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MOLECULAR GENETICS OF AUTISM SPECTRUM DISORDERS IN THE FINNISH POPULATION

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Academic Dissertation

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original articles, referred to in the text by roman numerals. In addition, some unpublished data are presented.

I Auranen M, Nieminen T, Majuri S, Vanhala R, Peltonen L, Järvelä I (2000) Analysis of autism susceptibility gene loci on chromosomes 1p, 4p, 6q, 7q, 13q, 15q, 16p, 17q, 19q and 22q in Finnish multiplex families. Mol Psychiatry 5:320-22.

II Auranen M, Vanhala R, Varilo T, Ayers K, Kempas E, Ylisaukko-oja T, Sinsheimer JS, Peltonen L, Järvelä I (2002) A Genomewide Screen for Autism Spectrum Disorders: Evidence for a Major Susceptibility Locus on Chromosome 3q25-27. Am J Hum Genet 71:777-90.

III Auranen M, Varilo T, Alen R, Vanhala R, Ayers K, Kempas E, Ylisaukko-oja T, Sinsheimer JS, Peltonen L, Järvelä I: Evidence for allelic association on chromosome 3q25-27 in families with autism spectrum disorders originating from a subisolate of Finland. Mol Psychiatry, in press.

IV Auranen M, Vanhala R, Vosman M, Levander M, Varilo T, Riikonen R, Peltonen L, Järvelä I (2001) MECP2 gene analysis in classical Rett syndrome and in patients with Rett-like features. Neurology 56:611-17.

ABBREVIATIONS

A	Adenosine
AGRE	Autism genetic resource exchange
AnS	Angelman syndrome
APA	American Psychiatric Association
AS	Asperger syndrome
AUTS1	a susceptibility gene locus for autism on chromosome 7q
AUTS2	a susceptibility gene locus for autism on chromosome 3q
ASDI	Asperger Syndrome Diagnostic Interview
ASSQ	Asperger Syndrome Screening Questionnaire
bp	Base pair
C	Cytosine
CARS	Childhood Autism Rating Scale
CF	Cerebrospinal fluid
CNS	Central nervous system
ChAT	Choline acetyltransferase
cM	centiMorgan
CLSA	Collaborative Linkage Study of Autism
DLD	Developmental language disorders
DNA	Deoxyribonucleid acid
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders
DZ	Dizygotic (twin)
EEG	Electro-encephalogram
FOXP2	Forkhead domain gene
G	Guanine
GABRB3	γ-aminobutyric $acid_A$ receptor subunit gene
HDAC	Histone deacetylase
5-HIAA	5-hydroxy-indoleacetic acid
HNPCC	Hereditary nonpolypotic colon cancer
5-HTT	Serotonin transporter gene
HRR-LRT	Likelihood based haplotype relative risk
HVA	Homovanillic acid
IBD	Identical by descent
ICD-10	International classification of diseases, 10^{th} edition
IQ	Intelligence Quotient
IMGSAC	International Molecular Genetic Study of Autism Consortium
LOD	Logarithm of odds
LD	Linkage disequilibrium
MAP-2	Microtubule associated protein 2
MBD	Methyl-CpG-binding domain
MECP2	Methyl-CpG-binding protein 2 gene
Mecp2	Methyl-CpG-binding protein 2
MLS	Multipoint lod score
MRI	Magnetic resonance imaging
MZ	Monozygotic (twin)
MR	Mental retardation
NLS	Nuclear localisation signal
NOS	Not otherwise specified
NPL	Nonparametric linkage analysis
P	P-value

PCR	Polymerase chain reaction
PDD	Pervasive developmental disorders
PSV	Preserved speech variant
RTT	Rett syndrome
SD	Standard deviation
SNP	Single nucleotide polymorphism
T	Thymine
TDT	Transmission disequilibrium test
TRD	Transcription repressor domain
UTR	Untranslated region
WHO	World Health Organization
XCI	X chromosome inactivation
Zmax	Maximum lod score

ABSTRACT

The purpose of this study is to detect chromosomal loci predisposing to autism spectrum disorders in the Finnish population. Families were collected and recruited through a nationwide collaborative project. Due to the rarity of autism and the small size of the Finnish population a wide phenotype was accepted including families with two or more affected individuals with autism, Asperger syndrome (AS) and/or developmental dysphasia.

This thesis consists of the molecular genetic studies on autism spectrum of disorders in Finland. We initiated the study by investigating the previously reported susceptibility regions with 17 families with autism and AS. In study I, we reported the findings for 10 chromosomal regions studied. Of these only the region on chromosome 1q showed slight evidence for linkage.

Most genome-wide reports on autism have been performed with mixed family material mainly from Caucasian populations. Taking into account the population history of Finland, we postulated that Finns may harbour novel susceptibility genes for autism. In study II, we report genome-wide linkage findings with 38 families with autism, AS and dysphasia. For analysis purposes we divided the material into three diagnostic criteria: (1) patients with autism, (2) patients with autism and AS and (3) patients with autism, AS and developmental dysphasia. The maximum multipoint lod score (MLS) of 4.81 was obtained on chromosome 3q25-27 in families with autism and AS. This locus was designated as AUTS2. This is a novel locus that needs to be analysed in other populations to confirm whether it is a rare locus unique to the Finns. Other regions with lod scores over 2 were detected on chromosomes 1 and 7, both overlapping with the results obtained from other populations. Association analyses supported the linkage finding on chromosome 1 in patients with autism.

Genealogical studies were performed in order to search for a possible founder effect in autism. In fact, 51% of the grandparents of the patients in the genome scan were shown to originate from late settlement region of Central Finland. In study III, 31 families originating from this particular region were analysed. Evidence for the extended region showing an association was obtained on chromosome 3q suggesting that this region may act as a major predisposing locus for autism in this subisolate.

During this thesis, the methyl-CpG binding protein 2 (MECP2) gene was identified to underlie Rett syndrome, a member of the autism spectrum disorders (Amir et al. 1999). In study IV, we screened 52 patients with Rett syndrome (RTT) for mutations in this gene. We found a disease-causing mutation in 39/39 of the classical patients and in one patient with preserved speech variant (PSV). A novel mutation, P127L, was detected in a patient with PSV in the MECP2 coding region. We also analysed a total of 12 atypical RTT patients, and no mutations were found in the MECP2 gene. The mutation spectrum of the Finnish patients did not differ from that of the non-Finnish patients. Our studies also included X chromosome inactivation analyses. No clear correlation was detected between the phenotype and/or X-inactivation status and the type of the mutation, which is in line with the previously published reports.

INTRODUCTION

The autism spectrum of disorders are severe developmental disorders of childhood that affect about 1% of populations worldwide (Folstein and Rosen-Sheidley 2001). The disease group consist of several diseases with varying severity, including classical autism, Asperger syndrome (AS), atypical autism, childhood disintegrative disorder and Rett syndrome (RTT). Characteristic features include abnormalities in social interaction and communication as well as repetitive and stereotypic behaviour. Classical autism is typically recognised before the age of three years whereas in AS the fully blown phenotype can be detected at the age of 5-8 (Gillberg 1998a). In AS, difficulties in social communication predominate while the development of the language and intelligence are normal. RTT, inherited as an X-linked dominant trait affects girls, who after normal development manifest in addition to autistic features deceleration of head growth, loss of acquired hand skills and language, and stereotypic hand movements (Hagberg 1993b).

Developmental dysphasia is a severe language disorder that affects about 5-10% of preschoolers (Tomblin et al. 1997; Rapin 1998). It is distinguished from autism by the absence of social or behavioural abnormalities. An association of this disorder with autism has been suggested, e.g. since the family members of autistic probands often possess milder disease traits similar to autism (Folstein and Mankoski 2000).

The etiology of autism spectrum of disorders is unknown. The genetic component of autism was confirmed by the first twin study in 1977 showing significantly higher concordance rates for monozygotic than for dizygotic twins (Folstein and Rutter 1977). Current estimates for the recurrence risk of autism in the siblings is ~3%, and the heritability estimate is over 90% (Folstein and Rosen-Sheidley 2001). Clearly, autism is among the multifactorial disorders with a strong genetic component.

The development of laboratory protocols and methods in the field of molecular genetics have facilitated the identification of genetic factors underlying multifactorial disorders including autism spectrum disorders. The candidate gene approach including the genes involved in the metabolic pathway of the neurotransmitter serotonin did not show supporting results, and studies to explore the whole genome were initiated. The first genome screen was published in 1998 (IMGSAC 1998), and since then a total of eight genome-wide screens have been reported. Potential susceptibility loci for autism (lod score >1) have been detected in nearly every chromosome reflecting the difficulty in mapping major disease gene(s), and the heterogeneity between populations under study. A couple of loci, chromosome 7q susceptibility region (AUTS1), chromosome 1q and chromosome 2q, have been replicated in two or more studies (IMGSAC 1998; Barrett et al. 1999; Philippe et al. 1999; Risch et al. 1999; IMGSAC 2001b; Buxbaum et al. 2001; Liu et al. 2001; Shao et al. 2002b).

The first gene underlying the autism spectrum of disorders was detected in October 1999. It is the methyl-CpG-binding protein 2 (MECP2) gene that causes RTT (Amir et al. 1999). The protein product of this gene acts in the process of chromosome modification and gene silencing. So far mutations have been identified in ~80% of cases, and also in patients with nonspecific mental retardation (Meloni et al. 2000; Orrico et al. 2000; Couvert et al. 2001) and atypical Angelman syndrome (Imessaoudene et al. 2001; Watson et al. 2001).
Molecular genetic analyses in autism spectrum disorders have not to date been performed in founder populations. This study is the first attempt to identify predisposing gene loci for autism spectrum disorders in the isolated Finnish population. Also the mutation spectrum of the MECP2 gene in Finnish Rett syndrome patients was analysed.

REVIEW OF THE LITERATURE

1. AUTISM SPECTRUM DISORDERS AND DEVELOPMENTAL DYSPHASIA

1.1. HISTORY

The word 'autism' is derived from a Greek word '*autos'* meaning the 'self'. The term was first used as a description for individuals that become absorbed in their own world and lost contact with other people. The first clinician to use the word 'autism' was a Swiss psychiatrist Eugen Bleuler (1857-1939) in 1916 to describe the desire of schizophrenic (in Greek: schizen = to divide, phren = the mind) patients to withdraw from public places. The nosological status between autism and schizophrenia remained controversial for a long time. Autistic disorders were first introduced under the diagnostic criterion of childhood schizophrenia in the Diagnostic and Statistical Manual of Mental Disorders (DSM-II) (American Psychiatric Association, 1968).

