SCT, Sex Chromosome Trisomie; TD, typically developing.

three study groups, [$F_{(2, 39)} = 1.76$, $p = 0.186$]. Similar at follow-up, the total proportion valid on-screen fixation duration is 94.3%, and do not significantly differ between the three study groups, [$F_{(2, 38)} = 1.76$, $p = 0.186$].

Training Effect

A RM ANOVA is conducted to analyze overall training effect, revealing no significant effect of Time x Group for social orientation to eyes [$F_{(2, 38)} = 0.22$, $p = 0.803$] neither to faces



A. Social orienting to eyes % fixation duration	*M (SD)* baseline	*M (SD)* follow-up	Δ-value	*p*-value	Cohen's *d*
SCT training	.11 (.23)	.12 (.26)	.01	.468	0.04
SCT no-training	.06 (.05)	.06 (.06)	.00	.758	0.00
TD control	.08 (.08)	.07 (.04)	-.01	.832	0.16

B. Social orienting to faces % fixation duration	*M (SD)* baseline	*M (SD)* follow-up	Δ-value	*p*-value	Cohen's *d*
SCT training	.25 (.10)	.24 (.09)	-.01	.773	0.11
SCT no-training	.22 (.12)	.21 (.10)	-.01	.448	0.09
TD control	.26 (.09)	.26 (.11)	.00	.931	0.00

FIGURE 7 | Social orienting to eyes **(A)** and faces **(B)** before (Baseline) and after completing the training (Follow-up). SCT, Sex Chromosome Trisomie; TD, typically developing.

TABLE 3 | Social validity of parents in the SCT training group on subscales of the Social Validity Questionnaire.

	Negative opinion	Neutral opinion	Positive opinion
	Strongly disagree/disagree	Neutral	Agree / strongly agree
Incorporation in daily life	8.3%	0%	91.7%
Easiness to learn the training	0%	33.3%	66.7%
Effectiveness of the training	16.7%	58.3%	25.0%

N = 13.

$[F_{(2, 38)} = 0.05, p = 0.948]$. These findings do not change when global intelligence and receptive language skills are added as covariates [eyes: $F_{(2, 36)} = 2.15, p = 0.131$; faces: $F_{(2, 36)} = 0.29, p = 0.741$]. See **Figure 7**.

Feasibility of the Emotion Training Program

Social Validity Parents

Response on the 5-point Likert scale of the 15 items of the Social Validity Questionnaire are recoded into three categories: negative opinion (value 1 and 2), a neutral opinion (value 3), and a positive opinion (value 4 and 5). **Table 3** presents parents' responses on the three subscales of the SVQ. In sum, parents report positive experiences after the training period. To illustrate, 91.7% of the parents report that the training is easy to implement in daily life, 66.7% reported that the training is easy to learn and to use and valuable for their child. Although 83.3% of the parents recognize positive changes in their child after intervention (Q11: This intervention provided a significant positive change for my child), a vast majority of the parents are neutral about the generalization of the intervention to other contextual situations (Q12: I noticed meaningful increases in my child's interaction with the people in his environment; Q15: Other people noticed a significant positive change in my child). Only 8.3% of the parent report positive increases in child's eye contact (Q14: I noticed meaningful increases in my child's eye contact with the people in his/her environment). Parents do report that they would recommend the intervention to other parents and that they are willing to continue using the intervention model in the future. See the **Supplementary Table 1**) for parents' response on all items of the SVQ.

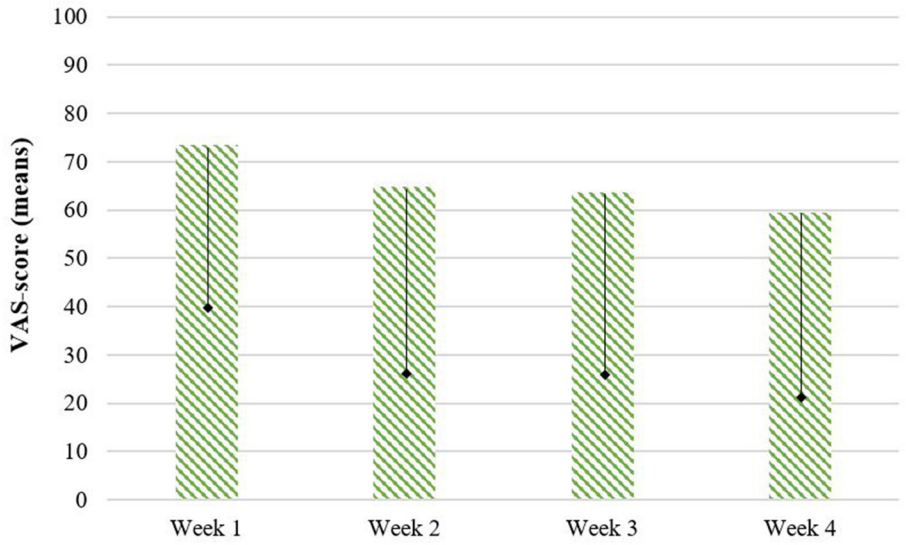


FIGURE 8 | Perception about the training during the training program, reported by children on a Visual Analog Scale (VAS). = *SD* (only lower bar depicted).

Perception of Children About the Training

Children were asked daily how much they liked the training, on a Visual Analog Scale. The mean VAS-score of children during watching the training episodes for the SCT training group is 65.2 on a scale of 0–100. There is no significant difference in VAS-scores of children reported between the first training week (M = 73.40, SD= 26.62), the second week (M = 64.76, SD = 30.31), the third week (M = 63.67, SD = 29.17), and the fourth week (M = 59.43, SD = 30.07), $F_{(3, 24)} = 1.70$, $p = 0.194$ (see **Figure 8**).

DISCUSSION

There is a great need for evidence-based interventions that support early development of young children with Sex Chromosome Trisomies (SCT). The current study aims to evaluate the effectiveness of a neurocognitive training in young children with SCT, aged 4–8 years. As social cognitive and behavioral vulnerabilities have been identified as among the key areas of vulnerability in SCT (6, 41), this neurocognitive training was targeted at improving the understanding of social cues from facial expressions.

Efficacy of the training was examined on key aspects of early social cognition that have proven to be vulnerable in young children with SCT: facial emotion recognition, Theory of Mind and social orienting (14). Three study groups were included in the study: 4–8 year old children with SCT, and two age- and gender-matched control groups that did not complete the training: one waiting list group with SCT and one typically developing group. Promising results regarding the effectiveness of the training were found, revealing that attending the 4-week home based neurocognitive training was effective in significantly improving the ability to identify and match basic and complex facial expressions in children with SCT, with a large effect size. These findings were irrespective of level of global cognitive functioning and expressive and receptive language abilities. After completing the training program, children with SCT show emotion recognition abilities to a level that could not be distinguished from the typically developing group at follow-up.

These findings illustrate that there are opportunities for positively supporting the development of emotion understanding in children with SCT, already at a young age. Given the evidence that in SCT early social cognitive vulnerabilities may emerge and present more profoundly with age (14), early support of early social cognitive development may alter adverse developmental trajectories of young children with SCT, reduce the negative long-term impact of SCT on social adaptive functioning (16, 42).

Improvements in facial emotion recognition were measured with a standardized task which required understanding of facial emotion of real human faces, different from the learned emotions attached on animated vehicles (35). Also, this standardized task gave no information of the emotion in terms of its context, supporting the notion that children with SCT were able to generalize their acquired knowledge during the training program on a distant generalization task. This is especially interesting, as other neurocognitive training programs aimed to enhance emotion recognition in other populations, often show limits in the generalization that were possible to achieve (see for example in ASD populations: (43, 44). The found training effects in the present study were independent of children's global cognitive functioning and their expressive or receptive language skills which are proven to be lower in young children with SCT (45), suggesting that neurocognitive training programs may be suitable and effective for a broad range of young children with SCT.

There were also areas of social cognitive functioning that did not change following the neurocognitive training program, as the study findings indicate that increased emotion recognition abilities after the training did not generalize to direct improvements in social orientation or Theory of Mind. These findings indicate a specific effect of the training on emotion recognition abilities which was the target ability to be trained in the program (27). However, the findings of the current study suggest that The Transporters training program is effective in training understanding of facial emotion in young children with SCT, rather than being effective in enhancing broad early social cognitive development.

Lastly, this study found positive parent and children's perceptions on the feasibility of the training program. The Transporters is an intensive program, expecting the child and parent to invest 45 to 60 min (i.e., three videos accompanied with the exercises from the parent guide) 5 days a week, for a total duration of 4 weeks. Nonetheless, parents were positive about the ability to incorporate the training program in daily life and report that The Transporters was easy to learn and easy accessible. These findings indicate that intensive involvement and guidance of parents during the training program is feasible, which has proven to be effective in generalization and maintenance of learned emotion recognition skills (30). In addition, our findings indicate an intrinsic motivation of children in the SCT training group to watch the animated series, as they reveal that children on average liked the training program from the beginning until the end. These results support the assumption that The Transporters training use intrinsically motivating animated media in a way that children like watching the episodes while learning about emotions in their context (27).

The current study was the first one, to our knowledge, to explore the effectiveness of a neurocognitive training in children with SCT. The inclusion of a training group and two control groups (a SCT waiting list group and a typically developing group), ensured the possibility to check for natural increases or learning effects in social cognitive functioning. Although further research is needed, the current results may contribute to improving clinical care in order to prevent negative long-term impact of SCT on social (cognitive) development. As neurocognitive training programs are easy accessible, cost-effective, and can be used without a clinical indication, support of early neurocognitive development can be preventively executed in home-based and school-based settings. Neurocognitive training programs can also be used as part of an integrative intervention program for young children with SCT at risk of specific social cognitive vulnerabilities, which have become visible based on individual neurocognitive assessment.

While the results of the present study are promising, future research is needed to address its limitations. First, the small sample size of this study especially when it comes to boys with 47, XYY limits the generalizability of the findings. Because of these small samples, we were not able to assess the specific contribution of karyotype (XXX, XXY, XYY) on the efficacy of the training program. Second, the present study only assessed post-training outcome, and did not have a follow-up period to investigate maintenance of the improved abilities and possible longer-term generalization effects. It remains for future studies to evaluate how support of early social cognition is related to functional outcomes, in order to prevent the detrimental impact of the presence of SCT. Replication is therefore necessary in future research, preferable in Randomized Control Trials studies with larger samples sizes and follow-up maintenance assessments, in order to investigate specific effects of neurocognitive training programs within the different karyotypes and longer term training effects. Another promising approach that could be used as a research method complementary to RCTs is the Single Case Experimental Design [SCED; (46)], the appeal of case-based time-series studies, with multiple assessments both before and after intervention. Benefits of SCEDs include the possibility to investigate the efficacy of early intervention in heterogeneous populations (e.g., populations with highly variable phenotypes such as SCT), and being able to test the effectiveness of treatment methods in the complex but real world practice of clinical work.

Taken together, the current study on the efficacy of a neurocognitive training in young children with SCT, an animated facial emotion training program, showed a significant improvement in facial emotion recognition abilities, with a large effect size. Moreover, encouraging results were found with respect to parents' and children's perception on the feasibility of the training program. These findings indicate that it is possible to (preventively) support the development of social cognition in children with an extra X- or Y-chromosome, which may reduce the negative long-term impact of SCT on social adaptive functioning. Additional research is warranted using a larger sample and follow-up maintenance assessments in order to further evaluate the effectiveness of the training for specific subtypes of SCT. Evidence based support of young children with SCT is of great importance given the need for preventive and early training programs, aimed to minimize neurodevelopmental impact.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

This study was approved by the Ethical Committee of Leiden University Medical Center, The Netherlands. Signed informed consent was obtained from the parents of all participating children, according to the declaration of Helsinki. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

NB: design, recruitment of participants, acquisition of data, analysis, interpretation of the data, and drafting. HS: conception, design, and final-approval of the manuscript. SvR: conception, design, interpretation of the data, and final-approval of the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2022.807793/full#supplementary-material

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Empowering Families Through Technology: A Mobile-Health Project to Reduce the TAND Identification and Treatment Gap (TANDem)

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Introduction: Tuberous Sclerosis Complex (TSC) is a multi-system genetic disorder with various TSC-Associated Neuropsychiatric Disorders (TAND) that significantly impact the mental health and wellbeing of individuals with TSC and their caregivers. TAND represents the number one concern to families worldwide, yet is highly under-identified and under-treated. The clinician-administered TAND-Checklist (Lifetime version, TAND-L) has improved identification of TAND in clinical settings. However, many individuals with TSC and their caregivers still have difficulty accessing suitable support for diagnosis and evidence-informed interventions. The TANDem study is a community-based participatory research project with a broad range of TSC stakeholders aimed at reducing the TAND identification and treatment gap.

Objectives: Participatory research identified three priority next steps: 1) development and validation of a self-report, quantified version of the TAND Checklist (TAND-SQ) and

building the TAND-SQ into a smartphone application, 2) generation of consensus clinical recommendations for the identification and treatment of TAND, to be incorporated as a TAND toolkit on the app, and 3) establishment of a global TAND consortium through networking, capacity-building and public engagement activities.

Methods: TANDem is a four-year project, and includes 24 consortium members from 10 countries representing all World Health Organization regions. Collaborators represent five stakeholder groups (family representatives, technology experts, clinical experts, non-profit organisations and researchers). Here we outline the project study protocol in detail, describing the scientific rationale, the project aims and objectives, the methods involved in participant recruitment, multi-site and multi-phase data collection, data analysis, ethical considerations including informed consent, data protection, privacy and confidentiality considerations related to the European Union General Data Protection Regulation and the USA Health Insurance Portability and Accountability Act. The expected outcomes and potential impact on the TSC community, implementation and dissemination of results, as well as future scale-up and scale-out plans are also discussed.

Conclusions: The TANDem project has the potential to transform the global TSC community by empowering families living with TSC through an easily accessible digital solution to allow them to document their own TAND needs linked to an evidence-informed toolkit to enhance personalised healthcare, and by providing healthcare professionals with consensus clinical recommendations to prevent, identify and manage TAND manifestations.

Keywords: tuberous sclerosis complex, TSC-associated neuropsychiatric disorders (TAND), digital technology, health app, personalised medicine, rare diseases, behavioural phenotypes

INTRODUCTION

Background

Tuberous Sclerosis Complex (TSC) is a rare genetic disease with multi-system manifestations including the brain, kidneys, skin, heart, and lungs (1). It has a birth incidence of approximately 1 in 5,000 to 10,000 live births (2, 3). Many have suggested that the prevalence of TSC has been underestimated due to factors such as under-recognition of less severe phenotypes, marked variability of symptoms and lack of genetic testing (4, 5). Appropriate management and coordination of medical specialist care are crucial throughout the lifespan of individuals with TSC to reduce morbidity and mortality (6, 7). Overall, clinical care has improved significantly over the last two decades, and clinical trials and evidence-based guidelines for many of the physical manifestations have led to better outcomes worldwide (7). TSC is also associated with a significant range of neuropsychiatric manifestations including developmental disorders (such as autism and intellectual disability), mental health problems (such as anxiety and mood disorders), scholastic difficulties, and specific neuropsychological deficits (1, 8). These neuropsychiatric manifestations lead to the greatest burden of care to families and are strongly related to psychosocial problems and family stress. Unfortunately, these are also the manifestations that are typically not identified and treated in TSC (1, 8–10).

In an attempt to raise awareness, to outline the multi-level manifestations, and to develop a 'shared language' for these neuropsychiatric manifestations we coined the term 'TAND' (TSC-Associated Neuropsychiatric Disorders) and recommended annual screening for TAND (7, 8). In response to the need of the TSC community, we next developed and pilot validated the TAND Checklist, a simple and free pen-and-paper checklist to guide conversations about TAND between clinicians and families (8, 11). The current version of the TAND Checklist (TAND Checklist-Lifetime version, TAND-L) has been implemented widely in the TSC community. At least 19 language translations of the TAND Checklist have been authorised to date and are available free-of-charge (www.tandconsortium.org) (8, 9). Unfortunately, the findings from the large-scale TOSCA natural history study of TSC showed that TAND manifestations

Abbreviations: ADOS-2, autism diagnostic observation schedule second edition; ADI-R, autism diagnostic interview revised; BCH, Boston Children's Hospital; BRIEF, behavior rating inventory of executive function; CBCL, child behavior checklist; CCH, Cincinnati Children's Hospital; COPPA, children's online privacy protection act; GDPR, general data protection regulation; GRADE, grading of recommendations assessment, development and evaluation; HIPAA, health insurance portability and accountability act; IQ, intelligence quotient; POPIA, protection of personal information act; TSC, tuberous sclerosis complex; TAND, TSC-associated neuropsychiatric disorders; TAND-L, TAND checklist (lifetime version); TAND-SQ, self-report, quantified TAND checklist; UZB, Universitair Ziekenhuis Brussel; UZL, Universitaire Ziekenhuizen Leuven.

were often not identified and psychiatric disorders were under-diagnosed or diagnosed late, even in expert TSC centres around the globe (10). Even though coining of the term 'TAND' and the development of the TAND Checklist have helped to raise awareness about TAND, the TOSCA findings therefore underlined the urgency of finding more empowering strategies to identify and treat TAND.

One of the real-life challenges of TAND is the fact that people with TSC seem to have vastly different and unique TAND profiles and, until recently, no data-driven investigations have been able to find any replicable TAND profiles. This observation of course underlines the importance of a personalised approach to treatment of TAND. Unfortunately, however, this 'overwhelming uniqueness' had also led to 'treatment paralysis' among clinicians (12). In order to manage the overwhelming uniqueness of individual TAND profiles, we proposed that data-driven methodologies would help us to identify a smaller number of 'natural TAND clusters.' In this context, a natural cluster is defined as a number of TAND manifestations that typically occur together. In a feasibility study of 69 individuals with TSC across all ages, we used TAND Checklist data and applied a range of cluster and factor analysis methods (13). We identified six natural TAND clusters, all with good face validity, and suggested that data-reduction may be feasible using the TAND Checklist (13). The findings were replicated in a slightly larger international study (14). We next applied comparable methods to an international group of 453 individuals with TSC from six international sites (including Belgium, South Africa, and the USA) and identified seven natural TAND clusters, very similar to the feasibility findings (15). Identified clusters included an autism-related cluster, a dysregulated cluster, an eat/sleep cluster, a mood/anxiety cluster, a neuropsychological cluster, an overactivity/impulsivity cluster, and a scholastic cluster (15). We proposed that these clusters could be used for psychoeducation of families and professionals and to develop tailored approaches to identification and treatment of TAND. In addition, we suggested that the different natural TAND clusters may point to potential differential aetiological underpinnings and responses to molecular and other treatments of TAND manifestations (16).

As part of the natural TAND cluster study, we incorporated focus group interviews with families from all over the globe. Focus groups were conducted in South Africa, USA, Europe and Australia with more than 50 TSC patients, parents/caregivers, family members and professional experts. Research partners included Tuberous Sclerosis International, European Tuberous Sclerosis Association, Tuberous Sclerosis Association (UK) and the TSC Alliance (USA). The main emphasis of the focus groups was to seek perspectives and recommendations from global TSC stakeholders about next step use of the TAND Checklist and natural TAND clusters (10).

Four main themes emerged from qualitative thematic analysis. Firstly, stakeholders felt that the TAND Checklist had provided the TSC community with a powerful tool to identify the range of TAND difficulties experienced by families on a daily basis. However, families expressed concern that the current version of the TAND Checklist was validated as an interview between a clinician and family. There was a clear desire to have a *self-report version* of the TAND Checklist that could be used by families even outside the context of a clinical visit. A number of families described how they had already used the TAND Checklist as a self-help tool to guide understanding of their family member's TAND profile. Secondly, stakeholders reported the desire for a *quantified version* of the TAND Checklist. Families described that the lifetime version of the TAND Checklist (TAND-L) focused on the lifetime occurrence of TAND manifestations. However, families were also keen to evaluate current needs and to quantify these needs, for instance, the severity of symptoms and/or their functional impact on daily life. Thirdly, a request from families was for a *toolkit of advice, information, recommendations, and general intervention strategies* to help them manage the TAND manifestations identified in their family member. This was the strongest and universal theme that emerged from the qualitative findings ('Can you tell us what we can do about TAND?'). Families described that most of them, regardless of where they were in the world, had struggled to access suitably-qualified therapeutic support, given barriers such as clinical care pathways, referral systems, waiting lists or funding. The fourth theme evident from data was a strong recommendation to use *digital technology* to make the TAND Checklist, quantification, and toolkit available to families across the globe (15).

Study Rationale, Aims, and Objectives

The aims of the TANDem project were shaped in partnership with the TSC user/caregiver community, and were therefore in direct response to the priority needs of individuals who live with TSC and their families. The need for TAND interventions had been articulated strongly in the TSC community, and recently also through a powerful participatory process funded by the King Baudouin Foundation in Belgium (17). Through a multi-step consensus-building process, a panel of people with lived experience in TSC and a panel of stakeholders with professional expertise in TSC generated 15 priorities for TSC research. In the study, TAND was identified as the most frequently endorsed area of concern. The first item on the priority list for research was the need for interventions for TAND, which will be addressed in the current project. In addition, the TANDem project will directly or indirectly respond to five other identified priorities: reducing the translational gap from research to real-world settings, determining additional evidence to be generated to substantiate treatment recommendations, early identification and treatment of manifestations, considering how (inter)national patient registries can be set up in sustainable ways, and identifying the most appropriate family support to guide acceptance and to mitigate the impact of TSC throughout the lifespan.

Given the significant identification, treatment and research gap for TAND, it was felt appropriate to address the priority requests from families and professional stakeholders through a multi-stakeholder participatory study, using a mixed-method approach.

The TANDem project aims and objectives are shown in **Table 1**. **Figure 1** provides a visual overview of the project.

TABLE 1 | TANDem project aims and objectives.

Aim 1: To develop and validate a self-report, quantified TAND Checklist (TAND-SQ), and to build it into a smartphone application (app).	Objective 1.1 Generate a self-report TAND Checklist
	Objective 1.2 Quantify the TAND Checklist
	Objective 1.3 Develop a smartphone app based on the TAND-SQ
	Objective 1.4 Validate self-completed TAND app data against expert clinical data
Aim 2: To generate consensus clinical recommendations for the identification and treatment of TAND, and incorporate these into the TAND app.	Objective 2.1 Scoping review of existing literature on interventions for TAND
	Objective 2.2 Generate consensus clinical recommendations for identification and treatment of TAND
	Objective 2.3 Generate a TAND toolkit based on literature and consensus recommendations
	Objective 2.4 Integration of the TAND toolkit into the TAND app
	Objective 2.5 Feasibility evaluation, including acceptability and appropriateness, of the final TAND app
Aim 3: To build a scalable and sustainable global TAND consortium through networking, capacity-building and public engagement activities.	Objective 3.1 Conduct networking activities between all global collaborators
	Objective 3.2 Capacity-building of emerging TAND researchers
	Objective 3.3 Public engagement activities to understand societal perspectives on TSC and TAND and to raise awareness of TAND and TSC
	Objective 3.4 Perform a multi-stakeholder review of the TAND app and integrated toolkit
	Objective 3.5 Plan and coordinate scale-up, scale-out and future TAND research

METHODS

TAND Consortium and Working Groups

TAND Consortium

The TAND consortium includes 24 members from 10 countries, representing all World Health Organization regions. Members include a broad range of stakeholder groups (including family representatives, non-profit organisations, researchers, technology experts, clinicians, social scientists, statisticians) and bring highly interdisciplinary skills to the project (including child and adolescent psychiatry, pediatric neurology, clinical psychology, educational psychology, speech and language therapy, special education, intellectual disability medicine, nephrology, biomedical engineering, biostatistics, veterinary sciences, behavioral sciences, neurosciences, and digital technology expertise).

This collaboration between individuals with lived expertise and professional expertise leads to cross-fertilisation and new synergies between established and young TSC clinicians and researchers, professionals from outside the TSC community, and family representatives from different parts of the world. The global map in **Figure 2** shows all consortium members and their geographical locations. The TAND consortium work together in various working groups, as illustrated in **Figure 3** and described under Working Groups.

Working Groups

Principal Investigator and Co-principal Investigator

The consortium is led by the principal investigator Petrus de Vries and co-principal investigator Anna Jansen who are responsible for the successful running and completion of the project. They are also responsible for all aspects of project oversight, financial management, human resource management, and ethical conduct of the TANDem project.

Action Group

The Action Group includes Petrus de Vries, Anna Jansen, Liesbeth De Waele (dissemination lead), Tosca Heunis (project coordinator), and Stephanie Vanclooster (postdoctoral researcher). They are responsible for the day-to-day running of the project and for coordination between all groups in the project. The Action Group meets on a weekly basis to discuss project matters.

Steering Group

The Steering Group includes all members of the Action Group as well as Chris Kingswood (family representative and representative of the Tuberous Sclerosis Association UK) and Shoba Srivastava (family representative and member of Tuberous Sclerosis Alliance of India). The Steering Group is responsible for oversight of the activities in the TANDem project and, in particular, provide a forum to bring a user/caregiver voice to the project.

Technology Group and Technology Partner

The Technology Group is led by the project coordinator, Tosca Heunis. The group includes Liesbeth De Waele, Chris Kingswood, Aubrey Kumm, and Peter Davis. The Technology Group, in close collaboration with the app development company, is responsible for all aspects related to development of the TAND Toolkit App. The app development requirements are further discussed under App Development.

Cluster Groups

The Cluster Groups are overseen by the postdoctoral researcher, Stephanie Vanclooster. Eight cluster groups were created to develop consensus recommendations for the assessment and treatment of TAND in TSC. Each cluster group (see **Table 2**) has a lead, a co-lead, and additional members, with at least one family representative. Cluster Groups are responsible for conducting a literature review and drafting of a 'cluster chapter' that focuses on the assessment and treatment of their assigned TAND cluster. These cluster chapters will form the basis of: (1) a book for families and practitioners (most comprehensive), (2) consensus clinical recommendations for TAND (summary of chapters), and (3) a toolkit to be built into the TAND Toolkit App (practical and specific elements from the consensus recommendations). The toolkit development is discussed further under Toolkit Development.

Validation Group

The Validation Group is responsible for the validation of the app-based TAND-SQ Checklist (a self-report, quantified version of

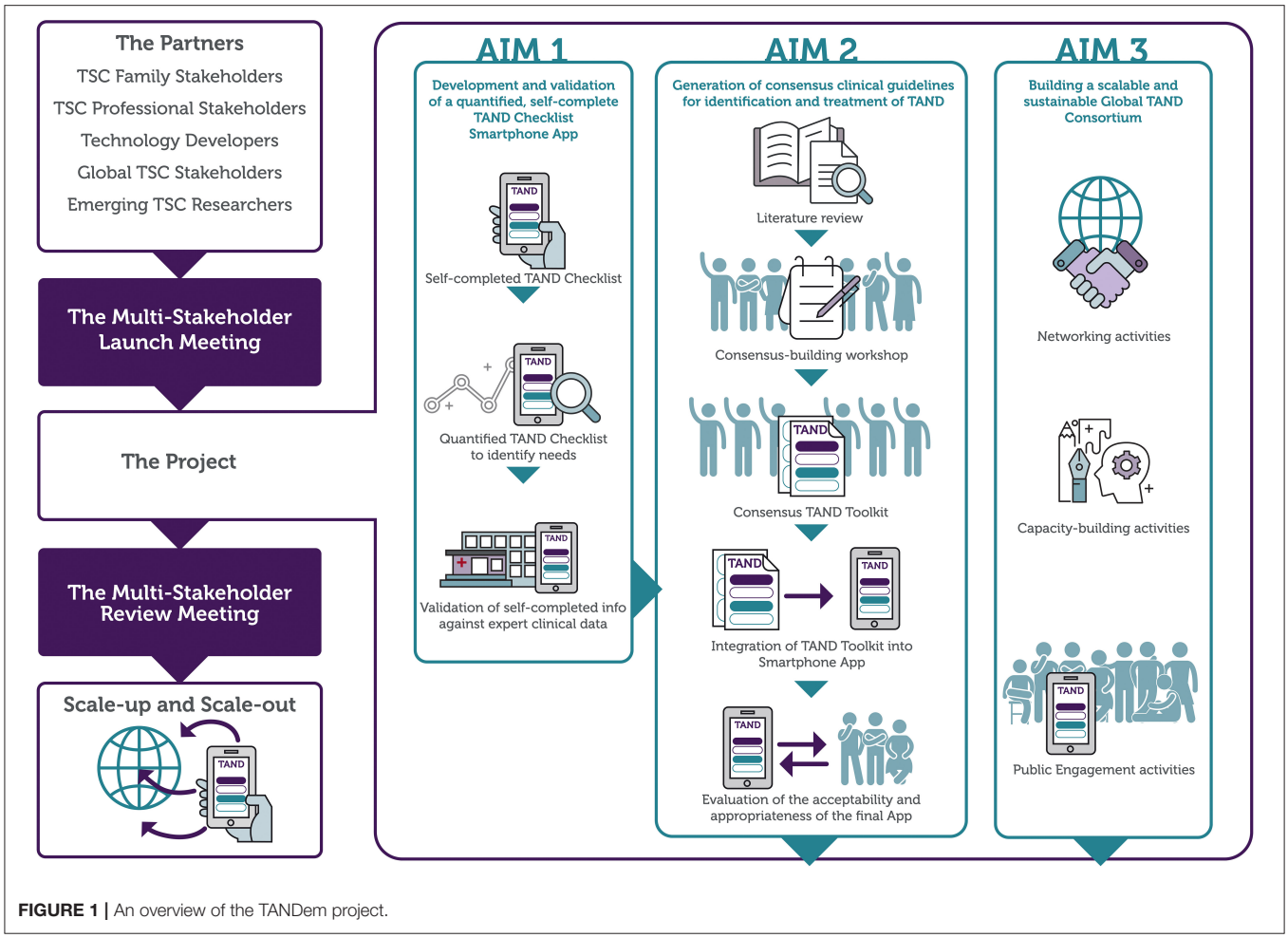

FIGURE 1 | An overview of the TANDem project.

the TAND-L Checklist) against expert clinical data. It includes the Steering Group along with Mustafa Sahin, Peter Davis, Darcy Krueger and representatives from the three validation sites at Boston Children's Hospital, Cincinnati Children's Hospital, and the TSC Alliance.

Emerging Researchers Group

Given that research mentoring and capacity building are goals of the TANDem project, a separate group was set up for self-identified emerging/early career researchers. This group is led by Stephanie Vanclooster. She is joined by Agnies van Eeghen, Peter Davis, Sebastián Cukier, Shoba Srivastava, Stacey Bissell, Tanjala Gipson, and Tosca Heunis. Their meetings are used as a forum to talk about career development, research and mentoring ideas.

Dissemination Group

This group is responsible for communication and dissemination of information about TAND and the TANDem project. The project has a website (www.tandconsortium.org), YouTube Channel and Twitter account. This group is led by Liesbeth De Waele and includes the Action Group and various other consortium members (Agnies van Eeghen, Aubrey Kumm, Jamie Capal, Katie Smith, Shoba Srivastava, Stacey Bissell, Sugnet Lubbe, and Tanjala Gipson).

Family Representatives Group

The TANDem project was fundamentally set up as a participatory project with multilevel stakeholders. For this reason, it was important to ensure that people with TSC and families who live with TSC have a clear and strong voice at all levels of the project. The Family Representatives Group therefore includes all TANDem members who have a lived experience with TSC: Chris Kingswood, Eva Schoeters, Jennifer Flinn, Megumi Takei, and Shoba Srivastava. Katie Smith, a global Tuberous Sclerosis International representative, is also part of this group. All of them wear multiple 'hats' as part of the project, either as scientists, educators, clinicians, or as leaders in global non-profit organisations. Family representatives are part of all other groups in the project to ensure that the project will deliver direct benefits to the TSC community.

App Development

The TAND Toolkit App will allow users to register in a secure way and to add a number of members to their 'TSC family' profile. Once registered, users will be able to capture a

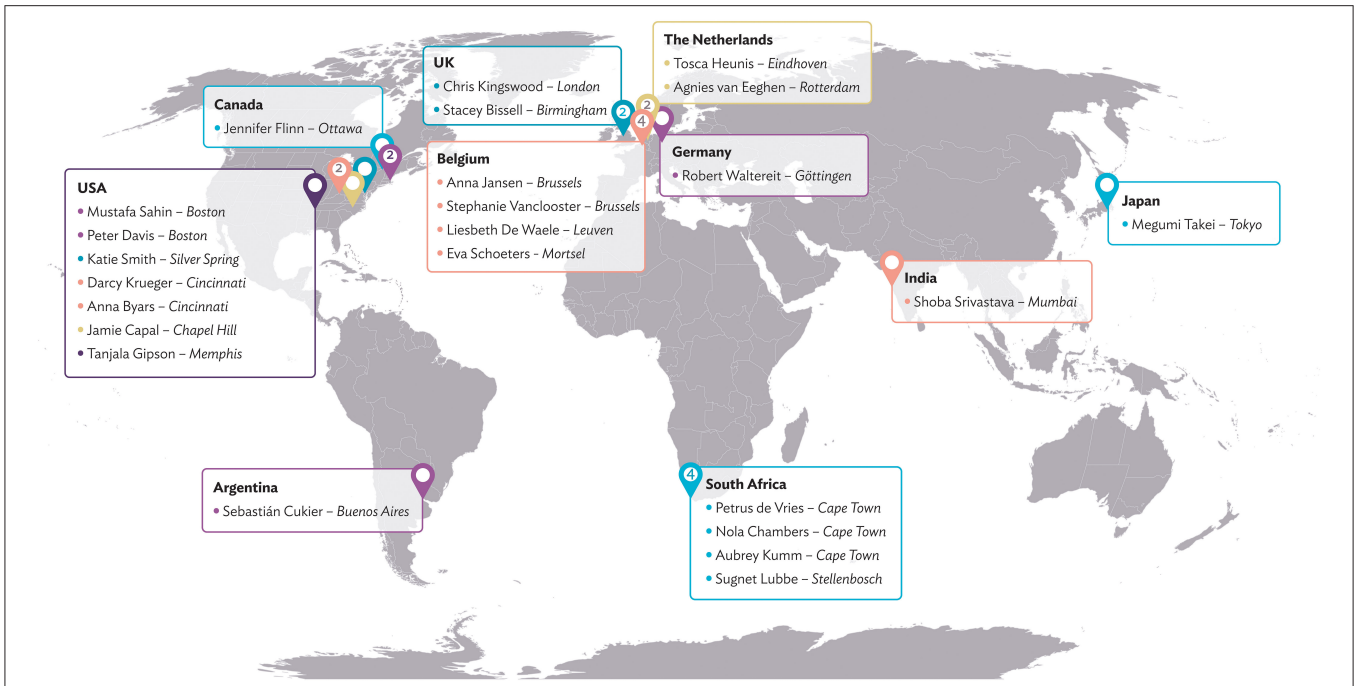

FIGURE 2 | TAND consortium.

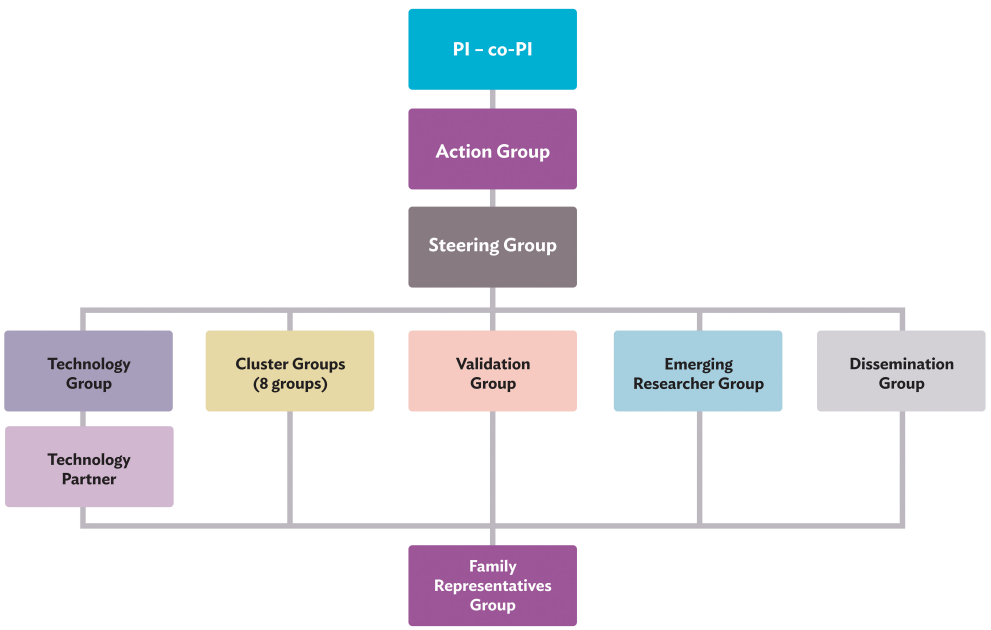

FIGURE 3 | TAND consortium working groups. PI, principal investigator; Co-PI, Co-principal investigator.

'TSC Story' (information about the TSC diagnosis), complete a self-report, quantified version of the TAND Checklist—the TAND-SQ (to identify and quantify the severity of TAND difficulties experienced), view the 'TAND clusters' profile (that illustrates how different TAND clusters are affected), and to view the 'TAND toolkit.' The TAND toolkit will provide evidence-informed information about each cluster, including an introduction to the cluster, what families should seek (e.g., assessment, diagnosis, and treatment by a professional), and what families can do themselves (e.g., self-help, home-based actions, strategies and interventions tips). The app also has a functionality to allow 'surveys' to be done (to collect

additional information from users about various aspects of TAND) at any stage during the use of the app. App development was divided into two phases—phase I of the app includes all development except for the toolkit, which forms part of phase II. The app will be accessible on Android and iOS mobile devices.

Toolkit Development

We will perform a scoping review of all primary literature on interventions for TAND using a standard scoping review methodology (18). The scoping review will serve two purposes: first, to describe the landscape of all TAND research to date in order to identify research gaps in the science of TAND; second, to act as background literature for the formulation of consensus clinical recommendations. In addition, cluster groups will perform expert targeted reviews of the TSC literature and of the broader scientific literature relevant to clusters. Each cluster group will draft a chapter to summarise the background literature and scientific evidence and will generate summary statements and recommendations based on the TAND literature and their expertise. Cluster chapters will be reviewed by two reviewers and presented to the consortium. Cluster chapters will represent a second source of evidence-base towards consensus recommendations development. All cluster summary statements and recommendations will be entered into an online form and consortium members will be asked to rate and rank statements and recommendations. These data will identify items with clear agreement/disagreement, in preparation for face-to-face discussions in a virtual consensus conference. Overall recommendations will be evaluated using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) framework rating tools (19).

Once consensus recommendations have been generated, a process will start to develop the 'toolkit' to be built into the app. The toolkit will include practical, useful yet evidence-informed information, advice and resources relevant to each cluster. Toolkit development will be supported by a series of 'creative sessions' with each cluster team to help translate consensus recommendations into app screens. All cluster app screens will be reviewed and revised by cluster teams and the whole consortium. On completion of revised cluster app screens, all information will be shared with the app development team to incorporate into the TAND Toolkit App, phase II of app development.

Study Design and Implementation

Study Design

The TANDem project is a multi-stakeholder participatory project that has included TSC family stakeholders from the project outset—individuals diagnosed with TSC, and parent/caregivers/families of individuals with TSC. As previously mentioned, TSC family stakeholders have already been instrumental in the prioritisation of TAND research goals and in focus group work to seek directions for TAND Checklist research. This 'lived expertise' input directly led to the project outlined here.

All TSC family stakeholders have participated in the multi-stakeholder launch meeting where the project was discussed in detail. Throughout the 4 years of the project, family stakeholders will participate in achieving the three project aims (see **Table 1**). In addition, all stakeholders will participate in the review meeting at the end of year three, where the final TAND Toolkit App and the TAND toolkit will be reviewed and where planning for 'scale-up and scale-out,' and future TAND research will take place.

Family stakeholders and technical partners will be treated equally for their time and input to the project. It should be clear that the philosophy of this project is one of 'nothing about us without us' (20). We firmly believe that only when 'lived expertise' and 'technical expertise' is brought together, truly impactful research in TSC can be conducted.

TABLE 2 | Cluster groups and members.

Autism spectrum disorder-like cluster	Nola Chambers (lead), Jamie Capal (co-lead), Eva Schoeters, Sebastián Cukier, Shoba Srivastava
Dysregulated behavior cluster	Tanjala Gipson (lead), Peter Davis (co-lead), Agnies van Eeghen
Eat/sleep cluster	Stacey Bissell (lead), Katie Smith (co-lead), Peter Davis
Mood/Anxiety cluster	Agnies van Eeghen (lead), Jamie Capal (co-lead), Megumi Takei, Robert Waltereit
Neuropsychological cluster	Anna Byars (lead), Jennifer Flinn (co-lead)
Overactive/impulsive cluster	Robert Waltereit (lead), Stacey Bissell (co-lead), Katie Smith, Megumi Takei
Psychosocial cluster	Stephanie Vanclooster (lead), Sebastián Cukier (co-lead), Chris Kingswood, Eva Schoeters, Katie Smith,
Scholastic cluster	Jennifer Flinn (lead), Peter Davis (co-lead), Shoba Srivastava

Study Population and Recruitment

The study population of this project consists of children, adolescents and adults diagnosed with TSC and their families (parents/caregivers and other family members). No children below the age of 13, individuals with intellectual disability or other groups of vulnerable participants will be included as direct participants in this study. However, to ensure applicability of the study to these groups, parents/caregivers of younger children and adults with intellectual disability will be invited to participate.

This study will be conducted in partnership with five organisations: Boston Children's Hospital (BCH, USA), Cincinnati Children's Hospital (CCH, USA), the TSC Alliance (USA), Universitair Ziekenhuis Brussel (UZB, Belgium), and Universitaire Ziekenhuizen Leuven (UZL, Belgium).

Recruitment will be done through the primary clinical contacts at the respective sites. The clinicians and their teams who will invite the participants all have expertise in working with patients with TSC, are all familiar with the clinical and personal needs of the families, and are therefore best positioned to determine whether study participation is appropriate. They are also all members of the TAND consortium and familiar with the TAND Checklist under investigation here.

Sample Size

The TANDem project will collect data in five steps (as outlined under Data Collection and Analysis). Pre-pilot data steps (1, 2, and 4) will include small sample sizes and will focus on feasibility of measures and methods. Step 3 (validation step) will include TAND-SQ data collection via the app and through a secure electronic portal, and comparison thereof with retrospective expert clinical/research data. Data will be used to replicate and expand previous cluster findings (13, 14, 16) and to examine the sensitivity, specificity and positive predictive value of the TAND-SQ in relation to expert clinical data. There is no consensus in the cluster analysis literature about the ideal sample size. With rare and extremely rare diseases the use of standard statistical methodology for sample size determination is not practical as the condition affects only a very small number of individuals. There is, however, reasonable consensus that a variable to sample ratio of 1:10 is good (i.e., if 10 variables = 100 participants) (13–15). The TANDem project will use 19 variables in analysis, suggesting a sample size of 190 participants. Given that we will have two different 'kinds' of data for validation (app data and portal data), the final sample size was set at $n = 100$ (portal data linked to real world clinical data), and $n = 100$ (app data linked to detailed research phenotyping data). Step 5, feasibility evaluation of the TAND Toolkit App, will be performed as a mixed-methods step with some quantitative data, but will predominantly involve qualitative data collection through individual interviews and/or focus group discussions. The sample size for this step was set at n = 40. We hope to collect data on a larger sample, but acknowledge the logistical, data transfer and ethical challenges of data collection across international borders.

Data Collection and Analysis

Overview of Study Steps

There are five steps of data collection and analysis involved, as outlined in **Table 3**. This table provides a summary of the participants, data collection sites, data to be collected, and data analysis to be conducted.

Step 1: The TAND-SQ Pre-pilot Study

Each participant and consortium member will be invited to complete a paper TAND-SQ Checklist, and to share their feedback via a checklist feedback form. Data will be used to perform mixed-method analysis of feasibility data. This will inform final TAND-SQ design.

Step 2: The App Phase I Pre-pilot Study

Each consortium member will conduct user acceptance testing of phase I of the app (i.e., all app functionality stated under App Development, excluding the 'TAND toolkit'), and complete an app feedback form. Each consortium member will collate their additional feedback on the app, e.g., screenshots and specific comments, in a PowerPoint or Word document and submit this to the project coordinator. All feedback collected will be reviewed by the Technology Group, Family Representatives Group and Action Group, either for immediate implementation or for future 'scale-up and scale-out' efforts (which fall beyond the scope of this current project). All feedback related to the assurance of data quality and integrity and optimal user experience will be implemented in the app before data collection step 3 commences to ensure that the app is built for accurate data collection and optimal user experience.

Step 3: The TAND-SQ Validation Study

Data collection from BCH and CCH participants will take place via the app, they will complete a TSC Story, TAND-SQ Checklist and an app feedback form. Pseudonymised app data will be linked to de-identified detailed phenotyping data collected at BCH/CCH as part of an ongoing rare diseases study (21). The de-identified data will encompass a broad range of phenotyping data including (but not limited to) standardised rating scale measures e.g., the Behavior Rating Inventory of Executive Function (BRIEF) (22, 23), and the Child Behavior Checklist (CBCL) (24, 25), formal Intelligence Quotient (IQ), neuropsychological and scholastic measures, as well as standardised clinical diagnostic tools e.g., the Autism Diagnostic Observation Schedule Second Edition (ADOS-2) (26, 27), and the Autism Diagnostic Interview Revised (ADI-R) (28).

Participants from the TSC Alliance will complete a TAND-SQ Checklist through a secure electronic portal. TAND-SQ data collected through the TSC Alliance portal will be linked to real-world clinical TAND data which is also collected through the portal as part of an ongoing longitudinal natural history study (29). Data will be shared with the study team through a secure electronic portal.

