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Ismo Makkonen

Childhood Autism

Aspects of Growth Factors and Monoaminergic Transporters in Etiopathogenesis

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ABSTRACT

Autism is a neuropsychiatric developmental disorder in which there is a dysfunction in social interaction and communication, and a combination of repetitive and stereotypic behavior. Disturbances in growth factors and monoaminergic neurotransmitter system have been postulated in autism. Selective serotonin reuptake inhibitors (SSRIs) have shown favorable effects in autism, and effects on growth factors elsewhere, but the relationships between these elements have not been investigated.

Prior to treatment, head circumference (HC) was registered and insulin-like growth factor-1 (IGF-1) was measured in cerebrospinal fluid (CSF) in 25 children with autism, aged 2 to 16 years. Serum brain derived neurotrophic factor (BDNF) was analyzed and serotonin transporter (SERT) and dopamine transporter (DAT) binding were determined by single photon emission computed tomography (SPECT) in 15 autistic children, aged 5 to 16 years. Control groups were composed of 16 and 10 age-matched children without autism, aged 1 to 15 years and 7 to 14 years, respectively.

Six-months' treatment with fluoxetine, an SSRI drug, was provided to 13 autistic children and symptoms were followed up using Autism Treatment Evaluation Checklist. The prior to treatment procedures were repeated 2 months after termination of treatment.

Before treatment, CSF-IGF-1 concentration was lower in children with autism under 5 years of age ($p=0.014$) than in controls. A positive correlation was detected between CSF-IGF-1 and HC in autistic children ($p=0.014$). BDNF displayed a bimodal distribution in autistic individuals; concentrations were very low or high as compared with controls.

At baseline, SERT binding capacity in SPECT was lower in autistic children in medial frontal cortex ($p=0.002$). Striatal DAT binding capacity was highest in the youngest autistic children, and then decreased with age, but in controls binding capacity increased with age.

Fluoxetine elicited positive effects in communicative skills, sociability, and sensory awareness, particularly in six autistic children. These good responders exhibited a decrease in BDNF ($p=0.03$) as well as in striatal DAT binding ($p=0.03$). CSF-IGF-1 increased ($p=0.003$) but no correlation was detected with rates of clinical response. Fluoxetine was well tolerated.

In conclusion, fluoxetine seems to modulate IGF-1, BDNF and the monoaminergic system, which are altered in autism. Fluoxetine could represent a useful adjunct therapy to conventional communication and behavioral therapies for children with autism.

National Library of Medical Classification: WS 350.8.P4, QU 107, QU 55.2, QV 126
Medical Subject Headings: Autistic Disorder; Brain-Derived Neurotrophic Factor; Child; Dopamine Plasma Membrane Transport Proteins; Fluoxetine; Insulin-Like Growth Factor I; Serotonin Plasma Membrane Transport Proteins

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TIIVISTELMÄ

Autismi on neuropsykiatrinen kehityksellinen häiriö, joka ilmenee sosiaalisen vuorovaikutuksen ja kommunikoinnin poikkeavuutena sekä toistavana ja kaavamaisena käyttäytymisenä. Autismiin on esitetty liittyvän kasvutekijöiden ja monoaminergisen välittäjäainejärjestelmän häiriöitä. Selektiivisten serotoniinin takaisinoton estäjien (SSRI-lääkkeet) hyödystä autismissa on raportoitu ja havaittu niiden vaikuttavan kasvutekijöihin, mutta näiden seikkojen välisiä yhteyksiä ei ole tutkittu.

Ennen hoitoa mitattiin päänympärysmitta (HC) sekä määritettiin insuliininkaltaisen kasvutekijä-1:n (IGF-1) pitoisuus selkäydinnesteessä (CSF) 25:ltä 2-16 vuotiaalta autistiselta lapselta. Seerumin aivoperäisen hermokasvutekijän (BDNF) pitoisuus määritettiin ja serotoniinin kuljettajaproteiinin (SERT) ja dopamiinin kuljettajaproteiinin (DAT) sitomiskapasitetti mitattiin yksifotoniemissiotomografiaa (SPECT) käyttäen 15 autistiselta lapselta. Kontrolliryhmiin otettiin 16 ja 10 vastaavanikäistä ei-autistista lasta.

Kuuden kuukauden fluoksetiinihoito (SSRI-lääke) toteutettiin 13 autistiselle lapselle joiden oireita seurattiin Autism Treatment Evaluation Checklist–menetelmällä. Ennen hoitoa tehdyt tutkimukset uusittiin kaksi kuukautta hoidon päättymisen jälkeen.

Ennen hoitoa CSF-IGF-1 oli alhaisempi alle 5-vuotiailla autistisilla lapsilla ($p=0.014$) kontrolleihin verrattuna. Autistisilla lapsilla havaittiin CSF-IGF-1:n ja HC:n välillä riippuvuus ($p=0.014$). BDNF-pitoisuudet jakautuivat autisteilla kaksihuippuisesti; pitoisuudet olivat joko hyvin matalat tai korkeat verrattuina kontrolleihin.

Ennen hoitoa SERT sitomiskapasiteetti oli autistisilla lapsilla matala otsalohkon sisemmällä kuorikerroksella ($p=0.002$). Striatumin alueella DAT sitomiskapasiteetti oli korkea nuorimmilla autistisilla lapsilla ja se väheni iän myötä. Kontrolleilla DAT sitomiskapasiteetti lisääntyi iän myötä.

Fluoksetiinihoidon myötä todettiin suotuisia vaikutuksia kommunikaatiotaidoissa, sosiaalisuudessa sekä sensorisessa tietoisuudessa. Vaikutus oli erityisen selvä kuudella autistisella lapsella; heillä BDNF-pitoisuus aleni ($p=0.03$) ja striatumin DAT sitomiskapasiteetin väheni ($p=0.03$). Fluoksetiini lisäsi CSF-IGF-1-pitoisuuksia ($p=0.003$), mutta riippuvuutta kliiniseen vasteeseen ei havaittu. Fluoksetiinihoito oli hyvin siedetty.

Fluoksetiini näyttää siis muokkaavan IGF-1:a, BDNF:a sekä monoaminergistä järjestelmää, joiden toiminta on muuttunut autismissa ja se saattaisi olla hyödyllinen lisä autististen lasten perinteisissä kommunikaatio- ja käyttäytymisterapioissa.

Luokitus: WS 350.8.P4, QU 107, QU 55.2, QV 126
Yleinen suomalainen asiasanasto: autismi; kasvutekijät; lapset

To Riitta, Katri-Kanerva and Aleksi

The effort is to think independently, or at least individually, in the endeavor to discover new truth, or to make new combinations of truth, or at least to develop an individualized aggregation of truth.

T.C.Chamberlin, 1890

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List of the original publications

This dissertation is based on the following original publications:

I Riikonen R, Makkonen I, Vanhala R, Turpeinen U, Kuikka J, Kokki H. Cerebrospinal fluid insulin-like growth factors IGF-1 and IGF-2 in infantile autism. *Dev Med Child Neurol 48:751-755, 2006.*

II Makkonen I, Riikonen R , Kokki H, Airaksinen MM, and Kuikka JT, Serotonin and dopamine transporter binding in children with autism determined by SPECT. *Dev Med Child Neurol 50:593–597, 2008.*

III Makkonen I, Riikonen R, Kuikka JT, Kokki H, Bressler J, Marshall C, Kaufmann WE. Brain derived neurotrophic factor and serotonin transporter binding as markers of clinical response to fluoxetine therapy in children with autism. *J Pediatr Neurol 9:1-8, 2011.*

IV Makkonen I, Kokki H, Kuikka J, Turpeinen U, Riikonen R. Effects of Fluoxetine Treatment on Striatal Dopamine Transporter binding and Cerebrospinal Fluid Insulin-Like Growth Factor-1 in Children with Autism. *Neuropediatrics 42:207-209, 2011.*

Contents

Abbreviations

ADHD	attention deficit hyperactivity disorder
ASD	autism spectrum disorder
ATEC	Autism Treatment Evaluation Checklist
BDNF	brain derived neurotrophic factor
CARS	Childhood Autism Rating Scale
CSF	cerebrospinal fluid
DAT	dopamine transporter
DSM	Diagnostic and Statistical Manual of Mental Disorders
HC	head circumference
ICD	International Classification of Diseases
IGF-1	insulin-like growth factor-1
IGF-2	insulin-like growth factor-2
IQ	intelligence quotient
MFC	medial frontal cortex
MRI	magnetic resonance imaging
PDD-NOS	pervasive developmental disorder – not otherwise specified
PET	positron emission tomography
SD	standard deviation
SERT	serotonin transporter
SPECT	single photon emission computed tomography
SSRI	selective serotonin reuptake inhibitor
TrkB	tyrosine kinase B

1 Introduction

Autism is a neuropsychiatric developmental disorder characterized by a core impairment in social interaction, abnormalities in communication, and a markedly restricted, repetitive and stereotyped behavior and repertoire of interests (World Health Organization, 1993, American Psychiatric Association, 2000). According to the diagnostic criteria of childhood autism, the clinical symptoms should appear by the age of three years. The first clinical symptoms and signs include typically disturbance in eye contact and facial expressions, and a delay in vocalizing (babbling) and using communicative speech. The parents and other caregivers become usually aware of the aberrant development during the child´s second year, but abnormal signs can often be recalled from the first year of life. There is a subgroup of autistic infants who first develop social and communicative skills with normal milestones, but then go through an autistic regression and become withdrawn and do not progress as normal.

Autism is currently considered as a continuum of pervasive developmental disorders, the autism spectrum disorder (ASD), rather than a constellation of separate entities. The etiology of autism is obscure but a heterogeneous concept with divergent backgrounds and relations, with alterations in genetic, neuropathological, neurophysiologic, immunologic and behavioral systems have been suggested (Muller, 2007). The impact of genetic factors is acknowledged based on family and twin studies (Folstein and Rutter, 1977), and on an overexpression of autism in several genetic syndromes (Miles, 2011).

Intrauterine and environmental conditions during pregnancy have been suspected since an increased risk for ASD has been detected in twins born with an affected co-twin, as compared with the risk for ASD in siblings born from separate pregnancies (Rosenberg et al., 2009). However, no evidence of any consistent perinatal or neonatal factor has been implicated with an elevated risk of autism (Gardener et al., 2011).

Abnormalities in brain growth patterns and neuropathological structures of brain have been reported in autistic individuals (Courchesne et al., 2003 and 2011). A diminished number of the cerebellar Purkinje cells have been found in post-mortem studies in autism. This suggests that the perturbation in the coordinating and inhibiting role of cerebellum may have an important impact in the pathogenesis of autism (Bauman and Kemper, 2005).

In autism, neurotransmitters, and especially the serotonin system have been an object of interest. Serotonin acts as a transmitter in the mature brain, but it is also a growth factor and a neuronal modulator in the prenatal and postnatal development of neuronal networks (Whitaker-Azmitia, 2001). The earliest observation in the 1960's of a possible dysfunction concerning serotonin in autism was can be traced in the detection of hyperserotonemia in one third of autistic subjects (Schain and Freedman, 1961).

Pharmacological agents with target on serotonin, including the selective serotonin reuptake inhibitors (SSRIs) have been used to relieve symptoms in several psychiatric and neuropsychiatric disorders, including autism (Kolevzon et al., 2006). The connections between the SSRIs and neurotrophins, the growth factors regulating the neuronal survival, differentiation and synapse formation, have been detected (Hodes et al., 2010, Aleman and Torres-Aleman, 2009). The effect of SSRIs in the behavioral features in autistic children has been studied (McDougle et al., 1996, DeLong et al., 2002, Hollander et al., 2005, King et al., 2009) but the influence on the neurotrophins has not been elucidated in autism.

The aims of the present study were to measure the neuronal growth factors (IGF-1, IGF-

2 and BDNF) and the monoaminergic neurotransmission system, serotonin transporter (SERT) and dopamine transporter (DAT), and to evaluate the effects of fluoxetine, an SSRI drug, in the above-mentioned factors and the clinical picture of autistic symptoms and behavior in a group of autistic children aged between 2 and 16 years.

2 Review of the literature

2.1 HISTORICAL ASPECTS AND DIAGNOSTIC CRITERIA OF AUTISM

2.1.1 History

The autistic disorder was first described by the Austrian born, American psychiatrist Leo Kanner (1943). In his visionary paper describing 11 pediatric patients with "autistic disturbances of affective contact", Kanner depicted out a spectrum of behavioral manifestations and diverse presence of these children. Kanner adopted the term "early infantile autism" to underline the fact that the origins of this disorder develop early in the infancy. Kanner suspected that pathology in the personality of the parents – especially emotional coldness of the mothers – would be responsible for the child´s development to become autistic (Kanner, 1949) and this impression lived for decades on.

In general, autism was considered as a part of a schizophrenic process in children (Cappon, 1953). In the diagnostic descriptions and classifications of diseases, autism was classified as a variation of childhood schizophrenia or atypical psychosis until for the first time it appeared as a distinct medical entity in the International Classification of Diseases (ICD) 9th revision published by the World Health Organization in 1979. ICD is a manual widely used in Europe, including Finland. The corresponding American manual, the Diagnostic and Statistical Manual of Mental Disorders (DSM) published by the American Psychiatric Association introduced autism as a separate disease in its 3rd revised version (DSM-III) in 1980.

In the most recent updates of these manuals, ICD-10 Classification of Mental and Behavioural Disorders: Diagnostic Criteria for Research in 1993 and DSM-IV Text Revision in 2000, the concept of autism was broadened to autism spectrum disorder (ASD), including childhood autism, Asperger syndrome, Rett syndrome, childhood disintegrative disorder, and pervasive developmental disorder not otherwise specified.

2.1.2 Diagnostic criteria and diagnostic tools in autism

There is no biochemical marker for autism or autism spectrum disorders. The diagnosis is entirely depending on the clinical symptoms and signs detected during the development of the child. The diagnostic criteria have three main domains; the functional impairments in social interaction, communication and behavior. The ICD-10 criteria for childhood autism are presented in Table 2.1.

The diagnostic criteria provide a base for a broad spectrum of diverse phenotypes; the patients may differ widely in the degree of impairment in the core symptoms, and also in aspects of their intellectual capacity and adaptive abilities. Furthermore, the individual manifestation may vary during the development from infancy to adulthood.

The diagnostic boundaries between the different autism spectrum disorders are defined by timing in the onset of symptoms, and the qualitative contents of the symptoms. The criterion separating most clearly childhood autism from Asperger syndrome is the development of language; in childhood autism there is a severe delay in the development

of spoken language before the age of 3 years, but in Asperger syndrome spoken or receptive language developes normally during the early years. Secondly, in childhood the cognitive development has a wide variation from severe mental retardation to high intelligence levels, but for criteria of Asperger syndrome, no general delay in cognition is allowed. There is a subgroup of ASD patients with regression in language and/or other communication skills after the age of 3 years or a repertoire of symptoms that do not completely fit the criteria of either autism or Asperger syndrome either, and this population is defined to present atypical autism or pervasive developmental delay not otherwise specified.

The most commonly used tests in clinical evaluation of childhood autism include Childhood Autism Rating Scale (CARS) (Schopler et al., 1988), Autism Diagnostic Interview –Revised (Lord et al., 1994), and Autism Diagnostic Observation Schedule (Lord et al., 2000). These tests are used by professionals.

In addition, there are tests, especially made for searching subjects with Asperger's syndrome or individuals with autistic traits in the general population, including Childhood Autism Spectrum Test or Childhood Asperger Screening test as it was called formerly (Williams et al., 2006), Asperger Syndrome Screening Questionnaire (Ehlers et al., 1999), and the Autism Spectrum Quotient (Baron-Cohen et al., 2001), typically used as screening tools, not as real diagnostic instruments.

The separation of entities in ASD may be difficult to determine in clinical practice: the diagnostic category the individual best represents may change over time. There has been discussion in the literature that especially the line is imaginary between the autistic patients with no cognitive developmental delay (the high functioning autism), and those with Asperger syndrome, despite the differences in their early language skills (Noterdaeme et al., 2010).

There has been proposal for the next revisions of DSM-5, scheduled in 2013, and ICD-11, scheduled in 2015, to remove the definitions between the subgroups and diagnose ASD as only a single entity (www.dsm5.org). However, there is no consensus in this and there is an on-going discussion (Wing et al., 2011, Mattila et al., 2011).

Table 2.1. International Classification of Diseases 10th revision (ICD-10) Criteria for Childhood Autism (F84.0).

A. Abnormal or impaired development is evident before the age of 3 years in at least one of the following areas:

1. receptive or expressive language as used in social communication;
2. the development of selective social attachments or of reciprocal social interaction;
3. functional or symbolic play.

B. A total of at least six symptoms from (1), (2) and (3) must be present, with at least two from (1) and at least one from each of (2) and (3)

1. Qualitative impairment in social interaction are manifest in at least two of the following areas:
   a. failure adequately to use eye-to-eye gaze, facial expression, body postures, and gestures to regulate social interaction;

b. failure to develop (in a manner appropriate to mental age, and despite ample opportunities) peer relationships that involve a mutual sharing of interests, activities and emotions;
c. lack of socio-emotional reciprocity as shown by an impaired or deviant response to other people's emotions; or lack of modulation of behavior according to social context; or a weak integration of social, emotional, and communicative behaviors;
d. lack of spontaneous seeking to share enjoyment, interests, or achievements with other people (e.g. a lack of showing, bringing, or pointing out to other people objects of interest to the individual).

2. Qualitative abnormalities in communication as manifest in at least one of the following areas:
a. delay in or total lack of, development of spoken language that is not accompanied by an attempt to compensate through the use of gestures or mime as an alternative mode of communication (often preceded by a lack of communicative babbling);
b. relative failure to initiate or sustain conversational interchange (at whatever level of language skill is present), in which there is reciprocal responsiveness to the communications of the other person;
c. stereotyped and repetitive use of language or idiosyncratic use of words or phrases;
d. lack of varied spontaneous make-believe play or (when young) social imitative play

3. Restricted, repetitive, and stereotyped patterns of behavior, interests, and activities are manifested in at least one of the following:
a. An encompassing preoccupation with one or more stereotyped and restricted patterns of interest that are abnormal in content or focus; or one or more interests that are abnormal in their intensity and circumscribed nature though not in their content or focus;
b. Apparently compulsive adherence to specific, nonfunctional routines or rituals;
c. Stereotyped and repetitive motor mannerisms that involve either hand or finger flapping or twisting or complex whole body movements;
d. Preoccupations with part-objects of non-functional elements of play materials (such as their odor, the feel of their surface, or the noise or vibration they generate).

C. The clinical picture is not attributable to the other varieties of pervasive developmental disorders; specific development disorder of receptive language (F80.2) with secondary socio-emotional problems, reactive attachment disorder (F94.1) or disinhibited attachment disorder (F94.2); mental retardation (F70-F72) with some associated emotional or behavioral disorders; schizophrenia (F20) of unusually early onset; and Rett Syndrome (F84.12).

