Emma van Daalen

Early diagnosis of Autism Spectrum Disorders

Project

A fruitful line of research might be
good manners
in animals.

Start with the big cats:
the jaguar that coughs
before it pounces,
or the one that lays the bones
of its victims in a neat pile
after each meal.

Or again, there's the ounce's
night cry with its
distinct undertone of apology

Christopher Reid

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Rudolf Magnus Institute of Neurosience,
Department of Child and Adolescent Psychiatry,
University Medical Center Utrecht,
Utrecht, The Netherlands

Vormgeving Karel Oosting
Omslagfoto Kees Rutten
Druk Thieme Almere

The studies described in this thesis were conducted at the Department of Child and Adolescent Psychiatry of the University Medical Center of Utrecht.

Early diagnosis of Autism Spectrum Disorders

De vroege diagnose van Autisme Spectrum Stoornissen
(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht
op gezag van de rector magnificus, prof. dr. J.C. Stoof, ingevolge het
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Contents

1

General introduction

Introduction

The general aim of this thesis is to evaluate the feasibility and the value of the diagnosis of Autism Spectrum Disorders (ASDs) in the preschool years and to determine behavioural and biological correlates that may endorse this process.

Autism or Autistic Disorder (AD) is a neurodevelopmental disorder characterized by deviant and delayed development of reciprocal social interaction, and of verbal and non-verbal communication, in combination with stereotyped and restricted behaviours, interests and activities, that lead to lifelong impairments. A further requirement for a classification of AD is that the delay or abnormal functioning starts before the child is 3 years [1].

In contrast to other fields in medicine, for most psychiatric disorders there are still no biological, psychological or genetic markers to validate the diagnostic process. As a consequence, diagnostic classification systems were introduced, to enhance agreement on a specific psychiatric diagnosis among clinicians. Two of the most well-known and widely used classificatory systems are the International Classification of Diseases (10th ed., ICD-10; World Health Organization [WHO], 1993)[2] and the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision, DSM-IV-TR (APA, 2000) [1].

The DSM included AD only in its third edition (DSM-III) (APA, 1980) [3], within a new category of disorder, Pervasive Developmental Disorder (PDD). After Kanner's original description of 'early infantile autism' in 1943 [4], the validity of this diagnostic concept was considerably disputed. The controversies included the role of psychological versus biological factors in the pathogenesis and the continuity between AD in childhood with schizophrenia in adulthood [5]. The DSM-III description of 'early infantile autism' had a strong focus on the infantile aspect resulting in just diagnosing the younger and more impaired children. As it was necessary to introduce a more developmental view in the classification of AD, especially because individuals continued to suffer from autistic symptoms after early childhood, the next version of the DSM, the DSM-III-R, enclosed criteria to encompass the entire spectrum of dysfunction (including both chronological age and cognitive level). One of the disadvantages of these changes was a higher rate of false positives. This in combination with the need to be more compatible with the other international classificatory system, the ICD-10, led to the development of the DSM-IV-TR [1], which included not only the ASDs, AD and Pervasive Developmental Disorder, not otherwise specified (PDD-NOS), in the category of PDDs, but also Asperger's Disorder, Rett's Disorder and Childhood Disintegrative Disorder, in congruence with ICD-10 [6].

PDD-NOS is developed as a sub threshold category for those disorders that are similar to AD but fail to satisfy the full set of criteria for the condition. Asperger's Disorder is characterized by a greater preservation of language skills at early age, when compared to AD. The essential feature of Rett's Disorder is the development of multiple specific deficits following a period of normal functioning after birth, and the essential feature of Childhood Disintegrative Disorder is a marked regression in multiple areas of functioning following a period of at least 2 years of apparently normal development [1]. The focus in this thesis is on the ASDs.

Considering children after preschool age, AD has always been one of the most reliably diagnosed disorders in child and adolescent psychiatry, provided that experienced clinicians have access to multiple sources of information [7]. However, there are challenges to the diagnostic evaluation of children with ASD, especially among less experienced clinicians. Syndrome expression can vary according to age, language delay and intellectual disability [8]. As a consequence a clinician needs training and experience to be able to recognize these different manifestations of AD in children with varying age and abilities [9]. The best clinical judgement of experienced clinicians is still considered as the 'gold standard' for the diagnosis of AD [8].

Prevalence

For many years the prevalence rates for ASDs were believed to be around 62.6 per 10.000 (95% CI 50.8-76.3) children, for AD of 16.8 per 10.000 children and for other ASDs of 45.8 per 10.000 children. These prevalence rates were established evaluating 15.500 children aged 2.5 to 6.5 years for developmental problems. The percentage of children with some level of intellectual disability in combination with ASD was 25.8% [11]. Baird and colleagues reported higher prevalence rates, measured in a total population cohort of 56.946 children aged 9-10 years: 116.1 per 10.000 (95% CI 90·4–141·8) children for all ASDs, 38.9 (29·9–47·8) for AD and 77.2 (52·1–102·3) for other ASDs [12]. Likewise, other recent studies reported higher prevalence rates for ASD [13,14]. In a review on 43 studies published since 1966 that provided estimates for the prevalence of all ASDs, a best estimate of 60 to 70/10,000 (equivalences = 6 to 7/1,000; or 0.6 to 0.7%; or one child in about 150 children) can be confidently derived for the prevalence of all ASDs [15].

The increase in prevalence rates is not well understood. Hypotheses about these increased prevalence rates are:

1) The age difference of the children evaluated in prevalence studies. A prevalence rate measured at school age instead of at preschool age is more likely to reveal all true cases, including children with ASD, who are functioning at a higher cognitive level.
2) The prevalence rates are influenced by changing diagnostic criteria and broadening of the concept of ASD, as mentioned before.
3) Different methods of measuring lead to different prevalence rates. In some studies children with ASD are identified through the registration system of health services, in other studies by screening the population followed by a diagnostic evaluation of the 'high risk' children with the accepted 'gold standard' practice of reaching a best-estimate clinical consensus diagnosis on the basis of combining information from standard research instruments of parent report, direct observation of the child, and independent information from school teachers [12].
4) Differences in prevalence rates between studies can be due to differences in the inclusion criteria for children with ASD who have co-morbid disorders.
5) Improvements in awareness of health care providers for ASD can lead to higher prevalence rates over time.
6) Also, the development and expansion of health care services for children with neurodevelopmental disorders can increase prevalence rates. And lastly, it can not be ruled out that the higher prevalence rate of ASD is accurate, and reflects a true increase over time [15].

The changing of diagnostic criteria over time has had an influence on the prevalence rates of intellectual disability in children with ASD. Since the introduction of the DSM-IV-TR, children who exhibit symptoms of ASD and are functioning at a higher cognitive level may be more likely to receive a diagnosis of ASD. As AD used to be associated with intellectual disability in 70-75% of cases, more recent epidemiological studies show much lower rates [17]. In a review of epidemiological studies on the prevalence of ASD the median proportion of children with ASD without intellectual disability is 30% (range: 0%-60%). For children with ASD and mild to moderate intellectual impairments this figure is 30% (range: 6.6%-100%), and for children with ASD and a severe to profound level of mental retardation this figure is 40% (range: 0%-81.3%) [11]. The male-to-female (M:F) ratio for AD is about 4:1 [16].

Children with ASD at preschool age may show a higher prevalence rate of comorbid intellectual disability compared to older children with ASD, as the delay in development may urge parents and primary health care professionals to present the child for a diagnostic evaluation.

Reasons for delay of an early diagnosis of ASD

In the 1994 U.S. birth cohort a median age of 5.7 years was measured as the age of the initial diagnosis of ASD in children [17]. ASDs are rarely diagnosed before 36 months of age, while the parents of most children with AD identify them as showing abnormalities or delays as early as the second year of life [11].

Factors that may cause the parents to hesitate in presenting the child to professionals are: first, parents of young children, especially when it is their first child, usually do not have detailed knowledge on every step in the normal development of young children. Furthermore, family and friends tend to reassure parents of young children, when they have concerns on the development of their child.

For professionals, the delay in acknowledging a deviant development in a very young child may be caused by: first, delays or abnormalities do not have to be present in all aforementioned domains, but may be recognizable in only one of the domains and second, it is very difficult to distinguish the often subtle differences between the abovementioned early signs of ASD and phases in normal development. In addition, primary health care professionals often have difficulties in acknowledging the delays or devi-

ances in the development of young children. They may lack the confidence and skills to recognize the subtle differences in the development of very young children with ASD, and it used to be common practice to attribute these differences to mild or transient problems in development.

Giacomo and Fombonne evaluated the first symptoms that aroused parental concern in children with AD, the age at which these first concerns were aroused, when they sought professional advice, and which factors influenced these variables. The mean age of parental concern was 19.1 months (SD = 9.4). The first professional advice was sought when the children were on average 24.1 months old (SD = 11.7). The most common concerns were for speech and language development, followed by abnormal socio-emotional response, medical problems and delay in milestones. Factors lowering the mean age of parental concern were the presence of an older sibling, the co occurrence of mental retardation, medical problems or a delay in milestones. Factors that were not of significance to the age of recognition were the child's gender, social class and place of residence [18].

The importance of an early diagnosis of ASD

The importance of an early diagnosis of a chronic disease, without the availability of a straightforward cure, needs to be clarified.

Delay in appreciating the fears and worries of parents and delay in the evaluation of the developmental difficulties of their child is known to increase parental stress. Furthermore, early detection guides parents to possibilities of early intervention, educational planning, development of a professional support system, and early genetic counseling for parents and other relatives. Several early treatment programmes report improved communication skills and social behaviour and diminished abnormal behaviour [19-22]. And there is also evidence that the earlier the intervention, the better the outcome [23]. Children with more severe cognitive deficits tend to respond less well to early intervention [23,24]. Unfortunately, children with ASD that are higher functioning are often diagnosed later than children with cognitive deficits [11,17,18]. In addition, as eligibility for financial support and participation in specialized programs for ASD is often limited to children with a formal diagnosis, the importance of an accurate early diagnosis of ASD becomes essential [25]. In conclusion, the efforts for early detection and early diagnosis of ASD, whether or not accompanied by intellectual disability, should be intensified.

Early presentation of ASD

Although the most common notion is that ASD is a neurodevelopmental disorder with substantial evidence of functional and morphologic abnormalities in the brains of subjects with ASD, abnormalities in functioning are often subtle in the first year, and therefore difficult to recognize for parents and professionals.

In a sample of 1512 children with AD, 62% of the parents reported to have noticed problems in the development of their child after the first birthday [26]. Problems in children with Asperger syndrome and in children with symptoms of ASD presenting after 30 months of age, therefore diagnosed as PDD-NOS, are usually identified even at a later age [27,28]. The most reliable information on very early symptoms of ASD comes from prospective studies of infants with an older sibling with ASD [7,29,30]. These siblings have a risk of 5% to 10%, a 20-fold higher risk of developing ASD in comparison to the general population [31]. The conclusion from the prospective studies was that children with a later diagnosis of ASD, in comparison to children with a typical development, show abnormalities or delays by 12 to 18 months of age in 1 or more of the following domains: [44]

1) visual (atypicalities in visual tracking and fixation on objects and prolonged visual inspection of objects [32-35]);
2) motor (decreased activity levels,33 delayed fine and gross motor skills [36,37], and atypical motor mannerism [38,39]);

3) play (delays in the development of motor imitation,33 limited toy play [32,37], and repetitive actions with toys [32-35,39]);
4) social-communication (atypicalities in eye gaze, orienting to name, imitation, social smiling, reactivity, and social interest and affect, with reduced expression of positive emotion [30,32-34,40,41]);
5) language (delays in babbling (especially back and forth social babbling), verbal comprehension and expression, and in gesturing, as measured with standardized assessment [30,38,34,37,42,43]); and
6) general cognitive development [44] (slower acquisition of new skills in a subset of toddlers subsequently diagnosed with ASD [33,34,37]).

Inter-rater reliability and stability

A lowering of the age of initial diagnosis for children with ASD presents new challenges [45]. AD may present itself with different symptoms at a young age. So, as mentioned before, results from studies in children older than three years of age concerning the value of an ASD diagnosis are not applicable to younger children. The value of an early diagnosis is reflected by its reliability and stability over time. The inter-rater reliability of a diagnosis made by clinicians refers to the consensus on the diagnosis between different psychiatrists. The stability of the diagnosis refers to the likelihood that the diagnosis at initial evaluation is the same as the diagnosis at the time of follow-up.

Many studies evaluated inter-rater reliability and stability in clinically referred children, younger than 5 years of age, with AD [25,46-56]. Overall, these studies indicate that a diagnosis of AD made at 2 years is stable in clinically referred samples measured at 3 years, and even up to 9 and 12 years. As in older children [6], less experienced clinicians have more difficulty differentiating AD and other ASD in children at such a young age [25]. In conclusion, clinicians experienced in diagnosing very young children with ASD can make reliable and stable ASD diagnoses at a young age.

Emphasizing the importance of identifying children with ASD at an earlier age, the American Academy of Pediatrics has recommended universal screening for ASD among toddlers at ages 18 and 24 months [57]. Hopefully this leads to the identification of children with ASD at a younger age. Next, the challenge will be to translate this expert research knowledge into practical, cost-effective approaches to be used by non-expert health professionals [58].

Early diagnosis of ASD and the DSM-IV criteria for AD

One of the most obvious choices for translating the expert knowledge on the diagnosis of ASD, in children younger than three years of age, into a practical cost-effective instrument is the DSM-IV-TR classification for AD. There are some difficulties to overcome, because not all the DSM-IV-TR criteria for AD might be applicable for all children at a young age [25]. Especially young children with AD may have intellectual disabilities, speech and language delays, or may simply, by virtue of their age, not reach a developmental level that is necessary for being able to fulfil all the criteria. If only a subset of the diagnostic criteria is applicable to young children, it seems plausible that a different algorithm might be warranted for this age group [25].

In one of the few studies into this subject, DSM-IV criteria from the social domain proved to be frequently applicable in young children with ASD. Particularly, 'impaired use of nonverbal behaviours' and 'lack of social or emotional reciprocity' seem to be very important to an early diagnosis of AD as well at 2 years as at 3 years of age. As expected, the criterion concerning 'relationships with peers' was difficult to evaluate in children with a mental age below 24 months of age. Behaviours within the communication domain were also very useful; of particular relevance in this area appears to be the 'delayed development of spoken language', which

was applicable to almost all young children with AD. Not applicable for all young children were 'limited conversational skills', and 'stereotyped language' [25]. There is a tendency in the literature that repetitive and stereotyped behaviour are not reported in children younger than three years of age [25,50,52]. It seems that these behaviours are not present in young children with the same consistency as behaviours within the other two domains and these activities show more variability from child to child. Within this domain, the most commonly reported behaviour was a preoccupation with stereotyped and restricted patterns of interest. Adherence to routines or rituals was reported very rarely for this group of young children [25]. Also later research, using the ADI-R, suggests that repetitive and stereotypic movements may be more common in this age group than insistence on sameness behaviours [59,60].

Considering the importance of the domains of the DSM-IV-TR criteria for AD to an early diagnosis of AD and the applicability of these criteria, an evaluation of the value of the DSM-IV-TR classification for AD in samples of young children with ASD is urgent. As mentioned before, separate criteria have been evaluated,[25] but not the usefulness of the DSM-IV-TR criteria for AD collectively, i.e. in the form of an algorithm.

Comparison of clinical diagnosis to standardized diagnostic instruments

There is an increasing tendency to use research instruments, such as the ADI-R (the Autism diagnostic interview-revised; ADI-R) [61], and the ADOS-G (the Autism Diagnostic Observation Schedule; ADOS-G) [62], for clinical diagnostic purposes, as they are considered to be the essential diagnostic tools by the National Institutes of Health National (Database for Autism Research). However, in normal clinical practice, during a typical single office visit to a clinical psychologist, a developmental paediatrician or a child psychiatrist without specific expertise in the field of very young children, such structured information is typically not obtained [63]. Besides, the administration and scoring* of both the ADI-R and ADOS-G require proper training and means to finance training and coding materials [46,57,64]. Also, in several studies standardized instruments, i.e. the ADI-R and the ADOS-G, applied in children at age two years were less successful in predicting clinical outcome than the clinical diagnosis of experienced clinicians [40,47,51]. This indicates that formal criteria for ASD as formulated in ADI-R or ADOS-G algorithms do not always apply to young children. Considering the professionals working in clinical practice with this very young population, the use of a more accessible diagnostic procedure, adjusted to young children with ASD, and without any additional training might prove relevant. As the ADI-R and ADOS-G have proved their relevance in research projects of older children with ASD, and furthermore as some services are beginning to regulate access with a diagnosis based on the ADI-R or the ADOS-G it is very important to compare the values of different diagnostic tools for an early ASD diagnosis.

Predictive power of biological correlates for an early diagnosis of ASD: head growth

One of the goals in this thesis has been to identify correlates that may have predictive power for an early diagnosis of ASD.

A possible candidate with predictive power in this age range comes from the combination of data from measurement of head circumferences, neuro-imaging and post-mortem studies showing that the brain is abnormally large in some, but not in all children with AD during postnatal development [65]. In comparison to the general population there seems to be no difference in head circumference at birth [65], as well as during foetal development in children with later ASD (Hobbs et al., 2007) [66]. The accelera-

* The administration and scoring costs 1 hour for the ADOS-G and 3-4 hours for the ADI-R in case of aproperly trained professional.

tion in head growth appears to start during the first year of life [67-69]. Two studies reported that growth in body length of children with ASD between birth and age 3 years was more abnormal than that of head circumference, and reported an increased growth of body length around 4 months [68] and between 2-3 years [70]. Because head growth is strongly related to brain growth during infancy and early childhood [67,71], an increased head size at a very young age is suggestive of early abnormal development of the brain in AD [72]. An interesting finding is that the odds that an infant will later meet criteria for ASD appear to be five times greater if the infant is macrocephalic by 5-12 months than if the infant is normocephalic [73]. As head growth is tightly linked to brain growth during infancy and early childhood, increased head size in infancy in AD is believed to be due to early abnormal growth of the brain [74]. As individuals with AD grow older, the difference in mean total brain volume in comparison to the normal population may progressively disappear [74,75].

Predictive power of biological correlates for an early diagnosis of ASD: genetics

Another candidate for being a biological correlate with predictive power for ASD at a very young age might be a genetic one.

As it is evident from heritability estimates for the narrow phenotype of AD being as high as 90%, the role of genetic factors is important in the development of ASD [31,76]. The search for specific risk genes for ASD, through linkage and association studies, has resulted in findings that only explain a very small part of the variance of the disease. Furthermore these findings have been very difficult to replicate. Resulting in the assumption that ASD is a disorder with a large and complex genetic heterogeneity [77].

It is generally agreed that about 10-15% of individuals with ASD have a known medical condition, namely a cytogenetic or single gene disorder that causes the disorder [78]. The prevalence of cytogenetic abnormalities in AD are estimated to be 3-5%, and can be found on most chromosomes [79-83]. Examples of the most prevalent cytogenetic abnormalities associated with ASD are duplication in the region of 15q11-13, and deletions in the region of 22q11. Deletions on the 15q11-13 regions are associated with two cytogenetic imprinting disorders, Angelman syndrome and Prader-Willi syndrome. The phenotype of subjects with Angelman syndrome is regarded as distinguishable from AD [77]. In subjects with Prader-Willi syndrome AD has been most commonly associated with a maternal uni-parental disomy with lack of the paternal copy of the 15q11-13 region, one of the three known major genetic mechanism that can cause the Prader-Willi syndrome [84].

The most common single gene disorders associated with ASD are tuberous sclerosis and fragile X syndrome. Rare single gene disorders associated with ASD are phenylketonuria and Smith-Lemli-Opitz syndrome. Neurofibromatosis was always thought to be associated with ASD, but does not occur with a higher frequency in subjects with ASD than in the general population [77].

The non-genetic medical conditions are considered to represent phenocopies of ASD [77]. Known non-genetic medical conditions are rare. Examples are: maternal use of thaladomide, valproic acid and alcohol during pregnancy [85-89]. Congenital rubella and cerebral palsy are associated with ASD [90,91]. The mumps-measles-rubella vaccine has been a candidate for causing ASD [92], but this has not been confirmed [93,94].

Predictive power of biological correlates for an early diagnosis of ASD: copy number variants (CNVs)

More recently, ASDs are hypothesized to be the result of at least two forms of genetic aetiology, i.e. common genetic variants with a small effect and rare genetic variants with a large effect on disease risk and phenotypic

presentation. It is still largely unknown in which proportion ASDs are accounted for by the different genetic variants, either alone or in combination [95,96]. Considering the rare genetic variants, recent advances of genomic array technologies have moved the identification of chromosomal abnormalities from the microscopic to the submicroscopic level. These genomic deletions and duplications, or dosage alterations, are often referred to as copy number variants (CNVs). CNVs can be inherited, or emerge *de novo* on paternal or maternal chromosomes [97]. As a result of the availability of these new techniques a fast growing body of literature has demonstrated that submicroscopic spontaneous genomic dosage alterations may possibly be associated with ASD in 7-27.5% [98-100].

However, at this moment little is known about the prevalence of CNVs among phenotypically normal individuals. It is essential to catalog this genomic variation in the normal population and to collect all the available information into a database, which will be a crucial resource in correlating these genomic variations with experimental findings and clinical outcomes [101]. Only CNVs that do not occur or only with a very low frequency in these normative databases and are reported in patients with ASD may have a clinical relevant meaning. Presently, the role of CNVs in causing or influencing the susceptibility to disease and genome evolution is still largely unknown [102].

The early screening of ASD: the SOSO project

The present thesis forms part of a larger study with the general aim of evaluating early signs of ASD and determining biological, behavioural, cognitive, and environmental correlates. This study, SOSO (Screenings Onderzoek Sociale Ontwikkeling), was implemented by the UMC Utrecht in close cooperation with preventive health services in the province of Utrecht (The Netherlands); Amant, Aveant, Vitras and Zuwe. Data from the SOSO project have been reported by Claudine Dietz in her thesis: 'The Early Screening of Autistic Spectrum Disorders', Fabienne Naber in her thesis: 'Toddlers with Autism: aspects of early behaviour', and Mijke Zeegers in her thesis 'Toddlers with autism'.

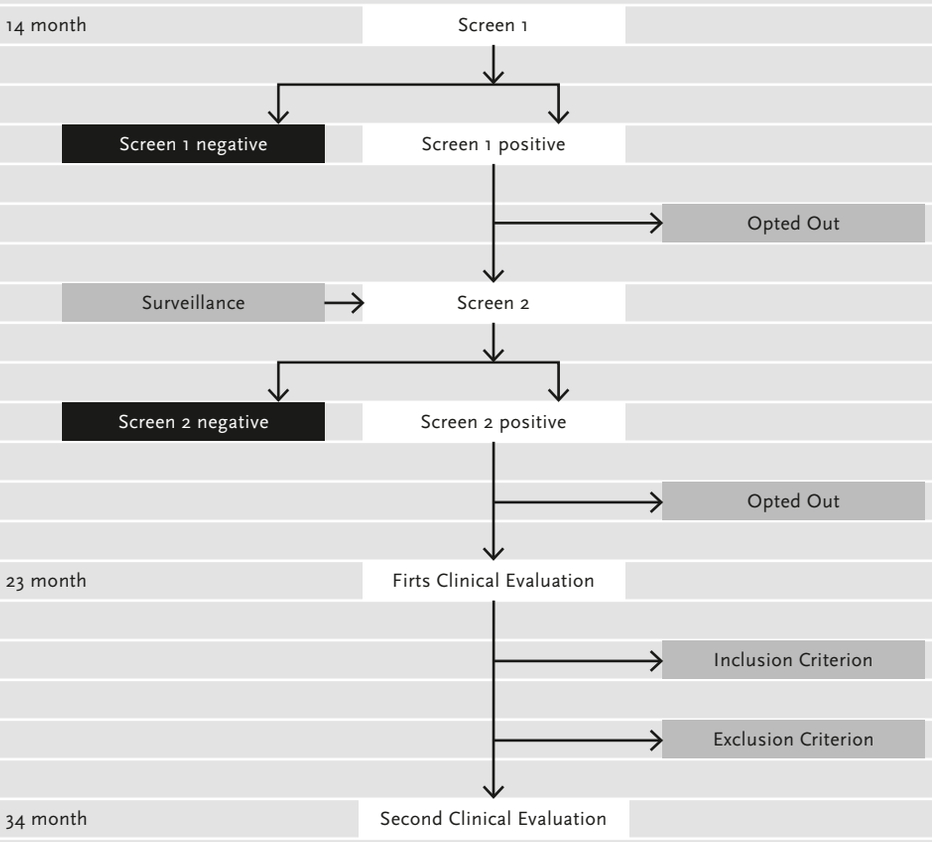
From October 1999 to April 2002, more than 31.000 children from the general population were screened by physicians at all well-baby clinics in the province of Utrecht using the 4-item Early Screening of Autistic Traits (ESAT) scale at their routine 14-month developmental check (Screen 1), see Figure 1: Design SOSO project. Parents were advised by the physician to continue with the screening procedure if their child failed at least 1 of 4 items of the ESAT and was considered screen positive. Children who scored positive at Screen 1 (population screening) and whose parents did consent and children aged up to 36 months identified by surveillance underwent Screen 2. Screen 2 consisted of the 14-item ESAT scale and was done at a home visit by an experienced psychologist (C.D.), a member of our research team. Also, the cognitive development of the child was examined at screen 2. Children who failed at least 3 items of the 14-item ESAT scale were considered screen positive. Children who scored positive at Screen 2 were invited for a first comprehensive psychiatric evaluation at the Department of Child and Adolescent Psychiatry of University Medical Centre Utrecht. A second, follow-up evaluation was performed when the children were on average 43 months old (range 34-64 months). The general outline of the screening procedure and diagnostic evaluations are portrayed in the Figure 1.

Aim of this thesis

The aims of the present thesis were as follows:

– To evaluate the inter-rater reliability and stability of ASD diagnoses in children identified through a screening procedure applied at 14-months of age.

Figure 1: Design SOSO project



– To evaluate the utility of a cheap, simple and easy to administer diagnostic instrument, the algorithm of the DSM-IV-TR criteria for AD, in terms of its power to predict a clinical diagnosis of ASD at 42 months (i.e. the gold standard).
– To explore the potential contribution of biological measures for the phenotype of ASD. We evaluated the value of head growth on the phenotype of ASD and furthermore performed a preliminary evaluation of the possible contribution of Copy Number Variants (CNVs) to the phenotype of ASD.

Outline of this thesis

The first goal of this thesis was to evaluate inter-rater reliability and stability of the diagnosis of ASD at a very young age (Chapter 2). The method of evaluating a clinical diagnosis, the 'gold standard' for ASD diagnosis, especially at such a young age was derived from the DSM-IV field trial. The first psychiatric evaluation of the children in the project took place at a mean age of 23 months (t1). The children were given a preliminary clinical diagnosis at t1 based on the clinical judgement of a child psychiatrist, experienced in the psychiatric evaluation of children younger than four years of age. In this preliminary diagnosis, the psychiatrist predicted whether the child was likely to have an AD, PDD-NOS, or another psychiatric diagnosis when he or she was 4 or 5 years old. The same evaluation procedure was repeated at the second psychiatric evaluation at 42 months (t2).

Inter-rater reliability was evaluated by comparing the diagnosis of two independent psychiatrists, who had not conducted the psychiatric evaluations. Stability of diagnosis was assessed comparing the consecutive diagnoses at t1 and at t2 of every individual child.

The next step consisted of an evaluation of the DSM-IV-TR criteria for AD on their usefulness at a young age (Chapter 3). At the first evaluation, the preliminary clinical diagnosis was assigned before the presence of the

diagnostic criteria was established and without using the classification algorithm specified in the DSM-IV-TR for AD. As it is the expectation that more young children will be referred for psychiatric evaluation in the near future the next step in this project was to examine the utility of a cheap, simple and easy to administer diagnostic instrument, the algorithm of the DSM-IV-TR criteria for AD, in terms of its power to predict a clinical diagnosis of ASD at 42 months (i.e. the gold standard).

