AUTISM SYNDROMES IN THREE BEHAVIOURAL PHENOTYPE CONDITIONS

A clinical psychiatric study of 76 individuals with Möbius sequence, CHARGE syndrome, and oculo-auriculo-vertebral spectrum

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Abstract

Objectives: (1) Examine the prevalence of autism syndromes in three different Behavioural Phenotype Conditions (BPCs), (2) examine background and associated factors/conditions, and (3) describe and evaluate diagnostic difficulties in this field of research. **Method**: As part of multidisciplinary surveys of Möbius sequence (Möbius) (n=25), CHARGE syndrome (CHARGE) (n=31) and oculoauriculovertebral spectrum (OAV) (n=20), the occurrence of autism symptoms was assessed utilizing the DSM-III-R and DSM-IV checklists for autistic disorder (AD), the Autism Diagnostic Interview-Revised (ADI-R), the Childhood Autism Rating Scale (CARS) and the Autistic Behavior Checklist (ABC). Mental level was evaluated using standardized IQ tests or the Vineland Adaptive Behavior Scales. Results from previously performed radiological imaging/laboratory tests, and data on pre/perinatal nonoptimal conditions and family factors were scrutinized. The applicability of the autism diagnostic instruments used in individuals with multiple disabilities (such as in these BPCs) was analysed. **Results**: There was a high rate of autism syndromes (Möbius 48%, CHARGE 68%, OAV 42%) across all BPCs. Severe behaviour disturbances with major impact on family life were common in the individuals with autism syndromes, especially in the CHARGE group. Learning disability (LD) was a common finding (Möbius 32%, CHARGE 72%, OAV 25%), possibly reflecting the link between autism syndromes and LD. Visual and/or hearing impairments affected only a few subjects with Möbius, but were very common and associated with autism syndromes in the CHARGE/OAV groups. Cerebral abnormalities were recorded in one fifth of radiologically examined individuals with Möbius, 74% with CHARGE, and 63% with OAV. Autism syndromes, LD and cerebral abnormalities tended to occur together in the same individuals. Cranial nerve dysfunction was present in all Möbius individuals, in 55% of the CHARGE, and in 60% of the OAV group. Pre-, perinatal and/or family factors of possible interest were recorded in several individuals in each BPC. The diagnostics of autism syndromes in these BPCs presented difficulties, notably due to sensory impairments, cranial nerve palsies and LD. The diagnostic difficulties increased with the number and severity of disabilities. **Discussion and conclusions:** This study suggests that autism syndromes always should be considered in subjects with Möbius/CHARGE/OAV. In the CHARGE/OAV groups, cerebral abnormalities occurred frequently in subjects with autism syndromes, indicating that autism symptoms were not only attributable to sensory impairments. The frequent occurrence of cerebral abnormalities in those with autism syndromes, together with the fact that the majority of those with LD in all the three BPCs had an autism syndrome, could be suggestive of a specific link between autism syndromes and Möbius/CHARGE/OAV. The associated overall clinical findings, including the frequent occurrence of cranial nerve dysfunction found in all three BPCs, implicate the early embryonic brain, including the brain stem, as a possible area of core dysfunction. The use of an extensive battery of autism diagnostic instruments is essential in individuals with multiple disabilities. Current autism diagnostic instruments are insufficiently tailored to deaf-blind individuals.

Key words: autism syndromes, behavioural phenotype conditions, Möbius, CHARGE, OAV, ADI-R, CARS, ABC, brain stem

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To Gunnar, Per and Axel

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LIST OF PAPERS

This thesis is based on the following publications, which will be referred to in the text by the Roman numerals I-IV.

I. Johansson M, Wentz E, Fernell E, Strömland K, Miller MT, Gillberg C. (2001) Autistic spectrum disorders in Möbius sequence: a comprehensive study of 25 cases. Developmental Medicine and Child Neurology 43: 338-345.

II. Johansson M, Råstam M, Billstedt E, Danielsson S, Strömland K, Miller M, Gillberg C. (2006) Autism spectrum disorders and underlying brain pathology in CHARGE association. Developmental Medicine and Child Neurology 48: 40-50.

III. Johansson M, Billstedt E, Susanna D, Strömland K, Miller M, Granström G, Flodmark O, Råstam M, Gillberg C (2007) Autism spectrum disorders and underlying brain mechanisms in the oculoauriculovertebral spectrum. Developmental Medicine and Child Neurology 40: 280-288.

IV. Johansson M, Gillberg C, Råstam M. (2007) Autism spectrum disorders in individuals with Möbius sequence, CHARGE syndrome and oculoauriculovertebral spectrum: diagnotic aspects (submitted).

ABBREVIATIONS USED IN THIS THESIS

A	Average intelligence; IQ ≥85
ABC	Autistic Behavior Checklist
AD	Autistic Disorder
ADD	Attention Deficit Disorder
ADHD	Attention Deficit Hyperactivity Disorder
ADI-R	Autism Diagnostic Interview-Revised
ALC	Autistic-like Condition
AT	Autistic Traits
ASC(s)	Autism Spectrum Condition(s)
BP	Behavioural Phenotype
BPC(s)	Behavioural Phenotype Condition(s)
CA	Childhood Autism
CARS	Childhood Autism Rating Scale
CHARGE	CHARGE syndrome (Ocular **C**oloboma, **H**eart anomaly, **A**tresia of the choanae, **R**etarded growth and/or development, **G**enital hypoplasia, **E**ar anomalies and/or hearing impairment)
CNS	Central Nervous System
CT	Computerized Tomography
DB	Deaf-blind
DNA	Deoxyribo Nucleic Acid
DSM-III-R	Diagnostic and Statistical Manual of Mental Disorders, 3rd Edition
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, 4th Edition
EEG	Electroencephalogram
FISH	Fluorescence in Situ Hybridization
GS	Goldenhar Syndrome
HFM	Hemifacial Microsomia
ICD-10	International Classification of Diseases, 10th Edition.
ICSI	Intra Cytoplasmic Sperm Injection
IVF	In Vitro Fertilization
IQ	Intelligence Quotient
LD	Learning Disability
MLD	Mild Learning Disability
MRI	Magnetic Resonance Imaging
NA	Near Average intelligence; IQ 70–84
OAV	Oculoauriculovertebral spectrum
PDD NOS	Pervasive Developmental Disorder Not Otherwise Specified
PLD	Profound Learning Disability; IQ <20
ROP	Retinopathy Of Prematurity
PSIV	Probably Severe Visual Impairment
SD	Standard Deviation
SLD	Severe Learning Disability; IQ 20-49 (CHARGE/OAV), IQ <50 (Möbius)
SVI	Severe Visual Impairment
US	Ultrasound Scan
VI	Visual Impairment
WAIS-R	Wechsler Adult Intelligence Scale-Revised
WISC-III	Wechsler Intelligence Scale for Children, 3rd Edition

INTRODUCTION

The concept of autism, autism spectrum conditions (ASCs) and autism syndromes

In 1943 Leo Kanner described a group of 11 children (8 boys, 3 girls) with abnormalities in social behaviour. He introduced the term "infantile autism" to describe a disorder with specific features and early childhood onset. Lorna Wing (Wing and Gould 1979), based on her epidemiological study of disabled children in the Camberwell borough of London, launched the idea of a triad of symptom-areas in autism (impairments in reciprocal social interaction and communication, and repetitive and restricted repertoire of behaviours), which has become generally accepted as the core features of the concept of autism. However, it was only with the development of the Diagnostic and Statistical Manual of Mental Disorders, 3rd Edition (DSM-III) criteria (APA 1987) (Table 1) that autism was strictly defined with a set of stringent criteria (as consisting of concomitant severe impairments) in this triad. The DSM-III-R criteria specify that a symptom shall only be considered to be present if the behaviour is abnormal for the person's developmental level, but do not include an age of onset criterion. Today, the concept of autism is strictly operationalized with a categorical menu approach in the DSM-IV, 4th Edition (APA, 1994) (Table 2) and in the International Classification of Diseases and Disorders-10 (ICD-10, WHO 1993), which dictate a set of criteria within the three symptom areas (impaired reciprocal social interaction, impaired reciprocal communication and restricted repetitive and stereotyped patterns of behaviour) (Table 2). Both the ICD-10 and the DSM-IV criteria include an age of onset criterion, specifying that symptoms must have been present during the first three years of life. In the DSM-IV, Autistic Disorder (AD) is included in the category of "Pervasive Developmental Disorders", a group of dysfunctions defined as having common characteristics such as severe difficulty in regulating sensory, attention, cognitive, motor and affective processes (APA, 1994). There are three other PDDs: Rett syndrome, disintegrative disorder and pervasive developmental disorder not otherwise specified (PDD NOS).

Over the years, the idea of the concept of autism has changed. What was once seen to be a unique disease (Kanner and Eisenberg 1956) is now subsumed under a wider "spectrum" or "continuum" of autism spectrum conditions (ASCs). The term ASC has been used to comprise terms such as autistic disorder (AD), Asperger syndrome (Gillberg and Gillberg 1989), PDD NOS (DSM-IV, APA 1994), atypical autism (ICD-10, WHO 1997), "autistic-like condition" (Steffenburg and Gillberg 1986, Swedish legislation regarding "autism and autistic-like conditions" (LSS), 1993: 387), and "autistic traits". Of these, the concepts of PDD NOS, atypical autism and autistic-like conditions have been used almost synonymously. All so called ASCs share the same common fundamental disturbances as seen in classic autism, but the clinical picture among ASCs vary considerably. The diagnostic terms PDD NOS and atypical autism are only loosely delineated in the DSM-IV and ICD-10 and the term "autistic traits" is not included at all in these manuals, whereas "autistic disorder" (AD) according to the DSM-IV, and "Asperger syndrome" according to Gillberg (1991) are rigorously operationally defined. Coleman (1976) and later Gillberg (Gillberg and Coleman 1992) have argued that because of the multiple aetiologies, and varying symptomatology of autism, the most reasonable term for the group of conditions in question would be "autistic syndromes". From the semantic point of view "autism syndromes" would appear to be the more adequate term.

Recently, on the basis of research at the behavioural, cognitive and genetic level, a behavioural fractionation of the three core diagnostic dimensions in ASCs (social

impairment, communication problems and rigid, repetitive behaviours) has been advocated (Happé et al. 2006). Still, the fact that current autism diagnostic criteria mark out a clearly recognizable group of individuals is not questioned.

For the purpose of convenience, and given our use of this term in some of the papers providing the basis for the thesis, the present thesis will be using the term ASCs throughout (while acknowledging the problematic nature of the label).

Table 1. Diagnostic criteria for DSM-III-R autistic disorder (APA 1987). Eight or more symptoms from (A), (B) and (C), with at least two from (A) and one each from (B) and (C) are required for a diagnosis to be made

Overall category	*Symptom criteria*
A. Severe qualitative impairment of social interaction	1. Marked lack of awareness of the existence of feelings of others 2. No or abnormal seeking of comfort at times of distress 3. No or impaired imitation 4. No or abnormal social play 5. Gross impairment in ability to make peer friendships
B. Severe qualitative impairment of communication	1. No mode of communication 2. Markedly abnormal non-verbal communication 3. Absence of imaginative play/interest in stories about imaginary events 4. Marked abnormalities in the production of speech 5. Marked abnormalities in form or contents of speech 6. Severely limited capacity to initiate/maintain conversation
C. Severe restriction of repertoire of activities/interests	1. Stereotypic body movements 2. Persistent preoccupation with parts of objects 3. Marked distress over changes in trivial aspects of environment 4. Unreasonable insistence on following routine in precise detail 5. Markedly restricted range of interests and a preoccupation with one narrow interest

Table 2. Diagnostic criteria for DSM-IV autistic disorder (APA 1994) (similar to ICD-10). Six or more symptoms from (1), (2) and (3), with at least two from (1) and one each from (2) and (3) are required for a diagnosis to be made.

Overall category	*Symptom criteria*
A. Symptoms	
1. Qualitative impairment in social interaction	(a) Marked impairment in the use of multiple non-verbal behaviours (b) Failure to develop peer relationships appropriate to developmental level (c) A lack of spontaneous seeking to share enjoyment, interests, or achievements with other people (d) Lack of social or emotional reciprocity
2. Qualitative impairment in communication	(a) Delay in, or total lack of, the development of spoken language (b) In individuals with adequate speech, marked impairment in the ability to initiate or sustain a conversation with others (c) Stereotyped or repetitive use of language or idiosyncratic language (d) Lack of varied, spontaneous make-believe play, or social imitative play, appropriate to developmental level
3. Restricted repetitive and stereotyped patterns of behaviour	(a) Encompassing preoccupation with one or more stereotyped and restricted patterns of interest, which is abnormal either in intensity or focus (b) Apparently inflexible adherence to specific, non-functional routines or rituals (c) Stereotyped and repetitive motor mannerisms (d) Persistent preoccupation with parts of objects
B. Delays/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 3. Symbolic or imaginative play
C. Not better accounted for by Rett's disorder or childhood disintegrative disorder	

Symptom patterns in autism

Even the complexity of the symptomatology of the narrower concept of autism, as defined in operationalized criteria, is striking. Sometimes, a distinction is made between individuals with autism with and without learning disability (LD), which have been referred to as “low-functioning” and “high-functioning”. Children with severe LD (SLD) are less likely to have speech, and children with higher IQ levels much more likely to display a variety of elaborate repetitive routines and islets of special abilities. There are also data indicating that there might be a need to revise the clinical prototype for autism in females (fewer special interests, slightly better superficial social skills, a greater tendency to avoid demands, better expressive language ability, less hyperactivity and violent behaviour), at least in those individuals functioning in the normal range of IQ (Kopp and Gillberg 1997). Further, the prevailing symptoms in autism, like in other developmental disorders, change with age (Gillberg 1990). In infancy and the first year of life non-specific symptoms such as lack of initiative, hyperactivity, sleep problems, and feeding difficulties often dominate the clinical picture, but more "autism specific" symptoms such as abnormal responses to sensory stimulation, autistic aloofness and abnormalities of behaviour and play are also often present (Dahlgren and Gillberg 1989, Gillberg et al. 1990). In many patients the typical autism symptom patterns are most evident from about two to six years of age. In addition, behavioural disturbances not included in the autism diagnostic criteria, such as temper tantrums, hyperactivity and sleep problems, are frequently prominent at this age. Inability to develop peer relationships is often a conspicuous concern in the early school years, but, in general, typical autistic symptoms as well as behavioural disturbances are less marked during this period. Epilepsy, aggravation of symptoms and additional psychiatric problems may set in during adolescence.

Behavioural Phenotype Conditions (BPCs)

Although the behavioural symptoms in ASCs share fundamental features, the aetiology is known to vary (Cohen et al. 2005). During the last decades, autistic symptoms have been shown to be associated with a growing number of other medical conditions, of which many have known genetic causes and may be referred to as behavioural phenotype conditions (BPCs). A behavioural phenotype (BP) can be defined as a characteristic pattern of motor, cognitive, linguistic and social abnormalities, which are associated in a way that is compatible with a biological disorder. There is evidence for characteristic BPs, in which autistic symptoms have been recognized to be a component, in a number of congenital conditions, including Rett syndrome (Sansom et al. 1993), Fragile X syndrome (Turk and Cornish 1998), Tuberous sclerosis (Bolton and Griffiths 1997), and Angelman syndrome (Steffenburg et al. 1996). The BP in Möbius sequence (Möbius), CHARGE syndrome (CHARGE) and oculoauriculovertebral spectrum (OAV), was not well explored at the time of the launch of the present study, even if autism and autistic symptoms had been described. Insight into the physiological and behavioural characteristics of conditions, known to be associated with autistic symptoms, may shed new light on the pathophysiology of autism.

Möbius sequence

This condition, also referred to as "Möbius syndrome", has more recently been designated Möbius sequence. The term "sequence" defines a cascade of secondary events after a single embryonic insult due heterogeneous causes, in contrast to the designation "syndrome", which implies a single cause. A variety of systemic and functional dysfunctions may occur in Möbius sequence, but the most accepted clinical criterion for

Möbius sequence is evidence of congenital sixth and seventh cranial nerve dysfunction. Commonly associated anomalies include other cranial nerve involvement, limb defects (usually of hypoplastic type), craniofacial anomalies involving the tongue and lip, and pectoral muscle defect (Poland anomaly). Möbius sequence is included in a syndrome complex characterized by cranial nerve palsies, limb anomalies and craniofacial malformations: "the oro-mandibular-limb-hypogenesis syndrome" (OMLH: Hall 1971, Gorlin 1990). Tentamy and McKusick (1978) referred to this group of syndromes as "terminal transverse limb defects with orofacial malformations" (TTV-OFM). Support for a genetic mechanism is given from reports of abnormal karyotypes in individuals with Möbius (Slee et al. 1991, Donahue et al. 1993), identical twins with Möbius, and families with dominant, autosomal recessive and X-linked inheritance (Verziljl et al. 2003). Infants with Möbius have been born after unsuccessful abortion attempts with misoprostol, a synthetic analogue of Prostaglandin causing uterine contractions (Gonzales et al. 1998), and after fetal exposure to thalidomide (Strömland et al 1994). However, most cases of Möbius appear to be sporadic with unknown aetiology (Gorlin 2001). The estimated rate of Möbius ranges from 0.02-0.2/10000 (Kuklik 2000, Verzijl et al. 2003).

