Prediagnostic and comorbidity factors in preschool children with autism

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Cover illustration: Tom Carlsson 3.5 years old

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To my beloved, big family

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ABSTRACT

The aim of the thesis was to investigate Autism Spectrum Disorder (ASD) diagnosed in the early years from different angles; screening, load of coexisting disorders, outcome at routine developmental surveillance, and to study a possible background factor (prenatal ultrasound).

The objective of **Paper I** was to investigate if the CHecklist for Autism in Toddlers (CHAT), when added to the routine 18-month developmental surveillance at Child Healthcare Centres (CHC), would result in earlier diagnosis and intervention for children with ASD. The study was carried out in southern Stockholm, and 18 - month-old children in northern Stockholm who underwent the same routine developmental surveillance at CHC, but not the CHAT- screening, served as a comparison group. Although a helpful tool, the use of CHAT in the investigated area did not lead to earlier diagnosis of ASD.

In the study reported in **Paper II**, records from the18-month routine surveillance at CHC of children later diagnosed with ASD were reviewed. The study group consisted of 175 of a total of 208 children with ASD who had been referred to the Autism Center for Young Children (ACYC) in Stockholm for intervention. More than a third of the total group of children with ASD and half of the group with ASD and concomitant intellectual disability (ID) had failed the 18-month routine developmental surveillance, compared to one in fifty in the general child population. When the presence of regulatory problems also was taken into consideration, the difference between ASD and the general child population was even more marked.

The aim of **Paper III** was to examine different coexisting disorders in children with ASD. From the total group of 208 preschool children referred to in Paper II, 198 had been followed over a two-year period and were the subject of this study. At this follow-up, including broad clinical examinations, 91% of the children were found to have at least one coexisting developmental disorder or problem; language disorder being the most common, followed by ID, motor control problems, and severe hyperactivity.

In the fourth study, reported in **Paper IV**, the research question was whether early (gestational week 12) or later (gestational week 18) prenatal ultrasound would be associated with an increased risk for ASD in the child. The population under study comprised approximately 29.000 pregnant women, randomized to early or later ultrasound. The proportion of their children with ASD (with and without ID) was found to be identical in the two groups, 1.2%.

Conclusion Pre-school children with ASD usually have a complex clinical presentation with many more problems than those subsumed under the ASD label. Many of these children, particularly those who also have ID, can be identified at 18 - month routine health surveillance. Adding the CHAT to such surveillance did not, in itself, appear to increase the uptake rate. The frequency of ASD was similar in the early and later ultrasound groups.

Keywords: Autism Spectrum Disorder, Intellectual Disability, ESSENCE, Children, Screening, CHAT, Surveillance, Child Healthcare Centre, Prenatal ultrasound

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SAMMANFATTNING PÅ SVENSKA

Syftet med avhandlingen har varit att belysa frågor rörande tidig upptäckt och riskfaktorer vid autism (Autism Spectrum Disorder, ASD) hos barn ur olika aspekter. Specifikt har frågeställningarna varit (1) om en screeningmetod på BVC skulle kunna bidra till att upptäcka barn med ASD redan vid 18 månaders ålder; (2) i vad mån den ”vanliga” 18- månadskontrollen på BVC skulle kunna identifiera barn som senare utvecklar ASD; (3) hur vanligt är det att förskolebarn med ASD också har andra funktionsnedsättningar och svårigheter; samt (4) om ultraljudsundersökning under graviditeten skulle kunna vara en riskfaktor för senare utveckling av ASD hos barnet.

Det finns idag stöd för att det är viktigt med tidig ASD diagnos – och tidiga insatser – i form av utbildning till föräldrar och till barnets förskolepersonal om funktionsnedsättningen och om metoder som främjar barnets utveckling. I den första delstudien prövades om man genom att addera en screeningmetod till BVC:s rutinkontroll för barn vid 18 månaders ålder skulle kunna spåra ASD tidigare. Screeninginstrumentet omfattade 9 frågor till föräldrarna och 4 lekmoment som BVC – sjuksköterskan genomförde med barnet. Efter positiv screening, och om misstanke på ASD kvarstod efter ytterligare bedömning av barnläkare på BVC, remitterades barnet för utredning med frågeställning ASD. Screeningmetoden användes i den södra delen av Stockholms län och barnen i den norra delen – där screeninginstrumentet inte användes – utgjorde en jämförelsegrupp. Det visade sig att screeningmetoden i sig själv inte bidrog till att fler barn med ASD kunde spåras.

I den andra delstudien ingick 208 barn som hade remitterats till ett habiliteringscenter i Stockholm, specialiserat för förskolebarn med ASD, för att få tidiga insatser och stöd. Gruppen var populationsbaserad, det vill säga representativ för små barn som tidigt utretts och fått diagnos ASD i den studerade regionen. För att avgöra i vad mån BVC:s ”vanliga” 18-månaderskontroll skulle ha kunnat spåra svårigheter som kunde indicera ASD analyserades barnens BVC – journaler. För 175 av de 208 barnen kunde BVC – journalen granskas. Studien visade att en tredjedel av barnen som senare fått diagnos ASD inte hade klarat det antal uppgifter som 98 % av barn i allmänhet klarar vid 18 månaders ålder. I den grupp av barn med ASD som också hade en intellektuell funktionsnedsättning hade hälften inte klarat 18 – månadskontrollen. Om man vid denna utvecklingskontroll även skulle ha beaktat andra problem hos barnet i form av sömnsvårigheter, skrikighet och mat/uppfödningssvårigheter skulle ännu fler barn med ASD ha kunnat spåras enbart med den nu tillgängliga utvecklingskontrollen vid 18 månaders ålder.

I den tredje delstudien följdes förskolebarnen med ASD från den just nämnda delstudien upp då de hade haft insatser från det specialiserade habiliteringscentret under två år. Vid uppföljningen, då 198 av de 208 barnen deltog, bedömdes i vad mån andra funktionsnedsättningar, utöver ASD, förelåg. Det visade sig att 91% av barnen hade ytterligare minst en ytterligare funktionsnedsättning, av vilka de vanligaste var språkstörning, intellektuell funktionsnedsättning och hyperaktivitet. Barn med ”autistic disorder” hade högre andel ytterligare funktionsnedsättningar jämfört med de barn som hade lindrigare former av ASD. Studien visar vikten av att alltid utreda barn med misstänkt ASD i ett brett perspektiv.

Vid ASD föreligger många kända orsaksfaktorer och kunskapen om viktiga genetiska faktorer har ökat starkt under senare år. Exempel på icke-genetiska faktorer är vissa infektioner, toxiner och läkemedel som kan skada fostret under graviditet. Forskare har också diskuterat om ultraljud under graviditet skulle kunna innebära en risk för fostret. Studier har t.ex. visat att barn som blivit undersökta med ultraljud under fostertiden oftare än förväntat varit vänsterhänta jämfört med barn som inte blivit undersökta med ultraljud. I den fjärde delstudien undersöktes om barn födda efter en tidig ultraljudsundersökning, i graviditetsvecka 12, jämfört med barn födda efter ett senare ultraljud under graviditeten, i vecka 18, hade ökad risk för att utveckla autism. Studien baserades på data rörande barn till ca 29 000 kvinnor som under graviditeten deltagit i en studie och randomiserats till antingen tidigare eller senare ultraljud. Barnen (11-15 år) följdes upp via uppgifter från Försäkringskassans register angående beviljade vårdbidrag. I både ”tidig” och ”senare” ultraljudsgrupp hade 1.2% av barnen fått diagnos autism, vilket innebär att ingen skillnad kunde påvisas.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. Höglund Carlsson L, Gillberg C, Lannerö E, Blennow M.A. (2010). Autism: screening toddlers with CHAT in a child health care programme did not improve early identification. Acta Pædiatrica. 99, 1897–1899.

II. Höglund Carlsson L, Westerlund J, Barnevik Olsson M, Gillberg C, Fernell E. (2015). Autism spectrum disorders before diagnosis - Developmental assessment at Child Healthcare Centres at 18 months. Submitted.

III. Höglund Carlsson L, Norrelgen F, Kjellmer L, Westerlund J, Gillberg C, Fernell E. (2013). Coexisting disorders and problems in preschool children with autism spectrum disorders. Scientific World Journal. 2013: 213979.

IV. Höglund Carlsson L, Saltvedt S, Anderlid B-M, Westerlund J, Gillberg C, Westgren M, Fernell E. (2015). Ultrasound in the first and second trimester and autism; a prospective randomized study. In manuscript.

CONTENT

ABBREVIATIONS

ABA	Applied Behavior Analysis
ACYC	Autism Centre for Young Children
ADHD	Attention-Deficit / Hyperactivity Disorder
ALC	Autistic Like Condition
APA	American Psychiatric Association
ASD	Autism Spectrum Disorder
ASSQ	Autism Spectrum Screening Questionnaire
BVC	Barnavårdscentral [in Swedish]
CHAT	Checklist for Autism in Toddlers
CHC	Child Healthcare Centre
CMV	Cytomegalovirus
CTG	Cardiotochography
DISCO	The Diagnostic Interview for Social and Communication Disorders
DSM	Diagnostic and Statistical Manual of Mental Disorders
DSM-IV	DSM, 4th edition
DSM-5	DSM, 5th edition
EIBI	Early Intensive Behavioral Intervention
ESSENCE	Early Symptomatic Syndromes Eliciting Neurodevelopmental Clinical Examinations
FDA	Food and Drug Administration
DNA	Deoxyribonucleic acid

DCD	Developmental Coordination Disorder
DQ	Developmental Quotient
Hz	Hertz = cycles per second
ICD	International Classification of Diseases
ID	Intellectual Disability
IQ	Intelligence Quotient
JA-OBS	Joint Attention OBervation Schedule
MI	Mechanical Index
M-CHAT	Modified CHecklist for Autism in Toddlers
M-CHAT R/F	Modified CHecklist for Autism in Toddlers Revised and with Follow-up
PDD-NOS	Pervasive Developmental Disorders-Not Otherwise Specified
PPV	Positive Predictive Value
PUS	Prenatal Ultrasound
SD	Standard Deviation
SLI	Specific Language Impairment
SSIA	Swedish Social Insurance Agency
TEACCH	Treatment and Education of Autistic and related Communication handicapped Children
TI	Thermal Index
US	Ultrasound
VABS	Vineland Adaptive Behavior Scales
WHO	World Health Organization

1 INTRODUCTION

Autism spectrum disorders (ASD) represent early-onset neurodevelopmental disorders, characterized by impairments in reciprocal social interaction/communication and a restricted range of interests and behaviours. The symptoms are usually associated with cognitive deficits in "theory of mind", central coherence and executive functions (1). The consequences of these cognitive impairments in daily life vary widely between individuals, depending on the severity of the ASD *per se*, on coexisting disorders and problems, and on underlying medical/aetiological factors.