The original work of an American psychiatrist Leo Kanner in 1943 was the basis for the modern definition and today's diagnostic criteria for infantile autism (Kanner 1943). He provided a detailed analysis of 11 children reported to possess 'autistic disturbances of affective contact'. Because of the early onset abnormalities observed in most children's behaviour, Kanner suggested the presence of an inborn, presumably genetic, disorder of affective contact (Kanner 1943). Later on Kanner noted that early cold relations between the parents and the affected children might be the root for autism. This led to speculations that problems in parent-child relationship could cause autism, an idea, which was later scientifically discarded. However, among lay people this belief was deeply rooted and the mothers of autistic children were especially blamed.

To specify the diagnostic criteria for autism as a separate syndrome and to make a distinction between other similar conditions Rutter reported criteria for the diagnosis of infantile autism (Rutter 1978). A year later in 1979 Lorna Wing delineated the diagnostic criteria for a group of children with autism and autistic-like condition (Wing and Gould 1979), and also pointed out the following triad of abnormalities: in socialisation, in social communication, and in social play. In the subsequent classification system, DSM-III, of the American Psychiatric Association (APA) a similar triad of symptoms was formulated to belong to pervasive developmental disorders (PDD) including autism, childhood-onset PDD, residual autism and atypical autism in 1980. In the revised version of the criteria (DSM-III-R) the diagnostic categories of autism were widened and the concept of 'pervasive developmental disorder not otherwise specified' (PDD-NOS) was introduced to contain other forms of PDD.

Shortly after Kanner's publication in 1944, an Austrian paediatrician Hans Asperger described four boys with normal cognitive and verbal skills, who showed marked difficulties in social interaction, unusual circumscribed interests and motor difficulties. He suggested that this condition almost never becomes evident before three years of age, and that similar traits can be observed in the relatives of the probands, particularly in the males.

It was not until the first English report by Wing appeared in 1981, that knowledge of the condition described by Asperger began to expand (Wing 1981). She widened the original diagnostic criteria by including the abnormal development of language or communication skills, mild mental retardation and female cases. The term Asperger syndrome (AS) was first inaccurately used, most commonly referred to as PDD-NOS. Detailed cross-sectional studies

defined the disease to belong to the PDD spectrum disorders (Gillberg 1989; Szatmari et al. 1989; Szatmari 1992; Klin et al. 1995). The new diagnostic definitions were outlined in the DSM-IV in 1994 and in the international classification of diseases (ICD-10) (World Health Organization 1993), deliberately made identical.

Developmental dysphasia belongs to diagnostic class of developmental language disorders (DLD), and is diagnosed in children that have early onset problems in language ability, but otherwise develop normally. The children have normal nonverbal IQs, no clearly identifiable neurological problems, no disabilities in hearing or articulation that would interfere with normal language acquisition (Ingram 1959; Rapin 1996). Cognitive deficit and a pervasive language disorder encompassing communication in general has been found to be an essential part of the syndrome of autism (Ferrari 1982; Bishop 1989; Tanguay et al. 1998) Comparative studies of clinical differences in groups of children with autism and developmental language disorders have been performed. It has been found that a distinction between these entities can clearly be made based on behavioural, language or cognitive features (Bartak et al. 1977). Interestingly, in a follow-up analysis of the Bartak sample the clinical outcome of the two patient groups was qualitatively found to be very similar (Howlin et al. 2000; Mawhood et al. 2000).

1.2. CLINICAL CHARACTERISTICS AND CLASSIFICATION

1.2.1. Infantile autism

The diagnostic criteria of classical autism are shown in Table 1. In addition, mental retardation (MR) is present in 40-75% (Rutter 1979) and epilepsy in 25-30% of cases (Volkmar and Nelson 1990). Only about 10% of individuals with autism are able to live and function relatively independently (Lainhart and Piven 1995), and most require lifelong assistance. The two strongest predictions of the clinical outcome later on are the IQ and the level of language development at the age of five years (Bailey et al. 1996).

Individuals with a diagnosis of autism have a recognisable medical syndrome in ~10-15% of the cases (Folstein and Rosen-Sheidley 2001). These known medical conditions include e.g. phenylketonuria, Fragile-X syndrome, tuberculosis sclerosis, neurofibromatosis 1, Rett syndrome, Down syndrome, Angelman's syndrome, cerebral palsy, Moebius syndrome and Cornelia de Lange's syndrome. Environmental factors reported to cause autism are infectious diseases occurring prenatally or after birth (rubella, herpes simplex virus encephalitis) and some toxic syndromes (fetal alcohol syndrome, fetal cocaine or valproate exposure, lead poisoning and thalidomide embryopathy).

The differential diagnosis of autism include e.g. other forms of PDD, developmental language disorders, mental retardation and deafness (Smalley et al. 1992; Rutter et al. 1994).

Table 1.

DIAGNOSTIC CRITERIA FOR AUTISM DISORDER (ICD-10) (WHO 1993)

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

1. Qualitative impairment in social interaction, as manifested by at least two of the following:
   a. marked impairment in the use of multiple non-verbal behaviours, such as eye-to-eye gaze, facial expression, body postures, and gestures to regulate social interaction
   b. failure to develop peer relationships appropriate to developmental level
   c. a lack of spontaneous seeking to share enjoyment, interests, or achievements with other people (e.g. by a lack of showing, bringing, or pointing out objects of interest)
   d. lack of social or emotional reciprocity

2. Qualitative impairment in communication as manifested by at least one of the following:
   a. a delay in, or total lack of, the development of spoken language (not accompanied by an attempt to compensate through alternative models of communication such as gesture or mime)
   b. in individuals with adequate speech, marked impairment in the ability to initiate or sustain a conversation with others
   c. stereotyped and repetitive use of language or idiosyncratic language
   d. lack of varied, spontaneous make-believe play, or social imitative play, appropriate to developmental level

3. Restricted repetitive and stereotyped patterns of behaviour, interests and activities, as manifested by at least one of the following:
   a. encompassing preoccupation with one or more stereotyped and restricted patterns of interest, which is abnormal either in intensity or focus
   b. apparently inflexible adherence to specific, non-functional routines or rituals
   c. stereotyped and repetitive motor mannerisms (e.g. hand- or finger-flapping or -twisting, or complex whole-body movements)
   d. persistent preoccupation with parts of objects

B. Delays or abnormal functioning in at least one of the following areas, with onset prior to age 3 years:
   1. Social interaction.
   2. Language as used in social communication
   3. Symbolic or imaginative play.

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

1.2.2. Asperger Syndrome

The different diagnostic criteria of AS currently in use are shown in Table 3. AS can be distinguished from autism and PDD-NOS on the basis of higher verbal IQs, higher rates of the disorder in the first degree relatives and different patterns of co-morbidity, especially depression (Volkmar et al. 2000).

Recently, a novel multidimensional system to classify autism spectrum disorders has been proposed (Szatmari 2000) that emphasises the developmental process of the disease. The key factor in this process is the timing of the development of fluent language that will canalise the further development to a certain level of functioning. PDD subtypes might be classified as different developmental pathways that differentiate at certain time points, rather than separate entities. This means that the diagnostic criteria need not be changed, but focus should be emphasised on the genetic, epigenetic and environmental factors that move the child from one PDD pathway to another.

1.2.3. Developmental dysphasia

Under ICD-10 criteria the disorder is classified either as expressive type of language disorder (F80.1) or mixed receptive-expressive type of language disorder (F80.2) (World Health Organization 1993). The diagnostic criteria are shown in Table 2.

Table 2.

DIAGNOSTIC CRITERIA FOR DEVELOMENTAL LANGUAGE DISORDERS (ICD-10) (WHO 1993)

EXPRESSIVE LANGUAGE DISORDER (F80.1)

A. Symptoms including markedly limited vocabulary, making errors in tense, having difficulties in recalling words or producing sentences with developmentally appropriate length or complexity
B. The difficulties with expressive language interfere with academic or occupational achievement or with social communication.
C. Criteria are not met for mixed Receptive-expressive language disorder or for PDD
D. If mental retardation, a speech-motor or sensory deficit, or environmental deprivation is present, the language difficulties are in excess of those usually associated with these problems.

MIXED RECEPTIVE-EXPRESSIVE LANGUAGE DISORDER (F80.2)

A. Symptoms include those for Expressive language disorder as well as difficulties in understanding words, sentences, or specific types of words such as spatial terms.
B. The difficulties with mixed receptive-expressive language interfere with academic or occupational achievement or with social communication.
C. Criteria are not met for PDD.
D. If mental retardation, a speech-motor or sensory deficit, or environmental deprivation is present, the language difficulties are in excess of those usually associated with these problems.

Table 3. The clinical criteria for AS.

CLINICAL FEATURE	ASPERGER (1944; 1979)	WING 1981	GILLBERG & GILLBERG (1989)	TANTUM (1988)	SZATMARI (1989)	ICD-10 (WHO, 1993)	DSM-IV (APA, 1994)
Social impairment	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
Poor nonverbal communication	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	Yes
Poor empathy	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	Yes
Failure to develop friendships	**Yes**	**Yes**	**Yes**	**Yes** (implied)	**Yes**	**Yes**	Yes
Language/ Communication							
Poor prosody and pragmatics	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	Not stated	Not stated
Idiosyncratic language	**Yes**	**Yes**	Not stated	Not stated	**Yes**	Not stated	Not stated
Impoverished imaginative play	Yes	**Yes**	Not stated	Not stated	Not stated	Yes	Not stated
All-absorbing interest	**Yes**	**Yes**	**Yes**	**Yes**	Not stated	Yes	Often
Motor clumsiness	**Yes**	**Yes**	**Yes**	**Yes**	Not stated	Yes (common)	Often
Onset (0-3 years)							
Speech delays/ Deviance	No	May be present	May be present	Not stated	Not stated	No	No
Cognitive delays	No	May be present	Not stated	Not stated	Not stated	No	No
Motor delays	Yes	Sometimes	Not stated	Not stated	Not stated	May be present	May be present
Exclusion of autism	**Yes** (1979)	No	No	No	Yes	**Yes**	**Yes**
Mental retardation	No	May be present	Not stated	Not stated	Not stated	Not stated	Not stated

Table adapted from Volkmar and Klin, 2001

1.3. EPIDEMIOLOGICAL STUDIES

Prior to 1988, the surveys conducted on children and adolescents estimated the prevalence of autism to be 4-5 per 10 000, and the prevalence of any of the PDD phenotypes 19 per 10 000 (Fombonne 1999). However, the latest epidemiological studies have reported increased prevalence rates for this group of disorders. In the Japanese population a prevalence rate of 21 per 10 000 has been found for autism among children at the age of five (Honda et al. 1996). In a recent study performed on autism in Northern Finland, similar figures were reported. The cumulative incidence was found to be highest, 20.7 per 10 000, in the age group of 5-7 year-olds and lowest, 6.1 per 10 000, in the age group of 15- to 18-year-old children, when the criteria of the ICD-10 and the DSM-IV were used (Kielinen et al. 2000), whereas in the United Kingdom a prevalence rate of 16.8 per 10 000 and 63 per 10 000 have been found for autism and PDD respectively in children younger than five years of age (Chakrabarti and Fombonne 2001).