CHARGE syndrome

CHARGE syndrome was initially defined as a non-random association (a non-random occurrence of anomalies, which, in contrast to the anomalies characterizing a syndrome, are not known to be pathogenetically or casually related). The acronym "CHARGE", suggested by Pagon et al. (1981), signified the association of colobomas, heart defects, choanal atresia, retarded growth and/or mental development, genital anomalies, and ear anomalies and/or hearing loss. During the years, the inclusion criteria have been refined and expanded to include i.e. cranial nerve dysfunction, orofacial cleft, tracheoesophageal fistula and a distinctive face (Davenport et al. 1986, Blake et al. 1998). Verloes (2005) reinforcing the weight of pathogenesis and specific embryological defects, suggested eight key features; three major (coloboma, choanal atresia and semicircular canal anomalies) and five minor (rhombencephalic anomalies, hypothalamo-hypophyseal dysfunction, external/middle ear malformation, malformations of the mediastinal viscera and LD). Recently, mutations in a member of the chromodomain gene family (CHD7) on chromosome 8 have been revealed in about 50-75% of individuals characterized as having CHARGE syndrome (Vissers et al. 2004, Jongmans et al. 2005, Aramaki et al. 2006, Lalani et al. 2006). Graham (2001) suggested that there exist a “true” well-delineated syndrome within "CHARGE association". The reported rate of CHARGE ranges from 0.1-1.2/10000 and depends on professional recognition (Blake and Prasad 2006). Severe visual and/or hearing impairment is common in CHARGE.

Oculoauriculovertebral spectrum

Branchial arch disorders comprise a great variety of developmental anomalies such as oculoauriculovertebral spectrum (OAV), hemifacial microsomia (HFM) and Goldenhar syndrome (GS). There is no general agreement as to the diagnostic criteria for these conditions, or as to delineation of these conditions one from another. Gorlin and co-workers (1963) employed the term OAV dysplasia, which was subsequently replaced by the denomination OAV spectrum, to comprise a complex of heterogeneous conditions (such as HFM and GS), affecting primarily ocular, auricular, mandibular, oral and vertebral structures. The main characteristics in HFM are aural, oral and mandibular underdevelopment. It occurs mostly unilaterally but may be bilateral with more severe expression on one side (Gorlin 2001). GS is considered to be a variant of HFM characterized additionally by vertebral anomalies and epibulbar dermoids (Gorlin 2001).

Familial occurrence of OAV have been explained by autosomal dominant/recessive inheritance, and several chromosomal anomalies with similar facial phenotypes as in OAV (Gorlin 2001, Josifova et al. 2004), have been reported. There are also several reports of monozygotic twins discordant for OAV/GS (Boles et al. 1987), and of infants with malformations considered typical of OAV/GS born after in vitro fertilisation (IVF) (Roesch et al. 2001), to diabetic mothers, and after fetal exposure to primidone, thalidomide and retinoic acid (Gorlin 2001). However, most cases are sporadic with unknown aetiology. The prevalence has been estimated to be about 1/5000 (Gorlin 2001). Severe visual and/or hearing impairment as well as cranial nerve dysfunction is common in OAV.

The concepts of ASCs viewed from different neuropsychological and neurobiological theories

Genes are extremely important in autism and the current thinking is that perhaps a hundred different genes or more increase the susceptibility for autism. Some specific genes – neuroligins and SHANK 3 in particular (Jamain et al. 2003, Durand et al. 2006) have been identified, but the genetic framework of autism is still poorly understood.

There have been theories suggesting that there might be a common denominator (at the neuropsychological or neurobiological level), other than the typical behaviours, of all cases of autism. During the 1970s and 1980s neurophysiologic dysfunctions, believed to involve the brain stem and leading to abnormal sensory processing and possibly even other more complex behaviours associated with autism (Ornitz et al. 1985), was debated. During the 1990s it was taken almost for granted that a basic deficit in development of "theory of mind" also referred to as “mentalizing” (the ability to attribute mental states such as desire, knowledge, and belief, to oneself and other people as a means of explaining behaviour) (Baron-Cohen 1985) was at the root of autism, and that some "final common pathway" in the central nervous system (CNS) should underlie these functions. However, from a purely phenomenological point of view, considering the range of all the mental and other neurological functions which are impaired in autism, the systems of the brain which subserve the development of all these functions are likely to be complex. It follows that unusual functioning in a variety of different patophysiological pathways could account for the behaviours referred to as “autistic symptoms”. During the very recent past, it has become generally accepted that several neuropsychological mechanisms, other than mentalizing, are impaired in autism. These include central coherence (overriding processes which provide overview and understanding of complex mechanisms, such as social interaction) (Frith and Happé 1994), executive functioning (abilities to find strategies and organise a behaviour with the help of internal goals and mental models) (Ozonoff et al. 1991), and joint attention (visually coordinating attention with a partner to an external focus, showing social engagement and an awareness of the partner’s mutual interest for the purpose of commenting rather than requesting) (Loveland and Landry 1986). Further, if one extends the scope to the wider concept of ASC, theoretically, the possible variety of neuropsychological and neurobiological correlates in different subtypes of ASCs would be even greater. For instance, Asperger syndrome has by some been conceptualized as a distinct subtype, distinct from "high-functioning autism", although the weight of recent evidence suggests few qualitative differences (Macintosh and Dissanayake 2004). However, though there has been some evidence for distinct subtypes within ASCs involving factors such as language ability and IQ (Fein et al. 1999), there is still insufficient evidence to draw strong conclusions on subtypes of autism.

Neurobiological correlates of autism

Basic neural structures and processes

There are numerous reports of abnormalities in brain volume, neurotransmitter systems, and neuronal growth in patients with autism.

Brain volume

Clinical, postmortem and MRI research combine to provide converging evidence that autism is associated with increased head size in childhood (Baumann and Kemper 1997, Fombonne et al. 1999, McCaffery and Deutsch 2005), although the persistence of this abnormality is unclear. However, it is important to make note of the fact that only one in five of all individuals with ASCs have macrocephalus (Gillberg and de Souza 2002). Generalized enlargement of grey and white matter cerebral volumes, and cerebellar, especially white matter, volumes have been shown in children with autism at 2 to 4 years of age, whereas there appear to be a reduction in size of the cerebellar vermis, which is largely grey matter (Courchesne et al. 2004, Hazlett et al. 2005, Courchesne 2005). There is evidence that autism is associated with overgrowth in childhood followed by a period of abnormally slowed growth (Courchesne et al. 2004).

Neuronal growth

There is some evidence that enlarged brain volume in children with autism may be due to increased neuronal growth or decreased neuronal pruning (McCaffery and Deutsch 2005). Cell migration errors, decreased dendritic branching and abnormal minicolumn formation have been detected in post-mortem research (Courchesne et al. 2005, Bauman and Kemper 2005).

Neurochemical correlates of autism

A number of neurotransmitter abnormalities have been reported in autism, including abnormal levels of serotonin, dopamine, norepinephrine, gamma-aminobutyric acid (GABA), glutamate, oxytocin, and opioid in blood and cerebrospinal fluid, (Lam et al. 2006). Glial fibrillary acidic acid (GFA)-protein and ganglioside abnormalities have also been documented in some studies (Nordin et al. 1998, Laurence and Fatemi 2005).

Gross brain structures and functions

There is now converging evidence for abnormalities in the medial temporal lobe, the frontal lobe, the cerebellum, the brain stem, the basal ganglia and the corpus callosum.

The temporal lobe

Temporal lobe abnormalities could account for a number of behavioural impairments associated with autism, including impairment of language, facial processing, and theory of mind, and difficulty taking context into account (Dawson et al. 2002). Functional MRI has suggested that theory of mind and empathy (the ability to infer emotional experiences) are mediated by specific temporal lobe regions, as regards empathy e.g. neuronal networks in the amygdalae (Völlm et al. 2006). Temporal lobe abnormalities (hippocampus, the amygdalae, the limbic system, the entorhinal cortex) have been shown to be present in post mortem cases of autism (Bauman and Kemper 2005). Case reports of autistic symptomatology in temporal lobe damage due to viral encephalitis (Gillberg 1986) and tuberous sclerosis (Bolton and Griffiths 1997), support the link between temporal lobe dysfunction and autism. Although MRI of the hippocampus and amygdalae have produced inconsistent findings, functional MRI research demonstrates abnormal activation of the

amygdala and other temporal lobe structures during facial processing, theory of mind, and language tests (Zilbovicius et al. 2006, Gaffrey et al. 2007).

The frontal lobe

The prefrontal cortex is implicated by four of the most prominent neuropsychological theories about central deficits underlying a range of autistic symptoms; namely the theories of deficits in theory of mind, joint attention, executive functioning and central coherence. PET scan and functional MRI studies have suggested that a circumscribed region in the medial prefrontal cortex is a crucial component in brain systems underlying normal understanding of other peoples' minds and empathy (Völlm et al. 2006). Although MRI studies have not consistently revealed frontal lobe abnormalities, thickened cortices, irregular laminar patterns, atypical minicolumn structure have been detected in some post mortem cases (Bailey et al. 1998, Casanova et al. 2002). In addition, there have been reports of abnormalities in cerebral blood flow, serotonin synthesis and dopaminergic activity (Ernst et al. 1997, Wilcox et al 2002, Lam et al. 2006). The mesial frontal cortex has an exceptionally high concentration of dopamine, which by numerous studies have been shown to have a regulatory role in motor and cognitive functions, and also by some have been suggested to play a role in emotional processes (Salgado-Pineda et al. 2005). There is evidence that the mesial areas in the frontal lobes have particularly close connections with three deeper brain areas; the thalamus (the site of perception), the caudate nucleus (one of the basal ganglia) and the brain stem.

The basal ganglia

Langen et al. (2007) found significantly enlarged caudate nucleus, even after correction for total brain volume, in high-functioning individuals with autism and Asperger syndrome. Decreased perfusion in the basal ganglia in individuals with high-functioning autism has been demonstrated using SPECT (Ryu et al. 1999).

Corpus callosum

Several autism MRI studies have reported reduction of the size of corpus callosum, especially of the posterior portion (Just et al. 2006). Reduced corpus callosum size in autism may be suggestive of aberrant lateralization (Nicolson and Szatmari 2003) and have been shown to be correlated with fronto-parietal disconnectivity (Just et al. 2007).

Cerebellum

There is some evidence that cerebellar abnormalities in autism are linked to deficits in shifting and orienting attention and cognitive operations such as procedural learning. A reduced number of Purkinje cells in cerebellum have consistently been reported in post mortem studies of "autistic brains". Abnormalities have also been detected in cerebellar nuclear groups (Bauman and Kemper 2005). Structural imaging research has suggested atypical vermal lobule volume in autism (Courchesne et al. 2004), and functional imaging has shown abnormalities in cerebellar activation (Allen et al. 2004).

The brain stem

Some core symptoms in autism, lack of eye contact and facial social responses, and delayed development of speech, may have explanations at the brain stem level. Rodier et al. (1996) noted almost complete absence of facial nuclei and shortening of the brain stem in a patient with autism. MRI studies (Hashimoto et al. 1995, Cody et al. 2002) have shown significantly smaller brain stem in patients with autism, and neuropathological studies olivary nuclear malformations (Kemper and Bauman 2005). Athough Auditory

Brain stem Evoked Responses (ABR) studies have yielded contradictory results, some reports have shown abnormal ABR in a subgroup of individuals with autism, indicative of brain stem dysfunction (Rosenhall et al. 2003).

Lines of research supporting a theory of early embryonic brain stem dysfunction leading to abnormal input to higher levels of the CNS in some cases of autism

While especially the frontal and temporal lobes, and the limbic system have been implicated in the dysfunctional brain in autism (Gillberg and Coleman 2000), neuronal systems arise in the brain stem which project into the limbic, cortical and cerebellar structures, meaning that disruption of brain stem connections, theoretically, could lead to dysfunction of these structures. Data supporting an initial injury restricted to the brain stem in autism has come from reports of patients with autism and thalidomide embryopathy (Strömland et al. 1994), and autism and valproic acid embryopathy (Moore et al. 2000). The pattern of malformations in the patients with autism and thalidomide embryopathy indicated injury at day 20-24 of gestation, the time of closure of the neural tube. The cranial nerve motor symptoms and ear malformations in these individuals have given rise to the hypothesis that thalidomide interfered with pattern formation for the rhombomeres from which the brain stem nuclei arise and/or neuron production for the cranial nerve motor nuclei. Individuals with valproic acid embryopathy have been shown to have limb and craniofacial anomalies resembling those in thalidomide embryopathy, and neural tube defects (Moore et al. 2000). In valproic acid exposed rats, a decreased number of neurons in the cranial nerve motor nuclei and the inferior olive, and shortening of the brain stem have been reported (Rodier et al. 1996). Thus, CNS injuries occurring just after neural tube closure have been hypothesized to lead to a selective loss of neurons derived from the basal plate of the rhombencephalon (Rodier et al. 1996). The time of production for the large cerebellar neurons appears to be soon after the production of cranial nerve motor nuclei (Bayer et al. 1993).

Background for the present study

In Möbius, CHARGE as well as OAV, early injuries to the embryo have been hypothesized to be associated with brain stem dysfunction. Cranial nerve dysfunction is mandatory in Möbius (constituting the diagnostic criteria) and has been reported frequently in CHARGE as well as HFM (Blake 1998, Carvalho et al. 1999). Brain stem hypoplasia and brain stem mineralization has frequently been described in Möbius (Verzijl et al. 2003, Dooley et al. 2004). There has been radiological evidence for hindbrain abnormalities in patients with CHARGE (Issekutz et al. 2005) and in GS (Pane et al. 2005) The specific pattern of malformations in Möbius, CHARGE and OAV, respectively, suggest their origin to be early during the first trimester, around the fourth to sixth week of development (Miller et al. 2005). Further, there is a similarity/overlap in some of the malformations in these conditions. Patients meeting diagnostic criteria for both Möbius and CHARGE (Byerli and Pauli 1993), for CHARGE and GS (Van Meter and Weaver 1996) and for both Möbius and GS (Preis et al 1996) have been described. Some authors have even proposed theories of identical aetiology in Möbius and GS, as well as in CHARGE and GS. Preis et al. (1996) delineated disruption of embryonic blood supply during the fourth to fifth embryonic week, caused by mechanical/toxic injury, or disturbed mesodermal proliferation/mesodermal-ectodermal interaction, as a possible aetiological mechanism in both Möbius and GS. Van Meter and Weaver (1996) suggested abnormal migration of neural crest cells, disturbance of formation of mesodermal cells or defective interaction between neural crest cells and mesoderm in the aetiology of CHARGE and GS.

In 1994, our group described four individuals with autism and thalidomide embryopathy associated with facial nerve and ocular motility dysfunctions as in Möbius (Strömland et al. 1994). Intrigued by this association, and by some case reports/reports of minor series of individuals with Möbius and autism (Ornitz 1977, Gillberg and Winnergård 1984, Gillberg and Steffenburg 1989, Larrandaburu et al. 1999), we decided to further explore this association with a multidisciplinary approach. After the Möbius study, the multidisciplinary team decided to study other conditions with craniofacial malformations and cranial nerve involvement, in which ASCs had been noted. We chose CHARGE and OAV because of the similarity/overlap in some of the malformations in these conditions, and because of case reports of autism and autistic symptoms in these conditions (Rapin and Rupen 1976, Davenport 1986, Wiznitzer et al. 1987, Jure et al. 1991, Harvey et al. 1991, Barton and Volkmar 1998, Fernell et al. 1999, Landgren et al. 1992).

Coexisting disabilities in ASCs – diagnostic aspects

Individuals with ASCs, especially those with concomitant defined other conditions, frequently also have other disabilities and signs of CNS dysfunctions such as LD (Gillberg and Coleman 2000), epilepsy (Steffenburg et al. 1991) and visual/hearing impairment (Steffenburg et al. 1991, Rosenhall et al. 1999). Patients with the three BPCs in the present study are frequently, in addition to the afore mentioned disabilities, affected by cranial nerve palsies, motor dysfunction, swallowing and feeding difficulties, impaired balance (especially CHARGE), decreased olfaction (especially CHARGE), recurrent ear infections, frequent hospitalizations and surgeries. To identify autistic symptoms in these groups poses considerable difficulties.

The problems in the diagnostics of autism in individuals with LD are well known. LD itself often leads to impairments in social and adaptive skills, and the lack of normal adaptation to the demands of daily life is part of the definition of LD. Many individuals with LD also have other autistic features, such as stereotyped body movements and repetitive and restricted ranges of interests, without meeting all criteria for a diagnosis of autism. Conversely, diagnostic criteria for autism might not be met in individuals with LD, because a certain developmental level is needed for some behaviours to emerge. However, Wing and Gould (1979) presented data indicating that children with LD differ in their ability to take part in two-way interaction, but that "sociability" could be found also among those with profound LD.

The fact that sensory deprivation blurs the clinical presentation of autistic symptoms in ASCs has attracted some attention. Autistic-like features in severely sensory deprived individuals are often considered to be explained by visual/hearing impairment per se. In severely visually impaired children, "blindisms" (eye-pressing, light gazing, flicking fingers in front of the eyes, rocking, spinning and twirling (Warren 1986) are considered to be due to loss of sensory input and secondary difficulties in expressive ability (Fraiberg 1977). In addition, communication abnormalities, self-isolation, abnormal play, and interpersonal relationships have frequently been described in individuals with severe visual impairment (Carvill et al. 2002). Delays in the development of social maturity have been observed in hearing-impaired children (Bailly et al. 2003), and deaf people have been described to more often have behavioural disorders, explosive and labile personalities, and psychiatric disorders such as anxiety disorder (Meadow 1981, Roberts and Hindley 1999). Further, there is evidence that development of theory of mind is delayed in both blindness and hearing impairment per se (Hobson 1993, Peterson and Siegal 1999).