Children with ASD may have exhibited symptoms already in infancy, including abnormalities of joint attention, eye contact, strange reactions to sound and regulatory problems, i.e., sleep problems, excessive crying and feeding difficulties (1-3). Common symptoms during preschool age are delayed and aberrant language development, peer relationship problems, hyperactivity or passivity, and insistence on sameness with associated temper tantrums.

A subgroup of children with autism has a different onset of symptoms. After apparently normal early development up to the age of approximately 18 to 24 months, sometimes up to 30 months, there is a loss of verbal and/or non-verbal skills, i.e., a regression in development (4). This regressive trajectory has to be carefully investigated and several medical disorders may underlie this clinical presentation.

Children with ASD and Intellectual Disability (ID) are usually assessed and receive their diagnoses during preschool years, while children with ASD and a general cognitive function within the "normal" variation may not present obvious developmental problems until school age when demands on cooperation with peers and school tasks increase.

1.1 Definitions and classifications of ASD

ASDs are usually defined according to the Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association, APA). The first DSM appeared in 1952 (DSM-I) and autism was at that time considered a childhood type of schizophrenic reaction, and in the next DSM, from 1968, (DSM-II) a similar term, schizophrenia, childhood type was used. In DSM-III

(1980) the term infantile autism was used and in DSM III-R (1987) there was a major change, to autistic disorder. During the study period of the present thesis, the DSM-IV (5), collecting different categories of ASDs under the umbrella term Pervasive Developmental Disorders, was used. The DSM-IV distinguishes between Autistic Disorder, Pervasive Developmental Disorders-Not Otherwise Specified (PDD-NOS), Asperger syndrome and Disintegrative disorder (which overlap with the regressive form of autism).

The fifth updated edition, DSM-5 (6), was introduced in 2013 and in this manual the subcategories have been collapsed into one subtype; Autism Spectrum Disorder. This change can be seen partly as a response to a considerable literature having found that diagnoses of the different subtypes (particularly Asperger syndrome and PDD-NOS) are not highly reliable across clinicians (7, 8).

A parallel system, used in medical diagnostic registers, is the WHO ICD (International Classification of Diseases) classification system, currently the ICD-10 version, 1992 (9).

1.1.1 DSM-IV (1994) and DSM-IV-TR (2000), diagnostic criteria

Autistic Disorder

A total of six (or more) items from (1), (2), and (3), with at least two from (1), and one each from (2) and (3):

(1) Qualitative impairment in social interaction, as manifested by at least two of the following:

Marked impairment in the use of multiple nonverbal behaviors such as eye-to-eye gaze, facial expression, body postures, and gestures to regulate social interaction

Failure to develop peer relationships appropriate to developmental level

A lack of spontaneous seeking to share enjoyment, interests, or achievements with other people (e.g., by a lack of showing, bringing, or pointing out objects of interest)

Lack of social or emotional reciprocity

(2) Qualitative impairments in communication as manifested by at least one of the following:

Delay in or total lack of, the development of spoken language (not accompanied by an attempt to compensate through alternative modes of communication such as gesture or mime)

In individuals with adequate speech, marked impairment in the ability to initiate or sustain a conversation with others

Stereotyped and repetitive use of language or idiosyncratic language

Lack of varied spontaneous make-believe play or social imitative play appropriate to developmental level

(3) Restricted, repetitive, and stereotyped patterns of behavior, interests, and activities, as manifested by at least of one of the following:

Encompassing preoccupation with one or more stereotyped and restricted patterns of interest that is abnormal either in intensity or focus

Apparently inflexible adherence to specific, nonfunctional routines or rituals Stereotyped and repetitive motor mannerisms (e.g. hand or finger flapping or twisting, or complex whole body movements)

Persistent preoccupation with parts of object

Delays or abnormal functioning in at least one of the following areas, with onset prior to age 3 years: (1) social interaction, (2) language as used in social communication, or (3) symbolic or imaginative play.

The disturbance is not better accounted for by Rett's disorder or childhood disintegrative disorder.

DSM-IV (1994) Pervasive Developmental Disorder-Not Otherwise Specified (PDD-NOS), diagnostic criteria

This category should be used when there is a severe and pervasive impairment in the development of reciprocal social interaction or verbal and nonverbal communication skills, or when stereotyped behavior, interests, and activities are present, but the criteria are not met for a specific pervasive developmental disorder. Presentations do not meet the criteria for autistic disorder because of

late age of onset, atypical symptomatology, or sub threshold symptomatology, or all of these.

DSM-IV (1994) Asperger syndrome

Asperger syndrome is according to DSM-IV defined with criteria pertaining to impairments in social interaction and restricted repetitive and stereotyped patterns of behaviour, interests and activities, but without qualitative impairments in communication.

1.1.2 DSM-5 (2013) Autism Spectrum Disorder

ASD, according to DSM-5, in short, is an umbrella term encompassing persistent deficits in social communication and social interaction, across multiple contexts, and restricted, repetitive patterns of behaviour, interests or activities. Severities and accompanying impairments should be specified.

1.2 Prevalence

Autism has previously been regarded as a rare disorder. The first prevalence study in England, by Lotter (1966) pointed to a prevalence of 4 in 10,000 children with childhood psychosis/autism (10).

In the late 1970s, the famous Camberwell study in England included children with classical autism as well as with milder variants of autism (11). Wing, Gould and Gillberg, found that more than 20 in 10,000 children had the "triad of social impairment", today subsumed under the ASD umbrella. However, Gillberg reported that 0.7% of seven-year-olds in Gothenburg already in the mid-1970s had Wing´s triad/ASD ("comorbid" with deficits in attention, motor control and perception) (12).

Prevalence rates of autism or ASD will vary across time due to diagnostic criteria in use. The registered prevalence has increased over time, which could be due to the increased awareness of the condition; awareness of its common association with intellectual disability (ID) and with medically identified syndromes. More attention has also been drawn to the occurrence of ASD in children and adolescents with "normal" intellectual level. In Sweden, increased register rates can probably also be partly accounted for the fact that a diagnosis of autism or ASD opens the door to support and interventions from habilitation and community services, under the act concerning support and services for persons with certain functional impairments (LSS in Swedish).

Today ASDs are identified in the general population of preschool children at approximately a rate of 0.6%–0.8%, and in school children and young adults at about 1.0% (13-18).

In a Swedish study covering a period of 10 years, a comparison of the annual prevalence of the autism symptom phenotype in children and of registered diagnoses for autism spectrum disorder was carried out. The prevalence of the autism symptom phenotype was found to have remained stable while the official prevalence for registered, clinically diagnosed, autism spectrum disorder had increased substantially. The authors concluded that the increased prevalence of ASD most likely is attributable to administrative changes, rather than to a "true" increase in the rate of ASD (19).

In the past, the girl:boy ratio in ASD was usually reported as 1:3-5 (20). However, there have been several more recent studies indicating that the male excess is probably less pronounced. Girls with social/communication problems often have a slightly different symptom presentation and therefore have not been considered sufficiently with regard to ASD – clinically or in research (21).

1.3 Coexisting disorders/"comorbidity"

Most developmental disorders occur in combinations. This is the rule not only for ASD, but also for, for example attention-deficit/hyperactivity disorder (ADHD) and ID (22, 23).

In the Swedish nationwide twin study, the coexistence of eight psychiatric disorders, known to coexist with ASDs, were investigated in nine-year-old children (24). It was demonstrated that 50% of the children with ASD had indications of four or more coexisting disorders, only 4% did not have any indication of concomitant disorder.

ASD and ID often co-occur and the rate of coexisting ID in children with ASD varies considerably between studied groups; 10-90% (2).

The relationship between specific language impairment (SLI) and ASD was systematically reviewed by Tomblin (25). The author discussed that many of the features reported to be characteristic of SLI were also found in other forms of neurodevelopmental disorders. ASD and what has been known as SLI very likely overlap, reflecting a complex mixture of similarities and differences.

To analyze comorbidity of neurodevelopmental disorders, including ASD, a community-based sample of children screening positive for language delay at 2.5 years were followed with regard to neuropsychiatric and neurodevelopmental outcome. At the age of seven years, 62% had a major neuropsychiatric diagnosis (autism, atypical autism, Asperger's syndrome, ADHD, or combinations of these). Another 10% had borderline IQ with no other major diagnosis. The study also showed remaining language problems at age six years to be strongly predictive of the presence of neuropsychiatric or neurodevelopmental disorders at age seven years (26).

A review (in Spanish) of 33 studies regarding ASD and coexisting ADHD showed that the prevalence of symptoms of ADHD in children with ASD was 33-37%. It was discussed that the clinical profile of ASD + ADHD should be considered to be more severe than that of pure ADHD or ASD (27).

Assessments of children with epilepsy in a defined geographical area in the south of the UK showed that 21% met criteria for ASD, and that 61% of those with ASD had other behavioural or motor disorders. It was concluded that features of ASD were common in children with epilepsy and this occurred regardless of cognitive ability (28).

In a Norwegian population-based study, parents of children with cerebral palsy completed the Autism Spectrum Screening Questionnaire (ASSQ). Nine-teen percent of the cohort had ASSQ scores at or above the 98th percentile of the general child population in the study area. It was emphasized that more attention should be given to autism spectrum symptoms in the regular follow-up of children with cerebral palsy in an attempt to enhance their social functioning (29).

The concept of ESSENCE (Early Symptomatic Syndromes Eliciting Neurodevelopmental Clinical Examinations) introduced by Gillberg in 2009 underscores the difficulties that often occur in trying to establish one specific definitive diagnosis and the marked changes of symptoms that may occur over time; sometimes symptoms become more evident and sometimes less prominent as the child gets older. ESSENCE focuses on the very common coexistences of developmental disorders. It stresses that clinicians have to consider not only the first presenting symptoms but also the "bigger picture", and identify other difficulties the child may display. ESSENCE covers all kinds of developmental disorders; ASD, ADHD, oppositional defiant disorder (ODD), tic disorders, developmental coordination disorder (DCD), ID, language impairment and also more “typical” neuropaediatric disorders, such as cerebral palsy, motor/muscular disorders and epilepsy as well as different types of

behavioural problems— tantrums, sleeping problems, feeding/eating problems, and sensory hyper- or hypersensitivities.