2.2 EPIDEMIOLOGY

The prevalence of childhood autism or ASD has been inconsistent in reports from different populations and at different times. The changing diagnostic criteria (or differences in interpretation and impression of the criteria) in different time periods have made it difficult to compare the results across the studies.

In early epidemiological studies up to 1990's, a prevalence rate of childhood autism was concluded to be from 4 to 5 per 10,000 children in reviews by Fombonne (1996) and Gillberg and Wing (1999). In his review, Fombonne (1996) estimated the minimum

prevalence of ASD as 20 in 10,000 children. Since then, the prevalence has been elevated in most reports, and in a review only a few years later (Charman, 2002) the prevalence of autism was reported to rise up to 40 in 10,000, and prevalence of all ASDs up to 70 in 10,000 children. The gender distribution was highly male-dominated; 73 to 88 percent of all ASD patients were males (Charman, 2002). In Sweden, in 1999 Kadesjö et al. reported a prevalence of ASD to be 1.2% in a cohort of 826 children born in 1985, at age of 7 years. All the 10 children with ASD (5 with autistic disorder, 4 with Asperger syndrome, and 1 with autistic like condition) were assessed as needing special education or personal assistants in normal class-rooms.

In Finland, the prevalence of autism was first published by Kielinen et al. (2000) presenting the prevalence of autism in the population of the Northern Finland born from 1979 to 1994. They reported the prevalence of "classic autism" 5.6 in 10,000 and that of "autism and autism-like syndromes" 14 in 10,000. The prevalence in boys was four times higher than that in the girls. The degree of autism was assessed by CARS and notably high proportion of the subjects had moderate or severe autistic features, 59% and 33% respectively (Kielinen et al., 2000).

In a preliminary report based on national hospital registers and concerning the complete Finnish population born between the years 1987 and 2005, a prevalence of 10 in 10,000 for childhood autism and 46 in 10,000 with ASD was estimated (Lampi et al., 2011). A 4:1 male-female distribution for childhood autism and for total ASD was detected in that study. In patients with Asperger syndrome, the male-domination is even greater: 5 males for 1 female (Lampi et al., 2011).

In a recently published epidemiological study targeting all 8-year-old children (n = 5,484) born in 1992 and living in a defined area of Northern Finland, reported the prevalence of ASD as 84 in 10,000 and that of autism 41 in 10,000 according to DSM-IV Text Revision criteria (Mattila et al., 2011).

In another Nordic country, Denmark, the prevalence for childhood autism in children younger than 10 years has been reported to be 12 in 10,000, and the prevalence for ASD as 35 in 10,000 (Lauritsen et al., 2004). In this report almost half of the ASD prevalence, 18 in 10,000, comprised of individuals with atypical autism or pervasive developmental disorder –not otherwise specified.

In the United States of America, a repetitive surveillance concerning children aged 8 years has reported the prevalence of ASD to have increased from 57 in 10,000 subjects in 2000 up to 90 in 10,000 subjects in 2006 (Centers for Disease Control and Prevention , 2007 and 2009). The Centers for Disease Control and Prevention reports did not separate the ASD cases more closely. Another recent report in the USA comparing the trends in developmental disorders in children aged 3-17 years elicited that prevalence in ASD had become more than threefold between the years 1997 to 2008, an increase from 19 to 74 per 10,000 individuals (Boyle et al., 2011). In the last-mentioned report (Boyle et al., 2011), the male-dominance in ASD was 3 to 5 for 1 female, in different 3-year age cohorts.

There are only a few epidemiological studies providing detailed information about the separate entities in ASD or comparing the epidemiology of autism with other psychiatric or neuropsychiatric disorders in children. A Danish cohort study published in 2007 discovered an increase in the prevalence in ADHD, Tourette syndrome and obsessive-compulsive disorder in childhood altogether, not simply increase in autism or ASD (Atladottir et al., 2007). In Massachusetts birth cohorts 2001-2005 the prevalence of the "classic" childhood autism remained the same (approx. 20 in 10,000) while the proportion of unspecified autism and PDD-NOS increased clearly (from 44 to 70 in 10,000) (Manning

et al., 2011), reaching a total ASD prevalence of 90 per 10,000 equal to the USA nationwide report in 2006 (Centers for Disease Control and Prevention, 2009).

In a British survey of children aged 5-9 years, a total prevalence of ASD was 113 in 10,000 children (Baron-Cohen et al., 2009). Interestingly, the researchers reported that during the study, they could detect 2 "new" cases meeting the ASD criteria for every 3 earlier diagnosed subjects. Two-thirds of the "new" ASD cases were Asperger syndrome or high-functioning autism, the distribution of subgroups in the earlier diagnosed ASD cases were not reported.

The increase in the number of reported diagnoses of autism, or ASD, has been under discussion during the past decades. Several factors are believed to be involved in this increase; improved awareness both in parents and in professionals in being able to recognize and detect autistic symptoms, as well as an increase in availability of diagnostic services might be responsible for part of the increase. In addition, since there are more broad diagnostic criteria in use, and accepting autism as a co-morbid condition with other medical conditions may explain the increasing and varying figures of prevalence in different studies (Baron-Cohen et al., 2009). It has also been stated that the differential diagnosis between the presentations of autistic traits in the general population and the diagnostic criteria for ASD can be unclear. The definition of the severity of the symptoms or the behavior interfering the daily functioning are dependent on the environmental demands. As the most recent studies from the United Kingdom and the USA indicate, the increase in ASD is mostly at the "milder" end of the spectrum, including Asperger syndrome, high-functioning autism, and pervasive developmental disorder not otherwise specified (Baron-Cohen et al., 2009, Manning et al., 2011). However, in spite of these explanations, some part of the increase in ASD prevalence remains unexplained and will require clarification.

2.3 GENETIC FACTORS IN AUTISM

The impact of genetics on autism has long been acknowledged based on several studies showing increased incidence of childhood autism and ASD in family members of the index person. According to a recent review, twin concordance in monozygotic twins has varied between 36 to 95%, and in dizygotic twins between 0 to 31% in different studies (Ronald and Hoekstra, 2011). The largest twin study so far published consisted of 67 monozygotic and 120 dizygotic twin pairs, reported a pairwise autistic spectrum concordance of 88% for monozygotic twins and 31% for dizygotic twins (Rosenberg et al., 2009). It was notable that the pairwise concordance was higher in male-male dizygotic twins than in female-female pairs. However, there was ASD concordance in all 9 female-female monozygotic pairs in that study.

The risk for ASD in siblings in a family with a child with autism or ASD has been reported between 3 to 10% (Chakrabarti and Fombonne, 2001, Lauritsen et al 2004). The stoppage effect, i.e. having no more children after ASD has been detected in one child may affect the overall risk in siblings. In a recent study, a higher risk of 19% of ASD recurrence in later-born siblings was detected, and the risk was higher in male (26%) than in female (9%) siblings (Ozonoff et al., 2011).

The increased prevalence of autistic traits or the broader autistic phenotype without the

diagnostic criteria being fulfilled has been detected in siblings, parents or other relatives of ASD patients (Ruta et al., 2012).

More than 100 disease genes and over 40 genomic loci have been linked with an increased risk of ASD (Betancur, 2011). The association between autism and fragile X syndrome was noticed already in the 1980´s (Brown et al., 1982). The genetic background for fragile X syndrome is a defect in fragile X mental retardation protein coding gene, the FMR1 gene (DeVries et al., 1998). Through FMR1 gene it has been possible to generate the first experimental animal model of autism, the FMR1 knockout mice (Dutch-Belgian Fragile X Consortium, 1994).

In fragile X syndrome, up to 60% of patients have been reported to meet the ASD criteria. Although it is the leading known cause of autism, fragile X syndrome comprises a mere 2% of all ASD patients. In tuberous sclerosis complex, the prevalence of co-morbid ASD is up to 90% in mentally retarded patients but less than 20% in patients with normal intelligence. The reason behind this discrepancy remains unclear (Abrahams and Geschwind, 2008).

Rett syndrome, a developmental disorder affecting almost exclusively females, is included in the autism spectrum in ICD-10 and DSM-IV. Rett syndrome was linked with mutations in the X-linked methyl CpG binding protein 2 (MeCP2) gene (Amir et al., 1999)

In Prader-Willi and Angelman syndromes, which are both affected by disequilibrium of the genes in 15q11-13 locus, the prevalence of ASD has been reported to be increased (Veltman et al., 2005). The risk of ASD seems to be higher (25%) in individuals Prader-Willi syndrome, and maternal uniparental disomy, or partial paternal deletion of the affected loci, than the risk of ASD (2%) in Angelman syndrome with paternal uniparental disomy or partial maternal deletion, respectively (Veltman et al., 20005). Interestingly, an overlap in the gene regulation pathways has been suggested in cases of Angelman syndrome, Rett syndrome, and (non-syndromic) autism (Jedele, 2007).

It has been postulated that the neurobiological defect in autism could be considered as a synapsopathy as the deficient genes in fragile X syndrome, Rett syndrome, Angelman syndrome, and tuberous sclerosis have been associated with protein synthesis and neuronal plasticity in the synapse. Several single autistic candidate genes without syndromic endophenotypes have also proposed to influence synaptic function; with Neuroligins 1-4, Neurexin 1, and SH3 and multiple ankyrin repeat domains 3 (SHANK3) being the most relevant (Ylisaukko-oja et al., 2005, Dolen and Bear, 2009).

There are several metabolic conditions in which there are increased prevalences of ASD. Mitochondrial disorders have been identified in selected autistic subgroups with concomitant atypical features as failure to thrive, epilepsy or intermittent episodes of regression (Haas, 2010). Inborn errors in creatine metabolism have been linked with an increased incidence of autistic symptoms, although mental retardation and seizures usually dominate the clinical picture (Newmeyer et al., 2007). Smith-Lemli-Opitz syndrome, a disorder in cholesterol metabolism, has a reported rate of ASD of up to 75%, but no correlation between the abnormal metabolite concentrations and autism severity has been detected (Sikora et al., 2006).

ASD patients with a “complex” phenotype, including dysmorphic features, microcephaly or alteration of early morphogenesis, are believed to be more likely, in approximately 25% of those individuals one finds, an autism associated syndrome or chromosome disorder in comparison with individuals with “essential” autism without the abovementioned features (Miles, 2011). It has also been stated that there are differences in the sex ratio, recurrence risk and family history between the complex (or “syndromic”)

autism and the essential autism, the latter having a higher male predominance, higher occurrence risk in siblings, and a greater likelihood of a family history of ASD (Miles, 2011).

Furthermore there are numerous gene loci variations listed as displaying an elevated frequency in autistic subjects, without any common syndrome features. These include variants in serotonin transporter gene alleles (Prasad et al., 2009) and BDNF gene polymorphism (Cheng et al., 2009).

2.4 ABNORMALITIES IN BRAIN GROWTH AND STRUCTURE

Already in his initial article Kanner (1943) mentioned that five of the examined 11 children had relatively large heads. Thereafter between 10 to 20% of autistic individuals have been reported to have macrocephalia, i.e. HC more than 2 standard deviations (SD) above average, and the macrocephaly trait has been observed also in the non-autistic family members (Miles et al., 2000).

However, in the neonates with later ASD, HC at birth has been normal or slightly below the average, and then followed by an early overgrowth during the first 1 or 2 years. However, growth is slowed down thereafter and the brain volumes have been reported to be smaller than those of normal controls in their adulthood (Courchesne et al., 2007). The evolution of neuroimaging has provided opportunities to accurately measure structure and volumes of different regions of the brain. In magnetic resonance imaging (MRI) studies, the brain overgrowth in autism appears to be most prominent in the frontal and temporal lobes and in amygdala, and more apparent in gray matter than white matter (Carper and Courchesne, 2005). The regional overgrowth in early infancy seems to be most remarkable in those brain regions whose cognitive function is most severely impaired in later life (Courchesne et al., 2007).

Akshoomoff et al. (2004) measured brain volumes of 52 autistic children aged 2 to 5 years, and 15 typically developing age-matched children. The autistic children were divided into subgroups of high functioning autism, low functioning autism and pervasive developmental disorder not otherwise specified. The whole brain volume, overall cerebral volume and cerebral gray matter volume were discovered significantly larger in children with low functioning autism than in controls. The overall cerebellar volume did not reach significance but cerebellar white matter volume and cerebellar vermis lobules I-V areas were larger in all autistic subgroups than in typically developing children (Akshoomof et al., 2004).

A recent longitudinal MRI study with multiple scans from 1.5 years up to 5 years of age on 41 children with low functioning autism confirmed that both gray matter and white matter volumes were enlarged by 2.5 years of age as compared with 44 typically developing controls (Schumann et al., 2010). The enlargement was most apparent in frontal, cingulate and temporal cortices with the percentage difference of 6%, 8%, and 9%, respectively. Total cerebral white matter volume was 10% larger and total cerebral gray matter volume 5% larger in autistic individuals than in controls. In that study, the growth pattern was more widespread and more severe in the 9 female autistic patients than in the 32 autistic males (Schumann et al., 2010).

A comparison between the HC and MRI studies has indicated that HC is an accurate

index of brain volume (and weight) in children less than 7 years of age (Bartholomeusz et al., 2002). In older children, the wide range of ventricular volume makes HC less precise.

Diffusion tensor imaging studies in autistic patients have been published since 2004. The very first paper examined 7 children and adolescents with high functioning autism revealing abnormalities in white matter tracts as compared with those of 9 typically developing control subjects (Barnea-Goraly et al., 2004). Reduced fractional anisotrophy, a measure reflecting how diffusion varies along different directions, was observed in brain regions implicated in face and gaze prosessing, emotional prosessing, and those activated in theory of mind tasks, i.e fusiform gyrus, anterior cingulate, amygdala, ventromedial prefrontal cortex, temporoparietal junction, and superior temporal sulcus (Barnea-Goraly et al., 2004). Recently, the same research group reported likewise abnormalities in the frontal, parietal and temporal lobes of 13 autistic children as well as in their 13 unaffected siblings as compared with 11 controls (Barnea-Goraly et al., 2010). In the last-mentioned study, the abnormalities were not restricted to regions with importance for social cognition (Barnea-Goraly et al., 2010). Consistent with the findings of Barnea-Goraly et al., Jou et al. (2011) reported abnormalities in long-range cortico-cortical connectivity involving several association, commissural and projection tracts important for social cognition in 15 autistic boys, aged 5-17 years, as compared with 8 typically developed controls with the same age distribution.

Macrocephaly persisting beyond the early childhood may present a familial genetic predisposition since the non-autistic family members of autistic children with macrocephaly have been reported to have even higher rates of macrocephaly (Fidler et al., 2000). In the cases where there is very noticeable macrocephaly, a genetic background with mutations in phosphatase and tensin homolog (PTEN) gene has been reported in 24 patients with autism (Conti et al., 2011).

Considering the neuronal structure of brain in autism, the most consistent and repeated finding has been the decreased number of Purkinje cells in cerebellum, the structure with an important role in modulating cognitive and motor functions (Bauman and Kemper, 1985 and 2005, Ritvo et al., 1986, Bailey et al., 1998,). The timing of the loss of the Purkinje cells has been estimated to occur before 28-30 weeks of gestation, according to earlier reported findings examining Purkinje cells′ connections to other brain structures (Rakic and Sidman, 1970).

Maternal use of valproic acid, an antiepileptic and mood-stabilizer, during pregnancy has been shown to increase the risk of autism in children (Rasalam et al., 2005). In an experimental model of autism, mice exposed prenatally to valproic acid have been found to display significant reductions in Purkinje cell number and aberrations in dimensions of cerebellar structures similar to those encountered in autistic human patients (Ingram et al., 2000).

A decrease in the number of neurons in the amygdala, a part of limbic system known to be important in processing emotions, learning ,and memory, has been reported in autism (Schuman and Amaral, 2006).

A disruption in the architecture of the neocortical minicolumns has been reported (Casanova et al., 2003). A theory proposing hyper-functioning neuropathology in the microcircuits has been presented, especially in the minicolumns but also in other brain structures (Markram and Markram, 2010). Experimental studies have suggested that there is a link between the prenatal effects of valproic acid and the neuropathology in the microcircuits, including the decreased number of inhibitory Purkinje cells (Ingram et al., 2000).

2.5 GROWTH FACTORS IN AUTISM

2.5.1 Insulin-like growth factors

The amino acid sequences of human insulin-like growth factors IGF-1 and IGF-2 were subsequently determined and reported in 1978 (Rinderknecht and Humbel, 1978a and 1978b). The broad spectrum of their stimulating effects on cellular proliferation and differentiation during fetal and postnatal development has been appreciated (Werther et al., 1998).

In the brain, IGF-1 was initially considered as a neurotrophic factor involved in brain growth. Later, the IGF-1´s role in regulating brain function as a whole has become more evident. At present it is acknowledged that IGF-1 has many effects on the modulation and plasticity of neuronal circuits and connectivity (Torres-Aleman, 2010). Associations between cognitive function, intelligence, and IGF-1 have also been presented (Creyghton et al., 2004, Aleman and Torres-Aleman, 2009). There is evidence that IGF-1 is important in cerebellar development (Torres-Aleman et al., 1998, Werther et al., 1998), and the most consistent neuropathological findings in autism have been detected in cerebellum (Bauman and Kemper, 2005). Low IGF-1 concentrations have been reported in CSF of children with autism (Vanhala et al., 2001). On the other hand, Mills et al. (2007) detected elevated serum concentrations of IGF-1 and IGF-2 in autistic children between ages of 4 and 8 years as compared with age-matched controls. They reported finding a positive correlation between IGF-1 and HC, but no correlation between IGF-2 and HC.

Patients with symptomatic infantile spasms have been reported to exhibit markedly low CSF-IGF-1 concentrations as compared with those of children with idiopathic infantile spasms or control children (Riikonen et al., 2010). Low CSF IGF-1 concentrations were associated with a history of early insults or stress, and a poor response to treatment and a poor cognitive outcome (Riikonen et al., 2010). An increased future risk for ASD has been reported in children who have suffered infantile spasms, and recently this risk has been especially connected with the symptomatic origin of the seizures (Saemundsen et al., 2008). In a rare Finnish genetic disorder, children with progressive encephalopathy, hypsarrhythmia (and infantile spasms), and optic nerve atrophy – the PEHO syndrome – have been reported to have low CSF-IGF-1 concentrations as compared with controls and "PEHO-like" patients without the typical neuroophtalmologic or neuroradiologic findings (Riikonen et al., 1999).

A reduced concentration of CSF-IGF-1 than in controls has also been reported in infantile neuronal ceroid lipofuscinosis, a progressive encephalopathy with severe developmental delay emerging by the age of 3 years (Riikonen et al., 2000).

No significant differences in serum IGF-1 concentrations were detected between children and adolescents with idiopathic epilepsy and controls (El-Khayat et al., 2010).

Two antidepressants, fluoxetine and venlafaxine, have been shown to increase the concentration of IGF-1 and several other proteins associated with neurogenesis in the hippocampus in experimental studies (Khawaja et al., 2004). In a recently published paper in adult depressed patients treated with antidepressants, including fluoxetine, increases in CSF-IGF-1 were detected (Schilling et al., 2011).