The third step was to explore the potential contribution of biological measures for the phenotype of ASD. We evaluated the value of head growth on the phenotype of ASD in the SOSO-cohort (Chapter 4). ASD is a neurodevelopmental disorder with many known functional and morphological abnormalities in the brain, but the pathophysiology and the aetiology of these disturbances are unknown. One of the most consistent findings in children with ASD older than 3 years of age is an increased rate (14-34%) of macrocephaly (head circumference >97th percentile) and a larger mean head circumference compared with normally developing children [65,72]. To evaluate head size in relation to body growth all data on head circumference, body length, and weight that were available from the well baby clinics were collected. Head circumference, body length, and weight were compared with the population norms of the Netherlands Organization for Applied Scientific Research database [103].

Furthermore, we performed a preliminary evaluation of the possible contribution of Copy Number Variants (CNVs) to the phenotype of ASD in a cohort of consecutive referred children with symptoms of ASD as detected at or before the age of four years that have been routinely evaluated by a specialized team of clinicians for both their psychiatric and clinical genetic phenotypes (Chapter 5 and Chapter 6). With regard to the aetiology of ASD genetic factors play an important role. More recently, ASDs are hypothesized to be the result of at least two forms of genetic aetiology, i.e. common genetic variants with a small effect and rare genetic variants with a large effect on disease risk and phenotypic presentation.

The focus in chapter 6 is placed on the second form of genetic variation in ASD, the rare genetic variants. For this purpose the probands were evaluated by a clinical geneticist and received an evaluation of peripheral blood and genomic DNA. Also, the children underwent a multidisciplinary evaluation including the Autism Diagnostic Interview-Revised (ADI-R) and the Autism Diagnostic Observation Schedule generic (ADOS-G). The clinical diagnosis was established by an experienced clinician. The genetic variants found in ASD patients were compared to variants found in the normal population. The rare variants found in probands are reported in chapter 6.

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Emma van Daalen, Chantal Kemner, Claudine Dietz,
Sophie H.N. Swinkels, Jan K. Buitelaar, Herman van Engeland

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Inter-rater reliability and stability of diagnoses of Autism Spectrum Disorder in children identified through screening at a very young age

To examine the inter-rater reliability and stability of autism spectrum disorder (ASD) diagnoses made at a very early age in children identified through a screening procedure around 14 months of age.

In a prospective design preschoolers were recruited from a screening study for ASD. The inter-rater reliability of the diagnosis of ASD was measured through an independent assessment of a randomly selected subsample of 38 patients by two other psychiatrists. The diagnoses at 23 months and 42 months of 131 patients, based on clinical assessment and the diagnostic classifications of standardized instruments, were compared to evaluate stability of the diagnosis of ASD.

Inter-rater reliability on a diagnosis of ASD versus non-ASD at 23 months was 87%, with a weighted κ of .74 (SE: .11). The stability of the different diagnoses in the autism spectrum was 63% for autistic disorder, 54% for PDD-NOS, and 87% for the whole category of ASD. Most diagnostic changes at 42 months were within the autism spectrum from autistic disorder to PDD-NOS and were mainly due to diminished symptom severity. Children who moved outside the ASD category at 42 months made significantly larger gains in cognitive and language skills than children with a stable ASD diagnosis.

The inter-rater reliability and stability of the diagnoses of ASD established at 23 months in this population-based sample of very young children are good.

Introduction

Autism spectrum disorders (ASD), which include autistic disorder or autism (AD), Asperger syndrome, and Pervasive Developmental Disorder, not otherwise specified (PDD-NOS), are characterized by deviant and delayed development of reciprocal social interaction, and of verbal and non-verbal communication, in combination with stereotyped and restricted behaviours, interests and activities, that lead to lifelong impairments. A further requirement for a classification of AD is that the delay or abnormal functioning starts before the child is 3 years [1]. However, in most children the diagnosis is made later [5,9,20], even though most parents report concerns about the development of their child as early as the second year of life or even earlier [10,18,19,20,25,34]. Problems in children with Asperger syndrome and in children with autistic symptoms presenting after 30 months of age, therefore diagnosed as PDD-NOS, are identified a later age than they are in AD [16,20].

Diagnosing ASD at an early age has several advantages. First, it facilitates starting early intervention, educational planning and development of a professional support system. Several early treatment programmes report improved communication skills and social behaviour and diminished abnormal behaviour [17,24,28,29]. Second, early diagnosis enables professionals to learn about the developmental trajectories of ASD in the early years and to identify predictors of outcome [46]. Lastly, given the importance of genetic factors in the aetiology of ASD, early diagnosis enables early genetic counselling for parents and other relatives.

Recognition of the importance of the early identification of ASD has spurred researchers to improve diagnostic procedures in the preschool years [3,7,15,16,18,21,25,27,31,33,39,40,42,43]. However, lowering the age of initial diagnosis presents new challenges [5]. For example, the phenotypic expression of ASD at 2 years of age or younger may differ from that at 3 years or older. Thus, severity and pattern of symptoms of ASD at a young age need to be established, as do the inter-rater reliability and stability of the early diagnosis.

The inter-rater reliability of a diagnosis made by clinicians refers to the consensus on the diagnosis between different psychiatrists. The stability of the diagnosis refers to the likelihood that the diagnosis at initial evaluation is the same as the diagnosis at the time of follow-up. The inter-rater reliability and stability of the diagnosis of AD have been examined in clinically referred samples of children older than five years and found to be excellent [30,45]. Studies that investigated inter-rater reliability and stability in clinically referred children, younger than 5 years of age, with AD are summarized in Table 1 [7,9,15,18,25,27,31,36,39,40,42,43]. Overall, these studies indicate that a diagnosis of AD made at 2 years is stable in clinically referred samples measured at 3 years, and even up to 9 and 12 years. Diagnostic stability, however, is less strong for PDD-NOS. Another result of these studies is that clinical judgement, when a child is 2 years of age, proved to be superior to the diagnostic algorithm of a standardized interview, the Autism diagnostic interview-revised (ADI-R) [24] or standardized observation, i.e. the Autism diagnostic observation schedule-generic (ADOS-G) [26] in predicting children's later diagnostic classification [9,25,27]. Diagnoses based on the ADI-R, appear to change significantly, particularly in younger and more intellectually disabled children [25]. Diagnostic thresholds from the ADI-R were crossed and recrossed between ages 2 to 7 years [9].

Although, standardized research instruments at age two years are inferior to the insight in the decision whether AD is present or not made by experienced, well-trained clinicians, this clinical insight proves not to be sufficient by itself. In conclusion, scores on these standardized research instruments also make real contributions beyond their influence on informing and structuring clinical diagnosis [27].

Inter-rater reliability for ASD diagnoses below age 3 year has been

Table 1: Descriptive Characteristics of Best-Estimate Diagnoses, Reliability and Stability of Clinical Diagnoses

Sample	N	Type of sample	Age at 't1', months (SD or range)			Age at 't2', months (SD or range)			Reliability at 't1', percentage agreement, (κ, p); percentage agreement, certainty rating				Reliability at 't2', percentage agreement, (κ, p)		Stability of diagnosis between 't1' and 't2', percentage agreement, (κ, p)		
Gillberg et al., 1990	28	CR		23.96	(9.3)		58		–					100%	AD: PDD-NOS: ASD:	95% 50% 88%	
Lord, 1995	30	CR		25-35			38-52		–					97%	AD: PDD-NOS:	87.5% –	
Sigman & Ruskin, 1999	50	CR		47.2	(12.1)		154	(3.74)	–					0.93	AD:	98%	
Stone et al., 1999	65	CR		31.4	(3.4)		45	(4.3)	ASD/non-ASD: AD/PDD-NOS:	88% 64%	.67 .28	<0.5 <0.5		– –	AD: PDD-NOS:	80% 42%	
Moore & Goodson, 2003	20	CR		34.0	(29-42)		53	(48-58)	–					–	AD: PDD-NOS: ASD:	87.5% 33.3% 100%	
Eaves & Ho, 2004	49	CR		33.0	(4.6)		59	(7.47)	–					–	AD: PDD-NOS: ASD:	91% 22% 93%	
Lord et al., 2006	192	CR	AD: PDD-NOS: Non-ASD:	29.1 29.1 28.8	(4.7) (5.6) (5.5)	AD: PDD-NOS: Non-ASD:	110.1 113.8 114.9	(15.7) (17.1) (11.8)	AD, yes/no: Cert. Rat.: –	92%	.89		AD : PDD-NOS: Non-ASD:	> 90% > 83% > 83%	Total agreement: ASD /non-ASD: AD/ non-AD:	67% 90% 76%	.47 .72 .51
Turner et al., 2006	25	CR		31.0	(3.8)		108.8	(7.9)	–				–		ASD: AD: PDD-NOS:	88% 89% 29%	
Sutera et al., 2007	90	CR	ASD/ASD: ASD/Non-ASD: Non-ASD/Non-ASD:	27.6 26.5 28.0	(4.7) (4.9) (3.9)	ASD/ASD: ASD/Non-ASD: Non-ASD/Non-ASD:	52.2 54.4 28	(6.6) (10.1) (3.9)	–				–		AD: PDD-NOS:	89% 61%	
Turner & Stone, 2007	26	CR		28.8	(3.4)	53.3		(3.5)	–				–		ASD /non-ASD: AD/ non-AD:	63% 68%	
Chawarska et al., 2007	31	CR	AD: PDD-NOS:	21.6 21.6	(3.2) (2.5)	AD: PDD-NOS:	34.8 38.1	(2.5) (8.3)	–				–		AD: PDD-NOS: ASD:	90% 100% 100%	
Cox et al., 1999	46	PB		20.7			42		–				–		AD: PDD-NOS: ASD:	67% 33% 100%	

ASD: Autism Spectrum Disorders
Non-ASD: No Autism Spectrum Disorder
AD: Autistic Disorder
PDD-NOS: Pervasive Developmental Disorder, not otherwise specified
CR: Clinically Referred
PB: Population Based
Cert.Rat.: Certainty Rating

examined in only two studies and found to be good to excellent for the distinction between ASD and non-ASD, and between presence and absence of AD, but poor for the distinction between AD and PDD-NOS (Table 1). A factor associated with more accuracy in an early ASD diagnosis is the experience of the clinician [39]. Less is known about reliability and stability of ASD diagnoses in population-based samples. In a population-based screening study of 17,173 children, using the Checklist for Autism in Toddlers (CHAT), in the United Kingdom, the stability of a clinical diagnosis of AD made at 20 months was very good, with no false positives for ASD at 42 months. The diagnosis of AD appeared to be more stable than that of PDD-NOS, see Table 1 [9]. In a follow-up sample of children, recruited using the CHAT, to a randomized control trial of a parent training early intervention [14], the stability of a clinical diagnosis of AD made at 20 months consistently proved to be good at seven years of age. Almost all of these children met ADOS-G algorithm criteria for ASD and half of these children met the full ADI-R algorithm cut-off for AD at age 7 [6].

The focus in recent studies has been on variability in outcome for children with an early diagnosis of ASD [6,37,38,40]. Although differences between children with an early diagnosis of ASD who retain the diagnosis and who lose the diagnosis as a toddler do exist, the two groups are very difficult to differentiate when diagnosed initially [40]. Diagnostic stability has shown to be significantly higher for children who were initially diagnosed after 30 months (87%) than for those who were initially diagnosed at 30 months or younger (52%) [42].

The aims of the present study were as follows. First, we set out to evaluate the inter-rater reliability and stability of ASD diagnoses in children identified through a screening procedure applied at 14-months of age [11,41]. Unlike the UK-study [2], this population-based sample included children with intellectual disability. Second, we examined the cognitive and language correlates of children with a stable versus an unstable diagnosis of ASD.

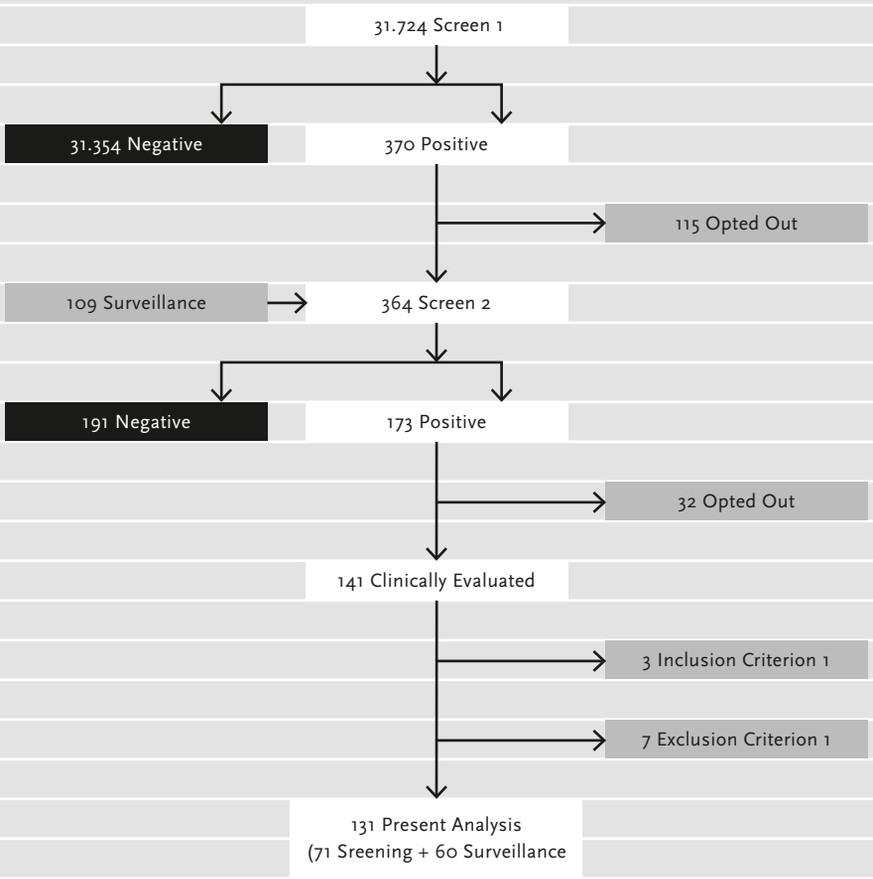
Method

Design

From October 1999 to April 2002, 31,724 children from the general population were screened by physicians at all well-baby clinics in the province of Utrecht using the 4-item Early Screening of Autistic Traits (ESAT) scale at their routine 14-month developmental check (Screen 1)* [41], see Figure 1. Parents were advised by the physician to continue with the screening procedure if their child failed at least 1 of 4 items of the ESAT and was considered screen positive. Children who scored positive at Screen 1 (population screening) and whose parents did consent (n = 255) and children aged up to 36 months identified by surveillance (n = 109) underwent Screen 2 [11]. Screen 2 consisted of the 14-item ESAT scale [41] and was done at a home visit by an experienced psychologist (C.D.), a member of our research team. Also, the cognitive development of the child was examined by the Mullen scales of early learning (MSEL) [32]. Children who failed at least 3 items of the 14-item ESAT scale were considered screen positive. The average (SD) age at screen 2 was 16 (2) months for children recruited by the population screening and 27 (6) months for the group detected by surveillance. Children who scored positive at Screen 2 were invited for a first comprehensive psychiatric evaluation at the Department of Child and Adolescent Psychiatry of University Medical Centre Utrecht. A second, follow-up evaluation was performed when the children were on average 43 months old (range 34-64 months). Because of limited resources, only children with a preliminary clinical diagnosis of ASD, intellectual disability, language or phonological disorder as a result of the first psychiatric evaluation, or at parental request were included in a follow-up evaluation. As a result, 141 young children received two comprehensive psychiatric evaluations, see Figure 1. Further details of the screening procedure can be found elsewhere [11,13,41].

* The routine developmental check is part of the system of surveillance for infants and toddlers as it is performed in the well baby clinics in the Netherlands [11-13,41].

Figure 1: Design - two level screening for ASD



Screen 1: 4-item Early Screening of Autistic Traits (ESAT) scale at routine 14-month developmental check.
Screen 2: 14-item ESAT scale.
Inclusion criterion 1: a first psychiatric evaluation before the age of 37 months and a second evaluation at approximately the age of 42 months, and no sooner than 12 months after the first evaluation.
Exclusion criterion 1: presence of a genetic or medical disorder that could be associated with specific phenotypes of psychiatric disorders.

Clinical measurements

The first psychiatric evaluation at t1 (at about 23 months) was scheduled in the preschool program at the department of child- and adolescent psychiatry. The preschool program consisted of a parent-interview and psychiatric evaluation of the child. The parent-interview included a developmental history, the Vineland social emotional early childhood scales [38,44], and the Wing autistic disorder interview checklist (WADIC), administered by the primary clinician [47]. The evaluation of the child consisted of an unstructured psychiatric evaluation by the primary clinician and an ADOS-G, a semi-standardized observation procedure, administered by a research associate, which were both videotaped.

The cognitive evaluation of the child was performed with the Mullen scales of early learning (MSEL) by trained psychologists. Some children with intellectual disability were evaluated with the psycho-educational profile revised (PEP-R) [35]. The first children in the project were assessed with the Bayley scales of infant development (BSID-II) [4], see Table 2. The MSEL and the BSID-II were used to calculate an overall cognitive score (CS), the PEP-R was used to calculate an age equivalent score. This last score was converted to an overall cognitive score (CS) to make the scores of the three instruments comparable.**

At t2, the parents of 18 children agreed to a psychiatric and an ADOS-G evaluation, but did not give consent for a cognitive evaluation. These were all children with a high level of intellectual disability. Eight of these children received a diagnosis of AD and three of these children a diagnosis of intellectual disability without an ASD. One of the children was diagnosed with ADHD and 2 of the children with a language disorder. Four children were diagnosed with a regulatory disorder and had been evaluated at the age of 24 months, and found to perform at an average cognitive level.

** A cognitive score was calculated from the PEP-R by dividing the mental age in months by the chronological age in months and then multiply this by 100.

Table 2. Distribution of number of participants by instruments used for cognitive evaluation, and by instruments used for standardized psychiatric evaluation at t1 and t2, number of participants at t1 and at t2 is 131

	t1	t2
Cognitive Instruments		
BSID-II	8	0
MSEL	117	88
MSEL-NV	5	2
PEP-R	0	23
No IQ	1	18
Total IQ	**131**	**131**
Standardized Diagnostic Instruments		
ADOS-G, module I	126	65
ADOS-G, module II	3	59
ADOS-G, missing	2	7
ADOS-G Total	**131**	**131**
ADI-R	–	98
ADI-R, missing	–	33
ADI-R Total	**–**	**131**

BSID-II: Bayley Scales of Infant Development..
MSEL: Mullen Scales of Early Learning.
MSEL-NV: Mullen Scales of Early Learning-Non Verbal Subscales.
PEP-R: Psycho-educational Profile-Revised.
No IQ: no cognitive evaluation performed.
ADOS-G: Autism Diagnostic Observation Schedule-Generic.
ADI-R: Autism Diagnostic Interview-Revised.
t1: first psychiatric evaluation.
t2: second psychiatric evaluation.

Children were given a preliminary clinical diagnosis at t1 on the basis of the judgement of the primary psychiatrist of whether the child was likely to meet the DSM-IV-TR criteria for Autistic Disorder, PDD-NOS, or another psychiatric diagnosis when he or she was 4 or 5 years old. The child psychiatrist used all available written and videotaped information with the exception of the results of the ADOS-G algorithm or individual item scores and classified the children according to the DSM-IV-TR diagnostic criteria they were likely to meet at 4 or 5 years of age. The same evaluation procedure was repeated at the second psychiatric evaluation at 42 months (t2). In addition, the parents were interviewed with the ADI-R by a research associate, see Table 2. The children were assigned DSM-IV-TR diagnoses, based on all the available clinical information, again with exception of the results of the ADOS-G and ADI-R algorithms. The diagnosis AD was reserved for these children meeting the algorithm for Autistic Disorder of the DSM-IV, the other diagnoses of ASD were given to children with serious and pervasive symptoms of ASD, but who are not meeting the threshold for AD. The ADI-R Diagnostic Algorithm specifies that the most prototypical autistic behaviour is seen at the ages 4 to 5 years, and that the ADI-R may be less specific or sensitive at younger ages [24]. Thus because the mean age of the children at t2 was 43.07 months (SD = 5.15), the instrument was not used as sole arbiter in the diagnostic process [9].

Children could have more than one diagnosis, but only the principal diagnosis, being the main focus of attention or treatment [1], was used for the scope of this article. For example, the diagnosis of AD took precedence in the case of a child with an AD and a phonological disorder. If only a phonological disorder was present, this was considered as being the principal diagnosis.

With regard to treatment, all children with an ASD diagnosis or another developmental disorder in our cohort went to a facility for challenged toddlers or a facility for children with a mental handicap for four days a week. These facilities offer a day-care program based on behavioural principles.

The facilities for challenged toddlers offer this approach in a group especially for autistic children. Children receive speech and language therapy in the facility or externally. For most children the frequency was limited to one hour in every two weeks. One of the children received an intensive treatment especially designed for autistic children in the facility for children with an intellectual disability. She was severely handicapped and later diagnosed with Rett's syndrome.

The effect of treatment was not assessed for the purpose of this article.

Statistics

To evaluate inter-rater reliability of diagnosis, Cohen's kappa was used. Kappa values were interpreted according to the criteria by Cicchetti and Sparrow [8]: excellent agreement (κ between 0.75 and 1.00); good agreement (κ between 0.60 and 0.74); fair agreement (κ between 0.40 and 0.59); and poor agreement (κ < 0.40).

Contingency tables were applied to assess stability of diagnosis between t1 and t2. Differences in age and cognitive scores between the different diagnostic groups were tested with analysis of variance, and if significant, followed by Bonferroni corrected post hoc tests. Comparisons of changes in cognitive scores between the stable and unstable groups were done using Student's T test for independent samples. In all cases P values < 0.05 were considered to be significant. All statistical analyses were performed using SPSS 12 for Windows.

Results

Participants

Children were only included for the present analyses if a first psychiatric evaluation, at t1, was performed before the age of 37 months and if a second evaluation, at t2, was carried out at approximately the age of 42 months, and no sooner than 12 months after the first evaluation. Accordingly, 138 children were selected from the 141 that were clinically evaluated after the screening procedures (Figure 1). In addition, children in whom the presence of a genetic or medical disorder that could be associated with specific phenotypes of psychiatric disorders was confirmed were excluded (Rett's disorder (N = 1), tuberous sclerosis (N = 2), neurofibromatosis (N = 2), 22q11.2 deletion syndrome (N = 1), and fragile X syndrome (N = 1)).

As a result, 131 children were left to be included in the analysis. Of these 131 children, 71 children originated from the population screening and 60 children originated from surveillance by the well baby clinics. These 131 children were on average 26 months old (SD = 6.2) at t1, and on average 45 months old (SD = 6.4) at t2. Accordingly, 53 out of the 80 children with a preliminary diagnosis of ASD were included for the present analyses.

Descriptive Data for Children at t1

The descriptive data for the remaining 131 children at t1 are reported in Table 3 by diagnostic category. Forty children were classified as having an AD by clinical judgement; 13 as having PDD-NOS, 20 as having an intellectual disability, without an ASD, 28 as having an expressive language disorder, 6 as having a mixed receptive-expressive language disorder, 7 as having ADHD, and 4 as having other axis I diagnoses of the DSM-IV-TR (i.e. sleeping disorder, separation anxiety disorder, stereotypic movement disorder, parent-child relational problem); 6 as having borderline intellectual functioning; 7 children were not classified according to the DSM-IV-TR. These children had severe regulatory disorders.

The diagnostic groups differed in chronological age at t1 [ANOVA, $F(8,122) = 4.69$, $P < 0.01$]; post-hoc Bonferroni tests revealed significant higher ages for children with an AD than children with an expressive language disorder, other axis I diagnoses, borderline intellectual functioning, or regulatory disorders; $P < .03$.

Table 3: Demographic Data for Children at t1 and t2

	t1						t2					
Diagnosis	**N**	**Gender**		**Chronological Age in months (SD)**	**N(CSS)[1]**	**CSS[1] (SD)**	**N**	**Gender**		**Chronological Age in months (SD)**	**N(CSS)[1]**	**CSS[1] (SD)**
		M	**F**					**M**	**F**			
AD	40	34	6	29.4 (5.6)	39	57.4 (15.0)	26	22	4	46.2 (5.0)	21	50.7 (18.5)
PDD-NOS	13	9	4	28.2 (5.2)	13	72.3 (18.5)	22	18	4	46.3 (9.8)	19	88.5 (22.6)
ID	20	15	5	26.4 (6.1)	20	60.4 (11.6)	13	8	5	44.6 (2.9)	10	54.0 (14.4)
ELD	28	24	4	24.1 (1.1)	28	83.8 (10.7)	6	6	0	43.0 (5.4)	5	93.6 (11.5)
MR-ELD	6	6	0	24.6	6	83.0 (10.4)	8	7	1	43.8 (6.1)	6	86.0 (5.2)
PhD	0	0	0	–	–	–	16	15	1	43.7 (2.8)	16	100.3 (16.9)
Other DD	0	0	0	–	–	–	2	2	0	40.1 (5.3)	2	103.0 (11.3)
ADHD	7	7	0	25.4 (5.7)	7	94.9 (8.5)	7	5	2	49.5 (9.2)	6	99.8 (20.0)
Other axis I	4	2	2	19.1 (1.0)	4	102.0 (6.2)	3	3	0	40.2 (2.8)	2	95.5 (9.2)
BIF/no axis I	13	7	6	20.8 (5.6)	13	88.5 (13.3)	28	18	10	43.9 (6.3)	25	106.8 (12.4)
Total	**131**	**104**	**27**		**130**		**131**	**104**	**27**		**112**	

AD: Autistic Disorder.
PDD-NOS: Pervasive Developmental Disorder, not otherwise specified.
ID: Intellectual Disability without ASD.
ELD: Expressive Language Disorder.
MR-ELD: Mixed Receptive-Expressive Language Disorder.
PhD: Phonological Disorder.
Other DD: Other Developmental Disorder.
ADHD: Attention-Deficit/Hyperactivity Disorder.
Other axis I, DSM-IV-TR: Other axis I diagnosis.
BIF/no axis I: Borderline Intellectual Functioning and no diagnosis on axis I, DSM-IV-TR.

t1: first psychiatric evaluation.
t2: second psychiatric evaluation.
M: male.
F: female.
SD: standard deviation.
N(CSS): number of children with an available cognitive standard score.
N(CSS)[1]: number of children with an available cognitive standard score.
CSS: cognitive standard score.
CSS[1]: cognitive standard score without correction for floor effect. For correction for floor effect, see text.

Children with an AD had a significantly lower cognitive score than the children in the other diagnostic groups (all $P < 0.03$), with the exception of the children with an intellectual disability without an ASD [ANOVA, $F(8,121) = 18.53$, $P < 0.01$]. In addition, children with PDD-NOS had a significantly lower cognitive score than children with ADHD and other axis I diagnoses (all $P < 0.02$). Ten children cognitively evaluated with the MSEL received the lowest possible score on the instrument and received a cognitive score of 49 (see Table 3). To correct for a possible floor effect, the one-way ANOVA for cognitive score was repeated without these ten children. Accordingly, children with an AD had a significant lower cognitive score than children in all the other diagnostic groups (all $P < 0.03$), with the exception of children with an intellectual disability without an ASD and children with PDD-NOS [ANOVA, $F(8, 111) = 16.13$, $P < 0.01$].

Descriptive data for Children at t2

The descriptive data for the 131 children at t2 are reported in Table 3 by diagnostic category. Twenty-six children were classified as having an AD by clinical judgement, 22 as having PDD-NOS, 13 as having an intellectual disability without an ASD, 6 as having an expressive language disorder, 8 as having a mixed receptive-expressive language disorder, 16 as having a phonological disorder, 2 as having another developmental disorder (developmental coordination disorder), 7 as having ADHD, 3 as having other axis I problems of the DSM-IV-TR (i.e. 2 as having a parent-child relational problem; 1 as having selective mutism); 28 were not classified according to the DSM-IV-TR. These children had severe regulatory disorders.