So far, three Swedish epidemiological studies have been performed on AS, the first of which conducted on 7 year old children reported a prevalence of 28.5 per 10 000 for cases fulfilling ICD-10 criteria (Gillberg and Gillberg 1989). In a cohort of 7- to 16-year-olds, the prevalence of 35.7 per 10 000 was obtained for AS fulfilling the author's own criteria (Ehlers and Gillberg 1993). The recent Swedish study reported a rate of 48 per 10 000 in 7- to 11-year olds (Kadesjo and Gillberg 1999). The variation between these studies might be explained by methodological issues and the ages of the study groups.

Autism is more common in males with the average male to female ratio of four to one (Bailey et al. 1995; Fombonne 1999). Twin studies on autism show elevated concordance rates for monozygotic twins (MZ) (36-95%) compared to dizygotic twins (DZ) (0-23%) (Folstein and Rutter 1977; Steffenburg et al. 1989; Bailey et al. 1995). Also, in an article of 166 affected sib pairs, an excess of twins was reported (12 MZ, 17 DZ and 1 unknown zygosity), which deviated from the expected values significantly ($P<10^{-6}$). The authors speculate that parental risk factors, either related to twinning or fetal development or other risk factors (genetic or environmental) may contribute to autism (Greenberg et al. 2001). Another study also found an excess of twins in a sample of 79 affected sib-pairs, however in their sample only MZ twins were over represented (Betancur et al. 2002). The data from a recent report with 465 patients from Western Australia strongly suggests that twinning itself is not a risk factor for autism. They demonstrate that the high proportion of twins can be explained by the high ratio of concordance rates in MZ twins versus siblings and the distribution of family size in the population studied (Hallmayer et al. 2002).

The data from the family and twin studies suggests that the phenotypic spectrum of autistic traits is broader than originally thought (Bolton et al. 1994; Bailey et al. 1998b). Family studies have estimated the recurrence rate of autism among siblings to be ~3-6% (Smalley 1997). Considering the recurrence risk for a broad phenotype including language-related cognitive disorders and/or deficits of social functioning some studies show an increased risk (between 12.4 and 20.4%) depending on the inclusion criteria (Bolton et al. 1994; Piven et al. 1997a). In most studies marked abnormalities in social behaviour are observed in the minority of the first-degree relatives of autistic probands, but milder abnormalities are seen in a large proportion (Bailey et al. 1998b). In the combined analysis of all published family studies cited above, the risk for autism in second-degree relatives is 0.18% and in third-degree relatives 0.12% (Szatmari et al. 1998). Given a family with more than two affected children, the recurrence risk might be as high as 25% (Folstein and Rosen-Sheidley 2001).

For developmental language disorders, an overall prevalence rate of 7.4% was found among monolingual English-speaking kindergarten children (Tomblin et al. 1997). Twin and family studies performed on DLD have reported concordance rates of 70% and 45% for MZ and DZ twins respectively (Lewis and Thompson 1992; Bishop et al. 1995; Tomblin and Buckwalter 1998). It has been proposed that the disorder has a biological basis, and is not only a consequence of abnormal social functioning (Rapin and Dunn 1997). Although autism spectrum disorders and DLD are classified as distinctive disorders, some evidence for an association between these entities has accumulated. An increased risk of language-related problems (articulation, pragmatic, spelling and reading difficulties) have been observed in the first-degree relatives of autistic individuals (Landa et al. 1992), as well as delay in the language development (Bailey et al. 1995).

A long-term follow-up study of two male groups, one with autism and the other with developmental receptive language disorder reported some degree of clinical overlap between the groups (Howlin et al. 2000; Mawhood et al. 2000). The significant problems observed in the autism group, abnormalities in stereotyped behavioural patterns and social relationships were also present in the language group. A total of 65% had moderate social problems and 25% were rated as being near/completely normal in social functioning. The two groups were first assessed in early childhood, when aged 7 to 8, and the study was completed when the participants were aged, on average, 23 to 24. These findings suggest that there are common traits in these disorders. The traits are more easily recognisable during childhood whereas in the adulthood though the abnormalities in language development are qualitatively similar, only the magnitude of the impairments is different. The boundaries of these two disorders are still to be characterised.

1.4. NEUROPATHOLOGICAL, BRAIN IMAGING AND METABOLIC STUDIES

In the neuropathological studies, no gross structural abnormalities have been reported. Interestingly, a recent report performed in nine patients with autism suggests that the minicoluns, consisting of neurons in layers VI to II of the brain, are significantly smaller and less compact in patients with autism (Casanova et al. 2002). Some studies have shown reduced Purkinje cell densities in the cerebellum, a diminished number of neurons in the cerebellar granule cell layer and cellular abnormalities in the areas of the limbic system (Bauman and Kemper 1985; Kemper and Bauman 1998). In a study by Bailey et al. the brains of six mentally handicapped patients with autism were analysed. Four of the subjects presented cortical developmental abnormalities, suggesting the involvement of cerebral cortex in autism (Bailey et al. 1998a).

In a recent neuroanatomical study of MZ twins discordant for autism one severely affected twin had markedly smaller caudate, amygdaloid and hippocampal volume, and smaller cerebellar lobules compared to his brother (Kates et al. 1998). In some patients the brains were observed to be megalencephalic (Lotspeich and Ciaranello 1993; Bailey et al. 1998a), and some studies have reported slightly smaller brain volumes in autism or no difference compared to control groups (Rosenbloom et al. 1984; Aylward et al. 1999). A literature review combining autopsy material of 21 patients with autism, the brain weight of the patients was found to be ± 1 SD of the normal range (Courchesne et al. 1999).

In brain imaging studies, abnormalities in the posterior fossa have been reported including hypo- and hyperplasia of the cerebellar vermal lobules (Courchesne et al. 1994; Hardan et al. 2001) further supporting the role of the cerebellum as a modulator of mental and social functions (Riva and Giorgi 2000). However, in some studies no differences in these structures

have been detected (Holttum et al. 1992; Kleiman et al. 1992). Other abnormal findings include reversed left/right asymmetry of the frontal lobe (normally the left frontal lobe is larger than the right), enlarged lenticular nuclei and the presence of gyria malformations (Lotspeich and Ciaranello 1993). A recent quantitative MRI-study reported abnormal regulation of brain growth in autistic boys. Early overgrowth with hyperplasia in cerebral gray matter and cerebral and cerebellar white matter was detected in 90% of autistic boys by the age of two to four years, whereas in older autistic boys and adolescents the enlargement was not such prominent (Courchesne et al. 2001). Although not being a follow-up study, the authors conclude that there is abnormal growth regulation in autism leading to slowed brain growth (Courchesne et al. 2001).

No specific marker for autism has been identified in the analyses of the blood or urine levels of different metabolites. Whole blood serotonin is elevated in about a quarter of autistic individuals as a consequence of increased amounts of serotonin in the platelets (Cook 1990), however no difference has been detected in the serotonin metabolite, 5-hydroxy-indoleacetic acid (5-HIAA) in the cerebrospinal fluid (CSF) (Narayan et al. 1993). Increased blood serotonin levels are also observed in other neuropsychiatric disorders, such as chronic schizophrenia, and mental retardation (Cook 1990). The role of serotonin in autism is supported by the finding that drugs inhibiting serotonin reuptake in the neurons alleviate aggressive behaviour and hyperactivity in patients with autism. No consistent findings of the studies measuring abnormalities in the dopaminergic, noradrenergic and the neuropeptide systems have been obtained (Bailey et al. 1996).

1.5. MOLECULAR GENETIC STUDIES

1.5.1. Chromosomal aberrations

A chromosomal aberration can be useful in localising a gene responsible for a particular syndrome. Autism has been associated with abnormalities of nearly every chromosome. The majority of them are located on the long arm of chromosome 15, which include the genes for Prader-Willi/Angelman syndrome and undergoes genomic imprinting, and the sex chromosomes (Gillberg 1998b). The most common abnormality associated with autism is fragile-X syndrome (Gillberg and Coleman 1996).

The results of several genetic linkage studies support a susceptibility locus on the long arm of chromosome 7 (IMGSAC 1998; Barrett et al. 1999; Philippe et al. 1999; Risch et al. 1999; IMGSAC 2001b; Liu et al. 2001; Shao et al. 2002b). Also, chromosomal abberrations on this region, such as familial paracentric inversion in 7q and other rearrangements have been reported in families with autism (Ashely-Koch et al. 1999; Vincent et al. 2000; Warburton et al. 2000; Yan et al. 2000; Tentler et al. 2001). Mutation analyses of a gene on chromosome 7q interrupted in a translocation t(7;13)(q31.3;q21) did not give evidence for sequence alterations in a group of autistic individuals (Vincent et al. 2000)

1.5.2. Candidate gene studies

Based on the finding of duplications on 15q11-13 in some patients with autism, association analyses have been conducted in order to study a putative candidate gene, γ-aminobutyric $acid_A$ receptor subunit gene (GABRB3) locating on this region. Evidence for linkage disequilibrium between 140 mostly singleton families with autism and 155CA-2 locating in the third intron of GABRB3 have been reported with P = 0.0014 (Cook et al. 1998). Also, positive results have been obtained on chromosome 15q11-13 in two of the genome-wide scans performed on autism (Barrett et al. 1999; Philippe et al. 1999).

Serotonin transporter gene (5-HTT) polymorphisms, consisting of a repeat in the second intron, and a deletion/insertion polymorphism that regulates the expression of the transporter (Heils et al. 1996) have been studied in autism with contrasting results. A transmission/disequilibrium (TDT) analysis of 86 families with autism detected no linkage nor association with the polymorphism in the second intron, however a preferential transmission of a short variant of 5-HTT promoter (P = 0.030) was found (Cook et al. 1997). Klauck et al. observed transmission of the long variant in 65 singleton families (Klauck et al. 1997). Similarly, a significant excess of the long/long 5-HTT promoter genotype was observed in the families as well as preferential transmission of the long allele of the 5-HTT promoter in a study with 33 autism families (Yirmiya et al. 2001). A further analysis did not show evidence for linkage nor associating of these polymorphisms (Maestrini et al. 1999). Also, no association have been detected between autism and the 5-HT2A receptor gene (Herault et al. 1996; Veenstra-VanderWeele J et al. 2002) nor between the $5\text{-}HT_7$ receptor gene or the pseudogene $5\text{-}HT_{7P}$ on chromosomes 10 and 12, respectively (Lassig et al. 1999).