Cranial nerve palsies may mimic some core symptoms of autism. Facial nerve palsy causes impaired facial mimicry and palsy of the abducens nerve causes impaired ocular motility, which may affect the impression of eye-contact. Rather surprisingly, problems in the diagnostics of autism in individuals with concomitant cranial nerve palsies, have received very little attention.

Autism diagnostic instruments

Since there is no direct way to diagnose ASCs or to definitely prove that a person has ASC, constellations of symptoms are tied together conceptually by postulating an underlying disorder. The concept of ASCs has to be operationalized into items to form diagnostic instruments/diagnostic classification systems, each instrument/set of diagnostic criteria reflecting somewhat different underlying theories. As the knowledge of ASCs has increased, there has been an appreciation of the need to develop standardized diagnostic instruments that gather clinical information in a fashion that is comparable from patient to patient and from one clinic to the other. Several autism screening and diagnostic instruments have been developed during the last decades. These fall into three main groups: (i) questionnaires, (ii) observational schedules (iii) interviews, and sometimes a combination of these. Different diagnostic instruments have been proved to be of great value in providing detailed descriptions of autistic symptoms in a systematic way, but the "gold standard" remains the clinical diagnosis (nowadays based on diagnostic criteria in combination with clinical judgement). Currently, the most widely used autism diagnostic instruments are the Childhood Autism Rating Scale (CARS, Schopler et al. 1980), the Autism Diagnostic Interview-Revised (ADI-R, Lord at al. 1994), the Autism Diagnostic Observation Schedule (ADOS, Lord et al. 2000), and the Diagnostic Interview for Social and COmmunication disorders (DISCO, Wing et al. 2002, Leekam et al. 2002). More recently, two new autism diagnostic instruments have been developed, the Developmental, Dimensional and Diagnostic Interview (3di, Skuse et al. 2004) for ASCs at all levels of intellectual functioning, and the Forerunners in Communication (ComFor) for low functioning individuals with ASCs (Noens et al. 2006). Current autism and screening diagnostic instruments have been validated for the differentiation of autism from other developmental disorders, especially LD, but they have not been validated for autism with concurrent sensory impairments or cranial nerve dysfunction.

AIMS OF THE PRESENT THESIS

The aims of the this thesis were to

- examine the rate of AD and other autism syndromes/ASCs in clinically defined groups of individuals with BPCs (Möbius, CHARGE and OAV) believed to be associated with brain stem dysfunction;

- analyse clinical and background factors possibly associated with ASCs in these groups;

- describe and analyse how specific physical and non-ASC behavioural peculiarities of these BPCs affect the diagnosis of ASC;

- analyse the applicability of the ADI-R, CARS and ABC in the diagnostics of ASCs in people with additional dysfunctions, particularly visual/hearing impairment and cranial nerve palsies

- examine the "profiles of autistic symptoms" in individuals with autism syndromes/ASCs in the Möbius, CHARGE and OAV groups.

SUBJECTS AND METHODS

Studies of three BPCs, Möbius (I), CHARGE (II) and OAV (III) (in the following referred to as the Möbius, CHARGE and OAV studies, designed as prospective multidisciplinary studies, were conducted at the Queen Silvia's Hospital for Children and Adolescents in Göteborg 1995-1998 (Möbius study) and 1998-2002 (CHARGE and OAV studies).

Table 3. Study groups and methods used in Möbius, CHARGE and OAV studies

	Möbius study (I)	*CHARGE study (II)*	*OAV study (III)*
Total group in multidisciplinary study	n=25	n=31	n=20
Number of patients diagnosed regarding ASCs	n=21	n=25	n=19
Male:female (total group) Male:female (diagnosed)	18:7 16:5	15:16 11:14	12:8 11:8
Total group: - Age range, yrs - Mean age (SD), yrs - CI yrs Diagnosed as regards ASCs: - Age range, yrs - Mean age (SD) yrs - CI yrs	 1 month-55 12:4 (11:7) 7:6-17:1 1:11-55 13:11 (11:5) 8:10-19:0	 1 month-31 8:11 (6:7) 6:6-11:4 2:4-31 9:1 (4:11) 7:1-11:2	 8 months-17 8:1 (5:3) 5:5-10:4 1:11-17 8:3 (5:1) 5:10-10:8
Autism diagnostic instruments	ADI-R (n=20) CARS (n=22) ABC (n=23)	ADI-R (n=28) CARS (n=28) ABC (n=28)	ADI-R (n=20) CARS (n=19) ABC (n=19)
Autism diagnostic criteria	DSM-III-R (n=22)	DSM-IV (n=28) DSM-III-R (n=28)	DSM-IV (n=19) DSM-III-R (n=20)
Measurements of mental development	Wechsler scales (n=9) VABS (n=19) *	Wechsler scales (n=12) VABS (n=16)	Wechsler scales (n=13) Griffith (n=1) VABS (n=5)
Radiological imaging and laboratory tests of special interest from neuropsychiatric perspective	MRI brain (n=6) CT brain (n=4) Chromosomal analysis (n=6)	MRI brain (n=17) CT brain (n=25) US brain (n=13) MR/CT temporal bone/inner ears/middle ears (n=9) EEG (n=18) Chromosomal analysis (n=25) FISH-test (n=6)	MRI brain (n=5) CT brain (n=10) US brain (n=7) MR/CT temporal bone/inner ears/middle ears (n=6) EEG (n=7) Chromosomal analysis (n=14) FISH-test (n=4)

* In some individuals with Möbius results form both standardized psychological IQ test and the VABS were available

Calls for patients were announced in the Journal of The Swedish Medical Association and through speciality organisations of Swedish physicians. The aims of the studies outlined in the calls were to survey the clinical picture in Möbius, CHARGE and OAV, respectively. ASCs were not mentioned. The patients were recruited from all over Sweden and examinded by multidisciplinary teams. Neuropsychiatry, neuropsychology, neurology, paediatrics, ophthalmology, otolaryngology, odontology, speech and language therapy, orthopaedics (Möbius study) and paediatric radiology (OAV study) were reprsented. The neuropsychiatric assessments presented, due to additional dysfunctions, notably learning disability (LD), impaired vision, hearing and cranial nerve function, diagnostic difficulties in all three BPC groups. A substudy was performed with the aim of analysing the applicability of the autism diagnostic instruments which were used: the ADI-R, the CARS and the ABC, in individuals with ASCs and dysfunctions as manifested in Möbius/CHARGE/OAV.

Subjects (I-IV)

Möbius study (I)
Twenty-five patients met the study criteria of Möbius sequence (18 males, 7 females). Ages ranged from 1 month to 55 years (mean age 12:4 years), of whom twenty patients were aged two to twenty years. Twenty-one subjects were 2 years or older and diagnosed regarding ASCs.

CHARGE study (II)
Thirty-one patients were included in the CHARGE study (15 males, 16 females). They were 1 month to 31 years old (mean age 8:11 years). Twenty-seven patients were between 2 and 20 years of age Twenty-eight subjects, 2 years or older, were assessed regarding ASCs. Three deaf-blind patients could not be reliably assessed for ASCs, leaving 25 subjects that could be reliably diagnosed as regards ASCs.

OAV study (III)
Twenty patients were identified who met the study criteria for OAV (12 males, 8 females). Age range was eight months to 17 years (mean age 8:1 years). Nineteen patients were between two and 17 years. One deaf-blind boy, eight months old at time of the study, was evaluated according to the study design at the age of 5 years, but could not be reliably assessed regarding ASCs. Thus, 19 subjects were diagnosed regarding ASCs.

Definitions of Behavioural Phenotype Conditions (BPCs) (I-IV)

Criteria for entry into the Möbius/CHARGE/OAV studies are shown in Table 4.

Definitions of ASC and subcategories of ASC (I-IV)

In the three BPC studies, three subcategories of ASC: childhood autism/autistic disorder (CA/AD), autistic-like condition (ALC)/atypical autism, and autistic traits (AT) were defined as shown in Table 5. In addition, patients who exhibited/previously had exhibited symptoms consistent with autistic symptoms, though too mild to warrant a diagnosis of an ASC at the time of the study, were given the label “autistic traits?” (AT?).

Table 4. Inclusion criteria for entry into the Möbius, CHARGE and OAV studies

Behavioural Phenotype Conditions (BPCs)	*Inclusion criteria*
Möbius sequence (Möbius)	Congenital bilateral or unilateral palsy of cranial nerves VI and VII with or without associated symptoms.
CHARGE syndrome (CHARGE)	Four or more of the six acronym characteristics (Ocular **C**oloboma, **H**eart anomaly, **A**tresia of the choanae, **R**etarded growth and/or development, **G**enital hypoplasia, **E**ar anomalies and/or hearing impairment) or three of these plus additional characteristics.
Oculoauriculovertebral spectrum (OAV)	Malformations in two of the four areas; oro-cranio-facial, ocular, auricular and vertebral.

Table 5. Definitions of ASC and subcategories of ASCs

Autism spectrum condition (ASC) and subcategories of ASC	*Möbius study*	*CHARGE and OAV studies*
Autism spectrum condition (ASC)	Severe impairments in social interaction *in combination with* restricted communication and/or behaviour.	As in Möbius study
Childhood autism/Autistic disorder (CA/AD)	DSM-III-R criteria for AD and ADI-R algorithm criteria for CA	DSM-III-R and DSM-IV criteria for AD and ADI-R algorithm criteria for CA
Autistic-like condition (ALC)/ Atypical autism	6-7 of DSM-III-R symptom criteria for AD (at least one criterion within the social domain)	6-7 of DSM-III-R symptom criteria for AD (at least one criterion within the social domain) and 5 of the DSM-IV symptom criteria for AD (at least one criterion within the social domain)
Autistic traits (AT)	3-5 of the DSM-III-R symptom criteria for AD (at least one criterion within the social domain)	3-5 of the DSM-III-R symptom criteria for AD (at least one criterion within the social domain), and 3-4 of the DSM-IV symptom criteria for AD (at least one criterion within the social domain)
Autistic traits? (AT?)	At least for a period of life impairments in social interaction, communication or behaviour, consistent with symptoms within the autism spectrum, though too few and/or too mild to warrant an ASC diagnosis	As in Möbius study

Definitions of learning disability (LD) and subcategories of LD (I-IV)

In the Möbius study, mental development was divided in four broad categories, as illustrated in Table 6. In the CHARGE/OAV studies some individuals was functioning on a very low level, why the category profound learning disability (PLD) also was used.

Table 6. Definitions of learning disability (LD) and subcategories of LD

Subcategories	*IQ (Intelligence Quotient) range*
Average intelligence (A)	≥85
Near average intelligence (NA)	70–84
Learning disability (LD)	<70
Mild learning disability (MLD)	50-69
Severe learning disability (SLD):	
- *Möbius study*	<50
- *CHARGE, OAV studies*	20-49
Profound learning disability (PLD)	<20

Clinical multidisciplinary assessment (I-III)

The participants were examined by a multidisciplinary team. All assessments except the neuropsychiatric/neuropsychological evaluations were performed during one day in all individuals.

General physical examination

A paediatric status, including weight, length, head circumference, ability to walk, gross neurological function and cardiopulmonary status was performed. In six individuals with Möbius corresponding data were collected from medical records. In the CHARGE/OAV groups the paediatric examination also included skeletal and genitourinary anomalies. Measures of height and length were transformed to normative values (CHARGE/OAV). Swedish normative values for height are available from birth to 16 years (Karlberg et al. 1976), for head circumference up to 2 years (Karlberg et al. 1988). For older children, normative values for Norwegian children (Knudtzon et al. 1988) were used and percentile values transformed into SD values.

Ophthalmologic examination

Anterior/posterior segments of the eyes, visual function, refraction and ocular motility was examined. Visual impairment was defined as visual acuity ≤0.3 (20/60), and severe visual impairment as visual acuity ≤0.1 (20/200) according to the definitions of The World Health Organization.

Otologic examination

In the Möbius study the patients were interviewed about hearing problems and otologic information was retrieved from medical records. In the CHARGE and OAV studies an otolaryngologist diagnosed external/middle/inner ear malformations. Ear canal, middle ear, and inner ear malformations were classified on the bases of previously performed conventional radiological imaging. Hearing loss had been assessed by air and bone conduction audiometry. Severity of hearing impairment was graded as follows: deaf >80 dB, severe 60-80dB, moderate 40-59 dB, minor 20-39 dB, normal hearing 0-19dB.

Rotation/caloric test was performed in participants with CHARGE.

Orthopaedic examination
In the Möbius group orthopaedic data, including limb anomalies, were determined by medical records or orthopaedic examination.

Neuroimaging and laboratory tests
No radiological imaging, laboratory or electrophysiological tests were performed at the time of the study. Results from previously performed Magnetic Resonance Imaging (MRI), Computerized Tomography (CT) Ultrasound scan (US) of the brain, MRI/CT of the temporal bones/middle ears/inner ears, chromosomal analysis, DNA-analysis (Fluorescence in situ hybridization test, FISH) and electroencephalogram (EEG) were retrieved whenever possible (Table 1). A paediatric neuroradiologist reviewed the MRI and/or CT films of the brain in the OAV patients. Results from radiological imaging, performed after the study, were obtained in three infants with CHARGE. Radiological imaging of the brain stem had not been performed in any subjects.

Orofacial examination
Dentists and a speech pathologist examined orofacial morphology, odontology, oral motor function and swallowing. Facial expression, oral motor function, and speech were recorded by a video camera.

Analysis or pre/perinatal nonoptimal conditions and family history
Information was obtained from parental questionnaires/interviews (history of diseases, bleedings, assisted fertilization, previous spontaneous abortions, twinning, use of drugs, alcohol, and smoking during pregnancy, and family history of possible interest) and maternal health care/delivery unit records.

Methods used in study of ASCs (I-IV)

The Autism Diagnostic Interview-Revised version (ADI-R)
The original Autism Diagnostic Interview (ADI) was intended for use in subjects with a chronological age of 5 years or above and a mental age of at least 2 years. Good psychometric properties for the ADI were provided 1989 by Le Couteur et al. The revised form, the ADI-R (Lord et al. 1994), was developed to be able to diagnose autism in preschool children. The ADI-R was translated into Swedish in 1993 by Nordin and Ehlers, and in a revised version in 1997 by Nordin.

The ADI-R is currently the most widely spread autism diagnostic interview. It has been shown to have very good psychometric properties in many studies and has by some been considered to be “the gold standard” in the diagnostics of classic autism. The ADI-R is described as a semi-structured, parental and highly investigator-based interview, designed to differentiate individuals with classic autism from individuals with other pervasive developmental disorders, such as LD and language impairments. The content of the ADI-R closely mirrors the descriptions of autism found in the DSM-IV and ICD-10.

Eighty-four items are divided into four sub-domains, corresponding to the four DSM-IV domains: early development (item 2-10), communication (item 11-41), social skills (item 42-69), and restricted repetitive and stereotyped behaviour (item 70-85). The ADI-R also comprises 27 items concerned with behaviour and skills, intended to elicit information of relevance for planning of habilitation and educational programmes. The ratings for the vast

majority of items are numerical codes arranged in a three- or fourfold hierarchy of severity. For some items the age of onset of atypical behaviour/achievement of skills is coded. Questions can be scored for their current manifestation, most abnormal manifestation between 4 and 5 years of age, and for their presence at any moment in the individual's history (ever). The ADI-R provides a diagnostic algorithm, based on separate thresholds for these four DSM-IV domains. The algorithm was created using items shown to have the best discriminant validity between individuals with and without autism. Cut-off points in all domains must be succeeded for a diagnosis of autism. Individuals with and without speech has different cut-offs on the Communication sub-domain.

For some items, the ADI-R provides special "not applicable codes" and instructions for when these should be used. With the exception of "undue general sensitivity to noise", the items do not appear to have been designed with sensory deficits in mind. The "not applicable codes" are given the same value as "not occurring codes" in the algorithm.

The Childhood Autism Rating Scale (CARS)
The CARS is one of the most best documented and used autism measures. It was developed to distinguish autism from other developmental disabilities in children (Schopler et al. 1980) and is used as a mixture between behaviour observation scale and interview. Recent professional consensus documents have described good psychometric properties. A Swedish translation of the CARS by Jakobsson and Gillberg has been used since 1990.

The CARS covers 14 domains plus an overall category of "impression of autism". Each item is rated on a four-graded scale with detailed behavioural descriptions for each point (1 indicating behaviour appropriate for age and 4 indicating severe abnormality). Use of midpoints between adjacent scorings (such as 1.5, 2.5 etc) yields a nominal scale of 7 classes for each item. Item scores are summed to a total CARS score ranging from 15-60, scores of 30 to 36 indicating "mild autism", and scores of 37 and above indicating "severe autism".

The CARS does not give any directions for when specific items should be considered as "not applicable" in individuals with other disabilities.

The Autistic Behaviour Checklist (ABC)
The ABC is a common and well-established instrument. It was developed by Krug et al (1980) to measure levels of autistic behaviour in individuals with severe disabilities. The ABC was translated in Swedish in 1980 by Gillberg.

Fifty-seven items are grouped into five subscales (Sensory, Relating, Body and Object use, Language, and Social and Self-Help skills. The items are assigned weighted scores from 1 to 4 depending on their relative power in predicting autism (4 indicating the most "autism specific" items). In the original study, the main total score for the sub-sample of individuals with autism was reliably differentiated from mean scores for individuals diagnosed as severely learning disabled, deaf/blind, severely emotionally disturbed or normal. A total score of 67 or above was considered to indicate autism with "high probability", and scores in the range 53 to 67 to indicate "suspected autism".