Data will be analysed to establish the external and predictive validity of the TAND-SQ in relation to two different datasets—one a 'real-world' clinical dataset, and the other a highly standardised research dataset. We plan to use expert data to code participants for each natural cluster (both categorically and in a quantified form) blind to the TAND-SQ data. We will then compare these data (deep phenotyping and clinic-derived) to the automated classifications from the TAND Toolkit App. In addition, we will examine external validity by comparing TAND-SQ cluster domain scores with specific instrument scores such as the BRIEF, CBCL and so on. The key question that we want to answer is whether or not the TAND-SQ Checklist is sensitive enough to identify TAND difficulties that had been picked up by expert evaluation. There is less of a priority on specificity, given that this is a *screening* tool (to lead to next-step actions) rather than a *diagnostic* tool.

Feedback form data will be analysed using descriptive and summative analysis to examine the clarity, comprehensiveness and ease of use of the app. These data may indicate user-interface or technical issues that may require further changes to be made to the app before continuing with step 4, App Phase II Pre-pilot Study.

All TAND-SQ data will be used to replicate and expand previous cluster work. We will apply the same clustering analysis technique developed by Leclezio et al. (13), de Vries et al. (14), and Leclezio (15) to explore the natural TAND clusters identified through TAND-SQ data. This will allow an opportunity to compare natural clusters derived from TAND-L data to TAND-SQ data. In addition, TAND-SQ data will be used to explore

TABLE 3 | Steps of data collection and analysis.

Step	Description	Participants	Data collection	Data analysis
Step 1	TAND-SQ pre-pilot study	± 20 participants ± 20 TAND consortium members	Each participant and consortium member will complete a paper TAND-SQ and a checklist feedback form.	Mixed-methods analysis of feasibility data. This will inform final TAND-SQ design.
Step 2	App phase I pre-pilot study	± 20 TAND consortium members	Each consortium member will conduct user acceptance testing of phase I of the app, and complete an app feedback form.	Mixed-methods analysis of app feedback data. This will inform final app design before validation (step 3).
Step 3	TAND-SQ validation study	± 100 participants from BCH ± 100 participants from CCH ± 100 participants from the TSC Alliance	BCH and CCH participants will complete a TSC Story, the TAND-SQ Checklist, and an app feedback form. TSC Alliance participants will complete the TAND-SQ Checklist via a secure electronic portal.	BCH/CCH data will be used to evaluate the external and predictive validity of the TAND-SQ by comparing app data to detailed phenotypic data collected as part of a TSC research project (21). TSC Alliance data will be used to evaluate the external and predictive validity of the TAND-SQ by comparing self-reported TAND-SQ data through the online portal with real-world clinical TAND data, also collected through the portal. All TAND-SQ data will be used to replicate and extend previous cluster findings. All feedback form data will be used for descriptive and summative analysis.
Step 4	App phase II pre-pilot study	± 20 TAND consortium members	Each consortium member will conduct user acceptance testing of phase II of the app, and complete a toolkit feedback form.	Mixed-methods analysis of toolkit feedback data. This will inform final app design before feasibility evaluation.
Step 5	App feasibility evaluation study	± 40 participants from BCH, CCH, TSC Alliance, UZB, UZL	Each participant will conduct user acceptance testing of the full app (phases I and II), complete an app feedback form, a toolkit feedback form, and participate in a focus group/semi-structured interview.	Mixed-methods analysis of app and toolkit feedback data and framework analysis of qualitative data.

BCH, Boston Children's Hospital; CCH, Cincinnati Children's Hospital; UZB, Universitair Ziekenhuis Brussel; UZL, Universitaire Ziekenhuizen Leuven.

how quantified cluster data could be utilised in the app and in future research.

Step 4: The App Phase II Pre-pilot Study

Each consortium member will conduct user acceptance testing of phase II of the app (i.e., the 'TAND toolkit') and complete a toolkit feedback form. Each consortium member will collate their feedback on the 'TAND toolkit,' e.g., screenshots and specific comments, in a PowerPoint or Word document and submit this to the project coordinator. The toolkit feedback form will be completed via a secure online portal. All feedback collected will be used to inform final app design, and will be reviewed and prioritised by the Technology Group, Family Representatives Group and Action Group, for either immediate implementation or prioritisation for future 'scale-up and scale-out' efforts. Changes requiring immediate implementation will be completed before proceeding with step 5, feasibility evaluation.

Step 5: The App Feasibility Evaluation Study

Each participant will conduct user acceptance testing of the full app (phase I and II), complete an app feedback form and toolkit feedback form. Each participant will also participate in a focus group / semi-structured interview, either in person or via a remote communication tool. Simple descriptive statistics will be used to summarise the quantitative comments, and thematic analysis will be used to summarise the qualitative comments. See Leclezio et al. (11) for detail of this methodology. The results from this feedback will be used to prepare 'scale-up and scale-out' recommendations for future improvement and implementation of the TAND-SQ Checklist and the TAND Toolkit App. The survey functionality in the app will also be used in future to get feedback from families, allowing us to learn more about TAND. It would be possible to add a short survey to, for instance, ask families to rate and provide feedback on specific toolkit recommendations.

Data Protection, Management, Storage, and Transfer

Given that this is a multisite project, with data collection sites located in the USA and Belgium, and data processing sites located in Belgium and South Africa, various data privacy regulations need to be considered: the Health Insurance Portability and Accountability Act (HIPAA) (30) and the Children's Online Privacy Protection Act (COPPA) (31) of the USA, the General Data Protection Regulation (GDPR) (32) of the European Union, and the Protection of Personal Information Act (POPIA) (33) of South Africa.

At present, the GDPR is seen as the most stringent standard for data protection. All data collection, protection, management, storage and transfer will be handled in strict compliance with the GDPR (32). The TAND Toolkit App will collect limited personal data, such as first name, year of birth, sex, personal pronouns and country. The app will not collect any personal identifiers as defined by HIPAA (30), such as full name, date of birth,

address, email address, mobile contact numbers or device serial numbers. In accordance with HIPAA (30), the limited personal data that the app will collect does not meet criteria for identifiable personal health information—the app is therefore exempt from HIPAA. COPPA relates to online data collection directly from minors under the age of 13 years (31). In the TANDem study, no children under the age of 13 years will use the TAND Toolkit App themselves, their families/caregivers will be invited to participate in the study and complete the app about their child; COPPA thus does not apply. POPIA is also not applicable as we will not be collecting any data from participants in South Africa (33).

During the course of the 4-year project we will continue to ensure that the app and the research it enables remain compliant with the highest level of data protection and security, in accordance with the GDPR. Informed consent is the legal basis for collecting, sharing and processing data. Risks of reidentification of individuals are mitigated through several processes. Pseudonymised data will be collected and transferred between the data providers and data processors. A unique user code will be assigned to each app user by the respective data collection sites (data providers). This user code will be used as the username, along with a user-chosen password, to log in to the TAND Toolkit App. Data processors will use this unique user code to link app data with expert clinical data for data analysis purposes, as described in step 3—the TAND-SQ Validation Study. A master key with identifiable information will be kept at the primary data collection sites. No personally identifiable information will be shared by the data providers. Data will be shared with the data processors through a secure electronic portal. All participating data collection sites (data providers) and recipient sites (data controllers and data processors) will sign a data transfer agreement. All researchers participating in data collection will have completed Good Clinical Practice training and will be bound by the clinical rules of ethics, including confidentiality of patient information.

The app itself will also be designed for GDPR compliance. It will be password protected and all necessary encryption protocols will be implemented by the app developers. Users will have complete control over and access to their own data, and will be able to delete their data from their mobile device and the data storage server. An electronic informed consent, privacy policy and terms of use will be developed for the app. The electronic informed consent is a requirement for both research ethics and GDPR, it describes the study objectives and expected outcomes, the risks and benefits of participating in the research, the option to withdraw consent at any time, and other details. The privacy policy stipulates how personal data is protected, what types of data are collected in the app, who are the data controllers, how the data will be processed and stored, and what the rights of the app users are with respect to the privacy of their data, etc. The terms of use is the agreement between the app user and the app owner, and explains what the app does, who owns which app content, how the content can be used by the app user and by the app owner, etc. Each participant or consortium member will be required to read and accept the electronic informed consent, privacy policy, and terms of use (accessed in the app) before they will be allowed to register as app users in the TAND Toolkit App. All app data will be hosted on GDPR compliant servers, and will be stored securely and indefinitely. All other research data will be stored securely for 10 years, or as per the ethics guidelines of the respective data collection sites.

Table 4 lists some important considerations when developing a GDPR compliant app for research purposes. Please note that this is not an exhaustive list of all requirements. The local research ethics committee and data protection officer will be able to provide guidance with conducting a data protection impact assessment and developing a data management plan. A data protection officer and a lawyer specialised in data protection legislation will be able to provide guidance with developing the data sharing agreement and the data processing agreement. The data management plan outlines the full life-cycle of the data, who the data collectors/providers are, who the data recipients/processors are, details on how the data will be managed, transferred and stored securely, what risks are involved, and how these risks will be mitigated. A data sharing agreement stipulates the purpose of data sharing, how the data will be used, what data will be shared, who the data providers, data controllers and data processors are, and what their respective data protection responsibilities are. The data processing agreement stipulates the scope and purpose for which the data will be used and how the data will be processed, as well as the data protection responsibilities for each data controller and data processor involved. These are legally binding agreements that need to be signed by all parties involved in the study.

Ethical Considerations

Participant Vulnerability

The TANDem project will include participants who may be considered to be of medium vulnerability. This includes adults with TSC (a rare genetic disease), adults with some degree of intellectual or developmental disability and young people 13–18 years of age. As outlined in this paper, all research will be led through expert TSC centres and organisations and all research recruitment will be done through these organisations. We are therefore confident that participants and families will be invited and selected in a manner sensitive to their level of potential vulnerability. In the spirit of distributive justice, it was important not to exclude young people and adults with developmental disabilities from this study, as they represent a fundamentally important stakeholder group in this study. For adults with more significant disabilities and children under the age of 13, we will recruit parents/caregivers to participate on their behalf.

Participant Risks

Given that all the research work will be qualitative and participatory, this project meets criteria to be regarded as a minimal risk study. As outlined in **Table 3**, participants will be asked to complete a paper TAND-SQ Checklist and checklist feedback form, and/or complete the TAND Toolkit App (capture a TSC Story, TAND-SQ Checklist, and an app feedback form), and/or complete the TAND-SQ via an online portal, and/or complete a toolkit feedback form, and/or participate in a focus group / semi-structured interview. Participants will also give permission for their retrospective medical records to be accessed

TABLE 4 | Considerations when developing a GDPR compliant app for research purposes.

1.	Conduct a data protection impact assessment to ensure that all risks are identified, assessed and mitigated
2.	Determine which data protection regulations are applicable based on the locations of all data collection and data processing sites involved
3.	Create a data management plan
4.	Create a data sharing agreement
5.	Create a data processing agreement
6.	Search for app developers who have expertise in developing and hosting apps in compliance with the GDPR
7.	Sign a non-disclosure agreement with the app developers and a contract that ensures that one retains ownership of the app, intellectual property and data
8.	Develop the app on an open-source platform rather than a proprietary/exclusive platform; this will allow one to more easily transfer the app development/support to another service provider in future should it be required
9.	Develop an electronic informed consent for the app; in the app the app user must be able to view and download/print this document
10.	Develop a privacy policy for the app; in the app the app user must be able to view and download/print this document
11.	Develop terms of use for the app; in the app the app user must be able to view and download/print this document
12.	The first step in the app is to have prospective app users read and agree to the electronic informed consent, privacy policy, and terms of use. Only once app users have provided this consent can any app user registration and other data be captured
13.	The app should be password protected
14.	The app and data administration panel must implement the necessary encryption protocols and strategies for data protection and security
15.	App users must be able to control, access and delete all of their own app data on the mobile device and the storage servers
16.	Multifactor authentication must be implemented for all means of accessing captured/stored data via the app data administration panel or secure cloud storage solution
17.	Ensure that the location of the app hosting servers and the applicable data protection legislation in that country/state/region meet the requirements for GDPR compliance
18.	Retain separate staging (testing) and production environments of the app data administration panel, this way all ongoing iterative development and testing can be done in the staging environment without affecting the app and live data in the production environment
19.	Ensure that only the data controllers/processors have access to the live (real person) data in the production environment. If support is required from an app developer, server manager, or other third party, ensure that a sufficient data processing agreement has been signed by all parties involved

and pseudonymised data to be shared for the analysis goals of this study. Importantly, the information in the app will also be pseudonymised on the app storage servers and will not collect any personal identifiers.

No interventional procedures will be performed. No children below the age of 13 will be included as direct project participants. Therapeutic misconception will be avoided at recruitment, because the purpose of the study will be made clear to families on recruitment and in the terms of use of the app. No other prospective evaluations or interventions will be performed.

Informed Consent

All consortium members and participants will be asked to provide informed consent before data collection. Consortium members will be provided with information sheets and informed consent forms by the postdoctoral researcher. Requesting informed consent from consortium members signals the participatory nature of this project, thus allowing, for example, consensus discussions to be recorded, collected and analysed in a systematic way as 'data' of this study.

At the clinical research sites (BCH, CCH, UZB, and UZL), individuals with TSC and/or their families/caregivers will be invited by the TSC clinic team to participate. The objectives, study set-up and expected outcomes of the project will be explained in detail to each prospective participant guided by the informed consent document. Individuals will be given time to think about whether or not they would like to participate, and to discuss the matter with friends/relatives. At any time, individuals with TSC and family members can ask questions or make comments.

Informed consent documents will be available in the native languages of the participants to be enrolled. The USA sites will conduct work in English. For the Belgian sites, the informed consent documents will be available in English and Dutch. During this project, the TAND Toolkit App and all feedback forms will only be available in English. Qualitative interviews will, however, be conducted either in English or Dutch, based on the preference of participants. Participants will be able to withdraw their consent at any stage. Parents/caregivers and individuals over 18 years will be asked for written informed consent. Individuals between the ages of 13 and 18 years will be asked for written assent with support from their parents/caregivers.

Individuals completing the app will also be required to provide electronic consent that they have read and understood the electronic informed consent, privacy policy, and terms of use documents within the app before being allowed to register as app users.

For data collection taking place through the TSC Alliance electronic portal, individuals will provide informed consent via the portal. Families/Caregivers of already consented participants of the TSC Alliance database will receive individual email invitations to participate. They will be able to complete the TAND-SQ Checklist online through the portal, and data will automatically be captured and saved to the TSC Alliance database.

DISCUSSION

In Need of Multidisciplinary Phenotyping and Treatment Design for Psychopathological and Neurocognitive Disorders in Genetic Syndromes

There are many thousands of rare genetic diseases where significant progress has been seen in recent decades in understanding of the molecular pathways in many syndromes, and in the identification and treatment of the physical phenotypes/manifestations associated with these syndromes. However, many genetic syndromes are also associated with a wide range of behavioural, psychiatric, intellectual, academic, neuropsychological and psychosocial difficulties and disorders. In the majority of syndromes these are highly under-identified and under-treated. This manuscript forms part of a special issue on the need of multidisciplinary phenotyping and treatment design for neuropsychiatric disorders in genetic syndromes—and the rationale for this pressing need is clear.

In this protocol paper, we focused on Tuberous Sclerosis Complex (TSC) and TSC-associated neuropsychiatric disorders (TAND) as example. Through participatory research with family stakeholders in the TSC community, we were able to identify priority areas to empower families and people living with TSC towards improved (self-)phenotyping, and to use technology as a tool to direct them to educational information, links and resources that would ultimately support patient/individual-centred health care. This is the primary aim of the TANDem project. Recognising the limited 'evidence-base' for interventions in TSC (and other genetic syndromes), our second aim is to generate consensus clinical recommendations for identification and intervention of TAND manifestations. We hope that this will also empower clinicians with up-to-date knowledge and information to support their clinical decision-making, which we acknowledge can be very complex in the context of rare genetic syndromes. Recognising the need for a 'next generation' of TAND researchers and for expanded knowledge and awareness about TAND in the broader community, our third aim is to have a strong focus on research capacity-building, public engagement and creation of a digital infrastructure for future research.

Impact on the TSC Community

The TANDem project has the potential to transform the landscape for TAND identification and intervention at a global level. A well-designed app can be made accessible internationally to empower families with relevant and up-to-date knowledge about TSC and TAND. An app provides the opportunity to translate information into various languages in future scale-out, and the toolkit can be updated as new evidence becomes available. Even without the technological elements of the project, a self-report, quantified TAND Checklist will provide a validated tool to support families in profiling, monitoring and intervening with TAND, while additional help is sought. Given that the burden of TAND is the most pronounced aspect of TSC and given the priority of interventions for TAND, empowering families with self-help tools, skills and information around TAND will have a major impact on their quality of life, quality of care and social participation. This project is designed in partnership with family stakeholders and a wide range of other stakeholders to ensure that the impact of the research will be direct, immediate, ongoing and relevant.

Apart from the direct benefits to individuals with TSC and their families, the project will create a digital infrastructure to collect big data that will allow for further optimisation of the TAND toolkit, for better TAND management for individuals with TSC and their families and for fueling global TAND research in the future. The digital technology, as will be developed in the TANDem project, will therefore create the potential for large-scale (and self-guided) phenotyping, that in turn can inform large-scale (and self-guided) intervention planning. **Figure 4** shows the impact loop of the overall study.

Future of the App

The overarching goal of this project is to develop an app for the TSC community with a good evidence-base and with accurate self-help information and consensus recommendations for the identification and intervention of TAND. During the 4 years of the project, the app will be used purely within the TAND consortium and will not be available to access by the public. One of the goals of the project in year 4 is to prepare for scale-up and scale-out of the app. This will include separate conversations about data curation, access, security, servers etc. The philosophy of the app is to make the tool freely accessible in as many languages as possible and to aim for global reach. Families/Caregivers/App users should never have to pay for the app, and we will ensure that the app is never used for any commercial purpose. This will be achieved (a) through a creative-commons license on materials, (b) through a non-disclosure contractual agreement with the app developers and (c) through development of the app on an open-source platform. At the end of the project, the app developers will hand over the full app to the TAND consortium who will be the 'owners' of the app. The TAND consortium will therefore be able to revisit and renegotiate contracts with the app developers or may move to other developers. This way the TSC community will retain control of the data collected, and app companies will only be used to support our goals rather than to have any ownership of TAND data.

How We Will Use the Data in Future

There are many potential uses of the data to be collected through the TAND Toolkit App. These could include refining and updating natural TAND clusters, collecting of preliminary data on new research ideas via the survey functionality in the app, informing app users of potential research studies for participation, and considering how and if the TAND-SQ could be a useful patient/self-reported outcome measure for future clinical trials of pharmacological or non-pharmacological interventions of TAND. Other research uses could include studying the natural history of TAND over the lifespan, or to assess response to TAND-focused interventions. On completion of the TANDem project, a clear data management and curation plan will have been drawn up and clear procedures will be put

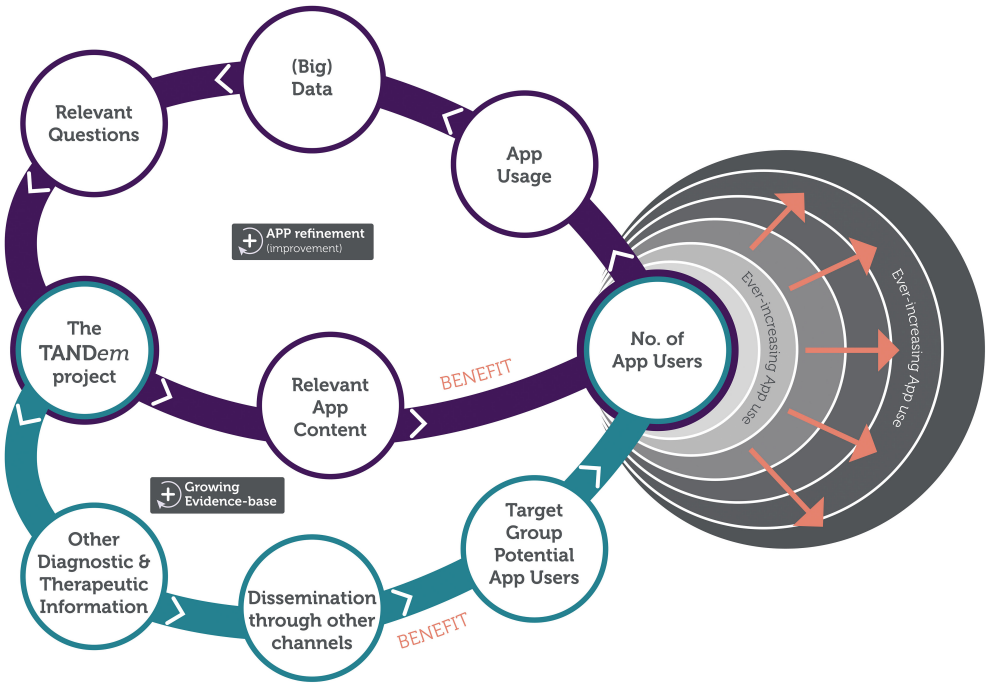

FIGURE 4 | TANDem project impact loop.

in place for application from internal or external researchers to apply for access to specific data to answer specific research questions. We will want to ensure that data access and future research using TAND Toolkit App data will be done in line with the spirit and philosophy of the overall TANDem project, including participatory principles, direct benefit of research to the community we serve, and socially responsive research. One of the fundamental principles which is easily managed through an app is the recognition that individuals and families are the ultimate 'owners' of their own data, and that they can at any point decide to remove their data from the app.

Are We Developing a Medical Device or a Health App?

There has been growing interest in the digital health literature about health apps vs. medical devices. Different countries and jurisdictions are developing their own sets of guidelines and regulations about medical devices, including definitions of medical devices. The USA Federal Drug Administration provided a very helpful list of mobile apps that are not medical devices (34). The TAND Toolkit App represents an app for general education and to facilitate access to reference information to those in the TSC community. All information provided in the app (TAND-SQ Checklist, clusters, consensus recommendations, etc.) will be published in the peer-reviewed scientific literature, and will be presented in the app in a user-accessible format. The app will filter information to people with specific characteristics (e.g., specific natural TAND clusters) in order to provide patient/individual awareness, education and empowerment. The app will, however, at no stage perform any diagnostic procedures or diagnostic analytics, provide any direct treatment or replace any clinician decision-making. We are therefore clear that the TANDem project aims to develop a health app but not a medical device. Ultimately, as outlined in the background section of this protocol paper, we aim to reduce the identification and treatment gap for TAND by empowering families with a digital tool that will provide them with a portal to access accurate information, help their own health decision-making in terms of what to seek as next step professional support, and by providing them with general and practical tips of things they can do at home to improve their journey with TSC and TAND.

AUTHOR CONTRIBUTIONS

TH, ACJ, and PJdV drafted the manuscript. All authors contributed to the design, drafting, revising of the TANDem study protocol, critically reviewed and revised the manuscript, and approved the final manuscript prior to submission.

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A Comparison of Adaptive Functioning Between Children With Duplication 7 Syndrome and Williams-Beuren Syndrome: A Pilot Investigation

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Interstitial deletions of 7q11.23 cause the well-known Williams–Beuren Syndrome (WBS), while duplication of the same region leads to duplication 7 syndrome (Dup7). Children with WBS share a distinct neurobehavioral phenotype including mild to severe intellectual disability, severely impaired visual spatial abilities, relatively preserved verbal expressive skills, anxiety problems, enhanced social motivation (i.e., hypersociable behaviors) and socio-communicative problems. Children with Dup7 syndrome exhibit some "inverted" features when compared to those of individuals with WBS, such as reduced social motivation and impairment of expressive language. Direct comparison of WBS and Dup7 represents a unique opportunity for the neurobehavioral characterization of the 7q11.23 section. However, most of the available data come from qualitative analysis between different studies. To the best of our knowledge, there are no studies directly comparing features of two matched samples of individuals with WBS and Dup7 syndromes. In this pilot study, we compare the adaptive functioning – measured with the Vineland Adaptive Behavior Scales, Second Edition – of two relatively small samples of children with molecularly confirmed diagnosis of WBS and Dup7 matched for IQ and chronological age, with a particular attention to socialization domain and expressive subdomain. Contrary to our assumption, we have not found any significant difference on socialization domain and expressive subdomains. This pilot investigation suggests that, when matched for chronological age and cognitive level, children with WBS and Dup7 share more similarities than expected. The inverted features that emerge in clinical settings on expressive language and social motivation seem not to differently interfere with the daily abilities to communicate and socialize with meaningful others during

daily lives. Differences highlighted by previous undirected comparisons could be due to general and non-specific factors such as cognitive level, which is more severely impaired in individuals with WBS than Dup7. Implications for assessment and treatment are discussed.

Keywords: behavioral phenotype, language impairment, adaptive functioning, cognitive functioning, rare genetic syndrome

INTRODUCTION

Interstitial deletions of 7q11.23 cause the well-known Williams–Beuren Syndrome (WBS) (MIM 194050), while micro-duplication of the same region leads to 7q11.23 duplication syndrome (Dup7). WBS was first described in 1961 by J.C.P. Williams (1) and since the moment in which this non-allelic homologous recombination was reported, it has been supposed that a syndrome with the opposite picture would have existed, even though there were still no information about the phenotype. The first case of Dup7 was described only in 2005, when Somerville and colleagues tested a patient who showed severe expressive speech delay – a feature that was opposite to the linguistic characteristics seen in WBS (2).

In principle, microdeletions and micro-duplications of this specific region on the long arm of chromosome 7 were supposed to occur at the same frequency. However, while the prevalence of WBS is 1 in 7.500 live births, the prevalence of Dup7 is still unknown. It has been proposed that *de novo* prevalence of Dup7 could be 1/12.000, while parental transmission prevalence 1/7.500 live births (3).

The comparison between WBS and Dup7 phenotypes may represent a unique opportunity to better understand the brain functions in which the 7q11.23 section is involved.

Suggestive findings of WBS can be heterogeneous, however, they generally include characteristic "elfin-like" facial features (i.e., periorbital fullness, a stellate/lacy iris pattern, large ear lobes, wide mouth, prominent lips), feeding difficulties in childhood and cardiovascular diseases (usually supravalvar aortic stenosis) and ocular, endocrine and gastrointestinal abnormalities (4). Furthermore, people with WBS share a distinct neuropsychological, cognitive and behavioral phenotype, which counts mild to severe intellectual disability (ID), developmental delay (5), severely impaired visual spatial abilities (i.e., preferential processing of local forms and details; deficits in face processing) (6), anxiety problems, enhanced social motivation (i.e., "hypersociable behaviors," lack of "stranger danger") (7), and socio-communicative problems (deficits in shared attention, the understanding of social relationships, etc.) (8). In addition, people with WBS exhibit a characteristic linguistic profile, with receptive skills usually more impaired than expressive ones (9). On the other side, even though language skills have always been considered a strength in the WBS profile, they fail during everyday life-communication and community living, as studies on adaptive functioning showed (10, 11), in particular in adolescence (11).

Children with the reciprocal microduplication do not share distinct physical features, even if some recurring characteristics have been reported, including macrocephaly, broad prominent forehead, elongated palpebral fissures, short philtrum, thin lip vermilion and microstomia (12, 13). Furthermore, children with Dup7 may exhibit developmental delay and ID (usually in the mild range), behavior problems (selective mutism, anxiety disorders), ADHD (Attention Deficit Hyperactivity Disorder) and extreme shyness around strangers (14); moreover, individuals with Dup7 demonstrate language delay, with wide variability ranging from mild to severe expressive impairment (2). The receptive abilities seem to be considerably better than the expressive vocabulary (15).

The comparison of WBS and Dup7 represents an outstanding possibility for the neurobehavioral characterization of the 7q11.23 section. For instance, some "common," "mitigated," and "inverted" features have already been revealed. Common features between the two syndromes include anxiety problems and the presence of autistic features. Mitigated characteristics count ID and adaptive impairment, usually mild in children with Dup7 when compared to the ones with WBS. Instead, social motivation and verbal skills are usually considered as inverted characteristics (16, 17). In fact, while children with WBS are described as hyperverbal, hyper-sociable and with relatively spared expressive skills, children with Dup7 usually show selective mutism, social anxiety and impairment in expressive skills. Visuospatial cognition skills seem to be inverted too (spared in patients with Dup7 and impaired in WBS patients) (17).

In order to provide a more realistic clinical picture and then a logical basis for individualized supports, over the last two decades assessment of cognitive functioning has been integrated with measurement of adaptive functioning, which is more representative of behavior during everyday life. Indeed, AAIDD (American Association on Intellectual and Developmental Disabilities) postulates that "*ID is characterized by significant limitations both in intellectual functioning and in adaptive behavior as expressed in conceptual, social, and practical adaptive skills. This disability originates before age 18*" (18). Therefore, adaptive behaviors should be always evaluated during ID assessment.

To our knowledge there are no studies comparing adaptive functioning of the above mentioned rare genetic conditions, therefore the main aim of this cross-syndrome study is to compare the adaptive functioning of two relatively small samples of children with molecularly confirmed diagnosis of WBS and Dup7, matched for chronological age and IQ.

The comparison between adaptive profiles in these syndromes could allow having further information on "common," "mitigated," and "inverted" features as expressed in daily

life and to better understand if the reciprocal duplication of the region deleted in WBS leads to a different adaptive functioning or if on the contrary the two syndromes have a comparable phenotype. Based on previous studies, we expect to find significant differences in socialization domain and expressive subdomains, where children with WBS should reach better scores.

MATERIALS AND METHODS

Participants

All participants have been recruited at the Child and Adolescent Psychiatry Unit of Bambino Gesù Children's Hospital. Specifically, our sample includes seventeen participants, nine with molecularly confirmed diagnosis of WBS and eight with molecularly confirmed diagnosis of Dup7 (**Table 1**).

Age (Mean [M]; Standard Deviation [SD], MED [Median]) of WBS group was 102.88 (±30.48, MED = 102.88, age range 51–148) months, while Dup7 group was 95.63 (±23.62, MED = 91.5, age range 66–135) months. Cognitive level of WBS group was 70.88 (±14.39, MED = 75, IQ range 49–87), while Dup7 group was 70.37 (±12.80, MED = 70.5, IQ range 47–85). Groups match for chronological age and IQ (P values always > 0.05).

Materials

Adaptive functioning was measured by means of VABS-II (19), a standardized tool developed to measure adaptive behavior and to support diagnosis of ID. VABS-II are widely used in clinical, educational, and research settings and are often considered the "gold standard" instrument for quantifying impairments in adaptive behaviors [i.e., see (20)]. The scale can be administered from birth to 99 years of age and has already been used in several populations, such as ASD [i.e., (21)], Fragile X Syndrome [i.e., (22)] WBS (23–25) as well as Dup7 (13). VABS-II is a semi-structured interview with the primary caregiver evaluating four domains: Communication, Daily Living Skills, Socialization and Motor Skills. Each domain is composed by specific subdomains: Communication (Expressive; Receptive; Written); Daily Living Skills (Personal; Domestic; Community); Socialization (Interpersonal; Play and Leisure; Coping Skills); Motor Skills (Fine Motor; Gross Motor). As Motor Skills domain is usually administered only to children younger than 6 years of age, it has not been included in the statistical analysis of this research.

Obviously, since the expression of adaptive behavior changes across lifespan, in the VABS-II every composite score is age-normalized. This instrument allows to calculating an overall composite score as well as domain- and subdomain-level constructs.

Cognitive level was measured by means of appropriate developmental tools. More specifically, Wechsler scales (WIPPSI-III and WISC-IV) were used with verbally fluent and behaviorally compliant children, while Leiter International Performance Scale was used with children with more severe speech impairment and behavioral difficulties. Given to the extend time of administration, we used two different editions of the Leiter assessment system (Leiter-R and Leiter 3).

WIPPSI-III (26) is an intelligence test designed for children between 2 years and 6 months and 7 years and 3 months age. It provides both Verbal and Non-Verbal IQ, as well as a Full Scale IQ, which is representative of general intellectual functioning.

WISC-IV (27) is a measure of intellectual performance of subjects aged between 6 years to 16 years and 11 months. It allows to calculating four main Reasoning Indices (Verbal

TABLE 1 | Cytogenetic and molecular characterization of our cohort of patients affected by 7q11.23 microduplication syndrome and WBS syndrome.

N	Gender	Syndrome	CGH array (start and end point) or FISH analysis	Length of duplicated region	Inheritance
1	M	Dup7	7q11.23 (72,726,578–74,339,044)×3	1,6 Mb	n.a.
2	M	Dup7	7q11.23 (72,726,578–74,339,044)×3	1,6 Mb	paternal
3	M	Dup7	7q11.23 (72,726,578–74,139,390)×3	1,4 Mb	maternal
4	M	Dup7	7q11.23 (72,726,578–74,119,570)×3	1,4 Mb	*de novo*
5	F	Dup7	7q11.23 (72,726,578–74,139,390)×3	1,4 Mb	n.a.
6	M	Dup7	7q11.22q11.23 (72,044,007–74,139,390)×3	2,1 Mb	*de novo*
7	F	Dup7	7q11.23 (72,283,565–74,134,911)×3	1,9 Mb	*de novo*
8	M	Dup7	7q11.23 (72,726,578–74,119,570)×3	1,4 Mb	paternal
9	M	WBS	FISH 7q11.23 deletion	FISH	*de novo*
10	M	WBS	FISH 7q11.23 deletion	FISH	*de novo*
11	M	WBS	FISH 7q11.23 deletion	WBS critical region deleted	*de novo*
12	F	WBS	FISH 7q11.23 deletion	WBS critical region deleted	*de novo*
13	F	WBS	FISH 7q11.23 deletion	WBS critical region deleted	*de novo*
14	M	WBS	FISH and array-CGH negative	frameshift mutation in *ELN* gene (c.205delG, p.Ala71ArgfsTer51)	*de novo*
15	M	WBS	FISH 7q11.23 deletion	WBS critical region deleted	*de novo*
16	F	WBS	FISH 7q11.23 deletion	WBS critical region deleted	*de novo*
17	M	WBS	FISH 7q11.23 deletion	WBS critical region deleted	*de novo*

N, number; M, male; F, female; Dup7, 7q11.23 Microduplication Syndrome; WBS, Williams Beuren Syndrome; Mb, megabases; n.a., not available.

Comprehension, Perceptual Reasoning, Working Memory, and Processing Speed) and a Full Scale IQ.

Leiter-R (28) and Leiter-3 (29) are non-verbal intelligence scales, widely used with people with expressive difficulties. In both versions neither the examiner nor the patient are allowed to speak. Leiter-R can be administered from 2 years and 0 months to 20 years and 11 months, while Leiter-3 covers an age range from 3 years to 75 + years.

Procedure

Current research is a cross-syndrome comparison study of adaptive profiles in children and adolescents with WBS and Dup7 matched for chronological age and developmental/cognitive level. Subjects included in this study were evaluated in Child and Adolescence Psychiatry Unit of Bambino Gesù Children Hospital from 2017 to 2020. Tests were administered during routine clinical activities, with assessment procedures usually lasting 3 working days. Assessment of cognitive and adaptive functioning of Dup7 group was part of a previous larger neurobehavioral investigation on clinical features of children with Dup7 (13). Thus, we selected 9 children with WBS from a database including 63 children evaluated from 2012 to 2020.

We chose those patients that received a full evaluation of cognitive and adaptive functioning and that could have been matched with the ones with Dup7 for age and IQ.

Since Wechsler scales provide a general estimate of intelligence, while Leiter assessment system measures only non-verbal intelligence, children from the two groups were matched also for the cognitive test that was used for the evaluation. In two cases, since the same assessment systems were not available, we used the Visual Spatial Index of WISC-IV and the Performance IQ of WPPSI-III, which provide an estimate of non-verbal intelligence and we matched it with the IQ provided by Leiter-3. Cognitive tests were distributed as follow: WBS (2 WISC-IV, 1 WPPSI-III, 4 Leiter 3, 2 Leiter-R), Dup7 (3 WISC, 5 Leiter 3).

Assessment was conducted by a team of trained and specialized child psychiatrists and psychologists and consisted of clinical observations, standardized evaluations and parent interviews. All parents signed an informed consent for research purpose.

The study was approved by Ethical Committee of Bambino Gesù Children's Hospital (number of protocol: 1125).

Statistical Analyses

Descriptive statistics (MED, M, min-max; SD) were elaborated for VABS Adaptive Behavior Composite (ABC), domains and subdomains scores. Raw scores of VABS domains and subdomains were converted to standard scores (IQ, ABC and VABS II domains scores have a M of 100, and SD of 15 while subdomains scores have a M of 15 and SD of 3). Normalized scores were used in all analyses.

To analyze differences between WBS and Dup7 groups in the domains and subdomains of adaptive behaviors measured with VABS II, Mann–Whitney *U* test was performed with Group (WBS vs. DUP7) as independent variable and VABS II domains and subdomains scores as dependent variables; then, a between – group design was used. A P value ≤ 0.05 was considered as statistically significant.

Furthermore, to detect strengths and weaknesses within subcomponents of VABS II in children with WBS or DUP7, the Friedman Test was conducted, with VABS II domains (Communication, Socialization, and Daily Living Skills) as within-subject factors. Then, Wilcoxon signed-rank tests were used to analyze differences between pairs of VABS II domains. Bonferroni's correction was applied to the Wilcoxon signed-rank tests in order to correct alpha (alpha corrected = 0.016). After correction, a P value < 0.016 was considered statistically significant. All data analyses were performed using STATISTICA Six Sigma, STATISTICA release 7 (StatSoft, Inc., 1984–2006).

RESULTS

Adaptive Domains and Subdomains in VABS II – Between Groups' Comparisons

All descriptive statistics of two groups are presented in **Tables 2**, **3**.

There was no significant group difference on overall adaptive outcomes and on VABS II domains and subdomains ($P > 0.05$). A slight slide toward significance ($P = 0.26$) was revealed in Communication – Expressive Skills Subdomain, which scores are higher in WBS group (MED = 9, $M = 9.45$) than in Dup7 one (MED = 8, $M = 7.88$). Another approaching but not reaching significance result was observed in Daily Life Skills – Community subdomain ($P = 0.19$), where Dup7 (MED = 7.15, $M = 8.13$) perform better than WBS people (MED = 5, $M = 6.22$). Finally, a statistical trend toward significance was found in Daily Living Skills – Personal Subdomain ($P = 0.09$), with WBS group (MED = 2, $M = 3.12$) performing worse than Dup7 one (MED = 4.5, $M = 6.63$).

Adaptive Domains in VABS II – Within Groups' Comparisons

Considering Dup7 group, no significant differences were found between Communication, Socialization and Daily Life Skills Domain in any of the three comparisons. P value always > 0.05 (see **Figure 1**).

Taking into account WBS group, no significant differences emerged between Communication and Daily Life Skills. On the other side, results of Wilcoxon signed-rank test revealed significant differences between Communication and Socialization Domains ($P = 0.045$); however, significance was no longer present after Bonferroni's adjustment (P 0.045 > 0.016). A significant difference emerged between Daily Life Skills and Socialization Domains ($P = 0.007$), that persisted also after Bonferroni's correction (P 0.007 > 0.016).

DISCUSSION

Despite on the greater or lesser knowledge on adaptive functioning on WBS and Dup7 alone, no cross-syndrome

TABLE 2 | Descriptive statistic and comparison between groups in VABS II Domains.

	Dup7 group				WBS group					
VABS-II Domains	**MED**	**M**	**Min-Max**	**SD**	**MED**	**M**	**Min-Max**	**SD**	***z* adjusted**	***P* level***
Communication	53.00	53.38	40.00–71.00	10.41	60.00	57.44	41.00–80.00	12.38	0.7	0.47
Daily living skills	65.50	66.25	42.00–107.00	22.30	50.00	51.78	42.00–73.00	10.11	−1.3	0.19
Socialization	69.00	70.75	54.00–87.00	11.72	66.00	69.00	61.00–85.00	7.11	−0.24	0.81
ABC	59.50	59.25	38.00–85.00	14.97	52.00	52.22	41.00–74.00	9.42	−1.05	0.29

*ABC, Adaptive Behavior Composite; MED, median; M, media; Min-Max, Minimum-Maximum; SD, standard deviation, *significant at P ≤ 0.05.*

TABLE 3 | Descriptive statistic and comparison between groups in VABS II Subdomains.

	Dup7 group				WBS group					
VABS II Subdomains	**MED**	**M**	**Min-Max**	**SD**	**MED**	**M**	**Min-Max**	**SD**	***z* adjusted**	***P* level***
Receptive	8.50	8.38	6.00–10.00	1.77	9.00	8.89	7.00–11.00	1.62	0.54	0.58
Expressive	8.00	7.88	4.00–12.00	2.80	9.00	9.45	7.00–13.00	1.81	1.11	0.26
Written	7.00	7.38	2.00–11.00	3.02	9.00	7.11	2.00–14.00	3.92	−0.29	0.76
Personal	4.50	6.63	2.00–13.00	5.13	2.00	3.12	2.00–10.00	2.62	−1.66	0.09
Domestic	11.50	12.75	9.00–20.00	3.73	12.00	11.44	9.00–14.00	1.81	−0.4	0.62
Community	7.50	8.13	4.00–15.00	3.27	5.00	6.22	5.00–8.00	1.48	−1.2	0.19
Interpersonal	8.50	8.63	4.00–14.00	3.50	8.00	8.44	6.00–13.00	1.88	−0.15	0.88
Play and leisure	9.00	9.00	7.00–11.00	1.31	10.00	9.11	7.00–11.00	1.36	0.19	0.84
Coping skills	9.00	9.63	7.00–13.00	2.13	9.00	8.67	7.00–12.00	1.50	−0.84	0.39

*MED, median; M, media; Min-Max, Minimum-Maximum; SD, standard deviation, *significant at P ≤ 0.05.*

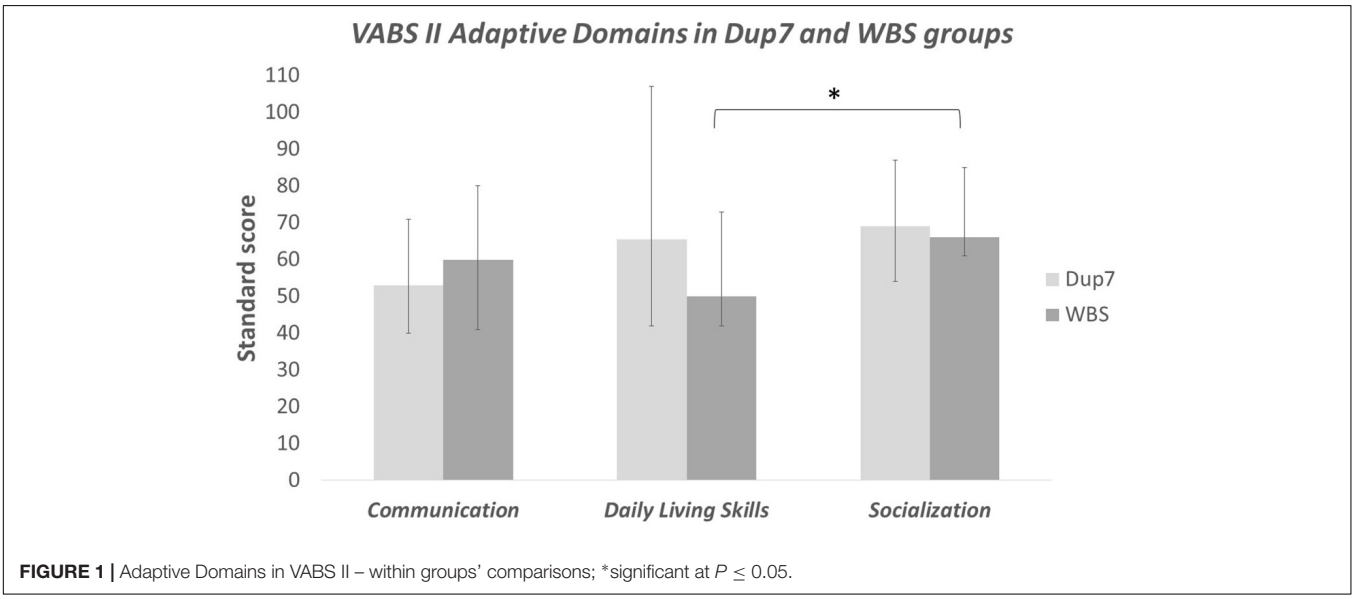

FIGURE 1 | Adaptive Domains in VABS II – within groups' comparisons; *significant at $P \leq 0.05$.

comparisons examining adaptive behavior have been carried out. However, delineating adaptive trends and potential phenotypic specificity between rare genetic syndromes is crucial to improve clinical-functional diagnosis and develop novel early interventions.

This brief report is the first attempt to investigate differences on adaptive functioning in two small samples of children with deletions of 7q11.23 and with microduplications of the same region.

Results showed that non-significant difference emerged between the two groups in domains, subdomains and in Adaptive Behavior Composite. These results are somehow unexpected, given the differences highlighted in the previous studies on neuropsychological and psychopathological features of the two syndromes [i.e., (15)]. On the other side, it has to be taken into account that the absence of significant results could be due to the small sample size; effectively, we selected a group of nine WBS to compare with a group of eight Dup7. Since these syndromes are

particularly rare and considering the methodological decision to match individuals not only for age, but also for IQ, our sample size was inevitably small. This specification has to be considered when interpreting results.

Our findings could suggest that differences between these conditions could be due to other features such as cognitive level rather than to the genotype. Previous observation reporting that children with WBS usually have a worsening functioning in adaptive skills when compared to children with Dup7 could depend on the widest presence of severely cognitive impairment in population of individuals with WBS (14). However, when children with WBS are matched with Dup7 for chronological age and IQ, these differences in adaptive level seems to disappear.

Analysis of domains and subdomains also revealed interesting information. The absence of difference in Socialization was not expected. The inverted hyper-sociable (WBS) versus inhibited/social anxious behaviors (Dup7) profile, which has been reported in previous studies, in our groups seems not to conduct to different patterns in establishing relationships with peers and adults.

Furthermore, even though in our sample there is not a significant difference in Communication domain and subdomains between WBS and Dup7, expressive subscale of WBS seems to be more preserved than the one of Dup7, consistently with literature considering expressive skills of children with WBS and Dup7 as opposite (relatively preserved in WBS and severely compromised in Dup7). However, reminding that both populations exhibit a performance below 2 SD in each subdomain of Communication, a possible interpretation could be that children with Dup7 are not so more impaired in expressive skills than the ones with WBS as usually thought. For this reason, assessment of speech and language characteristics should include also other information; in fact, high level of social anxiety and selective mutism, as well as Disruptive Behavior Disorders (typical of children with Dup7), could at least partially account for this discrepancy. Moreover, while children with WBS are generally compliant with the examiner, children with Dup7 may show oppositional or inhibited behaviors that could lead to a poorer performance in structured evaluations. Generalized anxiety could contribute to the worsening of performance on language tests as well.