One of the most important messages related to ESSENCE is the need for follow-up of the developmental trajectory of young children presenting with any kind of ESSENCE symptoms (30). All this means that there are many children affected with ESSENCE, about 5% of preschool children and about 10% of school children. It can be estimated that many more than half the group will still exhibit problems from these disorders as adults. To have this broad concept in mind in clinical assessments is decisive for providing the best possible intervention, treatment and support for the children and their families.

1.4 Background factors

A classification of genetic aetiologies can be divided into chromosomal abnormalities, monogenic disorders and multifactorial disorders. In each cell of the "normal" body, the cell nucleus contains 22 autosomal chromosomes together with the sex chromosomes, either two X in females or X and Y chromosomes in the males. The chromosome consists of DNA (deoxyribonucleic acid) encompassing the genes and the nucleus contains a total of about 2 metres of densely packed DNA! Each chromosome harbours some hundreds to several thousands of genes, amounting to about 20.000 genes in one cell.

Chromosomal abnormalities resulting in neurodevelopmental disorders, such as ASD, can be classified into numerical and structural. Numerical means that there is a loss of a chromosome or an extra chromosome and structural abnormalities mean that the structure of the chromosome is altered, such as loss or gains of part of the chromosome, i.e., deletions and duplications, implying gains or loss of genes. In the most common chromosomal disorder, trisomy 21, autism is a common feature. Many small deletions or duplications have been shown to cause or increase the risk for ASD.

Pathogenic mutations in specific genes underlie many monogenic disorders associated with ASD, each with its own phenotype, but often with a "core" of autism symptoms or ASD. Some of the most well-known of these are Fragile X syndrome, Tuberous sclerosis, Angelman syndrome, 15q13-syndrome, Rett syndrome, CHARGE syndrome, Cornelia de Lange syndrome, Myotonic dystrophy type I, Neurofibromatosis type I, Noonan syndrome, Pitt-Hopkins syndrome, Rubinstein-Taybi syndrome, Smith-Lemli-Opitz syndrome, Sotos syndrome and mutations in SHANK3 (2). It is of considerable interest that each of these disorders also has small or large subgroups that do not have

ASD. This implies that additional genes, epigenetic or environmental factors are needed to produce the full-blown picture of ASD in some of the cases.

Modern genetic technologies have increased our knowledge and possibilities to identify underlying medical diagnoses and causal pathways to ASD. With these technologies it has become possible to identify aetiological diagnoses in about 20-35% of children with ASD (31-33). It can be expected that this rate will increase in the near future with even more advanced techniques.

Multifactorial disorders or polygenetic disorders are caused by a combination of several genetic variants and environmental factors. The familial recurrence is high but there is no typical Mendelian inheritance. This is probably a common cause of ASD. Environmental factors also have the ability to modify the expression of genes, thus these disorders also engage the potential role of epigenetic mechanisms in the development of ASD (34).

In addition to complex genetic factors, there are other prenatal and also perinatal factors that contribute to ASD (35). The risk of prenatal rubella infection, not only causing malformations, but also psychiatric and behavioural consequences was highlighted during the 1970s (36). Due to immunization the risk that a woman acquires rubella infection during pregnancy has decreased considerably. Another prenatal virus infection, cytomegalovirus (CMV), has also been reported to be associated with the development of ASD (37).

Maternal intake of valproic acid, prescribed for epilepsy during pregnancy has been studied with regard to risk for ASD in the child. One such study, based on a population-cohort of children born in Denmark 1996 to 2006, revealed that maternal use of valproate during pregnancy was associated with a significantly increased risk of autism in offspring, even after adjustment for parental psychiatric disease and epilepsy (38).

Thalidomide (a sedative) can affect fetal development early in pregnancy, probably on days 20 to 24 after conception and is known to cause severe limb defects. In a population of 100 individuals with thalidomide embryopathy, at least four met full criteria for autistic disorder all of whom had been exposed during the fourth week of gestation (39).

Vitamin D has an important role in gene regulation and neurodevelopment and vitamin D deficiency during pregnancy has been suggested to be a possible environmental risk factor for ASD (40).

The most studied toxic substance during pregnancy is alcohol, with well-known risks of malformations and/or developmental disorders, including ID, ADHD and ASD in the child (41).

Extremely preterm born infants (birth before gestational week 28) have an increased vulnerability for cognitive deficits, including ASD. Their immature brain at birth implies several risks for altered developmental trajectories and future negative sequelae as regards behaviour and cognition (42, 43).

Findings from neuroimaging and genetic studies have provided many important insights into the pathological changes that occur in the brain in ASD. These studies have shown that ASD is accompanied by an atypical trajectory of brain maturation, which gives rise to differences in neurological anatomy, functioning and connectivity within the neural systems that probably mediate autistic symptoms and traits (44).

Williams and Casanova (45) discussed in a review the importance of synaptic genes coding for synaptic adhesion, and the role of connectivity. They also presented several teratogenic agents that may mimic or exacerbate effects of what is seen in single-gene syndromes. Among exogenic agents acting on the brain, the authors mentioned ultrasound, carrying a potential risk of altering the molecular environment of the brain.

In the last decade, magnetic resonance imaging has demonstrated decreases in connectivity in adolescents with ASD, possibly most pronounced between regions related to domain- specific circuits specialized for social processing (46).

1.5 Intervention and support

To support children with ASD or any developmental disorders, it is important to understand the basic cognitive deficits involved, as well as the treatments targeting those difficulties and their associated problems, e.g., motor impairment and epilepsy. They also need a medical work-up to identify possible underlying medical disorders and syndromes.

There are many intervention programs specifically focused on autism. The two major programs are those based on 1) theories of applied behavior analysis (ABA) focusing on principles of learning, motivation, and positive reinforcement and 2) Treatment and Education of Autistic and related Communication handicapped CHildren (TEAACH), which emphasizes visual work systems, positive routines, and structured teaching. Both types of programs

involve parents in the training procedures in addition to preschool and habilitation staff.

1.6 Outcome

The outcome of ASD is largely dependent on the types and severities of the coexisting disorders. The presence of ID has been shown to be the most important co-occurring disorder with regard to outcome (47). Furthermore, late speech development (after 5 years of age) is described as a negative prognostic factor (47). Many medical/aetiological disorders and the presence of epilepsy have also been found to correlate to adverse outcome but they also often, in and of themselves have a strong association with ID (48, 49).

1.7 Human brain development

The two major processes involved in human brain development are formation of the neural tube and cleavage of the prosencephalon, i.e., dorsal and ventral induction. These events constitute the neural component of the embryogenesis. The nervous system originates on the dorsal aspect of the embryo as a plate of tissue, differentiating in the middle of the ectoderm. Under the continuing inductive process the lateral margins of the neural plate invaginate and close dorsally to form the neural tube (Figure 1).

The peak time period for dorsal induction, resulting in the formation of the neural tube, is the third and fourth week of gestation.

During the ventral induction, with peak time during gestational week five to six, the face and forebrain are formed. This process involves cleavages of the prosencephalon, and the paired cerebral hemispheres and optic vesicles are formed during this period.

In the ensuing periods, intrinsic structures of the central nervous system develop, constituting the neural components of fetal development.

The event that follows after the first six weeks of gestation is proliferation of the brain´s total supplement of neurons and glia in the ventricular and subventricular zones of the brain. This process occurs during gestational week eight to 16.

Migration of the neurons to specific sites in the central nervous system, follows during gestational week 12 to 20. Neuronal migration refers to the complex series of events when millions of nerve cells move from their sites of

origin to the loci within the central nervous system where they form the cortex and will reside for life.

Thereafter, from about gestational week 20 to several years postnatally, organizational events occur, including alignment, orientation and layering of cortical neurons, dendritic and axonal ramifications and establishment of synaptic contacts.

The last period is myelination, starting in the second trimester of pregnancy and progressing most rapidly after birth and continuing into adult life. Myelination is characterized by the acquisition of the highly specialized myelin membrane around axons (50).

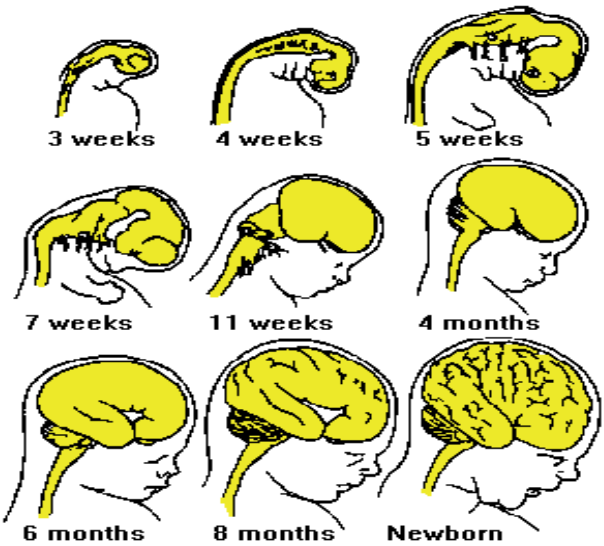

Figure 1. Human brain development

1.8 Ultrasound for imaging

Ultrasound (US) is an energy form produced by acoustic waves. The human ear can perceive frequencies around 20-20 000 hertz (20-20 kHz). Ultrasound used for medical purposes uses higher mega Hz (MHz). The lower the frequencies the deeper the waves can reach into tissues but higher frequencies create a more exact ultrasound image. US-energy has biological effects on human tissue. This is used therapeutically, for example to heal fractures by enhancing callus maturation (51-53) or to change skin permeability to get quicker pain relief with lidocaine cream (54).

When US is used exclusively for imaging in obstetrics it is important to avoid its biological effects on tissues (55).

1.8.1 Ultrasound safety in obstetrics

The American food and drug administration (FDA) used to set a safety limit of how much energy output the US-machines in obstetrics were allowed to produce. However, from around 1991, the FDA – in an attempt to increase diagnostic capabilities in a time period when pregnant women tended to become more obese – increased the allowed energy output from the machines from 94 to 720 mW/cm2 (= an 8 folded increase). With a view to still keeping the US-assessments safe, the machines were after 1991 required to show the safety thermal (TI) and mechanical indexes (MI). TI is the estimated effect needed to raise the temperature in the tissue one degree Celsius. MI reflects the risk for cavitations to occur in tissues. Risks also increase with exposure time (56).