The ABC does not give any directions for when specific items should be considered as "not applicable" in individuals with other disabilities.

DSM-III-R checklist
(Table 1) A list of all 16 DSM-III-R symptoms of autistic disorder was checked in all cases.

DSM-IV checklist
(Table 2) A list of all 12 DSM-IV symptoms of autistic disorder was checked in all cases.

Methods used in assessment of mental development (I-IV)

The Wechsler scales
The Wechsler intelligence scales for children (WISC-III, Wechsler 1992) and adults (WAIS-R, Wechsler 1981) are well-established IQ-tests, including full-scale IQ and subtests for performance and verbal IQ.

The Vineland Adaptive Behavior Scales
The VABS (Sparrow et al. 1984) is an informant-based measure of adaptive behaviour in which four major domains of adaptive functioning are assessed: communication, daily living skills, socialization and motor skills. The motor domain is included only for those under 6 years of age. Among many other things, the VABS yields a reasonable estimate of whether or not an individual belongs in the SLD category.

Diagnostic process (I-III)

Diagnoses of BPCs
Diagnoses of BPCs according to the study criteria of Möbius, CHARGE and OAV were assigned on the basis of the detailed descriptions of malformations and functional deficits, which was obtained during the examinations performed by each discipline, and completed by data from medical records.

Autism spectrum conditions (ASCs)
In the Möbius study one examiner independently completed the ABC, the CARS and the DSM-III-R checklist for AD, and another investigator, the author, performed the ADI-R. In the CHARGE and OAV studies one investigator independently performed the ABC, the CARS and the DSM-IV Checklist for AD and another examiner, the author, the ADI-R and the DSM-III-R criteria for AD. Diagnoses according to the DSM-III-R and DSM-IV criteria were made after completion of the diagnostic interview and the rating scales, on the basis of all available information. In all studies the ADI-R/CARS/ABC was performed by interviewing the parents/another principal care giver. During the CARS and ABC assessments the proband was in the room during the interview and was observed by the other investigator while playing and/or interacting with the parents/investigator. Since the neuropsychiatric/neuropsychological assessments required a considerable amount of time, these evaluations/parts of these evaluations in some patients had to be completed at another occasion. Diagnoses of AD were only assigned in patients for whom there was agreement between the two investigators, which in the Möbius study implied concordance in diagnoses according the DSM-III-R criteria and ADI-R algorithm, and in the CHARGE/OAV studies concordance according to the DSM-III-R, DSM-IV criteria and ADI-R algorithm. In the CHARGE/OAV studies, diagnoses of ALC and AT were only applied for individuals who met the postulated number of DSM-III-R and DSM-IV criteria. AD and ALC diagnoses in the Möbius study were assigned according to the DSM-III-R criteria.

Mental level
Formal standardized psychological IQ testing had been performed before the present study in many patients. The VABS (Möbius study: Swedish translation by Magne and Wahlberg 1961) were used to arrive at a social quotient and to estimate the cognitive development in most individuals participating in the Möbius study. In the CHARGE and OAV studies, the subjects who had not been tested with formal IQ tests, were whenever possible evaluated with the Wechsler Intelligence Scale for Children (WISC-III, Wechsler 1992), and in one instance with the Griffiths developmental scales (Griffith 1970). The VABS (CHARGE/OAV studies: Sparrow et al. 1984) were used to arrive at a social quotient and to estimate the cognitive development in patients who were too disabled (due to severe sensory impairments and LD) to perform standardized IQ tests. In one patient with OAV, attending normal school with average/above average performance without need of remedial teaching, evaluation of mental level was based on clinical observation and information about the educational situation.

Statistical methods used
In the CHARGE study Pitman nonparametric tests (Good 2000) were applied in analysing correlation between a number of background factors and severity of ASC/level of LD. Two tailed tests were used. The sample sizes, 25 and 20 patients, respectively, were considered to small to warrant such analyses in the Möbius/OAV studies.

Methods used: diagnostic aspects (IV)

For each of the autism diagnostic instruments used (the ADI-R, CARS and ABC), the frequency at which individual items had been considered unratable in each of the three BPCs was estimated. To analyse the impact of inclusion of omitted items in each condition, "possible maximum ADI-R, CARS and ABC scores" were calculated for each proband by adding maximal scores on omitted items to the total scores. The number of individuals in each BPC group, surpassing cut-off scores on each instrument when maximum possible scores on omitted items were added to the rated total scores, was recorded. ADI-R sub-domains (Social, Communication, Behaviour), CARS and ABC mean scores, before and after inclusion of maximum possible scores on omitted items, were calculated across diagnostic ASC groups in each BPC group. The diagnostic classification of autism according to each instrument (ADI-R/CARS/ABC)/criteria (DSM-III-R, DSM-IV) used was compared. The "profiles of autistic symptoms" in individuals with AD in the Möbius, CHARGE and OAV groups were analysed by comparing profiles of scores on ADI-R and ABC sub-domains in the patients with AD in each group. Profiles of ADI-R sub-domain scores in the subjects with AD in each BPC group were also compared with the corresponding profiles in a sample of individuals with "AD/AD only" (n=25, Lord et al. 1994), and profiles of ABC sub-domain scores in patients with AD in each BPC were compared with profiles in two samples of individuals with "AD" (Krug et al. (1980, n=172, Volkmar et al., 1988, n=94, respectively), and another sample of "mute individuals with AD or ASCs" (n=155, Miranda-Linné and Melin et al. 1997). (Description of comparison groups in Table 17 and Figure 4).

Ethics

Approvals for the studies forming the basis for this thesis were obtained from the Ethical Committee at the Medical Faculty, Göteborg University, and informed written consent was given by probands/principal caregivers in all instances.

RESULTS

Overall clinical findings (I-III)

(Table 7)

Möbius group

The most frequent malformations were facial nerve palsy (n=25), abducens nerve palsy (n=25), hypoglossal nerve palsy (n=16), limb malformations (n=10: club feet, n=7; hand-malformations, n=5; Poland syndrome (absence of the pectoralis muscle in combination with ipsilateral finger syndactylies or finger hypoplasia), n=2), mandibular hypoplasia (n=8), orofacial clefts (n=7), hypodontia (n=7) and microglossia (n=6).

Motor function was slightly/moderately impaired in five out of the 19 patients who were more than 2 years old. Severe eating and swallowing problems, dysarthria and drooling were common.

CHARGE group

The majority of patients with CHARGE were severely affected with multiple disabilities. Most individuals had bilateral anomalies of the eyes and ears. The most common defects were coloboma (n=28, iris colobomas, sometimes in combination with defects of the choroids/optic nerve), microtia (n=28), inner ear malformations (n=20, most commonly abnormal semicircular canals and/or cochlear hypoplasia), heart defects (n=16, most commonly persistent ductus arteriosus), short stature (height <-2SD, n=14), microphthalmus (n=13, of whom all had coloboma), genital hypoplasia/delayed puberty (10 boys, 2 girls), choanal atresia (n=11), facial nerve palsy (n=12), vertebral anomalies (n=8), middle ear malformations (n=7), orofacial clefts (n=6), limb anomalies (n=5), tracheoesophageal fistula (n=5), hypodontia (n=5), renal anomalies (n=4) and anal atresia (n=4). Several patients had impaired balance, including atactic gait (n=21). Seventeen of these were judged to have vestibular anomaly. Out of these seventeen, eight had abnormal semicircular canals on radiological imaging and nine had abnormal rotation/caloric tests

The quality of life was, especially for the younger children and their families, severely affected by sensory impairments, feeding and speech difficulties (due to orofacial clefts, tracheoesophageal fistula, bilateral choanal atresia, heart defects, tracheomalacia, cranial nerve dysfunction, frequent vomiting, hearing impairment).

OAV group

Some individuals with OAV were severely affected by multiple disabilities. The most frequently recorded defects were anomalies of the external ear and ear canal (n=18), microsomia (n=16), middle ear malformations (n=12), vertebral anomalies (n=10: scoliosis n=7), congenital heart defects (n=8: ventricular septal defect n=7), facial nerve palsy (n=9), short stature (height <-2SD, n=8), orofacial clefts (n=6), macrostomia (n=6), respiratory malformations (n=5), inner ear malformations (n=4), microphthalmus (n=4, of whom one also had iris coloboma), gastrointestinal malformations (n=3) and genitourinary anomalies (n=3).

Common functional deficits included sensory impairments (hearing impairment: n=16, visual impairment: n=6) feeding and speech difficulties. One boy with AD met criteria for a diagnosis of both CHARGE and OAV (this boy was included as participant only in the OAV study), and one girl with AT had Down syndrome and hypothyroidism.

Table 7. The most frequent malformations in Möbius, CHARGE and OAV groups (cerebral malformations are given in Tables 10, 11)

	Möbius n=25	*CHARGE n=31*	*OAV n=20*
Oro-cranio-facial	25	22	19
Cleft lip/palate/uvula	7	6	6
Cranial nerve dysfunction	25	17*	11
- NV	2	12	
- NVI	25		2
- VII	25	17*	9
-VIII			
- X		1	
- XII	16		
Choanal atresia		11	1
Ocular		28	15
Microphthalmus		13	4
Iriscoloboma		28	1
Dermoid			13
Auricular	1	28	20
External ear	1	28	18
Middle ear	1	7	12
Inner ear		21	4
Limb	10	5	2
Vertebral		8	10
Cardiovascular		16	8
Genitourinary		15	3
Gastrointestinal	1	5	3
Short stature, $\leq$-2SD	3	20	8

*Dysfunction of N. VIII was indicated in 17 individuals, described in Johansson et al. (2006), but further evaluation by the multidisciplinary team deemed the evidence for palsy-dysfunction of N. VIII to be insufficient

Neurodevelopmental background factors (I-III)

Neurodevelopmental background factors are summarized in Table 8. Patients with CHARGE and OAV were more often affected by sensory impairments and LD than those with Möbius, the patients with CHARGE being the most severely affected. ASCs and LD tended to occur together in all three BPCs. In the CHARGE and OAV groups, not only ASCs and LD but also visual and hearing impairments often occurred in the same individuals.

Learning disability

Möbius group

Thirty-five percent (8/23) had LD. All patients with LD had severe autistic symptoms, and all subjects with severe autistic symptoms had some degree of LD. However, within the group with severe autistic symptoms (AD and ALC) and Möbius, there was no indication of an association between autistic symptoms and degree of LD.

CHARGE group

Seventy-nine percent (22/28) in the CHARGE group had LD. Severity of ASCs and degree of LD was highly correlated ($p<0.001$). One boy with ALC had near average intelligence.

OAV group

Forty-five percent (9/20) in the OAV study had LD. Two of the three patients with severe autistic symptoms had LD. One boy with ALC had average intelligence. Six out of nine patients with LD had ASCs.

Sensory impairments

Möbius group

Marked visual impairment was not recorded in any of the patients ophthalmologically examined (the subgroup with the strongest suspicion of visual impairment). Hearing impairment was found in 5/19 examined subjects with Möbius (the subgroup with the strongest suspicion of hearing deficit).

CHARGE group

Visual impairment was shown to correlate independently to severity of ASC ($p<0.05$) and level of LD ($p<0.001$). Hearing impairment correlated independently and significantly to severity of ASC ($p<0.05$), but not to level of LD.

OAV group

All the three patients with AD/ALC, 2/8 with AT/AT? and 0/8 of those without ASC/AT? had visual impairment. All three patients with AD/ALC had bilateral hearing impairment, 6/8 with AT/AT? and 2/8 without ASC/AT?.

Gender

There was a predominance of males in the Möbius and OAV groups, 18:7 and 3:2, respectively. The male:female ratio in the CHARGE group was 15:16 (Table 3, 8). The male: female ratio among individuals with AD was 5:1 in the Möbius group, 2:3 in the CHARGE group and 1:1 in the OAV group. The male:female ratio among patients with cerebral abnormalities in the Möbius group was 1:1, in the CHARGE group 2:3, and in the OAV group 6:1.

Age

(Table 3) Mean age was somewhat higher in the Möbius group compared to the other two BPCs (Möbius: mean age 12:4 years, standard-deviation (SD)11:7 years, confidence interval (CI) 7:6-17:1 years, CHARGE: mean age 8:11 years, SD 6:7 years, CI 6:6-11:4 years OAV: mean age 8:1 years, SD 5:3 years, CI 5:5-10:4 years). For individuals who were diagnosed as regards ASC (Möbius n=21, CHARGE n=25 OAV n=19) the corresponding ages were: Möbius: mean age 13:11 years, SD 11:5 years, CI 8:10-19:0 years), CHARGE: mean age 9:1 years, SD 4:11 years, CI 7:1-11:2 years, OAV: mean age 8:3 years, SD 5:1 years, CI 5:10-10:8 years.

Table 8. Neurodevelopmental background factors

	Möbius n=25	*CHARGE* n=31	*OAV* n=20
Total group:			
- Age range, yrs	1 month-55	1 month-31	8 months-17
Sex ratio M:F	18:7	15:16	12:8
Cognitive level			
A	9	1	9
NA	6	5	2
MLD	3	10	4
SLD	5	3	3
PLD		9	2
Visual impairment	No subjects with	19/31	6/20
VI	PSVI/SVI. Data on	2	4
PSVI	number of subjects with	8	
SVI	VI not available.	9	2
Hearing impairment	5/19	31/31	16/20
Bilateral	Data on severity of	31	12
- minor	hearing impairment not	7	2
- moderate	available	1	5
- severe		23	5
Unilateral			4
-minor			4

ASCs in BPCs (I-III)

ASC and subcategories of ASC in each BPC group are summarized in Table 9.

ASCs in Möbius group

A total of 10 patients of the 21 comprehensively examined (48%) were diagnosed as suffering from ASCs. Six (29%) met the study criteria for AD, 1 (5%) for ALC and 3 (14%) individuals were classified as having AT. In one 19 months old boy with considerable developmental delay, considered as impossible to diagnose reliably as regards ASCs there were strong behavioural indicators that he might later meet criteria for AD.

ASCs in CHARGE group

Seventeen individuals out of the 25 who were diagnosed regarding ASCs (68%) had ASCs. Five (20%) met the study criteria for AD, 5 (20%) had ALC and 7 had AT (28%). Three deaf-blind (DB) participants showed several autistic features but were considered impossible to evaluate reliably as regards a diagnosis of ASC.

Three patients (5, 7, 13 years of age) diagnosed with AD and CHARGE had mental ages below 12 months, as estimated with the VABS (motor domain not included). Two of them had low socialization scores, intermediate communication scores and higher daily living scores. The 13 year old showed a relative strength in the daily living skills domain and deficits in the socialization/communication domains, although somewhat better socialization skills. They all walked unaided and displayed preoccupations with restricted

and repetitive quality/stereotyped body movements/repetitive manipulation of objects. Two of them exhibited ritualistic behaviour.

ASCs in OAV group
ASC was diagnosed in 8 out of the 19 patients (42%) who were diagnosed regarding ASCs. Two participants (11%) (of whom one boy also met criteria for a diagnosis of CHARGE, however not included in the CHARGE study) had AD, 1 (5%) had ALC, and five (26%) had AT. One boy had been characterized as deaf-blind as an infant. At the time for the neuropsychiatric assessment at age 5 years he showed several autistic traits but was considered as impossible to diagnose reliably as regards ASCs.

Table 9. ASC and subcategories of ASC in Möbius, CHARGE and OAV groups

	Möbius N=21	*CHARGE N=25*	*OAV N=19*
AD	6 (29%)	5 (20%)	2 (11%)
ALC	1 (5%)	5 (20%)	1 (5%)
AT	3 (14%)	7 (28%)	5 (26%)
ASC	10 (48%)	17 (68%)	8 (42%)

Neuroimaging findings (I-III)

Data pertaining to cerebral abnormalities are summarized in Table 10 and 11.

Table 10. Cerebral abnormalities in patients with Möbius, CHARGE and OAV

	Möbius N=10	*CHARGE N=27*	*OAV N=11*
Cerebral anomalies/abnormalities	2 (20%)	20 (74%)	7 (63%)
White/grey matter	1 (10%)	15 (56%)	7 (63%)
-Prosencephalon, midline	1 (10%)	10 (37%)	2 (18%)
-Rombencephalon		3 (11%)	1 (9%)
- Other		10 (37%)	5 (45%)
Enlarged intra-/extracerebral liquor rooms	1 (10%)	15 (56%)	4 (36%)

Möbius group
Cerebral abnormalities were recorded in two out of ten examined patients: corpus callosum agenesia in one girl with AD and SLD, and hydrocephalus in one boy with AD and MLD (Table 10).

CHARGE group
Cerebral abnormalities collapsed (white/grey matter, liquor rooms) were recorded in 74% (20/27) of examined patients, structural abnormalities of white/grey matter in 56% (15/27) and abnormalities of liquor rooms in 56% (15/27) (Table 10). Forebrain midline anomalies were common (Table 11). Neither severity of ASCs nor the level of LD was significantly correlated to "white/grey matter abnormalities collapsed"/forebrain midline anomalies. However, "white/grey matter abnormalities collapsed" as well as forebrain midline abnormalities occurred more frequently in those with AD/ALC than in the those with AT/AT?/no autistic symptoms, 8/10 versus 5/11 (n.s.), 5/10 versus 3/11 (n.s.). Hindbrain abnormalities (n=3) was not correlated with severity of ASC/level of LD/forebrain midline anomalies.