On the other side of the coin, children with WBS, which expressive abilities seem to be less impaired, when are evaluated with structured scales, show their real difficulties. For instance, they perform worse than people with Dup7 on tests that measure pragmatic and receptive skills that in WBS are usually more impaired than in Dup7. However, since VABS II do not deeply investigate pragmatic issues, potential significant differences may have not been emerged.

One previous study documented that communication skills measured by VABS II decrease during adolescence period in concomitance with enhancement of social demands, because of a specific worsening of receptive subdomain probably related to pragmatic skills (11). Due to the different language profiles, we would have expected differences between our two groups in the subdomains of communication but this did not happen. However, it should be noted that in our two samples the average age was about 10 years, so it cannot be excluded that the difference was not found due to the young age of our patients.

These considerations could suggest that assessment of language skills should always include batteries that measure pragmatic and daily-life usage of language. Likewise, evaluations should be conducted in a wider period in order to allow children with Dup7 to become more familiar with the clinical setting. In fact, only a more comprehensive evaluation could be able to clarify the reasons of eventual differences in language between those populations.

Furthermore, a slightly trend toward significance was found in Daily Life Skills – Personal Subdomain, which may indicate that WBS people probably show more difficulties in eating, dressing, self-care and personal hygiene than individuals with Dup7. Another difference, even though not achieving acceptable levels of statistical significance, was observed in Daily Life Skills – Community Subdomain, which may imply that WBS perform worse than Dup7 group in the ability to use money, to order food, to use technology and to read a clock. These tiny differences could be due to the visuo-spatial and coordination deficits largely described in WBS population [i.e., (30)].

Concerning within groups' comparisons, analysis of VABS-II domains did not show significant differences in Dup7 group. Previous studies underlined that Dup7 profile is characterized by a weakness in language abilities, in particular in expressive skills (15). Our results, even though failing to reach statistical significance, are consistent with this finding, in fact in Dup7 group Communication Domain is the most impaired than other domains (Communication MED $=$ 53, M $=$ 53.38; Socialization MED $=$ 69, M $=$ 70.75; Daily Living Skills MED $=$ 65.5, $M = 66.25$).

Taking into account WBS population, research has demonstrated that WBS is associated with deficits in adaptive functioning (31), where adaptive profile of children with WBS seems to be characterized by relative strengths in the Socialization and Communication domains and challenges in Daily Living Skills and Motor functioning (32). Our study is consistent with this line of evidence, in fact performance on Socialization Domain was significantly higher than Daily Living Skills one. This may indicate that the hyper-sociable behavior and the apparent expressive skills of WBS people may hide their real difficulties in adaptive functioning.

In conclusion, this pilot investigation suggests that, when matched for chronological and cognitive level, children with WBS and Dup7 share more similarities than thought. Differences that usually emerge in the clinical evaluation of expressive language and social motivation seem not to come up in the daily abilities to communicate and socialize with meaningful others. Then, differences popped out in previous indirect comparisons could be due to general and non-specific factors such as cognitive level, which is more severely impaired and inhomogeneous in individuals with WBS than in the ones with Dup7, rather than on syndrome-specific features. Therefore, this evidence could suggest that interventions for patients with WBS and Dup7 should target the same adaptive skills across all domains.

Our study has some limitations. First, the relatively small sample size may have not allowed to detect differences present in the general populations. Future studies on wider groups are then required to better compare the adaptive profiles of children with WBS and Dup7. Second, since adaptive behavior may change on the basis of different factors such as early interventions, it could be interesting to evaluate if other aspects influence the performance on VABS-II. Third, as we used developmentally appropriate cognitive assessment, different tests have been used to measure cognitive/developmental levels (i.e., Wechsler Scales and Leiter-3) that may have led to a heterogeneous evaluation. Then, future cross-syndrome studies should include a larger sample size and matched patients for the greatest number of features possible.

To conclude, using a cross-syndrome comparison approach revealed partially overlapping profiles in WBS and Dup7, despite the two groups being considerate opposite syndromes. However, as a whole, the two disorders remain distinct in the severity of their core difficulties. This study has some limitations, given the relatively small sample of evaluated subjects. Future studies on larger number of individuals with 7q11.23 deletion and duplication are necessary to support our data and deeper analysis is required to better investigate their adaptive functioning and to design innovative specific diagnostic measures and early novel interventions.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of the Bambino Gesù Children's Hospital number of protocol 1125. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

PA and FS conceived the study. PA, FS, and FAMM wrote the manuscript. CC and FAMM analyzed the data and assisted in the interpretation of findings. SV, PA, PB, and MLD critically reviewed the manuscript. All authors are agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved, they provide intellectual input and approved the final manuscript.

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Autism Symptoms in Children and Young Adults With Fragile X Syndrome, Angelman Syndrome, Tuberous Sclerosis Complex, and Neurofibromatosis Type 1: A Cross-Syndrome Comparison

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Objective: The etiology of autism spectrum disorder (ASD) remains unclear, due to genetic heterogeneity and heterogeneity in symptoms across individuals. This study compares ASD symptomatology between monogenetic syndromes with a high ASD prevalence, in order to reveal syndrome specific vulnerabilities and to clarify how genetic variations affect ASD symptom presentation.

Methods: We assessed ASD symptom severity in children and young adults (aged 0-28 years) with Fragile X Syndrome (FXS, $n = 60$), Angelman Syndrome (AS, $n = 91$), Neurofibromatosis Type 1 (NF1, $n = 279$) and Tuberous Sclerosis Complex (TSC, $n = 110$), using the Autism Diagnostic Observation Schedule and Social Responsiveness Scale. Assessments were part of routine clinical care at the ENCORE expertise center in Rotterdam, the Netherlands. First, we compared the syndrome groups on the ASD classification prevalence and ASD severity scores. Then, we compared individuals in our syndrome groups with an ASD classification to a non-syndromic ASD group (nsASD, $n = 335$), on both ASD severity scores and ASD symptom profiles. Severity scores were compared using MANCOVAs with IQ and gender as covariates.

Results: Overall, ASD severity scores were highest for the FXS group and lowest for the NF1 group. Compared to nsASD, individuals with an ASD classification in our syndrome groups showed less problems on the instruments' social domains. We found a relative

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strength in the AS group on the social cognition, communication and motivation domains and a relative challenge in creativity; a relative strength of the NF1 group on the restricted interests and repetitive behavior scale; and a relative challenge in the FXS and TSC groups on the restricted interests and repetitive behavior domain.

Conclusion: The syndrome-specific strengths and challenges we found provide a frame of reference to evaluate an individual's symptoms relative to the larger syndromic population and to guide treatment decisions. Our findings support the need for personalized care and a dimensional, symptom-based diagnostic approach, in contrast to a dichotomous ASD diagnosis used as a prerequisite for access to healthcare services. Similarities in ASD symptom profiles between AS and FXS, and between NF1 and TSC may reflect similarities in their neurobiology. Deep phenotyping studies are required to link neurobiological markers to ASD symptomatology.

Keywords: Fragile X Syndrome, Angelman Syndrome, Tuberous Sclerosis Complex, Neurofibromatosis Type 1, autism spectrum disorder, autistic traits

INTRODUCTION

Autism spectrum disorder (ASD) is a heterogeneous neurodevelopmental disorder defined by impairments in social communication, restricted or repetitive behaviors or interests, and hyper- or hyposensitivity to sensory stimuli. ASD occurs in around 1.7% of the general population (1). Despite the rapid discovery of genes related to ASD, and the high heritability estimates (64–91%), the exact etiology of ASD remains unclear (2, 3). Studying ASD symptoms in genetically homogenous groups could clarify the pathway from genes to behavior. Genetic syndromes with high ASD prevalence rates include Fragile X Syndrome (FXS), Angelman syndrome (AS), Tuberous Sclerosis Complex (TSC) and Neurofibromatosis type 1 (NF1) (4). Despite their unique genetic variation, these syndromes show similarities in their neurodevelopmental pathways. These syndromes are all affected by alterations in the mechanistic target of rapamycin (mTOR) pathway (5–10), which has also been related to non-syndromic ASD (nsASD) (10, 11). FXS and AS are similar in that both syndromes show atypical DNA methylation that results in increased levels of Activity-Regulated Cytoskeleton-associated protein (Arc), causing reduced synaptic plasticity and disruptions in cerebral development which often lead to intellectual disability (8). TSC and NF1 are similar in that they are both affected by genetic variations that inactivate tumor-suppressor genes. This inactivation leads to an over-activation of mTOR, which increases the risk of tumors in the nervous system that may affect brain development and function (7, 8). In these syndromes, a unique spectrum of ASD symptoms seems to be present (12–17). If differences or similarities in the affected pathways are also reflected by differences or similarities in ASD symptom presentation, this might help identify factors that contribute to the development of specific ASD symptoms, or the development of ASD in general. As a step toward linking ASD symptoms to specific neurobiological pathways, several studies have been conducted to describe ASD symptomatology in monogenetic neurodevelopmental disorders in detail.

FXS affects ∼1 in 4,000 males and 1 in 8,000 females (18, 19), and is one of the leading inherited causes of autism and developmental delay (4). FXS is caused by an CGG repeat expansion in the Fragile X Mental Retardation 1 (*FMR1*) gene, which leads to reduced synaptic plasticity, complications with dendritic development and problems with neurogenesis (20–24). Due to the X-linked nature of FXS, males are generally more severely affected than females (males: $20 < IQ < 70$ (25), females: $70 < IQ < 90$) (26, 27). It is estimated that about 15 to 36% of people with a full Fragile X Syndrome mutation meet the clinical criteria for an assessment-based ASD classification (4, 28–30). Even without meeting all the criteria of ASD, the majority of people with FXS express behavior related to autism (30–33). Studies have shown that, compared to nsASD, individuals with FXS and ASD show less impairment on social and communication domains (32–35), more social anxiety (16), more problems with restricted and repetitive behavior, and less compulsive and ritualistic behavior (32–35).

Angelman Syndrome (AS) can be caused by different genetic variations that affect the expression of the *UBE3A* gene in the chromosomal region 15q11-q13 (36). AS is characterized by cognitive impairments, lack of speech, motor dysfunction, and epilepsy (37). The intellectual development of individuals with AS usually does not exceed a mental age of 24 to 30 months, regardless of their chronological age (38, 39). The prevalence of assessment-based ASD classifications in AS ranges from 20 to 80% in the literature (4, 40–42). While several studies have found that genetic variation within chromosomal region 15q11-q13 is independently associated with ASD (43), a meta-analysis revealed no such relation. Therefore, the precise effect of *UBE3A* variations on the development of ASD remains unclear (44). Compared to people with nsASD, people with AS and ASD display significantly less impairment in areas such as social smile, facial expressions directed to others, shared enjoyment in interaction, response to name and unusual interests or repetitive behavior (40). Compared to FXS, individuals with AS appear to be more sociable (12), and while both syndromes have altered

sensory processing, their response to sensory stimuli is not similar (13).

TSC is caused by genetic variations in either the *TSC1* or *TSC2* gene, leading to mTOR overstimulation (45). This activation induces cellular and tissue dysplasia, causing tumorigenesis that can affect multiple organs (45, 46). Since the central nervous system is almost always afflicted, epilepsy and neuropsychiatric disorders are often seen (46, 47). While approximately half of individuals with TSC score within the normal range of cognitive ability, the other half shows mild to severe (14.5%, $25 \leq IQ < 70$) or profound (30.5%, $IQ < 25$) intellectual disability (48). The estimated prevalence of assessment-based ASD classifications in TSC ranges from 35 to 60% (4, 17, 47, 49, 50), of which the severity may be influenced by presence of epilepsy (49). Jeste et al. showed that impairments in social communication in children with TSC do not differ from those in children with nsASD (17). Recently, molecular target therapy with mTOR inhibitors has demonstrated a reduction of epilepsy symptoms in people with TSC (51, 52), and the reduction of autism symptoms in animal models (53, 54).

In NF1, a genetic variation in the *NF1* gene causes a neurofibromin deficiency, which inhibits the cell cycle and cell differentiation, and enables unrestricted cell growth (55). Tumor growth in NF1 can cause various neurologic, cardiovascular, gastrointestinal, endocrinal and orthopedic complications (56–60). However, the most common challenge for children with NF1 are learning and behavioral problems (61–63). The average IQ of individuals with NF1 lies around 90, with 6 to 7% having an IQ lower than 70 (15, 64). The prevalence of assessment-based ASD classifications in NF1 ranges from 10 to 39% (4, 15, 49, 65). Some studies have found that NF1 has a unique ASD phenotype with better eye contact, less repetitive behavior and more severe autistic mannerisms compared to individuals with nsASD (66, 67). However, others have shown that NF1 shows a symptom profile similar to that in both nsASD and TSC (49).

In summary, FXS, TSC, AS and NF1 have a high prevalence of ASD symptoms and are caused by unique and well-described genetic variations, making them ideal candidates to study genotype-phenotype relationships in ASD. A direct comparison of these syndromes, in which ASD symptoms are assessed in the same clinical setting and with the same diagnostic instruments, has not yet been done. The main aim of this large cohort study is to identify differences and similarities in ASD symptom severity between these monogenetic developmental disorders, as well as compared to a non-syndromic ASD group. Based on earlier studies, we expect the FXS group to display the most severe symptoms, especially on the domains of stereotypic behavior and limited interests, while children with TSC and NF1 will show less severity on these domains. In contrast, we expect less severe symptoms for the FXS and AS groups in social interaction. Based on the similarities in the neurobiological pathways between FXS and AS, and between NF1 and TSC, respectively, we will explore if and how these similarities may be reflected in the ASD symptom profiles of these groups.

Besides linking genetic pathways to behavior, the ASD symptom profiles in these syndrome groups could help us understand and value the information gathered with diagnostic instruments that are developed using non-syndromic norm groups. This may add to the discussion of whether a categorical approach in ASD diagnostics is appropriate in syndromic ASD as well as non-syndromic ASD, or whether a symptom-based approach is more suitable (68). A forced fit between symptom presentation and scoring procedures may result in a loss of clinically important information or the under- or over diagnosis of ASD within these groups, which may directly impact clinical decision making and an individual's access to health services (33). Additionally, as treatment of ASD symptoms requires a personalized stepped-care approach (69), the syndrome specific symptom profiles might reveal syndrome-specific targets for treatment and intervention.

METHODS

Participants

We included four groups of children and young adults (aged 0–28 years) with syndromes that have high ASD prevalence: FXS, AS, NF1, and TSC, see **Figure 1**. ASD symptoms and cognitive functioning were assessed as part of routine clinical care that is performed in all children seen at the ENCORE expertise center for genetic neurocognitive developmental disorders within the Erasmus MC Sophia Children's Hospital in Rotterdam, the Netherlands. All children with these syndromes were included, regardless of their ASD symptomatology.

In addition, we included an nsASD group as a frame of reference. For this group, we used data collected as part of the Social Spectrum Study, a clinical cohort study that aimed to identify children at risk for ASD by screening children who had been referred to six large child and adolescent mental health services in the South-West of the Netherlands. A detailed description of the sample characteristics and data collection procedure of this group can be found in the study design paper of the Social Spectrum Study (70). We included children that participated in the in-depth assessment (T1), which consisted of all children with an ASD classification on the Social Responsiveness Scale (SRS) (71) at initial screening (T0) ($N = 235$) and a random selection of children that screened negative at T0 ($N = 100$). From this group, we included the children with an ASD classification according to the Autism Diagnostic Observation Schedule (ADOS) (72) and/or the SRS in our subgroup analyses. None of these children were diagnosed with a genetic syndrome.

Measures

Autism Spectrum Symptoms

To assess ASD symptom severity, two diagnostic instruments were used: the ADOS and the SRS. Both instruments provide valuable insight into a child's behavior and are therefore often used together in clinical practice, which is also the case for children seen at ENCORE.

ADOS

The ADOS uses a semi-structured schedule of activities to allow researchers or clinicians to observe an individual's behavior in areas associated with a diagnosis of ASD: social

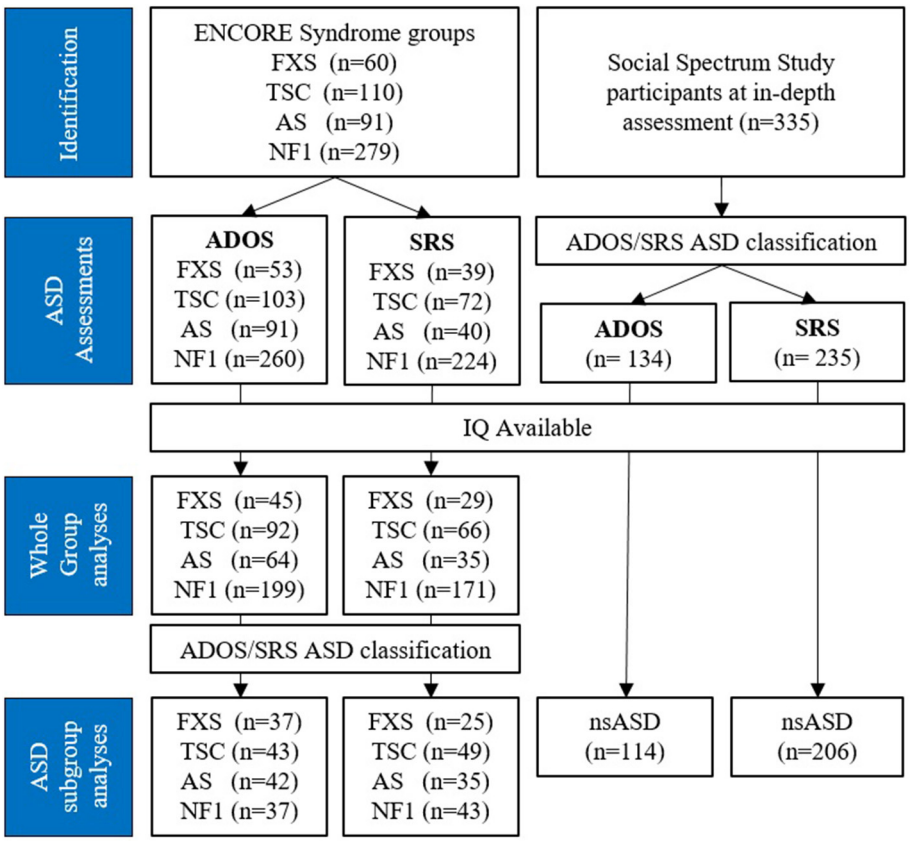

FIGURE 1 | Flow chart of inclusion process. ASD, Autism Spectrum Disorder; FXS, Fragile X Syndrome; TSC, Tuberous Sclerosis Complex; AS, Angelman Syndrome; NF1, Neurofibromatosis type 1; nsASD, non-syndromic ASD; ADOS, Autism Diagnostic Observation Schedule; SRS, Social Responsiveness Scale.

communication, reciprocal social interactions and restricted and repetitive behavior. To suit an individual's developmental level and language level, one of five modules is selected. Items vary across the modules. The sensitivity of the ADOS-2 is between 72 and 97% and the specificity is between 19 and 94%, depending on the module used (73).

Data was collected by certified clinicians, using the ADOS-G (3.6%) (72) and ADOS-2 (96.4%) (74). ADOS-G scores were converted to ADOS-2 scores *via* the manual (74). Calibrated severity scores (CSS) are computed for the total score, and for the domains Social Affect (SA) and Restricted and Repetitive Behavior (RRB). CSS correct for chronological-age and range between 1 and 10, with higher scores indicating more severe ASD symptoms. For children younger or older than the available norm groups we calculated scores based on the nearest available norm group. Based on the Total CSS, individuals classify as "non-spectrum" (scores 1–3), "ASD" (scores 4–5) or "Autism" (scores >5) (75, 76). A Total CSS > 3 was used as a cut-off for the ASD classification used in our analyses. It is important to note however, that the RRB CSS, scored 1 to 10, are converted from the raw RRB subscale scores that range from 0 to 7. The raw scores are converted to a 1–10 scale by making scores 2, 3, and 4 impossible to obtain. While this improves the ease of interpretation relative to the other CSS scores, it is problematic for data analysis (14). Therefore, we used the original CSS-RRB scores on a 0–7 scale in our analyses.

The ADOS groups items into five subscales (language and communication, reciprocate social interaction, creativity/play, restricted and repetitive behavior, and other behavior), but does not provide normed subscale scores. For our ASD profile analyses, we computed a "Weighted Subscale Score" (WSS) for each subscale that accounts for the differences in the number of items between both the subscales and the ADOS modules. The WSS were calculated by dividing the individual's total score per subscale through the subscale's maximum score, resulting in a score between 0 and 1, with higher scores representing higher autism severity. Because Subscale E includes 3 items that measure different constructs, we did not calculate a weighted score for this subscale.

SRS

In this 65-item ASD screening questionnaire, parents or caregivers rate their child's behavior over the past 6 months on a 4-point Likert scale, with higher scores indicating higher symptom severity. Each item belongs to one of five subscales: Social Awareness, Social Cognition, Social Communication,

Social Motivation, and Autistic Mannerisms. Age- and gender-normed T-scores can be computed for the total score (SRS_{TOT}) and for the domains "Social Communication and Interaction" (SRS_{SCI}, consisting of the first four subscales) and "Restricted Interests and Repetitive Behavior" (SRS_{RRB}, consisting of the Autistic Mannerisms subscale). In addition, T-scores can be calculated for all subscales. Based on the T-scores, ASD symptom severity is interpreted as non-clinical ($T < 60$), mild ($60 \leq T \geq 75$) or severe ($T > 75$). An SRS_{TOT} T-score $> = 60$ was used as a cut-off for the ASD classification used in our analyses. The sensitivity of the SRS-2 is 93%, with a specificity of 91% (38). Our sample included data from both the SRS and SRS-2. As items do not differ between the SRS and the SRS-2, all cases were classified using the SRS-2 classification methods, regardless of the questionnaire used. For children younger or older than the available norm groups we calculated scores based on the nearest available norm group.

Cognitive Functioning

Children with developmental delay are more likely to score in the clinical range of both the ADOS (77) and SRS (78). As developmental levels differ between the syndrome groups, we included intellectual functioning as a covariate in the group comparisons. Cognitive functioning was assessed using an age- or developmental level appropriate instrument. These instruments include the Wechsler preschool and primary scale of intelligence (WPPSI-III-NL) (79), the Wechsler intelligence scale for children [WISC-III-NL (80) or WISC-V-NL (81)], the Wechsler Adult Intelligence Scale (WAIS-III) (82), the Wechsler Non Verbal scale of Ability (WNV) (83), the Bayley Scales of Infant and Toddler Development third edition (Bayley-III) (84) and the Snijders-Oomen Non-verbal intelligence test (SON) (85). In some cases, the chronological age of the individual was higher than the available norm groups (e.g., when an 18-year-old with developmental delay was assessed using the WISC). In these cases, no full-scale IQ could be calculated and a developmental quotient (DQ) was computed instead (DQ = estimated developmental age/chronological age × 100, with M = 100, SD = 15).

Procedure

For the syndrome groups we included individuals with a complete ADOS and/or SRS assessment between May 2009 to March 2021. In case complete data from multiple time points was available for an individual, we selected the most recent time point. This resulted in 507 cases for the ADOS and 375 cases for the SRS. The nsASD group data consisted of 134 ADOS assessments and 235 SRS questionnaires, collected between May 2011 and December 2013.

Statistical Analysis

As each instrument has a unique focus, separate analyses were performed for the ADOS and the SRS data. First, we compared the syndrome groups on the prevalence of ADOS and SRS ASD classifications using Chi square tests. Next, we compared the main ASD severity scores (ADOS: CSS_{TOT}, CSS_{SA}, and CSS_{RRB}; SRS: T_{TOT}, T_{SCI}, T_{RRB}) between the syndrome groups using MANCOVA's with developmental level (IQ/DQ) and gender as covariates. We first did this for the syndrome groups as a whole. Then, we compared the syndrome groups again while only including individuals with an ASD classification on the ADOS or SRS, respectively, in order to include all individuals with a clinical score on that particular instrument irrespective of their classification status on the other instrument, with the nsASD group added as reference. We did this to reduce the expected within-group variability in ASD severity. Finally, for our ASD profile analysis, we also compared the ASD subgroups on subscales of the ADOS and SRS.

Data analyses were performed in IBM SPSS Statistics version 25 (86). We use an alpha level of 0.05 for all analyses and applied a Bonferroni correction for multiple comparisons in our *post-hoc* comparisons. In all tables the uncorrected-mean-scores, standard deviations, F-statistics, Bonferroni-corrected-*p*-values and the effect sizes (partial η^2) are provided. Without a suitable alternative, MANCOVA's were used despite the skewed data (see **Supplementary Figure 1**) and correlation between the covariates and the predictor, so our results should be interpreted cautiously.

Missing Data

A non-response analysis for missing IQ/DQ scores in the syndrome groups revealed no differences in ADOS or SRS ASD severity scores between individuals with and without available IQ/DQ scores. For the nsASD group however, severity scores were higher for those without an available IQ/DQ score than for those with an IQ/DQ score for the ADOS [$t_{(266)} = -2.087$, $p = 0.038$]. Because we expected the effect of IQ/DQ to be substantial in the syndrome groups we included IQ as a covariate nonetheless.

RESULTS

Descriptives

Sample characteristics are presented in **Table 1**. We found significant differences in ASD classification prevalence between the syndrome groups for both the ADOS and the SRS. According to both instruments, approximately a quarter of individuals in the NF1 group received an ASD classification, which was lower compared to the FXS, TSC, and AS groups in which at least half of individuals received an ASD classification. The ASD classification prevalence was highest in the FXS and AS groups. An overview of ASD classifications in the syndrome groups is provided in **Supplementary Figure 2**.

Cross-Syndrome Comparisons of Symptom Severity Scores

ADOS

ASD severity scores of the whole-group cross-syndrome comparison are presented in **Figure 2A** and **Table 2**. We found a significant main effect of group for the ADOS CSS scores collectively and individually. *Post hoc* pairwise comparisons revealed that for the total CSS, autism severity was higher in the FXS group compared to all other groups, and higher in the TSC group compared to the AS group. For the social affect domain, the FXS and TSC groups had higher severity scores than the AS

TABLE 1 | Sample characteristics.

	FXS	TSC	AS	NF1	nsASD	Cross-syndrome comparison		
						df	*X*	*p*
ADOS (*N* = 641)								
N	53	103	91	260	134			
Age M (SD)	9.00 (5.32)	9.54 (4.92)	8.85 (5.05)	7.22 (3.45)	6.82 (2.34)			
Age range (y)	2–28	2–19	2–21	1–18	2–12			
Males *N* (%)	41 (77.4)	54 (52.4)	47 (51.6)	143 (55.0)	113 (84.3)			
IQ/DQ								
N (%)	45 (84.9)	92 (89.3)	64 (70.3)	199 (76.5)	114			
M (SD)	49.6 (19.1)	63.9 (29.6)	19.0 (10.7)	87.0 (15.7)	92.2 (17.7)			
range	20–93	4–127	3–52	38–135	50–141			
ASD class *N* (%)	44 (83.0)$_a$	50 (48.5)$_b$	60 (65.9)$_{ab}$	52 (20.0)$_c$	–	3	112	<0.001
SRS (*N* = 635)								
N	39	72	40	224	235			
Age M (SD)	8.10 (5.50)	9.68 (5.16)	9.20 (4.70)	7.02 (3.34)	7.17 (1.97)			
Age range (y; m)	0;9–26	1–19	2–20	1–17	4–11			
Males *N* (%)	29 (74.4)	33 (45.8)	22 (55.0)	121 (54.0)	195 (75.0)			
IQ/DQ								
N (%)	29 (74.4)	66 (91.7)	35 (87.5)	171 (76.3)	206			
M (SD)	53.4 (20.9)	64.7 (26.3)	16.8 (10.6)	88.4 (15.7)	95.9 (16.7)			
range	22–93	7–127	3–52	38–135	50–145			
ASD class *N* (%)	35 (89.7)$_{ab}$	54 (75.0)$_b$	38 (95.0)$_a$	65 (29.0)$_c$	–	3	114	<0.001

For syndrome groups with the same subscript letter the prevalence of an ASD classification is not significantly different at the p = 0.05 level. ASD, Autism Spectrum Disorder; FXS, Fragile X Syndrome; TSC, Tuberous Sclerosis Complex; AS, Angelman Syndrome; NF1, Neurofibromatosis Type 1; nsASD, non-syndromic ASD; DQ, Developmental Quotient; y, years; m, months.

and NF1 groups. Lastly, the FXS group had higher severity scores on the restricted and repetitive behavior domain compared to all other groups.

SRS

The comparison of SRS severity scores also revealed a significant effect of group for the collective and individual T-scores, see **Figure 2B** and **Table 2**. Similar to the results of the ADOS, *post hoc* pairwise comparisons showed that the FXS group had higher severity scores than the NF1 and AS groups for all subscales, and higher severity scores compared to all other groups on the Restricted Interests and Repetitive Behavior domain. While for the ADOS the TSC group had higher ASD severity scores compared to the NF1 group specifically on the Social Affect domain, for the SRS the TSC group had higher severity scores compared to the NF1 group on all severity scores.

Cross-Syndrome Comparisons of Symptom Severity Scores in Individuals With an ASD Classification

ADOS

When including only individuals with an ASD classification on the ADOS, a group comparison of the ADOS severity scores again showed a main effect of Group for the CSS scores collectively and individually (see **Figure 3A** and **Table 3**). *Post hoc* pairwise comparisons showed that for both the Total and Social Affect CSS, severity scores in the individuals with an ASD classification in the FXS, TSC, and NF1 groups did not differ significantly from the severity scores of the nsASD group. Only individuals with an ADOS ASD classification in the AS group had lower severity scores compared to the nsASD group, as well as compared to all other syndrome groups. For Restricted and Repetitive Behavior, the severity scores were higher for individuals with an ADOS ASD classification in the FXS group compared to the nsASD group, as well as the AS and NF1 groups.

SRS

The group comparison of SRS severity scores in individuals with an SRS ASD classification again showed a significant main effect of Group for the collective and individual T-scores (see **Table 3** and **Figure 3B**). While for the ADOS only the AS group severity scores were lower compared to the nsASD group on the total and Social Affect severity scores, the SRS *post hoc* comparisons showed that the nsASD group had higher severity scores compared to individuals with an ASD classification in the TSC, AS and NF1 groups on these two subscales. Also unlike the ADOS, there was no difference in severity scores between individuals with an ASD classification in the FXS group and the nsASD group on the SRS Restricted Interests and Repetitive Behavior domain. The nsASD group did show higher severity scores on the SRS Restricted Interests and Repetitive Behavior domain compared to individuals with an SRS ASD classification in the NF1 and TSC groups.

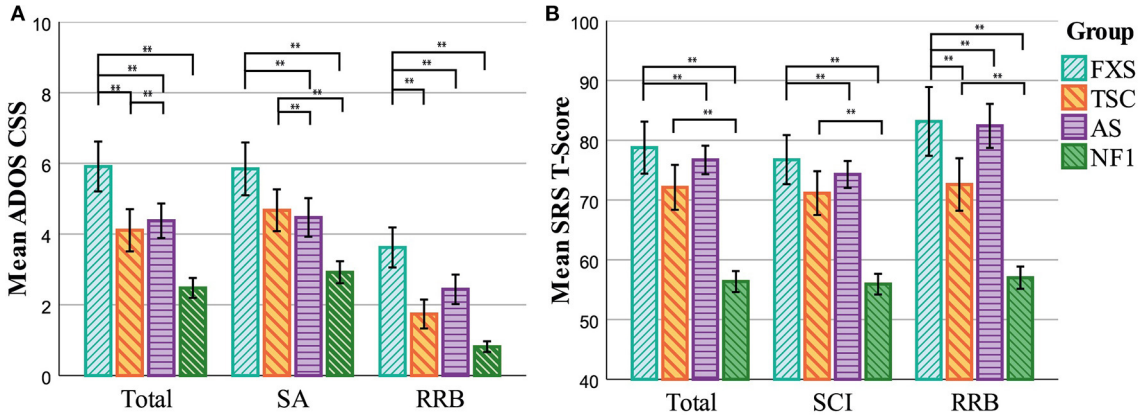

FIGURE 2 | ASD severity scores per syndrome group. **(A)** Means represent the ADOS scores of the whole group, regardless of the presence of an ADOS ASD classification. **(B)** Means represent the SRS T-scores of the whole group, regardless of the presence of an SRS ASD classification. FXS, Fragile X Syndrome; TSC, Tuberous Sclerosis Complex; AS, Angelman Syndrome; NF1, Neurofibromatosis Type 1; CSS, Calibrated Severity Score; SA, Social Affect; RRB, Restricted (Interests) and Repetitive Behavior; SCI, Social Communication and Interaction. The bars represent the uncorrected mean scores of the groups. Significant group differences of the MANCOVA *post hoc* comparisons are presented. Error bars represent 95% CI, $^{**}p < 0.01$, Bonferroni corrected.

TABLE 2 | Cross-syndrome comparisons of symptom severity scores.

Dependent variable	*df*	*df* error	F	η^2	FXS M (SD)	TSC M (SD)	AS M (SD)	NF1 M (SD)
ADOS CSS—Whole sample								
Multivariate	9	954	9.238**	0.066	*Wilks' Λ = 0.816*			
CSS_{TOT}	3	394	15.877**	0.108	$5.91\ (2.34)_a$	$4.11\ (2.89)_b$	$4.38\ (1.96)_c$	$2.48\ (2.02)_{bc}$
CSS_{SA}	3	394	12.654**	0.088	$5.84\ (2.34)_a$	$4.67\ (2.85)_a$	$4.47\ (2.92)_b$	$2.92\ (2.24)_b$
CSS_{RRB}	3	394	19.166**	0.127	$3.62\ (1.89)_a$	$1.74\ (1.97)_b$	$2.44\ (1.68)_b$	$0.81\ (1.79)_b$
SRS T-scores—Whole sample								
Multivariate	9	713.2	7.868**	0.074	*Wilks' Λ = 0.794*			
T_{TOT}	3	295	17.295**	0.150	$78.8\ (15.1)_a$	$71.7\ (15.2)_{ab}$	$77.5\ (6.5)_{bc}$	$55.7\ (12.4)_c$
T_{SCI}	3	295	16.341**	0.142	$76.7\ (14.3)_a$	$70.8\ (15.0)_{ab}$	$74.8\ (6.3)_{bc}$	$55.2\ (12.2)_c$
T_{RRB}	3	295	17.300**	0.150	$83.6\ (18.7)_a$	$72.0\ (17.7)_b$	$83.6\ (10.5)_{bc}$	$56.2\ (13.2)_c$

The means represent the uncorrected mean scores of the groups. For groups with the same subscript letter the mean CSS or subscale score is not significantly different at the p = 0.05 level (Bonferroni corrected). FXS, Fragile X Syndrome; TSC, Tuberous Sclerosis Complex; AS, Angelman Syndrome; NF1, Neurofibromatosis Type 1; CSS, Calibrated Severity Score; Tot, Total score; SA, Social Affect; RRB, Restricted and Repetitive Behavior; T_{TOT}, T-score SRS total score; T_{SCI}, T-score Social Communication and Interaction; T_{RRB}, T-score Restricted interests and repetitive behavior. $^{**}p < 0.01$.

When we compare the syndrome groups to each other, individuals with an ASD classification in the FXS group had higher severity scores on the total score compared to individuals with an ASD classification in the AS and NF1 groups, higher scores compared to individuals with an ASD classification in the AS group on the Social Communication and Interaction domain, and higher severity scores compared to all other syndrome groups on the Restricted Interests and Repetitive Behavior domain. Unlike for the ADOS, we did not find differences in SRS severity scores between individuals with an ASD classification in the TSC, AS and NF1 groups.

Cross-Syndrome Symptom Profile Analysis in Individuals With an ASD Classification

ADOS

The results of the cross-syndrome comparison of ASD symptom profiles are presented in **Figure 4** and **Supplementary Table 1**. Individuals with an ASD classification on the ADOS in the FXS and AS group had similar profiles for all subscales except creativity and play, for which the AS group had higher severity scores than the FXS group, as well as the NF1 group. The profiles of individuals with an ADOS ASD classification in the NF1 and TSC groups were similar to each other, as well as to the profile of the nsASD group, for all subscales.

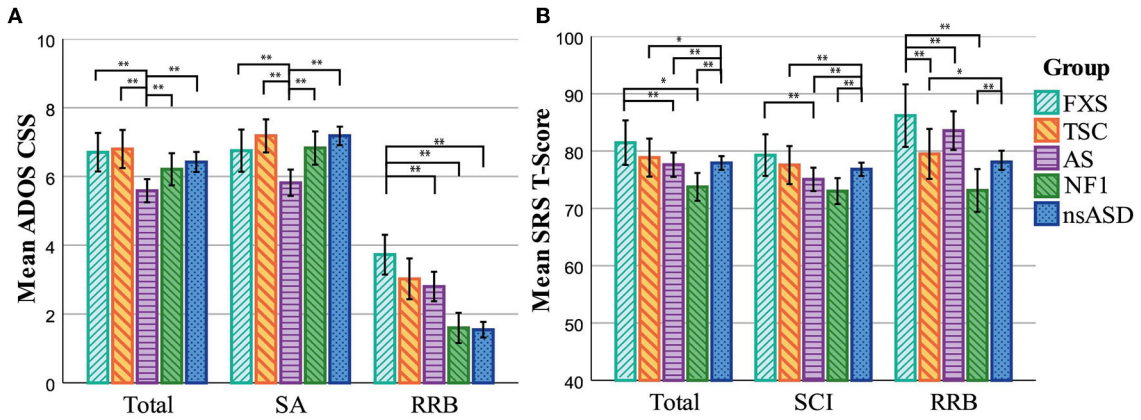

FIGURE 3 | Cross-syndrome comparisons of symptom severity scores in individuals with an ASD classification. **(A)** Means represent the ADOS scores. **(B)** Means represent the SRS T-scores. FXS, Fragile X Syndrome; TSC, Tuberous Sclerosis Complex; AS, Angelman Syndrome; NF1, Neurofibromatosis Type 1; nsASD, non-syndromic ASD; CSS, Calibrated Severity Score; SA, Social Affect; RRB, Restricted (Interests) and Repetitive Behavior; SCI, Social Communication and Interaction. The bars represent the uncorrected mean scores of the groups. Significant group differences of the MANCOVA *post hoc* comparisons are presented. Error bars represent 95% CI, $^{*}p < 0.05$, $^{**}p < 0.01$, Bonferroni corrected.

TABLE 3 | Cross-syndrome comparisons of symptom severity scores in individuals with an ASD classification.

DV	*df*	*df* error	F	η^2	FXS M (SD)	TSC M (SD)	AS M (SD)	NF1 M (SD)	nsASD M (SD)
ADOS CSS—ASD subsample									
Multivariate	12	701.4	4.951**	0.069	*Wilks' Λ = 0.860*				
CSS_{TOT}	4	267	5.757**	0.079	$6.68\ (1.91)_a$	$6.77\ (1.35)_a$	$5.50\ (1.79)_b$	$6.11\ (1.68)_a$	$6.36\ (1.69)_a$
CSS_{SA}	4	267	5.980**	0.082	$6.62\ (1.79)_a$	$7.14\ (1.91)_a$	$5.71\ (1.55)_b$	$6.73\ (1.79)_a$	$7.12\ (1.59)_a$
CSS_{RRB}	4	267	8.351**	0.111	$3.89\ (1.81)_a$	$2.95\ (2.13)$	$2.79\ (1.77)_b$	$1.57\ (1.56)_b$	$1.43\ (1.16)_b$
SRS T-scores—ASD subsample									
Multivariate	12	926.3	3.396**	0.037	*Wilks' Λ = 0.892*				
T_{TOT}	4	352	8.794**	0.091	$82.6\ (12.5)_{ac}$	$78.1\ (11.2)_a$	$77.5\ (6.5)_b$	$73.5\ (9.0)_b$	$78.4\ (9.1)_c$
T_{SCOM}	4	352	7.966**	0.083	$80.2\ (11.8)_{ac}$	$76.9\ (11.4)_a$	$74.8\ (6.3)_b$	$72.7\ (8.7)_a$	$77.3\ (8.9)_c$
T_{RRB}	4	352	6.438**	0.068	$87.8\ (16.0)_a$	$78.5\ (15.0)_b$	$83.6\ (10.5)_{bc}$	$73.4\ (12.6)_b$	$78.7\ (12.8)_{ac}$

The means represent the uncorrected mean scores of the groups. For groups with the same subscript letter the mean CSS or subscale score is not significantly different at the p = 0.05 level (Bonferroni corrected). No subscale letter means the group does not differ from any other group. DV, Dependent Variable; FXS, Fragile X Syndrome; TSC, Tuberous Sclerosis Complex; AS, Angelman Syndrome; NF1, Neurofibromatosis Type 1; nsASD, non-syndromic ASD; CSS, Calibrated Severity Score; TOT, Total score; SA, Social Affect; RRB, Restricted and Repetitive Behavior; T_{TOT}, T-score SRS total score; T_{SCI}, T-score Social Communication and Interaction; T_{RRB}, T-score Restricted interests and repetitive behavior. $^{**}p < 0.01$.

SRS

Contrary to the ADOS, the profiles of individuals with an ASD classification on the SRS in the FXS and AS groups showed little similarity for the SRS, as the FXS group showed higher severity scores compared to the AS group on all subscales except Social Awareness. Instead, the profile of the FXS group was similar to that of the nsASD group on all subscales. The AS group on the other hand showed lower severity scores compared to the nsASD group on the Social Cognition, Social Communication and Social Motivation subscales. Similar to the ADOS, individuals with an ASD classification in the NF1 and TSC groups shared a similar ASD profile for all subscales of the SRS, but their profiles were not similar to that of the nsASD group.

DISCUSSION

The main aim of this study was to identify differences and similarities in ASD symptomatology between monogenetic syndromes with high ASD prevalence—FXS, TSC, NF1, and AS—that may reveal how different genetic variations affect ASD symptom severity.

ASD Classification Prevalence

In line with the literature, for both instruments, the ASD classification prevalence was highest in the FXS group and lowest in the NF1 group. The prevalence of ADOS and SRS ASD classifications we found for the FXS group (around 80–90%), and the AS group (70–90%) fell on the high end of

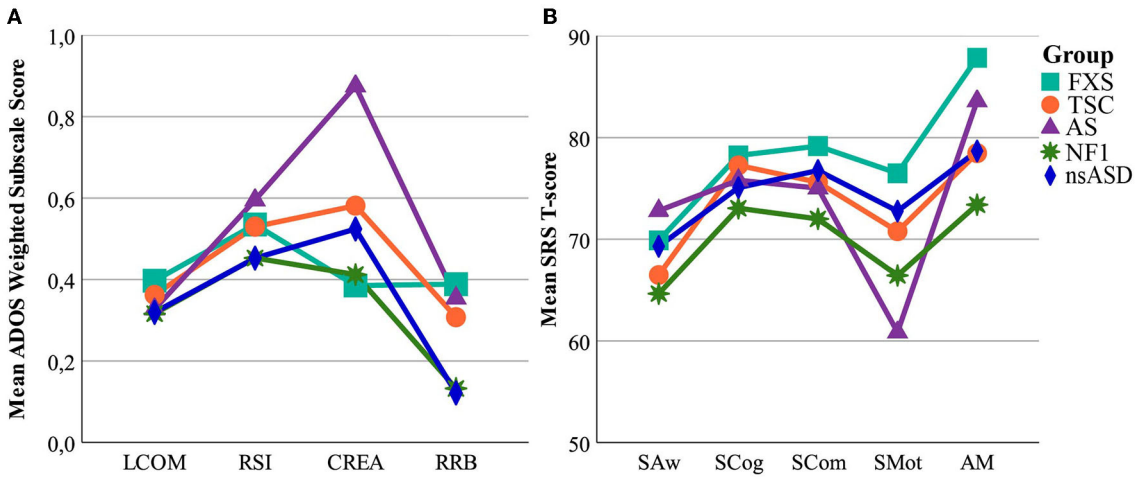

FIGURE 4 | ASD symptom severity profile per syndrome group in the ASD subsample. **(A)** Means represent ADOS Weighted Subscale Scores. **(B)** Means represent SRS subscale T-scores. FXS, Fragile X Syndrome; TSC, Tuberous Sclerosis Complex; AS, Angelman Syndrome; NF1, Neurofibromatosis Type 1; nsASD, non-syndromic Autism Spectrum Disorder; WSS, Weighted subscale score; LCOM, Language and communication; RSI, Reciprocal Social Interaction; CREA, Creativity and Play subscale; RRB, Restricted and Repetitive Behavior; SAw, Social Awareness; SCog, Social Cognition; SCom, Social Communication; SM, Social Motivation; AM, Autistic mannerisms.

the range found in the literature. Overall, ASD classification prevalence seemed to be higher for the SRS compared to the ADOS. This difference was especially high for the TSC (26% difference) and AS groups (29% difference), although we did not test this statistically as the samples contained different individuals. The difference in ASD classification prevalence between the ADOS and SRS is likely the result of the fundamental differences between the instruments. First, as a screening instrument the SRS is required to be highly sensitive by design. Secondly, the caregiver's perspective provided by the SRS may be more subjective. Thirdly, the setting and time period that is covered by these instruments may affect the scores, as some children may have learned to present more socially desirable behavior in unfamiliar surroundings than they would do in the comfort of their own home. Alternatively, they may experience more anxiety in the presence of an unfamiliar individual.