The diagnostic groups did not differ in chronological age [ANOVA, $F(9, 121) = 1.2$, n.s.]. Children with an AD had a significantly lower cognitive score than the children in the other diagnostic groups (all $P < 0.03$), with the exception of the children with an intellectual disability without an ASD [ANOVA, $F(9, 101) = 20.7$, $P < 0.01$]. In addition children with PDD-NOS had a significantly lower cognitive score than the children with no axis I problems $P < 0.02$, and a significantly higher cognitive score than children with an intellectual disability, without an ASD, $P < 0.01$.

The ADI-R and ADOS-G domain scores per diagnostic group at t1 and t2 are presented in Table 4. Children with a clinical diagnosis of AD received higher scores on all domains than children diagnosed with PDD-NOS or no autism spectrum disorder, indicating more or more severe symptoms. The mean score on the repetitive domain of the ADOS-G, module I, at two years of age for children diagnosed with an AD is 2.9 (SD: 1.5), indicating a high prevalence of restrictive and repetitive behaviours (RRBs) in our sample in this diagnostic group. In our sample, children with PDD-NOS and no ASD show a much lower prevalence at two years of age, 0.8 (SD: 0.9) and 0.7 (SD: 1.2) respectively.

Inter-rater reliability

The inter-rater reliability of the diagnosis established at t1 was measured in 38 children. Two psychiatrists, who had not conducted the psychiatric evaluations and parent interviews, assessed the children independently by reviewing the videotape of the psychiatric evaluation and the written reports of the parent interview and the evaluation of the cognitive development. They were not aware of the diagnosis made by the psychiatrist who conducted the initial evaluation.

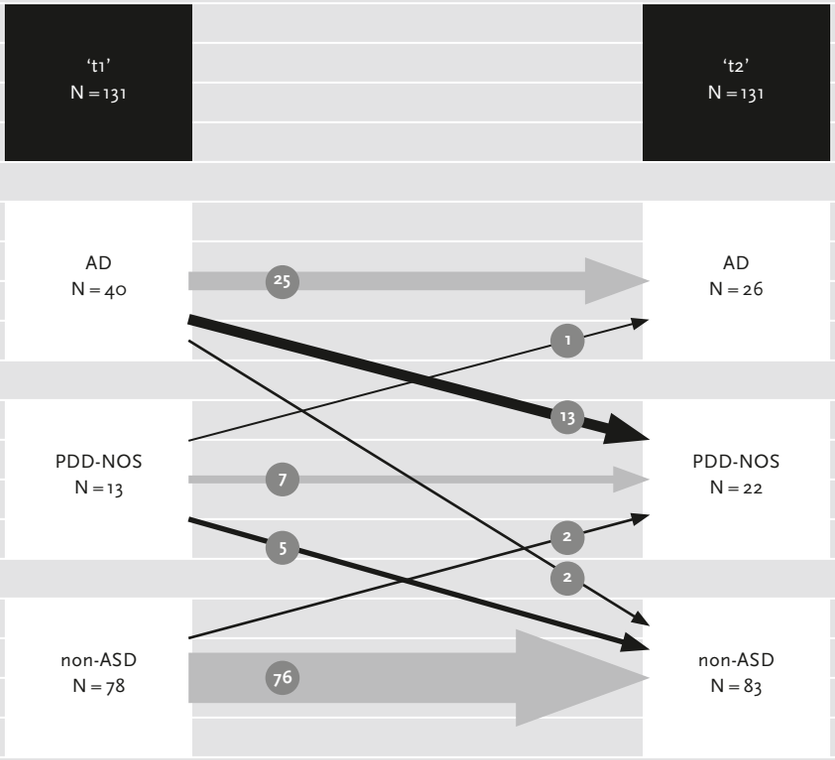
The agreement among psychiatrists regarding ASD diagnoses at t1 was 87%, 33 out of 38 cases, Cohen's kappa (κ), was 0.74 (SE: 0.11). The differentiation between ASD, intellectual disability without ASD, and other diagnostic categories was in 79%, 30 out of 38 cases, in conformity ($\kappa = 0.66$, SE: 0.10). Disagreement was for about 37.5%, 3 out of 8 cases, due to the distinction between ASD and an intellectual disability without ASD. Agreement regarding the distinction between AD and PDD-NOS was 75% ($\kappa = 0.51$, SE: 0.21).

Table 4: ADI-R and ADOS-G Scores by Clinical Diagnoses at t1 and t2

	Clinical Diagnosis, t1						Clinical Diagnosis, t2					
Variable	**AD (SD)**	**N**	**PDD-NOS (SD)**	**N**	**Non-ASD (SD)**	**N**	**AD (SD)**	**N**	**PDD-NOS (SD)**	**N**	**Non-ASD (SD)**	**N**
ADOS-G, social domain, Module 1	11.8 (2.6)	38	3.8 (2.7)	12	2.6 (2.6)	76	10.8 (3.5)	22	2.9 (2.0)	13	1.7 (2.2)	30
ADOS-G social domain, Module 2	8.0 (7.1)	2	5.0 (0.0)	1	-	0	11.5 (0.7)	2	3.4 (3.6)	8	1.0 (1.4)	49
ADOS-G communication domain, Module 1	5.5 (1.6)	38	2.3 (1.6)	12	2.3 (1.7)	76	5.9 (1.6)	22	1.8 (1.8)	13	1.3 (1.2)	30
ADOS-G communication domain, Module 2	5.5 (5.0)	2	4.0 (0.0)	1	-	0	5.0 (0.0)	2	2.4 (1.9)	8	1.3 (1.2)	49
ADOS-G repetitive domain, Module 1	2.9 (1.5)	38	0.8 (0.9)	12	0.7 (1.2)	76	2.6 (1.4)	22	0.8 (1.1)	13	0.6 (1.2)	30
ADOS-G repetitive domain, Module 2	1.0 (1.4)	2	3.0 (0.0)	1	-	0	2.0 (1.4)	2	0.9 (1.1)	8	0.2 (0.5)	49
ADI-R social domain	–	–	–	–	–	–	8.9 (3.4)	22	6.1 (3.4)	19	3.4 (3.4)	57
ADI-R nonverbal communication domain	–	–	–	–	–	–	5.3 (1.8)	22	3.9 (2.4)	19	2.7 (2.3)	57
ADI-R repetitive domain	–	–	–	–	–	–	3.9 (1.3)	22	2.7 (2.1)	19	2.1 (1.4)	57

ADOS-G: Autism Diagnostic Observation Schedule-Generic.
ADI-R: Autism Diagnostic Interview-Revised.
ASD: Autism Spectrum Disorders.
Non-ASD: No Autism Spectrum Disorder.
AD: Autistic Disorder.
PDD-NOS: Pervasive Developmental Disorder, not otherwise specified.
N: number of children of whom data are available on every separate domain of the ADOS-G or the ADI-R.
SD: standard deviation.
t1: first psychiatric evaluation.
t2: second psychiatric evaluation.

Figure 2: Stability of diagnoses between 't1' and 't2'



AD: Autistic Disorder.
PDD-NOS: Pervasive Developmental Disorder not otherwise specified.
Non-ASD: No Autism spectrum disorder.

Stability

Of the 40 children diagnosed with an AD at t1, 25 received the same diagnosis at t2 (see Figure 2), giving a stability of 63%. Of the 13 children diagnosed with PDD-NOS at t1, 7 had the same diagnosis at t2 (stability of 54%). The stability of a diagnosis of ASD between t1 (N = 54) and t2 (N = 47) was 87%.

In turn, sensitivity, that is the probability of a diagnosis of a specific disorder at t1 if the disorder is present at t2, was 96% for AD, 32% for PDD-NOS, and 96% for ASD. There were 7 false positives for ASD at t1. Only 2 children not diagnosed with an ASD at t1 were diagnosed with PDD-NOS at t2 (see Figure 2). Thirteen children (59%) diagnosed with PDD-NOS at t2 were classified as having an AD at t1, and 1 child (4%) diagnosed with an AD at t2 was diagnosed with PDD-NOS at t1.

Characteristics of children with an unstable ASD diagnosis

Forty-six children diagnosed with ASD at t1 had a stable ASD diagnosis at t2 (38 boys and 8 girls), and 7 other children (5 boys and 2 girls), diagnosed with ASD at t1 had a diagnosis other than ASD at t2, i.e. children with an unstable ASD diagnosis. The changes in cognitive scores between t1 and t2 of the children with a stable ASD diagnosis and of the children with an unstable ASD diagnosis were compared. Information about cognitive scores at both t1 and t2 were available for 35 children with a stable ASD diagnosis and 6 children with an unstable ASD diagnosis. The children with an unstable ASD diagnosis showed a significantly higher increase in cognitive scores (mean (M) = 37.2; SD = 13.1) than those with a stable ASD diagnosis (M = 7.4; SD = 22.4) (t (39) = 3.1; P = 0.003). The effect size of this difference is large (Cohen's d = 1.39). The change in cognitive scores between t1 and t2 on the different subscales of the Mullen scales of early learning for the two groups was also compared. The number of children with an evaluation with the Mullen Scales at both t1

and t2 was 14 for the stable ASD group, and 6 for the unstable ASD group. The children with an unstable ASD diagnosis (M = 25.8; SD = 7.9) showed a higher increase in scores on the expressive language subscale than those with a stable ASD diagnosis (M = 8.6; SD = 15.2). The difference is significant: t (18) = 2.6; P = 0.018. The effect size of this difference is large (Cohen's d = 1.27). The gender of the children in the stable and unstable group was compared and showed no significant difference.

Discussion

We found a good agreement (κ = 0.74) between psychiatrists in deciding whether 2-year-old children had an ASD or non-ASD diagnosis. This is in concordance with inter-rater reliability measurements of the distinction between an ASD or non-ASD diagnosis in very young clinically referred children [39], see Table 1. In our study, overall agreement for the finer distinction between AD and other ASD was fair, also comparable with the agreement obtained by experienced clinicians in a sample of clinically referred children [39], see Table 1. The inter-rater reliability in the DSM-IV Field Trial for AD was excellent (κ = 0.95) for clinically referred, older children, in deciding whether a child had an AD or a non-ASD diagnosis [22,45]. In contrast to our findings, we expected that clinician's ability to distinguish between ASD and non-ASD would be lower in very young children, given the possible diagnostic instability and the lack of age-appropriate diagnostic criteria for 2-year-old children. Also, we expected a lower inter-rater reliability in a population based sample in comparison to a clinical referred sample of ASD children, a lower inter-rater reliability for the finer distinction between AD and PDD-NOS. The DSM-IV Autistic Disorder Field Trial reported a kappa of 0.85 regarding the differentiation between AD and other ASD in older children for experienced clinicians, and reported a kappa of 0.59 for inexperienced clinicians [22,45]. Our findings show that the agreement between psychiatrists in deciding whether 2-year-old children have an ASD or non-ASD diagnosis is good, also in children, identified through screening and detected by surveillance [11]. Inter-rater reliability is lower, but still fair for the finer discrimination between an AD and PDD-NOS, as found earlier in clinically referred children. In our study, even experienced clinicians had most disagreement on the distinction between ASD and an intellectual disability without ASD. This illustrates that in the first two years of life the differentiation between delayed and deviant development remains clinically challenging.

The stability of the clinical diagnosis of AD between 26 months and 45 months in our study was 63%, a figure comparable to that of 67% found in the CHAT-study, the only other population-based study. These stability indices are lower than those obtained in clinically referred samples. This may be due to several factors, such as the older mean age of the clinically referred children at the first diagnostic evaluation in comparison to that of children in population-based studies, a factor of importance as found in recent studies [42,33]. Another factor might be that symptom severity usually is higher in clinically referred children compared to very young children selected from the population. The stability of the PDD-NOS diagnosis between 26 months and 45 months in our study was 54%, which is somewhat higher than the stability of PDD-NOS in the CHAT-study, i.e., 33%. The lower stability of the diagnosis of PDD-NOS relative to that of AD may indicate that the diagnosis of AD is based on a more well-defined symptom cluster than that of PDD-NOS. It might also reflect that the diagnosis of AD is reserved for children with more severe symptoms and social handicaps, who are therefore less amenable to change [39]. This is indeed the case in our study, see Table 4. The stability of the diagnoses of ASD overall is lower in our study, i.e., 87%, than that reported in the CHAT-study, i.e., 100%. This difference in overall stability of diagnosis of ASD can express that, unlike the CHAT-study [2], our study included children with an intellectual disability. Differentiating AD with severe intellectual disabilities from equivalent degrees of severe intellectual disabilities

without AD is much more difficult than differentiating AD from a generally less handicapped population [23,25], as was also found in our inter-rater reliability data. Neither the ADOS-G nor the ADI-R show a good specificity in diagnosing very young children with severe intellectual disability [25]. As it is likely that children with ASD who are referred at a young age to a diagnostic facility have intellectual disabilities as well, it is of great importance to improve specificity in diagnostic instruments for young children with AD with severe intellectual disabilities.

Earlier studies observed transitions between the subcategories AD and PDD-NOS, and found particularly that about 50% of children with an initial diagnosis of PDD-NOS around age 2 year received a diagnosis of AD at follow-up [27,39]. In contrast, our study found a reverse pattern that about a third of children with a first diagnosis of AD were diagnosed as having PDD-NOS at follow-up. This pattern was more consistent with another study with clinically referred children [42].

Our second aim was to explore differences in cognitive and verbal scores between children with a stable and unstable ASD diagnosis. The children in our study diagnosed as ASD at t1, and diagnosed as non-ASD at t2, the unstable ASD group, showed a substantial improvement in cognitive scores, especially verbal scores, between t1 and t2, that was significantly larger that the gain in cognitive scores found in the stable group. An increase in cognitive functioning has been reported in young children with a stable ASD diagnosis in earlier studies [6,15,42,48] and in our sample [12]. So far, there appear to be two groups of children with an early diagnosis of ASD identified with our screening instrument: a group of children who showed catch-up growth in language and other cognitive abilities, but still received a diagnosis of ASD at t2, and another group of children who had an even larger improvement in cognitive abilities, especially in the expression of language, but no longer fulfilled criteria for ASD at follow-up. It is essential for our understanding of ASD to follow these children in their further development to be able to determine whether these changes in cognitive and language scores and social functioning are temporary or lasting. Further, it is an important issue to examine whether the improvements of social interaction and communication drive the improvements of cognitive and language skills, or vice versa, whether the speed-up of cognitive and language development drives the changes in social repertoire.

Some limitations of our study should be noted. By our design of a prospective cohort study of children selected by screening from the population, we may have identified children that differ in clinical characteristics from those who are clinically referred. For example, we have screened for children with an early onset of autistic symptoms and early intellectual disabilities. This may have increased the subgroup of children with intellectual disabilities in our selection. The diagnosis of ASD in children who are high-functioning, in whom language milestones are not delayed, and whose cognitive skills are average or above average is likely to be delayed until school age [16,20]. Also, we do not know the sensitivity of our screening instrument, the ESAT. It may well be that we have detected a subgroup of children with ASD, and this needs to be established. Further, our follow-up period of two years is rather short. It is important for our understanding of developmental trajectories of young children with ASD to follow their development over the school age period. Also, in our sample, especially the parents of children with severe intellectual disabilities did not always give consent for a cognitive evaluation at t2, although they did give consent for a psychiatric re-evaluation. This is a general problem encountered in studies on early detection of ASD. Probably parents may be less likely to come in for an evaluation at t2 than at t1, since the child already has been diagnosed at t1 and might be receiving services, which are satisfactory to the parents [21]. In addition, we were not able to use the same measure of cognitive evaluation for all children at both moments of evaluation in time. Comparison of results from different instruments reduces the inter-rater reliability of these results. Also, means and standard deviations of the cognitive level of children in the different diagnostic

subgroups show large differences. Differences in cognitive and language findings between the stable and the unstable ASD group in our cohort should be interpreted with care and regarded as an exploratory finding. Subsequently, an exploratory finding that needs and awaits replication in other studies.

Conclusions

These results show that both AD and the broader category of ASD can be reliably diagnosed in very young children selected by means of a population screening procedure, as was earlier shown for samples of very young, clinically referred children. The stability of AD is higher than that of PDD-NOS. Given (1) the lower inter-rater reliability for the distinction between AD and PDD-NOS in our study, and in earlier studies [39] in very young children, and (2) the transition rate between AD and PDD-NOS and vice versa between the first and later assessments observed in our study and earlier work [27,39,40,42], one may question whether it is valid or useful to differentiate PDD-NOS from AD at age 2 year or below. For clinical practice, it might be more relevant to restrict prediction of a clinical diagnosis to ASD or non-ASD in children younger than two years and to be more careful in diagnosing ASD as a final diagnosis for all children at such a young age.

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3

Emma van Daalen, Claudine Dietz, Edwin P. Martens,
Sophie H.N. Swinkels, Herman van Engeland, Jan K. Buitelaar, Chantal Kemner

Submitted

Is using the dsm-iv algorithm for autistic disorder enough for diagnosing Autism Spectrum Disorders in toddlers?

It is unknown whether the diagnostic algorithm of the DSM-IV-TR for Autistic Disorder can be used to diagnose autistic spectrum disorder (ASD) in young children below the age of 36 months. We examined the predictive value of clinical judgement guided by the diagnostic algorithm of the DSM-IV-TR for Autistic Disorder to diagnose ASD in very young children, and compared this to the predictive power of an instrument that requires training, i.e. the ADOS-G.

From a random population of 31,724 children, 141 very young children with a high risk for ASD were evaluated twice, at a mean age of 26 and 45 months. These evaluations included the use of the DSM-IV-TR criteria for Autistic Disorder and the ADOS-G at the first evaluation, and a psychiatric diagnosis at the second evaluation (i.e. the gold standard).

The complete algorithm of the DSM-IV-TR criteria for Autistic Disorder at the first evaluation shows the best predictive validity (TPV = 0.76) for the clinical diagnosis of ASD at the second evaluation, and has, in comparison to the ADOS-G, a higher percentage of true positives and a lower percentage of false positives for ASD (P = 0.019).

The most reliable instrument for the evaluation of a possible ASD diagnosis in children at 2 years of age is clinical judgement guided by the complete algorithm of the DSM-IV-TR criteria for Autistic Disorder.

Introduction

Autism spectrum disorders (ASD), which include the DSM-IV-TR diagnoses of Autistic Disorder (AD), its subthreshold counterpart Pervasive Developmental Disorder – Not Otherwise Specified (PDD-NOS), and Asperger Disorder, represent a group of developmental disorders defined by deficits in 3 areas of functioning: reciprocal social interaction, communication, and stereotyped and restricted behaviors. For the AD diagnosis, each deficit must be present before 3 years of age.

Although parents often report concerns about the development of children with ASD as early as the second and sometimes the first year of life [1-3], many children receive a diagnosis much later [3,6]. This might be due partly to the lack of clear diagnostic criteria for children at a young age. As a consequence, the early detection of ASD is hindered, which delays the possibilities for early guidance, intervention through a professional support system, and genetic counseling. As several early treatment programs start to report improved communication skills and social behavior; and diminished abnormal behavior in children with ASD, it becomes more evident that psychiatric evaluation of these children at an earlier age is necessary [7-10] Furthermore, several studies have reported that it is indeed possible to reliably diagnose ASD at a younger age [4,6,11-22]. In these studies, a (clinical) diagnosis of ASD made at 2 years is reliable and stable in clinically referred samples up to 3 years, and in some studies up to 9 and 12 years. It has to be noted, however, that diagnostic stability is weaker for children that do not fulfill all criteria for AD (i.e. PDD-NOS), both in clinically referred samples [11,13-18], as well as in samples identified through screening [4,21]. The relative stability of clinical diagnoses at a young age, in the aforementioned studies is based on two factors, namely the use of extensive standardized diagnostic instruments and/or the (implicit) experience of experts in diagnosing early ASD. However, the lack of formal criteria that can be easily used in a clinical setting hinders the evaluation of ASD in young children by professionals in the field, who have less specific expertise in this population, and no access to research instruments. The awareness of the importance of early identification of ASD has increased [23,24]. Accordingly, a growing number of children will be referred to clinicians in the field of ASD at a much younger age than in previous years.

There is an increasing tendency to use research instruments, such as the ADI-R (the Autism diagnostic interview-revised; ADI-R)[25], and the ADOS-G (the Autism diagnostic observation schedule; ADOS-G)[26], for clinical diagnostic purposes, as they are considered to be the essential diagnostic tools by the National Institutes of Health (National Database for Autism Research). However, in normal clinical practice, during a typical single office visit to a clinical psychologist, a developmental pediatrician or a child psychiatrist without specific expertise in the field of very young children, such structured information is typically not obtained. [6] Besides, the administration and scoring of both the ADI-R and ADOS-G require proper training and the means to finance training and coding materials [26-28]. Also, in several studies these standardized instruments applied in children at age 2 years were less successful in predicting clinical outcome than the clinical diagnosis of experienced clinicians [4,6,12]. This indicates that formal criteria for ASD as formulated in ADI-R or ADOS-G algorithms do not always apply to young children. Furthermore, the instruments are not extensively evaluated in children at such a young age. Considering the large number of professionals working in clinical practice with this population, the use of a more accessible diagnostic procedure, adjusted to young children with ASD, without the need for any additional training, might prove valuable.

Probably the best known and most accessible diagnostic tool in clinical practice is the classification algorithm for Autistic Disorder of the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR)[29]. To receive a DSM-IV-TR classification of AD, an

individual's scores must meet separate cut-offs in the social interaction domain (at least 2 criteria met), the communication domain (at least one criterion), and the repetitive and stereotyped behavior domain (at least one criterion). Furthermore, six or more total criteria should be met, in addition to the age-of-onset criterion. With this construct, an assumption is being made that the DSM-IV-TR criteria represent the 'gold standard' for diagnosis of children older than four years of age. However, for younger children, i.e. two years of age, three of the twelve criteria prove irrelevant: the criteria evaluating peer relationships, conversational skills and stereotyped language [14]. In effect, the utility of these criteria or the algorithm, alone, in diagnosing very young children with AD is therefore not evident.

The main aim of the present study is to examine the utility of using clinical judgment together with the diagnostic algorithm of the DSM-IV-TR criteria for AD, in terms of their power to predict a clinical diagnosis of ASD at 42 months (i.e. the gold standard). Furthermore, we aim to directly compare the predictive power of a time-consuming and costly instrument, the ADOS-G, with that of the DSM-IV-TR algorithm. This will be done in very young children, i.e. two years of age, ascertained through screening and surveillance of a sample that includes children with developmental delay and other developmental disorders.

Methods

Study Design

A group of 141 children at 2 years of age with a high risk for ASD were selected. The first group of subjects was selected through a population screening procedure by physicians at all well-baby clinics in the province of Utrecht using the 4-item Early Screening of Autistic Traits (ESAT) scale at a routine 14-month developmental check. (The routine developmental check is part of the system of surveillance for infants and toddlers as it is performed in the well baby clinics in the Netherlands.) [21,30-33]. The second group at-risk children were selected via physician surveillance. Both groups subsequently underwent two comprehensive psychiatric evaluations at the Department of Child and Adolescent Psychiatry of University Medical Centre Utrecht, once at the initial referral at approximately 26 months of age (t1) and once when the children were on average 45 months old (t2). Because of limited resources, only children with a preliminary clinical diagnosis of ASD, intellectual disability, language or phonological disorder after the first psychiatric evaluation, or at parental request were included in a follow-up evaluation.

The ESAT-screening study was performed from October 1999 to April 2002, and 31,724 children from the general population were screened with this instrument. Further details of the screening procedure can be found elsewhere [21,30-33]. A written informed consent was obtained from all parents of the subjects included in the study. The Local Medical Ethics Review Boards approved all procedures.

Participants

A total of 192 very young children with a high risk of ASD were originally included in this study. The inclusion criteria were: 1) failing at least 3 items of the 14-item ESAT scale, 2) the occurrence of a first psychiatric evaluation performed before the age of 37 months and a second evaluation carried out at an approximate age of 42 months, and no sooner than 12 months after the first evaluation. Based on the inclusion criteria, 139 subjects were selected. Exclusion criteria included the presence of a genetic or medical disorder, e.g. Rett syndrome, fragile X syndrome, a deletion on chromosome 22q11.2, neurofibromatosis and tuberous sclerosis. After application of the exclusion criteria, 131 children were included in the analysis. Of these 131 children, 71 children originated from the population screening and 60 children originated from surveillance by the well baby clinics.

Demographics for the sample at the first and second evaluation, t1 and t2, are presented in Table 3, page 46, and the ADOS-G domain scores per diagnostic group at t1 and t2 are presented in Table 4, page 50.

Clinical measurements

The children were given a preliminary clinical diagnosis at t1 based on the clinical judgement of a child psychiatrist, experienced in the psychiatric evaluation of children younger than four years of age. In this preliminary diagnosis, the psychiatrist predicted whether the child was likely to have an AD, PDD-NOS, or another psychiatric diagnosis when he or she was 4 or 5 years old. In addition, all children received an evaluation by a clinical geneticist. The same evaluation procedure was repeated at t2. In addition, the parents were interviewed with the ADI-R by a research associate at t2. The children were assigned DSM-IV-TR diagnoses, based on all the available clinical information, again with exception of the results of the ADOS-G and ADI-R algorithms. The diagnosis AD was reserved for those children meeting the algorithm for AD of the DSM-IV-TR. The other diagnoses of ASD were given to children with serious and pervasive symptoms of ASD, but who did not meet the threshold for AD. The ADI-R Diagnostic Algorithm specifies that the most prototypical autistic behavior is seen at the ages of 4 to 5 years, and that the ADI-R may be less specific or sensitive at younger ages [4]. Further details of the diagnostic procedures can be found elsewhere [21].

Standard diagnostic instruments

At the first evaluation, a child psychiatrist evaluated the children without the systematic use of the DSM-IV-TR criteria before reaching a clinical psychiatric diagnosis, if appropriate. After the diagnostic assignment, each child was evaluated for the presence of each of the twelve DSM-IV-TR criteria for Autistic Disorder concerning deficits of social interaction, communication, or restrictive and repetitive behaviors, and on the fulfillment of the criterion concerning the start of the developmental problems before the age of three years. In this scenario, the preliminary clinical diagnosis was assigned before the presence of the diagnostic criteria was established and without using the classification algorithm specified in the DSM-IV-TR for Autistic Disorder. In addition, the ADOS-G was applied by a research associate. The ADOS-G is a semi-structured, standardized assessment of social interaction, communication, play, and imaginative use of materials for individuals suspected of having ASD. The observational schedule consists of four 30-minute modules, each designed to be administered to different individuals according to their level of expressive language.

Data Analysis

The complete algorithm and the algorithms of the subscales of the DSM-IV-TR criteria for AD, the complete algorithm and the algorithms of the subscales of the algorithm of the ADOS-G and the revised algorithms of the ADOS-G [34] were evaluated on their predictive power for a diagnosis of ASD at 42 months. The revised algorithms of the ADOS-G are a result of reviewing the ADOS Modules 1-3 item and domain total distributions for 1,630 assessments of children aged 14 months to 16 years with an ASD or with heterogeneous non-spectrum disorders. Reflecting recent research, the revised algorithm now consists of two new domains: Social Affect and Restricted, Repetitive Behaviors (RRB) [34].

Contingency tables were applied to assess the relationship between the algorithm of the DSM-IV-TR criteria for AD and the ADOS-G (both the 'classic' and the revised version) administered at two years of age, and the clinical diagnoses evaluated at 42 months, respectively. Sensitivity, specificity and positive predictive value for ASD at 42 months of the DSM-IV-TR algorithm for AD, the ADOS-G algorithms (classic and revised) for AD and the subscales social interaction and communication on this instrument evaluated at 26 months, were established. Differences in the rate of false

Table 3: Positive Predictive value of diagnostic instruments for ASD diagnosis at a young age

Instruments	Sensitivity	Specificity	PPV
DSM-IV-TR criteria for AD, complete algorithm	.88	.84	.76
DSM-IV-TR criteria for AD, subscale for social interaction, subscale algorithm	.96	.58	.57
DSM-IV-TR, criteria for AD, subscale for communication, subscale algorithm	.98	.24	.43
DSM-IV-TR, criteria for AD, subscale for repetitive behaviours and interests, subscale algorithm	.94	.42	.48
ADOS-G, criteria for AD, complete algorithm	.85	.70	.63
ADOS-G, criteria for AD, subscale for social interaction, subscale algorithm	.88	.68	.62
ADOS-G, criteria for AD, subscale for communication, subscale algorithm	.94	.40	.48
ADOS-G, criteria for AD, subscale social interaction, revised subscale algorithm (Gotham et al., 2007)	.85	.69	.62
ADOS-G, criteria for AD, subscale social communication + repetitive behaviour and interests, revised subscale algorithm (Gotham et al., 2007)	.87	.75	.67

ASD: Autism Spectrum Disorders.
DSM-IV-TR: Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision.
AD: Autistic Disorder.
ADOS-G: Autism diagnostic observation schedule-generic.
PPV: Positive Predictive Value.

positive predictions for ASD were assessed using the McNemar test. All statistical analyses were performed using SPSS 15 for Windows.