2.5.2 Brain derived neurotrophic factor

Brain derived neurotrophic factor (BDNF) was discovered in 1982 by Barde et al. who described it as a promoter of survival of neurons (Barde et al., 1982). BDNF belongs to the same family of neurotrophins as nerve growth factor and neurotrophin-3 and neurotrophin-4/5. BDNF and other neurotrophins bind to one or more of the tropomyosin-related kinase receptors, which are members of the receptor tyrosine kinase family (Patapoutian and Reichardt, 2001). BDNF has survival and growth promoting actions on many different types of neurons, including hippocampal and cortical neurons (Huang and Reichardt, 2001). Physiologic regulation of BDNF gene is important in the development of brain; both excess BDNF and blockade of BDNF signaling during a critical period of development of visual cortex can lead to abnormal functionality (Cabelli et al., 1997). BDNF appears to have more multifaceted properties. In addition to its contribution to survival and development of neurons; it has a major influence on molecular mechanisms in synaptic plasticity and neurogenesis. This makes its role most interesting in neurobiological processes concerning learning and memory. The hippocampus has a crucial role in long-term memory, and this is an important site of BDNF action (Yamada and Nabeshima, 2003). BDNF and its receptor tyrosine kinase B (TrkB) play a major role in the action of antidepressant medication; experimental studies indicate that there are no behavioral effects of antidepressants in mice lacking the BDNF gene (Saarelainen et al., 2003). BDNF infusion in hippocampus (Sirianni et al., 2010) and in raphe nucleus region (Siuciak et al., 1997) in rats has been able to mimick the effects of antidepressants**.** In contrast, rats receiving BDNF infusion in ventral tegmental area promoted a depression-like effect as compared with control animals (Eisch et al., 2003).

Nelson et al. studied (2001) BDNF in archived neonatal blood specimens from mandatory newborn screening, and detected increased concentrations of BDNF in those neonates who later were diagnosed with autism (n= 69) as well as in those with non-autistic mental retardation (n= 60), as compared with children with cerebral palsy (n=63) or controls with normal development (n=54). However, the recycled immunoaffinity chromatography method used in 2001 was later replaced with enzyme linked immune-sorbent assay technology and when the same samples were re-evaluated with the new method, BDNF concentrations did not distinguish any longer the children with autism from the controls (Nelson et al., 2006). The neonatal blood sample study has later been repeated by Croen at al. (2008) and no differences were detected in BDNF concentrations between children with autism, intellectual disability or those enjoying normal development. Elevated concentrations of serum BDNF have been reported in pre-school-aged children with autism and in childhood disintegrative disorder, a subgroup of ASD (Connolly et al. 2006). In another study, serum BDNF concentrations in school-aged children with autism and non-autistic mental retardation were significantly higher than those of adult controls (Miyazaki et al., 2004). There do appear to be differences between the serum concentrations of BDNF in autistic children and autistic adults. Hashimoto et al. (2006) have reported decreased BDNF in adult men with high functioning autism but no correlations were detected between clinical variables such as severity of autistic symptoms and BDNF concentrations.

At time when the present study was conducted, there were no reports on the possible differences in BDNF concentrations of pre-pubertal children and pubertal adolescents. In the recent report of Iughetti et al. (2011), plasma BDNF concentrations were reported to be

lower in pubertal boys than in pre-pubertal boys, and both in pre-pubertal and pubertal girls.

Additional support for the link between BDNF and autism has been provided by studies into those specific genetic disorders associated with autism. In Rett syndrome, the mutated protein MeCP2 regulates BDNF expression in complex manner. Cortical BDNF concentrations were reported to be reduced in a mouse model of Rett syndrome (Kaufmann et al., 2005). However, serum BDNF concentrations of Rett patients have not been shown to differ from those of healthy controls (Vanhala et al., 1998). In addition, BDNF polymorphism has been connected to modify disease severity in Rett syndrome (Zeev et al., 2009).

In the fragile X syndrome, BDNF has been reported to regulate the expression of the fragile X mental retardation protein (Castren et al., 2002), and furthermore, BDNF polymorphism has been reported to influence the severity of seizures in fragile X patients with epilepsy (Louhivuori et al., 2009).

2.5.3 Other growth factors

Abnormalities in other neurotrophic factors have also been reported in autism. In a study investigating postmortem cerebellar tissue samples of 8 autistic patients, the concentration of neurotrophin-3 was found to be elevated as compared with equivalent samples of 7 non-autistic control subjects (Sajdel-Sulkowska et al., 2009). The possible connection of neurotrophin-3 disequilibrium with the initial cerebellar overgrowth and a subsequent reduction of cerebellar Purkinje cells were also discussed in that report (Sajdel-Sulkowska et al., 2009). In the neonates who later had autistic development, serum neurotrophin was lower than control (Nelson et al., 2006). Serum neurotrophin-4 concentration was detected as being higher both in children with autism and children with non-autistic mental retardation than in adult controls (Miyazaki et al., 2004).

2.6 NEUROTRANSMITTERS IN AUTISM

The classical neurotransmitters are the small molecular weight compounds that transmit signals from one neuron to another on the other side of the synapse. Six of the major neurotransmitter systems are serotonergic, dopaminergic, noradrenergic, cholinergic, glutamatergic, and GABAergic systems, in view of the fact that the natural activating transmitters are serotonin, dopamine, norepinephrine, acetylcholine, glutamate and gamma-aminobutyric acid, respectively.

In the synaptic regulation mechanism of nervous transmission, two different types of proteins, receptors and reuptake transporters, are the essential structures. The receptors act as targets for the transmitters on the post- and presynaptic structures, although receptors have also been detected on the soma and dendrites of the neurons, as well as on non-neuronal glial cells in the central nervous system (Barnes and Sharp, 1999). The reuptake transporters take up the transmitter molecules back after they have been secreted from the axonal terminal into the synaptic cleft. In that way the transporters can modify the intensity and duration of the signal.

2.6.1 Serotonin

The serotonergic system has been the most intensively evaluated neurotransmitter system in autism. The serotonergic system seems to be involved in, or even to be responsible for many of the neuronal or behavioral perturbations observed in autistic individuals. Serotonin has been shown to act as an early regulator of brain development in experimental animals, and in humans (Whitaker-Azmitia, 2005). Schain and Freedman (1961) reported elevated blood serotonin concentrations in 6 out of 23 autistic patients. This has led to suggestions that high levels of serotonin may cause aberrations during the development of the neural circuits and connections and contribute to the emergence of autism. However, no consistent connection has been found between the blood serotonin concentration and autistic symptoms and behavior. However, there is some information about putative correlation between whole-blood serotonin concentration and cognitive functioning in autistic children and their close relatives (Cuccaro et al., 1993). Therapeutic methods aimed at reducing blood serotonin have not changed the clinical course in autistic children (Ritvo et al., 1971, Aman and Kern, 1989). As a matter of fact, serotonin does not cross the blood-brain-barrier in adulthood although it is suspected that this may occur during early embryologic and fetal development (Whitaker-Azmitia, 2005). Medications affecting the serotonergic system during pregnancy have been investigated, and recently an increased risk of ASD was detected in mothers who had been prescribed SSRI medication. Croen et al. (2011) investigated 298 children with ASD and discovered that if an SSRI had been used during the year before delivery (including the pregnancy), this doubled the risk for ASD. The use of SSRIs during the first trimester of pregnancy increased the risk more than threefold. Prenatal exposure to SSRI was reported in 20 ASD cases (6.7%) compared with 50 of the 1507 (3.3%) randomly selected controls. The statistically calculated increase in ASD incidence owing to prenatal use of SSRIs was about 2% in the Croen et al. study (2011).

Serotonin transporter (SERT) is a protein located mainly on the presynaptic terminal of serotonergic neurons, but it has been detected also along axons, soma and dendrites of them, and it is believed to be the most important determinant of the extracellular level of serotonin in the central nervous system (Hoffman et al., 1998). SERT is also present in peripheral cells which are specialized in serotonin storage or inactivation, for example the platelets present in the peripheral blood (Carneiro and Blakely, 2006). Serotonin does not cross the blood-brain-barrier but the same gene produces SERT molecules in brain and in periphery (Lesch et al., 1993).

The human SERT gene, SLC6A4 has been studied intensively in autism but it has not been possible to link common polymorphisms with the disease in families with autistic probands (Sutcliffe et al., 2005). Prasad et al. (2009) reported that rare functional variants in SERT gene may contribute to autism in some pedigrees, and there may be also an overlap with the obsessive compulsive disorder. One interesting finding was that the Gly56Ala variant of SERT gene evoked a selective effect on transmission only in males and the lack of transmission in unaffected females. Enhanced SERT activity was seen in several rare variants (Prasad et al., 2009). These observations are in concordance with the male predominance in autistic patients and the hyperserotonemia in blood and platelets in a minority of autistic individuals.

In a functional imaging study of serotonin metabolism by positron emission tomography (PET), Chugani et al. (1997) detected asymmetric alterations in serotonin

synthesis in frontal cortex, thalamus and dentate nucleus of cerebellum in 7 autistic boys but not in an autistic girl as compared with their 5 healthy siblings. These findings were believed to correlate with abnormalities in language production and sensory integration both of which are affected in autism. In another study, Chugani et al. (1999) evaluated the serotonin synthesis capacity in the brains of 30 autistic children as compared with their healthy siblings and epileptic children without autism. In non-autistic children, serotonin synthesis capacity reached its maximum at age of 5 years, being then about 2-fold compared to that of adults, and then slowly declined towards adult values. In autistic children, the early peak in serotonin synthesis capacity was missing. The serotonin synthesis capacity increased slowly until the age of 15 years, reaching then approximately values 1.5-fold of the adult values, thereafter slowly descending towards adult values. No gender dependence was detected, though the number of female autistic patients in the study was small – 6 out of 30. Furthermore, a linkage has been detected between the clinical developmental phenotype and the asymmetrical distribution of the aberrations in serotonin synthesis (Chandana et al., 2005); left sided cortical decrease in serotonin synthesis was associated with more severe language impairment. This may point to a link, and a disruption, in the development of the serotonergic system and the hemispheric specialization in autism.

Abnormalities in serotonin receptor 2A binding in the cerebral cortex have been detected by SPECT in Asperger syndrome patients (Murphy et al., 2006) and altered SERT and/or serotonin receptor 1A functionality in hippocampus in a mice model of autism (Gould et al., 2011).

In another experimental study, Boylan et al. (2007) induced a selective neonatal cortical serotonergic perturbation via injection of a neurotoxin into the medial forebrain bundle of the newborn mice. Neuroanatomic and behavioral changes resembling those observed in human autistic subjects were observed in the affected mice (Boylan et al., 2007). An early postnatal depletion of cortical and hippocampal serotonin concentrations was followed by recovery towards adulthood in the mice in the Boylan et al. study (2007), these findings resembling those detected in the abovementioned studies by Chugani et al. (1999). Several behavioral changes were detected in the serotonin depleted mice i.e. impaired impulse inhibition, impaired exploratory activity and spatial memory, and alterations in social interaction and fine motor performance. Perhaps even more interesting, the behavioral aberrations were expressed much more clearly in male mice than in females, mimicking the gender distribution in autistic human population.

Imaging SERT (and also dopamine transporter, DAT) by SPECT became possible in the early 1990's when a radioligand, ^{123}I-beta-CIT, became available (Brücke, 1993), and the method was rapidly adapted to use also in Finland (Kuikka et al., 1993). SPECT studies of SERT and DAT have been performed in several fields of adult neurology, including depression, panic disorder, anxiety disorder and eating disorders (Kuikka et al., 2001, Maron et al., 2004). The technique has also been introduced in pediatric neurology, including a study in fetal alcohol syndrome (Riikonen et al., 2005). However, no SPECT imaging of SERT in autistic patients had been performed until the present study.

2.6.2 Dopamine

Dopamine acts as a key neurotransmitter in several brain functions. Dopamine is involved

in the regulation of movement, reward and addiction, attentional mechanisms, cognitive functions, working memory processes, and in regulating and modulating emotional responses and social behavior (Nieoullon, 2002). Dopamine dysfunction has been reported to be related in several neuropsychiatric disorders, including Tourette syndrome, attention deficit hyperactivity disorder (ADHD), bipolar disorder, schizophrenia, mania, obsessive-compulsive disorder, substance abuse, and autism (Gillberg and Billstedt, 2000). Several important dopaminergic tracts innervate amygdala and cingulate and prefrontal cortex which are core locations of neuroanatomic aberrations detected in autistic patients as well as in experimental models for autism (see Chapters 2.4 and 2.5).
The characteristics of the motor disturbances in autism and their similarities with those in Parkinson's disease have been noted. Since a good response of L-dopa to motor components in Parkinson's disease had been detected, L-dopa treatment was also investigated in autistic patients (Ritvo et al., 1971). The decision to use L-dopa for treatment in autism was also linked to L-dopa's ability to decrease blood serotonin concentrations in experimental studies. Indeed, Ritvo et al. (1971) found blood serotonin concentrations decreased in 3 out of 4 children after 6 months of L-dopa treatment, but no change was detected in the motor function or in the autistic behavior.

The dopaminergic systems are considered to have major importance in the identification and modulating emotional stimuli, production and regulation of affective states and in the control of the flow of information from other areas of brain (Salgado-Pineda et al., 2005), and all those functions are suggested to be affected in autism. Prenatal genetically predisposed alterations in dopamine metabolism have been related with an increased risk of autistic development (Robinson et al., 2001). Furthermore, other environmental factors generating a hyperdopaminergic state have been postulated to act as an epigenetic factor to promote autistic expression (Previc, 2007).

Reduced pre-frontal dopaminergic activity has been detected in 14 autistic children in a PET study by Ernst et al (1997). They reported that the difference was especially prominent in the autistic children with IQ higher than 80. Nieminen-von Wendt et al. (2003) published a PET study of 8 adult male patients with Asperger syndrome using the same tracer as Ernst et al in 1997, and reported increased blood flow in the striatum and the frontal cortex.

Prior to the present study, there was only one SPECT study of imaging of DAT in autistic patients: Xiao-Mian et al. (2005) reported increased DAT binding in striatum in 10 autistic children aged 3-10 years, as compared to their age-matched controls.

However, several studies in other neuropsychiatric disorders with a high co-morbidity with autism have reported abnormalities in the dopaminergic system: in patients with obsessive-compulsive disorder, increased DAT binding in the basal ganglia was detected by Kim et al. (2003) and in a further study (2007) a decrease in DAT density was detected in all 10 patients with obsessive compulsive disorder who had been treated with SSRIs during the previous 16 weeks, achieving also a decrease in symptoms.

Increased DAT binding in basal ganglia has also been reported in children with ADHD (n=9), and in children with Tourette's disorder (n=9), as compared with healthy controls in studies published by Cheon et al. in 2003 and 2004, respectively.

PET studies suggest abnormalities in both serotonergic and dopaminergic functions (Rumsey and Ernst, 2000). Dopaminergic and serotoninergic pathways originate in brainstem but project their axons to the forebrain. Serotonin is believed to play a critical interactive and modulating role with both of these systems in autism. DAT is an essential element in the function of the dopaminergic system (Gadow et al., 2008, Hettinger et al.,

2008).

Significant clinical benefits have been achieved with treatment with dopamine-2 receptor antagonists, especially risperidone, emphasizing the importance of dopamine dysfunction being behind the autistic symptoms and behavior. Small doses of L-dopa have improved the sleep-wake cycle, aggressiveness and hyperkinesias in autistic individuals (Segawa, 2006), and this probably takes place through desensitization of the dopamine receptors.

2.6.3 Other neurotransmitters

Norepinephrine is a neurotransmitter synthesized from dopamine. Elevated plasma concentrations of norepinephrine have been reported in autistic children, especially in those with coexistent hyperserotonemia (Cook et al., 1990). However, most of the studies examining the norepinephrine system in autism have resulted in inconsistent findings and therefore it has been proposed that norepinephrine may have only a minor role in the expression and etiology of autism (Lam et al., 2006).

Acetylcholine is the neurotransmitter released in the cholinergic system, and it is found in several sites in the central nervous system. The cholinergic system has been considered to act in developing the ability to focus on the environment and helping the individual to achieve a coherent behavioral response (Janowsky et al., 1994). It has been hypothesized that alterations in this system could be linked with problems with attention or learning in autism. In post-mortem studies of autistic brains a significant reduction was detected in acetylcholine binding to nicotinic receptors in the parietal and frontal cortexes as well as to muscarinic receptors in the parietal cortex. The relation of these findings to clinical symptoms or the etiology of autism remains obscure (Martin-Ruiz et al., 2004).

2.7 OTHER FIELDS OF ETIOPATHOGENETIC RESEARCH

2.7.1 Immunologic aberrations in autism

The role of the immune system has been widely discussed considering the elevated risk of autism. Abnormalities in both innate and adaptive immune responses have been reported (Gupta et al., 2010) and the presence of various types of autoantibodies attacking nervous system components has been detected (Connolly et al., 2006, Singer et al., 2006). Damage to the blood-brain barrier and/or pathological activation of the brain immune response system due to unknown pathogenic factors has been proposed to be behind these aberrations (Meyer et al., 2006). Connolly et al. (2006) detected elevated levels of autoantibodies to brain endothelial cells, myelin basic protein, and BDNF in autistic pre-school aged children, but the findings were not specific to autism and occurred also in children with epilepsy or non-neurological illnesses. Autoantibodies to cerebellar neural cells have been detected in other studies (Wills et al., 2009, Goines et al., 2011).

2.7.2 Infections and vaccinations

Rubella and cytomegalovirus infections during pregnancy have been implicated as risk factors for autism in the developing child (Chess, 1971, Stubbs et al., 1984). Childhood immunizations given for the protection against several infectious diseases especially during the first and second year of life have been a cause of concern, due to beliefs that they may increase the risk of autism. The study of Wakefield et al., published in 1998, claimed that there was a link between measles, mumps, and rubella vaccination and autism. This attracted much media attention and caused considerable worry in parents and even mistrust of the public health services. Several epidemiological studies during the following years in different countries found no link between the measles, mumps and rubella vaccination and autism, and finally 12 years after its publication, the Wakefield study was found to have been fraudulent and it was retracted by the journal (Editors of the Lancet, 2010).

2.7.3 Prenatal and environmental risk factors

The incomplete concordance for autism in monozygotic twins and the increased risk in dizygotic twins compared with singleton-born siblings of an individual with ASD (Rosenberg et al., 2009) has raised interest in the environmental risk factors during the prenatal development.

There are several chemicals known to be toxic during human neurodevelopment, including valproic acid, thalidomide, misoprostol and ethanol (Dufour-Rainfray et al., 2011). The tragedy of thalidomide embryopathy although typically associated with severe limb anomalies has also been reported to be linked with an increased incidence of autism (Stromland et al., 1994). Misoprostol, used as an abortifacient agent, has led to a high proportion of autism in the children born following unsuccessful abortion attempts (Bandim et al., 2003).