An important factor that may explain the higher prevalence of ADOS and SRS ASD classifications in children with FXS and AS is their developmental level. Generally, individuals with FXS and AS experience more severe intellectual disability or developmental delay compared to NF1. Cognitive impairment is known to affect scores on ASD screening instruments (75), and has been related to reduced specificity of the ADOS, especially for young children (73). Cognitive impairment additionally impacts whether individuals qualify for a DSM-5 classification, which impacts their access to health care services (33). The DSM-5 requires that difficulties in social communication must be lower than could be expected based on the developmental level of the individual, so that the deficiencies in social skills cannot be attributed to developmental delay. Therefore, it is likely that difficulties in social communication in individuals with FXS and AS are more frequently attributed to developmental delay rather than to the presence of ASD. This means that children with intellectual disability or developmental delay can score in the clinical range on screening or diagnostic instruments but not qualify for a formal DSM-5 ASD classification, even though they may experience the same symptom severity. At the same time, children with intellectual disability or developmental delay who qualify for a formal DSM-5 ASD classification are likely to show more severe symptoms compared to children with a formal DSM-5 ASD classification without intellectual disability or developmental delay. Even though we controlled for IQ/DQ in our analyses, it remains unclear whether the symptoms we see in our sample are beyond their expected developmental level.

Given the wide variety of symptom profiles that exist, in both syndromic and non-syndromic populations, some researchers have raised the question whether an all-or-nothing ASD classification should be replaced by a multimodal symptom-based assessment of health care needs (68). Based on our findings, we would argue that, at least in children with a syndromic form of ASD, a symptom-based approach might be favorable. This would ensure that individuals who experience ASD symptoms are eligible for treatment of these symptoms regardless of their diagnostic status. However, further research is required to better understand the effect of developmental delay on social skills measured by ASD screening instruments. While an all-or-nothing diagnosis is still the standard, the differences between these instruments in our study highlight the importance of a multimodal assessment of ASD symptoms to diagnose ASD reliably in these children.

Cross-Syndrome Comparisons of Symptom Severity Scores

In line with our hypothesis, and in accordance with the ASD classification prevalence, ASD symptom severity overall was highest in the FXS group and lowest in the NF1 group when we compared the main subscales of the ADOS and SRS. Also in line with our expectations, the scores of the TSC and AS groups mostly fell between the FXS and NF1 scores. The TSC group showed higher symptom severity compared to the AS and NF1 group for the ADOS social affect domain, and higher scores than the NF1 group on the restricted and repetitive behavior domain of the SRS. Based on the similarities in the underlying pathways of FXS and AS, and of TSC and NF1, we hypothesized that we might also find similarities in symptom presentation between these groups. Contrary to our expectations, the overall ASD severity scores of the TSC group seemed to be more similar to those of the FXS group whereas the AS group scores appeared more similar to those of the NF1 group.

Cross-Syndrome Comparisons of Symptom Severity Scores in Individuals With an ASD Classification

By comparing the syndrome groups only including individuals with an instrument-based ASD classification we reduced the within-group variability and we were able to compare our syndrome groups to a non-syndromic ASD group. The severity scores of the syndrome groups differed from those of the non-syndromic ASD group on several domains. Especially on the SRS, the syndrome groups showed less ASD symptom severity compared to non-syndromic ASD in the social domain. On the restricted and repetitive behavior domains on the other hand, the nsASD severity scores were lower than those of the FXS group, and higher than those of the TSC and NF1 groups. Despite some slight differences, the results of the subgroup comparisons were broadly similar for the ADOS and the SRS. On the main scores of the ADOS and SRS, the scores that determine whether someone qualifies for an ASD classification, the syndrome groups seem to perform relatively well in terms of social interaction and communication compared to the non-syndromic ASD group. As we discussed earlier, the DSM-5 requires that social communication difficulties are more impaired than would be expected based on the developmental level of an individual. Our findings suggest that a combination of severe developmental delay and relatively good social communication skills may prevent individuals with these syndromes from receiving an ASD classification or even a clinical diagnosis, which influences their access to health care services.

Cross-Syndrome Symptom Profile Analysis in Individuals With an ASD Classification

Our comparison of the ASD symptom profiles revealed several syndrome-specific strengths and challenges. While the FXS group scored highest overall, our results demonstrated a specific relative challenge for this group in the restricted interests and repetitive behavior domain. On the other end of the spectrum, the NF1 group had the least severe ASD symptoms overall, and had a specific relative strength in the restricted and repetitive behavior domain. The low severity scores in NF1 could cause ASD symptoms to be more easily overlooked in clinical practice in this group compared to the other syndrome groups. The TSC group also showed more challenges in the restricted and repetitive behavior domain, but their scores in this domain were lower than the FXS group and the nsASD group. For the AS group we found a relative strength on the social communication and social motivation domains, and a relative weakness in the reciprocal social interaction and in the creativity and play domains of the ADOS. Despite the fact that for all children with AS the lowest module was selected, this effect is likely due to a discrepancy between the developmental level of the AS group and the demands that some ADOS items put on the children. Therefore, in clinical practice, all scores that require a certain cognitive level must be interpreted cautiously for individuals with severe developmental delay.

While the genetic variations in FXS and AS, and in NF1 and TSC, respectively, affect neurodevelopmental pathways in a similar manner, the syndromes are fundamentally different in their origin as well as their phenotypes. Therefore, it would be highly unlikely that these syndromes would show an identical pattern of ASD symptoms on all subscales. Despite several differences, the syndrome pairs did show similarities on a majority of the ASD symptom subscales. To further explore the relationship between the observed similarities in the ASD profiles and the genetic pathways affected in these syndromes deep phenotyping studies are recommended that, for example, include (neural) biomarkers.

Strengths and Limitations

A strength of our study is that our sample was relatively free of selection bias. All assessments were carried out in the context of regular clinical care and all children were assessed regardless of whether ASD was suspected. This was supported by the fact that we did not find a difference in ASD severity between the children with and without an IQ assessment. Nevertheless, a selection bias may still exist for the FXS group. As the somatic problems are often mild or absent in FXS—as opposed to NF1, TSC and AS—it is possible that only children with more severe (behavioral) problems choose to visit the ENCORE center of expertise.

Even though our sample is relatively large for a cross-disorder comparison between rare disorders, and the sample is relatively free of bias, we could not control for neurobiological variability within the syndrome groups (e.g., the deletion status in AS, the mutation type in TSC, the locations of neuronal tubers or abnormal tissue growth in NF1 and TSC, or the genetic mosaicism and gender differences related to the X-linked nature of FXS). We also did not account for other comorbidity within our sample, such as epilepsy, ADHD or anxiety disorders. While ASD, social anxiety and ADHD are distinct neurodevelopmental conditions, their symptoms do overlap and therefore comorbidity of ADHD and/or anxiety with ASD may influence scores on both the ADOS and the SRS. Especially in FXS, social anxiety is very common, in children with and without an ASD diagnosis (87). Future in-depth studies should explore these potentially contributing

factors in these individual syndromes and study their effect on the development and presentation of ASD symptoms. In the subgroup analyses the sample sizes were even smaller as we included only individuals with an ADOS or SRS ASD classification. We chose to use an instrument-based classification for both the SRS and ADOS separately, instead of DSM-5 criteria, or a combined ADOS and SRS classification, for our subgroup selection because we wanted to examine the broad spectrum of ASD symptoms regardless of whether individuals qualified for a DSM-5 classification, or whether they qualified on both instruments. Although conclusions on the presence of clinical ASD diagnoses within these syndrome groups could not be drawn with this approach, it enabled us to examine both clinical and sub-clinical symptoms present in these syndromes. While a reduction of sample sizes in our subgroup analyses was expected, given the prevalence of ASD in these syndromes, the results of the subgroup analyses should be interpreted cautiously. Therefore, replication of this study in a larger sample, while accounting for syndrome specific features, would be necessary to validate the results of this study. This may be achieved by combining data from large natural history studies, such as the FORWARD study on FXS (88) and the TSC Natural History Database (TSC Alliance) for example. Data from the FORWARD study shows that 87% of children with FXS would classify as having ASD on the SRS, irrespective of ASD diagnosis (89), which supports the findings of this study. However, because ASD is not as prevalent in all syndromes, ASD screening is not standard practice for all syndromes, so selection bias should be accounted for.

Regarding gender differences, males in general are more likely to receive an ASD diagnosis than females (90). In addition, we know that FXS is more prevalent in males, and that males with FXS are more severely affected, in terms of developmental level as well as ASD symptomatology. Therefore, we added gender as a covariate in our analyses. The proportion of males vs. females in our FXS group was high at ∼75%. Because the prevalence of ASD in males is higher than in females, in FXS (33) as well as in general (33, 91), including more males may have resulted in a higher ASD classification prevalence in our FXS sample compared to other studies, and a lower developmental level. Even though our sample size was relatively large for a cross-disorder comparison between rare disorders, the number of included females was not sufficient for stratification into gender subgroups. From studies in non-syndromic ASD as well as syndrome groups, we know that gender affects the pattern of ASD symptom presentation (90). Future studies should stratify their data in order to reveal the impact of gender on the presentation of ASD characteristics in males vs. females.

Another limitation of this study is the difference in developmental level between our groups, especially between the syndrome groups and the nsASD group. As our FXS group contained more males than expected, and the IQ of males with FXS is generally lower than that of females with FXS, the IQ of our FXS group may be relatively low compared to more balanced samples. It is known that the developmental level of children and young adults can influence the measurement of ASD symptom severity (77, 78). The ADOS allows clinicians to choose a module based on the developmental level of the participant, which is estimated based on the level of spoken language. However, the cognitive profiles of syndrome groups also show syndrome specific strengths and challenges. Individuals with similar levels of spoken language may vary greatly in other areas of cognitive and motor development. There are no specific norms available for the ADOS for samples with developmental delay or complex behavioral problems. In our study we compared symptom severity in the syndrome groups to a non-syndromic ASD sample in order to better interpret the group differences that we have found. However, it is important to keep in mind that the developmental level of this non-syndromic ASD sample was higher than that of our syndrome groups. We have attempted to reduce the effect of developmental levels on our results by adding IQ or DQ scores as a covariate in our analyses, but as IQ/DQ levels are likely to vary within and between our groups, our results should be interpreted cautiously.

CONCLUSION

The syndrome-specific strengths and challenges we found in FXS, TSC, AS, and NF1 provide a frame of reference to evaluate an individual's symptom severity relative to the syndromic population as a whole and to guide treatment decisions. Based on the overall ASD symptom profile, clinicians should closely monitor the development of ASD-symptoms, taking into account the syndrome-specific strengths and weaknesses within the ASD profile when selecting treatment methods. Similarities in ASD symptom profiles between AS and FXS, and between NF1 and TSC may be caused by similarities in their underlying neurobiological pathways. Deep phenotyping studies are required to link symptom patterns to specific neurobiological pathways more directly. Additionally, the variation in symptom severity within our sample also highlights the need to investigate patterns of ASD symptom severity within syndromes, which might reveal subgroups with a more homogeneous symptom presentation.

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DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Materials**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Medical Ethics Committee of the Erasmus University Medical Center, Rotterdam, Netherlands (MEC-2015-203 and MEC-2011-078). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

BD, LH, PN, JL, AR, the ENCORE Expertise Group, SM, and GD were involved in the design of the ENCORE database and supervised data collection. ES, KL, and DH gathered and prepared the dataset. KG-L provided the data of the Social Spectrum Study. KL performed the analyses. SM, GD, and MH were involved in the planning and supervision of the project. KL and ES prepared the manuscript in consultation with SM, BD, and GD. All authors discussed the results and contributed to the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2022.852208/full#supplementary-material

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The Feasibility and Effectiveness of a Novel, On-Line Social Skills Intervention for Individuals With Prader-Willi Syndrome

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Introduction: People with neurodevelopmental disabilities, including Prader-Willi syndrome (PWS), are at heightened risk for the negative sequalae of loneliness, including depression and anxiety. While societal factors such as stigma or limited social opportunities contribute to loneliness, so too do deficits in social cognition and social skills. People with PWS have specific difficulties recognizing affect in others, accurately interpreting social interactions, and taking the perspectives of others. These features, combined with hyperphagia, rigidity, and insistence on sameness conspire to impede the abilities of people with PWS to make and sustain friendships and reduce feelings of loneliness.

Methods: We developed and administered an intervention, Building Our Social Skills (BOSS), that aimed to improve social skill deficits in PWS. The 10-week intervention was administered on-line via Zoom to 51 young people with PWS in the U.S. (M age = 20.8, SD = 6.42). Two clinicians co-led groups of 6–8 participants in 30-min sessions, 3 times per week, and also trained 4 graduate students to co-lead groups with high fidelity. We used a pre-post intervention and 3-month follow-up design, with no control group, and mitigated this design limitation by triangulating across informants and methodologies. Specifically, parents completed the widely used Social Responsiveness Scale (SRS) and Child Behavior Checklist (CBCL), and participants were individually interviewed about their friendships and loneliness. Interview responses were reliably coded by independent raters.

Results: Repeated measure multivariate analyses, with baseline values entered as covariates, revealed significant pre-to post-test improvements in the SRS's social cognition, motivation and communication subscales (p's < 0.001), with large effect sizes ($n_p^2 = 0.920$, 0.270, and 0.204, respectively). Participant and parental reports of loneliness were correlated with the CBCL's Internalizing domain, specifically the Anxiety/Depressed subdomain. Over time, parents reported getting along better with peers, increased contact with friends, more friends and less loneliness. Participants also reported significantly less loneliness and more friends.

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Conclusions: This mixed method, proof-of-concept study demonstrated the feasibility of delivering an on-line social skills intervention to young people with PWS. As no differences were found between clinician vs. graduate student outcomes, the BOSS curriculum holds considerable promise for wider dissemination and implementation in the PWS community.

Keywords: building social skills in Prader-Willi syndrome, social cognition, social isolation, loneliness, COVID-19, telemedicine

"It is a good thing to have many friends. No one would choose to live without friends even if he possessed all other goods…True friends wish the good of each other."

Aristotle, Nicomachean Ethics, 349 BC

INTRODUCTION

Aristotle foretold what contemporary studies in psychology, sociology and social neuroscience have collectively confirmed: that the human brain is wired for social engagement and that friendships and connections to others are the cornerstones of human flourishing and well-being (1, 2). Yet some people struggle to develop friends and are at risk for social isolation or loneliness, especially those with intellectual disabilities (ID). Both societal factors and characteristics of ID hinder the ability of these individuals to successfully engage with others and to develop and maintain friendships (3).

At the societal level, people with ID often experience stigma, discrimination and restricted opportunities for community engagement (3). Relative to the general population, individuals with ID also experience elevated rates of bullying and abuse (4). At the same time, ID is defined by cognitive and adaptive deficits in conceptual, practical and social domains (5). Individuals may, for example, have problems communicating and taking the perspectives of others, as well as with performing such executive functions as focusing, attending to and evaluating pertinent stimuli, planning, self-regulating and controlling emotions (6, 7). Deficits in any these areas are likely to impede optimal social functioning.

Taken together, these societal forces and attributes of ID contribute to the increased rates of loneliness and social isolation in this population. Loneliness and social isolation are related but distinct concepts. Loneliness is conceptualized as a negative emotional response to the discrepancy between one's actual vs. desired quantity or quality of social interactions (8). In contrast, social isolation is an objective index that quantifies one's social contacts, often assessed through social network size. While isolation is a risk factor for loneliness, even those with enriched social networks may still feel lonely. Further, those who are isolated may be content with their solitude, without experiencing loneliness.

Both loneliness and social isolation have been studied in people with ID. Macdonald et al. (9) found that a full 73% of 310 individuals with cognitive impairments or other developmental disabilities indicated that they were lonely. In a review of studies that sampled over 11,000 adults with ID, Alexandra et al. (10) calculated an average loneliness prevalence rate of 44.7%. Despite variability in rates across these studies, people with disabilities experience loneliness to a greater degree than the general population (11). Further, loneliness in people with ID is associated with poor mental health, especially depression (10, 12).

Loneliness or social isolation may intensify in certain developmental periods. For example, once young adults with ID leave formal schooling, they are at heightened risk for social isolation or loneliness, as they have lost the built-in social connections, supports, and services that schools provide. Navigating the fragmented adult service system in the U.S. is challenging, and many adults with ID lack employment or meaningful social and recreational opportunities. As such, compared to others, adults with ID have fewer friendships and smaller social networks that are often limited to family members or paid care providers (13–16). Asselt-Goverts and colleagues (17), however, reported that the majority (73%) of their participants with ID were satisfied with the size of their social networks. Instead, they expressed desires to strengthen their existing relationships, as well as to bolster their skills interacting with others.

Although studies to date have focused on ID in general, people with Prader-Willi syndrome (PWS) have several phenotypic features that place them at even higher risk for loneliness or social isolation. PWS is a genetic, neurodevelopmental disorder caused by the lack of paternally imprinted genes on chromosome 15q11–15q13, either through paternal deletions that vary in size or through maternal uniparental disomy (mUPD), or when both copies of chromosome 15 are maternally inherited (18). Hyperphagia, often cast as the hallmark of this syndrome, begins in early childhood and is associated with aberrant neural networks involved in satiety and reward. Impaired satiety results in a state in which individuals are habitually hungry yet rarely feel full (19–21). People with PWS thus need external dietary controls and constant food supervision to avoid becoming morbidly obese (18). And, as food is readily available in most communities or family social gatherings, hyperphagia also restricts opportunities for engaging with others.

PWS is also characterized by mild to moderate intellectual disability, growth hormone deficiencies, temper outbursts, rigidity, insistence on sameness, and repetitive, compulsive behaviors (22–26). While approximately 12.3% of individuals with PWS have co-occurring autism spectrum disorder, many more show some degree of impairment in the quality or quantity of their reciprocal communication (24). Further, people with PWS often exhibit executive function difficulties, especially with

TABLE 1 | Demographic variables for 51 participants with PWS and their parents.

	M (SD) or %	
PWS demographics		
Age (M, SD)	M = 20.82 (4.63)	
Age range	14 to 33 years	
Male	45.1%	
Female	54.9%	
White	92%	
Black or asian	8%	
In high school	47.1%	
Graduated high school	52.9%	
Employed graduates	18%	
Living in family home	88%	
Living outside family home	12%	
Paternal deletion	70.6%	
mUPD	25.5%	
Imprinting defect	3.9%	
Parent demographics		
Maternal age	M = 51.01 (6.53)	
Paternal age	M = 53.33 (8.67)	
Education	Maternal	Paternal
High school	16.6%	37.5%
2-Year college	9.8%	8.3%
4-Year college	41.2%	20.8%
Professional/Graduate	32.4%	33.3%

attention and task switching (27). Many individuals thus have difficulties modifying their behavior to fit changes or nuances in social interactions and may instead respond to social situations with temper outbursts, impulsivity, and rigid thinking (27).

Given these phenotypic features, people with PWS often have significant problems sustaining friendships and getting along with peers or others (22, 24, 28). Such interpersonal problems are also associated with deficits in social cognition, or those processes that enable people to understand and successfully engage in the social world (29). These processes include recognizing emotional states in others, understanding what others are thinking (theory of mind), and using social cues to draw inferences about interpersonal or social situations (social perception). People with PWS often show deficits in these key relationship-building skills.

First, they have difficulties recognizing affect in others. Consistent across two studies (30, 31), participants with PWS readily identified happy, and were significantly better at identifying anger than sadness or fear. Examining affect recognition over a 2-year time period in 94 individuals with PWS, Dykens et al. (30) found that participants improved in their recognition of fear, but no significant gains were found for sadness. Further, even with some improvement, recognition of these negative emotions remained at chance levels, and sad was often mistaken for anger, and anger for sad.

Second, people with PWS show impairments in high-order theory of mind tasks. Administering false-belief theory of mind tasks to 66 children and youth with PWS, Lo, and colleagues (32) found that participants generally understood another person's mistaken belief, or so-called first order-beliefs. Similarly, Tager-Flusberg and Sullivan (33) reported that 10 children with PWS outperformed those with Williams syndrome on a first-order false belief task. Lo et al. (32), however, found that people with PWS struggled with more complex second-order tasks, or identifying what one person thinks about another person's beliefs.

Finally, just one study has examined the social perceptions of people with PWS, or how they use social cues to interpret interactions between people. Dykens et al. (31) longitudinally administered videotaped social perception vignettes to 94 individuals with PWS that depicted negative events with either sincere/benign or insincere/hostile interactions between peers. Participants made some gains over time detecting pertinent social cues, but not in using these cues to form correct conclusions about the intentions of others. They had consistent difficulties in accurately judging the sincere intentions of others, but over time performed better in correctly judging interactions involving trickery, deceit and lying.

Given such findings, interventions are sorely needed to improve social functioning in people with PWS. Group interventions teaching social engagement and communication skills have been deemed an evidence-based practice in individuals with autism spectrum disorder (34–36) and in other groups with impaired social skills [e.g., schizophrenia, (37–39)]. Yet no such interventions have yet been tried in people with PWS.

The first aim of this study, then, was to test the practicality and tolerability of a novel, on-line, group intervention aimed at improving social cognition and social engagement skills in adolescents and adults with PWS. Demonstrating feasibility was especially important as the intervention required a significant time commitment from both participants and group leaders; 30-min sessions were conducted 3 times a week for 10 consecutive weeks.

Our second aim was to determine how well participants with PWS responded to the intervention. We hypothesized that, on average, participants would show less social dysfunction but improved social engagement skills. We further expected that these gains would be associated such real-word outcomes as an increased number of friends, getting along better with others, and more sophisticated understandings of what friendships mean. We also hypothesized that loneliness would be associated with internalizing symptoms, and that feelings of loneliness as reported by both participants and parents would diminish over time.

METHODS

Design

As a proof-of-concept study, we used a quantitative and qualitative, mixed-method, pre-post intervention and 3-month follow-up design, with no control group. We mitigated the limitations of this quasi-experimental design by triangulating across informants and methodologies (38–40). Specifically, baseline, post-intervention and 3-month follow-up data were obtained from two sources: parents completed standardized measures of social and behavioral functioning; and participants

FIGURE 1 | Major topics addressed in the BOSS curriculum. Beginning with basic emotion recognition skills in one's self and others, the curriculum then emphasized regulating one's own emotions in order to accurately perceive the intentions and perspectives of others. Building on these skills, the program ended with lessons about conversing, listening and developing and maintaining friendships.

with PWS were administered semi-structured interviews regarding their friendships and loneliness. Although individual differences are often found between informant ratings of emotions or behaviors (41), triangulation increases the credibility of a study if similar findings are obtained from different informants and methodologies.

Participants

The intervention included 51 adolescents or young adults with genetically confirmed PWS aged 14 to 33 years who resided in the U.S. (M age = 20.82 years, SD = 4.63; 45.1% male). The study was posted via PWS-related social media outlets and included the need for prospective participants to have access to a computer and the internet. As shown in **Table 1**, participants varied in PWS genetic subtypes, with most (70.6%) having paternal deletions. We aimed to recruit participants both in and out of high school to determine if school status was associated with dependent measures or outcomes. Approximately half were still in high school (n = 24), and of the 27 high school graduates, just 5 were employed.

A power analysis was conducted using rates of loneliness in a separate population of individuals with PWS and anticipated rates of loneliness in our participants post-intervention. Setting the alpha at 0.05 and power at 0.80 yielded a sample size of 48. We over recruited as we anticipated some attrition. Indeed, an additional eight individuals were enrolled but then withdrew from the study either after baseline or in the first few weeks of the intervention. Reasons for withdrawal included scheduling conflicts, disruptive behaviors during sessions and/or an unwillingness to participate in the curriculum. No significant differences emerged between completers vs. non-completers in age, gender, genetic subtypes or baseline scores on dependent variables.

Procedures

Consent

The study was approved by the Behavioral Science Institutional Review Board (IRB) at Vanderbilt University (IRB# 16155). Consistent with IRB procedures, parents provided written, informed consent while individuals with PWS provided written, informed assent. We ensured that participants and their parents understood the time commitments involved in the study as well as the need for sessions to be recorded for training and research purposes.

Group Sessions and Leaders

Six to eight participants were enrolled in 30-min group sessions that met 3 times per week for 10 consecutive weeks via Zoom. To avoid scheduling conflicts and facilitate compliance, sessions were scheduled at the same time each week. All sessions were recorded.

Two clinicians with expertise in PWS co-led 46% of the group sessions. To mitigate "therapist effects," or the possibility that some interventionists consistently achieve superior outcomes than others (42), the two clinicians trained and supervised four graduate students (who had minimal exposure to PWS) to co-lead the remaining 54% of sessions. Students were supervised 2–3 times a week until they became comfortable with the curriculum and managing participants. Students then met with the supervising clinicians once-weekly or on an as-needed basis.

The clinical supervisors ensured high treatment fidelity by reviewing at least one graduate student led taped session weekly using well-accepted criteria (43). These included preparing materials for sessions; establishing rapport and group rules; adhering to curriculum lessons and content; appropriately engaging participants; reviewing content; and encouraging participants to practice specific lessons outside of group time.

BOSS Curriculum

The curriculum was based on the social skills deficits typically encountered in PWS. It was divided into three modules that functioned synergistically, with each module building on previous lessons. As shown in **Figure 1**, the curriculum began with teaching such basic social cognition skills as recognizing emotional expressions in others, taking another person's point of view, and correctly interpreting the intentions of others. The second module focused on recognizing affect in one's self, especially such strong negative emotions as anger, and how to best handle them via self-control, apologizing and taking responsibility. The curriculum ended with a module on making friends and such social engagement skills as starting a conversation, conversational turn-taking, listening to others, giving back, and moving from superficial exchanges to trusting one another and a deeper sharing of thoughts.

For one session toward the end of each module, participants were given an exercise to practice together based on lessons learned, without being guided by group leaders. Group leaders remained on Zoom to observe, provide feedback to individuals at the next session and assist as needed.

Participants were encouraged to practice specific skills at home or in the community, and to bring their experiences doing so to the next session. Parents were provided with brief descriptions of each week's curriculum, giving them the option of reinforcing concepts or practicing skills with their child.

Measures

Demographics

Parents completed a brief questionnaire regarding their child's age, gender, genetic subtype of PWS, and previous or current schooling and employment status. Parental age and educational status were also ascertained for descriptive purposes of the sample.

Social Responsiveness Scale-2

Parents completed this 64-item questionnaire (44) that assesses social impairments often seen in autism and other developmental disabilities. Items were rated using a 1 to 4 scale; 1 = Not true, 2 = Sometimes true, 3 = Often true, 4 = Almost always true. Seventeen items are reverse scored.

The SRS includes four social subscales and a repetitive and restricted behavior subscale. As the intervention did not target repetitive behaviors, this subscale was not included in analyses. The social subscales include: Social Cognition, 12 items that tap the ability to interpret social behavior (Cronbach's alpha = 0.75); Social Communication, 22 items that assess reciprocity in social interactions (Cronbach's alpha = 0.87); Social Motivation, 11 items that tap the extent to which individuals are motivated to engage and interact with others (Cronbach's alpha = 0.80); and Social Awareness, 8 items that measure social cue recognition. Unlike the other subscales, however, the Cronbach's alpha for the Awareness subscale was unacceptable (45) and eliminating two infrequently endorsed items did not improve the alpha. As such, we did not include this subscale in analyses. As recommended by Constantino and Gruber (44), in order to detect changes in social functioning analyses used raw rather than standardized scores. Higher scores indicate more symptoms.

Child Behavior Checklist

The CBCL (46) is a 113-item checklist of internalizing and externalizing problems completed by parents using a 3-point scale, 0 = not true; 1 = somewhat or sometimes true; 2 = very true or often true. In light of previous work on loneliness and internalizing problems, the study only used the Internalizing Problems Domain (Cronbach's alpha = 0.79), which consists of three subdomains (Anxious/Depressed, Depressed/Withdrawn and Somatic Complaints). Domain raw scores were used in correlational analyses; higher scores indicate more problems. The study also analyzed one question from a separate CBCL subdomain (Social Problems), specifically "Complains of feeling lonely."

The CBCL also includes a social competence domain. Three questions were analyzed from this domain that probed patterns of social interactions: (1) Aside from family members, how many close friends does your child have? (scored 0 = 0–1 friend, 1= 2–3 friends, 2 = 4 or more friends); (2) How often does your child visit friends outside of school/work time (scored 0 = < once a week, 1 = 2–3 times per week, 2 = 3 or > times per week); and (3) Compared to others his/her age, how does your child get along with family members and peers, and how well do they work or play alone (scored 0 = worse, 1 = average, 2 = better).

Semi-structured Interviews

Participants were individually interviewed by two graduate students who did not serve as group leaders. The interviews provided a structure for gathering data while also allowing interviewers to clarify or follow-up on comments. Interviews took from 10 to 15 min to complete and were audiotaped for subsequent transcription.

After introductions and rapport building, participants were asked about their friendships and feelings of loneliness. Starting with a general probe, "Tell me about your friends", interviewers followed up with specific queries: "How many friends do you have?", "What are their names?", "Where did you meet them?", "What do you like to do for fun with your friends?" and "What does being a friend mean to you?" or "How would you describe a friend?" Participants were also asked if they ever felt lonely and if so, if there were things that they do to help them feel less lonely.

Consistent with procedures for emergent content coding (47), transcribed interviews were read several times by two members of the research team in order to develop codes that best captured participants' responses. Most responses fell into objective or straightforward coding categories. Responses to the loneliness question, for example, were captured by codes of no, sometimes or yes. The number of friends was verified by asking for friends' names, or what they did together. Similarly, responses to where participants met their friends or what they did together were readily apparent. One question, however, involved making more subjective judgments, specifically "What does a friend mean to you?" or "How would you describe a friend?" As such, answers to this question

were independently coded by two members of the research team. Inter-rater reliability was very high at the pre, post and 3-month follow-up assessments (kappa's = 0.93, 0.86, and 0.89, respectively).

As we observed that responses to this question differed in tone, the same two individuals also independently rated the valence of participants' descriptions of friends as either: positive/neutral (fun, good, like them, I have friends) vs. negative. Negatively-toned responses included a loss of contact with friends (e.g., "I used to see them but not anymore", "They are too busy, I don't hang out with them that much") as well as backtracking from their initial responses (e.g., "Yeah, I got friends, but maybe not much really"). Codes were based on all responses over time, and inter-rater reliability was high (kappa = 0.79).

Statistical Analyses

Analyses included data from individuals who completed the program, without baseline values from non-completers. We justified using an "as-treated" vs. "intend-to-treat" approach as this is the first study to assess a novel intervention (48). Preliminary analyses found no significant effects of age, gender or PWS genetic subtype on dependent measures that would need to be controlled for in subsequent analyses. Similarly, we did not control for group leaders (clinicians vs. graduate students), as there were no significant leader effects in baseline or follow-up evaluations.

Repeated measure multivariate analyses were used to assess changes over time in SRS raw scores. For each analysis, the corresponding baseline score was entered as a covariate. If significant interactions with baseline scores were found, we determined if differential treatment effects were found in those with higher vs. lower baseline scores. Effect sizes were estimated by the partial eta^2 (η_p^2) and interpreted as: $\eta^2 = 0.01$ = a small effect; $\eta^2 = 0.06$ a medium effect; and $\eta^2 = 0.14$ a large effect (49).

Related Samples Cochran's Q Tests were used to assess changes over time in parent and self-reports of loneliness, number of friends and other CBCL social interaction variables. Cochran's Q, commonly used to analyze categorical longitudinal data, requires dichotomous variables. As study variables had 3 or more possible ratings, **Tables 3**, **4**, **7** note how data were meaningfully collapsed into 2 categories. For transparency, however, these three Tables present all ratings.

Spearman's rho correlations assessed relationships between the CBCL's Internalizing domain with parent and self-reports of loneliness. If significant, we followed up with correlations with the sub-domains subsumed under this domain.

RESULTS

Practicality and Tolerability

The intervention was well-tolerated by participants. They logged onto Zoom with minimal help yet did need occasional reminders to adjust their computer screens or microphones. Reliable internet connectivity was noted to be a challenge for two participants. Group leaders observed that participants were on time, prepared and engaged. Informal feedback from parents and group leaders indicated that individuals enjoyed meetings and took them seriously. Many participants stated that they looked forward to sessions and were disappointed when the intervention ended. Ways to address their disappointment and say good-bye were directly addressed in the BOSS curriculum.

Social Responsiveness Scale

Mauchly's Tests of Sphericity were significant for the three repeated measures ANOVAS; as such Greenhouse-Geisser corrections were applied to adjust degrees of freedom. Analyses revealed significant main effects of time for all three SRS subscales, with large effect sizes: Motivation $F_{(1.6,\ 81.2)} = 18.53$, $p < 0.001$, $n_p^2 = 0.270$; Communication $F_{(1.62,81.26)} = 12.84$, $p < 0.001$, $n_p^2 = 0.204$; and Cognition $F_{(1.67,83.57)} = 20.41$, $p < 0.001$, $n_p^2 = 0.920$. See **Table 2** for mean scores. In all analyses, baseline scores significantly differed from the end of the intervention and from the 3-month follow-up. In the Cognition subscale, the end of intervention also differed from the 3-month follow-up.

These main effects of time, however, were qualified by significant interactions with baselines scores, again with large effect sizes. **Table 2** summarizes the F and n_p^2 values and for these interaction terms. To help explain these interaction effects, participants' baseline scores were used to assign them into low, middle or high scoring groups for each subscale. Follow-up repeated measures ANOVAs were conducted with groups entered as a between-subjects factor. These were significant; Social Cognition, $F_{(3.5,\ 84.3)} = 4.47$, $p = 0.004$, $n_p^2 = 0.157$; Social Motivation $F_{(3.42,82.14)} = 10.35$, $p < 0.001$, $n_p^2 = 0.30$ and Social Communication $F_{(3.4,81.9)} = 3.40$, $p = 0.017$, $n_p^2 = 0.124$. As shown in **Table 2**, participants with high baseline scores showed more robust improvements than their counterparts with middle or low baseline scores. As well, most improvements occurred between baseline and the end of the intervention, with scores showing either stability or more modest improvements at the 3-month follow-up.

Even so, we observed individual differences within these three groups. As such, we also determined the percentage of participants in each group who showed improvements from baseline to the end of the intervention, and from baseline to the 3-month follow-up. Improvements were operationalized as a difference in scores that were at least one-half of each subscale's standard deviation. Summing across subdomains from baseline to the end of the intervention, improvements were found in 25.7% of the low baseline group, 54.8% of the middle group and 84.3% of the high group. Percentages were similar for differences from baseline to the 3-month follow-up (25.6%, 57.0%, and 84.3%, respectively).

Friendships

Number of Friends

As shown in **Table 3**, over time parents reported significantly more close friends in their offspring with PWS, Cochran's Q (2) = 25.90, $p < 0.001$, with baseline differing from the end of the intervention ($p < 0.001$) and from the 3 month-follow-up

TABLE 2 | Descriptive statistics, F's and p's for significant interactions between time and baseline raw scores on the Social Responsiveness Scale subscales.

SRS subscales	Baseline M (SD)	End of intervention M (SD)	3 month follow-up M (SD)	F, p	n_p^2
Social cognition total mean	25.23 (6.52)	22.76 (6.38)	21.44 (6.24)	7.13***	0.137
Low baseline	18.50 (2.68)	18.55 (4.11)	17.05 (4.59)		
Middle baseline	24.43 (1.31)	21.87 (3.98)	21.12 (3.98)		
High baseline	32.76 (3.42)	27.88 (6.46)	26.17 (6.00)		
Social motivation total mean	13.02 (5.98)	11.00 (5.45)	10.37 (4.27)	29.47***	0.367
Low baseline	7.31 (1.95)	7.62 (3.09)	7.68 (2.91)		
Middle baseline	12.25 (0.85)	10.62 (2.94)	9.43 (1.59)		
High baseline	19.52 (4.69)	14.78 (4.09)	14.01 (4.37)		
Social communication total	23.45 (7.51)	19.52 (7.79)	18.72 (7.77)	5.88**	0.107
Low baseline	14.38 (3.45)	12.61 (4.17)	12.31 (4.75)		
Middle baseline	21.55 (1.43)	20.90 (5.40)	19.55 (5.83)		
High baseline	31.44 (4.56)	25.05 (7.07)	24.31 (7.48)		

$^{**}p$ <0.01; $^{***}p$ < 0.001.

TABLE 3 | Parental- and self-reports of the number of participant's friends over time.

	Baseline	End of intervention	3-month follow-up
Parental reports			
0–1	62.7%	45.1%	30.0%
2–3	37.3%	51.0%	58.0%
4 or >	0	3.9%	12.0%
Participant reports			
0–1	36.8%	32.6%	22.4%
2–3	6.1%	18.4%	22.4%
4 or >	2.0%	6.2%	16.4%
Non-Specific+	55.1%	42.8%	38.8%

+Did not specify number or names of friends but stated had "lots" or "some" friends. For Cochran's Q, parental data were dichotomized into 0–1 friend vs. 2–3 and 4 or > friends. For Cochran's Q, self-report data were dichotomized into 1 or more named friends vs. the non-specific category. Analyses did not include the 2 individuals who reported no friends at any time point.

TABLE 4 | Parental responses over time regarding frequency of contact with friends and getting along with peers.

	Baseline	End of intervention	3-month follow-up
How often does your child see friends?			
< once a week	84.3%	66.7%	47.1%
2–3 times a week	13.7%	29.4%	47.1%
4 or > times a week	2.0%	3.9%	5.8%
How well does your child get along with Friends/Peers?			
Worse	49.8%	35.3%	22.4%
Average	46.3%	56.9%	65.8%
Better	3.0%	7.8%	11.8%

For Cochran's Q, variables were dichotomized into: < one a week vs. 2–3 and 4 or > times a week; and worse vs. average and better.

($p < 0.001$). Similarly, participants also reported having more friends over time, Cochran's Q (2) = 12.67, $p = 0.002$. See **Table 3**. Baseline values differed from the end of intervention ($p = 0.013$) and the 3-month follow-up ($p = 0.004$). At baseline, 55% stated that they had "some" or "lots" of friends but could not specify their names. At the 3-month follow-up, however, these non-specific responses declined to 38.8%, with more individuals naming friends or specifying what they did with them. Further, the number of individuals reporting 0 to 1 friend declined, with a concomitant increase in reporting 2 or more friends, from 8.1 to 38.8%. As participants, and not parents, offered non-specific responses, agreement between them was not assessed.

At Baseline, five individuals indicated that they had no friends, but subsequently named from 1 to 3 new friends at the end of the program or the 3-month follow-up. Two individuals stated that they had no friends at any time point. As all of these seven individuals were no longer in school, follow-up chi-square analyses revealed that participants in vs. out of school were also more apt to report having "some" or "lots" of friends (64% vs. 37.5%, respectively), X^2 (4) = 12.56, $p = 0.014$. No other differences were found in participants who were in or out of school on other outcome variables.

Contact With Friends and Getting Along With Others

As summarized in **Table 4**, parents reported significant increases in the amount of contact that participants had with friends outside of school/work, Cochran's Q (2) = 21.68, $p < 0.001$. Baseline and the end of the intervention differed from the 3-month follow-up ($p < 0.001$ and $p = 0.014$, respectively).

No changes were found in how well participants got along with parents or siblings, or when they played or worked alone. A significant improvement, however, emerged in getting along with peers (see **Table 4**), Cochran's Q (2) = 6.95, $p = 0.030$, with baseline differing from the 3-month follow-up ($p = 0.016$).

TABLE 5 | Frequency and examples of interview coded responses to "What is a friend?".

What is a friend? how would you describe a friend?		
Codes	**%**	**Examples**
Positive adjectives	37.7%	Nice, sweet, kind, funny, polite, friendly, adorable, caring, courteous, cool, energetic, fun
Be together/Have Fun	23.2%	Hang out, talk, have fun, play, stay in touch, do stuff, have conversations, laugh together, have same interests
Trustworthy/Loyal	15.2%	Someone you can trust, tell secrets to, loyal to me, I can rely on, dependable
Supports/Cares for me	13.5%	There for me, helps me out, cares for me, likes me, respects me, nice to me, understands me
Not mean	5.2%	Not take advantage of you, not saying mean things, doesn't bully you, doesn't talk back
Reciprocity	5.2%	You can talk to them and figure out what's wrong and then help them through the tough times; Being kind, respectful and supportive of one another; You stick up for each other and are there for each other in the good times and bad times

TABLE 6 | Percentage of interview responses to "What do you like do with your friends for fun?" and "Where did you meet your friends?".

What do you like do with your friends for fun?		Where did you meet your friends?	
Go to places, movies	25.7%	School	54.9%
Hang out, talk	25.0%	Special Olympics	10.8%
Play games, other activities	18.6%	Family, Neighbors	9.8%
Physical activities, sports	15.0%	Church	8.8%
Not much	6.1%	Job Training, Work	5.9%
Eat	5.3%	PWS Events	5.9%
Watch TV	4.3%	Camps	3.9%

Coded Descriptions of Friends

Coded responses to "How would you describe a friend?" are summarized in **Table 5**. Most participants, 49.7%, had 2 codes per response, 33.3% had just 1 code and 17.0% three codes. As no changes over time were found, **Table 5** presents the total average percentage across responses. The most frequent codes were positive adjectives (37.7%) and being together and having fun (23.2%). Two codes further reflected how friends benefited participants, being loyal to them (15.2%) and accepting and supportive of them (13.5%). Far fewer individuals, however, described being supportive or loyal to their friends (5.2%). As shown in **Table 6**, most participants met their friends at school (55%), Special Olympics (11%), and through their family or neighborhood (10%). **Table 6** also indicates that participants engaged in a variety of activities with friends.

TABLE 7 | Parental responses to "Complains of loneliness" and participant responses to "Do you ever feel lonely?" over time.

	Baseline	**End of intervention**	**3 month follow-up**
Parental responses			
Yes	7.8%	3.9%	0
Sometimes	43.2%	27.5%	23.5%
No	49.0%	68.6%	76.5%
Participant responses			
Yes	12.2%	6.1%	6.1%
Sometimes	38.8%	28.6%	26.5%
No	49.0%	65.3%	63.4%

For Cochran's Q, parental and self-reports were dichotomized into no vs. sometimes and yes.

The majority of respondents projected a positive valence about their friendships. Even so, 26% expressed that they had lost friends (e.g., "I have friends but I call them to hang out with them but they never get back to me cause they're too busy with college and what not", "Yeah, but I haven't seen them mostly for a long time," "I have friends from school, but they don't really call me back 'cause they don't have special needs like I do"). Unsurprisingly, those expressing a loss of friends also reported higher rates of loneliness than others (75% vs. 21.6%), X^2 (1) = 11.39, p <0.001.

Loneliness

Frequency of Loneliness

Table 7 depicts that over time, parents related significantly less loneliness in participants, Cochran's Q (2) = 13.65, p =0.001, with Baseline differing from the end of intervention ($p = 0.001$) and the 3-month follow up ($p < 0.001$). Similarly, participants with PWS also reported being less lonely over time, Cochran's Q (2) = 10.43, $p = 0.005$. Baseline values differed from the end of intervention ($p = 0.009$) and from the 3-month follow-up ($p = 0.003$).

Although both parents and participants reported diminished loneliness over time, agreement between them was relatively poor. Probing these low kappa's further, increases over time were found in parents and offspring agreeing that they were either lonely or not lonely (baseline agreement = 53.1%, end of intervention = 61.1%, 3-month follow-up$p$ = 75.5%). Across the three time points, disagreements occurred in both directions, when parents endorsed loneliness, but participants did not (48.1%) and when participants indicated they were lonely, but parents did not (53.0%).

Behaviors if Lonely

When feeling lonely, many (44%) participants reported engaging in activities that distracted them and made them feel better (e.g., listening to music, watching movies), an additional 25% played with their pets. Some (19%) reported that nothing really made them feel better, and just 12% reached out to others.

Correlates of Loneliness

Collapsing across all assessments, parental ratings of loneliness were correlated with the CBCL Internalizing domain,

$r_{(151)} = 0.46$, $p < 0.001$, specifically with the Anxious/Depressed subdomain, $r_{(151)} = 0.51$, $p < 0.001$. Similarly, participant self-reports of their loneliness were correlated with the Internalizing domain, and Anxious/Depressed subdomain, r's$_{(151)}$ = 0.27 and.31, p's = 0.007, and <0.001, respectively. Comparing correlations between informants using Fisher's r to z transformation confirmed that the strength of these relationships was stronger among parents vs. participants for both the Internalizing domain, $z = 0.1.89$, $p = 0.03$ and Anxious/Depressed subdomain, $z = 2.01$, $p = 0.019$.

DISCUSSION

This proof-of-concept, mixed-methods study is the first to explore the feasibility and impact of a social skills training program for young people with PWS delivered in an on-line, small group format. The BOSS intervention proved practical and well-tolerated, with excellent participant compliance. Regarding effectiveness, a convergence of findings across informants and methodologies were promising, reflecting improved social skills as well as increased numbers of friends and contact with them, ability to get along with peers and diminished loneliness. No differential effects of clinician vs. student group leaders were found, which bodes well for the implementation of the BOSS curriculum in the broader PWS community.

Over time, significant improvements, with large effect sizes, were found in the SRS's social motivation, communication and cognition subscales. Moreover, on average, participants appeared to maintain their gains in social skills at the 3-month follow-up. Main effects of time, however, were qualified by significant interactions with subscale baseline scores. Those with relatively high baseline SRS scores demonstrated more robust improvements than their counterparts, with 84.3% of this group showing improved scores. Even so, 57% of the middle and 27.4% of the low baseline groups also improved. On the one hand, those entering the intervention with high baselines have more room to improve relative to those that entered with less social dysfunction. Yet, given individual differences across baseline groups, it would be erroneous to conclude that only those who have more social impairments stand to potentially benefit from the BOSS intervention.

The study also included several real-world outcomes that directly bear on the well-being and quality of life for persons with ID, specifically having friends and keeping social isolation and loneliness at bay (3, 17). Regarding friends, both parents and participants reported an increased number of friends, as did participant's naming their friends or specifying what they did with them. Although getting along with family members did not improve, parents reported that participants were getting along better with peers from baseline to the 3-month follow-up. Admittedly, the BOSS curriculum emphasized peer interactions, yet because participants were encouraged to practice specific social skills at home, we had anticipated a possible "spill-over" effect with family members.

Further, from baseline to the 3-month follow-up, parents reported increased contacts with friends. It may be that increased contact with friends was a by-product of learning about and becoming familiar with Zoom as a user-friendly platform to engage with others. It is unclear, however, if increased contact occurred in person, via an online platform or if parents engaged in extra efforts to ensure contact with friends.