Table 11. Structural abnormalities in white/grey matter in individuals with CHARGE

Forebrain midline 10/27 (37%)	Agenesis/hypogenesis/abnormal configuration of corpus callosum (n=5), the hypophysis-sella /hypothalamus region (n=4) and the optic chiasm (n=2), olfactory tract (n=1), septum pellucidum (n=1),
Hindbrain 3/27 (11%)	Agenesis of the cerebellar vermis (n=1), cerebellar asymmetry (n=1), small pons (n=1)
Other 10/27 (37%)	Widened cerebral fissures (n=4), focal lesions (n=5) abnormal gyrii (n=2), small cerebral hemispheres (n=1), cerebral asymmetry (n=2), sparse white matter (n=2), pedunculus cerebri small (n=1), incomplete rotation of hippocampus (n=1)

OAV group
Cerebral abnormalities collapsed (white/grey matter, ventricles) were indicated in a total of 63% (7/11, 1 girl, 6 boys), structural abnormalities of white/grey matter in 63% (7/11) and abnormalities of liquor rooms in 36% (4/11). No detectable septum pellucidum on neonatal US, small frontal lobes and narrow interval between the anterior horns of the lateral ventricles, possibly indicating mild holoprosencephaly (n=1), occipital encephalocele and Arnold Chiari II anomaly (n=1), cortical dysplasia and polymicrogyria of the left hemisphere (n=1) and widened Sylvian fissures (n=3) were recorded. Four out of the seven patients in which cerebral abnormalities were shown had ASCs (one AD and three AT), two had AT? and one was deaf-blind. Cerebral imaging was more often available in patients with ASCs.

Brain stem (I-III)

Radiological imaging of the brain stem had not been performed in any patients. Cranial nerve dysfunction in Möbius, CHARGE and OAV group are summarized in Table 12.

Table 12. Cranial nerve dysfunction in patients with Möbius, CHARGE and OAV

	Möbius N=25	*CHARGE N=31*	*OAV N=20*
Cranial nerve palsy	25 (100%)	17[1] (55%)	12[2] (60%)
Facial nerve palsy	25 (100%) Bilateral paralysis of all mimic muscles in 16, unilateral with upper part of face more affected in 9	12[1] (39%) Unilateral, affecting both upper and lower face in all	9[2] (45%) Unilateral, affecting both upper and lower face in all
Facial nerve palsy ipsilateral to the more severely affected side of face			7 (78%)
Abnormal facial nerve canal	*	5 of 6 examined	2 of 4 examined
Abnormal lacrimation	7 (28%)		

[1] Two individuals were previously diagnosed with facial palsy but did not show this at the time of the study.
Cranial nerve dysfunction was indicated in 17 individuals with CHARGE, described in Johansson et al. (2006), but further evaluation by the multidisciplinary team deemed the evidence for palsy-dysfunction of N. VIII to be insufficient.
[2] One individual was previously diagnosed with facial palsy but did not show this at the time of the study
* No information

Möbius group
Sixteen patients had bilateral severe facial palsy (in other cases asymmetric palsy with the upper part of the face most affected). Severe bilateral facial palsy was recorded in 6/7 patients with AD/ALC, 5/6 with AT/AT?, and 4/8 without autistic symptoms. Four out of seven patients with AD/ALC had a clear history of abnormal tearing (lack of emotional tearing or tearing when eating), whereas 2/6 with AT/AT? and 1/8 without ASC/AT?. The hypoglossal nerve was affected in 4/7 patients with AD/ALC, 4/6 with AT/AT? and 5/6 without ASC/AT?. Severe dysphagia and velopharyngeal insufficiency suggested that the vagus nerves were affected in some of the subjects.

CHARGE group
Neither severity of ASC nor level of LD was correlated to cranial nerve dysfunction. Facial nerve palsy was in all instances unilateral and affecting muscles in both lower and upper parts of the face. Anomalous course of/difficulty to identify the facial nerve canal was recorded in 5/6 examined patients (four cochlear implantation candidates and two infants, thus possibly a subgroup of those with most severe hearing loss). Out of these five patients with indication on abnormal facial nerve canals, one had facial nerve palsy, two had no facial palsy at the time of the study, of whose one previously had been diagnosed with this, and two were infants. One girl with facial nerve palsy had normal facial nerve canal on radiological examination.

OAV group
Altogether, cranial nerve palsy was recorded in 11 patients (1/3 with AD/ALC, 5/8 with AT/AT?, 4/8 with no autistic symptoms, one deaf-blind boy). Facial nerve palsy was in all instances unilateral and affecting both lower and upper parts of the face. The facial palsy was ipsilateral to the more severely affected side of face in seven out of nine patients with current/previous facial palsy. Slightly abnormal course of/difficulty to identify the facial nerve canal was described in one boy with ALC and one with AT, none of whom had facial palsy. In the latter boy hypoplasia of the cochlear nerve was recorded, in another boy with AD it was difficult to identify the cochlear nerve. Two patients with no ASC/AT? had cranial nerve VI (abducens nerve) palsy.

Genetics/family factors (I-III)

Chromosomal analysis and FISH-test were normal in all patients with Möbius/CHARGE/OAV, in whom data from these analyses were available. Family factors of possible interest are summarized in Table 13.

Pre- and perinatal factors (I-III)

An overview of pre-/and perinatal risk factors is given in Table 14. Some possible pre-/perinatal risk factors, although of uncertain significance, were recorded in many patients.

Möbius group
Five of the seven patients whose mothers had vaginal bleeding during pregnancy had ASC (AD: n=3, AT: n=2). Drug abuse including benzodiazepines was recorded in the mother of a boy with AD. There was no trend for any other prenatal events to be more common among mothers of patients with ASC. All children in whom gestational age was known (n=24) were born at term.

CHARGE group
Both patients, whose mothers had diabetes mellitus, had ASC (AD: n=1, AT: n=1) and some of the more common events, first trimester maternal bleeding (ALC: n=2, AT: n=3, <2 years: n=1), more than one previous spontaneous abortion (2 with ALC, 1 with AT), smoking (AD: n=2, ALC: n=2, AT: n=4, deaf-blind: n=1), were recorded almost exclusively in patients with autistic symptoms. Six children were born in gestational week 34-36 and 24 had a gestational age between 37 and 42 weeks.

OAV group
The four assisted reproductive technology (ART) induced pregnancies (recorded in one mother of a child with AT and three mothers of children without ASC/AT?) were all (initially) twin pregnancies. There was no tendency for any recorded possible risk factors to occur more frequently in patients with ASC. Four children had been born after 31-36 weeks' gestation and the remaining 16 after 37-42 weeks.

Table 13. Family history of possible interest in Möbius, CHARGE and OAV groups

	CA/AD	*ALC*	*AT*	*AT?*	*No ASC*	*All patients*
Möbius		1[a]/1			1[b]/8	2/25
CHARGE	2[cd]/5	1[e]/5	5[fghi]/7	1[j]/3	1[k]/5	10/31
OAV	1[l]/2	1[m]/1	4[nopq]/5			6/20

Möbius group

[a] mother was characterized as learning disabled as a child, attended a special class at school

[b] father described himself as "somewhat withdrawn" with good mathematical skills, ability to immediately perceive the number of letters in all, even long, words and to unimpeded speak backwards, as hypersensitive to firm touch and with a tendency to have seizures in connection with fever and exposure to sun

CHARGE group

[c] father and brother with epidermiolysis bullosa

[d] mother with diabetes mellitus

[e] sister died in the newborn period due to congenital heart defect. Mother two spontaneous abortions.

[f] two probands with AT were siblings

[g] maternal half-brother with ADHD, MLD and AT

[h] mother with diabetes mellitus

[i] maternal half-brother with epilepsy and ADHD

[j] mother with gestational diabetes

[k] father had heart surgery at two months of age due to unknown heart defect

OAV group

[l] maternal uncle had Duchenne's muscular dystrophy

[m] mother had heart surgery as a child due to unknown anomaly of cardiac vessel

[n] brother has Down syndrome and autism, maternal grandmother had several spontaneous abortions

[o] father and sister have (surgically corrected) cleft lip and palate

[p] mother with severe adhesions of fallopian tubes and chromosomal structural abnormalities/fragility, high maternal/paternal age (40/51yrs)

[q] maternal cousin with hydrocephalus and hernia of spinal marrow

Table 14. Pre/perinatal factors

	Möbius n=25	*CHARGE* n=31	*OAV* n=20
Maternal bleedings	7 (28%)	6 (19%)	5 (25%)
First trimester	4	6	2
Second trimester	4		4
Third trimester	2		
>1 previous spontaneous abortions	6 (24%)	3 (10%)	1 (5%)
Previously induced abortion due to foetal malformation			1 (5%)
Assisted fertilization		3 (10%)	4 (20%)
ICSI		2	2
IVF			2
Pregnancy preceded by treatment with ovulation stimulating hormones		1	
Twin pregnancy		1 (10%)	5 (25%)
Spontaneous abortion of one twin		1	1
Other twin healthy			4
Suspicions of twin- to- twin transfusion syndrome during third trimester			1
Foetal diagnostics	1 (4%)	3 (10%)	1 (5%)
Chorion villus sampling	1	2	
Amniocentesis		2	
Several ultrasonographies			1
Maternal diabetes		3 (10%)	
Diabetes mellitus		2	
Gestational diabetes		1	
Other maternal disorder	4 (16%)	21(68%)	7 (28%)
Smoking >5 cigarettes/day	*	9 (29%)	6 (24%)
Drug abuse	1 (4%) (Benzodia-zepines)	1 (3%) (Alcohol in gestational week 6)	
Category C[1] drugs	1 (4%)	5 (16%)	3 (15%)

[1] Drugs considered risk factors for the foetus and/or the newborn without directly causing malformations. Recommendation in Sweden: to be avoided or used with caution during pregnancy.
* No information

Comparison of findings across groups (I-III)

ASC (AD/ALC/AT) were common in all BPCs, and most frequent in the CHARGE group (CHARGE group 68%, Möbius group 48%, OAV group 42%). Typical AD was most common in the Möbius group, affecting 29% of the individuals compared with 20% of those with CHARGE and 11% of those with OAV (Table 9). LD was also a common finding across groups (Möbius: n=8, 35%, CHARGE: n=22, 79%, OAV: n=9, 45%) (Table 8). Among those radiologically examined, cerebral abnormalities (white/grey matter, liquor rooms) were more common in patients with CHARGE/OAV (74%, 63% respectively) than in individuals with Möbius (20%) (Table 10). Patients with Möbius were more affected by limb anomalies (Möbius: n=10, 40%, CHARGE: n=5, 16%, OAV: n=2, 10%) (Table 7) and cranial nerve dysfunction (Möbius 100%, CHARGE 55%, OAV 60%) (Table 2, 12). Bilateral facial nerve palsy, abducens and hypoglossal nerve palsy were more frequent in the Möbius group (Table 7, 12). Individuals with CHARGE/OAV were more frequently affected by eye-, ear-, cardiovascular anomalies and short stature. Vertebral anomalies occurred only in patients with CHARGE/OAV and genitourinary malformations almost only in patients with CHARGE (Table 7). There was a trend for visual and hearing impairment to occur more often in patients with severe autistic symptoms in the CHARGE/OAV groups. Family factors of possible interest were recorded more often in subjects with CHARGE/OAV than Möbius (Table 13). Some mothers in all three BPCs had experienced first trimester vaginal bleeding. More than one previous abortion was recorded more frequently in the Möbius group. Twin pregnancy and assisted fertilization were only found in the CHARGE and OAV groups, most often in the OAV group (Table 14).

Omission of ADI-R, CARS and ABC items in each BPC (IV)

Some ADI-R, CARS and ABC items were, due to notably sensory impairments and cranial nerve dysfunction, difficult to rate in many subjects. Except for ADI-R items concerned with eye gaze and facial expressions in the Möbius group, there was a tendency that ABC items, especially in the CHARGE/OAV groups, were omitted more frequently than ADI-R and CARS items (Table 15 shows omission of ADI-R items).

ADI-R

The most frequently omitted ADI-R items in the Möbius group were those included in the subsection Failure to use nonverbal behaviours to regulate social interaction ("direct gaze" in 25%, "social smile" in 40%, "range of facial expressions used to communicate" in 45%). These items were omitted less often in the CHARGE/OAV groups, whereas ADI-R items pertaining to the sub-sections Failure to develop peer relationships, Lack of socio-emotional reciprocity, Lack of or delay in spoken language and failure to compensate through gesture and Lack of varied spontaneous make-believe or social imitative play, were omitted more often (individual items more frequently as well as a wider range of items) in patients with CHARGE/OAV (most frequently in the CHARGE group) than in those with Möbius.

Table 15. Omission of ADI-R algorithm items in Möbius, CHARGE and OAV groups

	Möbius (n=20)	*CHARGE (n=28)*	*OAV (n=20)*
QUALITATIVE IMPAIRMENTS IN RECIPROCAL SOCIAL INTERACTION			
Failure to use nonverbal behaviours to regulate social interaction			
42. Direct gaze	5 (25%)	6 (21%)	2 (10%)
43. Social smile	8 (40%)	3 (11%)	1 (5%)
52. Range of facial expressions used to communicate	9 (45%)	8 (29%)	2 (10%)
Failure to develop peer relationships			
64. Imaginative play with peers	1 (5%)	3 (11%)	2 (10%)
66. Interest in children	1 (5%)	3 (11%)	1 (5%)
67. Response to other children's approaches	1 (5%)		
68/69. Group play with peers/Friends	1 (5%)	5 (18%)	1 (5%)
Lack of socio-emotional reciprocity			
11. Use of other's body to communicate		2 (7%)	1 (5%)
49. Offers comfort		3 (11%)	2 (10%)
51. Quality of social overtures	2 (10%)	6 (21%)	2 (10%)
53. Inappropriate facial expression	1 (5%)	3 (11%)	
QUALITATIVE IMPAIRMENTS IN RECIPROCAL COMMUNICATION			
Lack of, or delay in, spoken language and failure to compensate through gesture			
30. Pointing to express interest	2 (10%)	2 (7%)	1 (5%)
31. Conventional instrumental gestures	1 (5%)	3 (11%)	2 (10%)
32. Nodding		4 (14%)	3 (15%)
33. Headshaking		6 (21%)	2 (10%)
Lack of varied spontaneous make-believe or social imitative play			
29. Spontaneous imitation of actions	1 (4%)	1 (3%)	1 (5%)
63. Imaginative play		3 (10%)	1 (5%)
65. Imitative social play			1 (5%)
Relative failure to initiate or sustain social interchange			
Inappropriate questions			1 (5%)
RESTRICTIVE, REPETITIVE AND STEREOTYPED INTERESTS AND BEHAVIOURS	--	--	--

CARS

The only omitted CARS item in the Möbius group was the one entitled "non-verbal communication" (in 9%). This item was scored in all CHARGE/OAV patients, but the "visual response"", "listening response" and "verbal communication" items were omitted in some (CHARGE in 14%, 32%, 18%; OAV in 5%, 15%, 20%).

ABC
Omitted ABC items pertained, in all BPCs, to the subsections Sensory, Language and Relating. In all BPCs the Language subsection accounted for a major part of omitted items. The range of excluded items and the frequency to which individual items were omitted was most pronounced in the CHARGE group and least pronounced in the Möbius group. The only omitted item included in the Relating sub-section in the Möbius group was the one entitled "has no social smile" (in 14%).

Discrepancies in diagnostic classification of autism according to DSM-III-R, DSM-IV, ADI-R, CARS and ABC (IV)

Möbius group
There was complete concordance as regards "DSM-III-R, ADI-R and CARS diagnoses of autism", whereas the ABC "under-diagnosed" autism in three patients as compared with the other instruments.

CHARGE group
(Figure 1) Complete concordance as regards diagnoses of autism was not observed according to any diagnostic instruments/criteria. The DSM-III-R criteria were most "restrictive" (five patients meeting DSM-III-R criteria for AD) and the ADI-R most "inclusive". Three individuals, who met algorithm criteria for autism according to the ADI-R did not meet DSM-III-R/DSM-IV criteria for AD and were diagnosed with ALC (n=2) and AT (n=1). One of these had a mental age below, and another slightly above, 18 months.

OAV group
There was complete concordance as regards "CARS, ABC, DSM-IV and DSM-III-R diagnoses of autism", whereas the ADI-R "over-diagnosed" one patient with AT and MLD.