However, several studies have shown that these indexes are not sufficiently applied in clinical practice (57). Moreover, the exposure time is not included in the safety indexes.

1.8.2 The ultrasound machines

Ultrasound (US) machines are now relatively inexpensive. The machines have become smaller, can be carried around and a diagnostic evaluation has become easier to perform.

The US equipment, including the transducer is sensitive for mechanical damage which may lead to an unanticipated incomplete image (58).

1.8.3 Prenatal ultrasound

From the 1970s, prenatal ultrasound (PUS) has been an established method to date the pregnancy, identify multiple pregnancies and detect malformations (Figure 2). It is considered to be a safe method, both for the child and the mother. Recently the earlier unexplained association with PUS and non-right handedness for boys is now shown to be statistically significant for both sexes (59). These studies were carried out in the 1970s and 1980s when prenatal ultrasound was mostly done once or twice in gestational week 17 or later and when the FDA was more restrictive as regards output levels. Currently, dating of pregnancy is considered to be more accurate in gestational week 12-14 when also malformations can be detected making this earlier period a common time point for prenatal US. US in gestational week 8, to confirm pregnancy, is another new way of using PUS. Other forms of prenatal US

devices used are transvaginal PUS (indicated when there is suspected premature labor or bleeding during pregnancy) and cardiotochography (CTG). CTG is used to assess the fetus´ heart rate during labour by adapting a small US machine on the mother's abdomen and another device to follow uterus contractions.
Lately, the technique has advanced further, and there is now pulsed and color Doppler to examine blood flow in fetal vessels or placenta. These examinations are usually performed with high energy intensities (60-62). PUS performed in early gestation and/or with longer exposure times and/or repeatedly, using the new machines has not been evaluated for safety with regard to fetus´ development (62).
In Sweden, there is no regulation requiring either prenatal US or length of exposure to be registered, but there are recommendations (61).

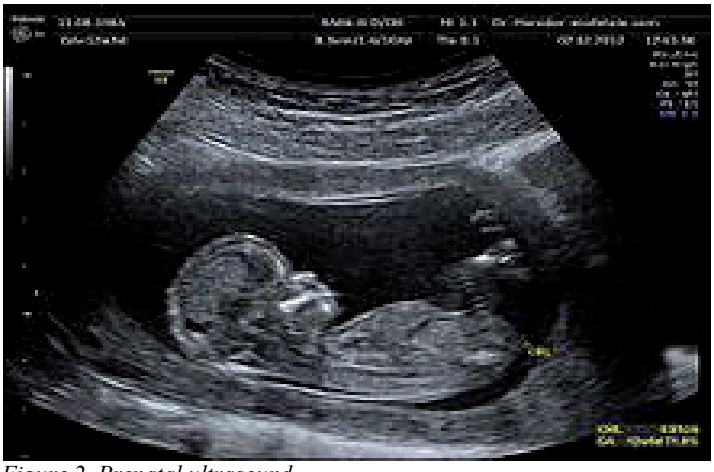

Figure 2. Prenatal ultrasound

1.9 Screening and surveillance

"The object of screening for disease is to discover those among the apparently well who are in fact suffering from disease" (63).

The term surveillance is often used as a synonym for screening, but there are useful and important distinctions between the two terms. In Webster's Third New International Dictionary (1966) surveillance is defined as "close and continuous observation", while the definition of "to screen" is "to examine methodically in order to make a separation into different groups" (63).

The American Academy of Pediatrics (64) has developed an algorithm for developmental surveillance and screening, in order to identify children who

may be at risk of developmental delays. The authors refer to "screening" as the use of standardized tools at specific intervals to support and refine the risk, whereas surveillance represents a “moving picture” of the child’s unfolding development. Screening represents “snapshots” of the child’s development at specific times. Developmental surveillance should occur at every preventive visit throughout childhood and includes attending to the parents’ concerns; maintaining a developmental history; making accurate and informed observations of the child; identifying the presence of risk and protective factors; and documenting the process and findings (64).

1.9.1 Screening

Screening is the procedure, not a diagnostic test, to detect pathology in a perceived healthy population and to raise suspicion of disease among people who seemingly have no problems. WHO, among others, has developed criteria for screening.

WHO Screening criteria

1) Important health problem
2) Accepted treatment for recognized disease
3) Facilities for diagnosis and treatment
4) Suitable latent and symptomatic stage
5) Suitable test or examination
6) Test acceptable to population
7) Natural history of condition understood
8) Agreed on policy on whom to treat
9) Cost of finding economically balanced with overall health
10) Case finding should be continuous process

Sensitivity is the screening test´s capacity to correctly detect the disease among individuals with the disease and specificity is the screening test´s capacity to correctly label a healthy person as healthy.

Ideally, the screening tests should have 100% sensitivity and 100 % specificity. In real life (when test values for those with and without the disease overlap) the rule is: the higher the sensitivity, the lower the specificity and vice versa.

1.9.2 CHAT-screening

Checklist for Autism in Toddlers (CHAT) was developed in a joint collaboration between England and Sweden as a screening test to detect young chil-

dren with ASD in a general population setting. It was one of the first screening instruments for this purpose and was evaluated in 2000 (65). The follow-up study yielded sensitivity as low as 20%, in contrast to its high specificity of 99%, when CHAT was used two times, as a 2-stage screening. The CHAT had previously been used in a high risk setting; siblings to children with ASD. In this setting, targeting siblings of children with ASD, the CHAT had a higher sensitivity: 38%.

The Modified Checklist for Autism in Toddlers (M-CHAT) is a 20 question ASD-screening for parents to answer before a routine developmental check-up around 16-30 months and was developed by Robins et al (66). Public screening among toddlers with M-CHAT is now used worldwide to screen for ASD. It is translated to many different languages and can be downloaded from mchatscreen.com.

It has now been revised, some questions have been changed and it is recommended that a (telephone) interview be performed as a follow-up in cases of screen positive children. By this 2-stage screening, M-CHAT-R/F, 94.6% of the screen positive children will later be diagnosed with ASD or other developmental delays (67).

In Sweden, the M-CHAT combined with an observation of the child's joint attention abilities (JA-OBS) by the nurses at CHCs showed promising results for early detection of autism (68).

1.9.3 CHC/BVC in Sweden

Swedish Health surveillance programmes started in the 1930s. The name (today Child Healthcare Centres) was initially "The Milk drop", due to its main purpose to serve milk without tuberculosis bacteria to children. Later the commitments for CHCs have increased considerably to include vaccinations, weight and height measurements, as well as surveillance on vision, hearing and general child development. CHCs have always been led by nurses with regular visits from a physician; a paediatrician or a general practitioner.

In Sweden, CHC´s have a high attendance rate; about 99% during the child´s first two years. The contact with the family often starts with a home visit. CHC offers developmental surveillance at 2, 6, 10 (by physician who also ensures a medical examination) and 18 months 2.5-3, 4 and 5 years of age (by nurse). The family often additionally contacts the CHC-nurse, by phone-calls or visits, several times to discuss other problems, for example feeding, sleeping and excessive crying, especially during the first year (3).

2 AIM

The aim of the thesis was to study young children with ASD from the point of view of early screening and detection, coexisting disorders, and possible prenatal risk. The specific aims were:

1) To study if the use of the CHAT - screening instrument, added to the routine 18-month developmental surveillance at CHCs would identify children with ASD at an earlier age;
2) To review findings from the regular 18-month developmental surveillance at CHCs in a representative group of preschool children, later diagnosed with ASD, with a view to establish whether or not many cases of ASD could have been picked up already at this routine developmental surveillance;
3) To explore the burden of co-occurring developmental disorders and problems in a representative group of preschool children diagnosed with ASD; and finally
4) To analyze whether the frequency of ASD, with and without concomitant ID, would differ in Swedish cohorts of children exposed to "early" (12th gestational week) or "later" (18th week) prenatal ultrasound.

3 PARTICIPANTS AND METHODS

3.1 Participants

The thesis is based on four different sets of data. The target groups are presented in figures below.

1. The group of children who had failed at least on one item at the 18-month routine developmental surveillance at the Child Healthcare Centre (CHC) in southern Stockholm County were chosen as the target group for the CHAT screening, n=6822.
2. The 175 of the 208 preschool children, referred to Autism Centre for Young Children (ACYC) in Stockholm County who met full criteria for ASD and had complete CHC-records were investigated with regard to their results at the 18-month routine developmental surveillance at CHC.
3. The 198 of the 208 preschool children with ASD in Stockholm County who had been referred to the ACYC for intervention and participated in a 2-year follow-up were investigated with regard to coexisting disorders and problems.
4. The 29 322 women, randomized either to early (gestational week 12) or later (gestational week 18) prenatal ultrasound were evaluated with regard to presence of ASD in their offspring.

3.1.1 Study I

The initial group in Paper I, participating in the 18-month routine developmental surveillance, consisted of all 18-month old children registered at the Child Healthcare Centres (CHC) in southern Stockholm County between January 1st 2005 and December 31st 2007. The 18-month old children in northern Stockholm County constituted the comparison group (38 062). The eligible 18 month-old population consisted of 37 630 children in the study area and of these, 35 990 (96%) participated in the surveillance. Of this group, 6822 children (19%) had failed at least one item at the developmental surveillance. This group was the target group for the CHAT study (Figure 3).