Results

DSM-IV-TR criteria

In congruence with previous research [14], three of the twelve DSM-IV-TR criteria for AD proved not applicable for the two-year-olds in our study, e.g. the criteria evaluating peer relationships, conversational skills and stereotyped language.

In our sample, 88% of the children with a diagnosis of ASD and 86% of the children with a diagnosis of PDD-NOS at t2 also fulfilled the algorithm of the DSM-IV-TR criteria for AD at t1. In consequence, 12% of the children diagnosed with ASD at t2 did not fulfil the DSM-IV-TR criteria for AD at t1 (false negative). Also, 16% of the children not diagnosed with ASD at t2 fulfilled the DSM-IV-TR criteria for AD at t1 (false positive). The results of the ADOS-G were as follows:

1) 85% of the children with a clinical diagnosis of ASD and 48% of the children with a clinical diagnosis of PDD-NOS, at t2, and 30% of the children not diagnosed with ASD at t2 received an ADOS-G classification of ASD (AD or PDD-NOS) at t1, and
2) 15% of the children diagnosed with ASD at t2 did not receive an ADOS-G classification of ASD (AD or PDD-NOS) at t1. The ADOS-G classification was significantly associated with a higher rate of false positive predictions compared to DSM-IV-TR criteria for AD based classification (P=0.019).

The sensitivity, specificity and positive predictive value of abovementioned scales and subscales are presented in Table 3. Algorithms of these complete scales have a slightly better positive predictive value than the algorithms of the individual subscales. The algorithm of the DSM-IV-TR criteria for AD at t1 has the best positive predictive value for the clinical diagnosis of ASD at t2, see Table 3.

Discussion

Algorithm for AD of the DSM-IV-TR

This study shows that the best positive predictive value for the ASD diagnosis at 42 months is provided by the complete algorithm of the DSM-IV-TR criteria for AD evaluated in children 26 months of age. Because three of the twelve DSM-IV-TR characteristics are rated not applicable for a substantial number of children [14], in order to be diagnosed with ASD, very young children have to score above the cut-off of the complete algorithm with fewer criteria available, i.e. fulfill 6 out of 9 criteria instead of 6 out of 12 criteria.

Further analyses of the subscale algorithms of the DSM-IV-TR criteria for AD or of the complete algorithm and algorithms of respective subscales of more elaborate instruments (the ADOS-G and revised algorithms) [34] evaluated at t1 were not able to improve the predictive power for the ASD diagnosis at 42 months. It is interesting to note that the complete algorithm of the DSM-IV-TR criteria for AD cannot be simplified to subscale(s) to improve the prediction of ASD. It has been reported in some research samples of young children with ASD that they exhibit more problems in the areas of socialization and communication than in the area of stereotyped behaviors and interests [4,5,12,14-16,35].

Noteworthy in our sample is the high prevalence of restrictive and repetitive behaviors (RRBs) at two years of age in children with AD (Table 2). For children diagnosed with AD at two years of age, the mean score on the repetitive domain of the ADOS-G, module I, is 2.9 (SD: 1.5). In contrast, children with PDD-NOS and no ASD show a much lower prevalence at two years of age, 0.8 (SD: 0.9) and 0.7 (SD: 1.2), respectively. One of the main differences between our study and a similar study detecting at-risk children through screening and surveillance at a very young age [4,5] is that our study included children with an intellectual disability, as will be the case in normal clinical practice. Thus, by including very young children with intellectual disability, our data show that the inclusion of all subscales results in the highest predictive power for an early diagnosis of ASD.

Growing awareness of the symptoms of ASD in young children among parents and professionals has resulted in a rapidly increasing number of very young children being referred for a diagnostic evaluation at specialized clinics [22]. It has been suggested that many ASD referrals will also be seen at less specialized clinics [6]. As such, less specialized professionals need a well known and easily accessible diagnostic tool to support the diagnostic evaluation of very young children with symptoms of ASD. For this purpose we evaluated the DSM-IV-TR diagnostic classification for Autistic Disorder as a potential candidate.

When the diagnosis at t2 is considered the final diagnosis, the complete algorithm of the DSM-IV-TR criteria for Autistic Disorder reliably diagnoses AD, but overdiagnoses PDD-NOS in children at two years of age. The ADOS-G, evaluated at t1, can reliably diagnose AD, but not PDD-NOS at t2. And more importantly, the ADOS-G diagnoses a higher percentage of children with a non-ASD diagnosis as ASD at t2. It is known that the transition rate in both directions between AD and PDD-NOS is high during development, as classifications change substantially more often in early years [6,14,16-18,20]. Additionally, in a large sample of children referred for evaluation of possible AD before 36 months of age, diagnostic change was primarily accounted for by movement from PDD-NOS to AD [6]. Consequently, it seems more preferable to overdiagnose children with PDD-NOS as AD at t2, than to overdiagnose children with non-ASD as AD. Therefore, it might be more relevant to restrict prediction of a clinical diagnosis to ASD or non-ASD in children younger than two years, and to be particularly cautious when diagnosing ASD as a final diagnosis for all children at such a young age [21]. This leads to the conclusion that the most reliable procedure for the evaluation of a possible ASD diagnosis in very young children is the use of clinical judgement guided by the complete

algorithm of the DSM-IV-TR criteria for Autistic Disorder. This procedure, in comparison to the ADOS-G, has a higher percentage of true positives and a lower percentage of false positives for ASD.

Limitations of this study include the selection method of the children in this cohort, by screening and surveillance at a young age. These children might not be representative of a cohort of children identified after referral at a young age. Early detection might also create a selective bias towards a subgroup of more developmentally challenged children and overlook the higher functioning ASD children [36].

In conclusion, clinical judgement guided by the DSM-IV-TR algorithm for Autistic Disorder is a feasible, inexpensive and effective procedure for evaluating an ASD diagnosis in very young children. In addition, in comparison to other standardized instruments, it has the highest predictive power for ASD in children at age 3-4 years.

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4

Emma van Daalen, Sophie H.N. Swinkels,
Claudine Dietz, Herman van Engeland, Jan K. Buitelaar

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Body length and head growth in the first year of life in autism

Data on the growth of the head in the first year of life in children with autism spectrum disorders are inconsistent. We measured head circumference and body length during the first year of life, and determined whether the head grew in proportion to body length. This is a case-control study nested in a population-based screening study of autism spectrum disorders. Longitudinal data for head circumference and body length of 53 children with autism spectrum disorders were compared with those of a control group and population norms, using univariate and multilevel statistical modeling. Growth of body length was accelerated, but growth of head circumference was normal in children with autism spectrum disorders compared with controls in the first year of life. The rate of macrocephaly we detected in the first year of life in our sample, 11.3%, fits within the 95% confidence intervals of macrocephaly rates in previous studies. Our findings suggest that autism spectrum disorder is due to a dysregulation of growth in general, rather than to a dysregulation of neuronal growth in the brain. It is unclear whether this early, disproportionate growth of children with autism spectrum disorders is specific to the disorder, and whether this growth could serve as a biomarker to delineate more homogeneous subtypes of autism spectrum disorders.

Introduction

Autism spectrum disorders (ASD) are neurodevelopmental disorders characterized by severe difficulties in social interaction and communication, and with restricted or stereotyped patterns of behavior and interests [1]. ASD include autistic disorder (AD), Asperger syndrome, and pervasive developmental disorder-not otherwise specified (PDD-NOS). The abnormal functioning generally starts before 3 years of age. Although there is abundant evidence of functional and morphologic abnormalities in the brains of subjects with autism spectrum disorders, the pathophysiologic processes underlying ASD are unknown [2]. One of the most consistent findings in children with ASD >3 years of age is an increased rate (14-34%) of macrocephaly (head circumference > 97th percentile) and a larger mean head circumference compared with normally developing children [3,4]. Because head growth is strongly related to brain growth during infancy and early childhood [5,6], an increased head size at a very young age is suggestive of early abnormal development of the brain in autism [3]. The rate of macrocephaly in children with ASD at birth is about 7%, higher than the expected value of 3%, but lower than that seen in older autistic children. Further, most studies report that the mean head circumference of autistic individuals at birth is normal [7-11] or even smaller [5] than that of control subjects. To examine the growth pattern of head circumference in children with ASD and to localize the timing of abnormal growth, longitudinal studies were performed in which information about head circumference between birth and age 3 years was extracted from medical records (Table 1). Four studies found an increased rate of head growth (three in the first year of life [5,10,11], and one between 2-3 years [12]), and two found that growth in body length of children with ASD between birth and age 3 years was more abnormal than that of head circumference, and reported an increased growth of body length around 4 months [9] and between 2-3 years [12].

However, these studies had a number of limitations, such as small sample size, the use of either a reference group or a normal control group but not both, the small number of measurements of head circumference and height per child, and the use of referred versus population-based samples. For these reasons, we investigated a population-based sample of children with ASD, and compared the increase in head circumference and body length with that of normally developing children from the same population, and with population norms. The study was based on the well organized infrastructure of well-baby clinics in the Netherlands, where children up to age 4 years are assessed at regular routine visits, and head circumference, body length, and weight are recorded.

Methods

Study Population

From October 1999 up to April 2002, 31,724 children from the general population were screened with the Early Screening for Autism Traits Questionnaire (ESAT) at their routine 14-month developmental check at well-baby clinics in the province of Utrecht [13]. Children who scored positive at the population screening, and children up to age 36 months identified as having social or communication problems by the monitoring system of the well-baby clinics, were invited for a comprehensive psychiatric assessment at the Department of Child and Adolescent Psychiatry of University Medical Center Utrecht. At this time, the children were on average 23 months old (range, 15-54 months: 74% of the children were aged ≤ 24 months). A second psychiatric assessment was performed when the children were on average 43 months old (range, 34-64 months). Further details of the screening study can be found elsewhere [13,14]. In this way, we identified a group of 76 young children diagnosed with ASD, but in this study we included only those children who met criteria for ASD at both psychiatric

Table 1: Summary of longitudinal studies of Head Growth in the First Three Years of Life in Autism versus Reference Samples; rates of head growth and rates of macrocephaly

	N	Macrocephaly	Mean Head Circumference at Birth	Mean number of measurements of head circumference / child	Growth of Head Circumference	Growth of Body Length	Reference Data
Courchesne et al. 2003	48	Birth: 0 % 6-14 months: 53%	Smaller than normal	2·0 (birth - 14 months)	Accelerated growth in first year of life	Normal growth	Center for Disease Control (2000); Fels Longitudinal Study sample (1988)
Torrey et al. 2004	15	Birth: 13·3% 6-14 months: 6·7% 15-28 months: 0 %	Normal	3·0 (birth - 12 months)	Normal growth	Accelerated growth at 4 months, otherwise normal	N = 49 348 population cohort
Dementieva et al. 2005	42	Birth : 5% 1-2 months: 12% 2-4 months: 18%	6-14 months : 20%	1·8 (birth - 12 months)	Accelerated growth in first year of life	Not measured	Center for Disease Control (2000)
Dissanayake et al. 2006	28	Not reported	Normal	8·6 (birth - 3 year)	Accelerated growth between 2-3 year, otherwise normal	Accelerated growth between 2-3 year, otherwise normal	N = 19 normally developing children
Dawson et al. 2006	28	Not reported	Normal	7·0 (birth - 3 year)	Accelerated growth in first year of life (length as covariate)	Not reported	Center for Disease Control (2002)
van Daalen et al. 2007	53	1-12 months: 11.3%	Normal (at 1 month)	6.0 (1-12 months)	Normal growth	Accelerated growth between 1-6 months	Netherlands Organization for Applied Scientific Research (TNO) (2000) N = 20 normally developing children

assessments. Exclusion criteria were non-Caucasian origin, premature birth or serious birth complications, and the presence of a genetic or medical disorder that could be associated with ASD, such as tuberous sclerosis, fragile X syndrome, and neurofibromatosis. The exclusion of children of non-Caucasian origin was based on the lack of population norms for head circumference of these children. In total, 53 children with ASD (44 boys and 9 girls) were included: 28 children diagnosed with an AD (24 boys and 4 girls), and 25 children (19 boys and 6 girls) diagnosed with a PDD-NOS.

For reference, we used the population norms of the database of the Netherlands Organization for Applied Scientific Research [15]. This database contains data on body length and height, weight, and head circumference for >14,500 boys and girls of Dutch origin aged 0-20 years. The data were collected in 1996 and 1997. To test for secular effects on head circumference between the study cohort and the reference cohort, we also included a comparison group from the study cohort, i.e., a group of normally developing children (n = 22: 9 boys and 13 girls). The study design and screening procedure were approved by the Medical Ethics Review Board of the University Medical Center Utrecht.

Procedures

Children diagnosed with autism spectrum disorders met the criteria for AD, or for a PDD-NOS, according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision [16]. The first comprehensive diagnostic assessment involved the administration of five tests over 5 weeks. At each weekly visit, the social and communicative behavior of the child was observed in a small group of very young children and their parents. The assessments also included a standardized parental interview, a developmental history, the Vineland social emotional early childhood scales [17], and the Autism diagnostic observation schedule-generic [18]. All children had a physical examination and medical workup. On the basis of the available information, an experienced child psychiatrist made a clinical judgment on whether the child was likely to meet the criteria of the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision, for specific categories of ASD (AD, or a PDD-NOS) by about 3-5 years of age.

The clinical diagnosis was re-evaluated when the children were on average 43 months old, using the same assessment protocol as before, but including the Autism Diagnostic Interview-Revised [19]. The inter-rater reliability of three child psychiatrists for distinguishing between autism spectrum disorder and no autism spectrum disorder was calculated. Agreement was reached in 87% of 38 cases: agreement corrected for chance was 0.74 (Cohen's kappa). Intelligence quotient was assessed with the Mullen scales of early learning [20] at the two psychiatric assessments. The subjects were divided into three categories, based on the results of both intelligence quotient assessments: intelligence quotient < 70, intelligence quotient between 70-85, and intelligence quotient >85. See Table 2 for a further description of the study samples.

Head circumference, body length, and weight were measured by well-trained health professionals during routine health examinations at well-baby clinics. In the Netherlands, visits are scheduled when the child is aged about 1 month, 2 months, 3 months, 4 months (facultative), 5 months (facultative), 6 months, 7 months (facultative), 8 months, 11 months, and 14 months. After the first year of life, body length and weight, but not head circumference, are measured during routine health examinations.

Head circumference was measured by placing a plastic, non stretchable tape measure, not too tightly, over the maximum occipital-frontal circumference. Length was measured to the nearest 0.1 cm with the infants in supine position, fully extended, with their heels in contact with the baseboard. Infants up to age 15 months were weighed naked, on calibrated baby scales.

Table 2: Participant's characteristics

Characteristic	ASD	ND
N	53	22
Age, months (mean ± SD) First assessment Second assessment	 30.9 ± 5.9 47.7 ± 8.2	
Boys : girls	44:9	9:13
Intelligence quotient categories, N (%) < 70 70-85 > 85	 33 (62.3%) 6 (11.3%) 14 (26.4)	 0 1 (4.5%) 21 (95.5%)
Duration of pregnancy, weeks (mean ± SD)	39.8 ± 1.35	40.2 ± 1.3
ADOS-G composite score (M ± SD) First assessment Second assessment	 14.3 ± 6.3 11.3 ± 7.5	
ADI-R composite score, impaired social interaction	12.4 ± 8.0	
ADI-R composite score, impaired communication	9.4 ± 5.7	
ADI-R composite score, stereotyped behaviour	3.7 ± 3.4	
z head circumference#, (mean ± SD)	−0.09 ± 0.94	0.01 ± 0.53
Number (%) of children with at least one z head circumference above 1.88 (i.e. >97th percentile)	6 (11.3%)	0 (0%)

ADI-R: Autism diagnostic interview-revised.
ADOS-G: Autism diagnostic observation schedule-generic.
ASD: Autism spectrum disorder.
ND: Normal development.
#,: Z values first averaged per child than per group.

Statistical Analyses

Head circumference, body length, and weight were compared with the population norms of the Netherlands Organization for Applied Scientific Research database [15]. Sex-normalized z-scores (measurements ex-pressed in standard deviations from the population mean) were calculated. As an indication of the ratio between head growth and whole-body growth, the z-score for length was subtracted from the z-score for head circumference for each child and for each time point at which both scores were available. The difference score expresses the extent to which head circumference is in proportion to body length (a positive value reflects that the head is relatively larger, and a negative value reflects that the head is relatively smaller).

Sex-normalized z-scores were analyzed in one-sample t tests against test value zero. This t test was performed for both subgroups (children with ASD, and children with normal development) and every time point separately. Multilevel analyses were then performed [21]. A two-level growth modeling approach was used to predict the difference score between the z-score for length and the z-score for head circumference. The z-scores of 'age in days' were used as time base at the first (within subject) level and at the second (between subjects) level. Because z-scores were used, the hypothesized function was a straight horizontal line through zero, repre-senting a growth curve completely in conformity to the norms. Polynomial functions were tested against this hypothesized or norm function. The ratio of the parameter estimate to the standard error was used to test the signifi-cance of fixed effects. As an additional measure in the model selection proc-ess, a deviance test was performed. An iterative generalized least squares algorithm was used as the maximum likelihood estimate of a model. The deviance test uses the difference in likelihood values (difference in iterative generalized least squares algorithm) of two models as a test statistic with an approximate $\chi 2$ distribution, with the number of different parameters as the degrees of freedom. All analyses were performed with the software packages SPSS version 12.0 and MlWiN [21].

Figure 1a: Age-related changes in head circumference during the first year of life (mean z-score and 95% confidence interval)

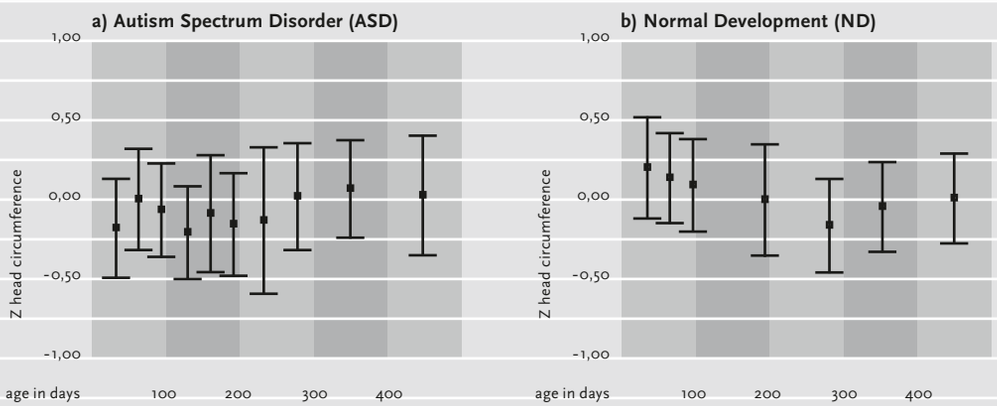
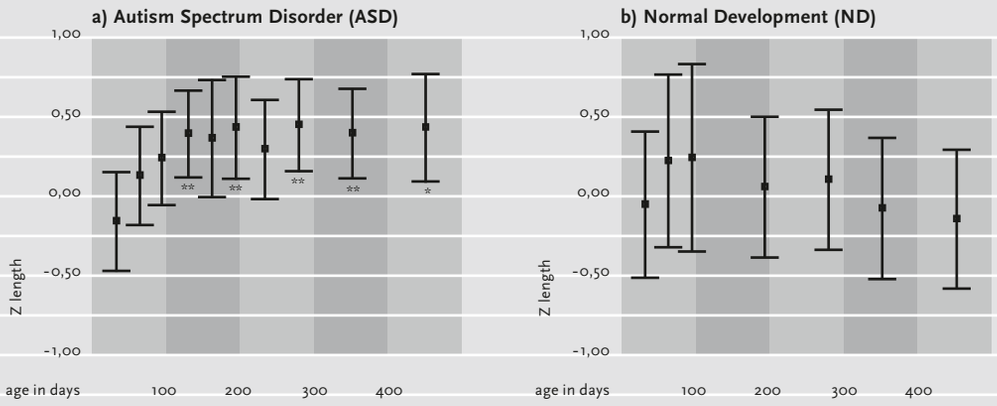


Figure 2: Age-related changes in body length during the first year of life (mean z-score and 95% confidence interval)



* One-sample t tests against test value zero, 0.05 > P > 0.01
** One-sample t tests against test value zero, 0.01 > P > 0.001

Results

Univariate Analyses

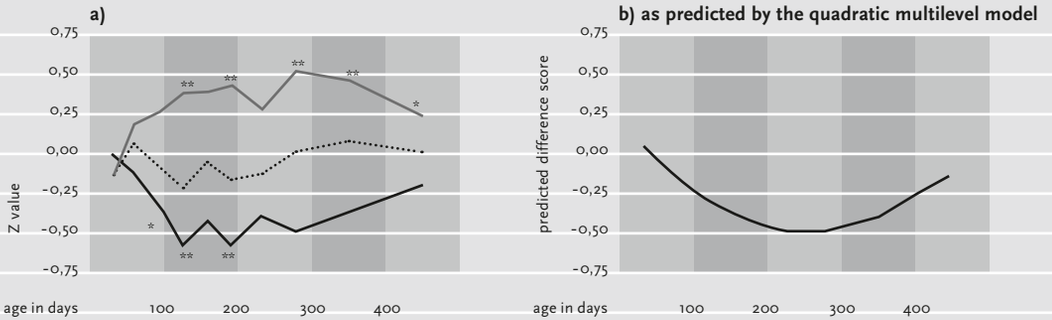
The head-circumference z-scores of children with ASD and of children with normal development were not significantly different from the population norms (Figure 1a,b). The z-scores for length of children with ASD were significantly higher than the population norms from age 4 months onward. The test statistic chance level was <0.01 at ages 4 months, 6 months, 8 months, and 11 months (Figure 2a). The z-scores for length of the control children with normal development did not retain this significant difference (Figure 2b). The z-scores for weight of children with ASD were significantly higher than the norm at ages 3 months ($t(47) = 2.42$, $P = 0.019$) and 4 months ($t(46) = 2.339$, $P = 0.024$).

The difference score (head circumference z-score minus body length z-score) of children with ASD was significantly below zero at ages 3 months ($t(36) = -2.329$: $P = 0.026$), 4 months ($t(31) = -2.912$: $P = 0.007$), 5 months ($t(19) = -3.789$: $P = 0.001$), 6 months ($t(31) = -2.911$: $P = 0.007$), and 8 months ($t(30) = -2.155$: $P = 0.039$). The difference score of z-head circumference minus z-weight yielded comparable results. Figure 3a depicts how the growth rate of head circumference and length changed in the first year of life in children with ASD.

Multilevel Approach

Within the group with ASD, a growth modeling analysis was performed, with the difference score of z-head circumference minus z-length as the outcome measure. The first and second models did not reach statistical significance. The three estimated mean growth parameters of the quadratic model did reach statistical significance, indicating that all three parameters are needed to mathematically describe the average develop-

Figure 3: Multilevel plot of the difference score of z-head circumference minus z-length for children with Autism Spectrum Disorders



Note: Only children with ASD are included in this analysis.

Table 3: Multilevel analyses on the difference score of z head circumference – z length for the ASD group

Final model	Estimate	SE	Statistical significance	IGLS
Fixed effects				
Constant	−0.290	0.115	*	859.1
Linear coefficient: z(day)	−0.684	0.102	*	
Quadratic coefficient: z(day by day)	0.619	0.102	*	
Random effects				
Variation between subjects (u)	0.744	0.144	*	
Variation within subjects (e)	0.262	0.019	*	

* P < 0.05: standard error; IGLS Iterative Generalized Least Squares.
IGLS: Iterative Generalized Least Squares algorithm.

ment trajectory of this difference score in the ASDgroup (Table 3). Adding another (cubic) term to the model did not significantly improve the fit. The deviance tests confirmed the choice of a quadratic model as opposed to a constant, linear, or cubic model. On average, at 4 weeks of age, the head circumference of children with ASDwas in proportion to their body length, but thereafter, head circumference grew more slowly than body length. At about 175 days of age, the growth of body length slowed, and head circumference became somewhat more in proportion to body length (Figure 3b).

Discussion

Our study was distinctive in several respects. We used a sample of children with ASD, identified through population-based screening and the monitoring system of well-baby clinics, and we had access to data on head circumference and body length measurements recorded several times throughout the first year of life. We compared these data with both population norms and data for normally developing children. Further, by using multilevel analyses, we took into account small differences in the timing of measurements and unbalanced data-sets, with different numbers of individuals per measurement and varying intervals between consecutive measurements.

We found that, during the first year of life, the head circumference of children with ASD did not deviate from the population norm or from that of children with normal development, whereas body length did. The normal growth of head circumference and body length in normally developing children was as expected, which supports the validity of our approach.

The finding of normal head growth in children with ASD is consistent with two earlier studies [9,12], but is in contrast with three other reports describing an increased growth of head circumference in the first year of

life in children with ASD [5,10,11]. These discrepancies may be a function of sample selection, composition, and size. We found that the accelerated growth of body length in children with ASD appeared to begin around age 1-2 months, leading to a significant difference at 4 months, but then slowed at about 6 months, which is in accordance with the findings of Torrey et al. [9], but not with those of Dissanayake et al. [12], who found that body length deviated between ages 2-3 years. This pattern of head and body growth in children with ASD was not observed in the normally developing children. In our study, 11.3% of the children had macrocephaly, i.e., at least one head circumference measurement with a z-score >1.88 (i.e., >97th percentile). The 95% confidence interval of this proportion is 4.7-23.7% (including continuity correction). Proportions of macrocephaly in previous studies, e.g., 20% [3,4] and 6.7% [9], all fall within this confidence interval. Because the rate of macrocephaly in our study did not differ significantly from that in other studies, it is very unlikely that our finding of normal head growth was due to a lower proportion of macrocephalic children.

The accelerated growth of body length in the first year of life in children with ASD suggests that ASD should be considered, as supported now by two independent studies, to stem from a general disorganization of growth rather than from a dysregulation of neuronal growth. Thus abnormalities of metabolism, growth factors, or hormone levels may be biological mechanisms underlying ASD. The finding of an increased head circumference in children with ASD has raised interest in neurotrophins, i.e., signaling molecules that promote neuronal growth and survival [22]. Increased levels of neurotrophins could lead to increased brain growth in autism [23], and in fact, increased levels of four neurotrophins and neuropeptides were measured in the blood of newborns later diagnosed with autism [24]. Neurotrophins act in several tissues as growth and survival factors [25]. They modulate body glucose metabolism by central regulation of satiety and food consumption, and by direct modulation of peripheral metabolic and endocrine pathways [25]. A candidate neurotrophin is insulin-like growth factor, which regulates somatic growth and metabolic pro-cesses and the growth, development, and myelinization of the brain [26]. Patients with autism have low levels of insulin-like growth factor and increased levels of insulin-like growth factor-binding protein in their cerebrospinal fluid [27,28].

Further research is needed to establish whether these very early abnormalities of head size in proportion to body length in children with ASD are specific. Hopefully they can thus serve as biomarkers to delineate more homogeneous subtypes of ASD. It will be a step forward for clinical and research purposes if ASD can be differentiated to more homogeneous subtypes on the basis of clinical correlates, course of the illness, brain pathology, or constellation of genetic and environmental risk factors.

Whereas infantile macrocephaly is associated with an increased risk of developing AD [29], data are inconsistent regarding the clinical relevance of macrocephaly in children with ASD. Macrocephaly was associated with better functioning compared with that of normocephalic children with ASD in one report [10], and with greater clinical severity in another report [5]. Longitudinal studies of the growth of head size and body length in other neurodevelopmental disorders, such as developmental language disorders, mental retardation without ASD, and attention-deficit/hyperactivity disorder, and in affected and nonaffected siblings of children with ASD, are warranted.