A positive association with autism has been detected with the c-Harvey-ras-1 (HRAS1) gene, important in cell growth, signal transduction, cell architecture and intracellular transport (Herault et al. 1995; Comings et al. 1996). Also, the major histocompatibility complex on chromosome 6 including a null allele of the C4G gene, extended haplotype B44-SC30-DR4 and the third hypervariable region (HVR-3) of certain DR beta 1 alleles have a strong association with autism (Daniels et al. 1995; Warren et al. 1996).

The involvement of the HOXA1 and HOXB1 genes, critical for hindbrain development in

autistic individuals was studid by analysing a sequence variant in the coding region of both genes (Ingram et al. 2000). A significant deviation from the HOXA1 genotype ratios expected from Hardy-Weinberg proportions (P = 0.005) was detected in autism on chromosome 7p. In a more recent study no association was detected between HOXA1 or HOXB1 gene variants and autism in 110 multiplex families (Li et al. 2002).

1.5.3. Genome-wide scans

A total of eight whole genome and one autosomal genome screens have been performed in individuals with autism spectrum disorders (Table 4). So far, the three best overlapping results on autism spectrum disorders have been obtained on chromosome 2 flanking marker D2S2188 (Philippe et al. 1999; IMGSAC 2001b; Buxbaum et al. 2001; Shao et al. 2002b), on chromosome 16 at ~20-30 cM (IMGSAC 1998; Philippe et al. 1999; IMGSAC 2001b; Liu et al. 2001), and on chromosome 7q, although the location estimates vary (IMGSAC 1998; Barrett et al. 1999; Philippe et al. 1999; Risch et al. 1999; IMGSAC 2001b; Liu et al. 2001; Shao et al. 2002b).

Table 4. A summary of the published genome screens on autism spectrum disorders. MMLS/het = Maximum multipoint heterogeneity lod score, NPL = nonparametric linkage

REFERENCE	YEAR	FAMILIES	ORIGIN	PATIENTS	BEST RESULT
IMGSAC	1998	99	Caucasian, mostly UK	Autism, AS and PDD	Chr 7q: MLS 3.55 (56 UK families)
Philippe et al.	1999	51	Caucasian, mixed European	Autism	Chr 6q: MLS 2.23
Barrett et al.	1999	75	Caucasian (CLSA families)	Autism	Chr13: MMSL/het 3.0
Risch et al.	1999	139	Mixed American	Autism	Chr1p: MLS 2.15
Buxbaum et al.	2001	95	Not stated	Autism and AS	Chr2: NPL-score 3.32 (49 families)
Liu et al.	2001	110	Caucasian (AGRE families)	Autism, AS and PDD	Chr5: MLS 2.55
IMGSAC	2001	83	Caucasian	Autism, AS and PDD	Chr2: MLS 4.0 (strict inclusion criteria)
Shao et al.	2002	99	Caucasian	autism	Chr X: MLS of 2.54

The first study, conducted by the International Molecular Genetic Study of Autism Consortium, found the best multipoint lod score (MLS) of 3.55 close to markers D7S530 and D7S684 in a subset of 56 UK affected sib-pair families (IMGSAC 1998). A further characterisation of the AUTS1 locus on chromosome 7q was recently completed on 170

multiplex families (IMGSAC 2001a). With the inclusion of more families and markers the peak of linkage (MSL of 3.37) was observed ~20 cM proximal to the initial peak at D7S477 with 153 sib pairs fulfilling stringent inclusion criteria. This locus was designated as the first autism susceptibility locus, AUTS1. Interestingly, the AUTS1 locus overlaps with the previously identified candidate gene region for a three generation family (KE) with severe speech and language disorders (Fisher et al. 1998). Recently, a mutation in the forkhead-domain gene (FOXP2) was reported in the affected members of this family (Lai et al. 2001). This gene encodes a putative transcription factor and a DNA-binding domain. In a recent study the involvement of this gene was excluded in families with developmental language disorder and in families with autism linked to 7q31 (Newbury et al. 2002).

Liu et al. performed a genome-wide screen in autism with families belonging to the Autism Genetic Resource Exchange (AGRE) (Liu et al. 2001). Based on the diagnosis of the patients the analyses were carried out using narrow diagnostic criteria including only patients with autism, and broad diagnostic criteria including patients with autism, AS and PDD. On two of the analysed loci, evidence for increased sharing was observed with an MLS of 3.59 combining markers D19S714 and DXS1047, with alleles in these loci tending to be maternally coinherited. This result suggests that a putative X-chromosomal locus may act in concert with an autosomal susceptibility locus/loci, explaining the increased male to female ratio observed in patients with autism (Skuse 2000).

In 83 IMGSAC sib-pairs, thirteen candidate regions found in the primary scan (IMGSAC 1998) were further analysed (IMGSAC 2001b). In this study patients with autism, AS and PDD were included. Of the previously reported 12 chromosomal regions showing MLS >0.82 (chromosomes 1, 2, 4, 7, 8, 9, 10, 14, 16, 17, 19 and 22) (IMGSAC 1998), only four regions (chromosomes 2, 7, 16 and 17) showed MLS >1.5 in the whole material (IMGSAC 2001b). The highest MLS of 3.74 was detected at D2S2188, increasing to an MLS of 4.80 when 127 sib-pairs fulfilling strict diagnostic criteria were studied (Table 5).

In the most recent genome scan, Shao et al. performed a genome-wide scan with 52 multiplex families with autism (Shao et al. 2002b). Eight promising candidate regions (on chromosomes 2, 3, 7, 15, 18, 19 and X) were studied in a total of 99 multiplex families. The best two point lod scores were detected on chromosome X with an MLS of 2.54 at DXS6789. Interestingly the peak on chromosome 2 (MLS of 1.30 at D2S116) is located ~12 cM from the peak of Buxbaum et al. (Buxbaum et al. 2001).

Table 5. The putative susceptibility loci for autism spectrum disorders. Only results with multipoint lod scores >1.5 are shown.

LOCUS	POSITION	MULTIPOINT LINKAGE RESULT	REFERENCE
D1S1675	149.2	2.15	Risch et al. 1999[a)]
D2S319	7.6	1.69	Buxbaum et al. 2001[b)]
D2S2188	180.8	4.80(strict)/3.74(all)	IMGSAC 2001b[a)]
D2S364	186.2	2.45	Buxbaum et al. 2001
D3S3680	361	1.51	Shao et al. 2002
D3S1267	139.1	1.91	Buxbaum et al. 2001
Close to D4S412	4.7	1.55	IMGSAC 1998
D5S406	11.9	1.65	Buxbaum et al. 2001
D5S2494	45-69	2.55 (B)	Liu et al. 2001[c)]
D6S309	14.1	1.65	Buxbaum et al. 2001
D6S283-D6S261	109.2-120.3	2.23	Philippe et al. 1999
D7S1813	103.6	2.2	CLSA 1999[d)]
D7S477	111.8	3.37	IMGSAC 2001a[a)]
D7S477	111.8	3.55(1/2)/3.20(all)	IMGSAC 2001b
D7S530-D7S684	134.5-147.2	2.53	IMGSAC 1998
D7S495	144.7	1.66	Shao et al. 2002
D7S483	165	2.13 (N)	Liu et al. 2001
D8S550	21.3	1.59	Buxbaum et al. 2001
D8S1179	134	1.66 (B)	Liu et al. 2001
D9S157	32.2	3.11(1/2)/2.02(strict)	IMGSAC 2001b
D9S283	94.9	1.72	Buxbaum et al. 2001
D9S1826	159.6	3.59(1/2)/2.23(strict)	IMGSAC 2001b
D9S158	161.7	3.16(1/2)/2.09(strict)	IMGSAC 2001b
D13S800	55.3	3.0	CLSA 1999
D13S217-D13S1229	17.2- 21.5	2.3	CLSA 1999
D15S129	34.1	1.49 (strict)/1.47(1/1)	IMGSAC 2001b
CYP19	40.46	2.21(1/1)/1.20(strict)	IMGSAC 2001b
D16S407-D16S3114	18.1-23.3	1.51	IMGSAC 1998
D16S3102	24.5	2.93(all)/2.61(strict)	IMGSAC 2001b
D16S2619	28	1.91 (N)/1.46 (B)	Liu et al. 2001
5HTTINT2	45.4	2.34(all)/1.87(strict)	IMGSAC 2001b
D19S714	42.3	2.53(N)/1.72 (B)	Liu et al. 2001
D19S433	52	2.46 (N)	Liu et al. 2001
DXS6789	62.5	2.54	Shao et al. 2002
DXS1047 –q tel	82	2.67 (N)	Liu et al. 2001

[a)] Multipoint maximum lod scores (MLS) calculated by ASPEX
[b)] Two-point NPL scores, only results with a P-value < 0.5 are shown
[c)] MLS calculated by MAPMAKER/SIBS
[d)] Maximum multipoint heterogeneity lod scores (MMSL/het)
(B) Patients with broad criteria including autism, AS and PDD
(N) Patients with narrow criteria including only autism
all: 152 sib-pairs
1/2: 84 case type 1/type 2 sib-pairs
strict: 127 case type 1/type 1 pairs + type 1/type 2 pairs
case type 1: clinical diagnosis of autism, history of language delay and IQ≥ 35
case type 2: clinical diagnosis of autism, atypical autism, Asperger syndrome or PDD NOS, no requirement for language delay

Based on the excess of affected male patients observed in autism spectrum disorders the involvement of sex chromosomes has been studied. The X chromosome was assessed in 38 multiplex families with autism by studying 35 microsatellite markers (Hallmayer et al. 1996). A moderate to strong gene effect was excluded on the whole X chromosome. Some evidence for linkage has been obtained in 59 multiplex families with autism on Xp (two-point maximum likelihood score of 0.89). A recent study in 31 families with two or more affected boys revealed no evidence for linkage on 16 evenly spaced X-chromosomal markers using the affected sib pair method (Schutz et al. 2002). Also, no similarities in Y chromosome haplogroups was detected in 111 autistic male patients compared to a control group (Jamain et al. 2002).

2. RETT SYNDROME

2.1. CLINICAL CHARACTERISTICS AND CLASSIFICATION

2.1.1. Classical Rett syndrome

RTT was first described in 1966 by Andreas Rett (Rett 1966a; Rett 1966b) a paediatrician working in Vienna. It is the second most common genetic cause for severe mental retardation in girls after Down syndrome affecting 1 in 10 000 to 15 000 females worldwide (Hagberg 1985; Skjeldal et al. 1997). The original description was largely ignored until the first English publication appeared in 1983 by Hagberg et al. describing 35 girls with a constellation of clinical features including developmental stagnation and rapid deterioration of higher brain functions, severe dementia, autistic features, loss of purposeful use of the hands, hand stereotypies, jerky truncal ataxia and acquired microcephaly (Hagberg et al. 1983). The diagnostic inclusion and exclusion criteria of RTT were formulated in 1988 (Table 6) (The Rett syndrome Diagnostic Work Group 1988). Moreover, there exist supportive criteria that are often present in girls with RTT including breathing dysfunction, EEG abnormalities and seizures, spasticity, peripheral vascular abnormalities, scoliosis, growth retardation and small, hypotrophic feet. Due to a lack of biological markers available at that time to support the diagnosis of RTT, the diagnostic criteria were formulated based on the phenotype of typical patients.