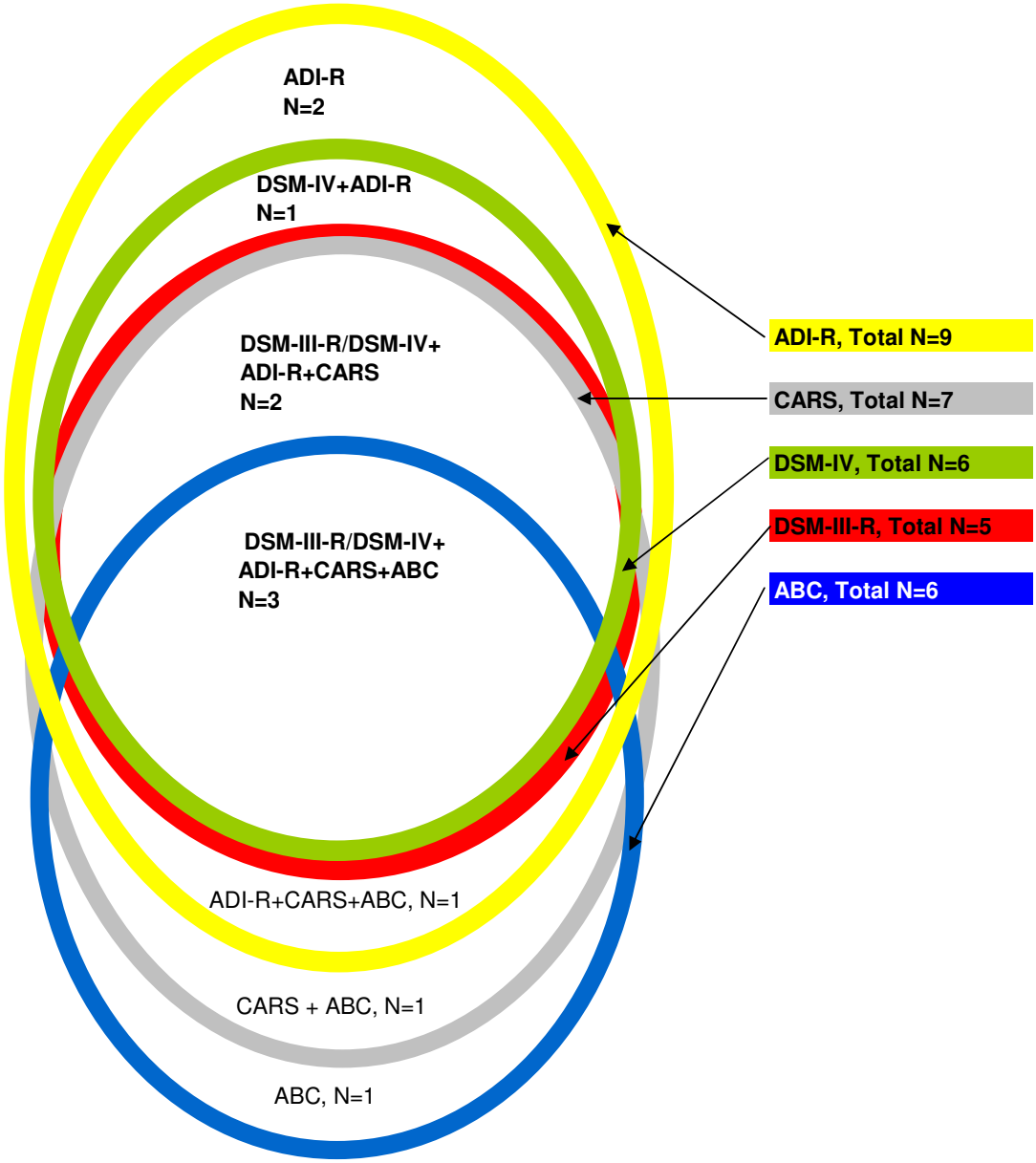
Impact of omitted items on "ADI-R, CARS and ABC diagnoses" in individual patients (IV)

(Impact of inclusion of omitted items is summarized in Table 16.) Except for the deaf-blind group, only very few subjects surpassed the respective cut-off scores when adding maximal possible scores on omitted items. The ABC cut-off was exceeded by more individuals than the CARS cut-off score, which in term was exceeded by more subjects than the ADI-R algorithm criteria for autism.

Table 16. Patients surpassing ADI-R, CARS and ABC cut-off scores after inclusion of maximal scores on omitted items in each BPC

	Möbius	*CHARGE*	*OAV*
ADI-R	-	N=3 3 DB	N=3 1 ALC, 1 AT, 1 DB
CARS	-	N=6 2 ALC, 1AT, 3DB	N=2 1 AT, 1 DB
ABC	N=2 2 AD	N=7 1AD, 2ALC, 1 AT, 3 DB	N=2 1 AT, 1 DB

Figure 1. Discrepancies in diagnostic classification of autism according to DSM-III-R/DSM-IV, ADI-R, CARS and ABC in CHARGE group



Distribution of ADI-R, CARS and ABC sub-domain scores and impact of omitted items (IV)

BPC groups excluding deaf-blind patients were compared. The deaf-blind patients with CHARGE (n=3) and OAV (n=1) were collapsed into one deaf-blind group.

ADI-R

In comparison between all those assessed with the ADI-R (Möbius: n=20, CHARGE: n=25, OAV: n=19), the CHARGE group had the highest rated mean scores across all sub-domains. The discrepancy between rated and maximum possible Social domain mean scores was most pronounced in the Möbius group (Figure 2). There was no discrepancy between rated and maximum possible Communication domain mean scores for individuals with AD across the BPCs (Möbius: n=5, CHARGE: n=5, OAV: n=2), nor for the subjects with ALC in the CHARGE group (n=5). Almost all of those with AD and ALC were classified as non-verbal according to the ADI-R algorithm. Rated and maximum possible Behaviour domain mean scores were equal across all diagnostic ASC groups in all the BPC groups (all items included in the Behaviour sub-domain were rated in all individuals, including the deaf-blind group).

The maximum possible Social domain mean score in the deaf-blind group (n=4) was more than three times as high, and the maximum possible Communication mean score more than five times as high, as the corresponding rated mean scores (7.75, SD=1.71 versus 26.25, SD=1.26; 2.00, SD=1.63 versus 11.50, SD =1.00, respectively).

CARS

In comparison between all those assessed with the CARS, the CHARGE group had the highest rated mean score (rated scores; Möbius, n=22: 24.14, SD=9.40, CHARGE, n=25: 26.16, SD=9.51, OAV, n=18: 20.72, SD=5.63). There were only minor discrepancies between rated and maximum possible scores across the three BPC groups, for all those assessed with the CARS (maximum possible scores: Möbius: 24.46, SD=9.24, CHARGE: 27.64, SD=10.30, OAV: 21.72, SD 6.62), as well as for individuals with AD across the BPCs (Möbius, n=6: 38.33, SD=3.71 versus 38.33, SD=3.71; CHARGE, n=5: 41.10, SD=5.98 versus 41.50, SD=6.04; OAV, n=2: 31.50, SD=0.00 versus 33.00, SD=2.12).

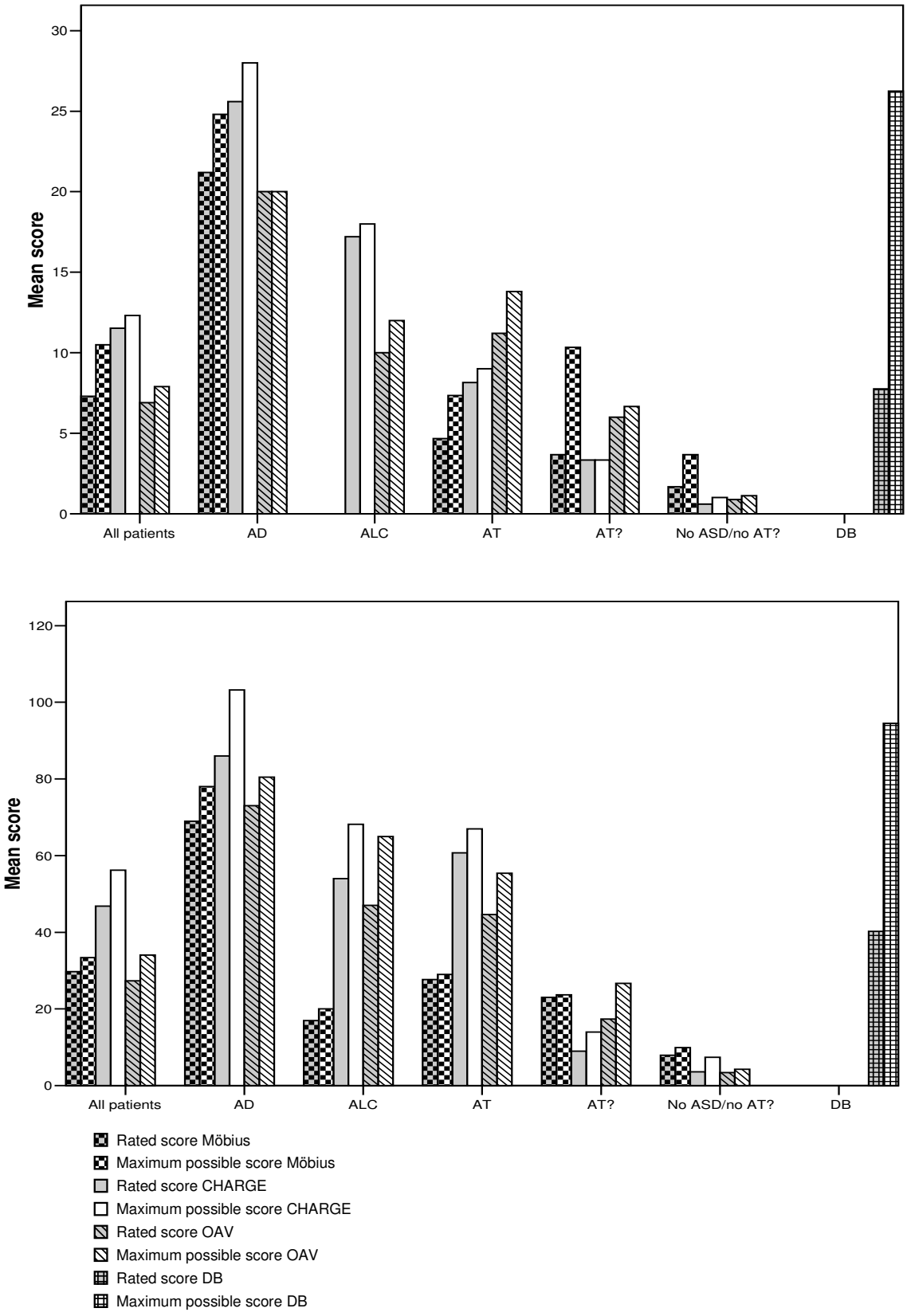
There was a considerable discrepancy between rated and maximum possible mean scores in the deaf-blind group (n=4, 21.88, SD=3.01 versus 33.38, SD=1.97).

ABC

(Figure 3) In comparison between all those assessed with the ABC (Möbius: n=23, CHARGE: n=25, OAV: n=16), the CHARGE group had the highest rated mean scores and the most pronounced discrepancy between rated and maximum possible ABC scores.

There was a more than twofold discrepancy between rated and maximum possible mean scores in the deaf-blind group (n=4: 40.24, SD=21.82 versus 94.50, SD=15.76).

Figure 2-3. ADI-R social domain (Figure 2) and ABC (Figure 3) mean scores across diagnostic ASC groups in Möbius, CHARGE and OAV groups.



ADI-R profiles in individuals with autism in Möbius, CHARGE, and OAV groups (IV)

The Social and Communication sub-domain mean scores were higher in the patients with AD in all three BPC groups (Möbius: n=5, CHARGE: n=5, OAV: n=2) as compared with the corresponding mean scores in a sample of 25 individuals with "AD/AD only" (Lord et al. 1994). The Behaviour sub-domain mean scores in the patients with AD and Möbius/CHARGE were slightly above, and in those with OAV and AD slightly below, that in the Lord et al. (1994) sample. In each BPC group the subjects with AD, achieved mean scores twice as high as the Lord et al. (1994) sample with "AD/AD only" on the Social domain sub-section Failure to develop peer relationships (Table 17).

ABC profiles in individuals with autism in Möbius, CHARGE, and OAV groups (IV)

(Figure 4) The Relating sub-domain mean score fell, in the individuals with CHARGE and AD (n=5), below/above the corresponding mean scores in two samples of individuals with "AD" (Krug et al. 1980, n=172, Volkmar et al. 1988, n=94, respectively), and above that in a sample of 155 "mute individuals with AD or ASCs" (Miranda-Linné and Melin et al. 1997). The Relating domain mean score in the patients with Möbius and AD (n=6) was lower than in the two samples of individuals with "AD", but higher than in the sample of "mute individuals with AD or ASCs". The Relating sub-domain mean scores in the two patients with OAV and AD was slightly below the corresponding mean score in the sample of "mute individuals with AD or ASCs". The Language sub-domain mean scores in the patients with Möbius/CHARGE and AD were similar to that in the Miranda-Linné and Melander (1997) sample of "mute individuals with AD or ASCs". The Body/object use mean score in the CHARGE subjects with AD was about/more than 1.5 folds higher than in the other two BPC groups as well as in the Krug et al. (1980), Volkmar et al. (1988), and Miranda-Linné and Melin (1997) samples.

Table 17. ADI-R sub-domain mean scores in individuals with AD in Möbius, CHARGE and OAV groups compared to individuals with "AD/AD only".

	Möbius n=5[r] Mean SD	*CHARGE n=5[s] Mean SD*	*OAV n=2[t] Mean SD*	*"AD/AD only", n=25[u] (Lord et al. 1994) Mean SD*
Social domain total	21.20 (3.90)	25.,6 (1.14)	20.00 (1.41)	19.00 (3.76)
B1. Failure to use eye-to eye gaze, facial expression, body gesture, and to regulate social interaction	2.00 (1.58)	4.40 (1.52)	4.00 (2.83)	3.52 (1.61)
B2. Failure to develop peer relationships	7.20 (1.30)	7.40 (0.89)	6.50 (0.71)	3.12 (0.93)
B3. Lack of seeking to share own enjoyment	5.20 (1.10)	6.00 (0.0)	4.0 (2.83)	5.00 (1.26)
B4. Lack of social emotional reciprocity and modulation to context	6.80 (1.30)	7.80 (1.10)	5.50 (0.71)	7.36 (1.93)
Communication domain total (verbal)	(20.00) (0.0) n=1		17.00 (0.0) n=1	16.33 (2.96)
Communication domain total (nonverbal)	12.00 (3.36) n=4	12.60 (1.67)	12.00 (0.0) n=1	11.62 (1.96)
C1. Delay or total lack of spoken language not compensated by gesture	7.00 (1.73) n=5	7.20 (0.84)	6.50 (0.71) n=2	6.60 (1.47)
C2. Relative failure to initiate or sustain conversational interchange[v]	6.00 (0.0) n=1		5.00 (0.0) n=1	3.22 (1.09)
C3. Stereotyped and repetitive use of language[a]	2.00 (0.0) n=1		2.00 (0.0) n=1	3.11 (1.90)
C4. Lack of varied spontaneous make-believe or social imitative play	5.00 (1.41) n=5	5.40 (0.89)	4.50 (2.12) n=2	3.44 (1.51)
Restricted repetitive behaviours and interests total	5.00 (0.71)	5.60 (1.82)	4.50 (0.71)	4.92 (1.80)
D1. Encompassing preoccupations	0.60 (0.89)	0.80 (0.84)	1.00 (0.0)	1.04 (0.93)
D2. Apparently compulsive adherence to non-functional rituals	0.80 (0.84)	1.00 (1.00)	0.00 (0.0)	1.16 (1.21)
D3. Stereotyped and repetitive motor mannerisms	0.80 (0.45)	2.00 (0.0)	2.00 (0.0)	1.36 (0.76)
D4. Preoccupation with part-objects and non-functional elements of materials	0.80 (0.45)	1.80 (0.45)	1.50 (0.71)	1.36 (0.57)

[r] Mean age 12:10 yrs, SD 7:2 yrs, SLD: n=4, MLD: n=1 (the ADI-R was not performed in 1 individual with Möbius and AD)

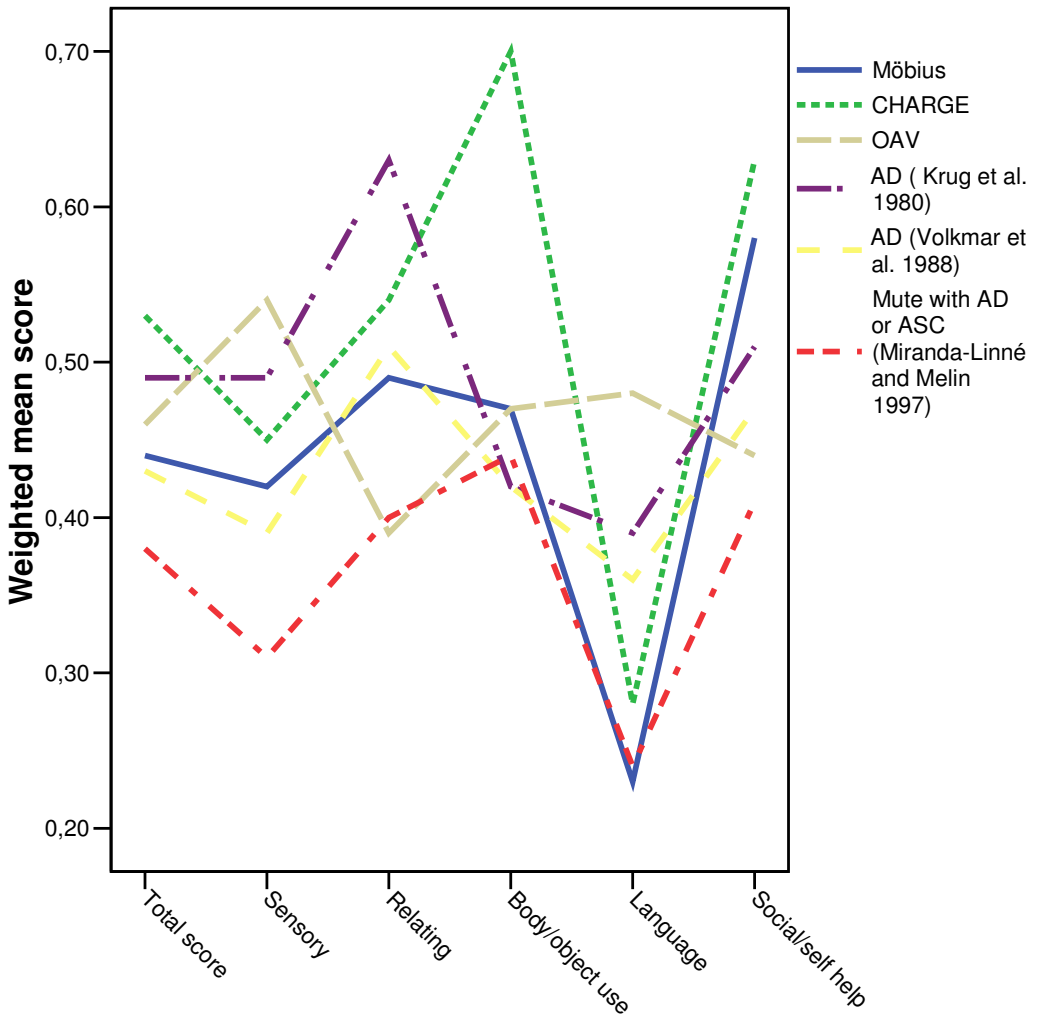
[s] Mean age 9:10 yrs, SD 4:10 yrs, PLD: n=4, MLD: n=1

[t] Mean age 10:2 yrs, SD 8:7 yrs, SLD: n=1, MLD: n=1

[u] Total sample of 25 individuals collapsed from two original samples; 10 subjects with mean age: 46.76 mths, SD=10.73, mean non-verbal, IQ/DQ:71.88, SD=21.33 (children who were non-ambulatory or had other marked motor impairments, had other than mild, remediable sensory impairments or identifiable syndromes, e.g. Down syndrome, Rett syndrome or were judged by their teachers to be functioning below the 12-month level were excluded in this sample); 15 subjects with mean age 48.9 mths, SD=12.2, mean non-verbal IQ/DQ: 64.12, SD =32.86

[v] Sub-domain only applicable for verbal individuals

Figure 4. Weighted ABC sub-domain mean scores in individuals with AD in Möbius, CHARGE and OAV groups compared to individuals with "AD" and "mute individuals with AD or ASCs".