Exploring how individuals with PWS meet with friends is especially important as many participants met their friends at school. At baseline, those out of school were more apt to report having no friends, and those in school indicated that they had "a lot" of friends. These findings underscore the importance of post-graduation venues for meeting friends such as Special Olympics, and religious or recreational organizations. As well, Fulford and Cobigo (50) found that adults with ID who were employed were twice as likely to report having friends than those who were unemployed. As only 5 adults in the current study were employed, working or volunteering are also promising avenues for adults with PWS to expand their social networks and make friends.

Participants engaged in a variety of activities with friends, and most described their friendships in positive terms. Even so, 26% noted a loss of friendships, typically with non-disabled peers, and higher rates of feeling lonely than their counterparts. Although friendship loss is not specifically mentioned, Mason et al. (51) found that negative experiences with friends in adults with ID were associated with stress and feelings of vulnerability. Lunsky and Benson (52) reported that distressful social interactions predicted future depressive symptoms and somatic complaints in adults with mild ID. Future studies are needed on the sequalae of both stressful interactions and friendship loss in people with PWS.

As children develop, they move from more egocentric ideas of friends (they do nice things for me or return a favor) to adolescent understandings that friendship involve empathy, mutual trust, reciprocity and shared support (53–55). Despite including these more sophisticated ideas of friends in the BOSS curriculum, participants did not grow in their understandings of what friendships mean. The majority of responses to "How would you describe a friend?" (66.7%) were captured by two or more codes, suggesting that most participants had at least some degree of complexity in conceptualizing friendships. Even so, the majority of participants' responses reflected the positive things that friends provided to them, not necessarily what they provided to their friends. Indeed, only 5.2% identified reciprocity in their views of friendships.

Reciprocity in friendships is associated with the cognitive ability to take another's perspective (54), which as previously noted, is a weakness for many with PWS. As such, future BOSS interventions may need to place more emphasis on these perspective-taking skills. Even so, it is critically important to emphasize the value of friendships at all levels of development in fostering happiness, well-being, psychological adjustment, self-esteem, and learning and refining interpersonal skills (56, 57).

Regarding loneliness, both parents and participants reported reduced loneliness over time, including at the 3-month follow-up. Agreement of loneliness status between participants and parents increased across assessments, from 53.1 to 75.5%.

The similar rates of disagreements between informants (when parents, and not participants, endorsed loneliness, and visa versa) raises the question of who is best suited to report on loneliness or other internal states. Given their cognitive and communication challenges, many researchers gather such data from parents or other informants. Yet loneliness is a subjective, internal state, and many have long argued that it best assessed in self versus informant reports, including in those with developmental disabilities (58, 59).

Interventions that reduce loneliness are critically important given the negative sequalae of loneliness on health and mental health. Loneliness in the general population is a potent predictor of such mental health problems as depression, anxiety and suicidal ideation, as well as poor physical health and reduced longevity (60–63). Similar associations between loneliness and mental ill health have been found in people with ID (10). Such relations may be amplified in people with ID as they are at higher risk than the general population for both loneliness and psychiatric, behavioral and emotional problems (10, 64). Heiman (12) found that loneliness was as a significant predictor of depressive symptoms in 310 adolescents with ID. Loneliness was associated with depression in 100 adults with Down syndrome (65), and loneliness in 99 adults with ID was associated with both depression and suicidal ideation (66). Similarly, in the current study, both parental- and self-reported loneliness were correlated with the CBCL's Internalizing domain and Anxious/Depressed subdomain, although such associations were stronger among parents. Further, participants reporting a loss of friends were more likely to report feeling lonely than their counterparts without such losses.

The need for strategies that reduce loneliness in people with PWS or other IDs are magnified by the COVID-19 pandemic. It is well-documented that people across the globe have experienced COVID-19 related spikes in such mental health problems as depression, anxiety, distress, loneliness and anxiety (67, 68). Yet people with ID are especially vulnerable to these and other negative sequalae of social distancing, lockdowns, disrupted daily routines, loss of contact with others, and closures of schools, religious, recreational and other community organizations (69, 70).

Although the BOSS intervention concluded prior to the onset of the COVID-19 pandemic, our research team led informal social groups with individuals with PWS during the first wave of the pandemic. Building on the BOSS curriculum, these informal groups emphasized how participants could connect to one another while also engaging in "good deeds" for group members, their families or communities. In doing so, group leaders stressed the need to adhere to rules (showing respect, common curtesy) and for parental involvement or supervision when individuals decided to form their own online social groups (71). As tele-therapy and other on-line social and behavioral health interventions continue to expand, (72, 73) (REF), future research needs to specify the advantages and disadvantages of these interventions for specific disability groups (74) (REF).

Several study limitations deserve mention. First, as a proof-of-concept study, we did not include a control group, which places limitations on how much we can attribute improvements to the BOSS intervention. We mitigated these limitations by triangulating across different informants and methodologies, with both parents and participants reporting positive effects. Such promising results thus lay the groundwork for further evaluation of the BOSS intervention using a more rigorous, controlled study design.

Second, we did not administer standardized measures of loneliness to participants, opting instead to gather self-reports of loneliness via semi-structured interviews. We did so for two reasons. First, we have found that individuals with PWS in our research programs have difficulty completing standardized questionnaires of their internal states (e.g., anxiety, depression), leading to unreliable data. Second, we have successfully used semi-structured interviews to explore the internal self-representations of young people with PWS (20).

An additional concern is that parental reports of loneliness were based on a single question. Single-item questions are widely used to assess loneliness in the general population (75) yet have met with some controversy. Comparing single- vs. multiple-item measures of loneliness in adults, Mund and colleagues (76) conclude that loneliness can indeed be reliably assessed with single-item questions, including the frequency of feeling lonely.

Relatedly, we did not administer a measure of social network size. Doing so would have added specificity to the types of friendships reported by participants or parents. Although informative, semi-structured interviews do not yield systematic data across individuals. For example, participants may or not offer such details as whether or not their friends also have a disability, if they are in a romantic relationship, or if they counted mentors or care-providers as their friends.

Despite these limitations, this proof-of-concept, mixed-method study justifies future work aimed at improving the social skills of people with PWS. Although challenged by their hyperphagia and food seeking (20), study participants learned social engagement, cognition, communication and motivation skills that furthered their friendships and reduced feelings of loneliness. Further studies are needed, yet findings bode well for the dissemination and implementation of the BOSS curriculum in the broader PWS community.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Behavioral Science Institutional Review Board (IRB) at Vanderbilt University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

ED conducted statistical analyses, wrote the first draft of the manuscript, and worked with ED and HH-H to devise the intervention curriculum. ER worked with H-HH and ED to devise the intervention curriculum, and also recruited participants, co-led the intervention and trained and supervised graduate students to co-lead the intervention. H-HH worked with ED and ER to devise the intervention, and also recruited participants, co-led the intervention and trained and supervised graduate students to co-lead the intervention. CM conducted inter-rater reliability analyses, entered data, conducted informal social groups, and assisted with data analyses. All authors contributed to the article and approved the submitted version.

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REVIEW
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Overall Rebalancing of Gut Microbiota Is Key to Autism Intervention

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Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder with unclear etiology, and due to the lack of effective treatment, ASD patients bring enormous economic and psychological burden to families and society. In recent years, many studies have found that children with ASD are associated with gastrointestinal diseases, and the composition of intestinal microbiota (GM) is different from that of typical developing children. Thus, many researchers believe that the gut-brain axis may play an important role in the occurrence and development of ASD. Indeed, some clinical trials and animal studies have reported changes in neurological function, behavior, and comorbid symptoms of autistic children after rebalancing the composition of the GM through the use of antibiotics, prebiotics, and probiotics or microbiota transfer therapy (MMT). In view of the emergence of new therapies based on the modulation of GM, characterizing the individual gut bacterial profile evaluating the effectiveness of intervention therapies could help provide a better quality of life for subjects with ASD. This article reviews current studies on interventions to rebalance the GM in children with ASD. The results showed that *Lactobacillus plantarum* may be an effective strain for the probiotic treatment of ASD. However, the greater effectiveness of MMT treatment suggests that it may be more important to pay attention to the overall balance of the patient's GM. Based on these findings, a more thorough assessment of the GM is expected to contribute to personalized microbial intervention, which can be used as a supplementary treatment for ASD.

Keywords: autism spectrum disorder, gut microbiota, gut-brain axis, prebiotics, microbiota transfer therapy

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INTRODUCTION

Autism spectrum disorder (ASD) is a group of developmental disorders characterized by impaired social interactions and communication together with repetitive and restrictive behaviors (Hsiao et al., 2013). At present, the diagnostic system for ASD is generally based on the Diagnostic and Statistical Manual of Mental Disorders (5th ed.; DSM-5) and International Classification of Diseases (11th ed.; ICD-11). Epidemiological studies have shown that the prevalence of ASD has been steadily increasing in recent years (Baio et al., 2018; Maenner et al., 2020). Moreover, the difficulty of early diagnosis and the lack of effective therapy method of ASD have brought a great economic burden to society and families (Wang et al., 2018).

Autism Spectrum Disorder (ASD) is multifactorial, mainly including genetic risk factors and environmental risk factors, and the clinical presentation of ASD is highly heterogeneous (Kim and Leventhal, 2015). In recent years, as a special environmental factor, gut microbiota (GM) has gradually attracted people's attention. Studies have shown that mental and neurological diseases, such as ASD, attention deficit hyperactivity disorder, depression, anxiety disorder, bipolar affective disorder, Parkinson's disease and Alzheimer's disease, are related to the imbalance of GM, and are usually accompanied by gastrointestinal (GI) disorders (Naveed et al., 2021). Clinical survey data shows that the risk of GI in ASD children is significantly higher than that in typical developing (TD) children, and the severity of autism is associated with the prevalence of GI (Alabaf et al., 2019). A prospective study has found that gut microbiome at age 1 can predict cognitive performance at age 2, especially in communication behaviors, suggesting a possible correlation between gut microbiome and delayed cognitive or language development (Carlson et al., 2018). Recently, the importance of genes such as *CHD8/chd8*, *Foxp1*, *Slc6a4,* and neuroligin-3 (*Nlgn3*) has been discovered in ASD patients with GI diseases (Niesler and Rappold, 2021). Many studies have proved that the disturbance of the microbiota-gut-brain axis plays an important role in the appearance and development of ASD.

With the importance of GM have been recognized, GM re-balance becomes a potentially effective therapy for ASD children, including oral antibiotics, dietary interventions, probiotics and prebiotics interventions, and fecal microbiota transplantation (FMT). Although these interventions are yielding favorable results in treating autistic behavior-related symptoms, standardized clinical studies will lead to more robust results. In this article, we not only reviewed the possible pathways leading to gut microflora dysbiosis in ASD, but also assessed the potential of gut microbiome in ASD screening. Finally, we evaluated the effects of different therapeutic approaches on GM, aiming to compare the effectiveness of GM rebalancing strategy from the behavioral manifestations, and to explore the correlation between species and behavior. In addition, this study also evaluated the presence of micro markers in ASD patients from the perspective of intervention.

GUT DYSBIOSIS IN AUTISM SPECTRUM DISORDER

In addition to neuropsychiatric characteristics, patients with ASD tend to suffer from GI problems. Functional constipation is the most common symptom (Marler et al., 2017), followed by abdominal pain, diarrhea, gas, and vomiting, etc. (Holingue et al., 2018). Many researchers have discussed the complex regulatory relationship (gut-brain axis) between GI system and central nervous system, and there are different views on the relationship between GM and autism (Mayer et al., 2015; Sampson and Mazmanian, 2015; Yap et al., 2021). The main reason for the debate is that the underlying mechanisms of GM affecting the central nervous system is unclear and hard to measure. Nevertheless, the GM still show great potential as non-invasive markers for the diagnosis and therapy of ASD.

Pathways of Gut Microbiota Affecting Autism Spectrum Disorder

The gut-brain axis indicates that the disorder of host intestinal microbiota may be one of the causes of ASD. Recently, Needham et al. (2020) summarized four approaches to how this "bottom-up" impact is carried out: vagus nerve, stimulation of endocrine cells (including enterochromaffin cells), immune-mediated signaling and transport of gut-derived metabolites from the circulation into the brain. And, they believe that all routes comprising the gut–brain axis are thought to be co-opted by the microbiota to impact brain activity and behavior, and signaling through any one of them may be intertwined with other routes (Needham et al., 2020). Based on these four pathways, this study listed the potential evidence of GM affecting ASD.

(a) The vagus nerve provides a direct neural communication pathway between the GM and the central nervous system (CNS), and promotes the regulation of the GM on the function of CNS. Previous studies have found that toxins produced by *Staphylococcus* and *Bacillus* (staphylococcus enterotoxin and glutenin) can send signals to the brain by stimulating the vagus nerve, so as to induce vomiting or other disease behaviors (Hu et al., 2007). Bravo et al. (2011) found that *Lactobacillus rhamnosus* could reduce anxiety and depression related behaviors only in mice without vagotomy, which further explained that neurotransmitters or other metabolites produced by GM could directly regulate vagal activity by stimulating vagal afferent sensory neurons.

(b) Studies have found that 90% of serotonin in the human body is produced by intestinal chromaffin cells, a secretory cell in the inner layer of the intestine (Gershon and Tack, 2007). Enterochromaffin cell production of serotonin impacts its circulating levels and has the potential to influence brain activity directly or indirectly (De Vedder et al., 2018). In addition, some studies demonstrated that some *Bifidobacterium* and *Clostridium* metabolites can also change the content of serotonin in the intestine (Yano et al., 2015; Tian et al., 2019). Improved performance in mouse models of depression have been shown by probiotic treatment with *Bifidobacterium* spp. in a study that concurrently observed an increase either in the levels of serotonin in the brain or in the secretion of serotonin precursor in enterochromaffin cells *in vitro* (Tian et al., 2019). Moreover, Colonic enterochromaffin cells do express receptors for, and respond to, various microbial metabolites, including microorganism-associated molecular patterns (MAMPs), short chain fatty acids (SCFAs), aromatic amino acid metabolites, and secondary bile acids (Kidd et al., 2008; Reigstad et al., 2015; Tsuruta et al., 2016; Lund et al., 2018).

(c) Studies have proved that there is a correlation between intestinal inflammation and immune dysfunction in ASD patients, such as abnormal balance of T cells in the intestine of ASD patients and increased GI problems in ASD patients (Navarro et al., 2016; Vuong and Hsiao, 2017;

Rose et al., 2018). Recently, it has been clearly demonstrated that high concentrations of pro-inflammatory microbiota in the gut can lead to increased intestinal permeability and inflammation, resulting in mild systemic inflammation and immune dysregulation (Felix et al., 2018). In addition, lipopolysaccharide (LPS), as an effective endotoxin in the cell wall of Gram-negative bacteria, has also been proved to induce disease behavior, cognitive impairment and acute depression like behavior in mice by activating systemic inflammation, and affect fetal brain development (Needham et al., 2020). Emanuele et al. (2010) found that the serum LPS level of ASD patients was significantly higher than that of healthy peers and was related to social behavior disorders, which further indicated that immune inflammation may play an important role in the intestinal brain axis.

(d) Many microbial metabolites produced in the gut can pass into systemic circulation at varying levels and rates. One example is SCFAs, where previous studies have shown that any interference in this signal transduction may have a direct impact on the central nervous system, which may lead to neurodevelopmental disorders and neurodegenerative diseases (Borre et al., 2014; Hill et al., 2014). Moreover, it has been proved that SCFAs metabolized by intestinal microorganisms can enter the circulatory system to regulate immune and inflammatory reactions, and then affect the neural function and development of human brain (Foley et al., 2014; Frost et al., 2014; Chambers et al., 2015). In spite of many studies support the health benefits of SCFAs, such as energy supply for epithelial cells, restoring epithelial barrier function, anti-inflammatory, and immunomodulating activities (Richards et al., 2016). However, it is important to note that excessive quantities of propionic acid (the main SCFA produced by *Clostridium*, *Bacteroides,* and *Desulfovibrio*) have been reported in irritable bowel syndrome, and necrotizing enterocolitis (Wang et al., 2007; Tana et al., 2010). In addition, the study found elevated levels of SCFA in the feces of children with autism (Wang et al., 2014). Although it needs to be established whether these elevated intestinal levels of SCFA are high enough to reach substantial levels in the brain, studies in rats have shown that exposure to propionic acid leads to significant deterioration of social behavior, which has shown that propionic acid may has harmful effects on neurological function (Thomas et al., 2012; Foley et al., 2014).

Potential of Gut Microbiome for Screening of Autism Spectrum Disorder

Children with ASD face the problem of difficult early diagnosis. Most children show some abnormal behavior symptoms only at about 18–24 months, while other specific functional characteristics may only be found at an older age (Borghi and Vignoli, 2019). Current studies indicate that there are multiple subtypes of ASD, potentially caused by different routes of pathophysiology and each with diverse comorbid psychiatric and medical conditions (e.g., gastrointestinal symptoms, allergies, sleep disorders) (Huang et al., 2021). However, this heterogeneity is not addressed by the conventional DSM5-based behavioral diagnostic criteria. Accordingly, it is particularly essential to discover effective objective physiological indicators as the basis for clinical diagnosis and evaluation of ASD. In the past decade, as the importance of GM in ASD has been identified, researchers have begun to investigate the microbial diversity of ASD patients, seeking to identify certain gut microbial characteristics as biomarkers for ASD. Unfortunately, the results of two recent meta-analyses show that these cohort studies have produced inconsistent results in exploring the intestinal microbiota of ASD children (Xu et al., 2019; Iglesias-Vazquez et al., 2020). The interactions between ASD and GM may be influenced by complicated factors such as genetic background, daily diet and the physiological status of the host, which may explain the conflicting results of these studies. However, it is worth noting that most studies have found that the overall diversity of GM (composed of archaea, bacteria, fungi, and viruses) in ASD children increases, while its fungal diversity decreases (Kuehbacher et al., 2006; Finegold et al., 2010; Zou et al., 2021). This suggests that there may be too many harmful bacteria in ASD children, such as *Clostridium* and *Desulfovibrio*, which are more common in ASD patients, also considered to be potential pathogenic bacteria of ASD (Parracho et al., 2005; Finegold, 2011).

In order to verify the claims on previous research concerning changes in the gut microbiome associated with ASD, Wu et al. (2020) performed Machine-learning based on feature selection and classification evaluation which were performed in the training cohort, the validation cohort, and independent diagnosis cohorts to evaluate the potential of the gut microbiome as a non-invasive biomarker for ASD. The results showed that *Prevotella*, *Roseburia*, *Ruminococcus*, *Megasphaera,* and *Streptococcus* may be potential biomarkers of ASD, especially *Prevotella* has significant differences between ASD patients and typical neurodevelopers (Wu et al., 2020), but this result is not consistent with the prediction model established by Zhai et al. (2019). One possible reason for this inconsistency is that the composition of intestinal microbiota is affected by the *in vivo* and *in vitro* environmental factors of its host individual, and the other influence could be the calculation method used in establishing the prediction model. Besides, the quality control conditions and methods of sequencing data might affect the results of the prediction model as well. Therefore, further studies may be required to explore the GM characteristics of ASD. In addition, previous studies mainly focused on the differences of GM between ASD patients and normal people, but rarely analyzed the changes of these biomarkers from the perspective of intervention. Thus, this review discusses different treatment methods, compares the changes of intestinal flora before and after intervention, and further looks into whether intestinal flora has great potential in ASD screening.

INTERVENTION METHOD OF AUTISM SPECTRUM DISORDER CHILDREN BASED ON GUT MICROBIOTA

Nowadays, internationally approved and recommended ASD therapies include rehabilitation, education and psychotherapy. In

addition, many alternative therapies have been tested, including antibiotics, probiotics, dietary intervention and gut microflora transfer therapy.

Antibiotics and Dietary Interventions

Although much research has shown that antibiotics can improve the GI and behavioral symptoms of ASD children, there are still some disputes about antibiotic treatment. In principle, antibiotics not only kill potentially harmful bacteria, but also kill beneficial bacteria in ASD patients, thus increasing the probability of GI diseases in ASD children (Vargason et al., 2019). Therefore, antibiotic therapy may not be an optimal intervention for GM rebalancing.

Recently, dietary interventions in children with ASD are very popular. Previous studies have shown that a simple, light and nutritious Mediterranean diet impacts the GM and associated metabolome as well as cardiovascular diseases and neurobehavioral health outcomes (Atladottir et al., 2012; Liu et al., 2017). Therefore, we summarized the studies of dietary intervention in ASD (**Table 1**). Many studies have shown that the ability of a ketogenic diet (KD, i.e., a high fat diet that has demonstrated beneficial effects on mitochondrial dysfunction and epilepsy) to mitigate some of the neurobehavioral symptoms associated with ASD in an animal model (Verpeut et al., 2016; Castro et al., 2017). Improvements in seizure control and neurobehavioral symptoms have also been reported in ASD children with mild-moderate types of ASD as a result of following a KD (Evangeliou et al., 2003; Herbert and Buckley, 2013; El-Rashidy et al., 2017; Lee et al., 2018; Żarnowska et al., 2018). In addition, the gluten-free and casein-free (GFCF) diet is also one of the most popular dietary therapies for ASD. Some publications report favorable results in the core or peripheral symptoms of autism after a GFCF diet: communication and language, social interaction, stereotyped behavior, hyperactivity, and gastrointestinal symptoms (Knivsberg et al., 2002; Elder et al., 2006; Whiteley et al., 2010; Johnson et al., 2011; Pennesi and Klein, 2012; Herbert and Buckley, 2013; Navarro et al., 2015; Ghalichi et al., 2016; El-Rashidy et al., 2017). However, data on the efficacy of a GFCF diet as a treatment for ASD in children are limited (Pusponegoro et al., 2015; Hyman et al., 2016; Gonzalez Domenech et al., 2019; Josw Gonzalez-Domenech et al., 2020; Piwowarczyk et al., 2020). Particularly in recent years, there have been many reports of an absence of behavioral improvement after such diets. Even recently, researchers have shown that dietary interventions could potentially have a harmful effect (Fattorusso et al., 2019). For example, restrictive diets further limit the variety of food intake since individuals with ASD already exhibit picky eating behavior, so restrictive diets can result in macronutrient and micronutrient deficiencies. Moreover, the food taken by this kind of diet method is usually expensive, which imposes an additional burden on the families of ASD children, and the standard of dietary intervention is extremely strict and does not apply to all ASD patients.

Probiotic and Prebiotic Interventions

Probiotics are defined as live microorganisms that, when administered in adequate amounts, benefit the host's health. Prebiotics refer to non-digestible fibers, such as oligosaccharides, that promote growth and improve the functioning of the probiotics in the GI tract by acting as a specific substrate. Initial evidence suggests that supplementing probiotics and prebiotics may have a good preventive effect on neurological and mental diseases such as Alzheimer's disease, Parkinson's disease, depression, and autism spectrum disorder (Yang et al., 2021). Moreover, some research has discovered that since some common abnormal genes between ASD and GI diseases, the abnormal genes may cause abnormal GM in ASD patients (Niesler and Rappold, 2021). Considering the two-way communication of gut brain axis, we believe that probiotic intervention in ASD infants may affect the expression of related genes and decrease the prevalence of ASD. However, the specificity of GM in different patients suggests that precision medicine may be the hope of the future, where treatment protocols will be tailored for specific subpopulations of patients. Therefore, in order to explore the effectiveness of different probiotic and prebiotic therapies on behavioral symptoms and GI symptoms of ASD patients, we summarized the existing probiotic and prebiotic interventions, which can be divided into single strain intervention (**Table 2**), mixed strain intervention (**Table 3**), single probiotic and probiotic plus prebiotic intervention (**Table 4**).

Single Strain Interventions

The strains used in single strain intervention mainly come from *Lactobacillus*. Parracho et al. (2010) previously found that taking *Lactobacillus plantarum* WCSF1 significantly increased the number of *Lactobacillus* and *Enterococcus* bacteria in the intestines of children with ASD, and significantly reduced the count of *Clostridium cluster* XIVa, a harmful bacterium. Moreover, after probiotic intervention, the scores of destructive behavior, anxiety, self-focused behavior and communication disorder in developmental behavior checklist (DBC) scale of ASD children were lower than the baseline level (Parracho et al., 2010). Kaluzna-Czaplinska and Blaszczyk (2012) found that *L. acidophilus* Rosell-11 can upgrade the ability of ASD children to concentrate and complete commands, but unlike *Lactobacillus plantarum* WCSF1, it does not affect the emotional or eye contact response of ASD children in social interaction. A similar conclusion was also reached by the Partty's research. By randomly giving 75 newborn infants *L. rhamnosus* GG (LGG) or placebo for 6 months, they found that after 13 years, attention deficit hyperactivity disorder (ADHD) or Asperger syndrome (AS) was diagnosed in 6/35 (17.1%) children in the placebo and none in the probiotic group. It can be seen that LGG plays an important role in the development of children's attention (Partty et al., 2015). Recently, *Lactobacillus plantarum* PS128 has also been proved to be effective in ASD intervention. Both cohort research found that taking *Lactobacillus plantarum* PS128 could reduce the scores of ASD children on the social responsiveness scale (SRS) and clinical global impressions (CGI) scale. In other words, *Lactobacillus plantarum* PS128 can improve the irritability, anxiety, hyperactivity, cognition, ring breaking behavior and communication behavior of ASD children (Liu et al., 2019; Kong et al., 2021). Moreover, Kong et al. (2021)

TABLE 1 | Dietary intervention studies.

Authors	Study design	Treatment	Effect on behavioral symptoms	Effect on GI symptoms
Knivsberg et al. (2002)	A randomized controlled trial of 20 ASD children aged 5–10 with abnormal urinary peptide levels	GFCF diet vs. RD for 12 months	The GFCF diet group improved more in LIPC, and the peer relationship and language communication were also better improved	–
Evangeliou et al. (2003)	A pilot prospective follow-up study on 30 children with autistic behavior from 4 to 10 years old	KD for 6 months	18 of 30 children (60%), improvement was recorded in several parameters and in accordance with the CARS	–
Elder et al. (2006)	A randomized, double blind repeated measures crossover design on 15 children with autism from 2 to 16 years old	GFCF diet for 6 weeks + RD for 6 weeks	Improvement of their language skills and reducing excessive tension and irritability, but the scores of CARS ($P = 0.85$) and ECOS ($P = 0.29$) decreased and the improvement of behavioral frequencies was not significant	–
Whiteley et al. (2010)	A randomized, controlled trial on 72 children with autism from 4 to 10 years old	GFCF diet vs. RD for 12 months	A significant improvement to mean diet group scores (time × treatment interaction) on sub-domains of ADOS, GARS and ADHD-IV measures	–
Johnson et al. (2011)	A prospective, open label, randomized, parallel groups design on 22 children with autism from 3 to 5 years old	GFCF diet vs. Healthy Control Diet vs. Omega 3 supplementation, for 3 months	Both treatment groups evidenced some gains across a range of variables, including measures of behavior, language, and ratings of the core features of ASD [in Mullen scales of early learning and CBCL]. No statistically significant differences were noted between treatment groups	–
Pennesi and Klein (2012)	A questionnaire analysis study on 387 children with ASD	The questionnaire survey of GFCF diet of ASD children	Improvement of their behavior symptoms, physiological symptoms and social behavior	
Herbert and Buckley (2013)	A case report of a child with autism and epilepsy	GFCF diet, then KD for 14 months	Improvement cognitive and social skills, language function, and stereotypies and reached seizure-free status	–
Navarro et al. (2015)	A randomized double-blind, placebo-controlled study on 12 children with autism from 4 to 7 years old	2 weeks of GFCF diet followed by 4 weeks of GFCF diet + supplement containing brown rice flour	Decrease in Inattention of CBCL-R; improvement in Irritability of ABC and Hyperactivity of ABC and CBCL-R	–
Pusponegoro et al. (2015)	A randomized, controlled, double-blind trial was performed on 74 children with ASD with severe maladaptive behavior and increased urinary I-FABP	Gluten–casein vs. placebo for 7 days	Administrating gluten–casein to children with ASD for 1 week did not increase maladaptive behavior	GI symptom severity did not increase
Hyman et al. (2016)	A case of 22 children with autism from 3 to 4 years old	GFCF diet vs. RD, for 18 weeks + Challenges occurred once per week for 12 weeks	Not find evidence of benefit from the GFCF diet	–
Ghalichi et al. (2016)	A randomized clinical trial, 80 children diagnosed with ASD from 4 to 16 years old	GFD vs. RD for 6 weeks	According to the scores of ADI-R, CARS-2 and, GFD intervention significantly decreased behavioral disorders and prevalence of gastrointestinal symptoms ($P < 0.05$)	Decrease in ROME III questionnaire scores, GI symptoms improved
El-Rashidy et al. (2017)	A case-control study on 45 children with ASD from 3 to 8 years old	KD vs. GFCF vs. RD, for 6 months	Both diet groups showed significant improvement in ATEC and CARS scores, KD group scored better results in cognition and sociability compared to GFCF diet group	-
Lee et al. (2018)	Cohort study of 15 children ages 2–17 years	Modified ketogenic gluten-free diet regimen with supplemental MCT for 3 months	Improved core autism features assessed from the ADOS-2	–
Żarnowska et al. (2018)	A case report of clinical on a 6 years child with autism	KD for 16 months	The patient's behavior and intellect improved (in regard to hyperactivity, attention span, abnormal reactions to visual and auditory stimuli, usage of objects, adaptability to changes, communication skills, fear, anxiety, and emotional reactions)	–

(Continued)

TABLE 1 | (Continued)

Authors	Study design	Treatment	Effect on behavioral symptoms	Effect on GI symptoms
Gonzalez Domenech et al. (2019)	A crossover clinical trial on 28 children with ASD	3 months GFCF diet + 3 months RD	Not find evidence of benefit from the GFCF diet	–
Piwowarczyk et al. (2020)	A randomized, controlled, single-blinded trial on 66 children with ASD from 3 to 6 years old	GFD vs. GD for 6 months	A GFD compared with a GD did not affect functioning of children with ASD	–
Josw Gonzalez-Domenech et al. (2020)	A crossover trial on 37 children with ASD	6 months GFCF diet + 6 months RD	No significant behavioral changes after GFCF diet	

I-FABP, Intestinal Fatty Acids Binding Protein; GFCF, Gluten-Free and Casein-Free; RD, Regular Diet; KD, Ketogenic Diet; Challenges, foods that contained gluten only, casein only, both gluten and casein, or neither (placebo); MCT, Medium-Chain Triglycerides; GFD, Gluten Free Diet; GD, Gluten Diet; LIPS, Leiter International Performance Scale; CARS, Childhood Autism Rating Scale; ECOS, Ecological Communication Orientation Scale; ADOS, Autism Diagnostic Observation Schedule; GARS, Gilliam Autism Rating Scale; ADHD-IV, Attention-Deficit Hyperactivity Disorder-IV scale; CBCL, Child behavior checklist; CBCL-R, Conners' Parent Rating Scale-Revised; ABC, Autism Behavior Checklist; ADI-R, Autism Diagnostic Interview-Revised; ATEC, Autism Treatment Evaluation Checklist; GI, gastrointestinal.

also found that the combination of *Lactobacillus plantarum* PS128 and serum oxytocin (OXT) showed a better effect in the treatment of ASD. In conclusion, these results suggest that a single strain (mainly *Lactobacillus*) can batter the symptoms of ASD to a certain extent, and there are similar conclusions in the study of mice. For example, recently researchers discovered that *Lactobacillus reuteri* can batter the anxiety and stereotyped behavior of Cntnap2 KO mice (an animal model of ASD) (Bellone and Luscher, 2021). However, it is worth noting that these strains do not show a consistent conclusion on the impact of these strains on the GM of ASD children, so it is difficult to explain the relationship between the improvement of behavioral symptoms and the regulation of GM balance.

Mixed Strain Interventions

Recently, many interventions no longer limited to a single strain, but mixed lactobacillus with *Bifidobacterium* and/or *Streptococcus* to intervene in ASD children. For example, Shaaban et al. (2018) found that after oral administration with the mixture of *Lactobacillus acidophilus*, *Lactobacillus rhamnosus* and *Bifidobacterium longum*, besides the colony count of *Bifidobacteria* and *Lactobacillus* in their intestinal tract increased, those ASD children had lower scores in the autism treatment evaluation checklist (ATEC), indicating that the verbal communication and social ability of children with ASD improved. Interestingly, this improvement in behavioral symptoms was also demonstrated in two intervention studies in which ASD patients were treated with a mixture of *Lactobacillus*, *Bifidobacteria,* and *Streptococci* (Tomova et al., 2015; Grossi et al., 2016). However, Tomova et al. (2015) found that probiotics significantly reduced the number of *Bifidobacteria* and *Lactobacillus* in the gut of ASD patients. Therefore, similar to the results of single strain intervention, probiotics mixed reagent has different effects on the GM of ASD patients, but it is worth noting that both of them can increase the relative abundance of *Lactobacillus* in the gut of ASD patients. In addition, probiotic supplements such as Delpro®, Vivomixx®, and VISBIOME (VSL#3) have been used to intervene in ASD patients with improved behavioral symptoms, especially verbal communication and social behavior, and VISBIOME even improved sleep quality and life quality of ASD patients (West and Roberts, 2013; Arnold et al., 2019; Santocchi et al., 2020). Nevertheless, the effectiveness demonstrated by these probiotic supplements did not show an advantage over the single *Lactobacillus* intervention. For example, although each package of VISBIOME probiotic supplement has a higher dose of bacteria than the single strain intervention used by Liu et al. (2019) (9×10^{12} CFUs vs. 3×10^{10} CFUs), it can be seen only from the SRS score before and after the intervention that the improvement effect of VISBIOME probiotic supplement on ASD children is not better than that of *Lactobacillus plantarum* PS128 when the intervention duration is 4 weeks (Arnold et al., 2019). In the study of Kong et al. (2021), it also proved that the improvement effect of single *Lactobacillus plantarum* PS128 on the scores of Irritability (S1), Social Withdrawal (S2), and Stereotypic Behavior (S3) in Autism Behavior Checklist (ABC) scale was better than that of VISBIOME, but this could not rule out the reason that the experimental intervention cycle of Kong et al. (2021) was longer. In addition, there is little evidence that taking probiotic mixed reagents can reduce the anxiety of ASD. Beyond that, few researchers have investigated whether there is synergistic or antagonistic effect of different strains in these mixed reagents in the gut of ASD patients. Moreover, at present, there is no standardized intervention cycle and dose for probiotic intervention, and researchers do not use a unified behavior scale for the detection of behavioral symptoms of ASD children. This brings great difficulty to the comparison of the effectiveness of different probiotic interventions. But anyway, it is certain that these interventions are at least harmless, and *Lactobacillus* is beneficial to ASD patients.

Single Probiotic and Probiotic + Prebiotic Interventions

Some researchers started to consider the overall balance of the GM ecosystem of ASD patients, and put forward the intervention therapy of the probiotics and the combination of probiotics and prebiotics. Grimaldi et al. (2018) found that the social behavior and sleep quality of ASD children were improved by giving

TABLE 2 | Single probiotic intervention studies.

Authors	Study design	Treatment	Effect on gut microbiota	Effect on behavioral symptoms	Effect on GI symptoms
Parracho et al. (2010)	Randomized double-blind placebo-controlled study on children with ASD from 3 to 16 years old	*Lactobacillus plantarum* WCSF1 vs. placebo, for 12 weeks	*Lactobacilli* and *Enterococci* increased significantly, the count of *Clostridium cluster* XIVa decreased significantly, and there was no significant difference in Chis150 (*Clostridium clusters* I and II)	Decrease in TBPS and DBC scores, in which the scores of disruptive antisocial behaviors, anxiety, self-focused behaviors and communication problems in probiotic group are lower than the baseline	GI symptoms improved
Kaluzna-Czaplinska and Blaszczyk (2012)	Cohort study of children with ASD from 4 to 10 years old	*L. acidophilus* Rosell-11 for 2 months	–	Improvement in their ability to concentrate and fulfill orders, with no impact on behavioral responses to other people's emotions or eye contact	–
Partty et al. (2015)	Randomized trial, placebo-controlled study on infants followed for 13 years	*L. rhamnosus* GG vs. placebo for the first 6 months of life	At the 6th month, the count of *Bifidobacteria* in children with ADHD / ASD was significantly lower than that in healthy children; At 18 months, the count of *Bacteroides* and *Lactobacillus-Enterococcus* group decreased; At 24 months, the count of *Clostridium histolyticum* group decreased	At the age of 13 years, 6 out of 35 (17.1%) children in the placebo group were diagnosed with ASD or ADHD, but none in the probiotic group	–
Liu et al. (2019)	Randomized, double-blind, parallel, placebo-controlled study of 71 patients with ASD aged 7–15 years	*L. plantarum* PS128 vs. placebo for 4 weeks	–	Decrease in CGI-S, CGI-I, ABC-T, SRS, CBCL and SNAP-IV scores, and anxiety, hyperactivity, rule violation, impulse and antisocial behavior were improved	–
Kong et al. (2021)	Randomized, double-blind, placebo-controlled study of 35 ASD patients aged 3–20 years	*L. plantarum* PS128 vs. placebo for 28 weeks, and from the 16th week, both groups received oxytocin	*Roseburia*, *Streptococcus* and *Veillonella* were observed only in the probiotic group, and the content of serum OXT (oxytocin) decreased in the probiotic group	Decrease in ABC, SRS and CGI scores, and irritability and cognitive ability were improved	–

TBPS, Teacher Beliefs and Practices Scale; DBC, Developmental Behavior Checklist; CGI, Clinical Global Impressions; CGI-S, Clinical Global Impression-Severity; CGI-I, Clinical Global Impression-Improvement; ABC-T, Autism Behavior Checklist-Taiwan version; SRS, Social Responsiveness Scale; SNAP-IV, Swanson, Nolan, and Pelham-IV; CBCL, Child behavior checklist; ABC, Autism Behavior Checklist; GI, gastrointestinal.

30 ASD children Bimuno® galactooligosaccharide (B-GOS®) prebiotic reagent for 6 weeks. Inoue et al. (2019) found that partially hydrolyzed guar gum (PHGG) improved irritability in ASD patients. These evidence suggested that the administration of prebiotics can cause the probiotics in the gut to generate specific metabolites, which is of great significance in balancing the entire GM ecosystem and treating ASD (Davies et al., 2021). Recently, a combination of probiotics and probiotics has been administered to ASD patients with good results. For example, after 1 month of constant supplementation of probiotics (*Bifidobacterium infantis* Bi-26, *Lactobacillus rhamnosus* HN001, *Bifidobacterium lactis* BL-04, and *Lactobacillus paracasei* LPC-37) and fructooligosaccharide (FOS, growth factors of *Bifidobacterium*) in ASD patients, there ATEC total score continuously decreased over 2 months, especially in which the scores of speech/language/communication and social interaction decreased significantly, indicating improvements in verbal communication and social behavior of those autism patients (Wang et al., 2020). Moreover, consistent with the results of previous studies, taking probiotics and FOS mixed supplements can increase the count of *Bifidobacteria* and *B. longum* in the intestine of ASD patients and decrease the amount of some harmful bacteria such as *Clostridium* and *Ruminococcus*. Sanctuary et al. (2019) found that although the total ABC scale score decreased for ASD patients receiving intervention therapy, along with improved stereotyped behavior and decreased sleepiness, the changes of GM varied from person to person. Interestingly, they also found that bovine colostrum

TABLE 3 | Mixed probiotic intervention studies.

Authors	Study design	Treatment	Effect on gut microbiota	Effect on behavioral symptoms	Effect on GI symptoms
Tomova et al. (2015)	Real-time PCR on fecal samples of 10 children with autism before and after probiotic administration	A mixture of *Lactobacilli*, *Bifidobacteria* and *Streptococci* given 3 times a day for 4 months	Normalization of *Bacteroides/Firmicutes* ratio and *Desulfovibrio spp.* abundance; the count of *Bifidobacterium* and *Desulfovibrio* decreased significantly, and the absolute amount of *Lactobacillus* decreased (relative amount increased), reaching the level of healthy subjects	Decrease in ADI scores, and restrictive and stereotyped behavior were improved	–
Grossi et al. (2016)	Case report of a 12 years old boy with ASD and severe cognitive disability	A mixture of *Bifidobacteria*, *Lactobacilli* and *Streptococci* given daily for 4 weeks	–	Decrease in AODS-2 scores, and social behavior and neurosexual behavior were improved	GI symptoms improved
Shaaban et al. (2018)	Cohort study of 30 children with ASD from 5 to 9 years old	*L. acidophilus*, *L. rhamnosus* and *B. longum* for 3 months	The count of *Bifidobacteria* and *Lactobacillus* increased significantly	Decrease in ATEC scores, and speech/language communication, sociability, sensory/cognitive awareness and health/physical/behavior were improved	Decrease in 6-GSI scores
West and Roberts (2013)	Cohort study of 33 children with ASD	Delpro [a] and Del-Immune V [b], for 21 days	–	Decrease in ATEC scores in 88% of children, and speech/language communication, sociability, sensory/cognitive awareness and health/physical/behavior were improved	Improvement of constipation and diarrhea, and GI symptoms improved
Arnold et al. (2019)	Randomized, double-blind, parallel, cross-controlled study of 10 ASD patients aged 3–12 years	Two groups were randomly assigned to receive 8 weeks each on VISBIOME[c] and placebo separated by a 3-week washout	The relative abundance of *Ruminococcaceae* and *Bifidobacteriaceae* decreased, and the relative abundance of *Bacteroidaceae* and *Verrucomicrobiaceae* increased, but not significant	Decrease in ABC, CSHQ, PRAS-ASD, PSI and SRS scores, and the sleep problems were significantly improved	Decrease in PedsQL GI scores, and GI symptoms improved
Santocchi et al. (2020)	Randomized, double-blind, parallel, placebo-controlled study of 63 patients with ASD aged 18–72 months	Vivomixx® [d] vs. placebo, two pack once a day for the first month; Vivomixx® vs. placebo, one pack once a day for the next 5 months	–	Decrease in ADOS-CSS scores, but not significantly; decrease in ADOS-CSS and VABS II scores in non-GI, with social and communication skills significantly improved	Decrease in 6-GSI scores, and GI symptoms improved

[a] *Delpro® is a probiotic supplement, including Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus delbrueckii, Bifidobacterium longum and Bifidobacterium bifidum.*
[b] *Del-Immune V® is a supplement containing stem fermentation cell lysate and DNA fragment of probiotic strain Lactobacillus rhamnosus V (DV strain).*
[c] *VISBIOME (VSL#3) is a probiotic supplement, including four strains of Lactobacilli (L. casei, L. plantarum, L. acidophilus and L. delbrueckii subsp. bulgaricus), three strains of Bifidobacteria (B. longum, B. infantis, and B. breve), one strain of Streptococcus thermophilus, and starch.*
[d] *Vivomixx® is a probiotic supplement, and each packet contained 450 billions of eight probiotic strains, including Streptococcus thermophilus, Bifidobacterium breve, Bifidobacterium longum, Bifidobacterium infantis, Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus paracasei, Lactobacillus delbrueckii subsp. Bulgaricus.*
ADI, Autism Diagnostic Interview; AODS-2, Autism Diagnostic Observation Schedule-2; CSHQ, Children's Sleep Habits Questionnaire; PRAS-ASD, Parent-Rated Anxiety Scale for ASD; PSI, Parenting Stress Index; ADOS-CSS, Autism Diagnostic Observation Schedule – Calibrated Severity Score; VABS II, Vineland Adaptive Behavior Scales-Second Edition; 6-GSI, 6-Gastrointestinal Severity Index; PedsQL, Pediatric Quality of Life Inventory; ABC, Autism Behavior Checklist; ATEC, Autism Treatment Evaluation Checklist; GI, gastrointestinal; SRS, Social Responsiveness Scale.

powder (BCP) alone could improve the behavioral symptoms of ASD patients more significantly than the combination of *Bifidobacterium infantis* and BCP (Sanctuary et al., 2019). This result convinces that the overall balance of intestinal micro ecosystem is more important than the change of single strain. Recently, a meta-analysis demonstrated that

TABLE 4 | Intervention studies of prebiotics and probiotics combined with prebiotics.

Authors	Study design	Treatment	Effect on gut microbiota	Effect on behavioral symptoms	Effect on GI symptoms
Grimaldi et al. (2018)	Randomized, double-blind, placebo-controlled study of 30 ASD patients aged 4–11 years	B-GOS, for 6 weeks	The diversity of gut microbiota increased, but there was no significant difference; the relative abundance of *Bifidobacterium* and *Veillonellaceae* decreased, while the relative abundance of *Faecalibacterium prausnitzii* and *Bacteroides* increased	Decrease the antisocial behavior score in ATEC; improve the sleep quality score in SCAS-P; decrease in AQ scores only for ASD patients on a restricted diet (gluten free casein free diet)	GI symptoms improved
Inoue et al. (2019)	Cohort study of 13 ASD patients aged 4–9 years	6 g PHGG every day, for 2 months or more	The relative abundance of *Acidaminococcus* and *Blautia* increased, while the relative abundance of *Streptococcus, Odoribacter* and *Eubacterium* decreased	Decrease the irritability subscale score in ABC-J	GI symptoms improved
Sanctuary et al. (2019)	Randomized, double-blind, cross-controlled study of 8 ASD patients with GI aged 2–11 years	Two groups were randomly assigned to receive 5 weeks each on the BCP alone and the combination of *Bifidobacterium infantis* and BCP separated by a 2-week washout	–	Decrease in ABC scores, especially when stereotyping behavior and sleep problems were improved, but the improvement was more pronounced when prebiotics were taken alone	Decrease in QPGS-RIII and GIH scores, and GI symptoms improved
Wang et al. (2020)	Randomized, double-blind, placebo-controlled study of 26 ASD patients aged 3–9 years	The combination of probiotics① and FOS vs. placebo, for 108 days	The relative abundance of *Bifidobacteriales* and *B. longum* increased, while the relative abundance of some harmful bacteria decreased, such as *Clostridium* and *Ruminococcus*	After the first 30 days, decrease in ATEC scores, but not significant; after the 30–60 days, decrease significant in ATEC scores	Decrease significant in 6-GSI scores, and GI symptoms improved

B-GOS®, Bimuno® galactooligosaccharide; PHGG, Partially hydrolyzed guar gum; BCP, Bovine colostrums powder; Probiotics①, is a probiotic blend, including Bifidobacterium infantis Bi-26, Lactobacillus rhamnosus HN001, Bifidobacterium lactis BL-04 and Lactobacillus paracasei LPC-37; FOS, fructooligosaccharide; SCAS-P, Spence's Children Anxiety Scale-Parent version; AQ, Autism Spectrum Quotient; ABC-J, Aberrant Behavior Checklist, Japanese Version; QPGS-RIII, Questionnaire on Pediatric Gastrointestinal Symptoms-Rome III Version; GIH, Gastrointestinal History; ABC, Autism Behavior Checklist; ATEC, Autism Treatment Evaluation Checklist; GI, gastrointestinal; 6-GSI, 6-Gastrointestinal Severity Index.