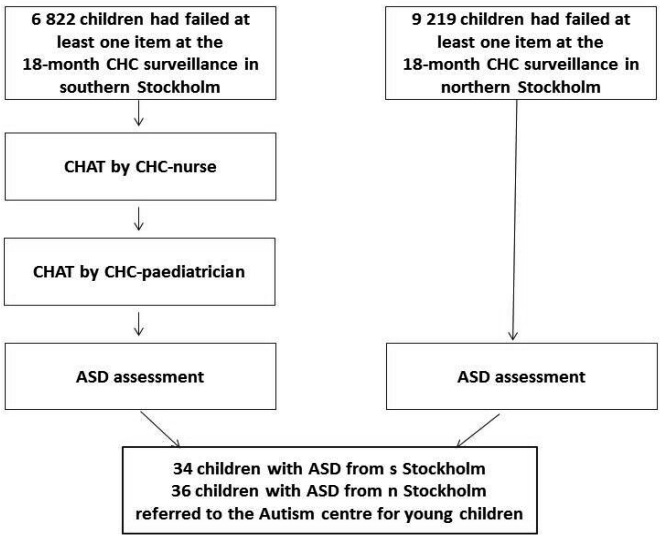

Figure 3. Inclusion of children and results in study I

3.1.2 Study II

The target group in Paper II consisted of those 208 preschool children (176 boys and 32 girls) with ASD, aged 20-54 months, when referred to the Autism Centre for Young Children (ACYC) to receive early intervention. The group derived from the 313 children who had been diagnosed with ASD in Stockholm County during 2005-2008. Of these, 288 children had been admitted to and registered at the ACYC and 25 children had been referred to one of the other 11 habilitation centres in the county mainly due to severe multi-impairments. Of the 288 children, 24 had been referred to ACYC before the project started and could not be included. The parents of the remaining 264 children were asked if they wanted to participate in the study with their child. Of these, parents of 37 children declined, for 15 children both parents could not communicate in Swedish or English and therefore had to be excluded, 2 children were referred to other habilitation centres because their needs were deemed to be better met at a general habilitation centre, and 2 had moved from Sweden. Of the remaining 208 children, CHC-records could be obtained for 196 children. Of these 196, 21 did not meet full criteria for ASD at the two- year follow-up, but had autistic traits with and without ID. This group of 21children was excluded, leaving a total of 175 children with autistic disorder, PDD-NOS and with Asperger syndrome in the study group (Figure 4).

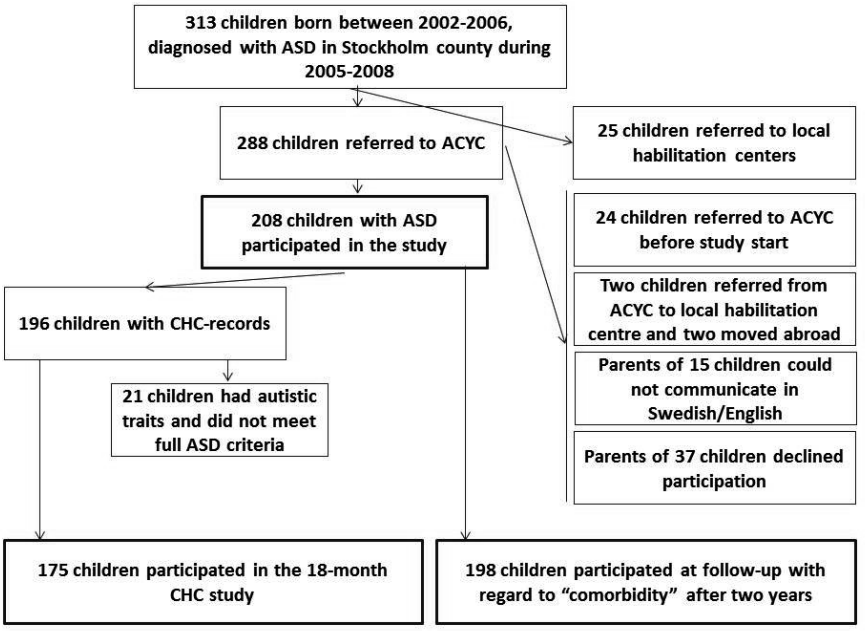

Figure 4. Flowchart demonstrating inclusion of children in study II and III

3.1.3 Study III

The investigated group in Paper III was the 198 preschool children with ASD (29 girls and 169 boys) derived from the same group as in study II (see figure 4), who participated in a follow up after two years of different types of interventions. This group was assessed by a multi-professional team with regard to coexisting disorders and problems.

3.1.4 Study IV

During 1999-2002, 39 572 pregnant women agreed to be randomized to ultrasound in gestational week 12 or 18 to compare a new strategy for prenatal diagnosis for Down syndrome (69). In the present ultrasound study, the 357 children with ASD, born after 29 322 of these pregnancies, constituted the study group, i.e., the children with ASD born to mothers that could be retrieved from the original 39 572 women. At follow-up these children were between 11 and 15 years of age (Figure 5).

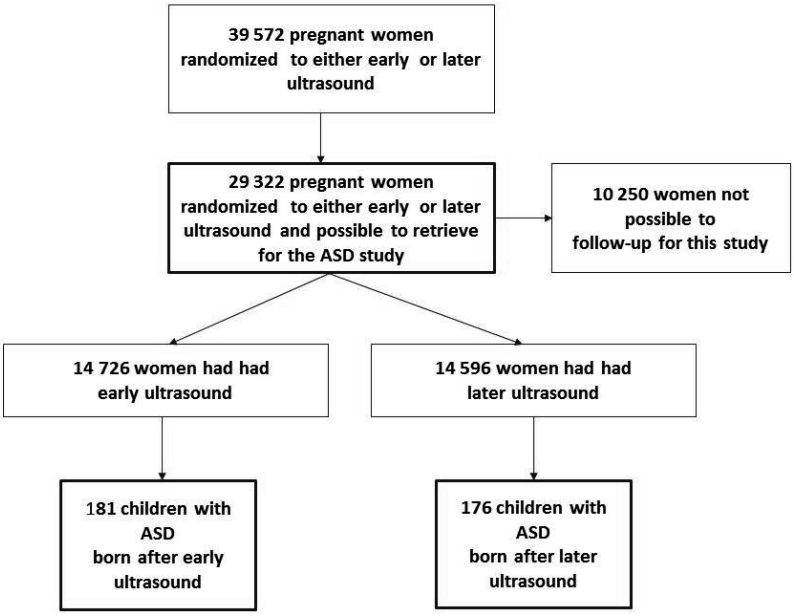

Figure 5. Flowchart demonstrating inclusion of children with ASD in study IV

3.2 Methods

3.2.1 Study I

All children had their 18-month developmental check-up at their local CHC. The children in southern Stockholm who did not pass one item or more were evaluated by CHAT-screening. If the child failed the two key items corresponding to the CHAT's "medium risk for autism" or three or more of any other items tested, a second CHAT evaluation and a general examination was performed by the paediatrician at CHC. If the paediatrician suspected that the child might have ASD, the child was referred to a neuropaediatric - or a child psychiatric clinic for evaluation.

Items in the CHAT test:

Section A: Ask Parent:

1. Does your child enjoy being swung, bounced on your knee, etc? YES/NO
2. Does your child take an interest in other children? YES/NO
3. Does your child like climbing on things, such as up stairs? YES/NO
4. Does your child enjoy playing peek-a-boo/hide-and-seek? YES/NO
5. Does your child ever PRETEND, for example, to make a cup of tea using a toy cup and teapot, or pretend other things? YES/NO
6. Does your child ever use his/her index finger to point, to ASK for something? YES/NO
7. Does your child ever use his/her index finger to point, to indicate INTEREST in something? YES/NO
8. Can your child play properly with small toys (eg cars or bricks) without just mouthing, fiddling or dropping them? YES/NO
9. Does your child ever bring objects over to you (parent) to SHOW you something? YES/NO

Section B: Observation:

i. During the appointment, has the child made eye contact with you? YES/NO
ii. Get child's attention, then point across the room at an interesting object and say 'Oh look! There's a (name of toy!)' Watch child's face. Does the child look across to see what you are pointing at? YES/NO*
iii. Get the child's attention, then give child a miniature toy cup and teapot and say 'Can you make a cup of tea?' Does the child pretend to pour out tea, drink it, etc.? YES/NO**
iv. Say to the child 'Where's the light?', or 'Show me the light'. Does the child point with his/her index finger at the light? YES/NO***
v. Can the child build a tower of bricks? (If so how many?) (Number of bricks:.....) YES/NO

* (To record YES on this item, ensure the child has not simply looked at your hand, but has actually looked at the object you are pointing at.)

** (If you can elicit an example of pretending in some other game, score a YES on this item.)

*** (Repeat this with 'Where's the teddy?' or some other unreachable object, if child does not understand the word 'light'. To record YES on this item, the child must have looked up at your face around the time of pointing.) (Copyright of MRC/SBC 1995)

3.2.2 Nurse survey

A written survey, including 18 questions about the nurses "attitude" to CHAT- screening was developed and conducted at the end of the study. The questionnaire included check answer questions, such as "how long time do you need for a CHAT-screening?" The last question was open for comments.

3.2.3 Outcome measure

The number of children diagnosed with ASD, less than three years old when registered at the ACYC to receive intervention, were registered and compared to the rate in the comparison group from northern Stockholm.

3.2.4 Study II

The physicians in the research team at the ACYC asked for the parents' permission and retrieved the children´s CHC-records, including results from the 18-month routine developmental surveillance. The surveillance consisted of eight items administered by the CHC nurse.

Items included in the routine developmental surveillance at CHC at 18 months:

1) Walks without support
2) Builds a tower of two to three cubes
3) Can make a scribble painting
4) Speaks at least eight to ten words
5) Understands more than eight to ten words
6) Enjoys playing with hiding objects
7) Points to body parts and
8) Retrieves objects when asked

In the CHC records the items are marked "+" for an observed skill, "-"when the skill is not observed at CHC or at home and "M" when the caregiver reports a skill to be present.
In accordance with the guidelines set up by the CHC in Stockholm County, the child had not passed the developmental surveillance, if she/he failed three or more of the skills or, could not walk independently (www.rikshandboken-bhv.se, in Swedish).

3.2.5 Registered regulatory problems

The CHC-nurse records all encounters concerning the child, for example if a mother or father had called for advice of how to improve the child's sleep. All contacts concerning problems with sleep, excessive crying or feeding during the first two years were registered.

3.2.6 A history of developmental regression

All parents had been interviewed by the physicians in the research team whether a history of developmental regression had occurred and this information was also checked in the child´s CHC record (70).

3.2.7 Study III

The 208 children in the Stockholm ASD study and their parents were invited to a two-year follow-up at the ACYC. Of these, 198 children participated. During this visit a clinical interview with at least one of the parents was carried out by one of the four physicians in the research team and a clinical observation and physical developmental examination of the child was performed. The clinical interview followed a structured questionnaire and included a detailed developmental history, questions about the child's current clinical symptoms and questions about co-existing disorders. The interview also included specific questionnaires pertaining to the child´s ASD and adaptive functioning. Epilepsy was documented when the child had a clinical diagnosis of epilepsy.