Children exposed to valproic acid have exhibited a variation of somatic malformation referred as the fetal anticonvulsant syndrome which carries also an increased risk of autism (Moore et al., 2000). In a recent study with 260 children with prenatal exposure to antiepileptic drugs, the risk for autism spectrum disorder with any of them was 1.9-4.6%, but the risk associated with valproic acid (alone or in combination with other antiepileptic drugs) was 11.7% (Rasalam et al., 2005). The possible mechanism of valproic acid in the pathogenesis of autism has been presented in chapter 2.4.

The connection between prenatal ethanol exposure and autism has been investigated in a recent study describing a 10% prevalence of autism among 21 children with fetal alcohol syndrome adopted from Eastern Europe (Landgren et al., 2010). However, no correlation between prenatal alcohol exposure and ASD was detected in a large populationbased study of more than 80 000 children and their mothers (Eliasen et al., 2010). The average alcohol consumption and episodes of binge drinking were very low in the latter study and the incidence of fetal alcohol syndrome was not investigated.

In the USA in 2003, a large study was started called the Childhood Autism Risks from Genetics and Environment (The CHARGE Study) which recruits children born in California, and living there at age of 2 to 5 years, to collect and evaluate information about

chemical and biological exposures, susceptibility factors and their interactions (Herz-Picciotto et al., 2006). The study population has been composed of children with a defined diagnosis of autism, other types of developmental delay, or typical development, and a total number of 2000 participants have so far been collected (Herz-Picciotto et al., 2006). Until September 2011, a total of 12 papers from the CHARGE study have been published including reports on some immunologic, cytokine or genetic profiles of the recruited children. The broad repertoire of factors covered in CHARGE study is represented by a report on potential effects of traffic-related air pollution in risk of autism (Volk et al., 2011). Volk et al. (2011) presented results based on demographic factors of 304 ASD cases, and 259 typically developing controls, and claimed that mothers with a residence near (< 309m) to a freeway during the third trimester, or at the time of delivery, had a 2.22-fold and 1.86-fold risk, respectively, for ASD in their newborn offspring compared with those mothers living further away from freeways or along other roads.

Recently, King and Bearman (2011) published a study on the socioeconomic status and the prevalence of ASD, with a large cohort of nearly 5 million birth records analyzed and almost 19,000 established ASD diagnoses in children born in California between 1992 and 2000. They detected a higher prevalence, and less severe cases of ASD in a community composition with higher education and higher property values suggesting that neighborhood dynamically interacted with the increase of more, but less severely affected cases of ASD.

2.8 TREATMENT

The treatment in autism is targeted at alleviating autistic symptoms and enhancing the functional capacity by speech and communication therapy, occupational therapy, behavioral training and other methods to improve daily living abilities (Levy et al., 2009). There are several clinical protocols of therapies used worldwide and describing them in detail is beyond the scope of the present review of the literature. Only a few randomized controlled trials on clinical treatments in autism have been carried out.

Rogers and Vismara (2008) reviewed the effects of different type of therapies in ASD, and scored the best category of effectiveness, possibly efficacious, only for three randomized controlled interventions. The first of those three trials was a combination of parent-training workshops, TEACCH (abbreviated from Treatment and Education of Autistic and related Communication-handicapped Children) model speech and language therapy and social skills training performed for 28 children with childhood autism for 12 months (Aldred et al., 2004). The second was a protocol reported by Drew et al. (2002) including an intensive discrete trial training of 33h/week along with standard speech and language and occupational therapies. The third approach described in the study of Jocelyn et al. (1998), with a 12-week intervention targeted not only at the children but also at the parents and the childcare workers. However, if one examines the findings in those three studies, only Aldred et al., (2004) reported any reduction in autism severity. Improvements in speech and communication were common achievements in all three studies.

Most of the pharmacological interventions in autism have been targeted rather towards adults rather than at children, and most often on associated behavioral symptoms like hyperactivity, aggression and motor stereotypies rather than the core deficits in autism: the deficiencies in communication and social interaction (Posey and McDougle, 2001).

As the origin of autistic development remains obscure, it is possible that the developmental period for the optimal interventions have already passed at the moment of diagnosis and therefore the treatment should be rather targeted at supporting the brain's response to developmental perturbations and enhancing neural plasticity (Bethea and Sikich, 2007).

2.8.1 Selective serotonin reuptake inhibitors

The function of SSRIs is based on inhibiting the reuptake of the neurotransmitter serotonin from the synaptic cleft by the SERT molecules present on the presynaptic neuron. However, effects on the serotonin receptors and autoreceptors during a long-term administration of SSRIs have been described (Gobbi et al., 1997). Furthermore, BDNF and its receptor TrkB have been proposed to be involved in the therapeutic actions of antidepressant drugs, including the SSRIs (Rantamäki et al., 2007).

In an open study with fluoxetine treatment in 129 children aged 2 to 8 years with childhood autism and continuing the treatment up to 6 years, DeLong et al (2002) reported positive effects in an excellent responder group of 17% - these subjects no longer meeting the diagnostic criteria of autism - and 52% with a good response showing improved social response, emotional stability, attention and awareness of understanding. Only one-third of the patients had a fair or poor response. The mean age of starting fluoxetine was 4½ years and the main duration of treatment was 36 months. In that study, a high prevalence, in 74% of the families, of either bipolar disorder or major depression, or both, was reported. In 85% of cases where children with autism had an excellent/good response to fluoxetine treatment, there was a family history of depression or bipolar disorder. In an earlier paper examining 37 autistic children DeLong et al. (1998) described more specifically the details of the therapeutic response; they reported marked responses within 2 to 3 weeks after starting medication and especially prominent progress in language abilities. They also reported that discontinuation of successful treatment almost invariably resulted in regression of both language and behavioral skills, although sometimes the regression occurred several months later.

In a double-blind placebo controlled study of 12-week fluvoxamine treatment in an adult population with autism, McDougle et al. (1996) detected a favorable response in 8 of 15 patients in the fluvoxamine group and none of 15 in the placebo group; the response was significant in terms of improved language usage, reduced repetitive thoughts, less repetitive or maladaptive behavior, less aggression, and improved social skills. However, the same research group could not obtain any clear response with the same treatment schedule in a pediatric population with autism, and there were more adverse events in the children than encountered in adults (Posey and McDougle, 2001).

A placebo controlled crossover study of 8-week fluoxetine treatment focused on the repetitive behavior in children and adolescents of 5 to 17 years of age Hollander et al., 2005). This revealed a significant decrease of symptoms on a Children's Yale-Brown Obsessive-Compulsion Scale and a trendtowards a reduction of global autism severity (Hollander et al., 2005). The side effects reported in this study were interesting; only anorexia and drowsiness were experienced more often during fluoxetine vs. placebo (15% vs. 11% and 18% vs. 11%, respectively) but anxiety, insomnia, agitation, diarrhea, weight gain or respiratory infections occurred with equal frequency, or were even more frequent

in the placebo group.

On the other hand there have been studies with SSRI therapy with less favorable results. In a randomized, blinded, multicenter study determining efficacy and safety of a 12-week citalopram treatment for repetitive behavior in 149 children and adolescents with ASD, no differences were found in the rate of positive responses between the citalopram-treated and the placebo groups (King et al., 2009).

Fluoxetine treatment has also been tested in an experimental model of autism i.e. a mouse strain presenting autistic-like behavior; sociability in the mice increased after administration of fluoxetine, but not after risperidone, an atypical antipsychotic agent (Chadman, 2011).

2.8.2 Atypical antipsychotics

The first generation of antipsychotic drugs (for instance chlorpromazine and haloperidole), or the typical antipsychotics, are considered to exert their effects through their potent dopamine type 2 receptor antagonism. However, these drugs may cause severe adverse effects, especially extrapyramidal symptoms, which can be irreversible in some cases. The second generation of antipsychotic drugs (e.g. risperidone and aripirazole), more commonly called the atypical antipsychotics, bind less effectively to the dopamine 2 receptors but have also some affinity for the serotonin type 2A receptors, having an antagonist effect. The abovementioned distinctions are believed to explain the milder spectrum of adverse events in the atypical antipsychotics as compared with the first generation antipsychotics (Seeman, 2002).

In autism, antipsychotics have been used to alleviate behavioral and mood disturbances such as aggression, agitation, and irritation. However, the benefit from the first generation antipsychotics has been limited by the relatively high frequency of adverse reactions (Posey et al., 2008). Risperidone, a second generation "atypical" antipsychotic, was the first drug to be used for the treatment of severe behavioral symptoms in autistic children and adolescents to be approved by the Food and Drug Administration in 2006 in the United States, and in 2009 also aripirazole was approved for the same indication. at present, neither of these drugs has gained official acceptance for the abovementioned indication in Finland, or elsewhere in the European Union. However, they have been approved for use in severe behavioral problems in mental disorders and in mentally retarded patients, aged from 5 years, or for schizophrenic patients, aged from 15 years.

McCracken et al. (2002) conducted a randomized study with 101 autistic children and reported that risperidone relieved aggression and other unwanted behaviors after 8 weeks of therapy. A positive response was reported in 49 children which was a statistically significant result. The favorable effects remained after 6 months in two-thirds of the patients with a good response rate at the 8-weeks' check-point.

In another study, after 3-months' therapy with risperidone, an increase in regional blood flow in frontal and prefrontal regions was detected, in parallel with a clinical improvement, in 11 autistic children aged 6 to 7 years (Ozdemir et al., 2009).

Another drug in this category, olanzapine has been reported to show improvement in core social and language impairment in pervasive developmental disorders, the broader aspect of the autism spectrum (Potenza et al., 1999, Hollander et al., 2006).

2.8.3 Other medications

Several other drugs with different mechanisms of action have been studied in autism, including, but not limited to, anticonvulsants, stimulants, alpha agonists and acetylcholine-esterase inhibitors.

The effect of anticonvulsant drugs on the autistic symptoms has been studied in autistic patients with or without concomitant epilepsy. Several antiepileptic drugs, including carbamazepine, valproic acid, lamotrigine and gabapentin have effects on serotonergic tone through several molecular mechanisms (Di Martino and Tuchman, 2001). Lamotrigine, an anticonvulsant attenuating glutamate release, has been reported to relieve the autistic symptoms of autistic patients with epilepsy (Uvebrant and Bauziene, 1994) but this finding has not been confirmed (Belsito et al., 2001). With the antiepileptic agent levetiracetam, a reduction in the amount of behavioral disturbances has been detected by some researchers (Rugino and Samsock, 2002) but others have not found any benefits (Wasserman et al., 2006). All these studies have been limited by small group sizes and also in the duration of the treatment.

As hyperactivity and inattention are commonly associated behaviors in autistic individuals it is not surprising that stimulants, with have gained status in attention deficit hyperactivity disorder (ADHD), have also been widely studied in autism. Methylphenidate is a reuptake inhibitor of the neurotransmitters dopamine and norepinephrine, increasing their availability at central synapses in brain. The clinical response to methylphenidate in ADHD patients has been linked to high striatal DAT availability before treatment, followed by a decrease in DAT availability during treatment (Krause et al., 2005). Recently, Jahromi et al. (2009) presented a study report about a 4-week randomized, double-blind crossover trial with methylphenidate in 33 children with ASD, of mean age of 7 years. They detected a significant positive effect on joint attention, self-regulation, and regulating affective state, while side-effects were rather rare (Jahromi et al., 2009).

Two alpha-2 adrenergic agonists, clonidine and guanfacine have been used to treat inattention, hyperactivity and aggression in autism but the benefits have been eroded by the high incidence of adverse events (Scahill et al., 2006).

Some preliminary studies in autistic patients have been performed with the acetylcholine-esterase inhibitors, donepezil and galantamine, but the results have been inconsistent; some improvements in the core social and language impairments were reported (Nicolson et al., 2006, Handen et al., 2011).

In Smith-Lemli-Opitz syndrome, a genetic disorder with a deficiency in cholesterol synthesis and which bears a high relationship to autism, early cholesterol supplementation started before the age of 5 years has been reported to reduce the risk of autism, but not if supplementation occurred later (Tierney et al. 2001).

In an experimental study, another potential treatment has been introduced in a mouse model of fragile X syndrome; reducing levels of striatal-enriched tyrosine phosphatase, an enzyme modulating synaptic plasticity and neuronal function, was associated with increased social behavior in the affected mice (Goebel-Goody et al., 2012). Furthermore, in a drosophila model of fragile X syndrome, administration of a glutamate receptor antagonist, methyl-6-phenylethynyl-pyridine has been reported to reverse abnormalities in brain structure and behavior, but only if given during early development (McBride et al, 2005). These findings are interesting with respect to autism, since in humans with

fragile X syndrome, the prevalence of ASD is increased (see Chapter 2.3).

2.9 PROGNOSIS

Autism and autism spectrum disorder are generally assumed to be lifelong conditions although the severity and the repertoire of the symptoms may change with time. The time or type of the onset of the symptoms does not appear to make any difference in later functioning or to the prognosis of the subject (Shumway et al., 2012).

In a recent review of studies investigating the course and prognosis of autism spectrum disorder, predictors of better outcome were normal intelligence level (IQ above 70), early communication and language abilities, especially spoken language at preschool age, verbal and nonverbal imitation skills and early joint attention skills, whereas the co-existence of genetic syndrome, mental retardation and epilepsy were unfavorable predictive factors (Helt et al., 2008).

In another study, there was no correlation between the age of onset of autistic symptoms and the clinical manifestation at age of 18 years in 38 autistic individuals with the historical diagnosis of autism who were followed by Tolbert et al. (2001), and furthermore no differences in IQs, proportion of males or need for institutional care was detected either.

Baghdadli et al. (2007) evaluated 219 children with childhood autism and atypical autism, and found that ability to speak at the age of 5 years predicted significantly the evolution of daily living skills in the next 3 years: those with speech at 5 years of age developed in their daily living skills but those without speech displayed a relative regression in skills.

Sigman and McGovern (2005) reported a follow-up of 48 autistic children with autism from preschool age to late adolescence measuring cognitive skill, language abilities and nonverbal communication. The improvement in cognitive and language skills was more evident in individuals with high functioning autism than in those with low functioning autism, and progress was more stable through the years in those with high function autism.

Lord et al. (2006) reported a 7 year follow-up of the stability of the autism spectrum disorder diagnoses established at 2 years of age. Of the children with an original diagnosis of childhood autism, 83 of the 84 still remained in the autism spectrum disorder at the age of 9 years. Those 46 children with an original diagnosis of pervasive developmental disorder not otherwise specified showed more variation at age of 9 years: 27 were best-estimated as having childhood autism, 14 pervasive developmental disorder not otherwise specified, and 5 no longer met any of the autism spectrum disorder criteria (Lord et al., 2006).

Cederlund et al. (2008) followed 76 males with Asperger syndrome and 77 autistic males for more than 5 years after the original diagnosis with the individual having been aged 16 to 38 years at the time of follow-up. They found that 8 of the patients with Asperger syndrome and one with autism no longer met the diagnostic criteria for autism spectrum disorder. Moreover, there was a great difference in the overall outcome between the two groups: at the age of 23 years or older, 64% in the Asperger syndrome group were living independently, but only 8% in the autistic group (Cederlund et al., 2008).

Szatmari et al. (2009) followed two patient groups, one with high functioning autism,

the other with Asperger syndrome from childhood to adolescence, and found that socialization, communication and daily living skills were better in the Asperger group already at ages of 4 to 6 years, and the difference remained significant at the end of the follow-up at 18 years of age.

Danielsson et al. (2005) followed up 120 individuals with autism from childhood up to ages ranging from 17 to 40 years, and reported epilepsy in 48% of autistic patients with severe mental retardation, in 20% of those with mild or moderate mental retardation, and in 16% of those with no mental retardation. However, the number of those with no mental retardation was very low in that study, only 6 subjects (Danielssson et al., 2005). In a recent study with 150 young adults with autism, Bolton et al. (2011) reported that 22% of patients were suffering from epilepsy, but those with severe or profound mental retardation were excluded. Epilepsy was associated with female gender, intellectual disability and poorer verbal skills, and the mean age of the onset of epilepsy was 13 years (Bolton et al., 2011).

3 Aims of the study

Neuronal growth factors and monoaminergic neurotransmission have attracted attention in autism because of the findings of abnormalities in growth and development of brain structures, and alterations in the serotonergic system. Experimental studies have pointed to an association between the growth factors and monoaminergic neurotransmission, and that certain antidepressant drugs might act on that interaction. The aim of this study was to investigate the possible correlations of growth factors and monoaminergic neurotransmission in autistic children and adolescents, and to evaluate the efficacy of serotonin reuptake inhibition on the core symptoms of autism. The specific aims of the present study were:

1. To measure IGF-1 and IGF-2 concentrations in serum and in CSF, and to evaluate correlations between those parameters and head circumference in autistic children, aged 2 to 16 years, as compared with healthy control children. (Publication I)

2. To measure SERT and DAT binding capacity in the brains of autistic children, aged 5 to 16 years, by SPECT and to compare the results with those of comparison children, aged 7 to 12 years, with non-autistic neurological symptoms. (Publication II)

3. To measure effects of a six-month treatment with fluoxetine, an SSRI drug, on concentrations of IGF-1 and BDNF in serum and in CSF, on SERT and DAT binding capacities by SPECT measurements, and on clinical response as assessed by the Autism Treatment Evaluation Checklist (ATEC) in autistic subjects, aged 5 to 16 years. (Publications III and IV)

4 Subjects and methods

4.1 ETHICS

The study protocol was approved by the Research Ethics Committee of the Hospital District of Northern Savo, Kuopio, Finland, and the Finnish Medicines Agency was notified. Written informed consent was obtained from the parents of the autistic subjects. For the BDNF components of the study, the protocol was explained to the control children and their parents and written informed consent was obtained. Those children able to understand the protocol gave assent.

The protocol for serum and CSF sample analysis of BDNF was also approved by the Johns Hopkins Medical Institutions' Institutional Review Board.

4.2 SUBJECTS

4.2.1 Autistic children

A total of 26 children and adolescents with childhood autism were recruited into the study. The flow chart of the patients is presented in Fig 4.1. No individuals with Asperger syndrome or pervasive developmental disorder not otherwise specified were included. None of the individuals with autism had either tuberous sclerosis, fragile X syndrome, or any major chromosomal defect. They did not have a history of perinatal asphyxia, severe infections of the central nervous system, nor a head injury requiring medical care. MRI of the brain was normal in all individuals. Autistic patients did not receive any medication with effects on serotonin and/or dopamine metabolism during the study or during the preceding 3 years.

The main study took place in the Department of Pediatrics, Unit of Child Neurology, Kuopio University Hospital, Kuopio, Finland, and 15 children and adolescents were recruited from the Northern Savo Province of Finland. The information of the children with a diagnosis of childhood autism, and aged less than 16 years by the end of year 2003 was gathered from the data system of Kuopio University Hospital, Kuopio, Finland. The families with autistic children were first contacted by phone, and then a personal meeting was arranged with the investigator (IM) for provision of more detailed information. These contacts were made for families with a total of 25 autistic children from 5 to 16 years of age. One family had 3 autistic children, in other families there was only 1 individual with childhood autism. Finally 15 families, with 1 autistic child in each, accepted to participate, and those 15 children and adolescents, aged from 5 to 16 years (mean age 8 years 8 months; 14 males, 1 female), were recruited into the study.