TABLE 5 | Intervention studies of FMT and MTT.

Authors	Study design	Treatment	Effect on gut microbiota	Effect on behavioral symptoms	Effect on GI symptoms
Linda et al. (2016)	Cohort study of 9 ASD patients (2, 3, 5, 5, 6, 8, 8, 11, and 21 years of age)	FMT	*Bacteroides, Barnesiella, Parabacteroides, Sutterella, Parasutterella, Clostridiales,* and *Erysipelotrichales* were most altered	Improved behavioral symptoms significantly of ASD children, with the exception of 21 years old subjects	–
Kang et al. (2017)	Cohort study of 18 ASD patients aged 7–16 years	MTT	Increased the diversity of bacteria in their gut, with the increased abundance of *Bifidobacterium*, *Prevotella,* and *Desulfovibrio*. And both of these changes persisted after treatment stopped (for 8 weeks)	Improved behavioral symptoms significantly of ASD patients (for 8 weeks)	GI symptoms improved (for 8 weeks)
Zhao et al. (2019)	Randomized, double-blind, controlled study of 48 ASD patients	FMT	Decreased the abundance of *Bacteroides fragilis*, and the gut microbiota of ASD patients gradually transferred to a healthy state. Changes of CARS were negatively correlated with *Coprococcus*	Decreased the CARS scores of the FMT group by a statistically significant 10.8% compared with a 0.8% decrease in the control group after the first FMT (F1), and still decreased slightly after the second FMT (F2)	Notable differences were also shown on GSI scores ($P < 0.05$) at F1 time point. 7 (29.2%) patients in FMT group reported adverse events such as fever, allergy and nausea, but all of them were mild, transient

FMT, Fecal microbiota transplant; MTT, Microbiota Transfer Therapy; CARS, Childhood Autism Rating Scale; GSI, Gastrointestinal Severity Index; GI, gastrointestinal.

prebiotics and probiotic-containing probiotics performed better than probiotic interventions in the treatment of ASD (Davies et al., 2021), further supporting our view that microbium-based interventions should focus on the overall balance of the patient's intestinal microecology.

In summary, we found that although probiotics and probiotics intervention showed certain effects in improving ASD behavioral symptoms, there were obvious differences in their effects on GM of ASD, and it was still difficult for researchers to give specific explanations on the biological mechanism of how probiotics and probiotics affected ASD behavior. In addition, alterations in gut microbiome composition have been confirmed in children with ASD, but few probiotics and prebiotics interventions have been designed for the gut microbiome characteristics of ASD. The effectiveness of prebiotic and mixed probiotic intervention compared with probiotic alone also shows that the overall balance of GM in ASD patients may need more attention during the intervention process.

Fecal Microbiota Transplant Therapy

Fecal microbiota transplant (FMT), consisted of transferring the fecal microbiota from healthy volunteers to patients with gut dysbiosis, may alleviate GI and neurobehavioral symptoms in children with ASD by rebalancing the physiological intestinal microbiota. We have summarized these studies in **Table 5**. Linda et al. (2016) used FMT to intervene ASD, and found that behavioral symptoms and GI symptoms improved in younger ASD patients, while there was no significant change in older patients (21 years old) before and after the intervention, proving the feasibility of FMT for the treatment of children with ASD. Based on this, Zhao et al. (2019) conducted a randomized controlled study of 48 patients with ASD. The FMT group received two FMT treatments (2 months apart) and the control group received only rehabilitation training. They found that after the first FMT treatment, the Childhood Autism Rating Scale (CARS) scores in the FMT group decreased by 10.8% (behavioral symptoms of ASD improved) compared with 0.8% ($P < 0.001$) in the control group. After the second FMT, the CARS scores in the FMT group continued to decrease slightly ($P = 0.074$), further demonstrating the efficacy of FMT. However, there are still some problems with FMT. For example, 7 cases (29.2%) in the FMT group had adverse reactions such as fever, allergy and nausea during the intervention. Therefore, some argue that the feasibility of this approach for all ASD children needs further validation.

In response to this, Kang et al. (2017) developed a modified FMT protocol (Microbiota Transfer Therapy, MTT) that consisted of 14 days of oral vancomycin treatment followed by 12–24 h of fasting bowel cleansing and then either oral or rectal administration of standardized human GM (SHGM) for 7–8 weeks. They found that 18 ASD children aged 7–16 years old not only improved their behavioral symptoms after treatment, but also increased the diversity of bacteria in their gut, with the increased abundance of *Bifidobacterium*, *Prevotella,* and *Desulfovibrio*. All of these changes persisted for at least 8 weeks after treatment ended. Furthermore, after 2 years of follow-up, the results showed that the ASD patients treated with MTT maintained a high diversity of gut bacteria and abundance of *Bifidobacterium* and *Prevotella*, and most of the improvement in gastrointestinal symptoms was also maintained. Importantly, behavioral symptoms were kept improved after 2 years of MTT treatment (Kang et al., 2019). The long-term effectiveness of this study shows that this treatment can maintain the remodeling of the gut of ASD patients, make their gut micro ecological system to achieve a healthy balanced state, and then improve the condition and the behavior level. The series of results confirmed an important role of overall gut microbes rebalancing during the process of intervention, which we believe to be an alternative and promising new approach for the treatment of GM dysbiosis in ASD.

CONCLUSION

This review summarizes the therapeutic interventions for ASD based on gut microbiome, including dietary therapy, antibiotic therapy, probiotic and prebiotic intervention, and microbial transfer therapy. By evaluating the changes of microflora and disease characterization in the intervention process of these methods, we proposed that probiotics and prebiotics intervention methods have good efficacy and high safety. Furthermore, through our summary of probiotic intervention studies, we discovered that *Lactobacillus*, particularly *Lactobacillus plantarum*, may play important roles in improving anxiety and social behavior symptoms in ASD children. In the study of mice, researchers found that *L. reuteri* may be of great significance in improving the social behavior of ASD, while *Bacteroides fragilis* is of great significance in improving anxiety. Therefore, we believe that there may be some probiotics that can specifically improve the different behavioral symptoms of ASD. Future studies of single probiotic interventions should focus on the mechanisms with which the corresponding behavioral symptoms are influenced.

At present, there is a lot of evidence implying that the intestinal microbiota of autistic children is specific. However, due to the few studies based on GM, there are few subjects, large regional differences and inconsistent sequencing methods. It is difficult to propose a broad and effective ASD intervention method based on the modulation of GM. Moreover, although the effectiveness of mixed probiotic reagent, prebiotic reagent, mixed reagent of prebiotics and probiotics, and MTT emphasizes the importance of the overall balance of gut microbial system. The specific biological mechanism of these interventions is not clear, which is also a major problem in the development of corresponding interventions. Therefore, we believe that evaluating the internal biological mechanism between microbiota change and behavioral symptom improvement from the perspective of intervention may be the first concern of researchers.

Although many studies have discussed the characteristics of GM in ASD, there are few studies to supplement the corresponding probiotics for intervention according to the characteristics of GM in ASD patients. It is known that Delpro®, Del-Immune V®, VISBIOME, Vivomixx®, B-GOS, and some other broad probiotic supplements, are not work for every ASD

child. However, as a special group, the GM of ASD patients is significantly different from that of healthy people or patients with other diseases. Therefore, subsequent intervention should develop specific probiotic and prebiotic reagents according to the characteristics of GM of ASD patients, and even develop corresponding personalized treatment schemes. Meanwhile, further research is still needed to prove the effectiveness and safety of probiotic and prebiotic therapy in the future.

Moreover, it is worth exploring that at present, researchers have different views on the association between GM and autism, including whether the microbiome differences found in the intestines of autistic children are due to their limited/specific dietary preferences related to the diagnostic characteristics of autism, or the reasons for their behavioral symptoms. The reason for this controversy may be that the internal mechanism of GM affecting the central nervous system is hard to measure and unclear. Therefore, we believe that while studying the specific biological mechanism of microbial-gut-brain axis, future research could focus on the changes of GM and behavioral symptoms of ASD patients during the intervention to help us have a deeper understanding of the relationship between microbiota and ASD.

AUTHOR CONTRIBUTIONS

CL, X-DJ, and JX conceived the project. JR, CF, and WW carried out the searches and synthesis. CL, JR, and JX interpreted the findings. CL and JR drafted the manuscript. CL and X-DJ approved the manuscript. All authors have read and approved the manuscript.

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Understanding Behavior in Phelan-McDermid Syndrome

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Background: Phelan-McDermid syndrome (PMS) or 22q13.3 deletion syndrome is a rare genetic disorder characterized by developmental delay, hypotonia and severely delayed speech. Behavioral difficulties are often reported in PMS, although knowledge of behavioral profiles and the interpretation of reported behavior remains limited. Understanding the meaning of behavior requires considering the context as well as other domains of functioning, for example the individual's level of cognitive, social and emotional development. Combining structured direct in-person neurodevelopmental assessments with contextual assessments to enable meaningful interpretations of reported behavior on functional dimensions across multiple units of analysis, as proposed by the RDoc framework, is essential.

Methods: In this article we present a structured multidisciplinary method of assessment through direct in-person neurodevelopmental assessments and assessment of contextual factors. Our study sample includes data of 33 children with an average age of 6.2 years (range 1.1 to 15.7) with PMS, obtained through individual in-person assessments in combination with parent informed questionnaires. We assessed developmental age using the Bayley-III, adaptive behavior was assessed with the Vineland screener, social-emotional development with the ESSEON-R and behavior by using the CBCL.

Results: Our results show a great deal of variability in phenotypic presentation with regard to behavior, symptom expression and symptom severity in individuals with PMS. The data on behavior is interpreted in the context of the individual's level of cognitive, adaptive development and the (genetic) context. Behavioral data showed high levels of withdrawn behavior and attention problems. More than half of the children showed borderline or clinical symptoms related to Autism Spectrum Disorder (ASD).

Conclusions: The interpretation of the meaning of certain behavior in PMS is often based on questionnaires and descriptions without taking the specific context of development into account. Combining questionnaires with direct in-person assessments measuring different domains of functioning should be considered a more accurate

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method to interpret the meaning of findings in order to understand behavior in rare genetic disorders associated with developmental delay such as PMS. Direct in-person assessment provides valuable and specific information relevant to understanding individual behavior and inform treatment as well as increase knowledge of the neurodevelopmental phenotype in individuals with PMS. More specific application of the proposed frameworks on behavior in PMS is desirable in making useful interpretations.

Keywords: Phelan-McDermid syndrome, neurodevelopmental phenotype, 22q13 deletion syndrome, behavioral difficulties, intellectual disability, contextual assessments

BACKGROUND

Phelan McDermid syndrome (PMS) or 22q13 deletion syndrome is a rare genetic disorder characterized by developmental delay, hypotonia and absent or severely delayed speech (1, 2). Specific behavioral issues (3, 4), minor physical anomalies, seizures (1, 3) and sleep disorders (5, 6) are often described in children and adults with PMS. Previous studies have shown that intellectual disability is a prominent feature of PMS and is mostly severe to profound (4, 7–9). Studies of neurodevelopmental and behavioral aspects in PMS however have often used assessments, methods, and tools more suited to assess mild to moderate intellectual disability (ID) (10). Behavior is often interpreted dichotomously as present or absent, but is rarely considered within the dimension of ID or psychosocial context. Soorya et al. (10) suggested a framework for assessing individuals with rare genetic disorders and Profound Intellectual and Multiple Disabilities (PIMD) and suggested PMS as an example of PIMD due to the severe to profound intellectual disability. Assessing neurodevelopmental aspects and behavior in PIMD, such as PMS, would therefore require a multidisciplinary and multimodal neuropsychological assessment. The framework suggested by Soorya et al. (10) is in line with the framework of Research Domain criteria (RDoc) proposed by the National Institute of Mental Health (NIMH) in 2009 (11). The RDoc framework aims at a better understanding of mental health issues opposed to current models like DSM-5 by the American Psychiatric Association. The RDoc criteria provide a framework that focuses on the full dimensional aspects of behavior and understanding behavior within the context rather than a description of psychopathological behavior being present or absent in an individual or group. The RDoc framework is an integrative model of different constructs within five domains that interact and are necessary to understand the meaning of behavior through multiple mechanisms. Domains within the RDoc are Negative valence systems, Positive valence systems, Cognitive systems, Systems for social processes and Arousal/modulatory processes. The RDoc framework focusses on underlying psychological constructs instead of systemizing behavior on a symptomatic level.

For example the degree of ID and other developmental domains as suggested in the RDoc criteria have a profound effect on behavior (12) and therefore on the interpretation of developmental and behavior measures in PIMD such as PMS. Esteves et al. (12) shows the correlation between adaptive functioning and behavioral problems including behavioral aspects of autism in individuals with ID. In PMS the delay in behavioral or social-emotional development is often more severe than would be expected based on the individuals' cognitive capabilities (4).

Oliver et al. (13) emphasize the importance of studying distinctive behavior in relation to the developmental perspective of specific groups of individuals with ID in their studies of phenotypes in specific syndromes. In PMS distinctive behavior has been described generally, but rarely within the perspective of the developmental delay. The developmental perspective on behavior in specific groups contributes not only to our understanding of behavior within that group but also on the possible etiology of this behavior in non-syndromic groups. For example expectations on mood regulation would differ enormously between a 3 year old child and a thirteen year old child. In the thirteen year old, tantrums can be a symptom of an oppositional defiant disorder, from the developmental perspective of a 3 year old, tantrums are normal behavior.

In 34 children with PMS between 0.7 and 14.8 years of age, Zwanenburg et al. (8) found that the average developmental level increased up to the calendar age of approximately 6 years, but not thereafter.

In this paper we reconsider data in part (Bayley-III and Vineland) previously described by Zwanenburg et al. (8) from the perspectives of the renewed frameworks suggested by Soorya et al. as well as the RDoc domains of functioning. We combined this previously described data with data on behavior and functioning gathered in the same timeframe. We advocate the use of the described perspectives on interpretation of behavior in rare genetic disorders such as PMS and propose adaptations in assessment of behavior that will enhance possibilities for interpretation. Domains of functioning described in this article are cognitive development, adaptive behavior, social-emotional development and behavior.

Zwanenburg et al. (8) found that the maximum developmental age equivalent (DAE) of the 34 children in this study was

Abbreviations: PMS, Phelan-McDermid syndrome; ASD, autism spectrum disorder; ADHD, attention deficit hyperactivity disorder; PDD, pervasive developmental disorder; ODD, oppositional deviant disorder; DAE, developmental age equivalent; CBCL, Child Behavior Checklist; Bayley-III, Bayley Scales of Infant and Toddler Development, third edition, Dutch Version; DSM, Diagnostic and Statistical Manual of Mental Disorders.

approximately 3 years, with one exception of a developmental level of 4.5 years.

Adaptive behavior can be described as everyday life skills on domains such as social, communication, motor and practical daily skills. Previous studies on adaptive behavior in children with PMS (14–16) had sample sizes ranging from 18 to 40 and age ranges across the three studies between 2 and 18 years (with one exception of 42 years) (15). The results showed adaptive behavior in the below-average range on all domains, with relatively high scores in the motor domain and low scores in the communication domain (14–16). Comparable results were found in another study of seven adults with PMS (7).

Behavioral problems associated with PMS are also described in persons with severe to profound ID and/or Autism Spectrum Disorder (ASD) but without PMS, e.g., mouthing behavior, social problems and stereotypies (3). Shaw et al. (15) found increased levels of mainly internalizing and maladaptive behavior, while other studies have found aggressive behavior and self-injury occurring in a little over 40% of people with PMS (6, 16). Self-injury, like hitting or biting oneself, seems to be associated with impulsivity and often serves the purpose of self-stimulation (16). Rahman (17) performed a study in 46 individuals with PMS between 2 and 27 years of age, both with and without ASD, and found few problems in the areas of anxiety, self-esteem and somatoform behavior in the whole sample. In a relatively large study involving 201 individuals with PMS between 0 and 64 years of age, behavioral difficulties appeared to decrease with age (18). In adults, difficulties in the areas of social relationships and anxiety are more prominent (7).

Vogels et al. (19) reviewed literature on behavior in PMS and found multiple psychiatric issues such as catatonia, bipolar disorder and ASD associated with PMS. ASD rates seemed to be depending on type of assessment. The rate of ASD characteristics is estimated up to 94% (20, 21). In a study involving 71 individuals with PMS between 0 and 40 years of age ($M = 7.5$, $SD = 2.5$), Sarasua et al. (20) found that 26% of participants older than 3 years of age had ASD. In a study of 201 individuals (18), ASD characteristics appeared to increase with age, from 19% in 3- to 4.9-year-olds to 60% in those over 18 (average 31%). Other psychiatric issues diagnosed in PMS are ADHD, psychosis and depression, and bipolar disorder (3, 15, 22). In adults, psychosis seems to occur more frequently than ASD (15). The average age of onset of psychiatric symptoms is between 15 and 20 years, but the range is large (4, 22). The study by Rahman (17) among 46 individuals found that comorbidity of PMS and ASD was related to greater impairment in adaptive behavior in the areas of socialization and communication.

Social-emotional development is described as learning how to relate to the social world and be able to differentiate, express and perceive emotions. Specific patterns of social-emotional development and behavior have been reported in specific genetic disorders like Down syndrome or Williams syndrome (23). In PMS little is known about the social-emotional development, only specific behavior like social communication are described. Size of deletions has been suggested to be related to level of development and behavior in children with PMS, but there is a large inter-individual variability (20). More severe developmental delay in the language, motor and cognitive domains appears to be associated with larger deletion sizes. This was found in the study by Zwanenburg et al. (8), the studies by Sarasua et al. (18, 20) and in a third study by Sarasua et al. (24) involving 79 individuals between 0 and 40 years of age ($M = 7.7$).

In this study we describe the findings on behavior, developmental domains and deletion size in the same sample of 33 children with PMS previously described by Zwanenburg et al. (8). We suggest modifications and a structured multidisciplinary approach in assessing and interpreting behavior and development in rare genetic disorders and PIMD such as PMS. Such a structured modified approach based on RDoc criteria and the framework Soorya et al. (10) proposed, leads to understanding the meaning of behavior in children with PMS. This informs interventions on care for individuals with PMS and allows comparison in behavior and levels of functioning within and between syndromes.

METHODS

Participants and Procedure

The sample included 33 children with PMS, due to a deletion 22q13.3, who were diagnosed at the University Medical Centre Groningen or had been referred from other medical centers in the Netherlands. This study examines data from the same sample described in Zwanenburg et al. (8), with the exclusion of child number 7 in that study. This child had a mosaic deletion, which is not comparable to the other deletion types. Zwanenburg et al. (8) previously described a subset of the data, the Bayley-III and VABS in their descriptive article. In this study we analyzed the data on behavior and social-emotional development and compared these results with the previously described data on the Bayley and VABS. The data on behavior and social-emotional development have not been published previously. This data was collected within the same timeframe as the previously published data on the Bayley-III and the VABS.

Our study population (see **Table 1** for details) consisted of 8 boys and 25 girls with an average age of 6.2 years (range 1.1 to 15.7). Twenty-eight children had a simple terminal deletion, with three also having an additional copy number variation. The other five children had a 22q13.3 deletion due to a ring chromosome 22. The average deletion size was 3.9 Mb (range 0.2–9.2 Mb). The calendar age presented is the age at the date of the Vineland test administration, which leads to slight differences with the age mentioned in our previous paper, which was based on the day of the Bayley-III assessment (see Section *Instruments*). An educational psychologist assessed the child in a familiar setting. A more detailed description of the sample and procedure can be found in Zwanenburg et al. (8). Characteristics of our study sample are shown in **Table 1**.

Instruments

We assessed cognitive development, using the Dutch Bayley Scales of Infant and Toddler Development, third edition (Bayley-III) (25). This instrument contains subscales for cognition, receptive and expressive language, and fine and gross motor development, which are assessed using a standardized in-person

TABLE 1 | Characteristics of the children in the sample.

ID no.	Age (mo)	Sex	Deletion type	Deletion size (Mb)	Walking unassisted (mo)	Medication at 1st assessment
1	13	M	Terminal	6.5	25 (crawling)	None
2	16	F	Terminal	2.1	12	None
3	22	F	Terminal	1.9	19	None
4	26	F	Terminal	7.7	36	Salbutamol (as needed)
5	22	F	Terminal + dup 13q (2.3 Mb)	7.3	39 (walking assisted)	None
6	17	F	Terminal	9.2	12 (rolling over)	None
8	37	F	Terminal	3.2	30	Valproic acid (for absence like periods)
9	37	M	Terminal	2.1	17	None
10	39	F	Terminal	182 kb	16	Not reported
11	41	F	Terminal	587 kb	24	Macrogol and omeprazole
12	42	F	Terminal	6.2	27	None
13	45	F	Terminal	6.6	20	None
14	45	F	Terminal	7.4	76	None
15	46	F	Terminal	6.2	25	None
16	47	F	Terminal + del 16p (761 kb)	3.0	42	Beclometason dipropionate, salbutamole, ipratropium bromide
17	47	M	Terminal	182 kb	18	Risperidone and clonidine
18	64	F	Terminal	183 kb	16	Macrogol
19	65	F	Ring 22	2.3	23	None
20	82	F	Terminal	1.6	17	None
21	96	M	Ring 22	3.1	28	None
22	99	F	Ring 22	3.4	31	None
23	92	M	Ring 22	2.7	24	None
24	92	F	Terminal[c]	n.a.	n.a.	Not reported
25	110	M	Terminal	6.1	43	Melatonin
26	105	F	Terminal	6.4	96	Omeprazole, alginic acid, domperidone, trimethoprim, melatonin
27	119	F	Terminal	377 kb	16	Melatonin
28	112	F	Terminal + dup 12q (5.1 Mb)	2.0	22	None
29	123	M	Terminal	7.8	48	None
30	129	F	Ring 22	3.4	32	None
31	118	F	Terminal	224 kb	15	None
32	142	M	Terminal	5.0	24 (walking assisted)	Alimemazine and melatonin
33	157	F	Terminal	3.5	32	None
34	188	F	Terminal	5.7	19	Lamotrigine (for fever-induced convulsions)

no, number; mo, months.

test administration. The test contains norms for children up to 42 months of age and is also used for older children with a developmental level up to 42 months. The test results of the children in the current sample were previously reported in Zwanenburg et al. (8).

The Dutch Vineland Screener 0-6 (26) an adaptation of the Vineland Adaptive Behavior Scales (VABS) (27) assesses adaptive behavior based on caregiver-report. This instrument can also be used for older children with a developmental level up to 6 years. The Vineland contains subscales for communication, social behavior, daily skills and motor skills. The parent indicates to what extent the child displays each of 72 descriptions of behavior using a 3-point Likert scale (yes, usually / sometimes or partially / no, never) and an additional response option "unknown." The raw score can be converted to a developmental age equivalent (DAE) ranging from 6 to 70 (communication), 1–70 (social behavior), 10–68 (daily skills), 0–58 (motor skills), and 2–68 (adaptive behavior total score) months.

We used the Dutch Child Behavior Checklist for children of 1.5–5 years (CBCL) (28), a questionnaire for assessing internalizing (anxiety/depression, somatic, withdrawn) and externalizing (attention problems, aggression) behavior. In addition, the CBCL yields scores for five problem areas, namely affective problems, anxiety, ASD (named *pervasive developmental*

disorder in the CBCL), ADHD and oppositional deviant disorder (ODD). It contains 100 items with short descriptions of behavior for which the respondent indicates if this suits the child on a 3-point Likert scale (not at all / a bit or sometimes / clearly or often). The questionnaire yields *t*-values ($M = 50$, $SD = 10$) for each subscale, for internalizing and externalizing problems, and for each of the five problem areas. *T*-values between 65 and 70 are in the borderline range. *T*-values above 70 are in the clinical range, indicating behavioral problems.

For all analyses involving the CBCL, we included all children with a calendar age of 18 months or older because the target group of the instrument starts at this age (28). As the CBCL can also be used for children with ID (29), we did include children with a developmental age below 18 months, after verifying that the descriptive results did not differ considerably from those of the children with a higher developmental level.

The ESSEON-R (30) is a questionnaire with 76 short descriptions of behavior for assessing social-emotional development of children with a developmental level between 0 and 14 years. The questionnaire yields a DAE per domain as well as a total DAE. The ESSEON-R is explicitly meant for assessing the social-emotional development of children with intellectual impairments or psychiatric problems.

All questionnaires were proxy questionnaires filled in by one or both parents or a care worker who was very familiar with the child and subsequently evaluated with parents or care workers by an educational psychologist. Deletion sizes were evaluated by a clinical geneticist. General principles on assessment as proposed in the framework of Soorya et al. (6) were applied in the assessments.

Data Analysis

Because of the small sample size, all the analyses reported in the current paper are descriptive in nature. We used both SPSS (Version 23) (31) and R (32) for the statistical analyses.

First an overview was made of the scores of the children in the sample on the tests and questionnaires. Second, we described the domains of the Vineland, ESSEON-R and CBCL on which the children obtained the highest scores. We visualized and/or described differences between subgroups of children based on scores on the Bayley-III, developmental level or deletion size. Regarding deletion size, we used the same groupings used in Zwanenburg et al. (8): <225 kb, 225 kb−6.7 Mb and >6.7 Mb. The reason for this size grouping is that children with a very small deletion have a higher developmental level, on average. The higher boundary of 6.7 Mb is downstream of the *PARVB* gene. Children were divided in two groups of calendar age with a cut-off at 6 years (72 months) based on the results of the Bayley-III ceiling effect described in Zwanenburg (8). The DAE on the Bayley-III cognition scale could be equally distributed in 2 comparable groups ($n = 12$ and $n = 13$) with a DAE of $\leq$ vs. >18 months. We also visualized the relationship between general behavior on the CBCL and adaptive behavior.

For visualizing behavioral characteristics as well as differences between subgroups therein, we used line graphs both for the individual children and for the group mean, so that individual differences would be reflected in the results [R Package "ggplot2" (33)]. Developmental age equivalents (DAE) in the figures are based on the cognitive domain of the Bayley-III.

As sex differences have not been found in the developmental or behavioral characteristics of persons with PMS (6), we did not take them into account.

RESULTS

Characteristics of our study sample are shown in **Table 1**. **Table 2** shows the scores of the children on cognition (Bayley-III), adaptive behavior (Vineland) and social-emotional development (ESSEON-R). **Table 3** shows the scores regarding behavior (CBCL). For a small number of children, data on the Bayley-III ($n = 1$), Vineland ($n = 3$), or CBCL ($n = 4$) were missing.

Adaptive Behavior

Figure 1 shows the individual as well as the average scores on the Vineland subscales. The average developmental age equivalent (DAE) for adaptive behavior was 17 months, with a range of 4 to 34 months. This wide range is partly explained by differences in calendar age. However, based on the DAEs shown in **Table 2** for the children with identification number 20 and higher, who all have a calendar age > 6 years, the inter-individual variation still is large. Variation is also large with respect to the direction and magnitude of the differences between the subscale scores. The level ranges from about 14 to 21 months between the subscales, with the highest averages for motor skills and daily skills. However, the subscale daily living skills has a relatively high baseline level, as the lowest possible DAE is 10, which influences the average. On the individual level, the subscale levels range from 2 to 42 months. A total of 13 of the children had their highest subscale score on motor skills, seven on daily skills, six on social skills and two on communication.

Comparing the Vineland profiles of children younger than 6 years old to children older than 6 years, did not show any clear differences. We therefore included a graph comparing four different age groups based on developmental milestones, see **Figure 1B**. Although the groups are small, this graph shows that the average developmental level in the area of adaptive behavior does increase with increasing age from 3 years up, the increase in adaptive behavior seems to plateau at the age of 6 years. **Figure 1C** illustrates the adaptive behavior profiles for children with a cognitive developmental level (as measured with the Bayley-III cognition scale) up to vs. >18 months. If the cognitive DAE is higher, the DAE for adaptive behavior is also, on average, higher. **Figure 1D** compares children with different deletion sizes, and a clear trend of decreasing level of adaptive behavior with increasing deletion size can be seen. We also compared children with different deletion types and found that children with a ring 22 deletion (median deletion size: 2.98 Mb) have slightly higher average levels of adaptive behavior (subscale averages ranging between 25 and 32 months) than children with a terminal deletion.

Behavior

Figure 2 shows the behavioral profiles of the 26 children >1.5 years of age on the basis of the CBCL subscale *t*-values

TABLE 2 | Developmental Age Equivalents (DAE) in months of cognition (Bayley-III), adaptive behavior (Vineland), and social-emotional development (ESSEON-R).

	Bayley-III	Vineland					ESSEON-R		
ID	**Cognition**	**Communication**	**Social skills**	**Daily skills**	**Motor skills**	**Sum score**	**Social**	**Emotional**	**Total**
1	3	6	6	10	2	4	6	6	6
2	8	9	12	18	20	13	30	18	24
3	8						12	12	12
4	5	8	6	14	14	9	18	12	15
5	13	11	10	10	6	7	30	18	24
6	1						12	12	12
8	7	6	19	10	17	12	18	18	18
9	13	16	24	12	29	19	30	12	21
10	23	18	17	25	30	22	48	36	42
11	8	9	3	12	17	9	6	24	15
12	16	14	15	10	14	12	12	18	15
13	22	16	28	25	24	22	30	24	27
14	7	11	17	14	7	11	12	24	18
15	18	14	23	28	27	22	36	24	30
16	24	14	14	18	20	15	36	24	30
17	22	18	21	23	30	22	36	24	30
18	38	33	28	39	30	32	72	36	54
19	25	28	39	34	37	34	48	36	42
20	22	18	12	41	37	26	12	24	18
21	21	21	26	32	38	29	36	18	27
22	21	19	24	21	29	23	30	24	27
23	25	19	12	23	30	20	36	18	27
24	8	6	3	12	20	9	12	18	15
25	10	13	15	16	15	13	30	24	27
26	6	6	8	12	7	7	3	3	3
27	24	6	10	19	25	14	6	12	9
28	21	8	14	14	22	13	12	6	9
29	14	6	8	16	17	10	6	18	12
30	30	38	23	27	24	27	36	24	30
31	52						72	84	78
32	7	9	12	14	4	8	18	24	21
33	25	23	37	34	27	30	30	24	27
34		9	6	12	12	8	6	12	9
Mean	17.1	14.4	16.4	19.8	21.0	16.7	25.4	21.5	23.5
sd	10.9	8.2	9.3	9.1	10.0	8.5	17.7	13.8	14.8

for behavior. Compared to the Vineland results, the inter-individual variation is large. As can be seen in **Figure 2A**, withdrawn behavior shows both the highest average *t*-value (in the borderline range) and the highest individual *t*-value ($t = 94$). For all other subscales, the average *t*-values are below the borderline range. On an individual level, two children scored in the borderline or clinical range for anxiety, five for somatic symptoms, 18 for withdrawn behavior, 14 for attention problems and six for aggression. Most children have the highest *t*-value for withdrawn behavior ($n = 15$) or attention problems ($n = 6$).

Figure 2B shows that there is no large difference between children up to vs. above 6 years old in terms of behavior. **Figure 2C** shows that in children with a DAE above 18 months on the Bayley-III cognitive scale, scores on somatic problems and aggression are slightly higher than under 18 months DAE. The group with a DAE under 18 months show a *t*-value within clinical range for attention problems opposed to the group above 18 months DAE. In both groups the *t*-value for withdrawn behavior is the highest score and within clinical range. **Figure 2D** compares children with different deletion sizes. Children with a deletion size below 225 kb have clearly higher average *t*-values for somatic problems, attention problems and aggression, which are all in the borderline range. When looking at deletion type, children with a ring 22 deletion ($n = 3$) have slightly lower average levels of behavioral problems (subscale averages below the borderline level, ranging between 50 and 64) than children with a terminal deletion (subscale averages: 53 to 75).

TABLE 3 | Scores on behavioral problems measured with Child Behavior Checklist (CBCL).

	CBCL (*t*-values)											
	Subscales					Main scales		Problem areas				
ID	Anxiety/ Depression	Somatic complaints	Withdrawn	Attention problems	Aggressive	Internalizing	Externalizing	Affective	Anxiety	PDD	ADHD	ODD
3	51	50	79	67	50	63	51	63	57	72	50	50
4	50	62	67	51	50	56	44	52	50	63	50	51
5	64	62	76	67	65	71	66	79	59	74	57	70
8	51	53	67	77	62	56	66	60	54	63	64	59
9	52	53	76	62	56	65	58	52	63	77	54	52
10	51	67	63	70	53	64	58	60	57	75	60	52
11	50	50	94	77	51	63	57	75	50	77	60	51
12	50	53	70	70	51	56	56	63	50	63	60	50
13	71	79	85	57	72	78	69	79	75	84	60	64
14	50	58	63	53	59	60	58	67	54	70	57	50
15	50	50	63	67	56	51	59	51	50	60	51	55
16	50	50	67	51	50	47	39	56	50	57	50	50
17	67	76	70	73	84	78	82	75	73	81	72	80
18	51	58	56	62	62	58	62	52	57	60	57	55
19	51	53	63	57	55	58	56	67	54	63	52	55
20	52	58	70	80	72	70	77	63	54	79	67	70
22	50	50	67	67	64	53	65	51	51	67	57	55
26	50	70	73	67	50	62	47	56	50	72	57	50
27	50	53	73	67	51	62	54	72	50	77	57	50
28	50	50	82	53	50	56	42	60	50	72	51	50
29	52	50	67	80	69	66	74	70	63	77	64	64
30	50	62	63	57	52	61	54	60	59	74	57	50
31	59	76	76	73	65	71	68	75	70	72	67	67
32	52	53	56	57	50	62	47	50	59	63	54	50
33	51	58	63	50	50	56	42	52	50	63	50	50
34	50	50	70	57	50	56	48	52	50	68	51	51
M	52.6	57.0	68.3	63.4	56.9	59.2	56.3	60.9	55.5	68.3	56.7	55.3
sd	5.4	8.8	9.7	9.3	8.9	10.3	11.7	9.6	7.2	8.8	5.9	7.9

PDD, pervasive developmental disorder; ADHD, attention deficit hyperactivity disorder; ODD, oppositional deviant disorder; M, Mean; sd, standard deviation; Clinical threshold = >70.

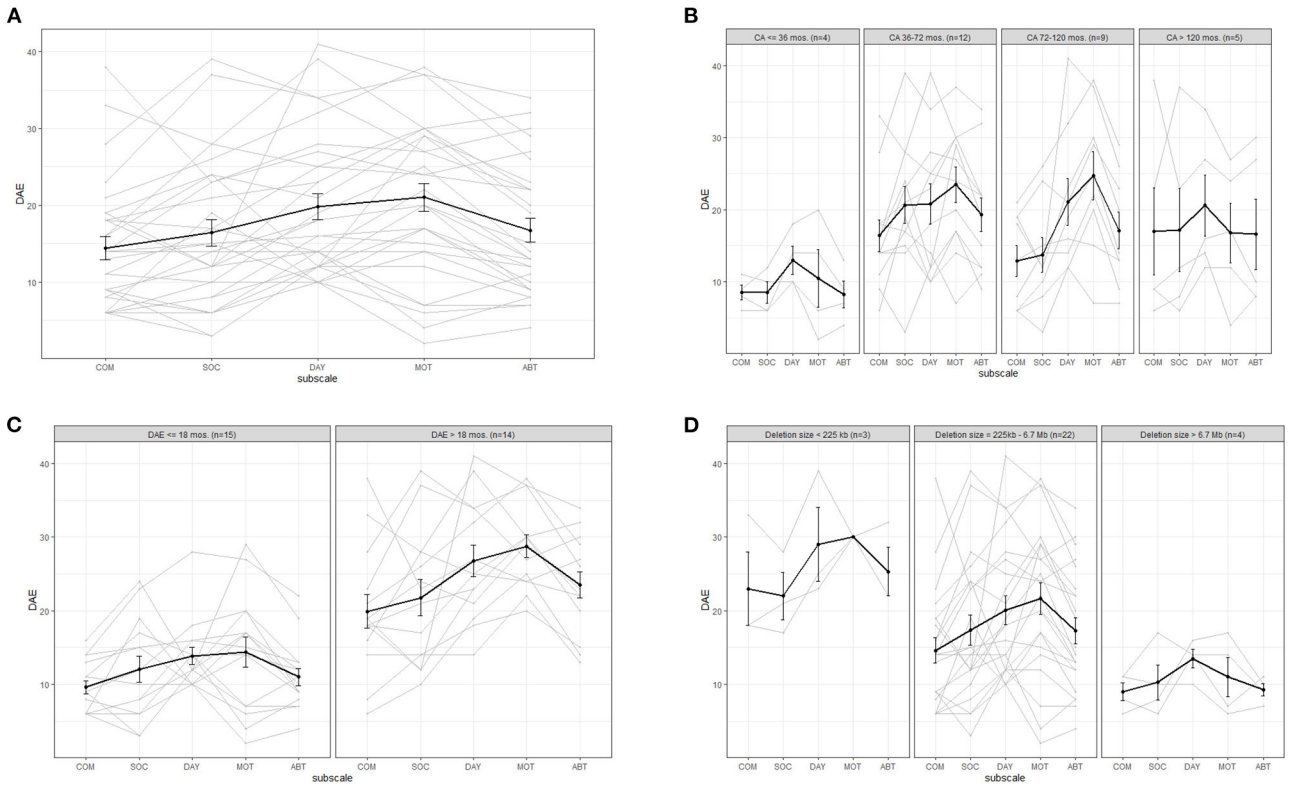

FIGURE 1 | Average (bold line) and individual behavior profiles on the Vineland for **(A)** the whole sample and for subgroups based on **(B)** calendar age (CA), **(C)** developmental age equivalent based on cognition scale in the Bayley-III (DAE) and **(D)** deletion size. COM, communication; SOC, social behavior; DAY, daily skills; MOT, motor skills; ABT, adaptive behavior total score. Please take into account: the average calendar age was 74 months (6.2 years).

Figure 3 shows the behavioral profiles for the CBCL-scales based on classification areas of the Diagnostic and Statistical Manual of Mental Disorders (DSM). The *t*-value for pervasive developmental disorder (PDD) has the highest average ($t = 70$) and the highest individual value ($t = 84$). All other average *t*-values are below the borderline range. On an individual level, five children scored in the borderline or clinical range for affective problems, three for anxiety, 17 for PDD, three for ADHD and four for ODD. Most children have the highest *t*-value for PDD ($n = 21$).

No clear differences can be observed between younger and older children (**Figure 3B**) or between children with a lower or higher cognitive developmental level (**Figure 3C**), although the variation in PDD-scores is clearly lower in children who are above 72 months of age. The average *t*-value for anxiety, ADHD and ODD is just below the borderline range for children with a deletion size below 225 kb, which is higher than that of children with larger deletion sizes. However, this subgroup is very small ($n = 4$, see **Figure 3D**). We found no clear differences when comparing children with different deletion types.

Social-Emotional Development

Figure 4 shows the levels of social and emotional development of the children, which is around 22 to 25 months, on average. The graphs of the individual children show large variation. The levels of social behavior do differ slightly from the levels of emotional behavior within the children. With the exception of one child with a clearly higher level of social behavior.

Figure 4B shows no clear differences between younger and older children. Among the children with a cognitive developmental level above 18 months there are more children with higher levels of social and emotional behavior (above 30 months) than in the group with a lower cognitive DAE (see graph c). Children with a deletion size smaller than 225 kb have higher levels of social and emotional development than children with larger deletion sizes, although the group is small ($n = 4$), see **Figure 4D**. We found no clear differences between children with a ring 22 vs. a terminal deletion.

ASD Symptoms and Adaptive Behavior

Children with a dual diagnosis of ID with ASD in general show a lower level of adaptive behavior on the social domain compared to intellectually disabled children without ASD (34). **Figure 5** shows the level of adaptive behavior, comparing children with and without a CBCL *t*-value in the clinical range ($t > 70$) on the PDD-subscale (indicating possible ASD). In children without a *t*-value in the clinical range, their profile is relatively balanced, with an average DAE around 20 months, except for the communication subscale, for which the average is around 16 months. Children with a PDD *t*-value within the clinical

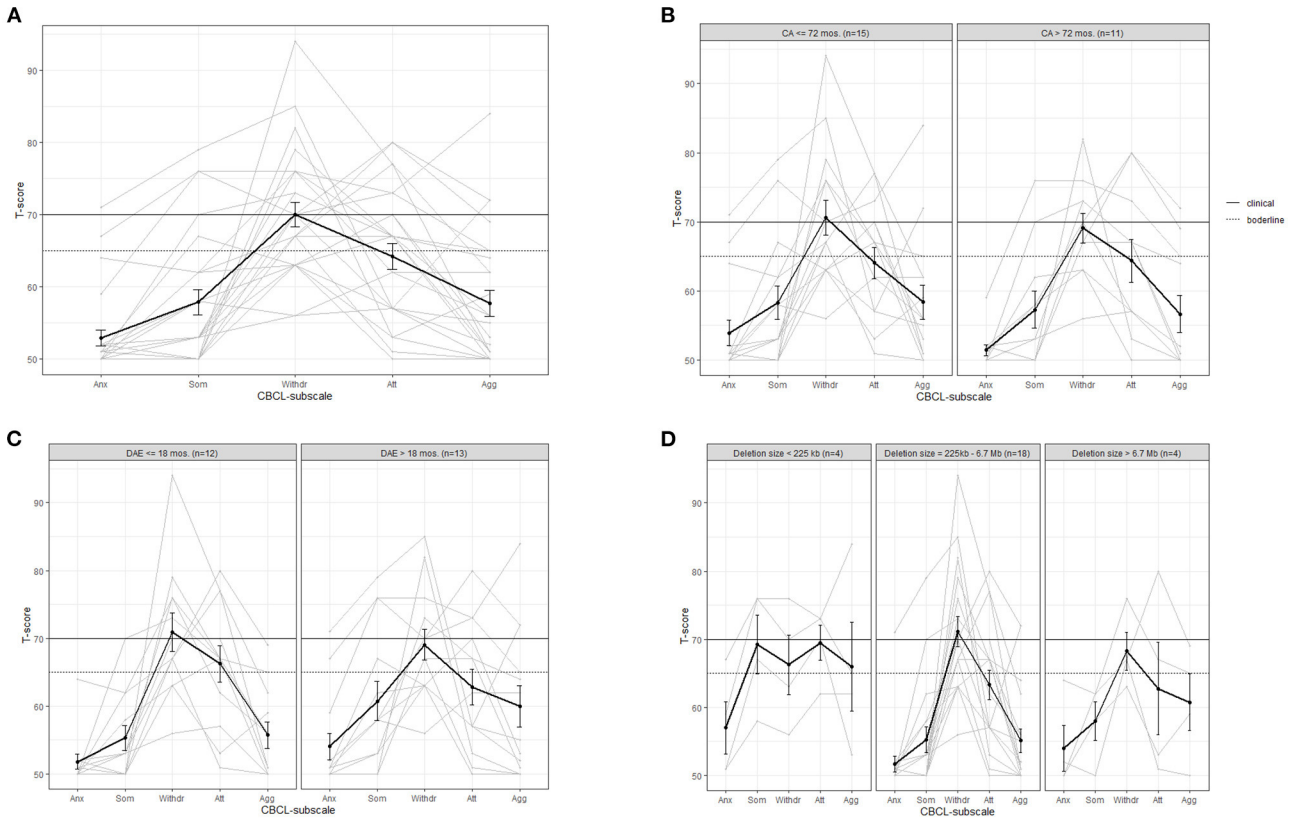

FIGURE 2 | Average (bold line) and individual behavior profiles on the CBCL subscales for **(A)** the whole sample and for subgroups based on **(B)** calendar age (CA), **(C)** developmental age equivalent (DAE) in months based on cognition scale Bayley-III and **(D)** deletion size. Anx, anxiety/depression; Som, somatic; Withdr, withdrawn; Att, attention problems; Agg, aggression.

range also have a lower score in the social domain, on average. The average level of communication skills does not differ much between the groups, but if we were to exclude child 30 (communication DAE = 38 months, PDD *t*-value = 74), this average would be lower in the group of children with PDD *t*-values in the clinical range. The average level of adaptive behavior in the domains daily living skills and motor skills does not differ much between the two groups. Within the group with clinical PDD *t*-values, fewer scores above the level of 30 months are obtained than in the other group.

DISCUSSION

This study followed the structured modified approach based on RDoc criteria and the proposed framework by Soorya et al. (10). We found large variations in cognitive development, adaptive behavior and social-emotional development. To understand the meaning of our results we interpreted developmental levels and behavior within the other domains of functioning. Cognitively the children showed severe developmental delay given the average calendar age of 6.2 years (range 1.11 to 15.7). The highest levels of adaptive behavior were found in the areas of motor skills and daily skills. The level was 17 months, on average, with a range of 4 to 34 months. The wide range of adaptive behavior can only partially be explained by differences in calendar age: levels of adaptive behavior seem to increase until the calendar age of approximately 36 months, and then appear to even out. Levels of adaptive behavior appeared higher with higher levels of cognitive development and smaller deletion sizes, which is consistent with a previous finding that relatively small deletions were related to a more favorable developmental phenotype (15).

The large variability and the observation of higher levels of adaptive behavior in children with smaller deletion sizes are also consistent with the earlier study into the development of children with PMS (8). We could not confirm previous observations of increased adaptive behavior than could be expected based on their cognitive developmental level (4, 5), except for the communicative domain. This last result could, however, be affected by limitations related to the validity of the communication domain (see paragraph adaptive behavior, communication below).