Table 6.

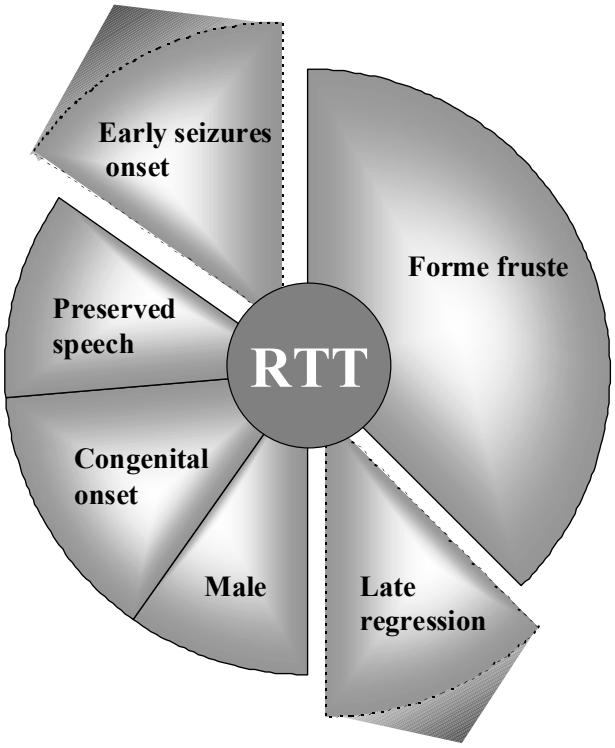
DIAGNOSTIC CRITERIA FOR CLASSICAL RTT
NECESSARY CRITERIA
Apparently normal pre- and perinatal development Developmental process within the normal range for the first 5-6 months (up to 18 months) Normal head circumference at birth Deceleration of head growth between the ages of 5 months and 4 years Loss of acquired purposeful hand skills between the ages 6 and 30 months, temporally associated with communication dysfunction and social withdrawal Development of severely impaired expressive and receptive language; severe psychomotor retardation apparent Stereotypic hand movements such as hand wringing/squeezing, clapping or tapping, mouthing and “washing” or rubbing automatisms appearing after purposeful hand skills are lost Appearance of gait apraxia and truncal apraxia/ataxia between the ages of 1 and 4 years Diagnosis tentative until 2 to 5 years of age
EXCLUSION CRITERIA
Evidence of prenatal onset of growth retardation or microcephaly Organomegaly or other evidence of storage disease Retinopathy of optic atrophy Existence of identifiable metabolic and other neurodegenerative disorder Acquired neurological disorder resulting from severe infection or trauma

The natural history of the classical RTT patient can be divided into four stages (Hagberg 1993b). Stage I starts at 6-18 months of age and is characterised by growth and head growth slowing and early stagnation of development. Stage II which starts at 1-4 years includes rapid developmental regression phase including the loss of purposeful hand movements, vocalisation and communication, and acquisition of hand stereotypies. At this point some autistic features may appear. After the regression the condition stabilises in Stage III and slight improvement may appear. Some of the supportive criteria may become observable at this stage. Motor deterioration usually appears after 10 years of age in Stage IV, and is characterised by loss of ambulation, muscle wasting, scoliosis or kyphosis. Most RTT patients survive till the age of 35, although their lifespan are often shortened due to sudden deaths possible because of cardiac arrhythmias or brainstem dysfunction.

2.1.2 Rett syndrome variants

Increasing evidence suggested that the phenotypic variability associated with RTT is much wider than originally thought. Hagberg et al. have delineated six different variants with a presence of combination of particular symptoms (Hagberg and Gillberg 1993c; Hagberg 1995).

Figure 1. RTT variants. To date no early seizure onset or late regression variants with MECP2 mutations have been characterised.



The term forme fruste is used for variants with a milder, incomplete and protracted clinical course (Hagberg and Gillberg 1993c). This is the most common variant constituting 10-15% of the cases. The girls initially show normal development with a regression phase following at the age of one to three years. Hand use may be preserved and hand stereotypies are not necessarily present. The abnormalities necessary for RTT are usually less striking, however as the patients grow older the diagnosis is indisputable.

In the course of classical RTT the patients lose their acquired speech in the regression phase (Stage 2). The preserved speech variants maintain the ability to speak some words or phrases later on, however these may appear unexpectedly and in inappropriate situations (Zappella et al. 1998).

The variant of RTT characterised by early seizure onset includes also cases with infantile spasms, which may blur the clinical picture and typical signs of RTT. In the Swedish series of Hagberg et al. seizures at or before the age of six months were seen in 6.7% of the RTT patients (Hagberg and Witt-Engerström 1990).

The congenital onset variant was first described by Rolando (Rolando 1985). The disease in this syndrome starts early on with typical RTT symptoms varying from mild to severe, and the necessary criteria for RTT, apparently normal psychomotor development during the first six months, is not fulfilled.

A couple of late childhood regression variants have been described with clinical features resembling simple nonspecific mental retardation from early infancy to early school age. However, later on the probands show the typical RTT features (Hagberg and Gillberg 1993c).

Males with some typical signs of the classical RTT syndrome have been characterised in the early 90's, but these cases did not fulfil all the necessary criteria for RTT (Coleman 1990; Eeg-Olofsson et al. 1990; Philippart 1990; Topcu et al. 1991; Christen and Hanefeld 1995). However, typical features of RTT have been described in males with Klinefelter's syndrome (47, XXY) (Vorsanova et al. 1996; Hoffbuhr et al. 2001; Leonard et al. 2001). In the few RTT families reported the existence of male patients with a severe form of encephalopathy raised the suggestions that the male RTT patients show a more severe clinical picture (Schanen et al. 1998). The clinical picture of these males is distinct from RTT as they are characterised by neonatal onset of hypotonia, seizures and apneic episodes. They developed microcephaly with profound developmental delay and died early at the second year of life.

2.2 NEUROPATHOLOGICAL AND METABOLIC STUDIES

Before the recent discovery of the mutations in the methyl-CpG binding protein 2 (MECP2) gene in the majority of the RTT patients, there were no specific findings in neuropathological nor metabolic studies warranting the diagnosis. Some typical changes were observed in the majority of the patients analysed.

Neuropathological and imaging studies have shown generalised brain atrophy including both cerebrum and cerebellum varying from ~12 to 34% in advanced RTT patients compared to age-matched controls (Armstrong et al. 1995). No evidence of cell loss, inflammation, gliosis or abnormalities in the migration pattern of neurons have been detected (Belichenko et al. 1994). Other morphological findings include the evidence of a decrease in neuronal cell size, and increased cell packing density throughout the brain (Bauman et al. 1995). These findings suggest that RTT is a neurodevelopmental disorder that has its greatest effects during the first few years of postnatal life (Hagberg and Witt-Engerström 1990) when dendritic extensions are increasing and synapse formation is occurring (Huttenlocher 1979).

Significant changes in the three-dimensional reconstructions of dendrites and dendritic spines of pyramidal cell layers II and III have been reported in RTT patients (Belichenko and Dahlstrom 1995). The reduction of dendritic aborizations is one of the most consistent abnormalities associated with genetic or nonspecific mental retardation (Kaufmann and Moser 2000) in addition to changes in dendritic spine density and morphology. A selective decrease of microtubule associated protein 2 (MAP-2) immunoreactivity was observed in RTT in multiple brain regions, especially in the superficial white matter neurons (Kaufmann et al. 1995). Also, decreased immunoreactivity of MAP-2 was seen in the soma and dendrites of pyramidal neurons in the layers V-VI. MAP-2 is expressed early on in embryonic brain development and is most abundant in the microtubules (Matus 1988). The changes observed in RTT reflect a disturbance in the early stages of cortical maturation. The abnormalities of MAP-2 expression may also reflect deficits of neurotransimitters, as it is regulated particularly by acetylcholine, dopamine and glutamate systems (Kaufmann et al. 1995).

In the neurochemical studies particular attention has been focused on the changes in neurotransmitters acetylcholine and dopamine. The consistent finding in the dopaminergic neural system in RTT is the reduction of the concentration of the intracellular neuronal pigment melanin in the substantia nigra (Jellinger and Seitelberger 1986). Hyperammonemia was detected in RTT patients in the original report by Rett (Rett 1966a), but subsequent studies have refuted this finding. Also, mitochondrial deficits have been suggested because of detectable lactatic/pyruvatic acidosis in some patients similar to mitochondrial diseases, and also elevated CSF lactate (Lappalainen and Riikonen 1994; Matsuishi et al. 1994; Haas et al. 1995). However, these changes are probably secondary to apneic periods common in some RTT patients (Matsuishi et al. 1994).

Choline acetyltransferase (ChAT) is a rate limiting enzyme for acetylicholine production, and is specific for cholinergic neurons. Decreased activity of ChAT has been observed in RTT in the basal ganglia, and also in the hippocampus and thalamus (Wenk et al. 1993; Wenk and Hauss-Wegrzyniak 1999).

2.3. MOLECULAR GENETIC STUDIES

2.3.1 Identification of the MECP2 gene underlying RTT syndrome

The mode of inheritance in RTT has been the subject of much debate. The disease is sporadic in the majority of cases and only ~0.4% represent familiar occurrences. Twin data suggests a full concordance rate for monozygotic twins while dizygotic twins are generally discordant (Zoghbi 1988).

Rett syndrome was thought to be an X-linked dominant disorder lethal to males. This assumption was supported by the observation of a skewed X chromosome inactivation (XCI) pattern in phenotypically normal/mildly affected female members in one family with recurrence of RTT in a maternal aunt and niece (Schanen et al. 1997). The observation of a random XCI pattern in a phenotypically normal carrier mother in the other family with maternally related half-sister pairs with RTT was explained by germ-line mosaicism (Schanen et al. 1997). A severely affected male with encephalopathy and early death in the former family also favoured an X-linked inheritance pattern (Schanen et al. 1997; Schanen and Francke 1998). These families were used in monitoring for shared segments on the X chromosome and narrowing the candidate region (Archidiacono et al. 1991; Ellison et al. 1992; Curtis et al. 1993; Schanen et al. 1997; Schanen and Francke 1998). Tentative exclusion mapping was performed also in full-sister pairs, but was interpreted with caution because of the possibility of paternal germline mosaicism for the mutation (Curtis et al. 1993). Identification of a Brazilian family with three affected daughters with RTT enabled the localisation of the defective gene to Xq28. The mother was found to have a highly skewed XCI pattern and was thus suggested to be a carrier rather than either of the parent being a gonadal mosaic (Sirianni et al. 1998). Systematic sequencing of the genes located in the region defined by linkage analyses led to the identification of mutations in the MECP2 gene in several RTT patients (Amir et al. 1999).