Families refusing to take part in the study had 10 children from 7 to 13 years of age (mean age 10 years; 6males, 4 females). The 3 siblings with autism in the same family were among those who refused to participate.

In addition, 11 autistic children from Southern Finland had been recruited earlier at the

Hospital for Children and Adolescents, Helsinki University Central Hospital, Helsinki, Finland. These 11 children were included in the first phase of the study concerning the measurements of HC and IGF-1. This group of autistic children has been examined earlier in a comparison with 11 children with severe neurological disorders; those results have been published in a report of Vanhala et al. in 2001.

Only those 15 individuals with autism recruited in Kuopio University Hospital participated all the phases of HC, IGF-1, IGF-2, BDNF, SERT, and DAT investigations - presented in detail later in this chapter - prior to fluoxetine treatment.

The diagnosis of the patients with autism in the present study was based on repeated multiprofessional studies, and the criteria of autism were as defined by ICD-10 (World Health Organization, 1993) and DSM-IV-TR (American Psychiatric Association, 2000) criteria.

In the 15 children and adolescents with autism recruited at Kuopio, the investigations prior to treatment included also CARS evaluation (Schopler et al., 1980) for the severity of autism and a psychological study conducted with the Leiter method (Roid and Miller, 1997) to define the intellectual capacity of the subjects. Other commonly used autism assessment instruments such as Autism Diagnosis Interview-Revised (Lord et al., 1994), or Autism Diagnostic Observation Schedule (Lord et al., 2000) were not utilized in the present study because the use of these methods had not yet been established in Finland. However, the basic information received from CARS and ICD-10 criteria were considered as being sufficient for the needs of the present study.

Into the fluoxetine treatment phase, 14 subjects with autism of the Kuopio study group went on; one male subject withdrew because of moving away from the province.

There was one drop-out during the fluoxetine treatment period; an autistic male did not finish this phase due to family request. The reason for withdrawal of consent was a family matter, not dependent on the efficacy or side-effects of the medication.

The phases of the study with autistic children and adolescents in each step are shown in Figure 4.1.

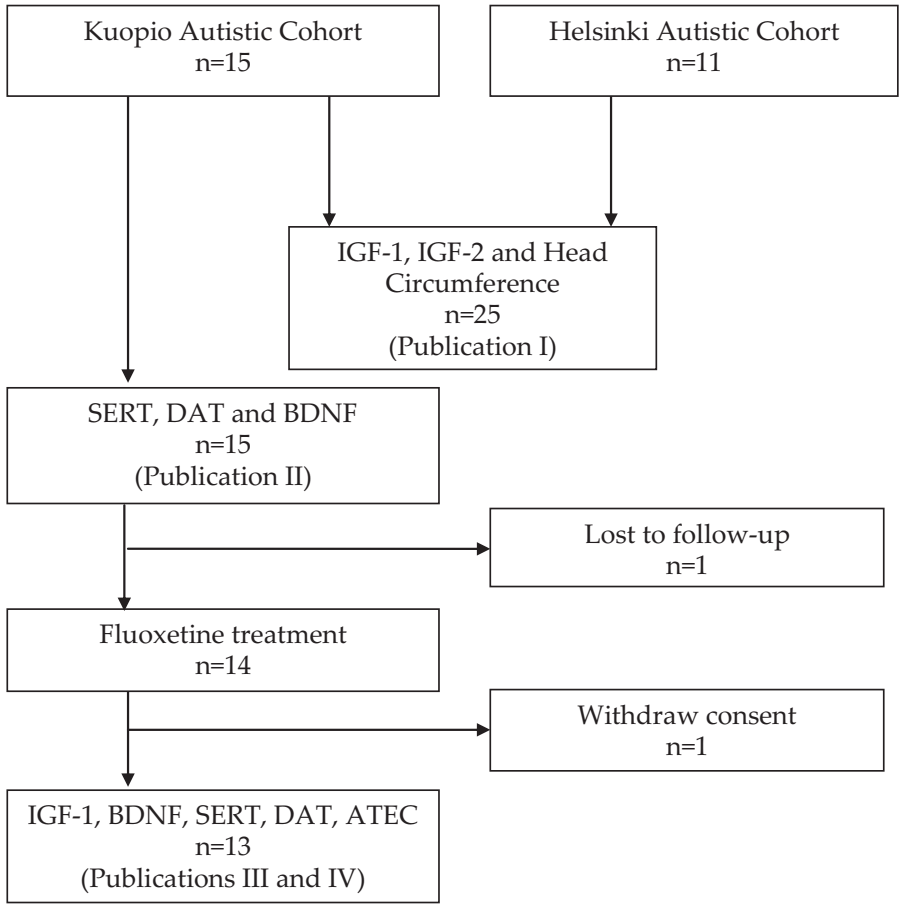

Figure 4.1. Flow chart of the autistic subjects in the study.

4.2.2 Control children

A total of 41 children and adolescents served as controls in the different study stages.
In the HC, IGF-1 and IGF-2 parts of the study 16 age-matched subjects were recruited from children and adolescents admitted to Kuopio University Hospital for surgery on the lower part of the body, to be performed under spinal anesthesia. The control children without autism or any other neuropsychiatric disorder did not have any symptoms or signs of nutritional, metabolic or endocrine disorder that might have influenced the IGF concentrations. The age of these control children was from 1 year to 15 years (mean 7 years 4 months; 8 males, 8 females), and they had following conditions requiring surgery;

herniotomy (n=6), orthopedic or traumatologic operations (n=6), urogenital procedures (n=4).

In the SPECT study, the comparison children were selected from a previous study (Riikonen et al., 2005). These 10 comparison children were examined originally due to their neurological symptoms, excluding autism spectrum disorders or intellectual disability. These children, aged 7 to 14 years (mean age 9 years 10months; 5 males, 5 females), had the following symptomatic diagnoses which warranted a SPECT examination: tremor (n=3), cephalalgia (n=2), tremor and cephalalgia (n=1), epilepsy (n=1), unexplained odd feeling (n=1), motor disturbance (n=1), and long-lasting tiredness (n=1).

In the BDNF studies, 15 neurologically healthy children were recruited from those admitted to hospital for lower body surgery to be performed under spinal anesthesia. These children without autism had no nutritional, metabolic or endocrine disorders that might have influenced the BDNF concentrations. These control children, aged from 4 to 15 years (mean age 8 years 8 months; 13 males, 2 females), had been admitted for the following indications for surgical procedures: inguinal herniotomy (n = 6), orthopedic procedures (n = 6), and operations of urogenital system (n = 3).

The phases of the study in aspect of the control children are demonstrated in a flow chart in Figure 4.2.

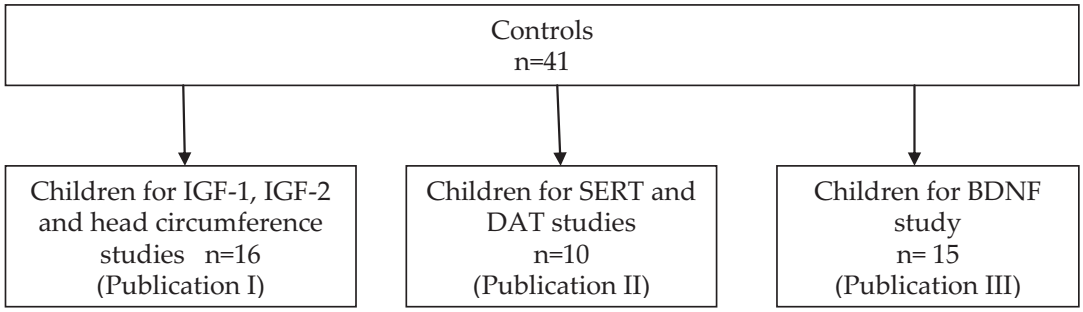

Figure 4.2. Flow chart of the control subjects in the study.

4.3 METHODS

4.3.1 Head circumference

Head circumference was measured at the time of sample collection for the IGF-studies. HC measures were expressed as z scores from the mean HCs of age and sex-matched individuals from the general Finnish population.

4.3.2 IGF-studies

Serum and CSF samples of children with autism were drawn when the patients were slightly sedated (with intravenous midazolam 0.1mg/kg and propofol/thiopental 1-2mg/kg) for the SPECT study and from the comparison children during the spinal anesthesia performed for the surgery on the lower part of the body.

The serum and CSF samples were frozen and stored until analysis. The concentration of IGF-1 and IGF-2 were determined by a radioimmunoassay method (Mediagnost GmbH, Reutlingen, Germany) in the Helsinki University Central Laboratories.

4.3.3 BDNF studies

Serum and CSF samples for BDNF studies were collected from the autistic subjects during sedation required for the SPECT. In the comparison children similar samples were drawn during the lumbar puncture performed as part of the spinal anesthesia for the lower body surgery. The samples were frozen immediately and stored at -70°C until analysis. BDNF analyzes were assayed by the sandwich-enzyme-linked immunosorbent assay method using a commercial kit for human BDNF (Chemicon, Temecula, CA, USA) following the manufacturer´s protocol and including a range of subject sample and technical controls. The sensitivity of the method is 7.8 pg/mL. The BDNF analyzes were performed in the Kennedy Krieger Institute, Baltimore, MD, United States of America.

4.3.4 SPECT imaging

Serial SPECT scans were obtained at 15 minutes, and at 6 and 24 hours after intravenous injection of 80 to 150 MBq of iodine-123 labelled N-(2-fluoroethyl)-2b-carbomethoxy-3b-(4-iodophenyl)-nortropane, ([^{123}I] nor-beta-CIT) (MAP Medical Technologies OY, Tikkakoski, Finland) on a dedicated Siemens MultiSPECT 3 gamma camera with fan-beam collimators (Siemens Medical Systems Inc., Hoffman estates, USA). The SPECT scans were decay-corrected, reconstructed with Butterworth-filtered back-projection and attenuation-corrected with Chang's algorithm (C_μ =0.11 cm^{-1}). Regions of interest were midbrain, temporal lobes, medial frontal cortex (MFC), and striatum. The cerebellum served as a reference region (i.e. a region with non-specific and free binding). The specific values of ligand binding in mL/mL for SERT (midbrain, MFC, and temporal lobes) and DAT (striatum) were calculated using a graphical plot. The slope of this plot is equal to the distribution volume ratio: (Region – Cerebellum) / Cerebellum. Radiation exposure to the participants was 4–5mSv (effective dose).

Sedation was required for the children with autism and adolescents, because they were unable to remain still during the 30 minutes of the SPECT scan. For the 15 minutes and 24 hours SPECT scan the children with autism were given per oral sedation with midazolam, at a dose of 0.3–0.5mg/ kg, (max. 7.5mg) (Dormicum® fluid for injection, 5mg / mL, Roche, Espoo, Finland). For the 6 hour SPECT scanning, intravenous sedation with midazolam

(0.05–0.1mg/ kg), propofol (1–2mg/ kg) and thiopental (1–2mg/ kg) was given. For the non-autistic individuals, the SPECT scans were performed without sedation or anesthesia, because the SPECT scan was done for clinical reasons and no lumbar puncture was performed.

4.3.5 Fluoxetine therapy

Fluoxetine (Seromex® 10 mg soluble tablets, Ratiopharm, Ulm, Germany) was given once daily in the morning. The trial was begun with a dose of 5 mg, which was raised by 5 mg (in children) or 10 mg (in adolescents) every 2 weeks until the highest tolerated dose or a maximum dose of 1 mg/kg or 40 mg total was reached. Children continued to receive special education and other interventions (i.e., speech and language therapy, occupational therapy) without any modification during the treatment trial.

Therapeutic doses ranged from 10–40 mg/day (0.4– 0.9mg/kg/day, mean 0.53mg/kg/day). The pharmacotherapy was given for 6 months, after which fluoxetine was discontinued by decreasing the dose gradually over a period of 2 to 4 weeks. Following this, all patients had a 2-month wash out period before SPECT scanning to exclude any acute effect of fluoxetine on SERTs.

4.3.6 Evaluation of clinical response

The clinical response to fluoxetine was evaluated with a follow-up checklist based on the Autism Treatment Evaluation Checklist (ATEC), an instrument developed by Rimland and Edelson in the Autism Research Institute, San Diego, CA, USA in 2000 and which is published in the Internet as for free use for clinical and research purposes (Website address updated in April 2012: http://www.autism.com/index.php/ind_atec_survey). There appeared to be no Finnish translation for the ATEC available, therefore for the purpose of the present study a translation was made by the first author (IM), and it was checked by the senior researcher (RR).

ATEC has four sections with multiple statements concerning the individual characteristics or abilities:

ATEC 1: 14 items on speech, language and communication
ATEC 2: 20 items regarding socialization
ATEC 3: 18 items on sensory or cognitive awareness
ATEC 4: 25 items on health, physical condition, or behavior

The adverse effects of the fluoxetine treatment were evaluated by including 12 questions in ATEC 4. These questions were selected considering the generally known adverse events of SSRI in children, adapted from a list from the British Columbia's Children's Hospital, Vancouver, Canada (Website address updated in April 2012: http://www.bcchildrens.ca/NR/rdonlyres/25643C99-5CE2-48F4-9757-0DDB45EED706/42895/SSRIteachingsheet1pageJun20091.pdf).

In the original ATEC, there are three alternative choices of answers for each phrase in ATEC 1, 2 and 3 (not true/not descriptive, somewhat true/somewhat descriptive, or very true/very descriptive) and four choices in ATEC 4 (not a problem, minor problem, moderate problem, or serious problem). In the present study, an adapted scale from 1 to 5 was employed in, which meant that the endpoint values were given the following definitions: 1= the statement/observation does not match the child at all, 5 = the statement/observations match the child perfectly.

Baseline information was gathered during an interview with the parents by the investigator (IM) by filling in the ATEC checklist together, this arrangement provided the parents the opportunity to ask clarifying questions if needed. During the treatment period, the parents reported the status every 1-2 weeks by filling out the checklist themselves.

They were encouraged to give more detailed information written in their own words if needed and they were advised to contact the investigators whenever they suspected drug intolerability or adverse effects in their child. After the treatment period, in the follow-up visit, the checklist was again filled out by the parents and the investigator together.

Table 4.1. Autism Treatment Evaluation Checklist - adapted version.

The additive statements or findings considering the possible adverse effects of fluoxetine are

ATEC 4 items 3, 7, 8, 9, 13, 14, 23, 24, 25, 26, 27 and 28.

ATEC 1. Speech/ Language/ Communication

1. knows own name
2. responds to ”no” or ”stop”
3. can follow some commands
4. can use one word at a time
5. can use 2 words at a time
6. can use 3 words at a time
7. knows 10 words or more
8. can use sentences with 4 or more words
9. explains what he/she wants
10. asks meaningful questions
11. speech tends to be meaningful/ relevant
12. often uses several successive sentences
13. carries on fairly good conversation
14. has normal ability to communicate for his/her age

ATEC 2. Sociability

1. seems to be in a shell – you cannot reach him/her
2. ignores other people
3. pays little or no attention when addressed
4. uncooperative and resistant
5. no eye contact
6. prefers to be left alone
7. shows no affection
8. fails to greet parents
9. avoids contact with others
10. does not imitate
11. dislikes being held/cuddled
12. does nor share or show (things meaningful for him/her, e.g.
13. does not wave ”bye bye”
14. disagreeable/ not compliant
15. temper tantrums
16. lacks friends/ companions
17. rarely smiles
18. insensitive to other’s feelings
19. indifferent to being liked
20. indifferent if parent(s) leave

ATEC 3. Sensory/ Cognitive awareness

1. responds to own name
2. responds to praise
3. looks at people and animals
4. looks at pictures (and TV)
5. does drawing, coloring, art
6. plays with toys appropriately
7. appropriate facial expression
8. understands stories on TV
9. understands explanations
10. aware of environment
11. aware of danger
12. shows imagination
13. initiates activities
14. dresses self
15. curious, interested
16. venturesome - explores
17. "tuned in" – not spacey
18. looks where others are looking

ATEC 4. Health/ Physical/Behavior/Adverse Effects

1. bed-wetting
2. wets pants/ diapers
3. other urinary problems
4. soils pants/diapers
5. diarrhea
6. constipation
7. stomach ache
8. sickness
9. sleeplessness
10. other sleep problems
11. eats too much
12. eats too little
13. loss of weight too much
14. increase of weight too much
15. extremely limited diet
16. hyperactive
17.lethargic
18. hits or injures self
19. hits or injures others
20. destructive
21. sound-sensitive
22. anxious /fearful
23. cramps or tics
24. tremble or shakes
25. headache
26. sweating
27. palpitation
28. eczema or rash
29. unhappy / crying
30. seizures
31. obsessive speech
32. rigid routines
33. shouts or screams
34. demands sameness
35. often agitated
36. not sensitive to pain
37. "hooked" or fixated on certain objects / topics
38. repetitive movements (stimming, rocking, etc.)

4.4 STATISTICS

The data of the present study are based on a total of 26 patients with autism who were available for this study. In the Kuopio cohort parents of 25 autistic children were asked for consent, but 10 declined to allow their children to participate. In order to validate the data obtained from autistics children, a comparison group of 16 age-matched children without autistic symptoms was enrolled to compare the correlations of HC measurements and concentrations of IGFs and BDNF in autistic children. For the SERT and DAT binding

capacity, the data of 15 patients with autism was compared with that of 10 age-matched children without autism. No formal sample size calculation was performed but these numbers of autistic children and controls were considered as providing sufficient data for the purposes of this study.

Before performing the statistical calculations, the continuous variables were tested for normal distribution with the Kolmagorov-Smirnov test. For normal distributed data, the unpaired Student's t-test was applied. For non-normally distributed data, Mann-Whitney U test, and Wilcoxon signed ranks test were used. To analyze the differences for the frequency data and the simultaneous group effects the Pearson's $\chi 2$ test and regression analysis were used.

The data were stored and the calculations were made using the Statistical Package for Social Sciences software (SPSS for Windows 17.0, SPSS Inc., Chicago, USA). In all statistical tests, a two-tailed p-value of less than 0.05 was considered statistically significant.

5 Results

5.1 HEAD GROWTH AND INSULIN-LIKE GROWTH FACTORS IGF-1 AND IGF-2

5.1.1. Prior to treatment (Publication I)

In this present study, none of the autistic subjects or the controls had either macrocephaly or microcephaly, i.e. the relative HC score was not beyond the borders of ± 2SD, at the moment of the study. The mean HC was +0.3 SD in the groups of both autistic and control children.

Detailed data about head growth from birth until the date of the present study was available in 14 out of 15 autistic children of the Kuopio cohort. The growth pattern in infancy showed an acceleration of head growth from birth to 3 months of age in 10 infants out of 14. Furthermore, 11 out of 14 had their peak relative HC before 6 months of age, and 2 of them had a single HC measurement with a relative HC score above +2 SDs (Above-mentioned details are not presented in the Publications).