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5

Ozgen HM, van Daalen E, Bolton PF, Maloney VK, Huang S, Cresswell L, van den Boogaard MJ, Eleveld MJ, van 't Slot R, Hochstenbach R, Beemer FA, Barrow M, Barber JCK, Poot M.

Clin Genet (2009)

Copy number changes of the microcephalin 1 gene (MCPH1) in patients with Autism Spectrum Disorders

Autism spectrum disorder (ASD) represents a set of neurodevelopmental disorders with a strong genetic aetiology. Chromosomal rearrangements have been detected in 5-10% of the patients with ASD, and recent applications of array comparative genomic hybridisation (aCGH) are identifying further candidate regions and genes. In this study, we present four patients who implicate microcephalin 1 (MCPH1) in band 8p23.1 as an ASD susceptibility gene. Patient 1 was a girl with a syndromic form of AD satisfying the Autism diagnostic interview-revised (ADI-R), Autism diagnostic observation schedule (ADOS) and Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria. Oligonucleotide aCGH (oaCGH) showed that she had a classic inv dup del(8)(qter->p23.1::p23.1->p21.2) containing at least three candidate genes; MCPH1 and DLGAP2 within the 6.9-Mb terminal deletion and NEF3 within the concomitant 14.1-Mb duplication. Three further patients with MCPH1 copy number changes were found using single-nucleotide polymorphism (SNP) array analysis in a cohort of 54 families with ASD patients. Our results show that ASD can be a component of the classical inv dup del(8) phenotype and identify changes in copy number of MCPH1 as a susceptibility factor for ASD in the distal short arm of chromosome 8.

Autism spectrum disorder (ASD) represents a set of chronic, progressive and severe neurodevelopmental disorders of childhood characterized by qualitative impairments in social interaction and communication skills,

accompanied by repetitive and stereotyped behaviours and interests. For the subtype of AD, these symptoms manifest in the first 3 years of age and show a lifelong persistence [1]. The prevalence of ASD is estimated to be approximately 1 in 150, with a male to female ratio of 4:1, making it one of the most prevalent medical conditions of childhood [2-4]. The prevalence of mental retardation in AD has been reported to range between 40% and 70% [5-7]. Family and twin studies have shown that ASD has a strong heritable component, but the pattern of inheritance is not straightforward and is likely to involve complex interactions between multiple genes and possibly environmental insults [4,5,8].

Numerous linkage and association studies have implicated several chromosomal regions in ASD, but none of the approaches have directly pinpointed ASD susceptibility genes, and replication of the findings has proven difficult using both approaches [9]. Cytogenetic abnormalities have been detected in 5-10% of the patients with AD [10] and, recently, copy number changes ranging from a few kilobases to several megabases in size have been reported [11-14]. These have facilitated the identification of reciprocal deletion/duplication syndromes and suggested candidate genes, including MCPH1, in patients with ASD [15-17].

Here, we describe a detailed clinical and molecular genetic analysis of four patients who share mental retardation, ASD and copy number changes of a single common gene: microcephalin (MCPH1).

Materials and methods

Clinical reports

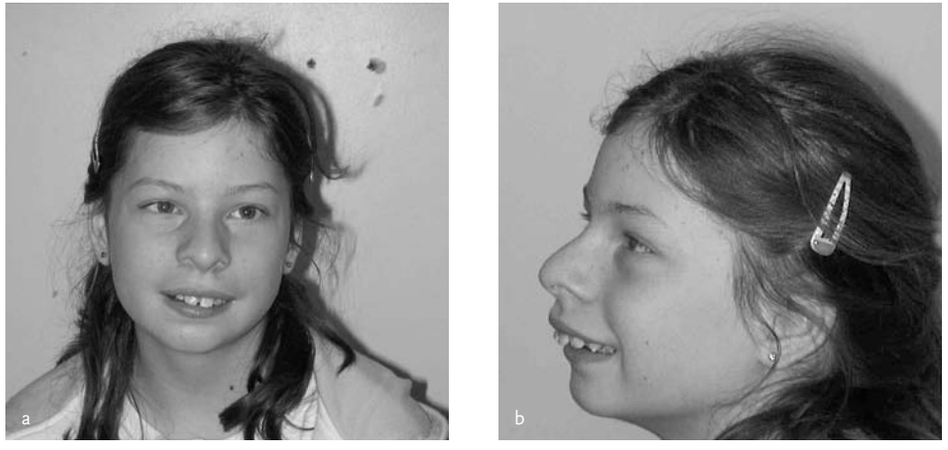
Patient 1 – family 1

The female proband was the second child of non-consanguineous parents. The family history was negative for congenital anomalies, recurrent miscarriages, or other individuals with ASD. Antenatal course was complicated by maternal hyperthyroidism that was treated by carbimazole from 25 weeks of gestation. She was born at term with an Apgar score of 9 at 1 min and 10 at 5 min. Her birth weight was 2800 g (10^{th} percentile). At 19 months of age, she was referred to the genetic clinic for further diagnostic evaluation. Weight, height and head circumference were all on 3rd percentiles. We also assessed morphological features as extensive evidence indicates a high prevalence of dysmorphic features in ASD [18]. Dysmorphic features included prominent metopic region (Figure 1a,b), upslanting palpebral fissures, prominent nasal root, large mouth, high-arched palate, micrognathia and bilateral clinodactyly. She had generalised hypotonia and developmental delay. Apart from tapering fingers and slender, flat feet, no other anomalies were of note on general examination. Brain magnetic resonance imaging (MRI) was reported as normal.

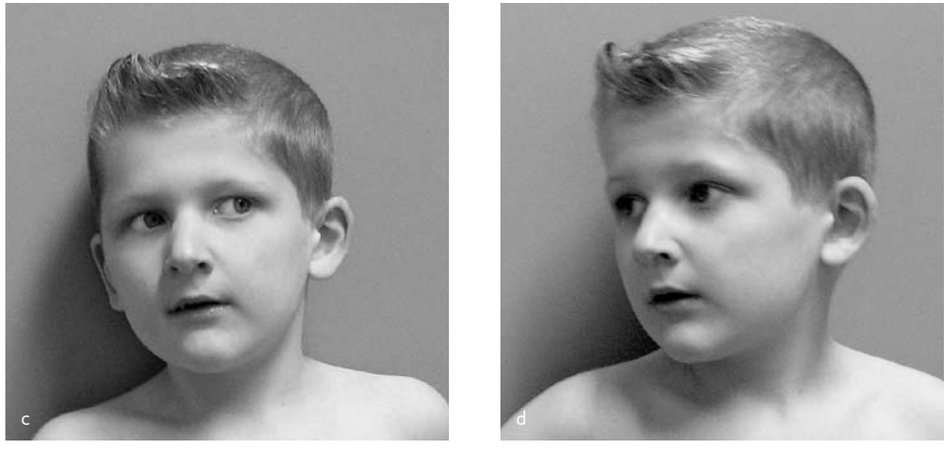
At 15 months of age, repeated episodes of febrile convulsions were observed, controlled with carbamazepine, until 11 years of age, no seizures having been observed after the age of 7 years.

She fulfilled Autism diagnostic interview-revised (ADI-R) [19] criteria for autism with above cut-off scores in all three domains. She was very interested in mirrors and she was echolalic. Non-verbal language skills, such as nodding and pointing to express interest, were limited. She fulfilled the criteria for AD according to the Autism diagnostic observation schedule-generic (ADOS-G) instruments [20]. Throughout most of the ADOS-G session, she exhibited meaningless chatter, speaking in short phrases. She used only limited gesturing, and her eye contact was very

Figure 1: Facial photographs of the patients from family 1 and 2



Frontal (a) and right lateral (b) craniofacial views of the patient from family 1 at the age of 12 years. Note asymmetry of the face with mild frontal bossing, upslanting palpebral fissures, prominent nose with a high bridge, large mouth and micrognathia.



Frontal craniofacial views (c) and (d) of patient 2 at age 10.

limited. Psychometric testing included investigations of receptive and expressive language abilities, non-verbal intellectual abilities and fine and gross motor skills. All tests suggested that her overall cognitive abilities and motor skills are equivalent to those of someone aged 2-3 years.

The overall diagnosis based on ADI-R, ADOS and DSM-IV criteria was AD. Written parental consent was obtained for use of her information in this case report.

Patient 2 – family 2

Patients 2, 3 and 4 were identified in a cohort of 54 families with at least one index patient, who is autistic and carries other clinical features (such as dysmorphisms). The male proband is the first child of non-consanguineous, Caucasian parents, who had no history of miscarriages. His mother developed hypertension during the pregnancy. At 32 weeks of gestation, the mother received pharmacotherapy to restrain preterm labour. The proband was born at 37 weeks of gestation, Apgar scores were 6 and 8. He received oxygen because of some respiratory distress.

He was admitted to the hospital with respiratory problems and tachypnoea at the age of 6 years and 10 weeks. He received physiotherapy for a torticollis. Dysmorphic features included cow's lick (Figure 1c,d), prominent nasal root, micrognathia and cup-shaped ears. His motor milestones showed no delay; he walked independently at 14 months of age. His hearing was adequate. He started making sounds at the age of 18 months and was able to use a few words at the age of 2 years, but he did not articulate well. He was referred for psychiatric evaluation at the age of 2 years with a language delay and received a clinical diagnosis of AD and developmental delay. Routine metabolic screening of urine and plasma was normal. His karyotype was: 46, XY; by fluorescent in situ hybridisation (FISH) no deletion 22q11.2 was found. He tested negative for an increased number of CCG repeats in the FMR1 gene. Results of brain MRI showed an asymmetry of the ventricles. Craniosynostosis was excluded by X-ray analysis of the skull. At 5 years of age, his overall diagnosis, based on ADI-R, ADOS and DSM-IV criteria, was AD.

Patients 3 and 4 – family 3

Patient 3 was born at 41 weeks of gestation as the second child of non-consanguineous, Caucasian parents, who had no history of miscarriages. His Apgar scores were 9 and 10. In the first month, he was admitted to the hospital with cardiac arrhythmias; an electrocardiogram showed no abnormalities, however. He reached motor milestones at appropriate time points. He walked without support at the age of 14 months. His hearing was well developed. He started speaking his first words at the age of 2 years and progressed very slowly. At 3 years of age, he was suspected of seizures and an electroencephalogram was made. No abnormalities were discovered. He suffered from frequent otitis, tonsillitis, hypersalivation, problems with articulation and swallowing. He had feeding problems. He suffered from problems with concentration, hyperactive behaviour and oppositional behavioural problems.

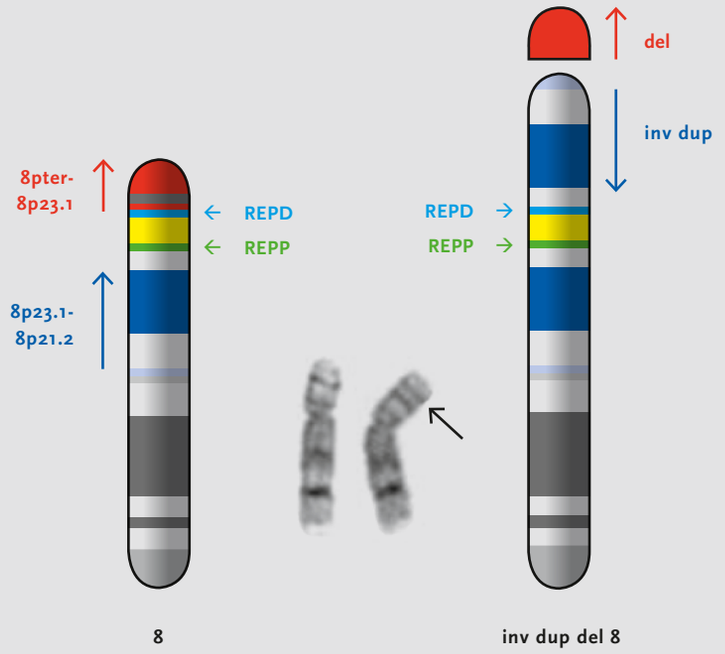
Routine metabolic screening of urine and plasma was normal. His karyotype was 46, XY; by FISH a deletion 22q11.2 was excluded. He tested negative for an increased number of CCG repeats in the FMR1 gene. At 4 years of age, his overall diagnosis, based on ADI-R, ADOS and DSM-IV criteria, was AD.

Patient 4, the sister of patient 3, was the first child of the family who was born at 41 weeks of gestation. Her Apgar scores were 9 and 10. As a baby, she was breastfed for 18 months without problems. She reached motor milestones at appropriate time points. Her language development progressed well. She had frequent otitis media and tonsillitis.

She showed problems in concentrating, hyperactivity and learning problems. She talked incessantly, but did not interact appropriately. Routine metabolic screening of urine and plasma was normal. Her karyotype was 46, XX; by FISH a deletion 22q11.2 was excluded. She tested negative for an increased number of CCG repeats in the FMR1 gene. At 4 years of age, her overall diagnosis, based on ADI-R, ADOS and DSM-IV criteria, was AD.

Figure 2: Illustrative idiograms and partial karyotype of the normal and inv dup del(8) chromosomes of the patient from family 1

The inv dup del(8) appears to have a single extra G-dark band at this level of resolution but the array CGH results show that this extra band is 8p22 within the duplicated region from proximal 8p23.1 (REPP in green) to 8p21.2 (dark blue). The deleted region (red) extends from distal 8p23.1 (REPD in light blue) to 8pter. The region between REPP and REPD (yellow) has a normal diploid copy number. The arrowed blue bar to the right of the normal chromosome 8 indicates the approximate extent of the overlapping duplication (15).



While the father of this family showed depression, timid behaviour and problems concentrating, the mother had learning problems, problems in social contact, articulation problems, compulsive behaviour, problems in concentrating, and hyperactivity. Both parents live in a very isolated situation and have no social network or contact with family members.

The core features of the patients are summarized in Supporting Information Table S1.

Cytogenetic, molecular cytogenetic and molecular analysis

G-banded chromosomes were analysed at the 550 or higher band level. In patient 1, dual colour FISH was carried out with nine Ensembl BACs spanning 8p21.2 to 8pter from the 1 Mb or 37k Sanger Institute clone sets (www.ensembl.org/homo_sapiens/cytoview) and the 8p sub-telomeric probe 2205a2. 22q11 deletions were excluded in families 2 and 3 using FISH with the LSI TUPLE1 probe (Abbott-Vysis, Abbott Park, IL). Repeat numbers in the FMR1 gene were determined by routine methods.

Oligonucleotide array comparative genomic hybridization (oaCGH) was performed in patient 1 with test and normal human male reference DNA using a customized 4x44K array (NGRL WESSEX CONSTITUTIONAL ARRAY CGH V1 design # 015543, Agilent) as previously described [21]. For HumanHap300 SNP array analysis in patients 2, 3 and 4 and their parents, we used 750 ng of patient DNA and followed the protocol as described by the manufacturer (Illumina Inc., San Diego, CA).

Results

Patient 1 – family 1

An extra G-dark band in distal 8p was found in the proband an thought to be a duplication of part of 8p22 or of 8p23.2 (Figure 2). Dual colour FISH with a panel of nine bacterial artificial chromosome (BACs) and the 8p sub-telomere probe indicated a classical inverted, duplicated and deleted chromosome 8p with a comparatively distal 8p21.1/2 breakpoint and normal copy number and orientation of the interval between REPeat proximal (REPP) and REPeat distal (REPD) (data not shown). Mosaicism was excluded in 200 inter-phases and 50 metaphases by looking for a single signal in each interphase or metaphase with the deleted BAC CTD-2629I16 from distal 8p23.1.

OaCGH was used to show that the terminal deletion from 8pter to REPD (in distal 8p23.1) has a minimum size of 6.91 Mb and a maximum size of 7.26 Mb (Figure 3) with a proximal deletion breakpoint within the 349 kb between oligo A-16-P38295006 at 6,907,624 bp and oligo A-16-P01857660 at 7,256,229 bp. The intervening normal copy number region extends for a minimum of 5.03 Mb from REPD in distal 8p23.1 to REPP in proximal 8p23.1 (Figure 3). The duplication has a minimum size of 14.1 Mb and a maximum size of 14.5 Mb (Figure 3). The distal duplication breakpoint is within the 341 kb between oligo A-14-P111014 at 12,285,464 bp and oligo A-16-P38308991 at 12,626,674 bp in REPP. The proximal duplication breakpoint in 8p21.1 is within the 521 kb between oligo A-14-P105065 at 26,711,713 bp and oligo A-14-P131635 at 26,763,834 bp in 8p21.2.

Parental chromosomes were normal, but the mother was heterozygous for the common inversion polymorphism between REPP and REPD, the father was homozygous normal (i.e. he had no inversion; data not shown). The karyotype of the proband was:

46, XX, dup(8)(p22-p23.1or p23.1-p23.3)dn.ish inv dup del(8)(qter->p23.1::p23.1->p21.2:)(141I17+, 395I14+,177H13+,529P14+,369E15+,809L8+, 433L7+,589N15+,211C9+,433L7+,809L8+, 369E15+,529P14+,177H13+,395I14+,2629I16-, 2205a2-).arr 8p21.2p23.1(12,626,674-26,711, 713++)x3,8p23.1pter(pter-6,907,624)x1.

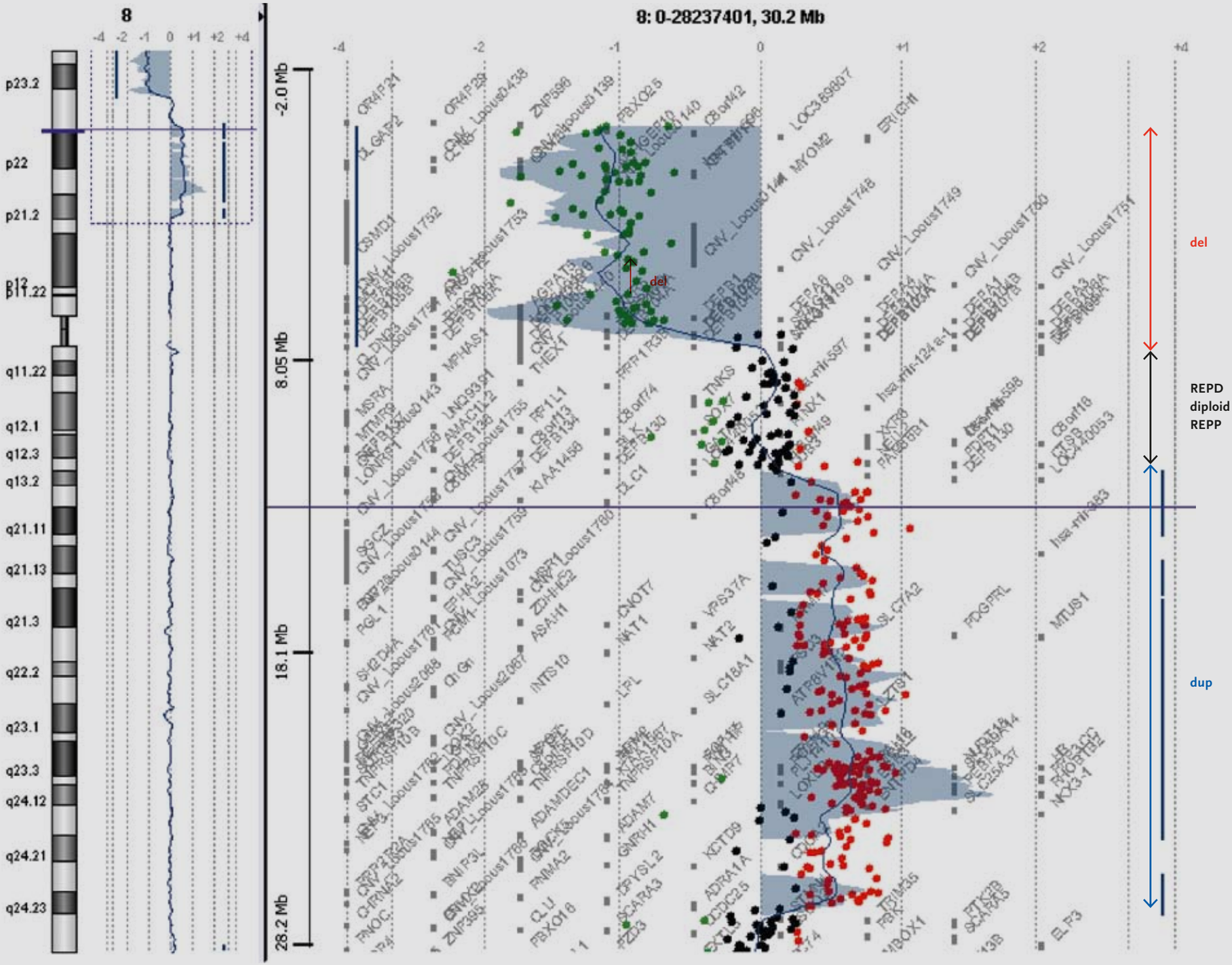


Figure 3: Customised Agilent 44K oligonucleotide array CGH analysis with the deletion (del) from 8pter to REPD in distal 8p23.1 (red arrow), the diploid central segment between REPD and REPP (black arrow) and the duplication (dup) between REPP in proximal 8p23.1 and the breakpoint in 8p21.1 (blue arrow). REPP and REPD are the olfactory receptor/defensin repeats.

Patients 2, 3 and 4 – families 2 and 3

All affected members of families 2 and 3 showed a normal karyotype and tested negative for DiGeorge (deletion 22q11) and Fragile-X syndromes. Patient 2 (family 2) carried a *de novo* hemizygous deletion of 66 kb from SNP rs4840940 up to rs2442502 (from nucleotide position 6,235,652 up to 6,301,894) flanked by the non-deleted SNPs rs2920616 (nucleotide position 6,219,845) and rs2440399 (nucleotide position 6,313,383) demarcating the promoter region and first nine exons of the MCPH1 gene [March 2006 human reference sequence (NCBI Build 36.1; hg18)] (Figure 4). He also shared with his unaffected younger sister a presumably neutral 269 kb copy number variant (according to the Database of Genomic Variants) consisting of 28 SNPs ranging from SNP rs574620 at 15,412,698 bp to SNP rs874836 (269,145 bp) at 15,681,843 bp in pericentromeric 22q11.1, which he inherited from his unaffected father.

Both patients from family 3 carried solely a maternally transmitted duplication of 149 kb from SNPs rs12681546 up to rs1968586 (nucleotide position 6,117,823 to 6,266,919) flanked by non-deleted SNPs rs4570185 (nucleotide position 6,108,358) to rs894888 (nucleotide position 6,266,316), demarcating a large DNA segment upstream from and including the first three exons of the MCPH1 gene [March 2006 human reference sequence (NCBI Build 36.1; hg18)] (Figure 4). Thus, patient 2 carries a *de novo* loss of 66 kb of the proximal part of MCPH1, whereas the patients from the third family carry a maternally inherited gain of 149 kb of the same gene. This maternally inherited duplication partially overlaps with a deletion detected in healthy subjects by McCarrol et al. [22] and by Conrad et al. [23].

Discussion

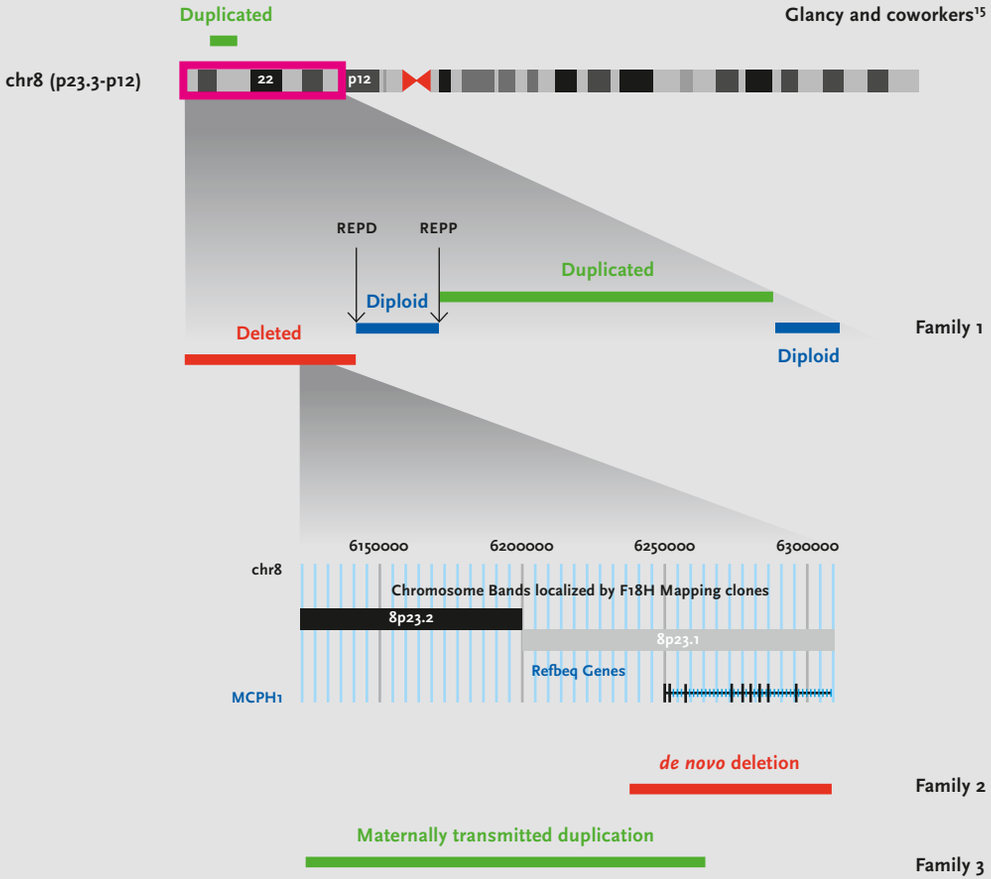
Here, we report on three families in which four patients had ASD and copy number changes affecting a single gene, MCPH1. These results are at odds with the apparently normal phenotype in the carrier parents of children with autosomal recessive primary microcephaly and loss-of-function mutations or partial deletions of MCPH1 [24,25]. However, primary MCPH1 microcephaly has, so far, been described in very few families [24,25] and it is conceivable that features of ASD might yet be found if a larger number of families was ascertained.

Diagnosing autism in children with a very low developmental age is challenging, and valid diagnostic tools were therefore used to support the clinical diagnosis. The ADI-R [19] and the ADOS [20], as used in all cases in this study, are considered to be the 'gold standard' in diagnostic evaluations for autism. The ADI-R is considered to be beneficial in obtaining reliable detailed descriptions of behaviour in a standardized, semi-structured way. According to the ADI-R manual, the instrument can be used appropriately for that purpose in children with mental ages below 24 months [ADI-R manual, Rutter et al. [26] p.3]. In addition, ADOS is validated for children with low developmental ages as well. A recent study of Gotham et al. [27] showed that the module 1 version of the ADOS, as used in our study, can be used in children even with a mental age below 15 months. In addition, in very young or low-functioning children, not able to use meaningful words but with non-verbal mental ages of >15 months, sensitivity and specificity of the ADOS classification is high: 97 and 91, respectively [Gotham et al. [27], Table 3, p.621]. These findings support previous reports of the validity of the ADOS in children with profound mental retardation [28,29]. Berument et al. used the ADOS in a group of children/adolescents with severe/profound mental retardation and concluded that 'even when the clinical picture is dominated by a lack of skills, there are qualitative features that are characteristic of autism' which can be elicited with the ADOS [Berument et al. [28], p.827].

Large inverted duplications of 8p with concomitant deletions are not often associated with AD but rather with developmental delay, mental retardation, facial dysmorphisms, agenesis of the corpus callosum and other problems including congenital heart disease [30,31]. Our patient 1 had a syndromic form

Figure 4: Summary of oaCGH and SNP array data

The large panel shows the 7.26-Mb terminal deletion in patient 1 and the insert depicts the deleted region (red bar) in patient 2 and the duplicated region (green bar) in patients 3 and 4 (for further explanation see text).



of AD satisfying the ADI-R, ADOS and DSM-IV criteria as well as developmental delay, severe learning difficulties, strabismus and dysmorphic features (Figure 1). However, she did not have other features associated with inv dup del(8p)s such as brachycephaly, the distinct facial appearance with prominent thick lips, joint contractures and/or hyper-extensibility, or cardiac defects; this may reflect the comparatively small size of the duplicated region due to the relatively distal 8p21.2 breakpoint compared with other inv dup del(8p)s. Interestingly, a similar case with an 8p21.2 breakpoint had, like our patient 1, seizures as the major presenting feature [32].