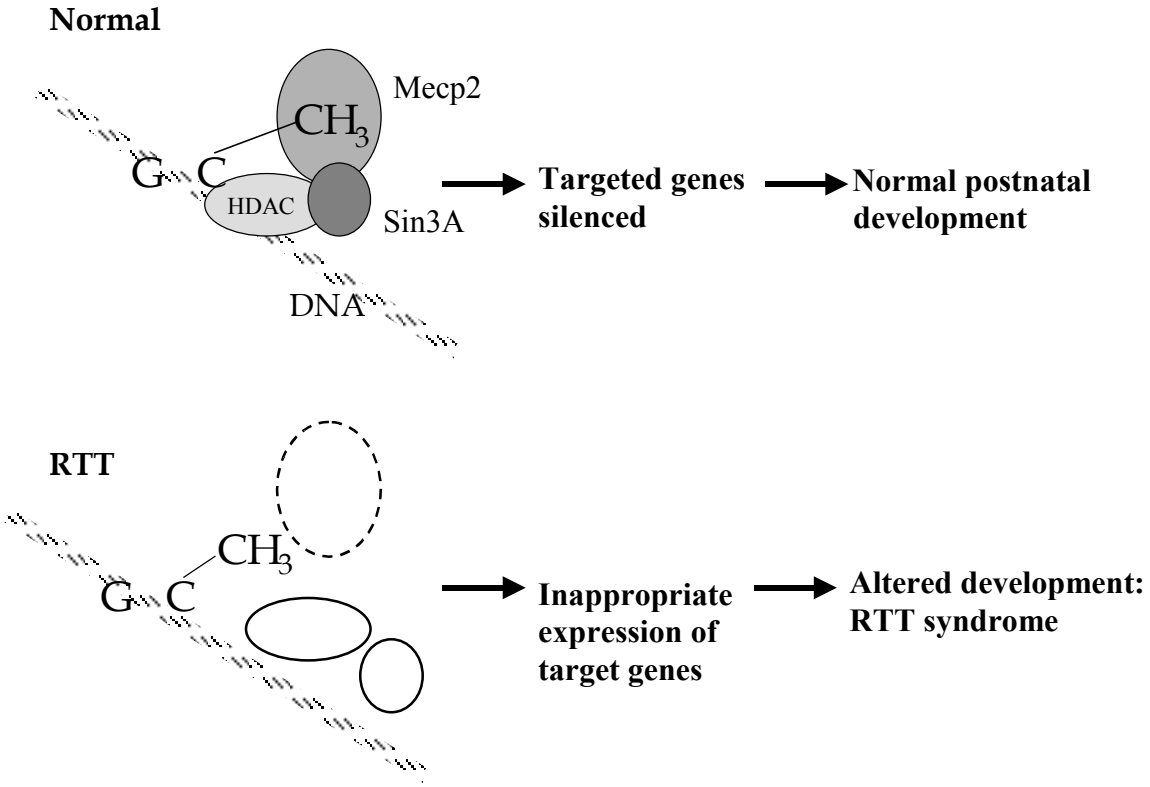
2.3.2. Structure and function of the Mecp2 protein

Mecp2, detected in 1992 (Lewis et al. 1992) is an abundant chromosomal protein. It is capable of binding to methylated CpG dinucleotides via its methyl-CpG-binding domain (MBD) and recruiting the histone deacetylase (HDAC) complex and corepressor Sin3A via its transcriptional repression domain (TRD) (Jones et al. 1998; Nan et al. 1998) (Figure 2). The MBD domain binds exclusively to a major groove of DNA that contains one or more methylated CpGs (Nan et al. 1993). In addition to specific binding to methylated DNA, Mecp2 also associates nonspecifically with non-methylated DNA (Meehan et al. 1992b) probably through short motifs that identify the minor groove of an AT-rich sequence, and are excluded from MBD (Meehan et al. 1992b; Nan et al. 1993). As it is a nuclear protein, Mecp2 contains a nuclear localisation signal (NLS) for transportation to the nucleus. The 3'-untranslated region (3'-UTR) in exon 4 is unusually long (8.5 kb) and it is well-conserved between humans and mice. According to what is currently known it does not contain any functional domains (A. Bird, personal communication).

CpG dinucleotides are concentrated on the heterochromatin regions, and promoters of human genes. Most of them undergo methylation of the 5-methylcytosine residues, which functions as an important mechanism of gene silencing associated with alterations in chromatin structure (Meehan et al. 1992a; Jones and Takai 2001). The epigenetic mechanism of gene silencing is important e.g. in X chromosome inactivation (Jeppesen and Turner 1993) and imprinting (Pedone et al. 1999). Gene silencing is thought to occur mainly indirectly, by repressor proteins that are recruited to methylated sites, although methylation alone can sometimes directly repress transcription (Siegfried et al. 1999). Mecp2 is capable of binding a

co-repressor complex consisting of the transcriptional co-repressor Sin3A and histone deacetylases 1 and 2 (HDAC1 and 2) via its TRD domain (Jones et al. 1998; Nan et al. 1998). Histone acetylation is one of the main determinants of chromatin structure, and it has been shown that the deacetylation or core histones renders the chromatin inaccessible to transcription (Eden et al. 1998). Several lines of evidence have shown that repression of histone deacetylation is crucial for transcriptional activation of neural specific genes during neuronal differentiation (Nan et al. 1998; Naruse et al. 1999; Pedone et al. 1999; Wade et al. 1999) Mecp2 is also able to repress transcription from a distance by interacting with the transcriptional machinery (Nan et al. 1997; Nan et al. 1998; Kaludov and Wolffe 2000).

Figure 2. Function of the Mecp2 protein in a normal individual and in a patient with RTT.



2.3.3. Mutation spectrum and clinical phenotype of the patients

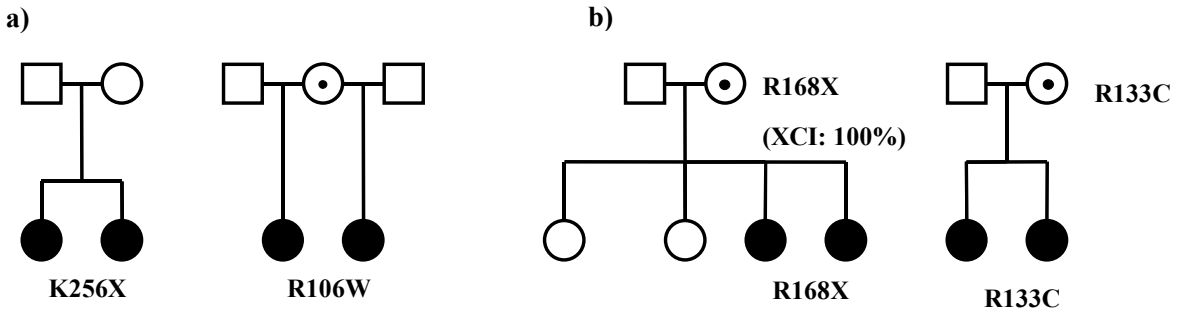
Since the original finding in 1999, over 20 reports have been published on the prevalence of the MECP2 gene mutations in RTT patients of various ethnic origins. To date, around 80 different mutations have been characterised in the coding region of the gene. The detection rate in classical patients varies (30-100%), with a mean value of ~80%. The MECP2 gene is composed of four exons with a coding sequence of 1461 nucleotides in exons 2-4. Almost all mutations are sporadic due to *de novo* mutation of the MECP2 gene, involving C→G transitions at CpG dinucleotides in exon 4. The majority of the mutations are nonsense mutations occurring mainly distal to MBD, whereas the missense mutations are concentrated on the MBD domain of the gene. A number of deletions of various size are found on the 3'-end of the gene that contains palindromic and quasipalindromic sequences. In addition, female RTT patients with a somatic mosaicism for a deletion in the MECP2 gene have been described (Bourdon et al. 2001).

The mutations can arise mainly by two different mechanisms:

- De novo, sporadic (common)
  - Parents are healthy
  - There is no family history of RTT
  - No gene mutations in the parent's DNA

- Familial mutations (uncommon)
  - Germline mosaicism have been described (Figure 3a)
  - Mutation is inherited as an X-linked dominant trait from an obligatory carrier woman with a 50% risk to the offspring (Figure 3b)
  - The XCI status affects the phenotype (Figure 3b, a totally skewed XCI pattern protects from the disease phenotype)

Figure 3. The familial mutations in RTT. a) An example of germline mutation. b) The obligatory carrier mothers with different MECP2 mutations. In the first example a totally skewed XCI pattern (100%) was observed in the carrier mother. In the second example the XCI status of the mother was not determined.



When comparing the type and the location of the mutation and the clinical phenotype several conclusions can be drawn. The same MECP2 mutation can result in a different clinical phenotype in the classical RTT patients (De Bona et al. 2000; Huppke et al. 2000; Nielsen et al. 2001). Cheadle et al. reported significantly milder disease in patients carrying missense mutations as compared with those with truncating mutations, and a milder disease was also associated with late rather than early truncating mutations (Cheadle et al. 2000). Similarly, Amano et al. found a milder disease in patients with a mutation which was located in the TRD domain rather than in the MBD domain (Amano et al. 2000). Amir et al. performed a broad correlation analysis and reported that awake respiratory dysfunction and high levels of CSF homovanillic acid (HVA) were more frequent in the patients with truncating mutations, however scoliosis was more common in patients with missense mutations (Amir et al. 2000). These patients with missense mutations also showed much more severe verbal abnormalities and more often had epileptic seizures.

In addition to the classical RTT patients, MECP2 mutations have been detected in RTT variants (Table 7) that are similar to those in the classical patients. To date, no mutations have been reported for early seizure onset or late regression variants (Figure 1).

Table 7. RTT variants with MECP2 mutations.

RTT VARIANT	AMINO-ACID/ NUCLEOTIDE CHANGE	MUTATION PREVALENCE	REFERENCE
Forme fruste	R255X	1/4	Orrico et al. 2000
	1162del26bp	3/3	Nielsen et al. 2001
	R133C		
	T158M		
PSV	R133C	3/3	Huppke et al. 2000
	R168X		
	P302A		
	?	1/1	Amir et al. 2000
	1157del41	2/3	De Bona et al. 200
	1159del44		
	R3026C	2/2	Obata et al. 2000
	R133C		
	T158M	1/2	Vacca et al. 2000
Congenital	1364-1365insC	1/1	Huppke et al. 2000

The spectrum of phenotype in male patients with MECP2 mutations is broad ranging from congenital encephalopathy and early death (Wan et al. 1999; Villard et al. 2000; Geerdink et al. 2002) to a severe form of MR (Meloni et al. 2000; Orrico et al. 2000; Couvert et al. 2001) (Table 8). Also, two studies have described two boys carrying somatic mosaicism in the MECP2 gene, one for P56R and the other for the R260X mutation (Clayton-Smith et al. 2000; Topcu et al. 2002). The clinical picture of the patients represented a non-fatal neurodevelopmental disorder and clinical similarities to RTT.