The mean concentration of CSF-IGF-1 (0.41μg/L [SD 0.18]) in 25 patients with autism was significantly lower than that in 16 patients in the control group (0.58μg/L [SD 0.27]; p=0.02, t-test). There was a significant correlation between the CSF-IGF-1 concentration and age both in the children with autism (r=0.68, p=0.002) and in the age-matched group (r=0.56, p=0.025; Fig. 5.1). A post hoc analysis revealed that in the children with autism who were less than 5 years of age (n=11) the mean CSF-IGF-1 concentration (0.27μg/L [SD 0.10]) was significantly lower than that in their age-matched control group (n=5; 0.44 μg/L [SD 0.15]; p=0.014).However, in the children who were 5 years of age or older, there was no difference in the mean CSF-IGF-1 concentration of the children with autism (n=14; 0.52μg/L [SD 0.16]) in comparison with the control group (n=11; 0.64μg/L [SD 0.29]; p=0.20).

The CSF-IGF-1 concentrations correlated significantly with head circumferences in the patients with autism (r=0.48, p=0.014) but not with those of the control group (r=0.06, p=0.85; Fig. 5.2).

There was no difference between the autistic and control groups in CSF-IGF-2 concentration; the mean was 19.1μg/L (SD 3.1) in 25 patients with autism and 20.5 μg/L (SD 4.4) in 16 patients in the control group (p=0.33). There was no correlation between the CSF-IGF-2 concentration and age in the group with autism (r=0.15, p=0.60) but a significant correlation was seen in the control group (r=0.55, p=0.027). There was no difference between the two groups in the correlation with head circumference and CSF-IGF-2 concentration (r=0.53 and r=0.56, respectively).

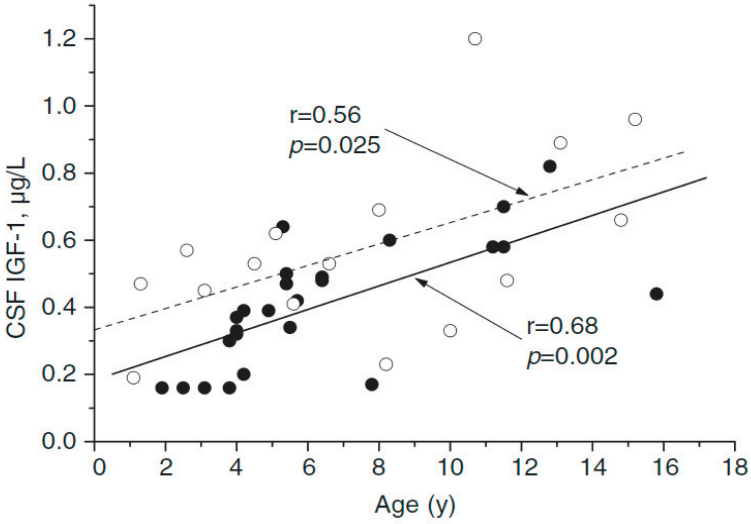

Figure 5.1. Concentrations of cerebrospinal fluid insulin-like growth factor-1 (CSF IGF-1) and age in children with autism (●; n=25), and their age-matched controls (○; n=16).

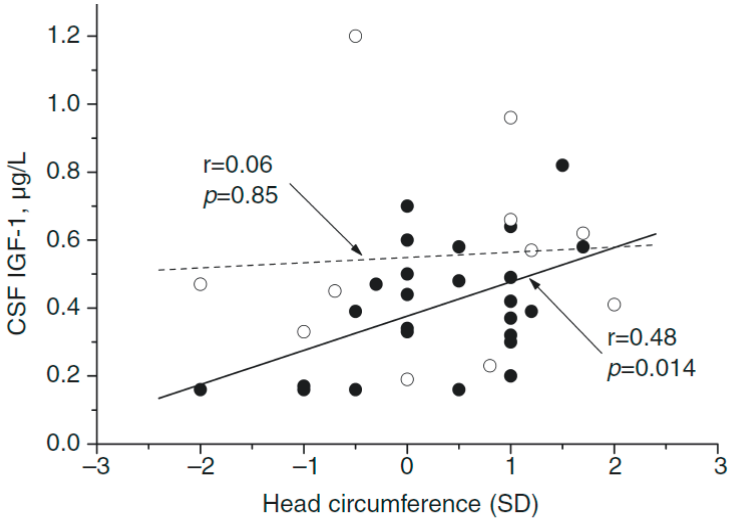

Figure 5.2. Concentration of cerebrospinal fluid insulin-like growth factor-1 (CSF IGF-1) with head circumference (SD) in children with autism (●; n=25), and in their age-matched controls (○; n=16).

5.1.2 After fluoxetine treatment (IV)

Cerebrospinal fluid IGF-1 concentration increased from baseline (CSF-IGF-1 mean 0.53 pg/mL, SD 0.18 pg/mL) to the end of fluoxetine treatment (mean 0.68pg/mL, SD 0.20

pg/mL, p= 0.003 paired samples t-test) in the autistic children. There was no significant correlation detected between the IGF-1 concentration and the clinical response (p=0.86). CSF-IGF-2 concentrations were not investigated after fluoxetine treatment.

5.2 BRAIN DERIVED NEUROTROPHIC FACTOR

5.2.1 Prior to treatment (III)

There was no group difference in serum BDNF concentrations between the autistic subjects (range <7.8–17149 pg/mL, [mean 6330, SD 6591 pg/mL]) and the control group (n = 15) (range 4575–14927 pg/mL, [mean 9652, SD 3223 pg/mL]) (p = 0.08). However, there was a bimodal distribution of BDNF concentration within the autistic group; subgroups had either a remarkably low concentration (n=8, mean 1497, SD 1651 pg/mL) or a concentration which was higher than the mean of the controls (n=5, mean 14062, SD 2026 pg/mL) (Table 5.1).

Table 5.1. Serum brain derived neurotrophic factor concentration and serotonin transporter binding in medial frontal cortex and midbrain, as compared with clinical response after fluoxetine treatment in autistic children.

Age (years)	Serum Brain Derived Neurotrophic Factor (pg/mL)		Serotonin transporter binding capacity (mL/mL)			
			Medial frontal cortex		Midbrain	
	Prior to treatment	After treatment	Prior to treatment	After treatment	Prior to treatment	After treatment
Good clinical responders *						
6	2,954	711	0.05	0.12	1.30	1.11
6	12,837	1,290	0.28	0.15	1.34	1.25
8	17,148	5,557	0.08	0.33	1.07	1.25
11**	11,760	1,728	0.11	0.06	1.00	1.02
13	1,375	1,229	0.23	0.29	1.07	1.10
16	14,320	1,821	0.16	0.20	1.09	1.17
Poor clinical responders *						
5	56	10,390	0.20	0.19	1.18	1.49
5	14,247	13,652	0.06	0.15	1.09	1.15
5	556	5,283	0.32	0.34	1.11	1.32
5	< 8	2,909	0.24	0.19	1.24	1.01
8	1,941	1,229	0.08	0.14	1.02	1.11
12	379	10,035	0.08	0.22	1.06	1.14
13	4,709	5,374	0.07	0.29	1.07	1.35

* According to Autism Treatment Evaluation Checklist Section 2 (ATEC 2); socialization

** Female autistic patient, all other are males

5.2.2 After fluoxetine treatment (III)

In autistic individuals, after fluoxetine treatment, the BDNF concentration was lower (range 711–13652 pg/mL, [mean 4891, SD 4194 pg/mL]) than that determined before the treatment, but the change was not significant statistically (p=0.44). BDNF concentration decreased in eight patients and increased in five, eliminating the bimodality observed prior to treatment.

5.3 SEROTONIN TRANSPORTER

5.3.1 Prior to treatment (II)

SERT binding capacity was significantly lower in autistic children in MFC (p=0.001, Mann–Whitney U test; Fig. 5.3) and in midbrain (p=0.02) than in the controls (Table 5.2). The differences between individuals with autism and the control group in SERT binding capacity in midbrain and in MFC remained significant also when adjusted for age. The regression coefficients were 0.11 units (95% CI: 0.03–0.20, p=0.014) and 0.18 units (95% CI: 0.10– 0.26, p<0.001) for SERT binding capacity in midbrain and in MFC respectively.

After the correction due to the assumed effect of sedation of the patients with autism during the SPECT scans, the difference in SERT binding remained significant in MFC (p=0.002, Mann–Whitney U test), but no longer in the other areas.

Table 5.2. Serotonin transporter (SERT) binding in different brain areas in 15 children with autism and in 10 control children.

Group	Age (years)	SERT (mL/mL)			
		Midbrain	Temporal Left	Temporal Right	Medial frontal cortex
Autistic children					
Mean (SD)	8.7 (3.8)	1.11 (0.09)	0.20 (0.09)	0.20 (0.06)	0.14 (0.10)
Controls					
Mean (SD)	9.8 (2.7)	1.22 (0.12)	0.29 (0.13)	0.29 (0.12)	0.32 (0.08)
p-value		0.02	0.23	0.09	0.001

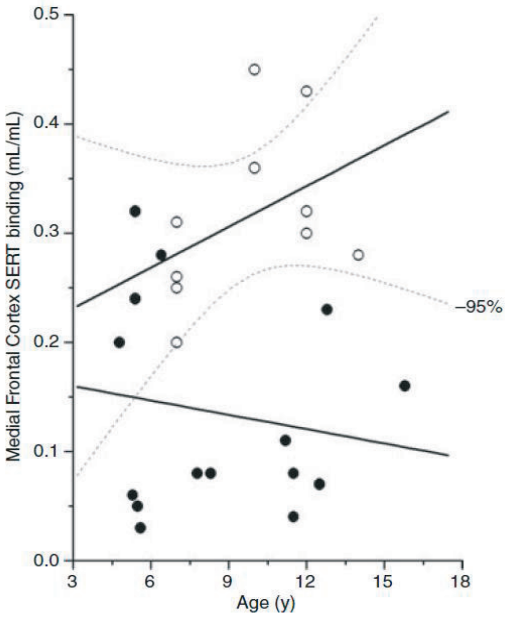

Figure 5.3. Serotonin transporter (SERT) binding in medial frontal cortex and age in patients with autism (•; n=15) and in control group (○; n=10).

5.3.2 After fluoxetine treatment (III)

After the fluoxetine treatment, there was an increased SERT binding capacity in both the midbrain and in the medial frontal cortex, but the change was not statistically significant (p=0.09 in both areas) (Table 5.1). There were no significant correlations between SERT binding capacity in the abovementioned areas and BDNF concentrations, either before or after the treatment.

5.4 DOPAMINE TRANSPORTER

5.4.1 Prior to treatment (II)

The baseline results of DAT binding detected no significant group difference between the autistic children and the control children (p=0.21). However, there was a trend towards a negative correlation between DAT binding capacity and age in the autistic subjects, whereas in the control group the correlation had a positive trend (Figure 5.4).

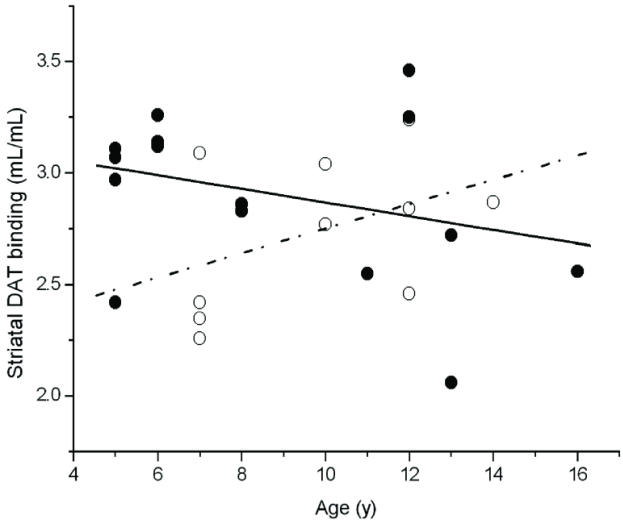

Figure 5.4. A divergent correlation between striatal dopamine transporter binding capacity and age in the autistic children (●) and control children (○).

5.4.2 After fluoxetine treatment (IV)

Six clinically good responders exhibited a significant decrease in striatal DAT binding (-0.25 mL/mL, SD 0.20, $p<0.05$) after the fluoxetine treatment. The other autistic children had a trend to an increase in both DAT binding and serum BDNF concentration after the treatment.

5.5 CLINICAL FINDINGS (III)

Before initiating the present study, the completion of the CARS was performed in the Unit of Child Neurology by paediatric neurologists and a multiprofessional team. The score of the CARS varied from 29 to 41 (mean 34, SD 3) with the IQ (according to the Leiter method) ranging from 70 to 109 (mean 88, SD 12); the latter demonstrating that this was a group of quite high functioning autistic individuals.

Baseline information for ATEC was gathered during an interview of the parents by the investigator (IM). The study design had been structured primarily to detect changes in the clinical condition, not to try to measure the absolute severity of a single feature or symptom. Since ATEC is basically a subjective scale, no further analysis of the baseline information was considered necessary in the present study.

In terms of clinical response, there was a statistically significant favorable effect, after fluoxetine, on 23 items out of the 77 in ATEC; seven items on speech, language, and communication (ATEC 1), eight items concerning socialization (ATEC2), and eight items on sensory/ cognitive awareness (ATEC 3) (Table 5.3). There were no statistically

significant changes in ATEC items dealing with health and physical conditions or behavior (ATEC 4). The positive effects found in the autistic cohort were mainly attributed to a subgroup of six subjects who had a particularly high frequency of favorable scores on the ATEC 2 scale (i.e., socialization). Therefore, subsequent analyses were carried out by dividing the autistic cohort into good (above the ATEC 2 median) and poor (at or below the ATEC 2 median) clinical responders (Table 5.1), as there was such a distinctive division in the autistic cohort.

Also in the ATEC 1 (Speech, language, communication) and ATEC 3 (Sensory/cognitive awareness) sections, the good response was led by the same subgroup of responders, 4 of them in ATEC 1, and 5 of them in ATEC 3, respectively. In ATEC 4, concerning behavior, no statistically significant responses were detected; however, a better response in stereotyped or repetitive behavior was detected in the good responders compared to the poor responders also in this section.

Post hoc analyses revealed that the six good responders displayed a significant decrease in serum BDNF concentrations compared to baseline (p=0.03), in contrast with the seven poor responders, who showed an increase in BDNF or no change. In fact, as a group, there was a trend towards increased BDNF concentrations among the poor clinical responders (p=0.09). SERT binding changes in the midbrain were less pronounced; good clinical responders showed minimal or no differences and most responders in the poor response group showed an increase. Figure 5.5 illustrates these BDNF and SERT binding profiles in terms of scores on the ATEC 2.

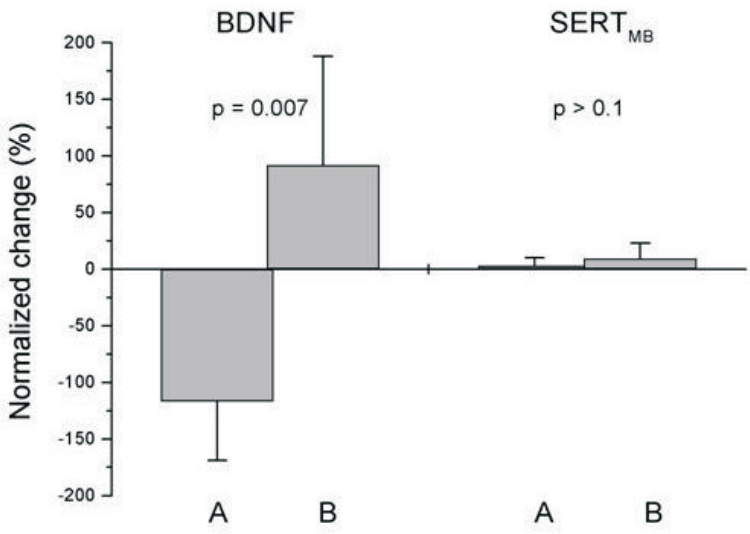

Figure 5.5. Change in serum brain derived neurotrophic factor concentrations and in serotonin transporter binding in the midbrain, in two groups with different clinical responses to fluoxetine, according to the Autism Treatment Evaluation Checklist Section Two, Socialization. Group A (good responders, n = 6) had the best clinical response, while group B (poor responders, n = 7) experienced a less beneficial clinical effect. Error bars indicate the standard deviations. The difference of brain derived neurotrophic factor between the groups was significant. Normalized change was calculated as follows: 100 % (treatment - baseline) / 0.5 (treatment + baseline).

There was no correlation detected between: changes in BDNF concentrations or SERT binding and the baseline cognitive function (i.e. Leiter scale of nonverbal intelligence), or severity of autistic behavior (i.e., CARS scores). However, these baseline measurements did not correlate with the clinical response during the study. There was also no correlation between fluoxetine dosage and any of the above-mentioned variables.

During the trial, symptoms representing possible drug intolerability or adverse effects were detected during the initiation and in the therapy course as the dose were elevated. Those symptoms included hyperactivity (n=5), increase in aggressive behavior (n = 5), and sleep disturbances (n=4). Reducing the dose or refraining from a further dose increase relieved the symptoms. Thus none of the patients needed to discontinue medication because of adverse events during the 6-month treatment period. The parents of nine autistic subjects requested restart fluoxetine treatment again after the washout period and the follow-up investigations had been completed, because in their opinion the positive clinical effects outweighed any adverse effects.

Table 5.3. Clinical response to fluoxetine in autistic children by the Autism Treatment Evaluation Checklist (ATEC).

Parameters	Prior to treatment *	After treatment *	*p-value* **
	Mean (SD)	Mean (SD)	
ATEC1: Speech/language/communication			
Can follow some commands	3.3 (0.8)	3.8 (0.7)	0.006
Knows 10 or more words	3.8 (1.2)	4.4 (0.9)	0.027
Explains what he/she wants	2.4 (1.1)	3.2 (1.1)	0.007
Asks meaningful questions	1.6 (1.0)	2.3 (1.6)	0.027
Speech tends to be meaningful/relevant	2.0 (0.8)	2.9 (1.2)	0.007
Often uses several successive sentences	1.7 (1.1)	2.1 (1.3)	0.041
Carries on fairly good conversation	1.3 (0.4)	1.6 (0.7)	0.038
ATEC 2: Socialization			
Seems to be in a shell - cannot be reached	2.5 (1.1)	2.0 (1.0)	0.026
Ignores other people	3.0 (0.7)	2.5 (0.9)	0.020
Pays little or no attention when addressed	2.5 (0.9)	2.0 (0.8)	0.007
No eye contact	2.5 (1.2)	2.1 (0.9)	0.028
Avoids contact with others	2.8 (0.9)	2.2 (0.7)	0.010
Does not imitate	2.8 (0.7)	2.4 (0.7)	0.050
Does not share or show	3.6 (1.0)	3.2 (1.2)	0.037
Insensitive to other's feelings	3.2 (1.0)	2.8 (1.1)	0.041
ATEC 3: Sensory/cognitive awareness			
Responds to praise	3.8 (0.7)	4.2 (0.5)	0.026
Aware of environment	3.1 (0.7)	3.4 (0.7)	0.041
Shows imagination	2.1 (1.1)	2.5 (1.3)	0.004
Initiates activities	2.7 (0.9)	3.2 (0.9)	0.006
Dresses self	3.8 (0.6)	4.2 (0.4)	0.011
Curious, interested	2.9 (0.8)	3.4 (0.8)	0.006
Venturesome – explores	2.7 (0.9)	3.1 (1.0)	0.010
"Tuned in" - not spacey	1.9 (0.8)	2.4 (1.0)	0.016

* ATEC scale ranged from 1 to 5; in ATEC 1 and ATEC 3 increasing values mean more favorable situation (or treatment response), in ATEC 2 decreasing values, respectively.** Wilcoxon signed ranks test

6 Discussion

6.1 THE STUDY DESIGN

This study arose from a review of previous research on the importance of the growth factors in autism spectrum disorder, including Rett syndrome (Vanhala et al., 1998, Riikonen and Vanhala 1999, Vanhala, 2000 and 2001). Another inspiration was the study of Riikonen et al. (2005) on imaging of SERT and DAT by SPECT in children with fetal alcohol syndrome, a syndrome with an increased risk of autism (Miles et al., 2003, Landgren et al., 2010).