Möbius: n=6, mean age 13:3 yrs, SD=6:6, SLD: n=4, MLD: n=2
CHARGE: n=5, mean age 9:10 yrs, SD=4:10, PLD: n=4, MLD: n=1
OAV: n=2, mean age 10:2 yrs, SD=8:7, SLD: n=1, MLD: n=1
Krug et al. (1980): "AD", n=172, no information about mean age or IQ ("autistic persons were compared with mentally retarded, emotionally disturbed, deaf-blind and normal non-autistic persons to demonstrate that the test could discriminate between the autistic persons and all other non-autistic populations")
Volkmar et al. (1988): "AD", n=94, mean age 17.99 yrs, SD=9.11, mean IQ 32.62, SD=19.88
Miranda-Linné and Melin (1997): "Mute with AD or ASCs", n=155, mean age 14:11 yrs in total sample of mute and speaking individuals, no significant age difference between mute and speaking individuals, no information about IQ

DISCUSSION

Overall summary of findings

ASCs were found in more than half of the patients with CHARGE and more than a third of the patients with Möbius/OAV. Twenty-nine percent (6/21) in the Möbius group met full criteria for AD. The corresponding rates in the CHARGE and OAV groups were 20% (5/25), and 11% (2/19), respectively. LD was also a frequent finding, possibly reflecting the link between ASCs and LD.

Cerebral abnormalities were recorded in one fifth of examined patients with Möbius, almost three quarters with CHARGE and more than half of those with OAV. Forebrain midline anomalies were common in the CHARGE group (37%). Midline cerebral malformations were also indicated in one individual with Möbius (corpus callosum agenesia) and two patients with OAV (mild holoprosencephaly, n=1, occipital encephalocele/Arnold Chiari II anomaly, n=1). ASCs, LD, and cerebral malformations often occurred together in the same individuals. All individuals in the Möbius group, 55% in the CHARGE group and 60% in the OAV group had cranial nerve involvement. Bilateral facial palsy was present in 64% of the subjects with Möbius but in none of those with CHARGE/OAV.

Visual and hearing impairments were recorded in a few patients with Möbius and several with CHARGE/OAV, affecting a majority of individuals with CHARGE. ASCs tended to be more common the more visual and hearing impairments observed.

Pre/perinatal and family factors of possible but uncertain interest were recorded in all three BPCs. One fourth of the mothers in the Möbius/OAV groups and almost one fifth in the CHARGE group reported vaginal bleedings during pregnancy. More than one previous abortion was recorded in almost one fourth of the mothers in the Möbius group. Assisted fertilization was recorded in the history of 10% of the subjects with CHARGE and 20% with OAV. Twenty-five percent of the individuals with OAV were twins (other twin healthy and discordant for OAV: n=4, other twin spontaneously aborted: n=1).

Use of the ADI-R, the CARS and the ABC presented, due to notably sensory impairments and cranial nerve palsies, rating difficulties in all three BPCs, and some ADI-R, CARS and ABC items in some individuals were considered as impossible to score. These difficulties increased with the number and severity of associated disabilities, and were most pronounced in the deaf-blind patients. The ADI-R “over-diagnosed” some individuals, whereas the CARS, and especially the ABC, both over- and under-diagnosed" some subjects, as compared with the DSM-criteria.

Diagnoses of BPCs

The range and severity of disabilities in the clinical presentation of Möbius, CHARGE and OAV, is indeed varying with some individuals being mildly affected while others are very severely disabled. Thus, the clinical pictures of these BPCs are far from always "clear-cut". There is still no universally accepted consensus regarding the diagnostic criteria for any of the three BPCs studied. The evaluation of the clinical representativeness of our Möbius/CHARGE/OAV samples is complicated by the fact that, to our best knowledge, there are no/very few population-based studies on these conditions. Since many of the individuals in our Möbius/CHARGE/OAV studies were referred from habilitation units

(thus perhaps constituting a selection of the most severe cases), and since knowledge and clinical awareness of Möbius/CHARGE/OAV has increased during more recent years, perhaps the proportion of mildly affected individuals with these BPCs would be larger in more representartive samples. The most reliable approach seemed to be to describe the clinical presentation in each individual as exactly as possible. This approach was applied for all the three BPCs studied.

Verloes et al (2005) suggested that there is a distinct clinical variant of Möbius, designated as "Robin-Möbius", characterized by extensive brain stem involvement, Robin complex (respiratory compromise secondary to CNS involvement, small mandible and cleft palate), hypotonia, club feet and acral anomalies, which appears to bear a higher risk of mental handicap. However, notwithstanding the frequent occurrence of ASCs and LD in our Möbius sample, we consider our cases of Möbius to be fairly representative of other cases in the literature regarding the type of different malformations.

As regards CHARGE, the discovery of a mutation in a CHD7 gene on chromosome 8 in 50-75% of cases (Vissers et al. 2004, Jongmans et al. 2005, Aramaki 2006, Lalani et al. 2006) has wiped out most doubt that this condition constitutes a distinct entity. No specific genotype-phenotype correlation has been noted in CHARGE, but a combination of colobomas, choanal atresia and abnormal semicircular canals has been suggested to be predictive for a CHD7 mutation, and that aplasia of the semicircular canals and hypoplastic uncus may be "probably the most specific anomalies in CHARGE syndrome" (Lalani et al. 2005, Sanlaville and Verloes 2007). Our study group of cases are undergoing CHD7-gene-analyses at the time of going to press with this thesis, and we cannot draw conclusions in this respect before the results of those examinations become available. Evidence for hypoplastic semicircular canals was only available in eight out of nine radiologically examined subjects in our CHARGE sample. Thus, if semicircular canals constitute one of the most characteristic malformations in CHARGE, and if one would speculate that there exist a "broader phenocopy" of CHARGE, caused by e.g. other gene defects and/or teratogenic agents, it might be that some individuals in our sample would be classified in this group. However, in respect of distribution of other features, which are characteristic of CHARGE, our sample is comparable to other samples reported in the literature.

The heterogeneity of OAV must be described as extreme and the diagnostic delineation of OAV is especially indistinct, rendering difficulties in defining the study entry criteria for this condition. The complete picture of GS (characterized by microtia, hemifacial microsomia, vertebral anomalies and dermoids) was present in one fifth of the patients with OAV, and three quarters had the type of ocular involvement (epibulbar dermoids) regarded as characteristic of OAV. Thus, it cannot be ruled out that the majority of our patients may have different characteristics compared to patients lacking ocular manifestations.

In agreement with other clinicians and researchers, we consider the clinical picture in Möbius/CHARGE/OAV to be characteristic, when enough signs are present. In all the three BPCs studies we have used the *clinical* criteria, which were the most widely accepted at the time of the present study.

Diagnoses of ASCs

The neuropsychiatric evaluations were performed according to what is generally agreed to be the gold standard diagnostics of ASCs, namely global judgment by experienced clinicians based on as much relevant available information as possible. Standardized autism diagnostic instruments/criteria, recognized to be the most valid and reliable when the present study was carried out (DSM-III-R, DSM-IV, ADI-R, CARS, ABC), were utilized. All patients were evaluated independently by two investigators. The DSM criteria were completed on the basis of all relevant information obtained (data from observations, interviews and medical records). Diagnoses of AD were only assigned in patients for whom there was agreement between the two investigators, which in the Möbius study implied concordance in diagnoses according the DSM-III-R criteria and ADI-R algorithm, and in the CHARGE/OAV studies concordance according to the DSM-III-R, DSM-IV criteria and ADI-R algorithm. In the CHARGE/OAV studies, diagnoses of ALC and AT were only applied for individuals who met the postulated number of DSM-III-R and DSM-IV criteria. AD and ALC diagnoses in the Möbius study were assigned according to the DSM-III-R criteria.

Appropriateness of methods used

The autism diagnostic instruments used are all considered to have good psychometric properties in individuals 2 years of age or older (all individuals under 2 years of age were considered as impossible to assess reliably as regards ASCs). However, none of the diagnostic instruments/criteria used in this study are validated for use in individuals with the kind of disabilities (often patterns of several disabilities blurring the recognition of one another) in Möbius/CHARGE/OAV. Pending the diagnostic difficulties and the lack of instruments for use in diagnostics of autism in individuals with sensory impairments and cranial nerve palsies, we used an extensive battery of autism diagnostic measures/criteria, and tried to describe exactly how the diagnoses were made by giving the scores obtained on each instrument, preferably for each individual.

The scoring difficulties increased with the range and severity of disabilities, and were most pronounced in individuals with both major hearing and visual loss. While in most cases it was possible to score the majority of items in patients with either severe visual or severe hearing impairments, deaf-blindness seamed to have major impact on practically all aspects of development. The scoring difficulties in the deaf-blind patients were reflected in very pronounced discrepancies between maximal possible and rated scores (most pronounced for the ADI-R social and communication domains; threefold and fivefold discrepancies, respectively).

Since there was a tendency for ABC items to be omitted more frequently than ADI-R and CARS items, it seems as if the rating of ABC items presented more difficulties for the investigators. This would seem to indicate the supremacy of the ADI-R and CARS to the ABC in individuals with multiple disabilities as in Möbius/CHARGE/OAV, but such an unambiguous conclusion would be too simple. The broader concepts of the ADI-R and CARS items have made it possible for the interviewers to consider alternative aspects of skills/behaviours when scoring ADI-R/CARS items, which involves an amount of arbitrariness (e.g. if imitation of sound and touch are scored in a blind child, the detailed guidelines given for scoring imitation in the ADI-R/CARS cannot be applied, since these are designed to be used in children with normal vision/hearing).

There were no or minor problems using the CARS and ABC in the Möbius individuals, who all had facial/abducens nerve palsy but no major visual/ hearing deficits (the ABC does not comprise any items regarding facial expressions, and the CARS has only one item dealing with gesture as well as facial expressions). The fact that more ADI-R items were omitted in Möbius than in CHARGE/OAV should not be taken as an indication of more problems with scoring ADI-R items in Möbius individuals. The discrepancy in omission rate is likely to reflect a more restrictive approach in omission of ADI-R items in the CHARGE/OAV subjects. Since major visual impairment may affect most aspects of social interaction, the interviewer has been forced to rate alternative aspects of behaviour, and thus to adapt the rating process to the conditions of each individual. This approach may also be the explanation of the fact that the ADI-R algorithm over-diagnosed autism in some subjects, as compared to the DSM criteria in the CHARGE/OAV groups. The fact that the ADI-R has lower specificity in individuals with mental ages below 18 months (Lord et al. 1993) may be another explanation.

CARS and ABC verbal communication items were omitted in several hearing impaired patients. The Language subsection accounted for a major part of omitted ABC items in all the BPC groups. Miranda-Linné and Melin (1997) speculated that ABC items concerned with expressive language might be weighted to heavily in regard to both the Language subscale and the total ABC score, since greater pathology scores are provided to verbal individuals than to mute. The fact that the ADI-R provides a special algorithm for nonverbal individuals may make it the instrument of choice in hearing impaired individuals who lack sign language, as well as in other individuals with no language. Further, the ADI-R yielded rich descriptions of various behaviours, which, in combination with structured clinical observation, were very valuable in our efforts to compile a full clinical picture of the subjects.

Problems pertaining to evaluation of ASCs in individuals with BPCs

Individuals with ASCs often have additional disabilities. Gillberg and Coleman (1996) estimated that about one in four cases of autism or autistic symptoms present with another concomitant medical condition. A range of other disabilities often obscure the presence of autistic symptoms and make the diagnostics of ASCs in individuals with BPCs much more complicated. In recent years, research on BPCs has focused on efforts to delineate specific BPs in different BPCs (Turk 1995). Children with CHARGE have been described as frequently exhibiting moderate to severe behaviour difficulties, and to often be diagnosed with obsessive-compulsive disorder, attention deficit disorder, Tourette syndrome, or autism (Hartshorne and Cypher 2004). The BPs in Möbius/OAV have received less attention, but recently Verzijl et al. (2005c), after a nation-wide call, in a group of 16 adult Dutch individuals with Möbius, found no evidence of attention/memory dysfunction, and that the rate of LD did not differ from that in the general Dutch population.

Hartshorne et al. (2005) found ABC scores in children with CHARGE to differ from ABC scores in blind children, and from scores in children with autism. However, variance of scores in the children with CHARGE was considerably greater than for the other groups and 27,5% of those with CHARGE "could be classified as autistic". Graham et al. (2005) used behavioural and personality assessments to compare boys with CHARGE syndrome to age matched boys with Down syndrome, Prader-Willi syndrome and Williams syndrome, and found the boys with CHARGE syndrome to have "behaviour that more resembled autistic spectrum disorder" but not to be "as socially impaired as in classic autism". However, these efforts to describe the unique BP in CHARGE have not or only

casually acknowledged the conception of ASCs as a continuum of severity. Further, neither the study by Hartshorne et al. (2005) nor the one by Graham et al. (2005) addressed difficulties in the use of the respective diagnostic instruments due to sensory impairments.

A very central issue is whether we are measuring symptoms of a "true ASC". Basically, this issue concerns the meaning of the concept of autism.

First, one must consider the possibility that we might be measuring autistic-like features due to sensory impairments instead of "true symptoms of ASCs". Major visual and hearing impairment are both considered to give rise to autistic-like features per se. On the other hand, the occurrence of ASCs has been shown to be increased in certain subgroups associated with neurological dysfunction and severe visual/hearing impairments, e.g. retrolental fibroplasia (Keeler 1958), Leber's amaurosis (Rogers and Newhart-Larson 1989), optic nerve hypoplasia (Ek et al. 2005), rubella embryopathy (often associated with both visual and hearing loss) (Wing 1969, Jure et al. 1991). It is easy to understand that quality of eye contact is difficult to assess in a visually impaired child and auditory response in a child with hearing loss. Furthermore, both visual and hearing impairment are bound to have impact on most aspects of social and communicative development, which is why the scoring guidelines for a wide range of autism diagnostic instrument items, based on the development of "normal" children, do not apply for children with major sensory impairments. There is a risk that ASCs may be over- as well as under-diagnosed in individuals with major sensory deficits. It has been hypothesized that the clinical picture of an "autistic-like syndrome" in blind children differs from autism in normally sighted children in respect of the quality of their social skills (Brown et al. 1997). However, according to my opinion, this line of argument, just as the efforts to delineate the unique BP in CHARGE described above, insufficiently acknowledge the concept of ASCs as a continuum.

Secondly, there is the possibility that we are measuring symptoms from a primarily neurodevelopmental dysfunction of different nature than that underlying "true ASCs". During more recent years it has been suggested that behavioural difficulties in CHARGE has a compulsive/impulsive/driven quality and may be best explained by specific deficits in executive functioning (Nicholas 2005). Executive dysfunction is not to be conceptualized as a singular disorder, but rather a variety of presentations involving one or more aspects of executive functioning, including a number of neurobehavioural disorders that reflect different aspects of executive dysfunction (Cicerone 2002). Executive deficits have, in addition to autism, been documented in hyperactive disorder (Pennington and Ozonoff 1996), Tourette syndrome (Bornstein 1990), obsessive compulsive disorder (Lichter and Cummings 2001). Some non-social impairments in autism, such as rigidity and preservation, are usually explained by deficits in executive functions, whereas theory of mind has been considered to underlie different aspects of autistic symptomatology pertaining to specific social and communication impairments (Hill and Frith 2003).

We may be moving towards a fractionation of the concept of autism implying measurement of the three aspects of the three core impairments in the autism syndromes; namely social interaction, communication and repertoire of activites/interests separately (Happé 2006), or separate measurements of neuropsychological functions such as executive skills and emotional recognition (Turk and Cornish 1998), instead of relying on global measures of severity of autism. However, the DSM-IV and ICD-10 criteria, not

comprising any criteria for excluding/classifying ASCs as secondary in the presence of sensory deficits or other neurological dysfunction, still constitute the prevailing norms.