3.2.8 Cognitive assessment

Each child had been invited to a cognitive assessment by a psychologist in the research team. Instruments used were Griffiths´ developmental scales (71) and/or WPPSI-III (72). Intellectual disability (ID) was defined as a total IQ < 70, combined with a corresponding level of adaptive functioning.

3.2.9 Speech and language assessment

Within the project, all children without ID (n= 101) had been invited to an assessment of receptive and expressive language, carried out by a speech and

language pathologist in the research team, using the tests: 1. Reynell developmental language scales III, 2. SPIQ, 3. Illinois test of psycholinguistic abilities (ITPA) and 4. Processability test (grammar screening) (73-76). A child was classified as having a language problem if the performance was below a set criterion in two or several of the tests. All children with ID were considered to have a language problem, without special testing.

3.2.10 ASD assessment

A detailed and semi-structured autism interview with one or both parents was conducted by a research member using The Diagnostic Interview for Social and Communication Disorders (DISCO) (77). The interview, proceeding over several hours, is a valuable tool for establishing if the child has ASD or not. All clinicians (a physician, a psychologist and when appropriate the speech and language pathologist) met and discussed their evaluations, looked at the DSM-IV criteria and in conjunction discussed and set the ASD-diagnosis.

3.2.11 Motor function assessment

A motor function problem was considered to be present if the score in the motor domain of the Vineland Adaptive Behavior Scales (VABS) (78) was below 70, that is, corresponding to below -2SD from the mean of 100.

3.2.12 Vision and Hearing assessment

A visual/hearing impairment was recorded when parents reported a diagnosis of visual/hearing impairment, verified by an ophthalmological/ hearing test of the child. Vision and hearing tests are included in the 4-year health assessment at the CHC. Children who cannot collaborate or fail in these assessments at CHC or when there is a suspicion of problems before this age, are referred for clinical ophthalmological and/or a hearing examination. A hearing evaluation had usually also been part of the primary ASD assessment.

3.2.13 Activity and behavioural assessments

Parents were asked if the child had behavioural disorders or problems, including severe hyperactivity or diagnosed ADHD, severe hypoactivity and/or problems with severe outbursts or severe sleeping problems. Activity regulation was also observed and considered by the research team members during assessments.

3.2.14 Study IV

To study the rate of children with ASD, born to mothers who had been randomized to either early (gestational week 12) or later (gestational week 18)

prenatal ultrasound, it was necessary to identify the children with ASD through their mother´s personal identification number, which was available in the ultrasound file.

In Sweden, parents of children with certain disabilities are offered economical support from the society. It was assumed that mothers of children with ASD to a high degree would apply for a "childcare allowance" from the Swedish Social Insurance Agency (SSIA). Childcare allowances are mainly registered based on the mother´s personal identification number and this method was chosen to enable identification of children with ASD.

Pregnancy outcome by analysis of the yield of childcare allowance is a new method and therefore a validation study was performed in 2013 in Stockholm County.

3.2.15 Identification of children with ASD – validation study for the US study

The validation study was carried out at habilitation centres in Stockholm County, i.e., resource centres for children with specific neurodevelopmental disorders, including ASD and intellectual disability (ID). Virtually all children with a diagnosis of ASD in the county are known to these services (15). For this validation study a) 100 children with ASD without ID, b) 100 children with ASD and ID were analysed with regard to granted childcare allowances.

The study revealed that in 96% of the children with ASD and ID the parents received a childcare allowance from the SSIA**.** Thus, childcare allowance, to a very high degree identified children with ASD combined with ID. With regard to children with ASD without ID, the corresponding rate was 69%.

3.2.16 Identification of children with ASD in the US study

Personal identification numbers of the 29 332 mothers in the ultrasound study were referred to the SSIA**.** Administrators at the SSIA performed a search to identify all children of these mothers registered with a diagnosis of ASD with or without ID. The medical reports of the children with such a diagnosis of ASD, which constitute the basis for allowance, were then shared with the research group. A diagnosis of ASD with or without ID, registered on these medical reports, is always preceded by a full clinical, paediatric or child psychiatric assessment. These medical reports were scrutinized by two of the authors (LHC and EF).

3.3 Statistical analyses

In study I descriptive statistics were used. In study II Fischer's exact test was used to test significance between the groups. In study III a between-subjects ANOVA followed by post hoc tests (Fisher LSD) was used to examine if the mean number of coexisting problems differed significantly between ASD groups. An alpha level of .05 was used. In study IV Fisher's exact test was used to test significance of difference between the groups.

3.4 Ethics

Study I was approved by the regional medical ethical board at Huddinge hospital: 671/2003. Studies II, III and IV were approved by the regional medical ethical board at Karolinska Institutet, Stockholm; 2006/61-31/2, 2011/1445-31/1 and / 2012/54-32/1.

4 RESULTS

4.1 Study I

4.1.1 Overall findings

The group screened with the CHAT and the non-screened comparison group did not differ with regard to the numbers of children diagnosed with ASD or their ages at diagnosis.

4.1.2 Numbers of children with ASD identified with the CHAT

In the whole of Stockholm, a total of 70 children with ASD had been diagnosed with ASD and been referred to the ACYC for intervention before 3 years of age during the study period. A similar number (taken into account the population numbers) was ascertained in the "CHAT-study area" (n = 34) and in the comparison area (n = 36). The average age at referral did not differ between the groups, nor did the ASD-diagnoses, with or without intellectual disability (ID). All children were born between July 1st 2003 and June 30th 2006, thus all had their 18 month surveillance at CHC during the study period; January 1st 2005 – Dec 31st 2007.

Of the 34 children with ASD living in the study area only18 (53%) had been screened with the CHAT. Sixteen of these were found to have been CHAT positive. The remaining two children had passed the initial CHAT-screen performed by the nurse but were, for other reasons, examined by the pediatrician and referred for evaluation and diagnosed with ASD.

4.1.3 CHAT-screening not performed at CHC as planned

Of the eligible population of 37 630 children in the study area during the 3-year study period, 35 990 (96%) performed an 18-month surveillance at CHC. Of these, 6822 (19%) children did not pass at least one item on the 18-month surveillance and were by the study protocol eligible for CHAT-screening. However, only 1230 children (18%) were further screened with the CHAT. The reason for the smaller than expected proportion of CHAT-screened children, was the CHC-nurse´s decision not to screen (only occasionally the parents´) – in spite of the study protocol.

4.1.4 The CHC nurse's attitude to the CHAT-screening

In the spring of 2007 a survey about the nurses' attitude to the CHAT-screening was conducted. A written evaluation was returned from 153 of the 225 nurses (68%). Of these, 40 (26%) had never done a CHAT-screening at all. Of the 110 nurses who reported that they had performed at least one CHAT-screening, 63% admitted that they had deviated from the study protocol at least once i.e., had not made a CHAT-screening according to the study protocol. The main reason, among the response options, was 'it was evident that the child was not autistic'. Nevertheless, many nurses stated that the CHAT was a useful tool in their work.

4.2 Study II

4.2.1 Overall findings

About one third of the children later diagnosed with ASD had not passed the 18-month routine surveillance at CHC. Of the children with ASD combined with ID about half the group had not passed. If also regulatory problems had been considered, more children with ASD would probably have been identified at this routine check-up at 18 months.

4.2.2 Results in the group of children later diagnosed with ASD

Of the total group of 208 children with ASD referred to the ACYC, 196 children (94%) had complete data from their 18-month surveillance at CHC. Of these 196 children, records from CHC were analyzed for those 175 children (89%) who met full criteria for ASD at the two-year follow-up. Of these, 89 children had ASD and ID and 86 had ASD without ID. A total of 63 of the 175 children (36%) had not passed the required number of items at the surveillance, in contrast to the corresponding rate in the general child population in the county of 2% (www.rikshandboken-bhv.se, in Swedish).

Of the 89 children who at follow-up were considered to meet criteria for ID in addition to ASD, 43 (48%) had not passed the check-up at 18 months.
Of the 107 children with the most severe subtype of ASD, autistic disorder, 47 (44%) had screened positive at the 18-month check-up. Corresponding data for those with Pervasive Developmental Disorder-Not Otherwise Specified (PDD-NOS) was 16/55 (29%) and for those with Asperger syndrome 0/13 (0%).

The 63 children who had not passed the required number of items at the 18-month check-up were registered at the ACYC significantly earlier than the children who had passed; M = 34.48 months, SD = 8.22 months and M = 43.32 months, SD = 7.28 months, respectively, i.e., a difference of about 9 months; $t173 = 7.36$, ($p < .001$).

4.2.3 Results in the group with ASD and ID who had passed the surveillance

Of the 46 children with ASD and ID who had passed the 18-month routine developmental surveillance, 18 had a reported developmental regressive trajectory occurring at or after the age of 18 months, mostly between 18-24 months of age. Thus, in 39% (18/46) children with ASD and ID it probably would not have been possible to identify their developmental problems at 18 months.

The CHC records of the remaining 28 children were reviewed with regard to regulatory problems as a possible further marker of developmental problems. This review revealed that nine children had had two or more consultations for regulatory problems (excessive crying, feeding and/or sleeping problems) and of these nine children, eight had had some reported problems at the 18-month check-up, but not sufficient to be regarded as a screen positive result.

Thus, in almost 60% of the children with ASD combined with ID, who had passed the developmental surveillance at 18 months of age (18 with regression and 8-9 with regulatory problems) there had been some markers of developmental disorders already at 18 months or a developmental regression occurring a few months later.

4.2.4 Measures taken at CHC after positive screening

For 58 of the 63 children who had not passed the 18-month check-up information about measures taken at the CHC were available. Eleven children (19%) were already undergoing assessments at the time for the 18-month check-up, 24 children (41%) had been referred to the pediatrician at CHC, 10 (17%) had been scheduled to the nurse for a new visit and in 13 children (22%) no reported measures had been taken.

4.3 Study III

4.3.1 Overall findings

The rate of coexisting disorders in children with ASD was high and occurred in more than 90% of the children. The most common recorded problem was related to receptive and expressive language.