As described in Chapter 4.2, it was not simple to recruit an autistic cohort for such a strenuous and long-lasting study as the present thesis study. To make the sample more homogenous, it was decided that the autistic individuals in the study should have neither antiepileptic pharmacotherapy nor any other psychotropic medication. All the patients had equally good educational and therapeutic facilities, and the present study did not attempt to modify those activities. Recruiting autistic individuals into such an intensive study is not easy. Diversity in patients´ symptoms and in the severity of their autism could not be avoided. It was noted that equalizing all the factors in an autistic population is impossible due to individual characteristics and needs. However, the small number of patients places certain limitations in the possibilities of evaluating the results, both in statistically and clinical respects.

With respect to fluoxetine therapy, a relatively long duration of the pharmacotherapy was regarded as an advantage because autism is a life-long disorder, and the effects of fluoxetine treatment may take a long time to become evident. At the end of fluoxetine therapy, a 2-month washout period was necessary to avoid that the measurements would be affected by the short-term effects in SERT and DAT binding instead of long-term – possibly constant – changes in these transporter proteins. However, the longer follow-up time also increases the possibility of confounding factors influencing the lives of the subjects being investigated, e.g. changes in day care or school.

Children and adolescents are vulnerable study subjects. Performing a SPECT scan on autistic children with low co-operative skills is challenging, and thus the need for sedation was inevitable. In control subjects, this kind of unnecessary sedation would be considered unethical. Thus, estimating the effects of sedation in autistic patients could not be avoided. However, during the repeated SPECT scan after the fluoxetine treatment, a similar type of sedation was used, hence minimizing the effect of sedation in the results.

6.2 HEAD GROWTH

The findings in the present study concerning the head growth patterns of the autistic children during the first year of life are consistent with the findings of increased brain (and HC) growth occuring during the first year of life in autism (Courchesne et al., 2003, Redcay and Courchesne, 2005). In a recently published report of nearly 9000 children

followed up to 3 years of age which sought for an association between head growth and autism, Barnard-Bark et al. (2011) could not detect any significant correlation, but at 9 months of age, the individuals who were diagnosed as autistic later had mean HC 1.3 cm larger than the typically developing children. This emphasizes the proposal that abnormal brain and head growth in autism takes place before the symptoms and signs of autism can usually be observed.

In the present study, a positive correlation between CSF-IGF-1 and HC in the autistic subjects was detected. In autism, a disruption of normal neurobiological mechanisms has been postulated, but it is not known which mechanisms might be involved in these processes. Pathological growth and arrest in brain growth are thought to be restricted to the first years of life. The present study was investigating older children (median age 5 y 5 mo) but even in these children a positive correlation was found between CSF-IGF-1 and head growth in autistic children, but not in controls. This suggests that IGF-1 may have an essential role in the pathogenesis of the aberrant brain growth in autism. No correlation was found between head growth and IGF-2 concentrations.

Further research should be focused on younger children to elucidate the process of brain pathology in autism. The present study suggests that IGF concentrations may play an important role in the pathophysiology of autism.

In general, one is unlikely to be able to obtain CSF samples for analysis of growth factors from young children under 2 years of age is, and autism is seldom diagnosed before 2 years of age, and invasive procedures in such young children always need special permission. In the future, it would be important to evolve non-invasive methods to investigate IGFs in young children with a suspicion of autistic development or an increased risk of autism, for example, baby siblings of an autistic child.

6.3 GROWTH FACTORS

6.3.1 IGF-1

In the present study, low concentrations of CSF-IGF-1 were detected in the children with autism of 5 years of age or younger. This is consistent with the proposal of an early perturbation in brain development during prenatal and early postnatal life. Anlar et al. (2007) reported lower urinary excretion of IGF-1 in autistic children between 2 to 5 years of age as compared with age-matched controls. They reported no correlation between the IGF-1 with the severity of the autistic symptoms, i.e. similar to the findings in the present thesis study.

Mills et al. (2007) have reported increased plasma concentrations of IGF-1 and IGF-2 in 71 autistic children aged 4 to 8 years as compared with age-matched controls. In the present study, no correlation was detected between the serum and CSF concentrations of IGFs, thus the findings of Mills et al. cannot be directly compared with those in the present study. However, interestingly Mills et al. (2007) found a positive correlation between IGF-1 and HC, but no correlation between IGF-2 and HC, similarly as the results of the present study.

The effects of recombinant IGF-1 in brain growth have been evaluated in children. Pediatric patients with Laron syndrome, dwarfism with insensitivity to growth hormone,

and very low concentrations of IGF-1, displayed a rapid increase in HC after initiation of IGF-1 therapy (Laron, 1999). This reflects the ability of IGF-1 to stimulate brain growth and indicates that IGF-1 therapy could be potentially useful in autism. However, further studies will be needed to confirm this hypothesis.

The role of neurotrophic factors, including the IGFs, has been investigated in several pediatric disorders involving the central nervous system; including neonatal asphyxia, infantile spasms, various white matter diseases, infantile ceroid lipofuscinosis, and autism (Riikonen, 2007). Treatment trials for these indications still remain to be conducted in pediatric patient populations.

In animal models, IGF-1 has been effective in treatment of cerebellar ataxia (Fernandez et al., 2005) which is interesting in relation to autism since both conditions share the characteristics of prominent damage of cerebellar Purkinje cells (Bauman and Kemper, 2005). The potential use of IGF-1 therapy has also been proposed in certain neuromuscular disorders such as muscular dystrophies and amyotrophic lateral sclerosis, yet transferring the results of experimental studies to practice in humans has not been done (Jablonka et al., 2011).

6.3.2 BDNF

In the present study, prior to fluoxetine treatment a bimodal distribution of BDNF concentrations was detected in the autistic cohort. Although the basis for this finding remains unknown, it may represent additional evidence of the heterogeneous etiology and pathogenesis of otherwise homogenous autistic behavioral phenotypes. In the present study, children with relatively higher baseline BDNF concentrations, who experienced a decrease in this biomarker after fluoxetine treatment, exhibited a more beneficial clinical outcome. Consistent with this, the behavioral response to antidepressant treatment is lacking in depressed individuals with either reduced brain BDNF concentration or inhibited signaling of its receptor TrkB (Castren et al., 2007). A certain level of BDNF might be required in order to obtain a positive response to SSRIs and this could, at least in part, explain the less favorable response in autistic children with low baseline BDNF concentrations.

The decrease in serum BDNF concentrations after fluoxetine treatment, seen in the good responder group, may reflect the correction of BDNF hyperactivity. Indeed, excessive BDNF can be deleterious for neuronal development, leading to a premature termination of cortical plasticity (Huang et al., 1999). Thus, in autism, BDNF may be a factor leading to precocious maturation, limiting the brain's ability to refine its synaptic processes. Therefore, a hypothetical target for early intervention in autism would be to decrease BDNF signaling via blockade of TrkB receptors or reduction in BDNF synthesis. This might possibly extend the period of synaptic plasticity and make it possible to overcome the atypical development seen in this disorder (Bethea and Sikich, 2007, Tsai, 2006).

The findings in the present study are important since excess of BDNF during critical periods of development might lead to precocious maturation and thereby limit the brain's ability to readjust to the changing environment and experiences later in life (Hanover at al., 1999, Huang et al., 1999).

Reports on serum BDNF concentrations have been quite inconsistent in the literature. In a recent report of serum samples collected at birth, BDNF concentrations were similar in

infants who were later diagnosed as autistic or had an intellectual disability, as compared with those who enjoyed normal development (Croen et al., 2008). Connolly et al. (2006) found elevated serum BDNF concentrations in preschool age children with autism or childhood disintegrative disorder, when compared with children with normal development or other neurological disorders. They also reported a positive association between immunological abnormalities, namely autoantibodies against BDNF and other antigens (e.g., myelin basic protein), and BDNF concentrations. These findings are consistent with those of Miyazaki et al. (2004), who found higher BDNF concentrations in children and in young adults with autism (and others with intellectual disability) as compared with those of healthy adult controls. However, another study demonstrated low serum BDNF concentration in adult males with autism (Hashimoto et al., 2006).

This discrepancy suggests that BDNF concentrations may be variable in autistic individuals and that they may also depend on their age. As far as is known, there have been neither any longitudinal studies of BDNF concentrations in autistic subjects nor evaluations of BDNF changes occuring after treatment of the disorder. For these reasons, the findings in the present study represent a contribution to the increasing body of literature on the role of BDNF in autism (Connolly et al., 2006, Croen et al., 2008, Nishimura et al., 2007).

In the present study, the genetic background of the autistic individuals was not evaluated except for ruling out major chromosomal disorders and fragile X syndrome. However, the importance of growth factors has been emphasized in autism models in some genetic disorders. In most cases of Rett syndrome, the mutated protein MeCP2 seems to regulate BDNF transcription, and cortical BDNF concentrations are reduced in mouse models (Kaufmann et al., 2005). Furthermore, BDNF polymorphisms appear to modify the propensity to seizures that are a key phenotypical feature in Rett syndrome (Zeev et al., 2009). However, in the study of Vanhala et al. (2001) no difference in serum BDNF concentrations was detected between autistic children and age-matched controls.

Experimental studies have revealed that BDNF can regulate the expression of the fragile X mental retardation protein in neuronal cell cultures, and an overexpression of BDNF receptors TrkB has also been detected in these cells (Castren et al., 2002). In patients with fragile X syndrome, an association between vulnerability to epilepsy and BDNF gene polymorphisms has been reported recently (Louhivuori et al., 2009). Tsai (2005) has postulated that early BDNF hyperactivity may represent a critical factor in the origin of autism as well as an explanation for the increased incidence of epilepsy in autism. In the present study, a bimodal distribution of BDNF concentrations was detected, but none of the autistic individuals had epilepsy, thus, one cannot draw any further conclusions about the role of BDNF and vulnerability to epilepsy.

The effects of BDNF are mediated mainly through its TrkB receptor (Binder and Scharfman, 2004). Tsai (2005) has postulated that overexpression of BDNF-TrkB would be implicated in the pathogenesis of epilepsy, mania and autism. Thus, TrkB might be a potential target for pharmacological compounds in the treatment of these disorders. Peptide molecules targeting TrkB as partial agonists with respect to BDNF have been developed and tested in cell cultures (O'Leary and Hughes, 2003), but it will be a long time before they reach clinical use.

In the present study, it would have been interesting to determine the BDNF concentration in CSF; in fact this was an initial aim. However, the detection level of the analytical method was not sufficiently sensitive for this measurement. Not surprisingly, there are no studies reporting successful measurement of CSF-BDNF in autistic children.

In patients with Rett syndrome, Vanhala et al. (1998) reported that CSF-BDNF concentrations were below the detection threshold. In another context, a recent report of adult patients with posttraumatic stress disorder claimed that the concentration of CSF-BDNF and CSF-IGF-1 did not differ between the patients and the controls (Bonne et al., 2011). Moreover, the concentrations BDNF and IGF-1 in CSF were not related to the clinical response to paroxetine, a drug in the SSRI family, given to the patients with post-traumatic stress disorder (Bonne et al., 2011)..

The bimodal distribution of BDNF concentrations detected in the present study has not been reported in earlier studies in autism and certainly needs to be confirmed in future studies. However, it is noteworthy that a wide range of inter-individual variation of BDNF concentrations has been detected in autism. Miyazaki et al. (2004) found a 7-fold variation in serum BDNF concentrations, but the inter-individual variation of the BDNF concentrations in healthy control children was almost equally wide. This may reflect either the general heterogeneity in BDNF concentrations or the variance in backgrounds of autism, but it does emphasize the difficulty in the interpretation of an individual measurement at one certain moment. Thus, BDNF concentrations in autism must be interpreted with caution until confirmatory studies with more subjects have been performed.

At the time when the present study was carried out, no reports on the possible effect of pubertal status in BDNF concentrations had been reported. Recently, Iughetti et al. (2011), detected lower plasma BDNF concentrations in pubertal boys as compared with pre-pubertal boys, or in girls regardless of their pubertal status. In this thesis study, 10 autistic children (9 boys and 1 girl) aged 11 years or below were clearly in pre-puberty, 4 boys aged 12-13 years were in mid-puberty, and 1 boy aged 16 years in late puberty. No statistical correlations with serum BDNF concentration and age or pubertal status could be detected in post-study calculations. Neither pubertal status nor body mass index was recorded in the controls.

Furthermore, lower serum concentrations of BDNF have been reported in typically developing children and adolescents who are overweight as compared with their normal-weighted peer (El-Gharbawy et al., 2006), but in the present study no correlation was detected between serum BDNF concentration and the body mass index.

6.4 MONOAMINE TRANSPORTERS

6.4.1 SERT

In children and adolescents with autism, there had been conducted no research into monoamine transporters, SERT and DAT, binding capacity with SPECT at time when the present study was designed and launched at the end of 2003. Later Nakamura et al. (2010) have reported SERT binding capacity in autism, but they investigated autistic adults with PET.

In the present study, prior to fluoxetine treatment, a lower SERT binding capacity was detected in MFC (equivalent to anterior cingulate cortex), in midbrain, and in temporal lobes of individuals with autism as compared with the non-autistic control children. The

difference became more evident with increasing age as SERT binding in autistic subjects decreased with age whereas in control subjects, SERT increased. Chugani et al. (1997) have evaluated the serotonergic system with PET in autistic children, and the results of that study can be contrasted with the findings of the present study. Chugani et al. (1997) indicated that children with autism lack an early peak in serotonin synthesis during the first 5 years of life. In the present study, the lower SERT binding capacity in brain in the autistic individuals may reflect a reduced serotonin synthesis capability during the preceding years. The observation of the ongoing decrease in SERT with age in the present study indicates that the delayed increase in serotonin synthesis towards adolescence found by Chugani et al. (1997) may not be sufficient to compensate for the early emerging deficit.

Another interesting relationship with the profile of serotonin synthesis capacity has been pointed out by Bethea and Sikich (2005); in post-mortem specimens from children, the profile of synaptic density during infancy and childhood demonstrated by Huttenlocher (1979) resembles that of the development of serotonin synthesis. There have been no studies into synapse density in brain tissue of young autistic patients.

The relationship between the serotonergic system and autism has been investigated in experimental studies. Boylan et al. (2007) reported that depletion of serotonergic neurons in the bilateral medial forebrain bundle in mice evoked a reduction in SERT density in cortex and hippocampus, and to social and behavioural impairments akin to those seen in autism. The observations by Boylan et al. (2007) support the interpretation that early serotonergic aberration in children with autism might lead to decreased serotonergic synapse density, and further to the evolution of the differences in SERT binding capacity. In the present study, SERT binding capacity was lower in all evaluated regions, before the estimated correction of effects of sedation was performed.

Findings consistent with the present study have been recently presented by Nakamura et al. (2010) in a PET study with adult men with high functioning autism. They found low SERT binding density in cingulate cortex (equivalent to MFC in the present study) and thalamus. Moreover, this reduced SERT binding displayed a positive correlation with impairments in social cognition and repetitive and/or obsessive behavior (Nakamura et al., 2010).

Incorporating a genetic study with polymorphism of SERT gene into the present study would have been interesting, because SERT gene polymorphism has been investigated in autism (Devlin et al., 2005). However, the resources in the present study were not available to undertake this kind of examination.

In the present study, after the 6-months of pharmacotherapy with fluoxetine and the 2-months washout period, a trend was detected towards an increase in SERT binding capacity both in MFC and in midbrain. The initial hypothesis in the present study was that fluoxetine might increase the serotonergic activity, and in that way also SERT binding capacity, through the effect of growth factors IGF-1 and/or BDNF. However, no correlation was detected between the changes in SERT binding and those of in IGF-1 and BDNF concentrations. Despite the lack of any significant relationship between fluoxetine treatment and the increase in SERT binding, it can be speculated that fluoxetine treatment did cause a trend towards changes in serotonergic metabolism in MFC and in midbrain. Serotonin and BDNF are associated with the pathogenesis of some neurological disorders, including Alzheimer's disease (Mattson et al., 2004). Thus, further studies evaluating BDNF and serotonin interactions should be conducted also in autistic populations.

There are several possible mechanisms that can be involved in SERT binding capacity;

the concentration of serotonin in the synapse, the density or affinity of transporters, or the actual number and density of synapses themselves. Based on the data about serotonin synthesis capacity in children and adolescents with autism, the most plausible interpretation of the results of the present study is that the decline in SERT binding capacity was due to a reduced number of serotonergic synapses and number of serotonin transporters.

6.4.2 DAT

Before the fluoxetine treatment, DAT binding in striatum of autistic children was highest in the youngest individuals, and then decreased towards adolescence, but in the age-matched controls DAT binding increased with age.

Xiao-Mian et al. (2005) reported increased striatal DAT binding in 10 autistic boys aged between 3 and 10 years as compared with normally developing controls. The findings in the present study in autistic individuals below 10 years of age are consistent with those in the study of Xiao-Mian et al. (2005). In a PET study, Nakamura et al. (2010) reported higher DAT binding in orbitofrontal cortex in adult autistic men.

The results of the present study, along with those of Xiao-Mian et al. (2005) and Nakamura et al. (2010), can be interpreted as being in agreement with with the theory of a hyperdopaminergic state in the etiology of autism as proposed by Previc (2007). In that theory, Previc (2007) introduces the idea that maternal and environmental risk factors during pregnancy, and soon after birth, create a hyperdopaminergic state that is devastating for the development of brain. Previc has proposed this hyperdopaminergic theory as an explanation for the rapid increases in the occurrence of autism. In the present study, an increase in CSF IGF-1 could have a neuroprotective effect against the neurotoxicity induced by the hyperdopaminergic state.