Regarding behavior, we found that withdrawn behavior, followed by attention problems were most frequent. The parents reported relatively little anxiety, which is in line with previous research (17). Although previous research in a sample including both children and adults described behavioral

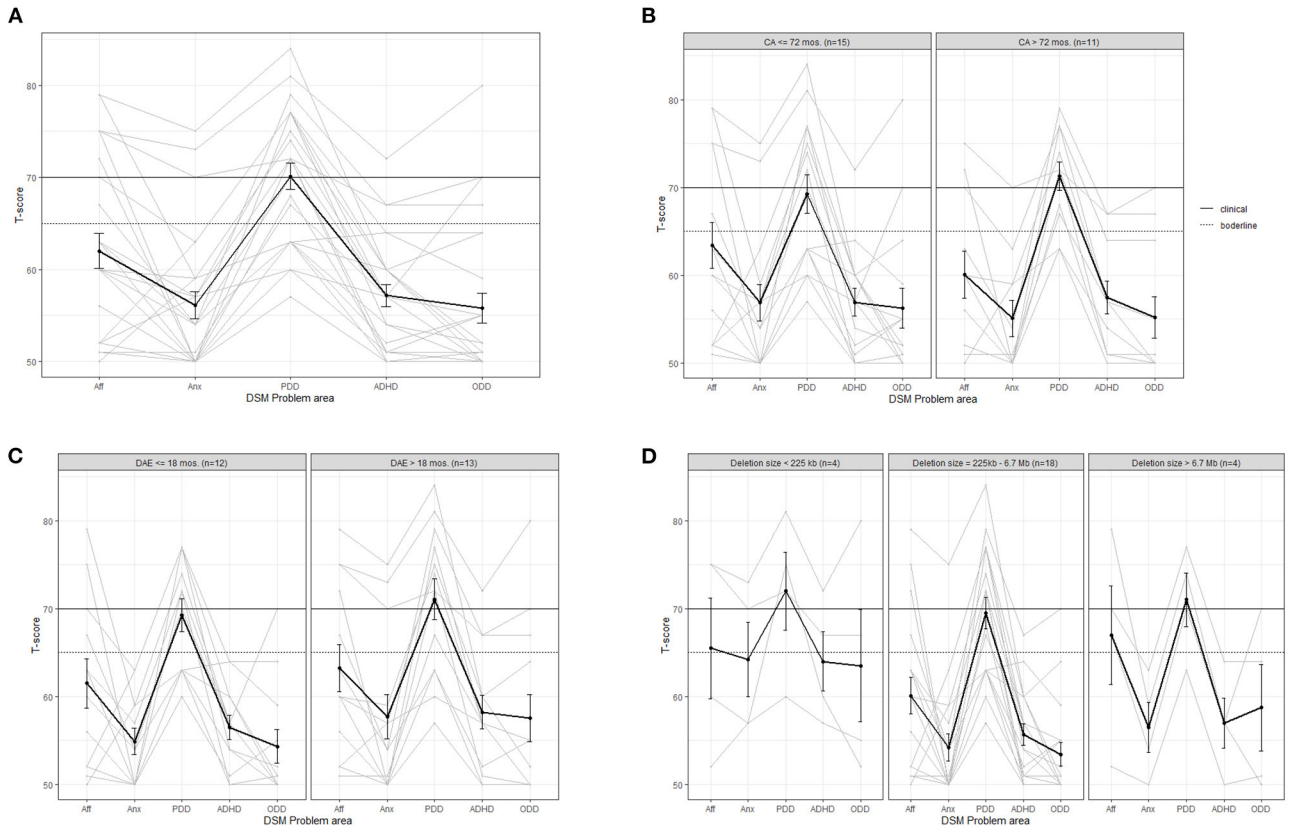

FIGURE 3 | Average (bold line) and individual behavior profiles on the CBCL DSM problem areas for **(A)** the whole sample and for subgroups based on **(B)** calendar age (CA), **(C)** developmental age equivalent (DAE) in months based on cognition scale Bayley-III and **(D)** deletion size. Aff, affective problems; Anx, Anxiety; PDD, pervasive developmental disorder; ADHD, attention deficit hyperactivity disorder; ODD, oppositional deviant disorder.

difficulties decreasing with age (18), we found no clear trend with respect to calendar age, which could be due to the limited age range up to 15 years and/or our small sample size. A higher cognitive developmental level and a smaller deletion size seem to be related to higher levels of somatic symptoms and aggression ($n = 4$). The increased average scores on these specific subscales could be specific to PMS.

Considering behavioral issues ASD-symptoms were most frequent, whereas small deletion size was related to increased symptoms of anxiety, ADHD and ODD. The level of reported ASD-symptoms is in line with previous findings (20, 21). Our findings of increased problems in the areas of withdrawn behavior, attention and ASD are comparable to those of a study in children with ASD (35). Of course, the fact that ASD-like symptoms co-occur with ID associated with PMS requires careful consideration whether these symptoms are more intense and frequent than expected for level of ID and merit an ASD diagnosis. Interestingly, our results show that few of the children with PMS show ASD symptom scores in the borderline or clinical range. Therefore, an additional ASD diagnostic trajectory can be helpful in understanding the meaning of behavior and differentiating between children and to identify underlying care needs.

High levels of supposed ASD symptoms were related to lower levels of adaptive behavior in the social domain. This is according to expectations of adaptive behavior in children with confirmed ASD diagnosis. All the children in the sample had somewhat lower levels in the communication domain, independent of their level of ASD symptoms and understandable within the context of the ID. As children with PMS have impaired language abilities (4, 5), this could explain the fact that the scores on the Vineland items measuring communication skills. These depend, to a large extent, on the verbal language skills of the children (e.g., "Does he/she have a vocabulary of at least 50 recognizable words?" or "Does he/she speak in full sentences?"), whereas this is not the case for the items measuring social skills (e.g., "Does he/she play with a toy or object, alone or with others?"). Possibly, the Vineland underestimates non-verbal communicative adaptive behavior in children with PMS in that the lower scores reflect low levels of expressive verbal language and do not sufficiently take into account non-verbal communication abilities.

The level of social-emotional behavior was around 23 months on average, but also showed a large inter-individual variation. A higher cognitive developmental level was related to higher levels of social-emotional behavior, but this was only true for the subgroup of children with a small deletion size.

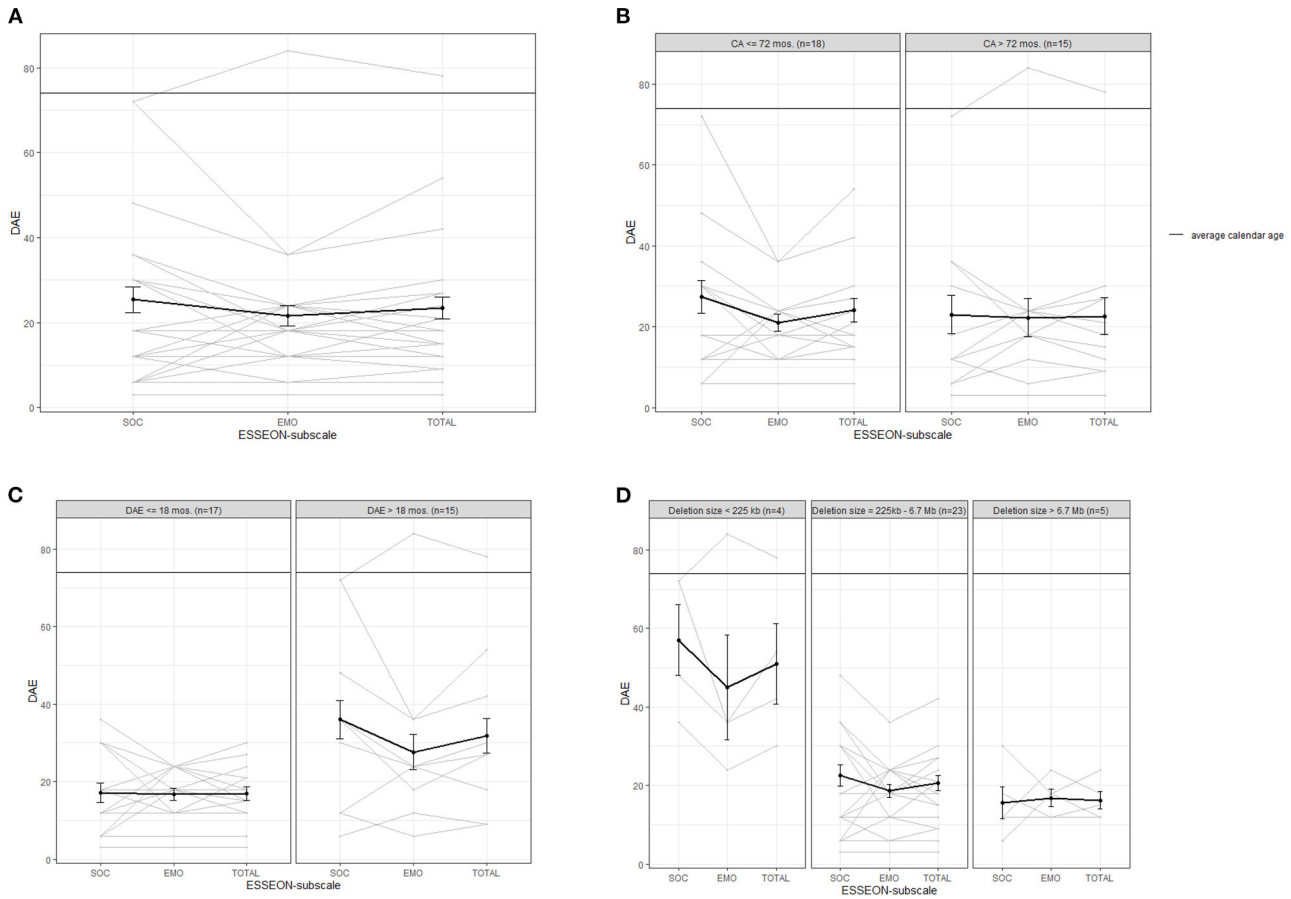

FIGURE 4 | Average (bold line) and individual behavior profiles on the ESSEON-R for **(A)** the whole sample and for subgroups based on **(B)** calendar age (CA), **(C)** developmental age equivalent (DAE) in months based on the Bayley-III cognition scale and **(D)** deletion size. SOC, social development; EMO, emotional development.

Consistent with earlier results (14, 18), smaller deletions and higher cognitive developmental level were related to higher levels of adaptive behavior, but also to more specific problems (somatic problems, attention problems, aggression and anxiety). This is unexpected, given the finding that lower intellectual ability level is related to having more CBCL scores in the deviant range (36). It may be that children who have a higher level of adaptive behavior are more aware of their limitations, are exposed to higher expectations due to their performance in adaptive behavior and therefore experience more stress, which could be expressed in the form of maladaptive behavior. Behavior like aggression and anxiety is very difficult to recognize in children with a younger developmental stage or age. Aggressive behavior is to some extent normal in younger stages of development and anxiety is related to developmental stage as well. Cognitive capacities are needed to comprehend possible danger and experience anxiety. Children with a higher developmental level might be better able to express themselves, this is also the case in expressing emotions. However, this hypothetical relationship between adaptive behavior and behavior is not clearly reflected in our results on the basis of the total score for internalizing and externalizing behavior.

The recognition and interpretation of behavior in children with lower developmental levels is also relevant in a more general sense in children with PMS and could play an explanatory role in our findings as proposed by the framework of Soorya et al. (10). The relatively high levels of withdrawn behavior and ASD symptoms in the sample were partly based on results on items describing behavior that can also be explained by the low level of cognitive and language development of children with PMS. More specifically, the items for which the answer category *clearly or often* was chosen most often (for more than half of the children) were: "Acts too young for his/her age," "Does not respond when others talk to him/her" and "Speech problems." This suggests that our results may include an overestimation of withdrawn behavior and ASD symptoms in these children. Children with a higher cognitive developmental level showed higher levels of somatic symptoms and aggression in our sample, which could imply that somatic symptoms and aggression are not easily recognizable in children with lower developmental levels (4, 7, 17) (**Table 4**). Even in a study using general principles for assessments in PIMD, as proposed in the framework of Soorya et al. (10) and the functional domains as proposed by the RDoc framework, interpreting behavior in PIMD such as PMS remains challenging.

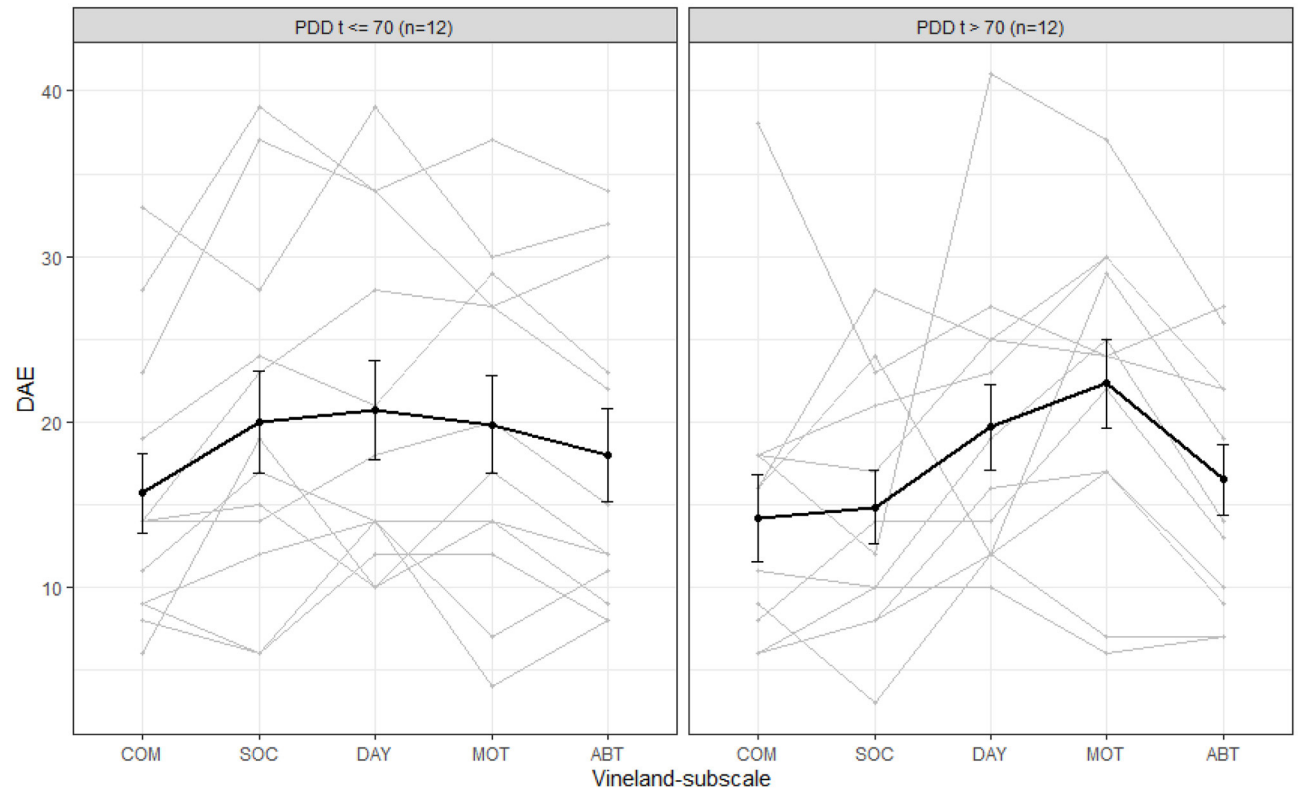

FIGURE 5 | Average (bold line) and individual behavior profiles on the Vineland for children with and without CBCL-scores in the clinical range for pervasive developmental disorder (PDD). COM, communication; SOC, social behavior; DAY, daily skills; MOT, motor skills; ABT, adaptive behavior total score; DAE, developmental age equivalent in months based on Bayley-III cognition scale. Please take into account: the average calendar age was 74 months (6.2 years).

Limitations and Directions for Future Research

The main limitation is the small sample size which is directly related to the rarity of PMS. The small sample and even smaller subgroup sizes mean that the results are highly sensitive to sampling variation and no firm conclusions can be drawn. However, our study adds to the body of evidence on development and behavior in children with PMS. A second important limitation is that the validity of the Vineland, CBCL and ESSEON-R for children with a PIMD as PMS has not yet been explicitly studied, and there are reasons to suspect measurement non-invariance. This means that the test validity might not be optimal for children with PMS in comparison to children without PMS and the same behavior (e.g., the measured construct). Previous research results about the validity of the CBCL for children with intellectual disability are inconsistent: one study found measurement invariance (29), while another found measurement invariance on the level of the total test score, but not for the subscales (37). As studying measurement invariance in relation to PMS is difficult due to the rarity of the syndrome, the descriptive results underpin the careful study and description of behavioral phenotype in PMS. In this study the general principles of assessment in PIMD (10) are applied in the assessments of the functional domains, but we argue these should also be applied in assessment and interpretation of behavior. The multi units of analysis based on the RDoc framework were used in this study and these should also be used when interpreting behavior in a PIMD as PMS. For example, expressive language (cognitive systems in RDoc framework) could be of influence on the scores regarding behavior. Our study endorses more explicit application of the other RDoc domains like negative valence, positive valence and arousal systems when assessing and interpreting behavior in PIMD like PMS. These domains could be of great use when interpreting documented behavior in children and adults with PMS.

When it comes to the CBCL, Koskentausta et al. (38) indeed found that the CBCL is less reliable to assess psychopathology in children with moderate, severe or profound intellectual disability, although this conclusion was based on descriptive statistics only. Another limitation is the fact that we used the CBCL, which can be used in children 1.5 years of age and up, although our sample included younger children. We solved this by excluding the children below 1.5 years of age from the analyses involving the CBCL, which means we cannot draw conclusions about the behavioral problems of these youngest children with PMS. In this study profiles of the scores have been analyzed instead of individual scores. The use of profiles limited the use of the

TABLE 4 | Previously published frequent somatic problems in PMS.

Frequent somatic problems in PMS
Hypotonia
Low pain perception
Sleep problems
Constipation
Regulating body temperature
Swallowing
Vision problems
Epilepsy

individual data but provide interpretation on possible underlying developmental aspects.

Future research should not only be focused on replicating the results in situations in which larger samples of children with PMS can be formed, studies should ideally use instruments that are widely used internationally so that data from multiple studies in various countries can be combined to overcome the problems related to small sample sizes. Research based on longitudinal data would also be valuable, all the more so because this would help overcome the sample size challenge by collecting more information per child. This would also help to answer questions about how the behavior develops over time within children. In addition, having multiple assessments per child enhances the reliability of the data in total because an unreliable assessment due to, for example, tiredness during testing can be identified if the results deviate greatly from the results of other assessments in the same child. The framework of Soorya et al. and the RDoc framework should be taken into account when assessing functional domains or behavior in children with PIMD.

Implications for Daily Practice

Our results have important implications for understanding behavior in PMS and adjusting the surroundings for children with PMS. Lower developmental levels and language skills are often reflected in difficulties adapting and responding to the environment, leading to stress. Stress reduces the possibilities for development, increases behavioral problems and decreases quality of life. Early identification of difficulties makes it possible to stimulate development and offer suitable support, to allow children with PMS to benefit from their environment. Difficulties need to be identified at an early stage so that suitable support can be given in the years where the children show the largest possibilities for development and emergence of more severe problems can be prevented. Children with PMS may need extra support in developing their expressive communication skills, for instance using visual communication or specific communication treatment programs.

Greater awareness of difficult to understand behavioral issues, psychiatric problems and underlying unmet needs, particularly in children with a low developmental level, is needed so measures can be taken to improve developmental opportunities and recognize unmet needs. In children with a small deletion size, the risk for behavioral problems in areas other than ASD is also increased.

Conclusions

Our results show a large variation between children with PMS in terms of adaptive behavior, behavior and social-emotional development. Moreover, large intra-individual differences were found between the various domains. Contrary to the general understanding, average levels of adaptive behavior in our sample were not lower than, but rather consistent with levels of cognitive development. Levels of adaptive behavior were highest in the areas of motor and daily skills. Levels of adaptive behavior seem to increase up to the calendar age of approximately 36 months, and then seem to level out. Problems were mainly found in the areas of withdrawn behavior, followed by attention problems. In children with a small deletion size, symptoms of anxiety, ADHD and ODD seem to be increased. Interpreting psychiatric symptoms and behavior in an PIMD such as PMS remained challenging despite the use of available frameworks. Specific diagnostic assessment with the use of valid instruments for the level of ID is very important. Findings should be interpreted by an multidisciplinary team.

A small deletion size seems to be related to higher levels of adaptive behavior and social-emotional development. The frequency of ASD symptoms appeared not to be related to deletion size. High levels of ASD symptoms seem related to lower levels of adaptive behavior in the social domain.

Altogether, these results add to those of earlier studies and help to define the development and behavior of children with PMS. The small subgroup sizes, large inter-individual variability, and the potentially limited validity of the assessments need to be taken into account when interpreting the results. The findings underline the importance of neuropsychological and behavioral assessments within the frameworks of PIMD and RDoc domains when it comes to interpreting behavior in PMS. Early identification and interventions in expressive communication within the context of developmental level could be helpful to optimize early developmental opportunities, prevent stress and prevent the emergence of specific behavioral problems in children with PMS.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available upon request by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Medical Ethical Review Board of the University Medical Center Groningen. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

RZ collected the clinical data, conducted the data preparation, and started with the data analysis. LV analyzed the data and drafted the manuscript. SR supervised developmental and behavior assessments, collected the data, and commented on the manuscript. BF collected pediatric clinical data, contributed to data interpretation, and commented on the manuscript. CvR-A coordinated the project and commented on the manuscript. AL and IvB interpreted all results, drafted, and finalized the manuscript. All authors read and approved the final manuscript.

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Family Matters: Trauma and Quality of Life in Family Members of Individuals With Prader-Willi Syndrome

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Background: Prader-Willi syndrome (PWS) is a potentially life threatening, genetic developmental disorder that requires lifelong medical treatment and behavioral management. PWS has a major impact on the patient's social environment. In this study, we have explored traumatic life events and symptoms of posttraumatic stress disorder (PTSD) in family members of individuals with PWS. We have also assessed quality of life in relation to trauma manifestations. In addition, we have evaluated demographic characteristics such as living setting of PWS patients as well as PWS symptom severity.

Methods: Data of this observational study were obtained by means of the Life Events Checklist DMS-5, the Posttraumatic Stress Disorder Checklist DSM-5, the abbreviated World Health Organization Quality of Life questionnaire, the Lancashire Quality of Life Profile questionnaire, and a short demographic inventory. The study sample includes 98 adults aged 19 to 80 years ($M = 49$, $SD = 15$), who are relatives of 69 individuals with PWS aged 0 to 58 years ($M = 19$, $SD = 13$). Participants were recruited via the two Dutch patient associations PWS and the Dutch Digital Center of Expertise PWS.

Results: Life time prevalence of traumatic events (93%) was higher in family members of PWS patients ("PWS relatives") than in the general Dutch population (81%). Of those who reported any traumatic event, almost half reported *PWS-related* events. The prevalence of probable PTSD was higher in PWS relatives (12.1%) than the general lifetime prevalence of PTSD (worldwide, and in the Netherlands 7.4%). Predominant trauma symptoms in PWS relatives were "negative changes in arousal and reactivity" and "negative changes in cognition and mood;" both significantly negatively related to quality of life. Symptom severity of PWS individuals, as well as the associated trauma symptom severity of their relatives increased with age of the PWS individual. The presence of trauma symptoms was less frequent among relatives of PWS individuals living in a care facility.

Conclusions: Having a relative with PWS is associated with higher prevalence of traumatic experiences and greater vulnerability to PTSD. Raising awareness in health care professionals of trauma symptoms in PWS relatives may contribute to effective treatment of their psychosocial stress. In addition, timely interventions might prevent family members from developing psychopathology like PTSD.

Keywords: trauma, PTSD, contextual neuropsychology, family, Prader-Willi syndrome (PWS), quality of life, systemic approach

INTRODUCTION

Prader-Willi syndrome (PWS; OMIM #176270) is a genetic neurodevelopmental disorder, caused by an anomaly in the paternally derived long arm of chromosome 15. PWS is characterized by multiple physical, cognitive, behavioral, and psychiatric symptoms. Hypotonia and pituitary hormone deficiencies, particularly hypogonadism occur most frequently (1–3). After birth, patients suffer from feeding difficulties, often necessitating tube feeding. Later in childhood, patients typically develop hyperphagia. In the absence of external control, this can result in excessive eating behaviors including pica, and consequent risks of developing obesity, diabetes mellitus, cardiopulmonary disease, and other serious somatic comorbidities (1, 4, 5). While PWS requires lifelong medical treatment and behavioral management, overall mortality rate is estimated at 3% per year and the average age at time of death is 40–50 years (6, 7).

As to cognitive and behavioral characteristics, PWS presents with mild to moderate intellectual disabilities, executive and social cognitive impairments and symptoms from the autism spectrum such as general cognitive inflexibility with perseverations, repetitive and ritualistic behaviors, temper tantrums, and self-injury (e.g., skin-picking). Challenging behaviors typically increase from teenage years up to their thirties, and affective disorders, mood instability or psychosis may frequently occur from adolescence onwards, albeit with varying severity (8–10).

PWS also has a major impact on PWS relatives. Lower levels of quality of life, increased burden of care and family problems have been reported in primary caregivers of young children with PWS (11–13). Furthermore, there is evidence of increased levels of distress and mood disturbances in family members (mostly mothers), as well as symptoms of posttraumatic stress disorder (PTSD) in siblings of young PWS patients (12, 14, 15).

So far, no information is available on trauma related distress and quality of life regarding wider family members related to PWS individuals in all chronological ages. This study aims to identify possible psychopathology and any underexposure of the issue in the group of family members. To this end, in those PWS relatives, we studied (a) traumatic life events and symptoms of PTSD, (b) trauma symptom severity and PWS-related stressors, (c) trauma symptom severity and Quality of Life, (d) age of PWS individuals and PWS symptom severity, the amount of PTSD symptoms, and Quality of Life respectively, and (e) living situation in relation to the occurrence of trauma symptoms, and content of reported trauma (see **Table 1** for the specific hypotheses).

MATERIALS AND METHODS

Participants and Procedure

The study sample included data of 98 adult family members aged between 19 and 80 years ($M = 49.5$, $SD = 15.0$). Of them, 67 participants were first-degree relatives (20 fathers, 46 mothers, and 1 stepfather) and 31 participants were second-degree family members (6 brothers, 19 sisters, 1 grandfather, and 5 grandmothers) of a patient with PWS. These participants were the relatives of 69 PWS patients between the ages of 0 and 58 years ($M = 20.4$, $SD = 13.6$; 50% females). All participants were Dutch speaking. **Table 2** presents additional information of both the family members and the PWS patients.

Participants were recruited via the websites of the Dutch Digital Center of Expertise PWS (provisioning public information by the cooperating PWS patient associations and designated PWS patient care organizations), and of the two Dutch patient associations PWS (the Prader-Willi Fund,

TABLE 1 | Hypotheses.

Research question	Hypothesis
(a)	Family members will experience PWS related events as a traumatic stressor and will suffer from higher levels of trauma symptoms than the general population, to an extent that they will meet the criteria of PTSD (DSM-5; American Psychiatric Association, 17).
(b)	Trauma symptom severity will be positively related to the experience of PWS-related trauma.
(c)	Trauma symptoms severity will be inversely correlated with experienced quality of life. This third hypothesis will be further investigated by studying the relationship between distinct clusters of trauma symptoms and domains of quality of life.
(d)	Relatives of PWS individuals aged 10 to 30 years will report higher degrees of PWS symptoms, more experienced PTSD symptoms, and lower quality of life than relatives of PWS individuals aged 0 to 9 years and PWS individuals aged older than 30 years.
(e)	Relationship between living situations and the occurrence (presence or absence) of trauma symptoms, and content of reported trauma (PWS-related or not) will be explored.

TABLE 2 | Characteristics of family members and individuals with PWS.

Variable	*n*	%
Family members		
Sex		
Female	69	70.4
Male	29	29.6
Nationality		
The Netherlands	88	89.8
Belgium	9	9.2
Switzerland	1	1.0
Living with the PWS patient		
Yes	49	50.0
No	49	50.0
Highest education completed with a diploma[a] (corrected to Verhage code)[b]		
No/special education (1)	0	-
Primary school (2)	1	1.0
Primary school and <2 years of low-level secondary school (3)	0	-
Low-level secondary school (4)	1	1.0
Average-level secondary school (5)	11	11.3
Average-level secondary school (5), High level secondary school (6)	21	21.7
High level secondary school (6)	44	45.3
University (7)	19	19.6
Paid job	66	67.3
Experienced PWS symptom severity over the last 2 weeks[c]		
0	2	2.3
1	6	9.3
2	5	5.8
3	6	7.0
4	6	7.0
5	13	15.1
6	8	9.3
7	6	7.0
8	19	22.1
9	12	14.0
10	3	3.5
Individuals with PWS		
Sex of the related PWS individual[d]		
Female	49	50.0
Male	49	50.0
Living setting of the related PWS individual[d]		
Care facility	38	38.8
No care facility	60	61.2

[a] n = 97. [b] Responses on question 1.4b of the Dutch version of the Lancashire Quality of Life Profile (16, 17). For the corrections for level of education, we used a seven-point scale ranging from 1 (primary school not completed) to 7 (academic degree) according to the Dutch educational system (18). This scale is comparable to the International Standard Classification of Education [UNESCO, (19)]. [c] n = 86. Scores on a eleven-point Likert scale (0 = no symptoms at all, 10 = symptoms to a very serious extent). [d] n = 69.

and the Prader-Willi Foundation). Further, invitation letters were deposited at the front desk for visitors of the Vincent van Gogh Centre of Excellence for Neuropsychiatry outpatient clinic, which provides shared care together with the Expertise Center for Adults with Rare Genetic Syndromes of the Erasmus University Medical Center. After the written provision of information about the research objectives and procedures, participants gave their written informed consent for voluntary and individual participation in the study. The study was performed in accordance with the Declaration of Helsinki and was approved by the Vincent van Gogh Institutional Review Board (Decision letter references: JE/hr/2020.012/; JE/hr/2021.004).

Questionnaires (provided with a reply envelope) were sent to the home addresses of the participants. Inclusion criteria were first degree (biological-, step-, and adoption parents) and second degree (biological siblings, step siblings, and grandparents) relationship with a PWS individual. Age criterion for those relatives was at least 18 years old, to legally ensure voluntary self-selection. Data about the related PWS individuals were obtained anonymously. As a convenience sample, data were collected in the period from September 2020 to July 2021.

Materials

Questionnaires were provisioned on hard copy forms and self-completed. The total completion time was (based on a prior try out) estimated at 60 to 75 min.

Demographic and PWS Information

Demographic data were collected partly by the Dutch version of the Lancashire Quality of Life Profile (LQoLP) (16, 17), and additionally by a ten-items questionnaire designed for this study. This form records participants' family relationship to and information of the PWS patient, such as chronological age and living setting. The latter evaluates the experienced severity of the PWS symptoms by relatives in the previous 2 weeks on a eleven-point Likert scale from 0 (*no symptoms at all*) to 10 (*symptoms to a very serious extent)*.

Trauma

To measure trauma symptoms according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-5; (20)) the Dutch versions of the Posttraumatic Stress Disorder Checklist for *DSM-5* (PCL-5-NL, (21, 22)) and the Life Events Checklist for *DSM-5* with extended A-criterion (LEC-5-NL, (21, 22)) were used. The first part of the LEC-5-NL screens for the occurrence of traumatic events in a participants' entire life. It consists of 17 items; assessing exposure to 16 major and/or stressful PTSD relevant events (e.g., natural disaster, sexual assault, life-threatening illness or injury) and one open item for reporting any other extraordinarily stressful event or experience that is not in the list. The second part comprises 9 items and checks the A criterion of PTSD (exposure to actual or threatened death, serious injury or sexual violence). If participants check for anything on the open item of part 1, first identification of that event is asked for (question A) in part 2. Subsequently, if more than one of the events are reported in part 1, a brief description of the currently worst event is asked for (question B) followed by the 7 remaining questions regarding that event of part 2. The third part is covered by the PCL-5-NL (see **Supplementary Material**).

The PCL-5 assesses the number and severity of PTSD symptoms in the past month, while keeping the worst event in mind, i.e., intrusion symptoms (cluster B; items 1–5), avoidance symptoms (cluster C; items 6 and 7), negative alterations in cognitions and mood (cluster D; items 8–14), and negative alterations in arousal and reactivity (cluster E; items 15-20). Answers on these 20 items have to be filled out on a five-point Likert scale from 0 (*not at all*) to 4 (*extremely*); reflecting symptom occurrence and severity. The score per cluster varies between 0 and 28 (cluster B: 0–20; cluster C: 0–8; cluster D: 0–28; cluster E: 0–24). Mean PTSD symptom cluster scores were calculated as subtotals of the corresponding items. Items can be summed to provide a measure for overall symptom severity (range 0 to 80). A cut-off score between 31 and 33 is considered to be indicative of probable PTSD across samples, and suggests that the patient may benefit from PTSD treatment. A cut-off point of 33 represents a good predictor of a PTSD diagnosis (23). The reliability and validity of the PCL-5 are considered to be strong (24). The LEC-5 has adequate psychometric properties as well (25).

Quality of Life

Quality of life was assessed using the Dutch abbreviated version of the World Health Organization Quality of Life Questionnaire (WHOQOL-BREF-NL) (26, 27). This is a self-report questionnaire containing 26 items. The first two items evaluate the subjective overall quality of life and general health, respectively. From the other 24 items seven cover the quality of life domain physical health, six psychological functioning, three social relationships, and eight environment. Answers have to be given on a five-point Likert scale from 1 (*very poor*) to 5 (*extremely*). A higher score corresponds to a better quality of life. The quality of life domain scores were calculated as means of the underlying items. WHOQOL-BREF reliability has been rated as "good" to "excellent" and validity as "good" (28).

To also gain insight into particular objective aspects of quality of life, also, the extensive Dutch version of the Lancashire Quality of Life Profile was used [LQoLP; (16, 17)]. The LQoLP consists of 126 items and distinguishes the following nine different domains of quality of life: Work and education, Leisure and participation, Religion, Finances, Living situation, Legal status and safety, Family relations, Social relations, and Health. Each domain comprises both subjective and objective questions. In this paper, only the objective items are used, which must be answered categorically (*yes/no*). Internal consistency, test-retest reliability and validity of the LQoLP are considered to be good (17).

Statistical Analyses

IBM SPSS version 27 for Windows was used for all statistical analyses. Due to missing responses, the number of participants differs across the various analyses.

First, life time prevalence of traumatic events was calculated based on Part 1 of the LEC-5-NL. To this end, the number of participants who had ticked one or more of the 17 options for experienced traumatic events were summated. Subsequently, the proportion of participants who reported PWS-related issues (e.g., traumatic birth, aggressive behavior, psychosis) as a traumatic life event on the forms was calculated (question A of Part 2 on the LEC-5-NL). Also, the proportion of subjects who considered the PWS-related event as currently the worst was established (question B of Part 2 on the LEC-5-NL). In addition, the proportion of subjects with a Total PTSD symptom on the PCL-5-NL at the cut-off point (33 and higher) was calculated.

Secondly, two groups were formed based on the content of the reported traumatic experiences on the LEC-5-NL: PWS-related trauma vs. other trauma. Subsequently, a two-tailed *t*-test for independent variables was performed with nature of trauma as (categorical) independent variable, and Total PTSD symptom score and the four PTSD symptom-cluster scores on the PCL-5-NL as dependent variables.

Thirdly, a Pearson's correlation coefficient was computed to assess the relationship between the Total PCL-5-NL score of trauma symptoms and the Total WHOQOL-BREF-NL score of the degree of quality of life in family members of patients with PWS. Additionally, the relationship between the four PTSD symptom clusters (B to E) on the PCL-5-NL and the four quality of life domains (physical health, psychological functioning, social relationships, and environment) on the WHOQOL-BREF-NL was analyzed. However, the PTSD symptom cluster scores are not assumed to be independent and are considered as non-categorial variables (range variables expressed as mean scores within the set of the real variable). Therefore, a GLM multivariate analysis of covariance (MANCOVA, Wilk's Lambda) test was performed with the distinct four mean PTSD symptom cluster scores as four covariates, and the distinct four mean quality of life domain scores as the linear combination of the dependent variables. A Bonferroni correction for multiple testing was applied to reduce the risk of a Type-1 error (29).

Fourthly, to investigate whether the perceived severity of PWS symptoms, experienced PTSD symptoms, and quality of life in family members differ between chronological ages of the PWS patient, subjects were divided into three age groups: family members of PWS individuals aged "0–9 years," "10–30 years," and "31 years and older." A one-way analyses of variance (ANOVA) with a *post-hoc* Bonferroni correction was performed, with perceived PWS symptom severity (item 10 on the short form), experienced PTSD symptoms (total score and four mean scores per trauma symptom cluster on the PCL-5-NL), and experienced quality of life (total score and four means scores per domain on the WHOQOL-BREF-NL) as dependent variables, and age group as independent variable.

Finally, a two level categorical variable was formed based on the occurrence of trauma symptoms on the PCL-5-NL: family members who did not score any trauma symptom (*absence*) an who scored one or more trauma symptoms (*presence*). Likewise, a variable was formed based on the reported content of trauma on part B of the LEC-5-NL (*PWS-related trauma, and other trauma*). Additionally, two categorical variables regarding the residential setting were selected ("Do you live together with the PWS patient?;" "Does your relative with PWS live in a care facility?" on the Demographic inventory; *no/yes*). Four chi-squared tests for independence were applied to analyze successively if occurrence of trauma symptoms and content of trauma were associated with the residential setting of the PWS

patients, and of the family members. *P*-values were calculated with Fisher's Exact Test.

RESULTS

Lifetime prevalence of traumatic events in family members of PWS patients was 92.9% ($n = 91$ out of 98). Seven participants (7.1%) reported no occurrence of any traumatic experience. Of those who reported traumatic events, 46.2% ($n = 42$) reported a PWS related experience as a traumatic event and 53.8% ($n = 49$) reported a traumatic experience of a different nature. Of the participants who reported a PWS-related traumatic event, 73.8% ($n = 31$) considered the PWS related event as currently the worst (currently bothering them the most). Furthermore, 11 of the 91 participants (12.1%) who reported traumatic events scored at or above the cut-off point of 33 on the PCL-5-NL, implicating that the severity of their current trauma symptoms probably indicates PTSD.

Participants who reported a PWS-related trauma scored significantly higher on both the total symptoms of PTSD of the PCL-5-NL, as well as on the four distinct clusters of PTSD symptoms of the PCL-5-NL compared to participants who experienced different, non PWS-related traumatic events. Results are displayed in **Table 3.**

There was a significant and substantial negative correlation between total PCL-5-NL score of trauma symptoms and the total WHOQOL-BREF-NL score of quality of life ($r_{(94)} = -0.57$, $p < 0.001$, $R^2 = 0.33$). There were significant differences in PTSD symptom clusters "cognition and mood" ($F_{(4,86)} = 5.589$, $p < 0.001$, $\eta_p^2 = 0.206$), and "hyperarousal and reactivity" ($F_{(4,86)} = 4.124$, $p = 0.004$, $\eta_p^2 = 0.161$). These two symptom clusters were further examined. Negative alterations in cognition and mood appeared to be inversely related with all four domains of quality of life (physical health, psychological, social relationships, and environment). Further, negative alterations in arousal and reactivity were negatively related to the quality of life domain physical health. These significant results are presented in **Table 4.**

TABLE 3 | T-test of independent variables on PTSD symptoms scores in PWS-related trauma vs. other trauma.

PTSD symptoms scores	PWS-related trauma		Other trauma		$t_{(91)}$	*p*-value	Cohen's *d*
	M	SD	M	SD			
Total	18.76	13.64	10.61	12.13	2.988	0.004**	12.853
Intrusion	0.90	0.90	0.49	0.71	2.404	0.019*	0.805
Avoidance	1.02	1.15	0.50	0.89	2.403	0.019*	1.017
Negative alterations in cognition and mood	0.88	0.74	0.52	0.71	2.379	0.020*	0.722
Negative alterations in arousal and reactivity	1.00	0.73	0.59	0.60	2.922	0.005**	0.666

*n = 91 (number of participants who experienced trauma during lifetime). Intrusion, Avoidance, Negative alterations in cognitions and mood, and Negative alterations in arousal and reactivity represent respectively the four distinct PTSD symptoms clusters B to E on the PCL-5-NL for DSM-5 (20). Mean PTSD symptom values are shown for the group who reported PWS-related trauma (n = 42) and the group who reported Other trauma (n = 49), as well as the results of the t test (assuming unequal variance) comparing the PTSD symptom scores between the two groups. *p < 0.05. **p < 0.01.*

TABLE 4 | Regression coefficients of associations between quality of life domains (WHOQOL-BREF-NL) and PTSD symptom clusters (PCL-5-NL) by MANCOVA statistics.

Variable	*B*	*SE*	*t*	*p*-value	95% CI	η_p^2
Negative alterations in cognitions and mood						
Quality of life domain						
Physical	−1.45	0.59	−2.46	0.016*	[−2.6,−0.3]	0.06
Psychological	−1.53	0.40	−3.81	0.000***	[−2.3,−0.7]	0.14
Social	−1.59	0.62	−2.57	0.012*	[−2.8,−0.4]	0.07
Environmental	−1.47	0.37	−3.99	0.000***	[−2.2,−0.7]	0.15
Negative alterations in arousal and reactivity						
Quality of life domain						
Physical	−1.79	0.59	−3.05	0.003**	[−3.0,−0.6]	0.10
Psychological	−0.48	0.40	−1.20	0.235	[−1.2, 0.3]	0.02
Social	−0.37	0.61	−0.60	0.552	[−1.6, 0.9]	0.00
Environmental	0.44	0.37	1.19	0.236	[−2.9, 1.2]	0.02

*n = 85. CI, confidence interval. Negative alterations in cognitions and mood, and Negative alterations in arousal and reactivity represent the PTSD Symptom Clusters D and E in the DSM-5 respectively (20). Cluster D symptoms comprise among others "having strong negative beliefs about yourself, other people, or the world;" "having strong negative feelings such as fear, horror, anger, guilt, or shame;" "loss of interest in activities that you used to enjoy;" "feeling distant or cut off from other people;" and "trouble experiencing positive feelings (for example, being unable to feel happiness or have loving feelings for people close to you)." Cluster E symptoms are for example "irritable behavior, angry outbursts, or acting aggressively," "being 'superalert' or watchful or on guard", "feeling jumpy or easily startled," "having difficulty concentrating," and "trouble falling or staying asleep."*p < 0.05. **p < 0.01. ***p < 0.001.*

Family members of PWS individuals reported more severe PWS symptoms in the PWS age groups "10–30 years" and "≥ 31 years," compared to PWS individuals aged 0–9 years. PTSD symptom scores among family members of patients aged 10–30 years were significantly higher than in the youngest age group, for the Total PTSD-score of the PCL-5-NL, as well as on its four trauma clusters (intrusion symptoms, avoidance symptoms, negative alterations in cognition and mood, and negative alterations in arousal and reactivity). The Total PTSD score and the level of arousal and reactivity symptoms were significantly lower in PWS individuals over 30 years than in those aged 10–30 years. The results are presented in **Table 5**. Quality of life did not differ between age groups.

A significant interaction was found between the occurrence of trauma symptoms in family members with PWS patients living in a care facility or not (χ^2 (1) = 6.02, $p = 0.021$). More often the absence of trauma symptoms (73%) than the presence (34%) was reported among relatives of PWS patients living in a care facility. Another significant interaction was found between the content of the experienced trauma (LEC-5-NL) and living setting of the family member (χ^2 (1) = 4.99, $p = 0.035$). Family members who reported a PWS-related traumatic event (64%) more often lived with their relative with PWS than family members who reported other traumatic events (41%).

DISCUSSION

This first study on trauma and distress in adult relatives of individuals with PWS shows an increased risk for traumatic experiences and greater vulnerability to PTSD. As expected, we have found higher life time prevalence of traumatic events in adult family members than in the general Dutch population (92.9 vs. 80.7%) (30). Of those who reported any traumatic event, almost half reported *PWS-related* events. Furthermore, the prevalence of probable PTSD in this study (12.1%) exceeded the general lifetime prevalence of PTSD (worldwide between 1.3 and 8.8%; (31)) and in the Netherlands (7.4%; (30)). These findings are in line with previous research which showed that a severe or chronic illness of a relative can be experienced as a traumatic stressor and has been associated with manifestations of PTSD symptomatology (32–34). In addition, as hypothesized, family members who reported *PWS-related* trauma scored higher on PTSD symptoms than participants who experienced a different, non PWS-related traumatic event. These findings should increase

TABLE 5 | Means, standard deviations, and one-way analyses of variance (ANOVA) between age of PWS patients on experienced PWS symptoms, and PTSD symptoms, in family members.

Measure	Age				ANOVA				
		M	*SD*	*n*	Effect	Mean difference	*F* ratio	*p*-value	η^2
Experienced PWS-symptom severity		5.85	2.72	86			6.112	0.003**	0.128
	Group 1	4.29	2.56	24	Group 2	−2.17		0.004**	
	Group 2	6.47	2.38	43	Group 3	0.04		1.000	
	Group 3	6.52	2.97	19	Group 1	2.13		0.025*	
Total PTSD-symptoms		13.81	13.33	96			6.542	0.002**	0.123
	Group 1	8.31	6.67	26	Group 2	−10.11		0.004**	
	Group 2	18.42	14.72	48	Group 3	8.14		0.042*	
	Group 3	10.27	13.33	22	Group 1	1.97		1.000	
Intrusion		0.64	0.82	96			4.903	0.009**	0.095
	Group 1	0.35	0.39	26	Group 2	−0.54		0.018*	
	Group 2	0.89	0.67	48	Group 3	0.46		0.081	
	Group 3	0.44	0.70	22	Group 1	0.08		1.00	
Avoidance		0.70	1.03	96			4.674	0.012*	0.091
	Group 1	0.35	0.68	26	Group 2	−0.66		0.022*	
	Group 2	1.01	1.22	48	Group 3	0.56		0.096	
	Group 3	0.46	0.71	22	Group 1	0.11		1.000	
Negative alterations in cognitions and mood		0.66	0.73	96			3.762	0.027*	0.075
	Group 1	0.40	0.42	26	Group 2	−0.46		0.031*	
	Group 2	0.85	0.79	48	Group 3	0.30		0.32	
	Group 3	0.55	0.80	22	Group 1	0.16		1.000	
Negative alterations in arousal and reactivity		0.76	0.69	96			6.030	0.003**	0.115
	Group 1	0.51	0.50	26	Group 2	−0.48		0.010**	
	Group 2	1.00	0.73	48	Group 3	0.44		0.031*	
	Group 3	0.55	0.65	22	Group 1	0.04		1.000	

Group 1 consists of family members of PWS patients aged 0 to 9 years. Group 2 consists of family members of PWS patients aged 10 to 30 years. Group 3 consists of family members of PWS patients aged 31 years and older. Intrusion, Avoidance, Negative alterations in cognitions and mood, and Negative alterations in arousal and reactivity represent the four respective PTSD symptom clusters B to E on the PCL-5-NL for DSM-5 (20). The mean difference is significant at the 0.05 level. $^{*}p < 0.05$. $^{**}p < 0.01$.

clinical awareness of signs of psychopathology, and result in a recommendation to systematically monitor the experience of traumatic events in relatives of patients with PWS.