4.3.2 Types and frequencies of coexisting disorders

Of the 198 children examined, 181 (91%) had at least one coexisting disorder or problem (Figure 6). In the group of 106 children with AD, i.e., the most severe subtype of ASD, the mean number of coexisting disorders or problems was 3.2 (SD 1.4) (range 0–6), in the 71 children with ALC/Asperger syndrome, the mean number was 1.6 (SD 1.3) (range 0–5), and in the 21 children with autistic traits but not a full ASD diagnosis, the mean number of coexisting disorders or problems was 1.6 (SD 1.2) (range 0–4). Post hoc test (Fisher LSD) revealed that the difference between the autism group and the ALC/Asperger group as well as the difference between the autism group and the Autistic traits group were significant ($p < .001$, for both comparisons) (Figure 7).

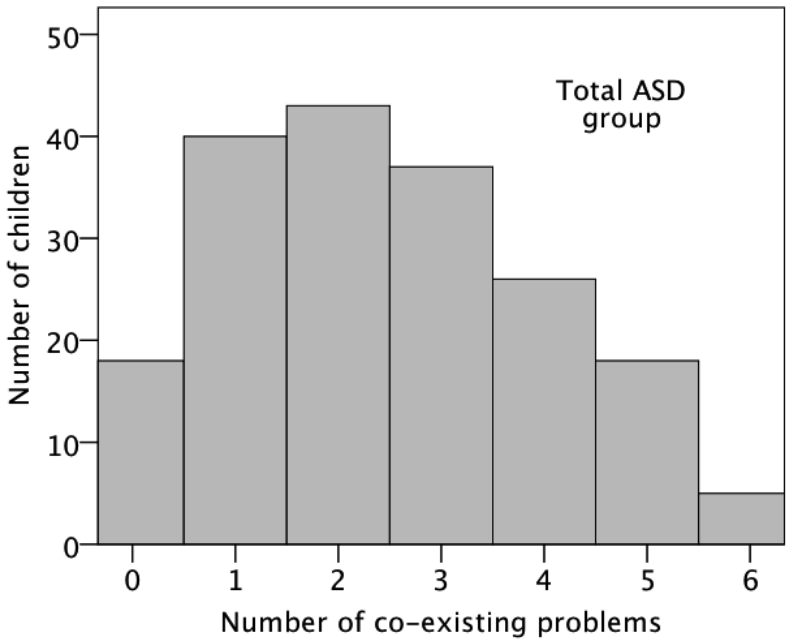

Figure 6. Numbers of coexisting problems/disorders in the total group of children with ASD

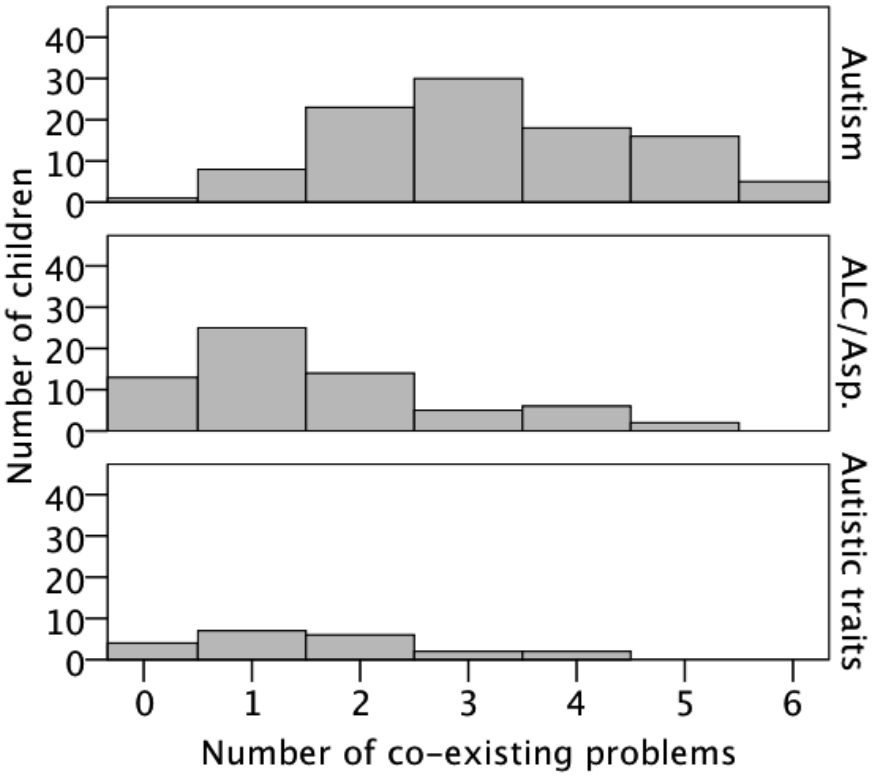

Figure 7. Numbers of coexisting problems/disorders in the three different ASD groups

Language: Of the 101children without ID, parents of 94 children agreed to let their children participate in a language assessment. Of these, 94 children, 53 (56%) had a definite language problem; that is, they fell below a set criterion in two or more of the language tests. The remaining 41 children either failed on one test only or passed all language tests. If all the 95 children diagnosed with ID were included by default they would belong to the group of children with a definite language problem, the rate of language problems was 78% (148/189).

Intellectual Function: Of the 196 who had a cognitive test in the project, 95 (49%) received a full DQ/IQ below 70. ID was more common in the group with AD, 80/105 children (75%), compared to the group with ALC/Asperger syndrome, 10/71 children (14%), and autistic traits, 4/21 children (19%).

Motor Function: 37 % (71/194) of the children who had Vineland interview data had a motor skills function below −2SD corresponding to a Vineland domain score below 70. Of these 71 children, 51 (72%) also had ID.

Activity Regulation: Severe hyperactivity or diagnosed ADHD was recorded in 63/198 children (32%) and severe hypoactivity in six children (3%). Of the 63 children with severe hyperactivity, 31 (49%) also had ID, and 39 (62%) also had AD.
Tantrums: Severe problems with tantrums were reported for 28/198 children (14%).
Sleeping Problems: Severe sleeping problems were reported for 24/198 children (12%).
Vision and Hearing: Any kind of visual impairment or strabismus was found in 21/198 children (11%). Of these 21 children, 10 (48%) had ID. A hearing impairment was recorded in one child (0.5%).
Epilepsy: 17 children (9%) had a diagnosis of epilepsy. Of these 17, 12 (71%) also had ID.

4.4 Study IV

4.4.1 Overall findings

No difference in ASD frequency between groups was found using the child care allowance from Swedish Social Insurance Agency as an outcome measure. A validation study prior to this study had revealed this method to cover about 96% of the children with ASD and ID.

4.4.2 Frequencies of children with ASD in the group with early vs later prenatal ultrasound

Identification numbers from 29 322 women could be retrieved. A total of 14 726 children had been born after early and 14 596 children after later ultrasound in 1999-2003 and of these, 181 (1.2%) (f:m=1:2.9) and 176 (1.2%) (f:m= 1:4.0) children, respectively, had been diagnosed with ASD. Mean age of the mothers at the child´s birth was 31 years and 30 years, in the early and later ultrasound groups, respectively. Of the 181 children, 17 had ASD with ID and of the 176 children, 14 had ASD with ID.

5 DISCUSSION

5.1 General Discussion

ASDs are heterogeneous conditions with regard to several aspects but with some important cognitive deficits in common. The overall presentation, that brings the child to an assessment, is the behavioural and overt developmental deviations and delays that are consequences of underlying cognitive deficits/peculiarities. These, in turn, will be dependent on the functional and morphological dysfunctions in the brain and the underlying genetic and/or environmental aetiologies. Such genetic and/or environmental factors can be ascertained/diagnosed in about 25-35% of children with ASD. With advancing medical/genetic technique this rate will increase in the near future. ASDs have been found not to be rare and most often coexist with other developmental disorders. It has also become obvious that the coexisting disorders are very important for outcome. ASD has a considerable impact on parents´ and children´s lives and early identification and different types of interventions and treatments are important.
In this thesis, four different aspects of ASDs have been studied; a screening instrument targeted for autism, the usefulness of the 18-month routine surveillance at Child Healthcare Centres to trace ASD, coexisting disorders in preschool children with ASD, and the possible risk of ultrasound during pregnancy for the development of autism.

5.2 Study I

There was a considerable increase with regard to the number of children being diagnosed with ASD before the age of 3 years during the study period. However, this increase was not limited to southern Stockholm but was also seen in the northern comparison area. There were similar numbers of children diagnosed with ASD in the study- and in the comparison area. Thus, the use of CHAT-screening in southern Stockholm could not in itself explain the increased numbers of children diagnosed with ASD. Other explanations could be that the awareness of ASD was increased all over Stockholm, and we cannot exclude that the screening instrument was used to some extent in the northern part.

Compared with 2001 and 2002 - when no child below 3 years of age had been diagnosed with ASD in southern Stockholm – i.e. the rise to 34 children detected in the area during the 3-year study period was surprising. The increased number was probably due to increased awareness of ASD in the

society and in child health at the time. When the study started it was known that CHAT, used as a 2-stage screening, only had a sensitivity of 20%; only every fifth child with ASD was likely to be identified by the CHAT. The power calculation prior to the study revealed that there would not really be sufficient power in the material to properly evaluate the CHAT, taken into account an ASD prevalence of less than 1%. However, the CHAT was chosen for the study since no alternative method was available; it was the only validated screening for ASD at the time. Also, the specificity was expected to be very good, almost 100 %. Therefore, it would be no risk for over-diagnosing ASD. The Autism parent organization in Stockholm was also eager to use the CHAT to enable young children to receive the diagnosis and intervention early and hopefully a better outcome compared to a situation with later diagnosis and start of intervention. It was also discussed that lectures about ASD and the use of CHAT would raise the awareness of ASD among the nurses working at CHCs. Taken together; the decision was to start the study.

5.2.1 Strengths and Limitations

During the study period all young children with diagnosed ASD throughout Stockholm County were referred to the ACYC for intervention, making it relatively "unproblematic" ethically to identify all children with ASD in this age group both in the southern and northern part of Stockholm.

At the completion of the study, it was found that only 18% of the children had participated in the CHAT screening. There are at least two explanations for this:

1) The nurses had limited time allocated for the routine surveillance. Therefore, in cases where they perceived that a CHAT screening was unnecessary – the child had no obvious signs pertaining to ASD – they refrained from the screening, which would have taken an additional 10-20 minutes of their time. This situation could have been avoided if more time had been allocated to preparations for the study; education and discussions with the 225 CHC nurses in the study area – about child development, ASD and the CHAT screening.