Couvert et al. reported the screening of the MECP2 gene in patients with mental retardation (Couvert et al. 2001). Out of 30 MR families studied, two different mutations, R168W and E137G, co-segregated with the disease phenotype in two different families with four and nine affected males respectively. In addition, four out of 185 patients found to be negative for the CGG expansions in the FMR1 gene which is defective in fragile-X syndrome carried a mutation (A140Vx2, P399L and R453Q) in the MECP2 gene. In patients with autism the frequent involvement of the MECP2 gene was ruled out in a group of 59 autistic patients (Vourc'h et al. 2001)

Interestingly, MECP2 gene mutations have been detected in patients with Angelman syndrome (AnS), which is considered as a differential diagnosis of RTT (Imessaoudene et al. 2001; Watson et al. 2001). AnS is mostly sporadic characterised by encephalopathy with microcephaly, epilepsy, absent speech, ataxia and inappropriate laughter (Williams et al. 1995).

Table 8. A summary of male patients with MECP2 mutations showing atypical features of Rett syndrome. OFC = Orbito-frontal cortex.

DATA	CLAES 1997; MELONI. 2000	ORRICO. 2000	YNTEMA 2001	WATSON 2001	LINDSAY 1996; KLAUCK 2002	COHEN 2002
MECP2 mutation	G406X (inherited)	A140V (inherited)	1161-1400del	241del2	A140V (inherited)	A140V (inherited)
Location of the mutation	3'-end	MBD	3'-end	5'-end	MBD	MBD
Familiarity	2 males (uncle and nephew)	4 brothers, one sister, mother with mild MR	A three-generation family (3 males, unaffected carrier female)	No	Three-generation family with 6 affected individuals	Unaffected mother
Age of onset	Infancy	?	?	Neonatal	Infancy	2-3 years
Age of death	39 yrs; not yet	Not yet (27-40 yrs)	?	Not yet (6yrs)	Not yet (>30 yrs)	Not yet (12yrs)
Growth parameters	?	?	?	?	Short stature(3/6)	Normal
OFC	75^{th}-90^{th} in adulthood	Normal in adulthood	?	10^{th}-25^{th}	10^{th}-90^{th}	?
Severe MR	+	+	Mild MR	+	Moderate, IQ~50	No
Seizures	+	?	?	+	1/6	-
Clinical findings	Distal muscular atrophy; ataxia; spasticity; no speech; unilateral choreoatetosis (1 case)	Resting tremors; slow movements; impaired language development	Nonspecific MR without any morphological or neurological features	Dg: AnS; repetitive midline hand movements; tremor; muscular atrophy in limbs; ataxia	Dg: PPM-X syndrome: MR, manic-depressive psychosis, pyramidal signs, parkinsonism, macro-orchidism	Dg: language disorder and schizophrenia. From 12 yrs psychotic symptoms
Purposeful hand skills	?	?	?	?	+	+
Loss of skills	+/-	-	?	+	-	During psychosis, after which slow recovery
EEG	Bradyarrythmia	?	?	Excess of slow wave activity	1/6 temporally abnormal	Normal
Brain imaging	Normal	?	?	?	?	Normal
Dysmorphic facial features	-	?	No	?	No	No, but bilateral pseudomembranous syndactyly of toes 2,3 and 4

AnS is caused by large de novo deletions exclusively of maternal origin on 15q11-q13 indicating that AS is caused by absence of a maternal contribution to the imprinted 15q11-q13 region (Knoll et al. 1989; Magenis et al. 1990). Recently, mutations in the UBE3A gene (coding for E6-AP, a ubiquitin protein ligase) have been described in the patients. However in ~15% of the AnS patients no cytogenetic or molecular abnormality on chromosome 15 can be detected (Kishino et al. 1997; Laan et al. 1999).

Imessaoudene et al. studied 78 patients diagnosed as possible AnS candidates with a normal methylation pattern in the UBE3A gene, and detected mutations in 4 female patients consistent with RTT (R106W, R255Xx2 and 803delG), one female with progressive encephalopathy of neonatal onset (R270X) and one male with non-progressive encephalopathy (G428S) (Imessaoudene et al. 2001). Recently, the G428S mutation was characterised to be a rare genetic variant (Laccone et al. 2002).

Out of 57 patients with a diagnosis of AnS Watson et al. found an MECP2 mutation in four girls with similarities to RTT (1230del44, P101R, 1230del52 and Y141X) and in one male patient with motor delay (241del2) (Table 8).

Furthermore, PPM-X syndrome characterised by psychosis, pyramidal signs and macro-orchidism has been shown to be due to a A140V mutation in the MECP2 gene (Klauck et al. 2002) (Table 8). Taking together, the diversity of phenotypes caused by mutations in the MECP2 gene is wide, and the factors affecting this variability are currently being studied.

2.3.4. Mouse models for RTT

Previous studies with Mecp2-null animals resulted in embryonic lethality, consistent with the idea that male patients hemizygous for the mutation would not survive (Tate et al. 1996). However, recently two groups generated Mecp2-null mice by using Cre-loxP cloning that allows the generation of spatially and temporally specific mutants that were viable (Chen et al. 2001; Guy et al. 2001). Guy et al. generated a deletion resulting in a protein containing only the eight amino-terminal amino acids, and Chen et al. a deletion with the loss of exon 3 (Chen et al. 2001; Guy et al. 2001). The features of these mice resembled the clinical symptoms observed in RTT (Table 9). Also, the finding that an identical phenotype is observed in mice carrying CNS-specific Nestin-Cre mediated Mecp2 deletion and in Mecp2 null mice indicate that the abnormalities observed are due to neuronal absence of Mecp2. Temporal Mecp2 mice mutants derived from a deletion of Mecp2 in postmitotic cells in the brain (CamK-Cre mediated deletion) show clinically and pathologically features which are similar to those of mice with Nestin-Cre mediated deletion. This result may indicate that Mecp2 is not indispensable for brain development, but the fact that it is needed for the maintenance of mature CNS neurons offers a potential opportunity for treatment in RTT (Chen et al. 2001). The notion that neurons are more vulnerable to Mecp2 mutation might be explained by a theory that neurons are more vulnerable to background transcriptional noise, or that Mecp2 is the primary controlling mechanism to regulate gene transcription in the CNS.

Table 9. Summary of the MECP2 null mice.

TYPICAL FEATURES OBSERVED	GUY ET AL. 2001	CHEN ET AL. 2001
Mecp2-null males and females	No initial phenotype at birth	No initial phenotype at birth
Age of onset	3-8 weeks of age	5 weeks of age
Phenotype observed	Stiff, uncoordinated gait; reduced spontaneous movement; limb clasping; low weight/fat deposition (depending on the mouse strain); internal testes; uneven wearing of teeth	Nervousness; body trembling; pila erection; occasional hard respiration, weight changes; physical deterioration
Age of death	~54 days	~10 weeks (70 days)
Pathological analyses	No obvious abnormalities in internal organs (including brain)	Brain: reduced brain size and weight; cell bodies and nuclei in hippocampus, cerebral cortex and cerebellum were of smaller size (15-25%), and more densely packed
Mecp2 deleted in the brain	Similar features as in the Mecp2-null mice	Similar features as in the Mecp2-null mice
Mecp2 $^{+/-}$ female mice	No initial phenotype at birth	No initial phenotype at birth
Age of onset	>3 months	~4 months
Phenotype observed	Inertia; hind limb clasping; breathing irregularities. Some asymptomatic at the age of one year	Weight gain; reduced activity; ataxic gait at later age
Age of death	No rapid deterioration; long-term stability	?

3. STATISTICAL APPROACHES

3.1. BACKGROUND

The uncovering of the human genome consisting of 2.9 billion base pairs (bp) of DNA sequence has made it possible to gain a global perspective of the structure of the genome (Lander et al. 2001; Venter et al. 2001). The human genome project working draft sequence, currently over 90% complete, is fully available for the public at http://genome.ucsc.edu/. On the basis of the current estimates there appears to be about 30 000-40 000 proteins coding genes in humans, which is about twice the amount found in worm or fly. The majority of the genome (75%) is intergenic DNA, and only ~1.1% of the genome constitutes of exons, the protein coding regions of the genes, whereas 24% is located in the introns, the sequence between the coding regions (Venter et al. 2001).

The analysis of individual variation has been facilitated by the availability of increasing amounts of single nucleotide polymorphisms (SNPs) offering tools for association analyses and disease gene discovery. According to the current estimates there exists one SNP per 1200 to 1500 bp nonrandomly distributed in the human genome (Venter et al. 2001). There are several databases that provide information on these variations (e.g. http://www.ensemble.com and http://www.ncbi.nlm.nih.gov/SNP/).

Microsatellite markers that have widely been utilised in disease gene mapping are 2-4 bp repeats occurring about in every 30 kb in the genome with a typical heterozygosity of 70% (Weber 1990; Hearne et al. 1992). Information about several thousands microsatellite markers is freely available in databases such as the Genome Database (http://gdbwww.gdb.org/), the Whitehead Institute (http://www-genome.wi.mit.edu/), the Marshfield Institute (http://research.marshfieldclinic.org/genetics/) and Généthon (http://www.genethon.fr/genethon_en.html).

The order of the markers in genetic maps is based on the recombination fraction between two loci. In general, 1% recombination is equivalent to about a 10^6 bp of DNA (1 Mb), which is defined as 1 cM. However, the rate of recombination varies depending on the chromosomal region, the frequency of which being higher in the telomeres and short arms of the chromosomes, and greater in females than in males (Lander et al. 2001; Venter et al. 2001). The physical maps quantify the distance in terms of kilobases (1 kb of DNA equivalents to 1000 bp).

For analysis purposes the markers are amplified by polymerase chain reaction (PCR), and the fragments are separated by denaturing acrylamide gels. Fluorescence labels can be conveniently used for fragment detection. Data scanning and analysis are currently highly automated.