The classical inv dup del(8p) in patient 1 contained at least three candidate genes for ASD. MCPH1 and DLGAP2 are both within the 6.9 Mb terminal deletion, distal to REPD, and NEF3 is within the concomitant 14.1 Mb duplication as well as the 6.1 Mb duplication of 8p21 reported in a further patient with syndromic autism [33]. The deleted region in patient 1 also partially overlaps with a transmitted 6.8 Mb duplication of 8p23.1 to 8p23.2 found in a boy with international classification of disease, version 10 (ICD-10) autism and speech delay as well as in his mother with epilepsy and learning difficulties [15]. Of the 19 genes, not affected by copy number variation, only MCPH1 was regarded as a plausible candidate gene [15].

In addition, we report three further affected children with MCPH1 copy number changes in two unrelated families from a cohort of 54 families with ASD (van Daalen et al., in preparation). These children share the behavioural perturbation of the autism spectrum and unspecified mental retardation. Patient 2 carried a *de novo* loss of 66 kb of the promoter region and exons 1 through 9 of MCPH1, whereas both the affected patients 3 and 4 carried a maternally transmitted gain of 149 kb of the promoter region and exons 1 through 3 of the same gene. The mother of patients 3 and 4 also had learning and behavioural problems. Thus, we found a total of four patients with copy number changes involving MCPH1, who share severe mental retardation and behavioural disturbances in the autistic spectrum.

The convergence of phenotypic features with chromosomal imbalances and copy number changes of the MCPH1 gene is intriguing. MCPH1 is expressed in the developing cerebral cortex of the foetal brain where it serves as a specific regulator of brain size by interacting with BRIT1 (BRCT-repeat inhibitor of hTERT expression) [24,34]. Although we did not find the microcephaly and premature chromosome condensation typical of autosomal recessive MCPH1 mutations (MIM:606858 and MIM:251200) [35] or homozygous 5' deletions [25] in any of our patients, it is interesting that impaired Ataxia telangiectasia and Rad3-related (ATR) signalling, implicated in primary MCPH1 microcephaly [34], has been associated with the heterozygous deletions that give rise to the three established haploinsufficiency disorders of blepharophimosis-ptosis-epicanthus inversus syndrome (MIM 110100), Miller-Dieker syndrome (MIM 247200) and Williams-Beuren syndrome (OMIM 194050) [36]. This suggests that MCPH1 may be a dosage sensitive gene in which heterozygous deletion or duplication of MCPH1, or the promoter and 5' exons, is sufficient to cause ASD without any apparent effect on brain size. A precedent for the gain or loss of the same gene or genes giving rise to a similar phenotype is provided by deletions and duplications of the Williams-Beuren critical region, both of which result in neuronal migration defect [37]. Precedents for different mutations in the same gene causing one or more conditions with different modes of inheritance are provided by the receptor tyrosine kinase-like orphan receptor 2 (ROR2) gene, which has been associated with both autosomal recessive Robinow syndrome and autosomal dominant brachydactyly type B (BDB1) [38], and Dymeclin (DYM), in which partial gene duplications and autosomal recessive mutations can both give rise to Dyggve-Melchior-Clausen syndrome [39].

It is also possible that copy number changes of truncated copies of the MCPH1 exert a dominant negative effect. This might be by perturbing the stoichiometry of MCPH1 encoded protein molecules with their putative partners or, as the c-terminal BRCT1 domains are essential for ionizing radiation-induced H2AX focus formation and centrosomal location of MCPH1 [40,41], impeding the link between DNA damage response and proper brain development provided by MCPH1 [42].

In summary, the data on our three families are consistent with the notion that altered gene dosage for MCPH1 causes a condition that is phenotypically distinct from the autosomal recessive disorders with which MCPH1 is commonly associated, and that dosage alterations of MCPH1 may be responsible for the shared autistic features and mental retardation in some patients with ASD. Further detailed study of MCPH1 in patients with ASD would therefore be worthwhile.

Databases

Ensembl tiling path (http://www.ensembl.org/homo_sapiens/cytoview).
OMIM (http://www.ncbi.nlm.nih.gov/entrez/query).
Database of Genomic VariantsEnsembl tiling path (http://projects.tcag.ca/variation/) (March 2006 human reference sequence (NCBI Build 36.1).
The Autism Chromosome Rearrangement Data-base (http://projects.tcag.ca/autism/).

Supporting Information
Table S1. Summary of the main clinical characteristics of four cases with ASD.
Table S2. Genes with transcript expression in the central nervous system located within the duplicated/deleted region of chromosome 8 of our patients.
Supporting information is available as part of the online article at http://www.blackwell-synergy.com

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6

Emma van Daalen (MD), Chantal Kemner (PhD), Nienke E. Verbeek (MD), Maretha V. de Jonge (PhD), Trijnie Dijkhuizen (PhD), Patrick Rump (MD), Renske Houben (MSc), Ruben van 't Slot, J.A.S. Vorstman (MD/PhD), Bert van der Zwaag (PhD), Wouter G. Staal (MD/PhD), Frits A. Beemer (MD/PhD), J. Peter H. Burbach (PhD), Hans Kristian Ploos van Amstel (PhD), Ron Hochstenbach (PhD), Eva H. Brilstra (MD/PhD), Martin Poot (PhD/Dr.med.habil)

Submitted

A systematic approach to evaluating the clinical significance of inherited and *de novo* CNVs in families with idiopathic ASD patients

Recent array-based studies have detected a wealth of Copy Number Variations (CNVs) in patients with Autism Spectrum Disorders (ASD). Since CNVs also occur in healthy individuals their contributions to the patient's phenotype remain largely unclear. To systematically evaluate the clinical significance of CNVs we used two independent strategies in a cohort of ASD patients. First, we performed gene prioritization analyses of the genes covered by those CNVs containing at least one gene known to be transcribed in the brain. Hence, genes involved in ion transport ($KCNMB_3$, $KCNMB_4$), cell communication ($CNTN_5$, CNTN6, $KCNMB_4$, PIK_3CA, RAB_{21}) and regulation of neurotransmitter levels ($KCNMB_4$, TPH_2) were overrepresented. Second, by taking into account the results of the Social Responsiveness Scale (SRS) obtained with both parents, CNVs were distinguished into four categories of potential phenotypic impact. We propose that *de novo* CNVs in families with both parents having normal SRS scores, and CNVs inherited from a carrier parent with deficiencies in reciprocal social interaction, are the most likely to be causally related to ASD. The genes in these two categories participate in previously identified biological pathways associated with ASD, such as contactin-based cell communication ($CNTN_5$, CNTN6) and phosphoinositol signaling (PIK_3CA). Thus, the outcomes of both gene prioritization and SRS-based classification converge on overlapping biological pathways. Our study shows that the scope of genome wide CNV profiling can be extended beyond *de novo* CNVs in sporadic patients, and thus constitutes a first

step toward uncovering the missing heritability in genome wide screening studies of complex psychiatric disorders.

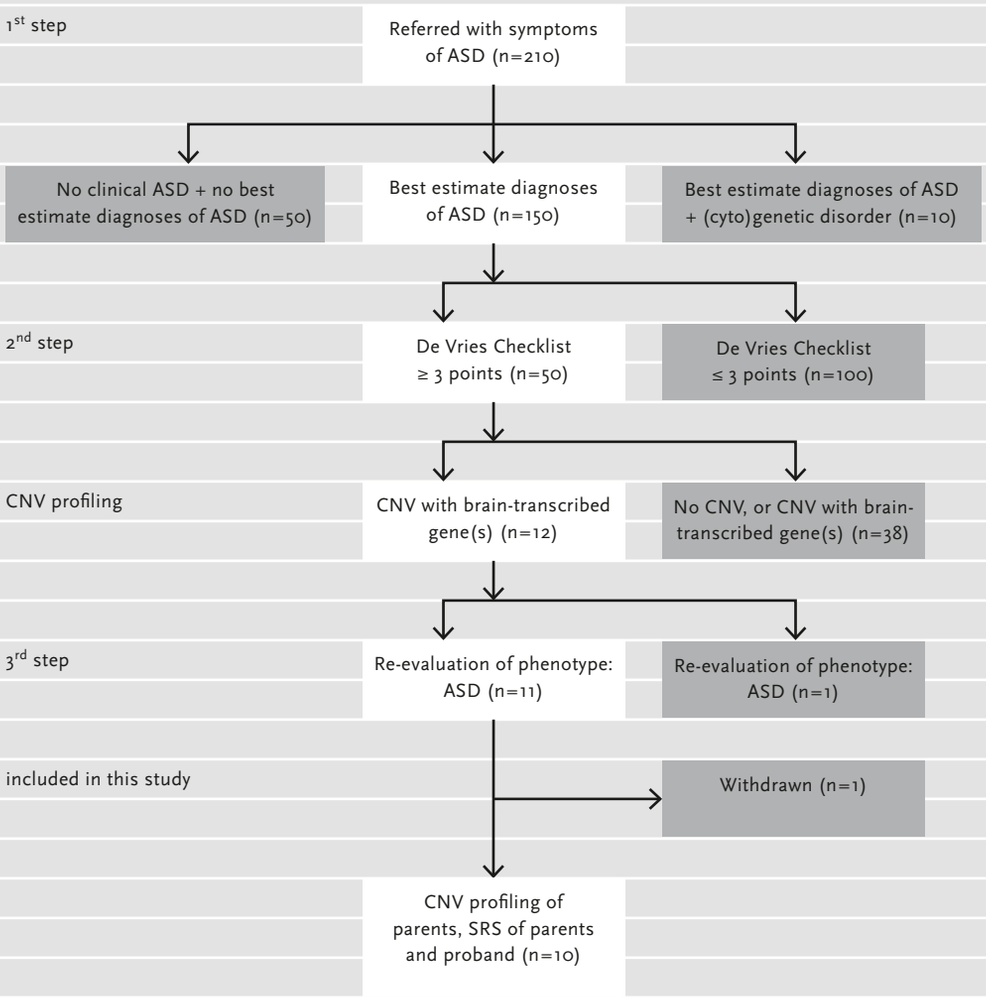
Introduction

Autism Spectrum Disorders (ASDs) represent a group of neurodevelopmental disorders, which involve deficits in up to 3 areas of functioning: reciprocal social interaction, communication, and stereotyped and restricted behaviors. Autistic disorder (AD) is defined as concurrent deficits in all three domain of functioning with at least one defect in one area being detected before completion of 3 years of age [1]. ASDs occur either sporadically or as familial cases, with an estimated prevalence of one in 150 children [2]. Males are four times more frequently affected than females [3]. Comparisons of monozygotic and dizygotic twins suggested a heritability as high as 90% for the narrow phenotype of AD [4,5,6]. Analyzing autism risk in multiplex families from the Autism Genetic Resource Exchange (AGRE) Zhao and co workers (2007) found strong evidence for dominant transmission to male offspring. They hypothesized that two types of families may exist: low-risk families with sporadic autism that is mainly caused by spontaneous mutation with high penetrance in males and relatively poor penetrance in females. Second, are high-risk families in which ASD probands receive a dominant mutation, most often from females, who carry this mutation but are themselves unaffected [7].

ASD has emerged increasingly as a genetically heterogeneous disorder [6]. Searching for specific ASD risk genes by genetic linkage and association studies identified only few of such genes and contributed little to explain the phenotypic variability among ASD patients. Replication of results proved to be difficult [6,8,9,10]. Genome wide segmental aneuploidy profiling revealed submicroscopic structural genome alterations, named Copy Number Variations (CNVs), being either inherited or emerging *de novo* in 7 to 27% of ASD patients under investigation [8,11,12,13,14,15]. It is frequently assumed that a CNV occurring concomitantly with ASD indicates a causal relationship between the CNV and the phenotypic characteristics. Given that CNVs frequently occur in the healthy population, this assumption is questionable [16]; discrimination between neutral variants and pathogenic events becomes an increasingly difficult and challenging task. Therefore, we studied the relationship between genotype and phenotype, not only in patients with ASD, but also in their parents [17].

In order to evaluate the clinical significance of inherited and *de novo* CNVs we selected from the population of ASD patients referred to our institution a cohort of 42 families with a single ASD patient and 8 families with multiple affected children. The index patients of this cohort were subsequently investigated for CNVs using a genome-wide SNP array platform. In families in which the index patient carried a CNV containing at least one gene that is transcribed in the human brain, the patients and their parents were subsequently evaluated for behavioral phenotypes using the Social Responsiveness Scale (SRS) [18,19,20,21]. We propose that CNVs inherited from a parent with a high SRS score and *de novo* CNVs in families in which both parents score low on the SRS are more likely involved in the ASD phenotype of the proband. In addition, we submitted the genes within the CNVs known to be transcribed in the brain to gene prioritization algorithms. Using the data obtained by both approaches we tested the hypothesis that genes contained within CNVs that, by SRS outcome, are more likely to be involved in the ASD phenotype of the probands participate in biological pathways, which may be causally related to ASD. We discuss our findings in terms of a recently proposed model for inherited and sporadic forms of ASD [7].

Figure 1: Flow chart outlining our patient selection procedure.



Material and Methods

Ethics statement

A written informed consent was obtained from all parents of the children included in the study. The Medical Ethics Review Board of the UMC Utrecht approved all procedures.

Psychiatric and clinical genetic evaluation and patient selection

During a three-year interval 210 children, being consecutively referred with symptoms of ASD as detected at or before the age of four years have been routinely evaluated by a specialized team of clinicians for both their psychiatric and clinical genetic phenotypes. A this age a best estimate diagnosis of ASD was obtained by combining a clinical diagnosis of ASD with an ASD classification based on the Autism Diagnostic Observation Schedule-Generic (ADOS-G) [22]. Of those, 50 patients did not reach a best estimate diagnosis and were excluded from this study (Figure 1). In addition, children having a genetic disorder involving ASD (e.g. Rett syndrome, tuberous sclerosis, neurofibromatosis, Smith Lemli Opitz syndrome, 22q11.2 deletion syndrome, and fragile X syndrome; listed in Supplementary Table 1), as well as children with cytogenetic abnormalities as ascertained by routine (see below) karyotyping and molecular genetic diagnosis were also excluded at this first step (Figure 1).

The remaining 150 probands with a best estimate diagnosis of ASD and being evaluated before the age of 4 years, but no (cyto)genetic disorder involving ASD, were included in this study (Figure 1). Patients presenting with a family history of ASD and/or intellectual disability, pre- and postnatal growth disorders, dysmorphic features and congenital anomalies have frequently been found to carry structural genome rearrangements [23]. Therefore, we investigated the retained 150 patients for these symptoms using the checklist for indication of screening for subtelomeric aberrations devised by de Vries and co workers (2001), with an adaptation for

Table 1: CNV's and phenotypes of probands

Characteristics of the proband and family				Genotype of the proband			Psychiatric phenotype of the proband				Clinical characteristics of the proband							Phenotype of the parents	
Proband Number	Gender	Age (years /months)	Multiplex or simplex	Region	CNV Type	Origin	DSM-IV-TR Classification	ADOS-G Classification	ADI-R Classification	SRS-score	CS	FH	IU GR	PG	FD	MM CA	N	SRS-score of parents	
																		Father	Mother
1	Male	5 / 2	Multiplex	2p16.1	Gain	DN	AD	AD	AD	76	NVCS: 102 VCS: 80	–	–	–	+	–	–	21	73 ••
2	Male	6 / 5	Simplex	3p26	Gain	DN	AD	AD	AD	90	75	+	–	–	+	+	–	21	26
3	Male	7 / 2	Multiplex	3p26	Loss	MAT	AD	AD	AD	90	NVCS: 65 VCS: 91							72 *	91••
4	Male	6 / 8	Simplex	3p14.1	Loss	DN	AD	AS	AD	90	69	–	–	–	–	–	–	21	42
4	Male	6 / 8	Simplex	7p22.1	Loss	DN	AD	AS	AD	90	69	–	–	–	+	+	–	21	42
5	Male	6 / 8	Simplex	3q26.32	Loss	DN	AD	AS	AD	90	96	–	–	+	–	–	+	29	37
6	Male	7 / 0	Multiplex	7q31.1	Loss	PAT	AD	AD	AD	84	75	+	–	–	–	–	–	34	95 ••
7	Male	8 / 7	Multiplex	8p23.1	Loss	DN	AD	AD	AD	90	NVCS: 73 VCS: 61	–	–	–	–	–	–	98 **	45
8	Male	9 / 0	Multiplex	11q22.1	Loss	PAT	AS	AS	AS	69	100	+	–	–	+	–	–	66 *	41
9	Male	8 / 10	Simplex	12q15	Loss	DN	AD	AS	AD	76	NVCS: < 50 VCS: 50	–	–	–	–	–	–	27	46 •
10	Male	15 / 10	Multiplex	12q24.11	Gain	DN	AD	AS	AD	90	< 50	+	–	–	+	+	–	103 **	8

Multiplex: multiple affected children in a family
Simplex: single affected child in a family
DN: *De Novo*
MAT: Maternal
PAT: Paternal
DSM-IV-TR: Diagnostic and Statistical Classification of Mental Disorders, 4th edition, Text Revision
ADOS-G: Autism Diagnostic Observation Schedule-Generic
ADI-R: Autism Diagnostic Interview-Revised
SRS: Social Responsiveness Scale AD: Autistic Disorder
AS: Autism Spectrum Disorder

CS: Cognitive Score (Mullen, 1995)
NVCS: non verbal cognitive score
VCS: verbal cognitive score
FH: Family History of ASD and/or intellectual disability
IUGR: Intrauterine Growth Retardation
PG: Postnatal Growth Disorder
FD: Facial Dysmorphic Features
MMCA: Minor Malformations and Congenital Anomalies
N: Neurological Disorder
* : SRS-score for males between 54.6-75.5
** : SRS-score for males above 75.5
• : SRS-score for females between 45.7-63.8
•• : SRS-score for females above 63.8

family history (A positive family history for intellectual disability or ASD, was scored for 1 point) [23]. Probands were included if they scored three points or higher on this checklist (see Table 1 and Figure 1). In this step, a cohort of 50 probands, 42 from simplex and 8 from multiplex ASD families, was selected for genome wide CNV profiling with Illumina Infinium HumanHap300 genotyping beadchip SNP arrays.

In case a CNV contained at least one gene transcribed in the human brain (according to the UCSC Genome Browser; http://genome.ucsc.edu), the proband was re-evaluated for phenotype, based on a clinical evaluation in combination with a standardized interview, the Autism Diagnostic Interview-Revised (ADI-R) [24]. At this third step (Figure 1) one family withdrew from this study, and one child was no longer considered to fit the ASD phenotype, such that 10 families participated in the final phase. Now both parents and the proband were evaluated with the Social Responsiveness Scale (SRS); the probands by parent report and the parents by spouse report. The result of the SRS for parents can be evaluated using the 'parent rating raw score means in the general population': females: 27.6 (SD 18.1), males: 33.7 (SD 20.9) [20,21]. Scores in parents above 2 SD suggest a more severe interference in everyday social interactions and suggest that the parent is affected. Scores in parents between 1 SD and 2 SD indicate deficiencies in reciprocal social interaction and suggest that the parent is partly affected.

In addition, a psychometric test, the Mullen Scales of Early Learning (MSEL) [25] was administered to the proband by a licensed psychologist. The MSEL was used to calculate an overall cognitive score (CS). All phenotypic data of this final subset of probands and their parents are summarized in Table 1.

Karyotyping and Molecular Genetic analyses

We ascertained the patient's karyotype at the 700 band level in cultured peripheral blood lymphocytes according to standard procedures. To confirm segmental aneuploidies detected by the SNP array (see below) BAC-based array CGH [26] or fluorescence in situ hybridization (FISH) with region-specific probes was performed according to Liehr and Claussen (2002) [27].

Illumina infinium HumanHap300 genotyping beadchip SNP array

Infinium HumanHap300 Genotyping BeadChip SNP array analyses were performed according to the protocol of the manufacturer (Illumina Inc., San Diego, CA, USA). Data were analyzed as described before [28].

Results

Genome wide CNV profiling of 50 probands, 42 from simplex and 8 from multiplex families with the Infinium HumanHap300 Genotyping BeadChip SNP array, resulted in a total of 58 copy number variant loci, indicating a wide distribution of CNVs among patients. All CNVs occurred only once and just one of them was flanked by segmental duplications. The precision of breakpoint determination afforded by the SNP arrays (median probe spacing 8 kb) allows unambiguous identification of all genes in a CNV. After exclusion of those CNVs that contained no genes with transcripts in the brain or those that had previously been found in healthy individuals (listed in the Database of Genomic Variants; http://projects.tcag.ca/variation) 10 patients with a total of 8 genomic losses and 3 gains remained. CNVs in regions 7q31.1 (containing IMMP2L) and 8p23.1 (containing MCPH1) have been reported before [29,30] (see Table 1 and Table 2), whereas 6 losses and 3 gains have not been reported before.

Thus, 8 losses and 3 gains were considered to potentially contribute to the ASD phenotype of the index patient, since they contained genes transcribed in the brain. This is by itself not sufficient to establish a contribution to the AD phenotype [17]. To evaluate the potential contribution of a particular CNV we studied the relationship between the CNV and the

Table 2: CNV's of selected probands

Characteristics of the proband			Genotype of the proband			Location of the CNV (as defined by SNP markers)				Genes of interest	Validation	Reference
Proband Number	Gender	Age (years/ months)	Region	CNV Type	Origin	Flanking	Proximal	Distal	Flanking	Brain-transcribed genes	Validation method	Author, Year
1	Male	5 / 2	2p16.1	Gain	DN	rs12464899	rs1056445	rs2589077	rs2864830	CCDC85A	Inheritance	
2	Male	6 / 5	3p26	Gain	DN	rs17047538	rs1357614	rs1846466	rs3772371	CNTN6	Inheritance	
3	Male	7 / 2	3p26	Loss	MAT	rs1159106	rs4684741	rs3772324	rs10510169	CNTN6	Inheritance	
4	Male	6 / 8	3p14.1	Loss	DN	rs7614311	rs704374	rs6798742	rs76298742	ATXN7	FISH	
4	Male	6 / 8	7p22.1	Loss	DN	rs12154986	rs2689420	rs13224907	rs10242703	KDELR2, ZDHHC4, ZNF12,	FISH	
5	Male	6 / 8	3q26.32	Loss	DN	rs4955793	rs6804195	rs2032700	rs12491673	PIK3CA, KCNMB3, ZNF639	FISH	
6	Male	7 / 0	7q31.1	Loss	PAT	rs10258236	rs12671676	rs2969502	rs1894753	5"part of IMMP2L	Inheritance	Maestrini et al. 2009
7	Male	8 / 7	8p23.1	Loss	DN	rs2920616	rs4840940	rs2442502	rs2440399	5"part of MCPH1	FISH	Özgen et al. 2009
8	Male	9 / 0	11q22.1	Loss	PAT	rs2047165	rs2407047	rs518677	rs10894961	CNTN5	Inheritance	
9	Male	8 / 10	12q15	Loss	DN	rs3741600	rs12810179	rs9988925	Rs1516275	KCNMB4, RAB21, TPH2	FISH + BAC-array	
10	Male	15 / 10	12q24.11	Gain	DN	rs7970490	rs3847953	rs616668	rs648997	ATXN2	Inheritance	

DN: *De Novo*.
MAT: Maternal.
PAT: Paternal.

Table 3: Classification of CNVs according to their mode of inheritance and the SRS scores of the parents of the probands

SRS score of parent	Origin of CNV	
	inherited	***de novo***
High (1 SD above the mean)	3: loss of part of CNTN6	1: gain of CCDC85A
	8: loss of CNTN5	7: loss of part of MCPH1
		9: loss of KCNMB4, RAB21, TPH2
		10: gain of part of ATXN2
Low (1 SD below the mean)	6: loss of part of IMMP2L	2: gain of part of CNTN6
		4: loss of KDELR2, ZDHHC4, ZNF12, ATXN7
		5: loss of PIK3CA, KCNMB3, ZNF639

number = family number (see Tables 1 and 2).

ASD phenotype in parents and probands. A strong contribution to the ASD phenotype is presumed if a CNV occurs *de novo* concomitantly with SRS scores below 1 SD above the mean in both parents, or when a CNV is inherited from a parent with a SRS score higher than 1 SD above the mean. A weak to moderate contribution to the autistic symptomatology is presumed in cases in which a *de novo* CNV occurs concomitantly with at least one of the parents scoring higher than 1 SD above the mean on the SRS, and in cases in which a CNV is inherited from a less than 1 SD above the mean scoring parent, while the other has an SRS score higher than 1 SD above the mean. Thus, we distinguish four different categories of ASD families (Table 3).

In our first category, comprising *de novo* CNVs in patients with both parents scoring below 1 SD above the means on the SRS, we retrieved four patients. Those have a gain of part of the CNTN6 gene (in 3p26), a loss of the ATXN7 gene (in 3p14.1) concomitantly with a loss of the KDELR2, ZDHHC4 and ZNF12 genes (in 7p22.1), and a loss of the PIK3CA, KCNMB3 and ZNF639 genes (in 3q26.32).

The second category includes probands with a CNV inherited from a parent with a SRS score higher 1 SD above the mean. Under this category are subsumed losses of CNTN5 (in 11q22.1) and of part of CNTN6 (in 3p26) inherited from a father and a mother, respectively each scoring higher than 1 SD above the mean on the SRS.

A third category, containing probands with *de novo* CNVs who have at least one parent scoring higher than 1 SD above the mean on the SRS, holds *de novo* CNVs of CCDC85A (in 2p16.1), of MCPH1 (in 8p23.1),of ATXN2 (in12q24.11), and of the KCNMB4 and TPH2, RAB12 (in 12q15) genes.

In the fourth category, probands with one parent scoring higher than 1 SD above the mean on the SRS, who inherited a CNV from the other, unaffected parent, only one patient fits: he had a mother scoring higher than 1 SD above the mean on the SRS and inherited from his father scoring lower than 1 SD above the mean scoring on the SRS a loss of part of the IMMP2L gene (in 7q31.1).

The dense probe spacing on our SNP arrays allows precise determination of which genes are contained within a given CNV. By submitting the genes in all four categories (Table 3) to gene prioritization clues regarding some of the biological processes involved in developmental disorders can be obtained [15,31,32]. We, therefore, entered all genes into the GATHER tool for gene prioritization [33]. In a single family we found a *de novo* loss containing PIK3CA, a gene involved in the phosphoinositol pathway of cell signaling, which has already been proposed as a pathway involved in ASD [15]. In addition, 2 genes involved in ion transport (KCNMB3, KCNMB4) appeared as a common theme among our CNVs ($p = 0.03$; Bayes factor 2). Also genes relating to processes of cell communication (CNTN5, CNTN6, KCNMB4, PIK3CA, RAB21) were found in CNVs in our probands, although their level of overrepresentation was borderline significant ($p = 0.01$; Bayes factor 1). Finally, genes related to the regulation of neurotransmitter levels (KCNMB4 and TPH2) were found to be significantly overrepresented ($p = 0.0002$; Bayes factor 5) among the CNVs in our probands.

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In the fourth category, probands with one parent scoring higher than 1 SD above the mean on the SRS, who inherited a CNV from the other, unaffected parent, only one patient fits: he had a mother scoring higher than 1 SD above the mean on the SRS and inherited from his father scoring lower than 1 SD above the mean scoring on the SRS a loss of part of the IMMP2L gene (in 7q31.1).