2) Compliance would probably have been better if three, rather than one, failed items on the 18-month surveillance had been the proposed cut-off for CHAT-screening.

5.2.2 Conclusion

In conclusion, the study itself led to increased awareness and knowledge at CHCs about ASD in young children. However, the CHAT-instrument *per se*

identified an equal number of children with ASD, as did the routine surveillance without the CHAT in the comparison region.

5.3 Study II

Although the 18-month routine developmental surveillance at CHC is not aimed specifically at identifying ASD, it is geared to identifying developmental deviations; motor, speech and language, social interaction and general cognitive ability. Statistics from the child health authority in Stockholm county show that 2% of the child population does not pass the required numbers of tested items, i.e., fail in at least three of the items being tested at the routine surveillance. The study revealed that more than a third of the children with ASD in the study group had not passed the required number of items, and among children with ASD and ID, nearly half had not passed.

Data from CHC records and from the parental research interview at the ACYC showed that a subgroup of children who had passed had had a regressive trajectory and lost skills at an age between 18 and 24 months. This group amounted to 18 children (20% of the total group with ASD and ID) and for this group it would not have been possible to identify ASD at the 18-month routine surveillance.

In a previous study (3) we had demonstrated that early regulatory problems (feeding-, crying- and sleeping problems) had occurred significantly more often in children later diagnosed with ASD, in contrast to a comparison group.

The presence of such regulatory problem symptoms could have identified another small subgroup of about 10% with ASD and ID who had passed the 18-month check-up – but had some developmental deviations at the routine 18-month check-up –and had not had a later developmental regression.

This means that in only about 20% of the children with ASD and ID it was not possible to explain why they had not been identified at the 18-month surveillance at CHC. Some of the children in this group may have had only reported skills, not observed at CHC, making them pass the surveillance. Parental delay might be a reason why children with ASD/ID are identified late.

5.3.1 Strengths and Limitations

Strengths of the study included the community-based design of the original study group, the comprehensive clinical assessments that had been performed in all children at study start and after two years, and that data from the 18-month check-up at CHC were available in 94% of the children.

Limitations of the study were that the data only cover children who had received the ASD diagnosis before age 4.5 years, and no information was possible to obtain from children who had received their ASD diagnosis at a later age. Also, we did not have information regarding the rate of children from the same birth cohorts, who had/had not screened positive at the 18-month check-up, but who received diagnoses of ID without ASD, or other developmental diagnoses or who in the long term perspective developed normally.

5.3.2 Conclusion

The conclusion of this study was that 1) the 18-month routine developmental surveillance at CHC would probably identify approximately half of the children who will later be diagnosed with ASD and ID and 2) if an item regarding regulatory problems had been added at this check-up, and a question about developmental regression at the ensuing speech and language screening at ideally 2.5 years of age at CHC, more children with developmental disorders, including ASD, would be traced at their regular CHC check-ups.

5.4 Study III

This study demonstrated that coexisting disorders and problems, in areas of many developmental and cognitive domains; speech and language, intellectual function, behaviour, motor function and epilepsy, were very common, and occurred in more than 90% in a group of young children with ASD. The more severe the ASD symptoms, the higher the rate of co-occurring disorders. Children with AD had significantly more coexisting disorders compared to the group with autistic like condition/Asperger syndrome and those with autistic traits.

The three most common co-occurring disorders were language disorder/problems, ID and ADHD. Language disorder/problems occurred in 78% of the total group. Much of this high rate was accounted for by the presence of ID in about half the study cohort, and that all the children with ID (although not always personally assessed by a speech and language pathologist) were considered to meet criteria for a language problem. However, the group of children classified as having language problems also included a group that had been assessed by a speech and language pathologist (approximately half the cohort, all without ID) and had been found to exhibit a language disorder.

In many children with autism, language delay is the presenting symptom of ASD. The importance of considering language delay in a wider developmental perspective was demonstrated by Miniscalco and collaborators who followed children identified with language delay at the child health screening at 2.5 years of age. At follow-up at the age around school start, 72% of the children were found to have a major neuropsychiatric or learning disorder (26).

Almost half the group of preschool children had ID in combination with ASD. In the group with AD, the rate of ID was 75%. A correlation between autism and low intellectual level has been demonstrated and many of the behaviours that characterize autism overlap with behaviours common in learning/intellectual disability (79).

A substantial degree of overlap between ASD and ADHD was found in the study, providing support that there are common genetic influences across ASD and ADHD (80). Both disorders are relatively common with ADHD showing a prevalence of 5% and ASD a prevalence of about 1% in the general population. Comorbidity of ADHD in patients with ASD has been found to be around 30% (81).

The importance of considering ADHD in children with ASD has implications for pharmacological/stimulant treatment of ADHD, which may contribute to improved attention and adaptive behaviour in the child (82).

5.4.1 Strengths and limitations

Strengths of the study include the representativeness of the cohort and that all children were personally examined by the research team.
One possible limitation stems from the fact that only a proportion of the group was assessed by a speech- and language pathologist at follow-up; the reason was limited resources for the project.

5.4.2 Conclusion

Conclusions of the study were that 1) more than 90% of the children in our cohort of preschool children with ASD exhibited other problems than the ASD per se, and 2) coexisting conditions should always be looked for in the developmental as well as the medical assessments of young children with ASD.

5.5 Study IV

Studies from different researchers (83-85) have reported that exposure to ultrasound during pregnancy might pose risks for fetal development.

The Food and Drug Administration (FDA) changed their policy of obstetric US-machines energy in 1991. Instead of stating an absolute intensity level for the machines, not allowed to be exceeded, they introduced an ”Output Display Standard”. This standard means that the examiner has to understand and manage the thermal and mechanical indexes, to use the ALARA (=As Low As Reasonably Achievable) principle and to keep the US examination safe for the fetus. Unfortunately very few studies on safety have been performed at all (86) and this has not changed after the change of intensity level in the machines and their more frequent use in obstetrics.

A large Swedish ultrasound study had randomized 39 572 pregnant women to either early (first trimester) or later (second trimester) US between 1999 and 2002 to evaluate a new regimen to detect Down syndrome (69). In 2011 a collaboration started with a view to analyze if any of the time periods for ultrasound, early or later during pregnancy, could entail adverse effects, i.e., increase the risk for ASD. Little more than 29 000 pregnant women were included in this study.

To identify children with ASD in these two cohorts, it was necessary to search the children through their mothers´ personal identification number. Based on the assumption that a very high rate of parents/mothers of children with ASD apply for a childcare allowance from the Swedish Social Insurance, this agency was approached. The procedure was approved ethically and all mothers in the two cohorts from the ultrasound study were searched for in the register at the Swedish Social Insurance with regard to whether they had a granted childcare allowance. The childcare allowance for ASD was used as the outcome measure.

The study did not reveal any significant difference in ASD prevalence (ASD with and without ID) between the two groups. However, there was a difference with regard to female:male ratio between the early and later US groups; 1:2.9 and 1:4.0, respectively. A recent study reported a higher death rate of female fetuses during early pregnancy, explaining the 50-50 girl-boy ratio at conception changing to a slight excess of boys at birth (87). Maybe female fetuses are particularly vulnerable during the early part of gestation. Our finding of a possibly slight tendency ($p = .104$, Fisher exact test, one-tailed, not significant) towards a preponderance of girls with ASD in the early US-group could, speculatively, be linked to such vulnerability. This gender

issue needs to be looked into in future studies of possible effects of ultrasound on human development.

5.5.1 Strengths and limitations

Strengths include the large randomized ultrasound groups, comprising about 29.000 women. Our outcome measure, childcare allowance gave a prevalence rate of autism close to what is expected in the general population and we therefore consider this search procedure to be valid.

Our data regarding the children is limited to whether or not they had received a diagnosis of ASD before the age of 11 to 15 years. No other information could be obtained; i.e., school achievements.

It can be assumed that children with ASD, but without ID are underreported. Our preceding validation study showed a higher rate of childcare allowances granted for parents of children with ASD combined with ID, compared to the group with ASD without ID. There may also be children with milder forms of ASD without ID who have not been assessed and diagnosed.

5.5.2 Conclusion

The conclusion was that our study could not find evidence of a specific time period for US (early or later during pregnancy) being associated with increased risk for ASD. Our results are based upon the use of B-mode US that was used during the study period and may not apply with today´s use of other high intensity US devices, like Doppler.

6 SOME CLINICAL CONSIDERATIONS AND A FUTURE PERSPECTIVE

With regard to screening for ASD it is important to consider the heterogeneity of ASDs, i.e., onset of symptoms at different ages, different symptom presentations, severities, comorbidities and aetiologies. Therefore it is unlikely that one screening method, used at a certain age, would be suitable for the identification of *all* children with ASD, and ASD *only*. However, results from a recent study in Sweden found the M-CHAT to be useful, particularly when *combined* with a certain joint attention observation. It probably would make more sense to perform broader developmental screens that would pick up children with ASD *as well as* several other neurodevelopmental/early onset neuropsychiatric problems/ disorders.

The 18-month routine surveillance at CHC, which is just such a broader screen, proved to be a useful "screening for ASD" when evaluated more comprehensively and with consideration of ASD-associated regulatory problems. It is likely that if a question regarding the occurrence of developmental regression were to be added to the speech-and language screening that is often performed at 30-36 months, then the majority of cases with the combination of AD and ID would be possible to identify before age 3 years.
Comorbidities, or co-occurring/coexisting conditions are obviously extremely common, perhaps even universally occurring, in young children with ASD. These disorders, that actually often predict the longer-term outcome for children with ASD better than the "autism load" in itself, need to get at least as much attention as the ASD per se. Concomitant ID and ADHD, epilepsy, motor coordination and other medical disorders that require specific support and treatment need to be diagnosed whenever present. A clinically highly relevant problem in Sweden today is the strong focus specifically only on autism, and the fact that many other developmental disorders and combinations of disorders do not guarantee any specific support from society or habilitation services.

The US study, in which no significant negative effect of early versus later US was found, included children with clinically impairing ASD, with and without ID**,** i.e. children with rather severe impairments. A future study, analyzing early and later US exposure, including children with milder developmental disorders - such as ADHD and speech and language disorder without ASD and ID - would be valuable. It is suggested that the weak and non-significant trend for an effect of early US in girls should be taken into consideration in the design of future studies looking at the effects, if any, of prenatal US on the neurodevelopmental trajectories of children.

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Love, Lotta

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