Strengths

To our knowledge, these studies, with their multidisciplinary approach including in-depth clinical assessments of groups of patients, are the most comprehensive investigations performed to date of individuals with Möbius/CHARGE/OAV (especially with regard to the neuropsychiatric work-up). Several studies of behaviour disturbances, "autistic-like behaviour" and ASCs in CHARGE have been published during recent years, and knowledge about a special behavioural phenotype in CHARGE is beginning to emerge. Most of these studies have reported on case series of small to intermediate size, and/or assessments of ASCs have been based on information from one/only a few questionnaires/interviews with care givers, (and have not included clinical evaluations), or have been based on not specifically designed for diagnostics of ASCs. The behavioural phenotypes in Möbius and OAV have attracted less attention, although some case reports/reports of autism or other neuropsychiatric disturbances in small groups of patients have been published (Gillberg and Steffenburg 1989, Bandim et al. 2003, Landgren et al. 1992, Verzijl et al. 2005).

Limitations

This was not a population-based study and the sample size in each sub-study was relatively small (25, 31 and 20 participants, respectively). The number of individuals considered to be reliably diagnosed as regards ASC was even smaller (21, 25, and 19, respectively). The mode of recruitment with referrals from habilitation units in many cases may have contributed to overrepresentation of severe cases in each condition.

The male:female ratio in the OAV sample was in agreement with the previously estimated male:female ratio 3:2 in OAV according to a recent review (Gorlin 2001). However, the same review quoted a male:female ratio for Möbius sequence of 1:1, which is strikingly different from the male preponderance found in the present study. Estimations of the male:female ratio in CHARGE is, to our best knowledge, insufficient. Prevour reports of series of individuals with CHARGE diverge concerning gender ratios, e.g. 33:26 in Wyse et al. (1993) versus 17:30 in Tellier et al. (1998). The male:female ratio was not reported in the Canadian population-based survey of CHARGE (Issekutz et al. 2005). It has been proposed that previously generally accepted CHARGE criteria (used in the present thesis), might lead to over-inclusion of boys, due to the "sex-dependent" inclusion of (non-specified) genital hypoplasia (Verloes 2005).

In order to obtain reasonable sample sizes of these rare BPCs, it was impossible to compile samples that were more homogenous as regards age. However, after omitting the very young children, almost all subjects were children and adolescents.

Given that radiological imaging was not performed during the present study, cerebral malformations could not be documented systematically. Cerebral imaging was more often available in patients with ASCs, meaning that the frequency of such anomalies in patients without ASCs in our results may be underestimated. Further, only results from radiological imaging in OAV patients were uniformly reviewed by one and the same paediatric radiologist.

None of the diagnostic instruments in this thesis were validated for use in patients with the constellations of dysfunction that are typically seen in these BPCs. This does not only apply for the autism measures/criteria, but also for assessments of vision, hearing, cognitive ability and adaptive functioning.

Implications for clinical practice

The frequent occurrence of ASCs in the three BPCs studied demonstrates that awareness of ASCs is important in the habilitation of children with these conditions. ASCs occurs frequently in individuals with visual (Steffenburg et al. 1991) and hearing deficits (Rosenhall et al. 1999), and behaviour disturbances in children with Möbius, CHARGE and OAV might be the manifestations of an ASC. In fact, our data indicate, in concordance with the report by Carvill and Marston (2002) that most individuals with severe sensory impairments and LD are on the autistic spectrum. This conclusion stands out as particularly important in the light of the fact that we frequently came across the opinion that behaviour disturbances in children with sensory impairments *should not* be conceptualized as being part of an ASC. Several parents in the study (especially those in the CHARGE sub-study), whose children were diagnosed with severe autistic symptoms and LD, stated that they had been informed that their children, taking their sensory impairments into account, were developing "normally". Some parents had been concerned because educational methods which seem to be successful with other severely sensory impaired children appeared to be relatively unsuccessful with their children. When behavioural/educational interventions for children with autism seem to work in a child with sensory impairments and LD, the distinction between "autistic-like symptoms" (causal indicators defining an "autistic–like syndrome") and ASCs (effect indicators of specific neurological disturbances in ASC) appear to be more of academic nature than of vital clinical relevance.

Thus, educational approaches for children with autism should be tried in individuals with Möbius/CHARGE/OAV *and* autistic behaviour, who are not progressing well in programmes for the visually/hearing impaired. Early development of alternative communication systems is considered to be of significance in the habilitation of multi-sensory deprived children, but that is also the case for children with autism, who have special communicative difficulties.

Evaluation of ASCs in children with disabilities such as in these BPCs need to be based on information from as many sources as possible and an extensive battery of diagnostic instruments, including both structured clinical observation and in depth parental interviews should be used, preferably by two independent clinicians.

When the ADI-R, the CARS and the ABC are used in assessment of individuals with disabilities such as in Möbius/CHARGE/OAV, blurring the recognition of symptoms in ASCs, some items will be difficult to score. For certain reasons, decisions about omission of items cannot be straight forward. First, none of these instruments provide clear directions for when specific items should be considered as "not applicable" in disabilities such as in these BPCs. Secondly, not only evaluation regarding ASCs, but all assessments requiring reciprocity, will be more difficult in subjects with multiple disabilities such as in these BPCs. Given that multiply disabled individuals with LD and autism are frequently passive and do not respond to visual or auditory stimulus, the degree of hearing/visual impairment may be difficult to establish with conventional audiometric/visual acuity testing. Therefore, decisions about omission of items and applicability of recommended

scoring instructions need to be evaluated separately in each case and, to a considerable degree, be based on parental knowledge of the child.

Not surprisingly, interpretation of VABS scores in multiply disabled subjects may entail difficulties. Three individuals diagnosed with AD in our CHARGE sample had mental ages below 12 months, as estimated with the VABS (motor domain not included). Two of them presented a pattern of adaptive behaviour as measured with the VABS, which has been shown to be characteristic for individuals with autism (low socialization scores, intermediate communication scores and higher daily living scores) (Burack and Volkmar 1992), whereas the third subject showed a relative strength in the daily living skills domain and moderate deficits in the socialization/communication domains. The motor and planning skills in these individuals indicated mental ages of at least l2-18 months. Carter et al. (1998) highlighted the need of special population norms for the VABS for individuals with autism, and within those in the autism spectrum for those with and without verbal language. According to assessment with the VABS, fine-motor skills were delayed in the pre-lingually deaf (without cognitive impairment) (Horn et al. 2006). Smith et al. (2005), in a group of 13 individuals with CHARGE (presence of ASCs symptoms considered moderate to strong in 6/10 who were above 4-5 years of age) noted lower daily living scores, relative to other scores than is typically seen in autism, and concluded that sensory impairments, poor motor and balance skills seem to have a dramatic impact on daily living skills in individuals with CHARGE.

Implications for research

Diagnostics of ASCs in BPCs

For a better understanding of the validity of autism diagnostic instruments there is a need for studies on different sub-populations, (e.g. groups with different BPCs, different levels of intellectual functioning, different types and levels of sensory impairments). The results of the present study indicate a need for further development of autism diagnostic instruments, rooted in the knowledge of development in children with sensory impairments. Further, since recent research indicate that a subgroup of ASCs is associated with dysfunction at the brain stem level (Rodier 2002), the influence of cranial nerve palsies on mimicry and eye contact in individuals with ASCs need to be studied. Another more complicated mission would be to delineate the course of "normal" social development in individuals with Möbius, CHARGE and OAV.

Brain stem and cerebral abnormalities underlying ASCs in Möbius/CHARGE/OAV

There is a great need for basic research (radiological, neurophysiological and post-mortem studies) regarding the possible brain stem link in ASCs, and also in subgroups of individuals with Möbius/CHARGE/OAV with and without ASCs. These studies need to take account of the possibility that other portions of the central nervous system might be affected alongside any abnormalities noted at the brain stem/pontine level.

The hypothesis that brain stem dysfunction plays a role in the pathogenesis of ASCs, has been put forward since the 1960s when it was shown that postrotatory nystagmus was often lacking in autism (Ritvo et al. 1969). Early embryonic brain stem damage was suggested to be at the root of such dysfunction by findings in the early 1990s from Gillberg's group that exposure to thalidomide during neural tube closure (the earliest stages of brain development) increases the risk of ASCs (Strömland et al. 1994). Möbius, CHARGE and OAV are considered to arise during early embryonic development, around the 4th to 6th gestational weeks (Miller et al. 2005). This, together with the occurrence of cranial nerve

palsies and ASCs in all these BPCs might be suggestive of early insults affecting brain stem structures, which in term may secondarily involve higher cortical centers not yet formed, in which functions known to be impaired in autism are located.

The most commonly proposed pathogenesis in Möbius is a local destructive process of brain stem nuclei. Verzijl et al. (2005 d) pointed out the distinction between hereditary congenital facial palsy (described as a usually autosomal dominant disorder characterized by isolated congenital facial palsy and in some instances deafness and hypoplasia of the facial motor nuclei and nerves), and Möbius syndrome (described as a usually sporadically occurring developmental disorder of the rhombencephalon, characterized by congenital facial palsy, impairment of ocular abduction, a range of other congenital defects, and complex anomalies of the posterior fossa with hypoplasia of the entire brain stem including the traversing long tracts). Clinical, radiological and electrophysiological studies of individuals with Möbius have yielded evidence for defects at three levels (supranuclear, nuclear and peripheral) (Verzijl et al. 2003, 2005a, b, d). Abnormal lacrimation (lack of emotional tearing, tearing when eating or booth) was recorded in seven of our subjects with Möbius sequence. Abnormal tearing is considered to be caused by aberrant innervation of the lacrimal gland, which during early embryonic stages is anatomically close to the sixth and seventh cranial nerve nuclei in the brain stem.

Even if there are some reports of brain stem malformations in CHARGE/OAV (Issekutz et al. 2005, Pane et al. 2005), there is not as much radiological evidence that lesions at the level of the brain stem underlie cranial nerve palsies in these conditions. In our CHARGE and OAV groups, facial palsy was always unilateral and affecting both upper and lower parts of the face. Further, anomalous course of/difficulty to identify the facial nerve canal were recorded in most examined patients in the CHARGE/OAV groups. Facial palsy ipsilateral to the more severely affected side of the face in the vast majority of the individuals with facial palsy was documented in the OAV group. All these findings point towards nuclear or more peripheral origin. Bassila and Goldberg (1989) reviewed 50 cases of HFM and described facial palsy on the more hypoplastic side of the face in all but one case. Abnormal tearing was not recorded in any individuals with CHARGE/OAV. Thus, the evidence for brain stem damage in CHARGE/OAV was weaker than in Möbius, and the data that exist suggest damage in the lower portion of the brain stem (at the nuclear level) or more peripherally in the cranial nerves themselves.

However, there are *clinical* indications in CHARGE which might reflect brain stem dysfunction, abnormal autonomic response to stress and cortical vision impairment (thermal dysregulation, sucking/swallowing difficulties, periods of altered consciousness, fluctuating hearing/vision, “spells” with slowed heart/respiratory rate, facial rash/red watery eyes/fever in response to stress, cool clammy skin) (Lauger et al 2005, Sanlaville and Verloes 2007). Tellier et al. (1998) stressed the signs of complex dysregulation of brain stem functions (cranial nerve palsies, feeding difficulties, pharyngo-oesophageal dysmotility, respiratory problems, vagal overactivity) in neonates and infants with CHARGE. Anomalies of cranial and CNS midline structures, hypophyseal disorders, and brain stem dysfunction have been emphasized as emerging problems hidden beyond the historical cardinal features in CHARGE (Verloes 2005). Rhombencephalic maldevelopment (brain stem dysfunctions, cranial nerve VII to XII palsies, neurosensory deafness) and hypothalamo-hypophyseal dysfunction (including growth hormone/gonadotropin deficiencies) were included in the inclusion criteria suggested by Verloes (2005).

Considering the frequent finding, especially in individuals with ASCs, of forebrain midline anomalies in our CHARGE group, the pineal-hypothalamus-pituitary-adrenal axis, might be considered in the pathogeneses of ASCs in CHARGE (Chamberlain and Herman 1990). Ek et al. (2005) reported AD and ALC in blind children with bilateral optic nerve hypoplasia and normal intelligence, of whom all had neuroimaging/hormonal evidence of midline malformations. In this context, it may be worth noting that short stature, which is one of the characteristics in CHARGE but previously only rarely reported in OAV (Sensi et al. 1996), was a frequent finding in our study, not only in the subjects with CHARGE, but also in those with OAV. It might also be worth noting that some individuals with CHARGE and OAV, of whom several had ASCs, had abnormalities of N. Opticus/retrogeniculate visual pathways or were described to show some, seemingly abnormal autonomic symptoms, such as profuse perspiration.

The disturbances of balance recorded in CHARGE are generally considered to be caused by vestibular dysfunction (Jongmans et al. 2006). Atactic gait was common among the subjects with “disturbances of balance” in our CHARGE group, possibly indicating cerebellar or higher cerebral involvement, especially since some of the patients with impaired balance in our study had no indication on hearing/vestibular impairment.

Possible interplay between genes, environmental factors and ASCs in these BPCs
Summarizing the reported adverse pregnancy events and family factors in our BPC samples, no common etiologic factors were found, even if there were some indications of possible risk factors. The findings in some subjects of maternal bleedings (mostly in early pregnancy), a history of more than one previous spontaneous abortion, assisted reproductive technology (ART), twinning, and family history indicative of malformations of the type seen in these BPCs, and of neuropsychiatric disturbances, may be suggestive of some kind of predisposition or vulnerability. However, the significance of such factors is uncertain, given that they all occur in the general population. Maternal bleedings have been noted in about 20% of all pregnancies (Williams et al.1991), and more than one previous spontaneous abortion in 3% in a control series (Hagberg and Fernell 1988). At present, 3% of children born in Sweden are the product of in vitro fertilization (Finnström and Nygren 2005). The rate of spontaneous monozygotic twinning in live births is about 1 in 330 (Hall 2003), and of dizygotic twinning 1 in 100. Nevertheless, maternal bleedings have been reported to be significantly associated with autism (Gillberg and Coleman 2002), and there is an increased frequency of twins among affected siblings with autism, especially monozygotic twins (Betancur et al 2002).

Some of the recorded adverse pregnancy events may be regarded in the context of "environmental risk factors", albeit of uncertain significance. Daily maternal smoking during early pregnancy was reported to be linked to autism in a large case-control epidemiology study (Hultman et al. 2002). The increase in anomalies and imprinting defects after in vitro fertilization, described by some authors, may be caused by the ART technique itself. In Möbius and OAV both familial occurrence and teratogenic factors have been implicated, but no common genetic abnormality has yet been revealed. The CHD7 gene has been shown to explain most but not all cases of CHARGE, leaving the possibility that there exists a "broader phenocopy" which might be caused by other gene defects and/or environmental factors. Thus, documentation of possible prenatal, perinatal and family factors, including neuropsychiatric history, may help clarify the role of genetic/epigenetic predisposition as well as environmental factors in these BPCs.

It would be particularly interesting to know whether CHD7-abnormality in CHARGE is specifically related to the occurrence of autism symptoms. In early embryos of chicken the ortholog of the human CHD7 gene has been shown to be expressed in the neural tube, followed by expression in the telencephalon, diencephalon, mesencepahlon, metencaphalon, myelencepahlon, optic and otic primordiums (Aramaki et al. 2006). If CHD7-abnormality is linked to autism symptomatology in CHARGE, the animal studies might help guide the examination of the chain of events that underlie the development of autism in humans.

CONCLUSIONS

ASCs were very common in our Möbius, CHARGE and OAV samples. More than half of the subjects with CHARGE and one third of those with Möbius and OAV were affected. Classic AD was most common in the Möbius group, affecting 27% of individuals the individuals compared with 20% of those with CHARGE and 11% of those with OAV. Many individuals diagnosed with ASCs in our Möbius/CHARGE/OAV subgroups exhibited severe behaviour disturbances, often with major impact on family life. Awareness of the coexistence of ASCs in Möbius/CHARGE/OAV is important in the habilitation of those individuals. It is inappropriate in many cases to attribute the autism symptoms to the BPCs, and to claim that there is no need for a separate diagnosis of ASC. Many of the individuals seen in the present study had not been given appropriate educational "autism-friendly" interventions, until the separate diagnosis of ASCs had been made.

LD was a frequent finding. Most individuals with severe ASCs had LD, and vice versa, possibly reflecting the link between ASCs and LD.

Visual and hearing impairments were very common in individuals with CHARGE/OAV; in both groups the level of autistic symptomatology exhibited increased with the degree of visual and hearing impairment. However, the frequent occurrence of cerebral anomalies in individuals with ASCs in these groups indicated that ASCs would be unlikely to be attributable to sensory impairments "alone".

Cranial nerve involvement occurred in all individuals with Möbius and somewhat more than half of those with CHARGE/OAV.

The more specific association between AD and Möbius than between AD and the other two BPCs, and the fact that there was no association between sensory deprivation and AD in the Möbius group, seems to contradict a causal connection between sensory deprivation and autism.

The diagnosis of ASCs in all three BPCs studied was complicated by additional disabilities, notably sensory impairments, cranial nerve dysfunction, and LD. The diagnostic difficulties increased with the number and severity of associated disabilities. Use of an extensive battery of diagnostic instruments, including both observational schedules and parental interviews, is essential in the diagnostics of ASCs in individuals with multiple disabilities. Current autism diagnostic instruments are insufficiently tailored to deaf-blind individuals. There is a need for further development of such instruments, which are more firmly rooted in knowledge of normal/deviant development in children with sensory impairments.

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