The dense probe spacing on our SNP arrays allows precise determination of which genes are contained within a given CNV. By submitting the genes in all four categories (Table 3) to gene prioritization clues regarding some of the biological processes involved in developmental disorders can be obtained [15,31,32]. We, therefore, entered all genes into the GATHER tool for gene prioritization [33]. In a single family we found a *de novo* loss containing PIK3CA, a gene involved in the phosphoinositol pathway of cell signaling, which has already been proposed as a pathway involved in ASD [15]. In addition, 2 genes involved in ion transport (KCNMB3, KCNMB4) appeared as a common theme among our CNVs ($p = 0.03$; Bayes factor 2). Also genes relating to processes of cell communication (CNTN5, CNTN6, KCNMB4, PIK3CA, RAB21) were found in CNVs in our probands, although their level of overrepresentation was borderline significant ($p = 0.01$; Bayes factor 1). Finally, genes related to the regulation of neurotransmitter levels (KCNMB4 and TPH2) were found to be significantly overrepresented ($p = 0.0002$; Bayes factor 5) among the CNVs in our probands.

Discussion

Copy number variations (CNVs) are the most frequently detected type of structural genome alterations in ASD patients [12,13,14,15], yet their contribution to the ASD phenotype of an individual patient is not *a priori* clear [17]. It is frequently assumed that only *de novo* CNVs occurring concomitantly with ASD bear a causal relationship with the ASD phenotype [12,13,14,15].

Since a significant proportion of ASD patients may have inherited ASD risk alleles, in particular from their healthy mothers [7], such inherited, but clinically relevant, CNVs may inadvertently get excluded. Therefore, it is pivotal to ascertain whether parents, although not being diagnosed with an outright ASD, may still show some phenotypic features of ASD. For ASD an increased rate of less severe, but similar impairments, termed the broader autism phenotype, is found in 12.4 % of the siblings and in 10-45% of parents of children with ASD [5,6]. Therefore, it is conceivable that in such families the ASD of the patient may have resulted from interaction of an inherited allele from a moderately impaired parent with a *de novo* or inherited CNV.

To understand such distinct patterns of genetic interaction in families and to resolve the potential contribution of CNVs to the ASD phenotype of probands we devised a systematic approach taking into account both the nature of the CNV (inherited or *de novo*) and the outcome of the SRS of both parents. Thus, a priori four categories can be distinguished. First, families in which the proband carries a *de novo* CNVs and both parents scoring 1 SD below the mean on the SRS. In these families the CNVs may by themselves be sufficient to elicit ASD in the proband. Second, families in which the proband inherited a CNV inherited from a parent with a SRS score 1 SD above the mean. Such inherited CNVs may represent examples of dominantly inherited ASD risk alleles, as has been inferred from pedigree analyses [7].

Gene prioritization analyses showed that the CNVs in all four categories appear to be enriched for genes in three shared biological pathways: ion transport (KCNMB3 KCNMB4), cell communication (CNTN5, CNTN6, KCNMB4, PIK3CA, RAB21) and regulation of neurotransmitter levels (KCNMB4 and TPH2). Strikingly these genes are covered by the CNVs presumed to strongly contribute to the ASD phenotype in the top left and bottom right quadrants of Table 3. Thus, the outcomes of both gene prioritization and our family-based CNV categorization converge on the same sets of genes and biological pathways. Therefore, these two independent approaches mutually cross-validate each other.

Thus, our data further support proposals regarding a pathogenic contribution of the phosphoinositol pathway [15]) and of the contactin based-networks of cell communication [34]. In addition, no genes involved in glycobiology were covered by the CNVs found in our patient cohort [32]. It is conceivable that the ASD phenotype in probands without additional minor malformations and congenital abnormalities is caused by a distinct biological mechanism. The pathways identified in this study also contrast with those found in two studies of patients with mental retardation and multiple congenital anomalies [35,36]. Apparently, our approach to evaluate both *de novo* and inherited CNVs in ASD patients allowed us to identify biological pathways distinct from those involved in mental retardation and congenital anomalies and likely to be related to a specific subset of patients with ASD.

In our third category, the proband had a *de novo* CNV, while one of the parents scored higher than 1 SD above the mean on the SRS. It is conceivable that the *de novo* CNV adds a further deleterious effect to an, as yet unidentified, genetic factor transmitted by the affected parent. Such constellations, in which two or more loci may interact to cause ASD, have been reported earlier [14,26]. It remains to be seen, however, whether in such two-loci constellations the two affected genes act in an additive way (i.e. in a single pathway) or synergize in two mutually compensating pathways [26]. Such a case may be proband 7, who carries a *de novo* loss of part of the MCPH1 gene, which has recently been implicated in ASD [30, 37]. Considering the fact that the father of proband 7 has an SRS score 1 SD above the mean may prompt the suggestion that in this case the ASD in the son is the result of two or more etiological factors. Following our family-based interpretation strategy, the previously identified CNV in region 7q31.1 may consequently contribute only moderately to the ASD phenotype in the affected sons [29].

Supplementary Table 1: Patients with (cyto)genetic disorders in the initially referred patient population

Disorder	OMIM	Number of patients
Angelman syndrome	105830	1
Neurofibromatosis 1	162200	1
Tuberous sclerosis	1911000	2
Rett syndrome	312750	2
Smit-Lemli Opitz syndrome	270400	1
Deletion syndrome 22q11.2 (VCF)	192430	1
Fragile X mental retardation syndrome	300624	1

Systematic evaluation of CNVs by taking into account data on gene content and transcription, mode of inheritance and the outcome of the SRS in probands and parents allowed us to distinguish four categories of CNVs and to attribute a relatively strong or an only moderately impact on the ASD phenotype of the proband. Thus, our approach extends the scope of genome wide CNV profiling beyond *de novo* CNVs in sporadic patients. The latter constitutes a first step toward uncovering the missing heritability in genome-wide screening studies of complex disorders [38]. Considering the relatively small size of our sample and that it refers to a specific subset of patients with ASD, our study does not yet allow for exhaustive conclusions [39]. Nevertheless, future replication of our systematic, family-based approach to CNV evaluation followed by gene prioritization may enhance our insights into the impact of rare genetic variants on the etiology of ASD and other complex psychiatric disorders with a high heritability, such as schizophrenia, and idiopathic mental retardation [38].

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7

Summary and general discussion

Summary

The general aim of the SOSO-project (Screenings Onderzoek Sociale Ontwikkeling), which provided the basis for the largest part of this thesis, was to evaluate the early signs and symptoms of Autism Spectrum Disorders (ASDs) in children identified through screening and by surveillance and to determine their potential biological, behavioural, cognitive, and environmental correlates.

This particular thesis has its focus on the early diagnosis of ASDs. This subject is divided into 3 separate parts; the first part centers on the feasibility and the value of a diagnostic evaluation of ASDs around two years of age (Chapter 2). The second part is aimed at providing professionals working with this population with a more accessible diagnostic procedure (Chapter 3). Thirdly, we explored the value of biological measures for the phenotype of ASD (Chapter 4, Chapter 5 and Chapter 6). The research data discussed in Chapter 2, Chapter 3, and Chapter 4 are based on the evaluation of children in the SOSO-project. The data presented in Chapter 5 and Chapter 6 originate from a cohort of children, referred with symptoms of ASD before the age of four years as an outpatient to the Department of Child and Adolescent Psychiatry of the University Medical Centre Utrecht.

Evaluation of the predictive value of a clinical ASD diagnosis in two-year-old children for the diagnosis of ASD at 42 months

When well-trained clinicians are given multiple sources of information, Autistic Disorder (AD), diagnosed after three years of age, is one of the most reliably diagnosable disorders in psychiatry [1]. A valuable asset to researchers and clinicians working with patients with autism spectrum disorder (ASD) is the availability of standardized instruments used to inform diagnosis [2], including the Autism diagnostic interview-revised (ADI-R) [3] and the Autism diagnostic observation schedule-generic (ADOS-G) [4]. Commonly, researchers in ASD apply a best-estimate diagnosis, which is derived by the process of reaching consensus on the diagnosis by the researchers/clinicians who consider the independent clinical diagnosis, the ADI-R and ADOS algorithm scores, and the cognitive, language, and adaptive test scores. However, when children are evaluated for ASD before three years of age, a clinical diagnosis, based on the clinical judgement of a well-trained clinician, proved to be superior to the diagnostic algorithm of the ADI-R or the ADOS-G in predicting children's later diagnostic classification [5-7]. Studies have investigated inter-rater reliability and stability in clinically referred children, younger than 5 years of age, with AD, and have indicated that a diagnosis of AD made at 2 years is stable in clinically referred samples measured at 3 years, and even up to 9 and 12 years [5-16]. Less is known about the reliability and stability of ASD diagnoses in population-based samples, such as the children derived through the SOSO-project.

Accordingly, as a first step in the process of providing a procedure for the evaluation of ASD in children younger than three years of age, we started to determine inter-rater reliability measurements of the ASD diagnoses, based on clinical judgement. We expected that clinician's ability to distinguish between ASD and non-ASD would be lower in very young children, given the possible diagnostic instability and the lack of age-appropriate diagnostic criteria for 2-year-old children. As a result, we found an agreement of $\kappa = 0.74$ between psychiatrists in deciding whether 2-year-old children had an ASD or non-ASD diagnosis. Inter-rater reliability was 0.51 for the finer discrimination between an AD and PDD-NOS. In our study, clinicians had most disagreement on the distinction between ASD and an intellectual disability without ASD. This illustrates that in the first 2 years of life the differentiation between delayed and deviant development remains clinically challenging.

As a second step, we investigated the predictive value of the clinical ASD diagnosis in two-year-old children for the ASD diagnosis at 42 months. As mentioned before, when the child is older than three years of age the diagnosis is considered as very reliable, and in consequence can be used as the 'gold standard'. Our study showed that the stability of a clinical diagnosis of AD made at 20 months was 63% with no false positives for the broader diagnosis of ASD at 42 months. We reported a stability of 54% for the diagnosis of PDD-NOS, and of 87% for the diagnosis of ASD. This means that if a diagnosis is limited to an ASD diagnosis without differentiating this diagnosis into subcategories, it can be done safely at 20 months of age.

Interestingly enough, earlier studies observed transitions between the subcategories AD and PDD-NOS during the development of individual children, and found that about 50% of children with an initial diagnosis of PDD-NOS around age 2 year received a diagnosis of AD at follow-up [7,8]. In contrast, our study found a reverse pattern that about a third of children with a first diagnosis of AD were diagnosed as having PDD-NOS at follow-up. This pattern was consistent with another study with clinically referred children [15].

Our conclusion, regarding the stability of the ASD diagnoses is that given 1) the lower inter-rater reliability for the distinction between AD and PDD-NOS in our study, and in earlier studies [8] in very young children, and 2) the transition rate between AD and PDD-NOS and vice versa between the first and later assessments observed in our study and earlier work [7,8,14,15],

one may question whether it is valid or useful to differentiate PDD-NOS from AD at age 2 year or below. For clinical practice, it might be more relevant to restrict prediction of a clinical diagnosis to ASD or non-ASD in children younger than two years and to be more careful in diagnosing ASD as a final diagnosis for all children at such a young age (Chapter 2).

Part 2

The efficacy of standard instruments in diagnosing ASD at age 2 years

The awareness of the importance of early identification of ASD has increased [17,18]. Accordingly, a growing number of children will be referred to clinicians in the field of ASD at a much younger age than in previous years. The relative stability of a clinical diagnosis of ASD at a young age is based on the (implicit) experience of experts in diagnosing early ASD. However, the lack of formal criteria that can be easily used in a clinical setting hinders the evaluation of ASD in young children by professionals in the field, who have less specific expertise in this population, and lack access to research instruments. There is an increasing tendency to use research instruments, such as the ADI-R (the Autism diagnostic interview-revised; ADI-R) [3], and the ADOS-G (the Autism Diagnostic Observation Schedule; ADOS-G) [4], for clinical diagnostic purposes, as they are considered to be the essential diagnostic tools by the National Institutes of Health (National Database for Autism Research). However, in normal clinical practice, during a typical single office visit to a clinical psychologist, a developmental pediatrician or a child psychiatrist without specific expertise in the field of very young children, such structured information is typically not obtained [7]. Additionally, the administration and scoring of both the ADI-R and ADOS-G require proper training and the means to finance training and coding materials [3,4]. Considering the large number of professionals working in clinical practice with this population, the use of a more accessible diagnostic procedure, adjusted to young children with ASD, without the need for any additional training, might prove valuable.

Probably the best known and most accessible diagnostic tool in clinical practice is the classification algorithm for Autistic Disorder of the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR) [19]. An assumption is being made that the DSM-IV-TR criteria represent the 'gold standard' for the diagnosis of AD in children older than three years of age. However, for younger children, i.e. two years of age, three of the twelve criteria prove irrelevant: the criteria evaluating peer relationships, conversational skills and stereotyped language) [8]. In effect, the utility of these criteria or the algorithm, alone, in diagnosing very young children with AD is therefore not evident.

The focus in Chapter 3 is on the utility of using clinical judgment together with the diagnostic algorithm of the DSM-IV-TR criteria for AD, evaluated at two years of age, in terms of their power to predict a clinical diagnosis of ASD at 42 months (i.e. the gold standard). Furthermore, the aim was to directly compare the predictive power of a time-consuming and costly instrument, the ADOS-G, with that of the DSM-IV-TR algorithm.

The sensitivity, specificity, and positive predictive power of the complete algorithms of the DSM criteria and of the ADOS-G for ASD are .88, .84 and .76, and .85, .70, and .63, respectively.

Because three of the twelve DSM-IV-TR characteristics are rated not applicable for a substantial number of children [8] in order to be diagnosed with ASD, very young children have to score above the cut-off of the complete algorithm with fewer criteria available, i.e. fulfil 6 out of 9 criteria instead of 6 out of 12 criteria.

In conclusion, our study shows that the most reliable standardized instrument for the evaluation of a possible ASD diagnosis in children at age 2 years is the use of the complete algorithm of the DSM-IV-TR criteria for Autistic Disorder (Chapter 3).

Evaluation of the contribution of biological measures to the phenotype of patients with an Autism Spectrum Disorder; head growth and Copy Number Variants (CNVs).

Considering that ASD is a neurodevelopmental disorder with many known functional and morphological abnormalities in the brain of which the pathophysiology and the etiology are unknown, an effort is made in Chapter 4, 5 and 6 to examine the potential contribution of biological and genetic measures to the phenotype of ASD, such as head growth and Copy Number Variants (CNVs).

Head growth

In Chapter 4 we present data that indicate that, during the first year of life, the head circumference of children with ASD did not deviate from the population norm or from that of children with normal development, whereas body length did. As reference, we used the population norms of the database of the Netherlands Organization for Applied Scientific Research (TNO) [20]. To test for secular effects on head circumference between the study cohort and the reference cohort we also included an additional comparison group from the study cohort: a group of normally developing children. The normal growth of head circumference and body length in normally developing children was as expected, which supports the validity of our approach.

We found that the accelerated growth of body length in children with ASD appeared to begin around age 1-2 months, leading to a significant difference at 4 months, but then slowed at about 6 months, which is in accordance with the findings of Torrey et al. [21], but not with those of Dissanayake et al. [22], who found that body length was significantly increased, being most evident after 52 weeks of age.

The finding of normal head growth in children with ASD is consistent with two earlier studies [21,22], but is in contrast with three other reports describing an increased growth of head circumference in the first year of life in children with ASD [23-25]. These discrepancies may be a function of sample selection, composition, and size.

In conclusion, the accelerated growth of body length in the first year of life in children with ASD suggests that ASD should be considered, as supported now by four independent studies, as being due to a general disorganization of growth rather than to a dysregulation of neuronal growth. Thus abnormalities of metabolism, growth factors, or hormone levels may be biological mechanisms underlying ASD.

Copy Number Variants (CNVs): submicroscopic alterations in gene dosage

In Chapter 5 and in Chapter 6 we explored the potential contribution of Copy Number Variants (CNVs) to the phenotype of ASD. These studies are based on a cohort of children, referred with symptoms of ASD before the age of four years as an outpatient to the Department of Child and Adolescent Psychiatry of the University Medical Centre Utrecht.

In Chapter 5 we present four patients of three different families who implicate microcephalin 1 gene (MCPH1) in chromosome band 8p23.1 as an ASD susceptibility gene. The data from our three families are consistent with the hypothesis that altered gene dosage for MCPH1 causes a condition that is phenotypically distinct from the autosomal recessive disorders with which MCPH1 is commonly associated, and that dosage alterations of MCPH1 may be responsible for the shared autistic features and intellectual disability in some patients with ASD.

In Chapter 6 we present an initial evaluation of the clinical significance of inherited and *de novo* CNVs in a cohort of 42 families with a single ASD patient and 8 families with multiple affected children, selected from a population of ASD patients referred to our institution. In families in which the index patient carried a CNV containing at least one gene that is transcribed in the human brain, the patients and their parents were

subsequently evaluated for behavioural phenotypes using the Social Responsiveness Scale (SRS) [26-29]. In addition we submitted all genes being transcribed in the brain to gene prioritization algorithms. To understand distinct patterns of genetic interaction in families and to resolve the potential contribution of CNVs to the ASD phenotype of probands we devised a systematic approach taking into account both the nature of the CNV (inherited or *de novo*) and the outcome of the SRS of both parents. The outcomes of both gene prioritization and our family-based CNV categorization converge on the same sets of genes and biological pathways. Therefore, these two independent approaches mutually cross-validate each other.

General Discussion

Part 1: Evaluation of the predictive value of a clinical ASD diagnosis in two-year-old children for the diagnosis of ASD at 42 months

The first aim of this thesis was to evaluate the diagnosis of ASDs up to three years of age regarding feasibility and predictive value (Chapter 2).

Our study showed that the stability of a clinical diagnosis of ASD, evaluated by a well-trained clinician, made up to three years of age was 87%, with a sensitivity of 96%, and a specificity of 92% for ASD. For AD the stability, the sensitivity and specificity were 63%, 96%, and 86%. For PDD-NOS they were 54%, 32%, and 94%. On the basis of these findings we can conclude that if this diagnosis is limited to an ASD diagnosis without differentiating into subcategories, it can be done reliably at 20 months of age.

One of the main reasons for obtaining an early diagnostic evaluation of children with developmental problems is to provide parents and children with means to facilitate the start of early intervention, educational planning, the development of a professional support system, and advice on family planning. With regard to the children, several early treatment programs report improvements in communication skills and social behaviour, and a decline in abnormal behaviour [30-33]. Our findings indicate that a reliable subdivision of ASD below age 3 is questionable, and that mental health care systems that require such a subdivision as a ticket to services are problematic from a scientific point of view.

Part 2: The efficacy of standard instruments in diagnosing ASD at age 2 years

The second aim of this thesis was to provide other professionals working with this population with a more accessible diagnostic procedure (Chapter 3).

Concerning standardized instruments for a diagnosis of ASD in children younger than three years of age, the most widely used standardized instrument for parent report, the ADI-R [6] tends to over-diagnose ASD in children with nonverbal mental ages of less than 2 years or with severe to profound mental retardation.

The complete algorithm of the DSM-IV-TR criteria for Autistic Disorder, in comparison to the ADOS-G and the revised version of the ADOS-G, has a higher sensitivity and specificity at two years of age for ASD in our sample.

The ADOS-G has a limited value for children with nonverbal mental ages below 16 months. For this age group, the ADOS Module 1 algorithm showed to be over-inclusive, classifying about 81% (19% specificity) of children with intellectual disabilities and/or language impairments as having an Autistic Disorder (AD) or ASD when they are not considered as ASD by clinical judgment. A modification of the ADOS, the ADOS Toddler Module for children under 30 months of age who have minimal speech (ranging from no spoken words to simple two-word phrases), have a nonverbal age equivalent of at least 12 months, and are walking independently [34], has a more promising sensitivity and specificity, but needs to be more widely investigated to prove its value.

As to providing other professionals working with this population with a more accessible diagnostic procedure we concluded that as yet, the most reliable standardized instrument for the evaluation of a possible ASD diagnosis in very young children is the complete algorithm of the DSM-IV-TR criteria for Autistic Disorder. Because three of the twelve DSM-IV-TR characteristics are rated not applicable for a substantial number of children [8], in order to be diagnosed with ASD, very young children have to score above the cut-off of the complete algorithm with fewer criteria available, i.e. fulfil 6 out of 9 criteria instead of 6 out of 12 criteria.

Considering the results of the different procedures used to evaluate ASD, in our sample of 2-year-olds, identified through screening and by surveillance, the sensitivity and specificity for ASD of an evaluation by a well-trained clinician is as yet superior to a classification derived from standardized instruments. However, for all clinicians the complete algorithm of the DSM-IV-TR criteria for Autistic Disorder is a well known and easily accessible diagnostic tool to support the diagnostic evaluation of very young children with symptoms of ASD. Although, more time-consuming and costly standardized instruments, like the ADOS-G, tend to improve the structure of the clinical evaluation, and therefore can be helpful to less experienced clinicians in evaluating such young children.

An important aspect of evaluating very young children with developmental problems in order to predict ASD is to differentiate them from children who develop disorders with a symptom-complex with some similarity to ASD, like language disorders, intellectual disability without ASD, Attention-Deficit and/or Hyperactivity-Disorder (ADHD), or attachment disorders. In our reliability study, even experienced clinicians had most disagreement on the distinction between ASD and an intellectual disability without ASD (Chapter 2). This illustrates that in the first two years of life the differentiation between delayed and deviant development remains clinically challenging. The two children that were false negative for ASD at two years of age were diagnosed with an intellectual disability without ASD, and with ADHD. Of the seven children that were false positive for ASD at two years of age, two children were diagnosed with a phonological disorder, one child with an expressive language disorder, and four children were not diagnosed with a disorder on axis I of the DSM-IV-TR. The false positives for ASD in our study, the unstable ASD group, showed a substantial improvement in cognitive scores, especially verbal scores, between two and three years of age, that was significantly larger than the gain in cognitive scores found in the stable ASD group. An increase in cognitive functioning has been reported in young children with a stable ASD diagnosis in earlier studies [10,15,35,36] and in our sample [37]. In our study we concluded that there appear to be two groups of children with an early diagnosis of ASD identified with our screening instrument: a group of children who showed catch-up growth in language and other cognitive abilities, but still received a diagnosis of ASD at three years of age, and another group of children who had an even larger improvement in cognitive abilities, especially in the expression of language, and no longer fulfilled criteria for ASD at follow-up. It is essential for our understanding of ASD to follow these children in their further development to be able to determine whether these changes in cognitive and language scores and social functioning are temporary or lasting. Further, it is an important issue to examine in which way these and other developing skills possibly mediate each other in the development of social interaction, communication, cognitive and language skills in typically developing children, children with ASD, and also in children with dysfunctions in some, but not all the domains that are affected in children with ASD, like children with language disorders.

In conclusion, a diagnosis of ASD is reliable and stable in two-year-old children. However, is this achievement appreciated by parents?

In her thesis on the SOSO-project: 'The Early Screening of Autism spectrum Disorders' Claudine Dietz evaluated parental compliance after screening for ASD was performed at 14 months of age at the well-baby

clinic. Her results suggested that screening after routine surveillance is more effective than general population screening, resulting in a faster and higher parental compliance. Her results suggest that the next step to the early detection of ASD needs to be on providing effective routine surveillance for children younger than three years of age, rather than establishing the focus on early population screening.

In several countries, the age at diagnosis of children with ASD has, on average, decreased during the last two decades from age 5 to 6 years to age 3 years or even less [44,46]. Nonetheless, the median age of 5.7 years for detection of ASD in the 1994 U.S. birth cohort stresses the need for attention for early detection and diagnosis of ASD in the United States and other countries [50]. Research into factors that influence parents and professionals timing of identification of ASD is necessary [51].

In conclusion, efforts are needed to provide effective routine surveillance for all children up to three years, so that an early diagnostic evaluation can be accomplished.

Part 3: Evaluation of the contribution of biological measures to the phenotype of patients with an Autism Spectrum Disorder; head growth and Copy Number Variants (CNVs)

We explored the potential contribution of biological measures for the phenotype of ASD. We evaluated the value of head growth on the phenotype of ASD in the SOSO-cohort (Chapter 4) and furthermore performed a preliminary evaluation of the possible contribution of Copy Number Variants (CNVs) to the phenotype of ASD in a cohort of consecutive referred children with symptoms of ASD as detected at or before the age of four years that have been routinely evaluated by a specialized team of clinicians for both their psychiatric and clinical genetic phenotypes (Chapter 5 and Chapter 6).

Head growth

One of the main questions considering head growth in relation to the phenotype of ASD is the question whether this biological measure should be considered as an endophenotype for ASD. The concept of 'endophenotype' is used in the context of complex neuropsychiatric diseases, and refers to measurable components linking disease and genotype. An endophenotype may be neurophysiological, biochemical, endocrinological, neuroanatomical, cognitive, or neuropsychological in nature. Endophenotypes can contribute to the understanding of an etiology of a disease by giving more clues on determining its genetic basis than the phenotype of the disease itself. The assumption is that psychiatric diagnoses can be decomposed or deconstructed, which can result in more accessible, and successful, genetic analysis [38].

In our study of head growth we concluded that the accelerated growth of body length in the first year of life in children with ASD suggests that ASD should be considered, as supported now by four independent studies, as being due to a general disorganization of growth rather than to a dysregulation of neuronal growth. In addition, Mraz and colleagues compared growth of head circumference, length and weight of children who had a stable ASD diagnosis with children who lost the ASD diagnosis and to typically developing controls [39]. In their study growth of head circumference and weight were significantly greater in both stable and unstable ASD groups compared to controls with no significant differences between the ASD groups. When length and weight were controlled for, accelerated head growth remained significant in the children who lost their diagnoses. The authors suggest that both the stable and unstable ASD groups show similar head circumference, length, and weight growth trajectories during infancy, although subtle differences in body growth between groups may exist.

With regard to our hypothesis that head growth might be an endophenotype for ASD, our data suggest that it is not head growth per se that is specific to ASD. Moreover, the findings by Mraz and colleagues further

discredit the hypothesis, because to be considered as an endophenotype, the measure has to be associated with illness in the general population. In conclusion, not head growth per se, but body growth might be a candidate endophenotype for ASD.

Copy Number Variants (CNVs):
submicroscopic alterations in gene dosage

Another measurable component to understand ASD is high resolution segmental aneuploidy detection using DNA-microarrays. Because some segmental aneuploidies also occur in normal individuals, (Copy Number Variants or CNVs), their clinical significance is as yet not completely understood [40]. Clinicians and researchers working with this diagnostic technique should consider the following issues: firstly, what do we know regarding the prevalence of CNVs in the normal population, or more precise, which ones of those are contributing to a clinical phenotype? Secondly, are CNVs only linked to disease or also to traits that are not clinically relevant? Thirdly, higher detection rates may lead to more findings without clinical significance, and in that way may potentially harm patients, by over-treatment, under-treatment or stigmatization or may confer a false sense of safety. Fourth, an adequate informed consent implies that the clinician can adequately inform the patient, which is as yet not the case for the clinical consequences of all possible CNVs [40].

We have carried out a pilot study to elucidate the clinical significance of CNVs, transcribed in the brain, and found in our patients, by administering the Social Responsiveness Scale (SRS) to the index patients and to both parents (Chapter 6). By doing so, CNVs containing at least one gene, being transcribed in the brain, were distinguished into four categories of descending relevance for the ASD phenotype of the patient. In addition, we performed gene prioritization analyses of the genes covered by these CNVs. Thus, the outcomes of both gene prioritization and our family-based CNV categorization converge on the same sets of genes and biological pathways. In this way, these two independent approaches mutually cross-validate each other. Our approach extends the scope of genome wide CNV profiling beyond *de novo* CNVs in sporadic patients, and thus constitutes a first step toward uncovering the missing heritability in genome-wide screening studies of complex psychiatric disorders.

In addition, we detected a possible connection between altered gene dosage for MCPH1 and ASD (Chapter 5). Interestingly, MCPH1 is expressed in the developing cerebral cortex of the foetal brain where it serves as a specific regulator of brain size. Autosomal recessive MCPH1 mutations lead to microcephaly [41]. However, in our four patients with an altered gene dosage for MCPH1 this effect on head growth was not found, emphasizing differences in phenotypic outcome of mutations and dosage alterations in a gene.