The present study is the first to have undertaken striatal DAT measurements in response to fluoxetine treatment in autistic individuals. After fluoxetine treatment, interesting changes were detected in striatal DAT binding. The six autistic patients with the favorable clinical response had a decrease in DAT binding capacity. In contrast, those 7 children out of 13 who gained less benefit from pharmacotherapy displayed no change or a slight increase in DAT binding capacity. As there are no comparable pharmacotherapy studies in autism, comparisons have to be done with studies performed in other neuropsychiatric disorders. Kim et al. (2006) reported a decrease in DAT density in the basal ganglia in 10 adult patients with obsessive compulsive disorder after a 16-week treatment with SSRIs, including fluoxetine and all patients were reported to have enjoyed a clinically good response. The results in the present study are consistent with those findings. As in the patients with obsessive compulsive disorder, a decrease in DAT binding capacity was detected in the autistic children with a good clinical response to fluoxetine. However, DAT binding capacity prior to treatment was not a predictive value in this autistic population.

SSRI therapy also affects the dopaminergic system: Hood et al. (2010) reported a study of adults with social anxiety disorder, introducing an experiment with a dopamine activation condition (with pramipexole) or a dopamine blockade condition (with sulpiride). Those patients that had not received any preceding SSRI pharmacotherapy displayed increased anxiety in both dopamine activation and dopamine blockade

condition, but the patients with SSRI therapy had significantly less anxiety during the same tests. Hood et al. (2010) concluded that SSRIs probably desensitize dopamine receptors in the limbic system, and therefore they alleviate the patients' anxiety response to dopaminergic stress. On the other hand, Segawa (2006) has presented a theory of supersensitization of dopamine receptors in autism, and they reported improvement in undesired awakenings during night sleep, decrease in aggressiveness and panic states, and less hyperkinesia in autistic children after administration of small doses (0.5mg/kg/d) of L-dopa. Segawa interprets the beneficial effects of L-dopa to be mediated probably through desensitization of dopamine receptors.

In conclusion, there is growing evidence for an interplay between SERT and DAT functioning. It is important to remember that SSRIs like fluoxetine act on both serotonin and dopamine systems.

6.5 CLINICAL EFFECTS OF FLUOXETINE

The main purpose of providing fluoxetine therapy was to investigate the connections between the growth factors and monoamine transporters, as reported in the previous chapters.

In the present study, significant favorable effects were detected on 23 out of the 77 items in ATEC although it took several weeks for the positive response to emerge. Mostly, the favorable responses were seen at 12 weeks after the initiation of fluoxetine treatment, and the response was most evident at the end of the 6-month treatment period.

The variety in favorable response was wide ranging; positive effects on sociability (ATEC 2) and sensory/cognitive awareness (ATEC 3) were more prominent than those in speech, language and communication (ATEC 1). The results in the present study were somewhat different from those reported by DeLong et al. (1998 and 2002) in autistic children, as they reported positive effects of fluoxetine mainly on language and communication. In addition, DeLong et al. (1998) described marked responses already during the first weeks of treatment, and in some individuals, the improvement was so great that they reported that those patients no met the criteria of autism. In the present study, the onset and degree of beneficial responses was less significant and all subjects still met the criteria for autism after fluoxetine therapy.

The time span of several weeks between the start of fluoxetine treatment before the emergence of the clinical response is consistent with the findings in studies with other patient groups, for example subjects with depression. It has been postulated that the response may take time because of changes in neuronal networks need time to become established and consolidated. In these mechanisms, growth factors, especially BDNF, are believed to play a critical role (Castren, 2004, Castren and Rantamäki, 2010).

The positive effects found in the autistic cohort were mainly attributable to a subgroup of six subjects, the good responders, who had a particularly high frequency of favorable scores on the ATEC 2 scale (socialization). In the seven subjects with less beneficial clinical response, symptoms concerned with obsessive-compulsive-like behavior or stereotypic behavior did not differ during the baseline or at the end of treatment. Thus, it was not possible to repeat the recent observations by Hollander et al. (2005) as they reported a decrease of repetitive behaviors in children with autism after an 8-week fluoxetine

therapy. In another recent placebo-controlled study with citalopram (an SSRI drug) therapy for 12 weeks, there was no significant response detected in repetitive behavior symptoms in children with ASD (King et al, 2009).

The true clinical significance of the therapy responses measured by ATEC and the actual relief experienced during fluoxetine therapy are difficult to estimate and quantify: change of one point in ATEC cannot be compared between subjects precisely. However, the general improvement during fluoxetine treatment is emphasized by the fact that the parents of nine autistic subjects requested that fluoxetine treatment should be reinstalled again after this study had been completed. They stated that the positive clinical effects and experiences during the fluoxetine therapy clearly outweighed any adverse effects or the trouble of remembering the daily dose of medicine. The favorable clinical response for fluoxetine treatment was evident, but the lack of placebo group leads to uncertainty about whether one can generalize those results.

6.6 FUTURE PERSPECTIVES

Investigating correlations between the genetic factors and the inter-individual variation in the anatomical and physiologic findings in brain might provide a way of making rapid progress in clarifying the etiology of autism. It can be seen already now that the realization that those genetic disorders in which there is increased incidence of autism have revealed the involvement of some putative novel mechanisms in the pathogenesis of autism. A minority of autistic subjects can be identified who present a syndromic background like fragile X syndrome, tuberous sclerosis or major anatomical defects of central nervous system, but more detailed genetic susceptibility or more sophisticated neuroanatomical, and metabolic connections have been described (Miles, 2011, Ecker et al., 2010, Benvenuto et al, 2009, Schiff et al., 2011). However, the great majority of autistic individuals do not have any of those single-gene syndromes for which tests are available today. Thus, mapping more genes in non-syndromic autistic patients could have great importance. Genetic testing is also much easier to performe than using different imaging methods or neurophysiological tests.

However, genetic factors alone are most unlikely to provide all the solutions. The development of new non-invasive methods for the examination of the nervous system, such as functional MRI, transcranial magnetic stimulation and diffusion tensor imaging might represent new possibilities since they could lower the threshold lower to undertake examinations for individuals with a reduced ability to co-operate, such as children with autism. New generations in SPECT and PET scanners with higher capacity and thereby more rapid imaging with less radiation exposure would also be helpful. The non-invasive and patient-friendly methods would make it possible to expand the studies to include the youngest children in whom there are initial suspicions of autism, or even simply an increased risk of autism, such as siblings of autistic children. Thus, clarifying the early phases of autistic development could be possible.

Autism is a heterogeneous entity and the additive nature of different factors often makes the results difficult to interpret. Therefore, more details about the clinical characteristics of autistic individuals could help to achieve more relevant results, even in respect of the genetic or neurophysiologic studies: e.g. it is probable that the

heterogeneous phenotypes of autism have also heterogeneous genotypes. Thus, gathering clinical information by interviewing parents and undertaking observations during diagnostic studies or follow-up visits should record the individual characteristics collecting as many specific details as possible. This would make different studies more comparable and contribute to developing more detailed study designs in the future.

It is not easy to obtain a carefully selected study sample of autistic patients. In Finland there is no distinct research center specializing in autistic individuals. Childhood autism is a rather rare disorder, and the population of Finland is rather small, 5.4 million inhabitants. According to the latest predictions, the yearly incidence will be about 60 new cases of childhood autism, and 180-240 with other autism spectrum diagnoses in Finland. As the population is scattered over a relatively large area and transportation from the home to a research center could be troublesome for autistic patients, good collaboration between physicians and hospitals would benefit the research in this field of science.

In the near future, new revisions of both DSM and ICD will be published. However, a new diagnostic methodology can make it difficult to compare the results of studies performed during different decades, as patients would have been selected with different criteria. In Finland, Mattila et al. (2011) have recently published an epidemiological study comparing DSM-IV Text Revision (American Psychiatric Association, 2000) and DSM-5 draft criteria (www.dsm5.org) for autism spectrum disorders, screening an age cohort of more than 5,000 children aged 8-years in Northern Finland. By applying the DSM-5 draft criteria in the cohort which had been earlier diagnosed with DSM-IV Text Revision criteria, the researchers reported a remarkable decrease in number of subjects meeting the criteria, especially in those subjects with high functioning autism or Asperger syndrome. This emphasizes the challenge in defining the diagnostic criteria of ASD.

Another interesting aspect in autism research is the difficulty in making a differential diagnosis between ASD and the presentation of autistic traits in general population. This seems to be due to the fact that in individuals with Asperger syndrome or pervasive developmental disorder not otherwise specified, the degree of symptom severity or behavioral impairment shows an interaction with environmental factors (Baron-Cohen et al., 2009). Individuals with autistic traits, like family members and relatives of individuals with ASD, might represent an interesting population for autism research, and they would be much more cooperative than the autistic patients themselves.

In the future it would be interesting to combine pharmacologic and conventional therapeutic methods with neurobiological research targets. However, this would require a precise selection of patients and detailed psychological studies prior to the therapeutic intervention. A multidisciplinary study group would be necessary for conducting these kinds of projects. However, small clinics with limited resources can succeed with well focused studies with relatively small patient populations, as the present study demonstrated.

7 Conclusions

The first aim of the present study was to measure growth factors IGF-1, IGF-2, and to correlate them with head growth, secondly to evaluate monoaminergic neurotransmission, through evaluation of SERT and DAT as determined by SPECT, and thirdly to evaluate BDNF in serum and in CSF, in autistic children and adolescents, and thereafter to undertake 6-months of pharmacotherapy with fluoxetine, and to evaluate the effects of fluoxetine on levels of IGF-1, BDNF, SERT, DAT, as well as in the clinical response.

The main conclusions that can be drawn from this study are:

1. Cerebrospinal fluid IGF-1 concentration was lower in the younger autistic children under 5 years of age compared with controls. The lower IGF-1 concentration might account for the aberrations in neurobiological mechanisms and be an etiopathogenetic link to loss of cerebellar Purkinje cells.
   There was a positive correlation between the HC and CSF-IGF-1 concentration in autistic children but not in the children with normal development. This might reflect a different response to IGF-1 in autistic individuals as comparedwith normally developing children.

2. Serotonin transporter binding capacity was lower in the SPECT imaging in MFC and in midbrain of the autistic children compared to age-matched non-autistic comparison children. This finding confirms the suspicions that there is disruption to the serotonergic dysfunction in autism.
   Prior to fluoxetine treatment, striatal DAT binding displayed a different correlation with the age of the individuals in the autistic group and in the comparison group. The DAT binding was highest in the youngest autistic patients and then decreased with the age but in the comparison children, DAT binding increased with age. This is pointing to the existence of an early hyperdopaminergic state in autism which might provide inappropriate conditions for neuronal development and survival.

3. Prior to treatment, a bimodal distribution was detected in the serum BDNF concentration in the autistic subjects, and the inter-individual variation in BDNF concentrations was higher in the autistic subjects, as compared with the controls, highlighting the heterogeneity in autistic individuals.

4. After 6-months of fluoxetine treatment:

   a. A positive clinical response in several aspects of communicational, social and cognitive functions was detected. This beneficial response was most evident in a subgroup of six autistic patients, the good clinical responders.
   b. In the good clinical responders, the beneficial effects of fluoxetine were associated with a decrease in serum BDNF concentration.

c. A slight increase in SERT binding capacity was detected both in MFC and in midbrain, but this change was not significant. The changes in SERT binding capacity did not correlate with the clinical response.
d. In the good clinical responders, DAT binding decreased, but remained unchanged or even increased in those with poor clinical response.
e. An increase in the CSF IGF-1 concentration was detected but the change exhibited no correlation with the clinical response. The increase in CSF-IGF-1 could have a neuroprotective effect against the neurotoxicity induced by the early hyperdopaminergic state.

However, the extensive inter-individual variation in the concentrations of growth factors and in the binding capacities of SERT and DAT was too large to have any direct implications on the everyday practice with autistic individuals.

A favorable clinical response for fluoxetine treatment was detected, but the lack of placebo group makes it difficult to generalize the results. Fluoxetine treatment was associated with only a low incidence of adverse events, evidence that fluoxetine treatment was well tolerated. Fluoxetine could be considered a safe choice of pharmacotherapy in selected patients; it may improve sociability, communicative skills, and sensory awareness in children and adolescents with autism. However, no medication is believed to be able to improve the symptoms and behavior of the autistic individuals alone, but in combination with behavioral, occupational, psychological, and speech therapies, it could represent a valuable adjuvant in a condition with few treatment options available.

The results of this study suggest that fluoxetine may modulate the monoaminergic system and neuronal growth factors, which are disturbed in autistic individuals. By acting on these factors fluoxetine may promote recovery and restore connections of cerebellar Purkinje cells. This finding indicates that fluoxetine may have an influence on the development of communication and social interaction skills, both of which are seriously disturbed in autism.

Future research focusing on the early process of brain pathology and relevant genes, it will likely be critical in elucidating the etiology of autism and in developing rational treatments. Treatment should be given as early as possible to support the normal synaptic development and the survival of neurons and their connections, including the cerebellar Purkinje cells.

8 References

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APPENDICES

LAPSUUSIÄN AUTISMIN FLUOKSETIINIHOIDON SEURANTALOMAKE

ORIGINAL PUBLICATIONS (I-IV)

Kuopion Yliopistollinen Sairaala
Lastenklinikka / Lastenneurologian yksikkö

LAPSUUSIÄN AUTISMIN FLUOKSETIINIHOIDON SEURANTALOMAKE

Merkitään väitteen tai havainnon sopivuus lapseen asteikolla 1-5 siten, että
1= ei sovi lainkaan, 5= sopii täysin (myös desimaalilukujen, esim. 3,5 käyttäminen on mahdollista, jolloin etenkin seurannan aikana mahdollisesti tapahtuvan muutoksen merkitseminen helpottuu)

Potilas _____ Lääkitys_____

Alkamispäivämäärä_____

Lääkitys

päivämäärä									
annos									

I. Puhe/ Kieli/ Vuorovaikutus

1. tuntee oman nimensä									
2. reagoi "ei" tai "seis" sanoihin									
3. pystyy noudattamaan joitakin kehotuksia									
4. osaa käyttää yhtä sanaa kerrallaan									
5. osaa käyttää kahta sanaa kerrallaan									
6. osaa käyttää kolmea sanaa kerrallaan									
7. tuntee enemmän kuin 10 sanaa									
8. osaa käyttää yli 4 sanan lauseita									
9. osaa kertoa sen mitä haluaa									
10. osaa tehdä kysymyksiä, jotka ovat mielekkäitä									
11. puheen sisältö asiaan liittyvää ja merkityksellistä									
12. käyttää usein puhuessa useita lauseita peräkkäin									
13. pystyy juttelemaan/ keskustelemaan ikätasonsa mukaisesti									
14. pystyy ikätasonsa mukaiseen normaaliin vuorovaikutukseen									

II. Sosiaaliset taidot

1. on kuin kuoren sisällä tavoittamattomissa									
2. jättää toiset ihmiset huomioimatta									
3. ei kiinnitä lainkaan tai vain vähän huomiota sitä häneltä aktiivisesti pyydettäessä									
4. ei suostu yhteistyöhön tai vastustaa voimakkaasti									
5. ei tule katsekontaktiin									
6. jää mieluummin yksin kuin on toisten kanssa									
7. ei osoita kiintymystä									
8. ei tervehdi / ilahdu vanhempien tullessa paikalle									
9. välttää ottamasta kontaktia toisiin ihmisiin									
10. ei matki / jäljittele toisten toimintoja tai leikkejä									
11. ei pidä sylissä olemisesta tai hellimisestä									
12. ei esittele toisille tai jaa toisten kanssa itselleen tärkeitä asioita (esim. lelut, leikit, harrastukset)									
13. ei vilkuta hyvästiksi "hei hei"									
14. vaikeasti suostuteltavissa, "vastarannan kiiski"									
15. saa kiukkukohtauksia tai "raivareita"									
16. ei kavereita kerhossa tai koulussa									
17. hymyilee vain harvoin									
18. ei reagoi toisten tunteisiin									
19. ei ole kiinnostunut siitä, että toiset hänestä pitäisivät									
20. suhtautuu vanhempien poistumiseen erotilanteissa välinpitämättömästi									

III. Sensorinen/ Kognitiivinen tietoisuus

1. reagoi omaan nimeensä									
2. reagoi kehumiseen									
3. katsoo kohti ihmisiä ja eläimiä									
4. katselee kuvia ja televisiota									
5. piirtää ja värittää									
6. leikkii leluilla tavanomaisella tavalla									
7. kasvonilmeet kuvastavat sisäisiä tunnetiloja									

8. ymmärtää TV-ohjelmista juonen									
9. ymmärtää selityksiä									
10. tiedostaa ympäristönsä									
11. tiedostaa vaaran									
12. osoittaa mielikuvitusta									
13. aloittaa toimintoja/ leikkejä omatoimisesti									
14. osaa itse pukea									
15. on utelias, kiinnostunut asioista									
16. tutkii itse, ottaa selvää									
17. on muiden kanssa "samalla aaltopituudella", ei "ulkona"									
18. katsoo sinne minne muutkin katsovat									

IV. Yleinen terveydentila / Käyttäytyminen/Lääkityksen mahdolliset sivuvaikutukset

1. yökastelua									
2. päiväkastelua									
3.muita virtsaamisvaivoja									
4. tuhrimista (kakkaa housuun)									
5.ripulia									
6.ummetusta									
7.vatsakipuja									
8.pahoinvointia									
9.unettomuutta									
10.levoton uni/ painajaisia									
11.syö liikaa									
12.syö liian vähän									
13.liiallista painonlaskua									
14. liiallista painonnousua									
15. ruokavalio erittäin rajoittunut									
16. motorista levottomuutta									
17 liian hidasliikkeinen									
18. lyö tai vahingoittaa itseään									
19. lyö tai vahingoittaa muita									
20. hajottaa tavaroita / leluja									
21. yliherkkä äänille									
22.rauhaton, hermostunut tai pelokas									
23.lihaskramppeja tai nykimisoiretta									
24.vapinaa									
25.päänsärkyä									
26.hikoilua									
27.sydämentykytystä									
28.ihottumaa									
29 onneton tai itkuherkkä									

30. kohtausoireita (jos niin millaisia?)									
31. pakonomaista puhumista/ääntelyä									
32. jäykkiä rutiinitoimintoja									
33. huutaa tai kirkuu									
34. vaatii samanlaisuutta, vastarinta kaikille muutoksille									
35. usein kiihtynyt tai ärtynyt									
36. ei reagoi kivulle lainkaan tai poikkeavan vähän									
37. äärimmäisen kiintynyt tai sitoutunut joihinkin esineisiin tai asioihin									
38. toistaa samoja liikkeitä tai eleitä, maneereja									

Lisätietoja (päivämäärä ja kohta johon viitataan, esim. III/13):

Ismo Makkonen

Childhood Autism

Aspects of Growth Factors and Monoaminergic Transporters in Etiopathogenesis

In autism, abnormalities in growth and development of brain structures have been detected as well as alterations in the serotonergic system. This study investigated the relationships between growth factors, monoaminergic transporters and autism in children. The efficacy of fluoxetine, a selective serotonin reuptake inhibitor, on these parameters was evaluated. Positive effects on the clinical symptoms of autism were detected, indicating that fluoxetine might represent a useful adjunct to other therapies in children with autism.

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