As expected, there was a negative relation between trauma symptoms and quality of life; particularly with respect to negative alterations in arousal and reactivity (cluster E), and in negative alterations in cognition and mood (cluster D). Cluster E symptoms were specifically negatively correlated to physical quality of life. The physical aspects of (acute and chronic) stress (high adrenaline and/or cortisol levels, sleep issues) could contribute to this relationship. Cluster D symptoms were negatively and fairly evenly related to all quality of life domains, affecting the experienced physical health, psychological health, social relationships, and living environment simultaneously. These results encourage awareness of clinicians regarding reciprocal influences of quality of life and the forementioned particular trauma symptoms in family members of PWS individuals. Cure of PTSD symptoms in family members might be supported by care in different life domains.

Further, results showed that higher chronological age of the PWS individual was related to increased PWS symptom severity evaluated by the family members. Both the total trauma score as well as the respective scores in all different trauma symptom clusters (B-E) were significantly higher in family members of the PWS patient age group 10 to 30 years (adolescents and young adults), compared to the age group 0 to 9 years (childhood). These findings regarding chronological age, PWS symptom severity and PTSD symptom severity did not fully demonstrate the expected quadratic relationship, since the expected PWS- and trauma severity differences between the age groups 2 and 3 did not reach significance. A possible explanation could be admittedly lower degree of challenging behaviors in PWS patients in group 3 (35), but higher levels of physical problems in this PWS patient age group (36), which may also cause stress and psychological complaints in caregivers (37). Nevertheless, as expected, increasing age of the PWS patient (from 10 years and up) was related to both PWS symptom severity and PTSD symptom severity negatively. This finding endorses the importance of clinical attention to both advanced care planning for the individual PWS patient, and timely support for their family members.

Finally, living together with their PWS relatives increased the risk of trauma symptoms, also of PWS-related trauma. Follow up research could explore trauma related symptoms in family members in relation to the intensity of their involvement in daily care of an individual with PWS, and the experienced caregiver burden.

Several limitations can be identified in the current study that may limit the generalizability of the results. At least the following two have to be mentioned here. Firstly, while selection-bias is difficult to avoid in research on genetic developmental disorders, due to participant recruitment by volunteer sampling, an over-representation of family members who felt attracted to the research topic may have occurred. Secondly, the sampling period was during the COVID-19 pandemic, which may have had a pre-existent negative impact on the experienced quality of life and health (38). Nevertheless, this applies to all participants who participated and leaves aside the differences we have found within our study population. Notwithstanding these limitations, the strengths of the study concern the considerable sample size, and the wide age range of related PWS individuals (from infancy to late adulthood), enabling cross sectional analyses. Furthermore, participation concerned all adult family members, instead of parents only. Demographic data regarding participants' educational levels indicate a fairly distributed representation, but a tendency to the higher end. At the same time, a majority of female relatives (∼2/3) was involved in this study. Both aspects could both under- and over-estimate the results, since distress can be considered a product of a dynamic transaction between individuals (including cognitive, physiological, affective, psychological, neurological systems) and their complex environment; consistent with the transactional model of stress and coping (39).

The results presented in this study warrant further, preferably longitudinal, research. When compared to other rare genetic developmental disorders, such as Williams syndrome, Fragile X syndrome, or Smith-Magenis syndrome, parents of patients with PWS have shown higher levels of stress (40, 41). Future research could compare our results with those on trauma and quality of life in relatives of patients with other genetic developmental disorders.

CONCLUSION

Having a relative with PWS is associated with higher prevalence of experienced traumatic events, and of PTSD, affecting the wider family system. Raising awareness in health care professionals of the typical presentation of trauma symptoms in PWS relatives may contribute to effective treatment of their psychosocial stress. In addition, timely, transdisciplinary (medical-psycho-social) attention to all relatives might prevent them from developing psychopathology like PTSD.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be available from the corresponding author on reasonable request.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Vincent van Gogh Institutional Review Board (Decision letter references: JE/hr/2020.012/; JE/hr/2021.004). The participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AB-R, EW, LG, and JE designed and planned the study. AB-R and AB performed data collection and executed statistical analyses. AB-R drafted the manuscript. EW, LG, and JE revised and critically reviewed the manuscript for important content. All authors read and authorized the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2022.897138/full#supplementary-material

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Early Developmental Trajectories in Infants With Neurofibromatosis 1

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Objective: To examine the trajectories of cognitive, motor and behavioural development in infants with NF1 compared to infants without a family history of neurodevelopmental difficulties.

Study design: Infants with NF1 and low-risk controls were recruited from 5 months of age and followed longitudinally. Data from standardised tests was gathered at 5, 10 and 14 months and developmental trajectories of motor, language, behaviour, sleep, social development and parent–infant interaction were examined. Linear mixed modelling was used to estimate group differences in cognitive and behavioural measures over time.

Results: No group differences were observed on Mullen Scale of Early Learning, overall adaptive functioning, temperament or behavioural measures. There were no group differences observed on measures of social communication or parent–infant interaction. Over the course of development, the NF1 group slept less and took more time to settle to sleep as compared to the control group. Maternal education was significantly associated with cognitive and behavioural developmental outcomes in both groups.

Conclusion: Cognitive, social and behavioural impairments are a cause of significant functional morbidity in children with NF1. This report is the first study to investigate the trajectories of cognitive, motor and behavioural development in infancy in NF1. Our results demonstrate that overall cognitive and behavioural developmental trajectories of the NF1 group in the infancy period are similar to controls. Given previous reports of delayed development in the NF1 cohort by 40 months, early clinical interventions strategies to promote sleep hygiene may be beneficial to optimise developmental outcomes.

Keywords: NF1 = neurofibromatosis type 1, autism, natural history, cognition, behaviour

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Abbreviations: NF1, neurofibromatosis type 1; ASD, autism spectrum disorder; ADHD, attention deficit hyperactivity disorder; Mullen, Mullen Scales of Early Learning; Vineland, Vineland Adaptive Behaviour Scales; IBQ, Infant Behaviour Questionnaire; ITSP, Infant-Toddler Sensory Profile; AOSI, Autism Observational Scale for Infants; SSQ, Sleep and Settle Questionnaire; MACI, The Manchester Assessment of Caregiver–Infant Interaction; MCDI, MacArthur-Bates Communication Development Inventory.

INTRODUCTION

Neurofibromatosis 1 is a common autosomal dominant single gene neurodevelopmental disorder, with birth incidence of 1:2700 (Evans et al., 2010). Approximately 50% of the cases are inherited and the rest are caused by a *de novo* mutation of the NF1 gene on chromosome 17q11.2, which has an important role in intracellular signalling, learning and synaptic plasticity (Costa et al., 2002). Diagnosis of NF1 may be established if two out the seven National Institute of Health (NIH) defined clinical criteria are met; these include (i) presence of ≥6 café-au-lait macules, (ii) ≥2 neurofibromas or ≥, (iii) freckling in the inguinal or axillary regions, (iv) optic pathway glioma, (v) ≥2 Lisch nodules, (vi) a distinctive osseous lesion, and (vii) a first- degree relative with NF1. In families with history of NF1, genetic tests on umbilical cord blood may be used to diagnose NF1 early in infancy. Whilst the disorder is known for its cutaneous manifestations, substantial morbidity in the paediatric population is due to cognitive, social and behavioural impairments. Specific learning impairments are common, although overall cognitive ability or IQ is in the low average range, with approximately 5–10% of individuals in the learning disability range (Lehtonen et al., 2015). Studies suggest a high prevalence of both ASD of approximately 25% and ADHD of about 50% (Garg et al., 2013).

Our understanding of the emergence of behavioural phenotype in NF1 is limited. Much of what is known is drawn from cross-sectional studies in school-age children with only handful of studies in the preschool population (Brei et al., 2014; Klein-Tasman et al., 2014; Lorenzo et al., 2015). These studies suggest that the NF1 gene mutation confers a general vulnerability for cognitive, motor and language difficulties that is observable in the preschool period. In a study of 40 children with NF1, aged 3–6 years compared to matched controls, impairment in at least one area (verbal, non-verbal or spatial skills) was found in 45% of the sample (Klein-Tasman et al., 2014). Similarly, a cross-sectional study of 39 toddlers aged 21–30 months with NF1 found poorer cognitive, motor and language development in the NF1 group compared to age matched controls. Mean cognitive development in the NF1 group was one standard deviation below controls, with a third of the NF1 cohort with low average motor development and parental responses indicated delayed receptive and expressive language development in over 70% (Lorenzo et al., 2011). Brei et al. (2014) assessed language abilities in 30 children with NF1 aged 4–6 years and found impairments in core language skills in a third of the sample, which were not fully accounted for by attentional impairments (Brei et al., 2014).

Whilst these cross-sectional studies suggest a vulnerability to cognitive impairment in the preschool period, little is known about the developmental trajectories of these behavioural phenotypes. Understanding the natural history of development in NF1 is important for several reasons. Longitudinal studies can offer insights into brain development and identify early predictors of later neurodevelopmental outcome. Further, neurodevelopmental disorders comorbid with NF1 such as ASD and ADHD, which are diagnosed based on behavioural assessments in school-age children likely emerge through a complex developmental cascade of interactions between the gene, brain, behaviour and the child's interaction with the environment. Early interventions targeted in the prodromal period (before behavioural symptoms of ASD and ADHD emerge) could ameliorate the later emergence of behavioural symptoms (Green et al., 2017). Targeted pharmacological treatments reverse the NF1 associated cognitive impairments in animal models but translational clinical trials in humans have so far had mixed results (van der Vaart et al., 2013; Stivaros et al., 2018). Understanding the developmental trajectories in NF1 will allow identification of treatment-sensitive early markers as surrogate endpoints in treatment trials. Lastly, diagnosis and intervention approaches in NF1 are complicated by the inter- and intra-familial variability in phenotypic expression (Sabbagh et al., 2009). Mapping individual developmental trajectories of development may provide insight into causal mechanisms and the role of modifying genes in phenotypic expression in NF1.

Two preliminary studies have examined trajectories of symptoms in NF1 in toddlerhood. In a longitudinal study of 39 children with NF1 and matched control assessed at 21, 30 and 40 months, the trajectory of cognitive development in NF1 diverged from the control groups over time, and early productive vocabulary was a significant predictor of later language skills (Lorenzo et al., 2015). Using parental reports of developmental progression, Wessel et al. (2013) reported that children aged 0–8 years with NF1 shifted between delays and typical performance in all areas of cognitive functioning, and showed persistent gross motor function delays from toddlerhood (Wessel et al., 2013). Both studies illustrate cognitive differences in NF1 emerge as early as the second year of life, but little is known about how these trajectories evolve in infancy. The aim of the present study was to examine the natural history of cognitive, motor and behavioural development in infants with NF1 compared with a group of low-risk infants with no familial risk of neurodevelopmental difficulties. Our primary objective was to determine how NF1 genetic variance might manifest in early development including in cognitive, social and behavioural development. A preliminary case series (Kolesnik et al., 2017) published from a subset of the current sample of infants at 10 months indicated early emerging differences in language and motor skills, confirming the importance of studying developmental abilities in the first year of life. Here we examine longitudinal trajectories of development from 5 to 14 months in a larger sample of infants, compared to low-risk controls. Based on previous studies, we expected that the infants with NF1 would have lower motor and early language development as compared to the control group.

MATERIALS AND METHODS

The Early DEvelopment in NF1 (EDEN) study is a prospective longitudinal study of infants with NF1. Participants were recruited via regional genetic centres and national NF charities in the United Kingdom. Inclusion criteria for the NF1 group included (i) infant <12 months at time of recruitment, (ii) confirmed diagnosis of NF1 via molecular testing of cord blood samples or clinical diagnosis based on NIH consensus

criteria (National Institutes of Health Consensus Development Conference, 1988). Inclusion criteria for the control group were (i) infants <12 months with no first-degree relatives with a diagnosis of ASD or ADHD or known genetic disorders; (ii) no developmental concerns reported by parents; (iii) full-term birth (gestational age greater than 36 weeks). Participants in the control group were recruited from a volunteer database at the Centre for Brain and Cognitive Development, Birkbeck, University of London (STAARS study). Exclusion criteria for both groups included (i) conditions that may make harder for an infant to participate (including any serious physical complications due to NF1 as judged by the referring clinician), (ii) significant vision or hearing abnormalities, (iii) significant prematurity, (iv) parents with evidence of significant learning difficulties or who are unable to give informed consent. The NF1 and the control groups over a 4-year period between 2016 and 2019. The sample size calculation was based on our previous studies comparing infants at high familial risk for ASD to controls [e.g., $n = 17$ (Elsabbagh et al., 2013), $\eta^2 = 0.17$; $n = 19$ (Elsabbagh et al., 2009), $\eta^2 = 0.16$] and was based on detecting differences on EEG biomarkers rather than behavioural measures as reported in this study.

Procedure

The study assessment took place at the Centre for Brain and Cognitive Development, Birkbeck, University of London. Written informed consent was provided by the parent prior to the commencement of the study. The testing only took place if the infant was in a content and alert state. Participant families were reimbursed expenses for travel, subsistence and overnight stay if required. Behavioural measures described below were administered as part of a more extensive experimental protocol.

Measures

Cognitive ability at 5, 10 and 14 months was assessed through the *Mullen Scales of Early Learning* (MSEL), an observational measure that assesses gross and fine motor skills, expressive and receptive language, and visual reception; analyses used raw scores for each domain (Mullen, 1995). Adaptive skills were assessed at 5, 10 and 14 months using the *Vineland Adaptive Behaviour Scale* (VABS), a parent-report measure that assesses socialisation, communication, motor behaviour and daily living skills and provides an overall composite score referred to as Adaptive Behaviour Composite (ABC); analyses used standard scores per domain (Sparrow, 2011). Language development at 10 and 14 months was assessed using parent reported MCDI (Fenson et al., 2007). The questionnaire is comprised of approximately 800 items, summed to produce a receptive and expressive vocabulary score. The resulting score was log transformed to base 10 for analyses. Autism symptoms were assessed using the AOSI, a 19-item interactive observation schedule designed to capture early signs of ASD and measures aspects of visual attention, social-communication, development of sensory and motor skills. Absence/presence of behaviours is rated 0–3, where 0 signifies typical function, and higher values suggests increasing deviation from the typical behaviour expected at the age of assessment (Bryson et al., 2008). Temperament was assessed at 5, 10 and 14 months using IBQ a widely used parent-report measure comprised of 14 subscales, grouped into three factors: Surgency (child's tendency to show excitement, positive affect and approach), Negative Affect (tendency to cry, be avoidant or otherwise fussy), and Regulation Capacity (ability to regulate their mood and behaviour) (Putnam et al., 2014). The SSQ (Matthey, 2001) was used to assess parental perception of infant sleep patterns. Parental reports of total sleep duration, number of night awakenings and time taken to settle (in minutes) are reported in this study. Parent–infant interaction was assessed at the 10- and 14-month visits using the MACI (Wan et al., 2017). A video-recorded caregiver–infant free play interaction session with toys was coded by two independent raters, blinded to family information. The coding scheme comprised of eight 7-point scales, from which we focused on four scales of areas of parent–infant interaction that were affected among infants with familial likelihood of ASD (Wan et al., 2013): caregiver sensitivity, caregiver non-directiveness, infant attentiveness to caregiver, and dyad mutuality (the amount and degree of dyadic reciprocity, closeness and sharedness). Maternal education was classified as primary, secondary, undergraduate or postgraduate. Further details of this measure and inter-rater reliability are provided in the **Supplementary Materials**.

Statistical Analyses

Statistical analyses were performed using R version 3.6 (R Core Team, 2019). Linear mixed modelling was used to estimate change in the cognitive and behavioural measures across time using the 'nlme' package. For each outcome, the overall group differences were modelled using fixed effects of predictors (group, participant age in days at time of assessment and maternal education) and random effect of individual variation. Because of the sample size, predictors and interactions were limited and models were of the general form: *Model <- lme (outcome ~ age*group + maternal education, random = 1| ID)*. Maternal education was included as a predictor as it is strongly associated with cognitive development (Jackson et al., 2017). The control group were treated as baseline and parameters were estimated for the NF1 group. Missing data were handled with the maximum likelihood approach.

A p-value of <0.05 was considered significant. Removal of maternal education from the models did not alter overall results.

RESULTS

Thirty-three infants with NF1 and 29 typically developing infants were enrolled. Due to flexible enrolment and variability in compliance, the sample size at each time point varied as shown in **Figure 1**.

At the 5-month assessment, the NF1 group was significantly older than the control group ($t = -3.09$, $p = 0.004$) but there were no significant age differences between the groups at 10- or 14-month assessments. There was a significant difference in maternal education with mothers in the control group more likely to have a graduate or post graduate education (Median NF1: 2, Control: 4 $\chi^2 = 24.78$, $p < 0.001$). There was no significant sex difference between the groups at any time point. Within the NF1 group, NF1

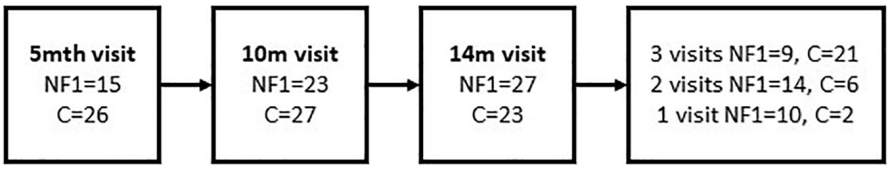

FIGURE 1 | Flowchart showing the number of NF1 and Control (C) infant participants at each time-point in the study.

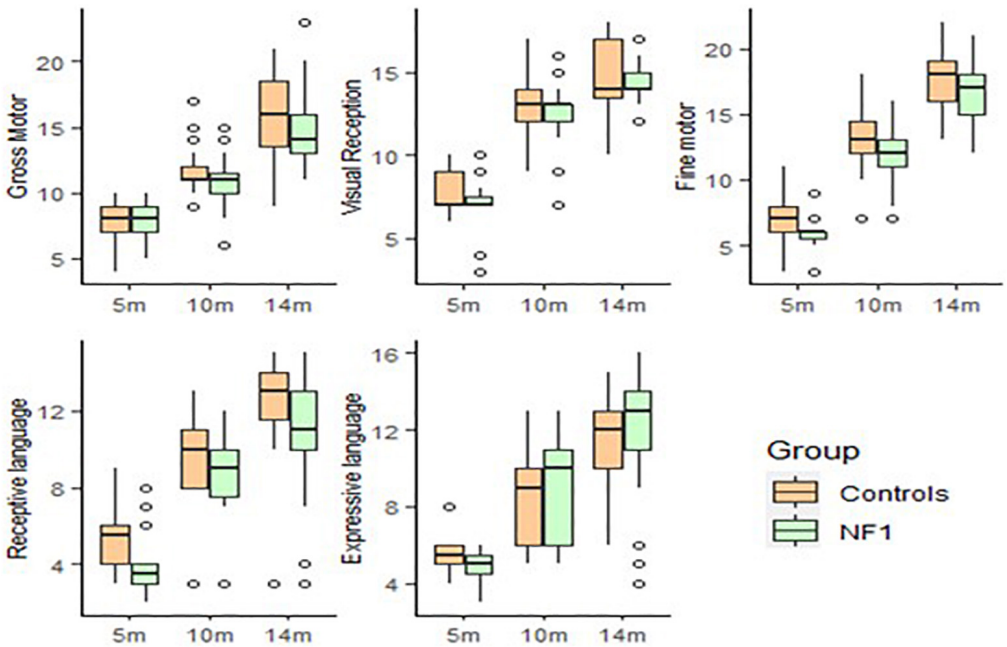

FIGURE 2 | Box-whisker plots of the NF1 and control groups on the Mullen Scale of Early Learning.

was inherited in 30 participants; *de novo* in 2 participants and inheritance was unknown in 1 participant. Further details of the demographic, clinical characteristics are provided in **Table 1**.

Trajectories of Cognitive, Motor and Behavioural Development

Cognitive skills (Mullen) improved significantly with age in both groups [$t(72) = 16.31$, $p < 0.001$]. There were no intercept differences observed between the NF1 and control groups in Gross Motor [$t(53) = 0.34$, $p = 0.73$], Visual Reception [$t(53) = -0.60$, $p = 0.55$], Fine Motor [$t(53) = -1.02$, $p = 0.31$], Receptive Language [$t(52) = -0.52$, $p = 0.60$] or Expressive Language [$t(53) = -0.75$, $p = 0.46$]. No group differences were observed in developmental trajectories of Gross Motor [$t(72) = -1.06$, $p = 0.29$], Visual Reception [$t(72) = 0.64$, $p = 0.52$], Fine Motor [$t(72) = 0.30$, $p = 0.77$], Receptive[$t(72) = -0.45$, $p = 0.66$] or Expressive Language [$t(72) = 0.97$, $p = 0.34$]. Higher maternal education was associated with higher scores only on Visual Reception [$t(72) = 2.56$, $p = 0.01$] and Fine Motor [$t(72) = 2.69$, $p = 0.01$] skills (**Figure 2**).

For adaptive functioning (Vineland), there were no significant group differences at intercept in Communication [$t(51) = 1.58$, $p = 0.11$], Daily Living skills [$t(51) = 0.18$, $p = 0.85$], Socialisation [$t(50) = -0.76$, $p = 0.45$], Motor skills [$t(51) = -0.20$, $p = 0.84$] and overall Adaptive function [$t(50) = 0.83$, $p = 0.41$]. No significant group differences emerged with age in Communication [$t(58) = -1.51$, $p = 0.14$], Daily Living skills [$t(57) = -0.27$, $p = 0.79$], Socialisation [$t(57) = -0.16$, $p = 0.87$], Motor skills [$t(57) = -0.79$, $p = 0.43$] or overall Adaptive function [$t(51) = -0.86$, $p = 0.39$]. Higher maternal education was related to better Communication [$t(58) = 2.53$, $p = 0.01$], Socialisation skills [$t(57) = 1.99$, $p = 0.05$], Motor skills [$t(57) = 2.02$, $p = 0.05$] and Adaptive function [$t(51) = 2.53$, $p = 0.01$] but not with Daily Living skills [$t(57) = 0.68$, $p = 0.50$].

Significant increase in Receptive [$t(28) = 5.30$, $p < 0.001$] and Expressive vocabulary [$t(29) = 4.35$, $p < 0.001$] as measured with the MCDI was observed with age. No group differences were observed at intercept in Receptive vocabulary [$t(47) = 1.54$, $p = 0.13$] or Expressive vocabulary [$t(47) = 1.04$, $p = 0.30$]. Similarly no significant group differences were observed in developmental trajectories of Receptive vocabulary [$t(28) = -1.65$, $p = 0.11$] or Expressive vocabulary [$t(29) = -1.05$, $p = 0.30$]. There was no effect of maternal education (**Figures 3, 4**).

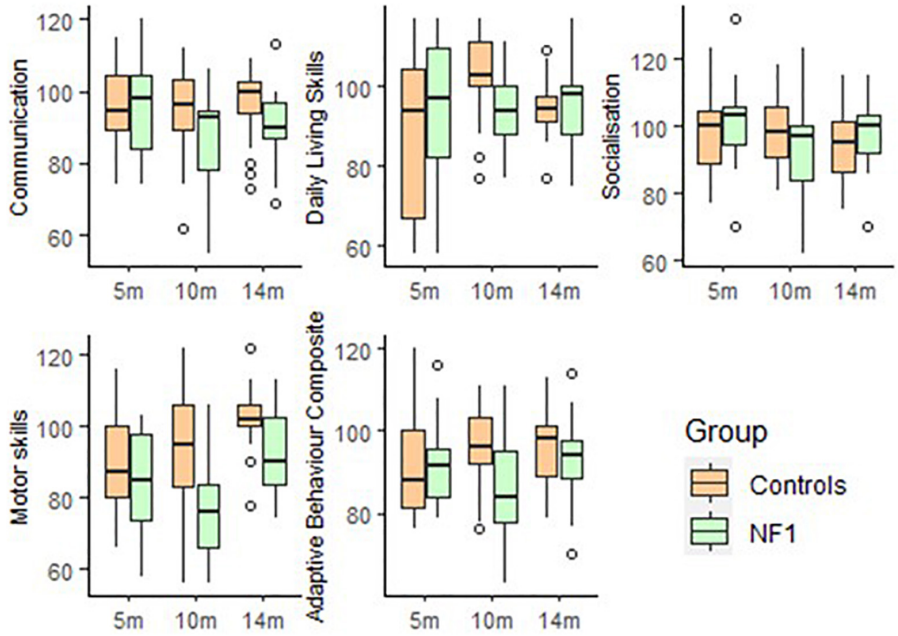

FIGURE 3 | Box-whisker plots of the NF1 and control groups on the Vineland Adaptive Behaviour Scale.

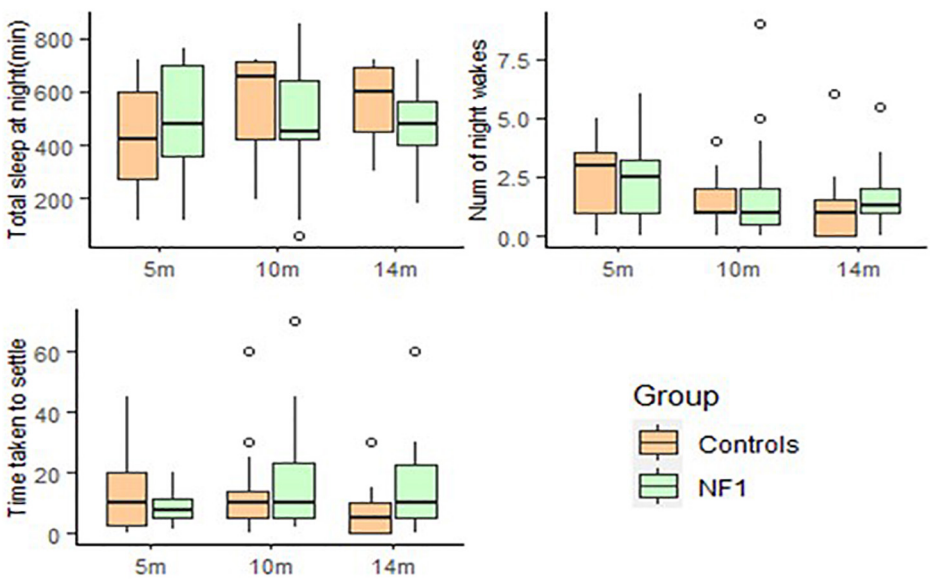

FIGURE 4 | Box-whisker plots for the NF1 and control group on the Sleep and Settle Questionnaire.

For temperament (IBQ), there was a significant increase in Surgency [$t(53) = 6.52$, $p < 0.001$], decrease in Regulation Capacity[$t(56) = -2.19$, $p = 0.03$] with age but no significant change in Negative Affect [$t(57) = 1.56$, $p = 0.12$]. No significant group intercept differences were observed for Surgency [$t(48) = 1.12$, $p = 0.27$], Negative Affect [$t(50) = -0.63$, $p = 0.53$], or Regulation Capacity [$t(50) = 0.80$, $p = 0.43$]. Similarly group trajectories showed no significant differences in Surgency [$t(53) = -1.40$, $p = 0.17$], Negative Affect [$t(57) = 0.49$, $p = 0.62$], Regulation Capacity [$t(56) = -0.05$, $p = 0.96$]. Higher maternal education was associated with better Regulation Capacity [$t(56) = 2.46$, $p = 0.02$] but not with Surgency [$t(53) = -0.28$, $p = 0.78$] or Negative Affect [$t(57) = 0.49$, $p = 0.08$]. No significant group differences were observed on the AOSI total scores at 14 months [$t(37) = -0.09$, $p = 0.93$]. Higher maternal education had a marginally significant association with lower AOSI scores [$t(37) = -1.97$, $p = 0.06$].

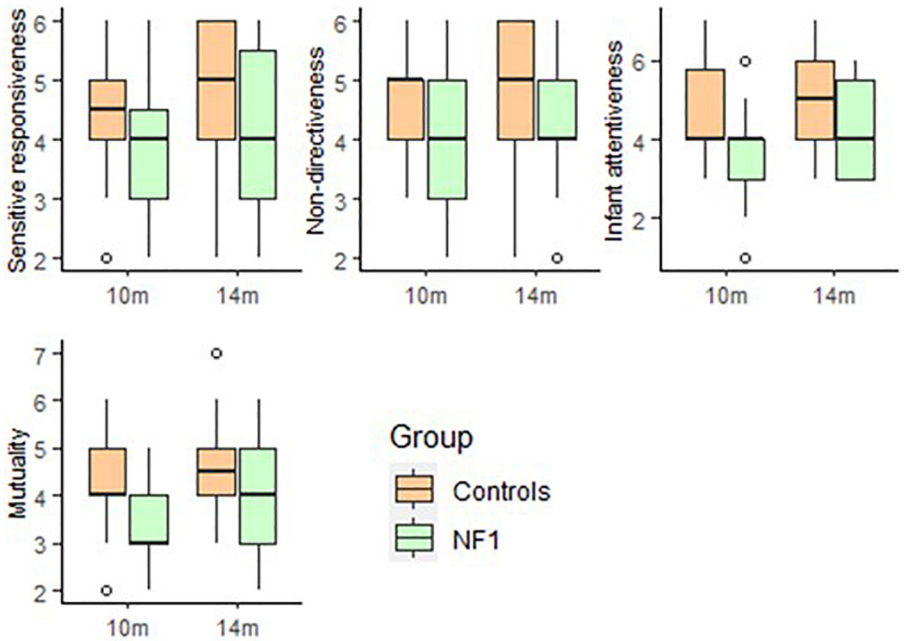

FIGURE 5 | Box-whisker plots for the NF1 and control group on the Manchester Assessment of Caregiver–Infant Interaction (MACI).

The total amount of sleep at night [$t(57) = 2.97$, $p = 0.004$] increased with age, whilst the number of night awakenings [$t(59) = -3.45$, $p = 0.001$] and time taken to settle [$t(56) = -2.35$, $p = 0.02$] reduced with age. Total amount of sleep at night was marginally higher for NF1 group at intercept [$t(49) = 1.94$, $p = 0.06$] but significant group differences emerged over development with the NF1 group sleeping less than the control group [$t(57) = -2.09$, $p = 0.04$]. There were no group differences in the number of night awakenings at intercept [$t(50) = -0.59$, $p = 0.55$] or over development [$t(59) = 0.55$, $p = 0.58$]. No group differences were observed in the time taken to settle at intercept [$t(49) = -1.49$, $p = 0.14$] but the NF1 group took more time to settle over development [$t(56) = 2.17$, $p = 0.03$]. Infants with higher maternal education slept more at night [$t(57) = 2.40$, $p = 0.02$] but there was no effect of maternal education on the number of night awakenings [$t(59) = -1.61$, $p = 0.11$] or the time taken to settle [$t(56) = -0.54$, $p = 0.59$].

For parent–child interaction (MACI) there were no significant group differences at intercept in parent Sensitive Responsiveness [$t(46) = -1.42$, $p = 0.16$] or Non-Directiveness [$t(46) = -0.61$, $p = 0.55$] but a trend of lower Infant Attentiveness [$t(45) = -1.76$, $p = 0.08$] and Mutuality [$t(45) = -1.82$, $p = 0.07$] was seen in the NF1 group. No significant group differences were observed over development in Sensitive Responsiveness [$t(23) = 1.16$, $p = 0.26$], Non-Directiveness [$t(23) = 0.33$, $p = 0.74$], Infant Attentiveness [$t(23) = 1.34$, $p = 0.19$] or Mutuality [$t(23) = 1.27$, $p = 0.21$]. There was no significant effect of maternal education on any of the MACI subscales (**Figure 5**).

The linear mixed modelling parameters are summarised in the online **Supplementary Materials**.

DISCUSSION

In this prospective longitudinal study, we describe the natural history of cognitive and behavioural development during infancy in NF1 infants, as compared to infants with no family history of neurodevelopmental disorders. To our knowledge, this is the first study to report developmental trajectories and examine the quality of parent–infant interaction in infants with NF1. Our results demonstrate that overall cognitive, motor and behavioural developmental trajectories of the NF1 group in the infancy period are similar to controls. Over the course of development, sleep difficulties in the NF1 cohort, including taking longer to settle to sleep and overall reduced sleep were noted. This data adds to the current body of literature by extending it to the infancy period and including prospective longitudinal assessments of infants with NF1.

In a previous prospective longitudinal study of NF1 children aged 21–40 months, Lorenzo et al. (2015) found diverging trajectories of cognitive development over time with lower scores in the NF1 group as compared to controls (Lorenzo et al., 2015). Cross-sectional studies confirm significant cognitive differences between the NF1 and control groups in the pre-school years, suggesting children with NF1 are at a significant disadvantage when they start school (Klein-Tasman et al., 2014). Contrary to findings described by these earlier studies, our results indicate that cognitive and motor function in the NF1 group in the infancy period are similar to controls, with subtle early differences emerging only in sleep functioning. We observed a trend of lower motor function at 10 and 14 months and parent–infant interactive differences mainly at 10 months in the NF1 group. Whilst behavioural differences were not prominent in

TABLE 1 | Descriptive statistics including means and SDs for the NF1 and control group at the three assessment time points.

	5 months				10 months				14 months			
	Control group		NF1		Control group		NF1		Control group		NF1	
	n	*Mean (SD)*	*n*	*Mean(SD)*	*n*	*Mean(SD)*	*n*	*Mean(SD)*	*n*	*Mean(SD)*	*n*	*Mean(SD)*
Age (days)	26	179.19(14.05)	15	194.73(17.85)	27	321.93(16.70)	23	327.0(17.11)	23	447.61(18.42)	27	449.74(23.41)
Male gender	26	18	15	8	27	16	23	12	23	13	27	13
Maternal education (*n*) (secondary/undergrad/postgrad)	24	2/9/13	11	7/2/2	26	2/10/14	22	14/5/3	23	2/7/14	25	14/8/3
Mullen Scale of Early Learning T scores												
Gross motor	26	43.69(9.41)	15	37.40(9.73)	27	34.89(11.78)	23	30.0(10.25)	23	36.74(13.37)	27	31.96(12.75)
Visual reception	26	47.27(6.33)	15	37.47(11.53)	27	48.85(7.99)	23	43.96(8.08)	23	35.09(8.88)	27	33.67(5.17)
Fine motor	26	42.92(10.46)	15	32.80(6.99)	27	51.63(12.88)	23	42.13(11.29)	23	49.65(12.18)	27	43.85(11.07)
Receptive language	26	36.88(11.28)	14	25.71(9.31)	27	39.26(8.96)	23	34.87(7.73)	23	32.87(6.50)	27	28.22(6.01)
Expressive language	26	41.85(7.45)	15	35.13(6.35)	27	36.85(9.89)	23	39.95(12.57)	23	37.09(8.80)	27	38.19(10.18)
Vineland Adaptive Behaviour Scale												
Communication	22	94.95(10.91)	14	95.71(18.26)	21	92.86(12.76)	23	86.13(14.91)	19	96.11(10.49)	24	91.04(10.08)
Daily living skills	22	86.32(19.86)	14	92.71(18.26)	22	102.45(10.76)	23	93.74(9.14)	18	93.44(8.39)	23	95.52(10.85)
Socialisation	23	98.70(12.26)	14	101.36(14.21)	22	98.77(10.04)	21	92.76(14.59)	18	94.22(9.79)	23	97.96(9.43)
Motor	21	88.67(12.99)	14	85.07(13.37)	21	95.76(16.86)	23	76.91(14.86)	18	101.67(9.49)	24	93.42(11.13)
Adaptive behaviour composite	19	90.63(11.92)	14	92.29(11.13)	20	96.80(10.44)	21	85.86(12.85)	17	95.29(9.73)	23	95.29(9.36)
MacArthur Communication Inventory												
Receptive vocabulary					21	15.38(15.52)	22	36.05(48.97)	19[a]	77.47(68.37)	22[a]	74.64(72.61)
Expressive vocabulary					21	0.33(0.97)	22	1.18(1.99)	19[b]	10.53(13.09)	23	7.96(10.52)
Infant Behaviour Questionnaire												
Surgency	17	4.16(0.82)	10	4.42(0.77)	22	4.80(0.46)	21	4.83(0.72)	19	5.18(0.60)	25	4.96(0.59)
Negative affectivity	21	3.17(0.72)	14	2.96(0.68)	21	3.50(0.66)	21	3.73(1.08)	18	3.43(0.66)	26	3.7(1.00)
Regulation capacity	19	5.09(0.51)	13	5.08(0.67)	20	4.76(0.47)	22	4.80(0.89)	19	4.80(0.67)	26	4.79(0.76)
Sleep and Settle Questionnaire												
Sleep at night (Min)	25	454.80(194.55)	11	480.0(228.57)	22	555.5(185.86)	22	479.32(212.86)	19	570.26(136.14)	22	469.77(149.24)
Number of night wakes	25	2.5 (1.45)	12	2.62(1.85)	22	1.32 (1.20)	23	1.72(2.09)	19	1.11(1.50)	24	1.60(1.34)
Time taken to settle	24	15.81(24.79)	12	23.20(49.63)	22	11.79(13.36)	20	17.47(16.76)	19	8.40(10.60)	23	16.74(16.88)
Total confidence	25	9.36(1.36)	13	9.23(1.36)	22	9.09(1.27)	23	9.00(1.35)	19	9.53(0.70)	25	9.04(1.17)
AOSI total score									18	10 (5.13)	24	12.13(4.88)
MACI												
Sensitive responsiveness					22	4.50(0.96)	19	3.68(1.16)	16	4.75(1.18)	19	4.21(1.08)
Non-directiveness					22	4.59(0.96)	19	4.0(1.11)	16	4.88(1.20)	19	4.21(1.08)
Infant attentiveness					22	4.55(1.22)	19	3.58(1.17)	16	4.94(1.06)	19	4.37(1.21)
Mutuality					22	4.36(0.95)	19	3.37(0.83)	16	4.63(0.96)	19	4.05(1.31)

The number of participant data available for each measure is indicated.
[a]*One data-point each in NF1 and control group removed as >4 SD above mean.*
[b]*One data-point in control group removed as >4 SD above mean.*

this infancy period, our previous work in this cohort of infants found atypical low-level auditory processing in the NF1 group as compared to controls (Begum-Ali et al., 2021). Using an auditory habituation paradigm, we found that although infants with NF1 were able to discriminate between different auditory stimuli, there were developmental differences in the pattern of neural responses to auditory stimuli in the NF1 group as compared to controls, suggestive of auditory processing delays. We could speculate therefore that the NF1 mutations may affect low-level cognitive processes with cascading and cumulative effects on development over time (Karmiloff-Smith, 2006). Whilst more direct measures of brain function such as EEG (Begum-Ali et al., 2021) show early differences, it is possible that behavioural difference only become evident in the second year of life (Lorenzo et al., 2015). Further follow-up of this cohort will be important to (i) identify when the developmental trajectories in the NF1 group start to diverge from control groups, and (ii) to identify whether any early predictors might identify children most at risk for later cognitive and behavioural difficulties.

Consistent with other pre-school studies we find no difference on measures of temperament, behaviour or sensory processing (Klein-Tasman et al., 2014; Lorenzo et al., 2015). Both the direct assessment using the AOSI and parent reported VABS socialisation suggest that the developmental trajectories of social communication skills in NF1 are comparable to the control group during infancy. This is an interesting finding given recent reports of social communication deficits and ASD in children with NF1. In the general population, early signs of altered social communication in infants with later autism emerge between 12 and 18 months and may include reduced gaze following, deficits in social referencing (Cornew et al., 2012), repetitive play (Loh et al., 2007), motor and attentional atypicalities (Elsabbagh et al., 2013). It is plausible that these behavioural signs emerge later on in the developmental period for some children with NF1 who may then subsequently meet criteria for ASD.

Sleep disturbances particularly with sleep initiation and sleep–wake transitions are well known in NF1 both in animal model (Bai et al., 2018) and human studies (Johnson et al., 2005). We found that the NF1 infants take longer to settle and show overall reduced sleep over development as compared to the control group. In a cross-sectional study of 129 children aged 2–19 years compared to unaffected siblings, Licis et al. (2013) found that the NF1 group were significantly likely to have reduced nightly sleep durations, longer sleep onset latency and greater number of night awakening (Licis et al., 2013). The results in our study suggest that the sleep disturbances are evident in the infancy period based on parental report. Sleep disturbances impact on cognitive function, daytime functioning at school and may impact on mood and anxiety (Lavigne et al., 1999). Executive function skills, often impaired in NF1, may be particularly vulnerable to the effects of sleep disturbance. Simple early intervention approaches such as parental support and advice about sleep hygiene measures may be helpful in ameliorating sleep difficulties, which in turn may have a positive impact on cognitive development.

A significant finding in this study, both for the NF1 and control groups, was the impact of maternal education on cognitive and behavioural development. In a longitudinal study of cognitive development in children aged 6–18 years with NF1, Hou et al. (2020) found that higher parental education related to higher IQ, Maths, reading and cognitive scores (Hou et al., 2020). Two recent studies have also examined the cognitive and academic differences in children with inherited versus *de novo* NF1 mutations. One study suggested that lower IQ in the inherited NF1 group was largely mediated by lower socio-economic status in families with NF1 (Biotteau et al., 2020). Conversely another study found that having a parent with NF1 was related to lower academic and cognitive skills despite adjusting for socio-economic status (Geoffray et al., 2021). The authors speculate that the psychological and physical morbidity caused by NF1 may impact parenting ability and the provision of cognitive stimulation conducive to the development of cognitive abilities in children. Further research investigating the impact of parental NF1 on children development will be important, and early interventions may be targeted towards at risk NF1 populations; such as those with socioeconomic adversity or family history of NF1.

Limitations of this study include the relatively small sample size and the incomplete data for some of the participants over the three assessment periods. Unless confirmed via cord blood testing, NF1 is usually diagnosed based on clinical features, which become more prominent as the infant develops (Elsabbagh et al., 2013) with only 30% meeting the NIH criteria by 12 months (Pinti et al., 2021). It is therefore difficult to recruit infants younger than 12 months, particularly with *de novo* mutations into the infancy longitudinal study as the clinical features may not be apparent. Our analytic approach using linear mixed modelling provides a way to handle missing data, explicitly modelling change over time, accounting for correlations of observations within subjects.

In conclusion, this is the first longitudinal study that reports cognitive behavioural development in the infancy period in NF1. Our results suggest overall similarities in trajectories of cognitive, behavioural, sleep development in the NF1 and control groups. Given that children with NF1 are at substantially increased risk of cognitive and academic impairments by the time they start school, our work suggests that early clinical surveillance may be helpful, especially for those with a family history of NF1 and intervention approaches to promote sleep hygiene may help promote overall development.

DATA AVAILABILITY STATEMENT

Data is available from through the BASIS network through a set of data sharing procedures that comply with the ethical permissions under which this highly sensitive data was collected. Available at: https://www.basisnetwork.org/.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Greater Manchester Central Research Ethics committee (16/NW/0324) and National Research Ethics Service London Central Ethical Committee (16/EE/0167 and 06/MRE02/73). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

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The STAARS team includes: Leila Dafner, Teodora Gliga, Amy Goodwin, Rianne Haartsen, Rebecca Holman, Sarah Kalwarowsky, Luke Mason, Laura Pirazzoli, and Chloë Taylor.

The EDEN team includes: *Manchester NF1 service*: Grace Vassallo, Emma Burkitt-Wright, Judith Eelloo, D. Gareth Evans, Siobhan West, Eileen Hupton, Lauren Lewis, and Louise Robinson; *Yorkshire Regional NF1 service:* Angus Dobbie, Ruth Drimer, and Saghira Malik Sharif; *Alder Hey NF1 clinic*: Jamuna Acharya and Zahabiyah Bassi; *Edinburgh Genetic Service:* Wayne Lam; *Sheffield NF1 clinic:* Alyson Bradbury, Neil Harrower, and Oliver Quarrell; *Newcastle NF1 service:* Helen Bethell, Rachel Jones, Susan Musson, Catherine Prem, and Miranda Splitt; *Sunderland NF1 clinic:* Karen Horridge; *Warrington NF1 clinic*: Shaheena Anjum; *Wirral University Hospitals NF1 clinic:* Christine Steiger.

AUTHOR CONTRIBUTIONS

SG contributed to the study conception and design, identified and recruited the participants, led the analysis, and drafted the manuscript and revisions. MWW led the analyses of parent–child interaction data, contributed to interpretation of findings, contributed to drafting of the manuscript, and commented on the revisions. JB-A led the fieldwork assessments, led data entry and initial analysis, led the quality control of phenotypic data, and contributed to drafting and critical review of the manuscript. AK-T contributed to the fieldwork assessments, data entry and initial analysis, and contributed to drafting and critical review of the manuscript. JG conceptualised the study and design, led the recruitment, and critically reviewed and revised the manuscript. MJ conceptualised and designed the study, and critically reviewed the manuscript. EJ conceptualised the study, contributed to the design, supervised the field-work, contributed to drafting of the manuscript, and critically reviewed and revised the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyg.2022.795951/full#supplementary-material

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