Conclusions

We determined a reliable procedure for the evaluation of ASD in children younger than three years of age, with a high sensitivity and specificity, even in a population-based sample. Furthermore, we provided other professionals working with children under three years of age with a more accessible diagnostic procedure, also with a good sensitivity and specificity. In conclusion, we have accomplished the availability of a reliable and stable procedure for the evaluation of ASD in children up to three years of age. Considering the median age of 5.7 years for detection of ASD in the 1994 U.S. birth cohort [50], all our efforts are needed to provide effective routine surveillance for all children up to three years of age.

Furthermore, we have evaluated the hypothesis that head growth is an endophenotype for ASD. In conclusion, not head growth *per se*, but body growth might be a candidate endophenotype for ASD.

In addition, we have been able to develop a method as a first step in

clarifying the clinical significance of CNVs for the etiology of ASD. These finding are especially important for the parents of children with later diagnosed ASDs, who consistently have worries relating to the development of their child as early as the first [42] or the second year of life [43,44]. An early evaluation and a subsequent diagnosis of ASD may provide parents with a clear description of the abilities and problems of their child. In consequence, this may lead to an explanation of the daily hassles parents experience in assisting their child with its developmental tasks. Also, it may provide them with feelings of acknowledgement, along with exculpating them from feelings of causing the disorder, and thus reducing unnecessary stress.

Methodological issues

Limitations of the sample derived from the SOSO-project include the selection method of the children in this cohort, by screening and surveillance at a young age. By our design of a prospective cohort study of children selected by screening from the population, we may have identified children that differ in clinical characteristics from those who are clinically referred. For example, we have screened for children with an early onset of autistic symptoms and early intellectual disabilities. This may have increased the subgroup of children with intellectual disabilities in our selection. The diagnosis of ASD in children who are high-functioning, in whom language milestones are not delayed, and whose cognitive skills are average or above average is likely to be delayed until school age [45,46]. Also, we do not know the sensitivity of our screening instrument, the ESAT (see Introduction). It may well be that we have detected a subgroup of children with ASD, and this needs to be established. Further, our follow-up period of two years is rather short. It is important for our understanding of developmental trajectories of young children with ASD to follow their development over the school age period.

Limitations of the sample of children, consecutively referred with symptoms of ASD before the age of four years as an outpatient to the Department of Child and Adolescent Psychiatry of the University Medical Centre Utrecht, are presented by our inclusion criteria and our sample size. In our study we focussed on children with ASD in combination with multiple congenital anomalies and dysmorphic features. As reported [47] our patient cohort was compared to a cohort of probands without these co-morbid features, similar to the original assumption by de Vries et al. 2001 [48], we hypothesized that these phenotypical differences may reflect a difference in the genomic defects underlying the disorder. However, no significant difference in the number or size of CNVs was observed between these patient groups, suggesting that the genes within these regions are responsible for the difference in phenotypes, not the total number of genes that are affected by genomic gain or loss. Considering our cohort of probands, the conclusion that our findings should be regarded as possibly representative only for this unique subgroup is warranted. Furthermore, our sample is too small to be representative for this subgroup in general.

Future directions

After the completion of the SOSO-project, we have conducted a semi-structured interview by telephone with all participating parents after the second psychiatric evaluation of their child (after 42 months) [49]. 97 percent of the parents proved to be in favour of an early evaluation of their child for developmental problems. Early acknowledgement of their worries, the chance to start early intervention for the developmental, and medical problems, and for problematic behaviours, were reported as their considerations. In addition, parents reported to perceive the environment as more supportive when the child received a formal diagnosis.

Another interesting focus is to learn more about the change in symptom patterns in children with ASD over time. Some symptoms may dimin-

ish; others may become more invalidating over time. An example might be the prevalence of restrictive and repetitive behaviors (RRBs) at different ages. A study differentiating RBB's in children at 2 years of age into groups of behaviours that have been shown to cluster together in factor analyses of items scored in the ADI-R describes two separate factors: 1) Repetitive Sensorimotor (RSM) factor, and 2) Insistence on sameness (IS) factor [52]. The RSM factor comprised of repetitive use of objects, unusual sensory interests, hand/finger mannerisms, and other complex mannerisms. The IS factor comprised of compulsions and rituals, difficulties with changes in routine, and resistance to trivial changes in the environment. Unusual preoccupations, unusual attachment to objects, sensitivity to noise, and abnormal/idiosyncratic response to sensory stimuli did not consistently load on either factor. IS behaviours appear not to be associated with ASD at a young age, but may indicate ASD in older children [53,54]. In this study, the behaviours that loaded on the RSM factor were more common and more severe in children with ASD in comparison to children with an intellectual disability without ASD or children without developmental problems. But these behaviours were also relatively common in the group with intellectual disability without ASD. Behaviour that loaded on the IS factor were relatively uncommon and had the same prevalence in all three groups of children. In conclusion, any one RSM behaviour is not indicative of ASD, but having several of these behaviours might be. Also, severe forms of these behaviours, especially RSM behaviours, are indicators of ASD at a young age [52]. Also, symptom clusters ascertained at a certain age may be prognostic for the outcome in adulthood for children with ASD, and may indicate necessary treatment at a young age.

A promising field for further research is the cognitive development of children in the first two years of life who later develop ASD. Klin and colleagues discovered in a serendipitous observation [55], that an infant with AD failed to recognize displays of biological motion, but was instead highly sensitive to the presence of a non-social cue that occurred within the stimuli by chance. This observation raised the possibility that perception of biological motion may be altered in children with AD from a very early age. Developmentally, these results identify an alternative path of neural and behavioural specialization in children with ASD, already before two years of age [56].

Efforts to investigate the potential contribution of biological measures for the phenotype of ASD can lead to the recognition of subgroups within the broad category of ASD. With the example of the 22q11 deletion syndrome, which is already known for two decades, research on this specific group of children has revealed more knowledge on health risks, treatment needs, prognosis, educational needs, and information for family planning [57,58]. Our efforts into subcategorizing ASD could result in a more appropriate appliance of diagnostic and treatment measures, reducing investment of time and energy for parents and their child, and being more cost-effective.

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8

Samenvatting

De vroege diagnose van Autisme Spectrum Stoornissen

Dit proefschrift is grotendeels gebaseerd op het Screenings Onderzoek Sociale Ontwikkeling, het SOSO-project genaamd. Het doel van het SOSO-project was het doen van onderzoek naar vroege signalen en symptomen van autisme spectrum stoornissen (ASS) bij kinderen uit de algemene populatie die op de leeftijd van 14 maanden gescreend werden op problemen in hun sociaal functioneren. Een tweede doelstelling van het SOSO-project was het onderzoek naar biologische, gedragsmatige, cognitieve en omgevingsfactoren die mogelijk een relatie hebben met het ziektebeeld.

Autisme spectrum stoornissen of pervasieve ontwikkelingsstoornissen is de benaming van een groep ontwikkelingsstoornissen, die gekenmerkt wordt door een afwijkende ontwikkeling op het gebied van de sociale interactie, de (non)verbale communicatie en door rigiditeit en stereotiepe interesses en gedrag. Deze afwijkende ontwikkeling leidt tot een verstoring van het dagelijks functioneren van de kinderen, jeugdigen of volwassenen die er aan lijden. Het is een stoornis, waarvan de symptomen van vorm en ernst kunnen veranderen gedurende het leven, maar waarbij altijd een zekere vorm van handicap aanwezig blijft. De verdeling van deze groep stoornissen over mannen en vrouwen laat zien dat mannen vier maal zo vaak zijn aangedaan als vrouwen. De ontwikkelingsstoornis met symptomen op zowel het gebied van de interactie, als de communicatie en met de aanwezigheid van stereotiepe interesses en gedragingen wordt autisme

genoemd. Autisme blijkt bij epidemiologisch onderzoek 32% van de totale groep patiënten met ASS uit te maken. In 67% van de gevallen gaat autisme samen met een verstandelijke beperking. De ontwikkelingsstoornissen die aan autisme verwant genoemd worden, zoals het syndroom van Asperger en de aan autisme verwante stoornis maken voor 17% en voor respectievelijk 51% deel uit van deze groep stoornissen. De aan autisme verwante stoornissen gaan in 12% van de gevallen samen met een verstandelijke beperking [1].

Dit proefschrift heeft als hoofdthema de diagnose van autisme spectrum stoornissen bij kinderen die jonger zijn dan drie jaar.

In dit proefschrift worden drie verschillende onderdelen van dit thema besproken. In het eerste deel wordt verslag gedaan van het onderzoek naar de waarde en de haalbaarheid van het diagnostisch onderzoek naar autisme spectrum stoornissen bij kinderen op de leeftijd van twee jaar (Hoofdstuk 2). Het tweede deel is gericht op de professionals die met deze jonge kinderen werken en heeft als doel om hen te voorzien van een eenvoudige en toegankelijke procedure voor diagnostiek (Hoofdstuk 3). Het derde deel is het verslag van het onderzoek naar biologische factoren die mogelijk gerelateerd zijn aan het fenotype, of de uiterlijke verschijningsvorm, van autisme spectrum stoornissen, ontstaan uit de samenwerking van erfelijke aanleg en factoren uit de omgeving (Hoofdstuk 4, 5 en 6). De wetenschappelijke gegevens, die de basis vormen voor Hoofdstuk 2, 3 en 4, zijn gebaseerd op de klinische gegevens verzameld in het kader van het eerdergenoemde SOSO-project. De wetenschappelijke gegevens die besproken worden in Hoofdstuk 5 en 6 zijn gebaseerd op de klinische gegevens van een groep kinderen die verwezen is naar de polikliniek Kinder- en Jeugdpsychiatrie van het Universitair Medisch Centrum te Utrecht op een leeftijd jonger dan vier jaar.

Deel 1

Evaluatie van de voorspellende waarde van de klinische diagnose autisme spectrum stoornis, gesteld bij kinderen op de leeftijd van twee jaar, voor diezelfde diagnose, gesteld op de leeftijd van drie en een half jaar.

Als goed opgeleide clinici informatie uit verschillende bronnen voorgelegd krijgen, dan blijkt de diagnose autisme, gediagnosticeerd bij kinderen die ouder zijn dan drie jaar, een van de meest betrouwbaar te stellen psychiatrische diagnoses te zijn [2]. Voor clinici en wetenschappelijk onderzoekers is de beschikbaarheid van goed gevalideerde, gestandaardiseerde instrumenten voor diagnostiek, zoals het Autisme Diagnostisch Interview Revised (ADI-R) [3] en het Autisme Diagnostisch Observatie Schema (ADOS-G) [4], een waardevolle aanvulling voor het diagnostische proces [5].

Voor wetenschappelijk onderzoek op het gebied van autisme wordt vaak gebruik gemaakt van een diagnose die gebaseerd is op de gecombineerde resultaten van psychologisch onderzoek, van deze gestandaardiseerde instrumenten en van een evaluatie van de patiënt door een ervaren clinicus. Bij kinderen jonger dan drie jaar blijkt een ervaren clinicus beter in het voorspellen van een latere diagnose in het autisme spectrum dan de algoritmes van de eerder genoemde gestandaardiseerde instrumenten [6-8]. Er is wetenschappelijk onderzoek gedaan naar de betrouwbaarheid en de stabiliteit van de diagnose autisme bij kinderen die verwezen zijn voor diagnostiek toen ze nog geen vijf jaar oud waren. Uit dit onderzoek blijkt dat de diagnose autisme op de leeftijd van twee jaar, zowel betrouwbaar te stellen is, als stabiel blijft tot het derde en in één studie tot het negende en in sommige gevallen zelfs tot het twaalfde jaar [6-17]. Er is veel minder bekend over de betrouwbaarheid en stabiliteit van de diagnose autisme spectrum stoornis bij jonge kinderen die na screening geselecteerd zijn uit de algemene populatie, zoals in het SOSO-project gebeurde.

De eerste doelstelling van het SOSO-project was het vroeg onderkennen van autisme spectrum stoornissen door middel van een screening uitgevoerd bij kinderen uit de algemene populatie vroeg in het tweede levensjaar.

De eerste doelstelling beschreven in dit specifieke proefschrift was het onderzoek naar de betrouwbaarheid van het stellen van de klinische diagnose autisme spectrum stoornis bij kinderen geselecteerd door screening en vroege opsporing op de leeftijd van gemiddeld twee jaar. De betrouwbaarheid van een diagnose wordt bepaald door de mate waarin verschillende clinici bij hetzelfde kind tot eenzelfde diagnose komen. De verwachting was dat clinici meer moeite zouden hebben met het onderscheiden van kinderen met en zonder een autisme spectrum stoornis op tweejarige leeftijd vanwege het ontbreken van diagnostische criteria die geschikt zijn voor kinderen op die leeftijd. De beschikbare criteria zijn gevalideerd bij kinderen ouder dan drie jaar. De bij deze onderzoeksgroep gestelde diagnoses door drie verschillende psychiaters lieten in ons onderzoek een goede mate van overeenkomst ($\kappa = 0.74$) zien. Die overeenkomst was minder goed ($\kappa = 0.51$) als de psychiaters gevraagd werd autisme en aan autisme verwante stoornissen bij kinderen op die leeftijd te onderscheiden. Clinici hadden de minste overeenstemming over het diagnosticeren van een autisme spectrum stoornis bij kinderen met een verstandelijke beperking. Kennelijk is het voor clinici vooral moeilijk om een vertraagde en een afwijkende ontwikkeling bij kinderen van die leeftijd van elkaar te onderscheiden.

Vervolgens hebben wij onderzocht wat de voorspellende waarde is van het stellen van de diagnose autisme spectrum stoornis op de leeftijd van twee jaar, voor diezelfde diagnose, gesteld op de leeftijd van drie en een half jaar. Uit wetenschappelijk onderzoek blijkt dat de diagnose autisme heel betrouwbaar te stellen is bij kinderen die ouder zijn dan drie jaar en dat die diagnose dus als ijkpunt of 'gouden standaard' te gebruiken is. In ons onderzoek is de stabiliteit van de diagnose autisme, gesteld bij kinderen op de leeftijd van 20 maanden, 63%. Bij kinderen met een diagnose aan autisme verwante stoornis is de stabiliteit 54%. Als de diagnose stoornis in het autisme spectrum niet gespecificeerd wordt is de stabiliteit 87%. Hieruit kan geconcludeerd worden dat de ongespecificeerde diagnose stoornis in het autisme spectrum op deze leeftijd de grootste stabiliteit heeft. Dit leidt tot de conclusie dat het bij kinderen op de leeftijd van 20 maanden het meest veilig is om een ongespecificeerde diagnose stoornis in het autisme spectrum te stellen.

In ander wetenschappelijk onderzoek is bij kinderen die op jonge leeftijd een diagnose autisme of aan autisme verwante stoornis kregen, op latere leeftijd een verschuiving te zien tussen de subcategorieën in het autisme spectrum. De helft van de kinderen die rond hun tweede jaar een diagnose aan autisme verwante stoornis gekregen hadden, kregen bij een nieuwe evaluatie op latere leeftijd de diagnose autisme [8,9]. In ons eigen onderzoek en dat van anderen zagen we juist een verschuiving in de andere richting [16].

Onze conclusie is dat het differentiëren tussen autisme en een aan autisme verwante stoornis bij kinderen die jonger zijn dan drie jaar niet voldoende betrouwbaar kan gebeuren. Op die leeftijd is het beter een ongespecificeerde diagnose stoornis in het autisme spectrum te stellen. Deze conclusie is gebaseerd op het gegeven dat de betrouwbaarheid van de diagnoses autisme en aan autisme verwante stoornis bij kinderen die jonger zijn dan drie jaar lager is dan die van een ongespecificeerde diagnose stoornis in het autisme spectrum [9]. Daarnaast komt deze conclusie voort uit het aantal verschuivingen tussen de gespecificeerde diagnoses in het autisme spectrum op latere leeftijd bij kinderen die voor het eerst gediagnosticeerd zijn op een leeftijd beneden de drie jaar [8,9,15,16] (Hoofdstuk 2).

De effectiviteit en bruikbaarheid van gestandaardiseerde instrumenten voor het diagnosticeren van autisme spectrum stoornissen bij kinderen op de leeftijd van twee jaar.

De vroege onderkenning van een stoornis in het autisme spectrum krijgt steeds meer aandacht [18,19]. Dit heeft als consequentie dat er de komende jaren steeds meer kinderen die jonger zijn dan drie jaar bij hulpverleners terecht zullen komen voor diagnostiek van een stoornis in het autisme spectrum. De relatieve stabiliteit van deze diagnose, zoals gevonden in ons onderzoek, wordt in de huidige praktijk bepaald door de impliciete kennis en ervaring van de desbetreffende clinicus. Het feit dat er geen formele criteria beschikbaar zijn die de clinicus, zonder veel ervaring met autisme spectrum stoornissen of toegang tot gestandaardiseerde instrumenten, kunnen helpen met het stellen van deze diagnose bij heel jonge kinderen is dan een beperking. Gestandaardiseerde instrumenten, zoals de ADI-R [3] en de ADOS-G [4], die vaak worden gebruikt voor diagnostiek in het kader van wetenschappelijk onderzoek, worden toenemend gebruikt voor diagnostiek in de klinische praktijk. Maar in de gewone klinische praktijk is er vaak niet de tijd of zijn er niet de middelen om de informatie via deze instrumenten te verzamelen [8]. Het afnemen en scoren van de ADI-R en de ADOS-G. vereisen opleiding en financiële middelen [3,4]. Voor het grote aantal clinici, dat met jonge kinderen werkt, is het belangrijk om een instrument als ondersteuning van de diagnostiek naar autisme spectrum stoornissen te ontwikkelen, waar geen extra training en financiën voor vereist zijn.

Het meest bekende en toegankelijke instrument voor de diagnostiek van de autisme spectrum stoornissen is het algoritme voor de classificatie van autisme volgens het Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR) [20]. Hierbij wordt aangenomen dat deze criteria de gouden standaard zijn voor het stellen van de diagnose autisme bij kinderen ouder dan drie jaar. Bij kinderen, die jonger zijn, zijn drie van de twaalf criteria niet goed toepasbaar. Dit zijn de criteria met betrekking tot het beoordelen van de relaties met leeftijdgenoten, het vermogen een gesprek te voeren en stereotiep taalgebruik [9]. Als gevolg van deze beperking is niet bekend in hoeverre het algoritme of de criteria op zichzelf een rol kunnen spelen bij het diagnosticeren van kinderen met autisme spectrum stoornissen jonger dan drie jaar.

In Hoofdstuk 3 wordt de bruikbaarheid van het diagnostische algoritme van de DSM-IV-TR criteria voor autisme voor de diagnostiek van autisme spectrum stoornissen bij kinderen, jonger dan drie jaar, onderzocht. De bruikbaarheid hangt af van de mate waarin dit algoritme een diagnose in het autisme spectrum op de leeftijd van drie en een half jaar kan voorspellen. Daarnaast wordt het resultaat van dit algoritme vergeleken met de mate waarin de ADOS-G een diagnose in het autisme spectrum kan voorspellen. De ADOS-G is een instrument wat veel meer investering kost op het gebied van tijd en geld.

De sensitiviteit, specificiteit en positieve predictieve waarde van het algoritme van de DSM-IV-TR voor autisme en van de ADOS-G voor autisme spectrum stoornissen blijken in ons onderzoek respectievelijk 0.88, 0.84 en 0.76, en 0.85, 0.70, en 0.63 te zijn. De positieve predictieve waarde van het gehele algoritme van de DSM-IV-TR voor autisme is hoger dan die van afzonderlijke onderdelen. Omdat drie van de twaalf criteria niet toepasbaar zijn bij een groot aantal kinderen jonger dan drie jaar9 betekent dit dat kinderen op deze leeftijd aan meer dan zes van de negen criteria moeten voldoen in tegenstelling tot oudere kinderen, die aan zes van de twaalf criteria moeten voldoen.

De conclusie van ons onderzoek, zoals beschreven in Hoofdstuk 3, is dat het volledige algoritme van de DSM-IV-TR classificatie voor autisme het meest betrouwbare en bruikbare gestandaardiseerde instrument is om een clinicus te ondersteunen bij het stellen van een diagnose in het autisme spectrum bij kinderen op de leeftijd van twee jaar.

Onderzoek naar biologische, gedragsmatige, cognitieve en omgevings-factoren die mogelijk een relatie hebben met het fenotype van de autisme spectrum stoornis; hoofdomtrek en copy number variants (CNVs).

Autisme spectrum stoornissen zijn ontwikkelingsstoornissen met een onbekende etiologie, waarbij vele functionele en morfologische afwijkingen in de hersenen gevonden worden. In Hoofdstuk 4, 5 en 6 wordt de mogelijke bijdrage van enkele biologische en genetische factoren, zoals hoofdomtrek en copy number variants (CNVs) aan het fenotype van de stoornissen onderzocht.

Hoofdomtrek

In hoofdstuk 4 laten we zien de hoofdomtrek van de kinderen met een later gediagnosticeerde autisme spectrum stoornis in hun eerste levensjaar niet afwijkt van de algemene populatienorm en ook niet van kinderen zonder een psychiatrische stoornis uit onze onderzoeksgroep, terwijl de lichaamslengte van deze kinderen dat wel doet. Als populatienorm hebben we de groeicurven van de Nederlandse Organisatie voor toegepast-natuurwetenschappelijk onderzoek (TNO) gebruikt [21].

Om de invloed van de effecten over de tijd op de hoofdomtrek te ondervangen zijn de kinderen met een autisme spectrum stoornis uit de onderzoeksgroep qua groeimaten ook vergeleken met een groep kinderen zonder psychiatrische diagnose uit hetzelfde onderzoekscohort. Deze kinderen weken op de onderzochte maten niet af van het gemiddelde op de Nederlandse groeicurven, de populatienorm, wat de validiteit van onze methode aantoont.

In onze onderzoeksgroep begon de lengte van kinderen met een autisme spectrum stoornis op de leeftijd van 1-2 maanden positief af te wijken van de populatienorm. Op de leeftijd van 4 maanden week de lengte van deze kinderen significant af van de norm. Deze versnelde groei nam weer af op de leeftijd van 6 maanden. Deze bevindingen zijn conform de bevindignen van Torrey et al. [22], maar niet conform de bevindingen van Dissanayake et al. [23]. In dat laatstgenoemde onderzoek is gevonden dat de lichaamslengte van de kinderen met een autisme spectrum stoornis fors verhoogd is vooral na de leeftijd van 52 maanden in vergelijking met de norm.

In ander wetenschappelijk onderzoek bleken kinderen met een autisme spectrum stoornis ook een hoofdomtrek volgens de norm in het eerste levensjaar te hebben [23,24]. Maar ook is een versnelde groei van de hoofdomtrek in het eerste levensjaar bij deze groep kinderen gerapporteerd [24-26]. Deze inconsistenties in gerapporteerde groei van het hoofd kunnen samen hangen met de grootte, de selectie en samenstelling van de onderzoeksgroep.

Wij concluderen dat de bevindingen van een versnelde lengtegroei van kinderen met een autisme spectrum stoornis in het eerste levensjaar gerapporteerd in vier onafhankelijke studies eerder wijst op een dysregulatie van de algemene groei dan op een dysregulatie van de neuronale groei in die fase van de ontwikkeling. Dat betekent dat afwijkingen in de stofwisseling, in de groeifactoren of de hormoonhuishouding een aandeel kunnen hebben in het ontstaan van deze stoornissen.

Copy Number Variants (CNVs):
submicroscopische veranderingen in doseringen van het gen.

In Hoofdstuk 5 en in Hoofdstuk 6 wordt de mogelijke bijdrage van copy number variants (CNVs) aan het fenotype van autisme spectrum stoornissen onderzocht. Dit onderzoek is gedaan bij een groep kinderen die verwezen is naar de polikliniek Kinder- en Jeugdpsychiatrie van het Universitair Medisch Centrum te Utrecht op een leeftijd jonger dan vier jaar.

In Hoofdstuk 5 presenteren we vier kinderen met een autisme spectrum stoornis, komende uit drie verschillende families, die allen een gewijzigde dosering van het microcephalin 1 gen (MCPH1) in chromosoom band 8p23.1 hebben. Op basis van deze bevinding is dit een gen met

een mogelijke associatie met het ziektebeeld autisme spectrum stoornis. De bevindingen bij deze kinderen laten zien dat een gewijzigde dosering van dit gen geassocieerd is met een ander fenotype dan gevonden wordt bij de autosomale recessieve ziektebeelden waar MCPH1 meestal mee in verband gebracht wordt. Wijzigingen in de dosering van MCPH1 zouden mogelijk een relatie kunnen hebben met de symptomen van autisme en de verstandelijke beperking die bij sommige kinderen met een autisme spectrum stoornis gevonden wordt.

In Hoofdstuk 6 presenteren wij een eerste aanzet tot het evalueren van de klinische betekenis van overgeërfde en *de novo* (nieuw verworven) copy number variants in een cohort van 42 families met één aangedaan familielid en 8 families met meerdere aangedane familieleden. De kinderen uit deze families zijn geselecteerd uit een groep die op jonge leeftijd verwezen is voor diagnostiek naar een eventuele stoornis in het autisme spectrum. Als in het erfelijke materiaal van de patiënt een wijziging in de dosering van een gen gevonden werd, waarvan bekend was dat het tot expressie komt in het brein, hebben wij vervolgens de ouders van deze patiënt onderzocht op de aanwezigheid van gedragskenmerken passend bij het fenotype van autisme spectrum stoornissen met behulp van de Social Responsiveness Scale (SRS) [27-30].

Daarnaast hebben we alle, op deze wijze geselecteerde genen, onderzocht met een algoritme voor gen prioritering. Wij hebben een methode ontwikkeld waarbij zowel de wijze van overerving van de gewijzigde dosering van het gen als de gedragskenmerken van de ouders meegewogen worden. In ons onderzoek blijken zowel de resultaten uit de gen prioritering als uit de inventarisatie van gedragskenmerken in familiair verband te wijzen in de richting van dezelfde genen en sequentie van biologische reacties in het lichaam. Op basis van dit resultaat blijken onze onafhankelijke methodes elkaar te valideren.

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Biography

Emma van Daalen (Almelo, 03-03-1961)

Training and affiliations

1973-1979	Gymnasium ß, Amsterdam.
1979-1984	Medical study, University of Amsterdam (UvA).
1982-1989	Assistent-teacher: dept. of Medical Psychology, Family Medicine and Social Medicine, University of Amsterdam, faculty of Medicine.
1983	Field research: compliance of multiple drug treatment in patients with leprosy, Kigoma Region, Tanzania (Dr. Armand van Deun).
1985	Clinical training Liaison Psychiatry: Memorial Sloan Kettering Cancer Center, New York, USA (Prof. Jimmie Holland).
1987	Follow-up research: course of schizophrenia in young adolescents, University Medical Hospital of Amsterdam (Prof. Dr. D.H. Linszen).
1982-1989	Assistent-teacher: dept. of Medical Psychology, Family Medicine and Social Medicine, University of Amsterdam, faculty of Medicine.
1989	Medical Degree, University of Amsterdam.
1989-1991	Resident in Psychiatry: Dept of Psychosis, long stay, Psychiatric Hospital Endegeest, Oegstgeest (Dr. R. van Meer). Dept. of Child and Adolescent Psychiatry, University Hospital of Amsterdam (Prof. Dr. J.A.R. Sanders-Woudstra).
1991	Clinical training Dept. of Child and Adolescent Psychiatry, Maudsley Hospital, Institute of Psychiatry, Londen, GB (Prof. M. Rutter, Prof. C. Dare, Prof. E Taylor).
1991-1994	Resident in Adult Psychiatry: Haags-Leids Opleidings Consortium (Prof Dr. H.G.M. Rooijmans).
1994-1996	Resident in Child and Adolescent Psychiatry University Medical Hospital Utrecht (Prof. Dr. H. van Engeland)
1996-current	Child and Adolescent Psychiatrist, Dept. of Autism and Psychosis, University Medical Hospital Utrecht.

Fields of interest

Behavioral phenotype of Autism, especially in preschool children and children with intellectual, psychiatric and somatic comorbidity.

Emma van Daalen, MD
Child and Adolescent psychiatrist
Rudolf Magnus Institute of Neurosciences, Utrecht, the Netherlands
Department of Child and Adolescent Psychiatry, University Medical Centre, Utrecht, the Netherlands
P.O. Box 85500
3508 GA Utrecht
The Netherlands
E.vanDaalen@umcutrecht.nl