3.2. LINKAGE ANALYSES

The measure of genetic linkage is the recombination fraction, theta ($0 \leq \theta \leq 0.5$), which is defined by the frequency that a crossing over event occurs between two loci during meiosis. The closer the two loci are to each other the smaller is the chance for recombination. An estimate of $\theta = 0.5$ is consistent with the two loci being unlinked. Two traits are considered to be linked when they fail to be transmitted to the offspring independently from each other. In human Mendelian monogenic diseases tests of linkage are usually performed by the likelihood ratio approach also called parametric lod score analysis, which is defined by the following formula (Ott 1976, Morton, 1995):

$$Z(x) = \log_{10} \left(\frac{L(\text{pedigree given } \theta = x)}{L(\text{pedigree given } \theta = 0.5)} \right)$$

L: likelihood function
Z(x): two-point lod score in which x presents a particular value of θ within the range of recombination fractions.

Traditionally, an odds ratio of more than 1000:1 (corresponding to a lod score of more than 3) is considered as a statistically significant demonstration of linkage in monogenic disorders. The two-point parametric lod score utilises the information of the pedigree structure and it is directly additive between the families. For the calculations, computer based package software programs have been developed, such as LINKAGE (Lathrop and Lalouel 1984; Lathrop et al. 1986) that uses prespecified parameters: a defined genetic model of inheritance, penetrance of the disease and gene frequency.

In complex diseases, linkage analyses are dependent on large number of multiplex families or pedigrees with a given trait. In contrast to monogenic diseases the inheritance pattern in complex diseases is in most cases unknown. The misspecification of a genetic model may lead to false positive (type α-error) and false negative (type β-error) linkage results. Consequently, model-independent, nonparametric linkage analysis methods have been developed that do not require definition of the model of inheritance, such as GENEHUNTER (Kruglyak et al. 1996), MAPMAKER/SIBS (Kruglyak and Lander 1995) and SIMWALK (Sobel and Lange 1996) programs, and SIBPAIR program for sib-pair analyses (Kuokkanen et al. 1996).

In order to map the underlying genes in complex diseases different strategies have been used. If relevant candidate genes are available, disease-causing mutations have been detected by direct sequencing of the candidate genes (Stone et al. 1997) or analysing SNPs in a gene in order to detect association (Perola et al. 1995). Alternatively, to locate new candidate gene loci for the disease phenotype under study a random genome screen is performed by analysing linkage of a trait to 300-400 polymorphic markers evenly spaced in the genome (Risch and Merikangas 1996). Falsely positive associations and linkage findings are excluded by replication studies performed in different populations or patient material with the same phenotype. According to Lander & Kruglyak genome-wide significance levels should be distinguished from pointwise significance levels (Lander and Kruglyak 1995). They suggest a lod score of 3.3 (being equivalent to a P-value of 4.9×10^{-5}) to be considered as a significant evidence for linkage. This higher value corresponds to a genome-wide false positive rate of 5% and would compensate for the testing of multiple markers.

3.3. ASSOCIATION STUDIES

Linkage disequilibrium (LD), the nonrandom association between alleles of linked markers is a powerful method for the high-resolution mapping of monogenic disorders (Hästbacka et al. 1994). Several factors influence the level of observed LD, such as the chromosomal region under study, the age and mutual distance of the markers, the age and history of the population (genetic drift, population growth and structure, admixture or migration) (Ardlie et al. 2002). The younger the mutation the more extensive is the observable region of LD (Varilo et al. 1996).

The strength of association between genotype and phenotype depends on the allelic diversity of the disease in a given population sample. Considering the rare Mendelian disorders in the Finnish population, the affected individuals typically share a specific chromosomal haplotype, which can extend up to several cM in young populations (Varilo et al. 1996, Kere 2001). In contrast the pattern of genetic variation underlying complex disease traits is much more complicated. To simplify, there exist two classes of models. The "complex trait-rare variant" model assumes that individually rare genetic variants that probably are population spesific cause the disease phenotype (Zwick 2000; Risch 2000). Being recent in origin the variant may be confined to a subpopulation. The other model, "complex trait-common variant" predicts that common disease variants are few but relatively common. These variants are more likely to be found globally (Zwick 2000; Risch 2000).

In complex human disorders, not so promising results have been obtained on pedigree-based linkage analyses, and consequently whole genome based association studies have been suggested (Risch 2000). Association analyses with SNPs that have lower mutation frequency compared to microsatellite markers have now gained growing interest. So far, the studies have been hampered by the approximations for predicted LD between an SNP and the disease mutation. First approximations suggested that LD would be limited to ~3 kb (Kruglyak 1999). However, recently published studies have shown that the extent of LD varies among populations, being wider in European than in African populations, and chromosomal regions under study (Ardlie et al. 2002, Taillon-Miller et al. 2000).

Current data suggests that LD is highly structured into discrete blocks of sequence separated by hot spots of recombination (Daly et al. 2001; Miller and Kwok 2001; Reich et al. 2001). Data about the structure of the haplotype blocks demonstrate they exist in blocks of ~22 kb or larger in African and African-American populations and ~44 kb or larger in European and Asian populations (Gabriel et al. 2002). Within each block there exist only a few common haplotypes, which are highly correlated across populations. Taking together, the extent of LD useful for mapping studies has been suggested to be ~10-30 kb for northern European populations (Ardlie et al. 2002) requiring as many as 300 000 well-chosen SNPs for genome-wide assocation mapping of complex diseases (Gabriel et al. 2002).

Interestingly, an SNP haplotype extending over 250 kb on 5q31 region was found to confer an increased risk of 2.0 for Crohn disease in Canadian families. However, association analyses provided no means of selecting the SNP responsible for the increased risk out of the many SNPs uniquely associated. The at-risk haplotype extended over 250 kb (Rioux et al. 2001).

4. STRATEGIES IN COMPLEX DISEASE MAPPING

The mapping of genetic predisposing factor(s) for common and/or complex disorders has not been as successful as in simple Mendelian disorders due to the multifactorial etiology of these diseases. The true inheritance pattern and the number of genes involved are unknown, and the effect of environmental factors has not been specifically characterised. The genetic component of a phenotype (segregating in families) is assessed by family and twin studies. The search for a predisposing factor begins with a careful definition of the disease phenotype and the collection of the family material. The different approaches besides conventional linkage analysis, also include studies with sib-pair material and case-control association studies. Genetic heterogeneity, different causes for the same phenotype, is one of the most important blurring factors in mapping studies. One way to simplify the phenotype is to focus on the most severe forms of the disease, or potential rare familial cases (Sawa and Snyder 2002).

In the characterisation of the affection status there exist several confusing factors: phenocopies (environmentally caused phenotype that mimics the trait under study), incomplete penetrance (phenotypically healthy patients carrying the mutation) and inter- or intrafamilial variation in the expression of the phenotype. Patients with somatic mosaicism carry the mutation in a certain population of cells and often express a milder form of the disease. As the mutation is limited to the cells present in the germline a phenotypically normal carrier can transmit the full disease phenotype to its offspring. Also, genomic imprinting (parent-of-origin differences in the gene expression) and mitochondrial inheritance (transmission occurs through affected females) are exceptions to the common vertical transmission (Haines and Pericak-Vance 1998).

The utilisation of population isolates compared to outbred populations may be advantageous based on the reduced genetic and environmental heterogeneity due to a founder effect (Hovatta et al. 1999; Peltonen et al. 2000; Paunio et al. 2001; Shifman and Darvasi 2001, Kere 2001). However, also opposite opinions exist (Eaves et al. 2000; Jorde et al. 2000). The population history of Finland is characterised by a small number of initial founders, several bottlenecks caused by famine and war, and rapid expansion during the last 80-100 generations that has enabled the creation of a unique genetic background (Nevanlinna 1972; Norio et al. 1973). Up to the 16th century only the coastline of Finland and the southeast and southwest areas were inhabited, the region nowadays called the early settlement or ‘old Finland’. Thereafter, the immigration of farmers mostly originating from a small area of South Savo to the inland established the late settlement region or ‘new Finland’. The founding population created small regional subisolates that were characterised by little further immigration (Kere 2001).

So far, the mapping for a predisposing loci in the Finnish population has been advantageous e.g. in colon cancer (Peltomaki et al. 1993), diabetes (Mahtani et al. 1996), multiple sclerosis (Kuokkanen et al. 1997), schizophrenia (Ekelund et al. 2000) and asthma (Laitinen et al. 2001). Similar examples of other isolated populations exist, such as mapping of Hirschsprung's disease in a Mennonite pedigree (Puffenberger et al. 1994), bipolar disorder either in a genetically isolated population of the Central Valley of Costa Rica (Freimer et al. 1996) or in the Amish population (Ginns et al. 1996). Also, studies on schizophrenia in Canadian-Celtic (Brzustowicz et al. 2000) and the non-insulin-dependent diabetes mellitus in Mexican American and Northern European families (Horikawa et al. 2000) have resulted in

promising findings.

The power of isolated populations in haplotype mapping might be stronger due to long range LD. Populations with shorter-range LD and greater haplotype diversity may be more advantageous for use in the fine mapping phase. The extent of common haplotypes in the vicinity of predisposing loci for different complex disorders is currently under investigation. Potentially, a greater number of markers and larger sample size are needed for genome-wide association studies with outbred populations than with isolated ones (Shifman and Darvasi 2001).

AIMS OF THE PRESENT STUDY

When this thesis was started the advances in the field of molecular genetics had made the mapping of complex disorders feasible. At that time the first genome-wide screens performed in multiplex families with autism have just been published. In order to localise the susceptibility genes for autism in the isolated Finnish population, we started a collaborative project together with skilled clinicians to collect a nationwide sample of Finnish families with autism spectrum disorders. During these studies, the gene responsible for RTT was identified, and the role of this gene was analysed in the Finnish RTT patients.

The detailed aims were:

1. To analyse the role of previously reported candidate loci for autism in the Finnish families with autism and AS.

2. To localise susceptibility regions for autism spectrum disorders in the Finnish families using a genome-wide scan approach.

3. To analyse the candidate regions of genome for autism spectrum disorders in families originating from a subisolate of Central Finland.

4. To study the role of the MECP2 gene in Finnish classical and atypical RTT syndrome patients.

MATERIALS AND METHODS

5. PATIENT MATERIAL

5.1. AUTISM SPECTRUM DISORDERS AND DEVELOPMENTAL DYSPHASIA

The family material was obtained through a nationwide collaboration of child neurologists and other clinicians. Raija Vanhala, MD, Lennart von Wendt, Professor, and Taina Nieminen-von Wendt, MD, from Helsinki University Central Hospital, Reija Alen, MD, from Jyväskylä Central Hospital and Raili Riikonen, MD, and Salme Majuri, MD, from Kuopio University Central Hospital were the main clinical collaborators. Information about the research was also provided in the magazine of the Finnish association for autism and Asperger syndrome.

Although developmental dysphasia is classified as a separate disease entity, a strong genetic component has also been reported in this group of disorders. Based on the coexisting diagnoses of autism spectrum disorders and developmental dysphasia in the same families we assumed that these disease entities might partly share a common genetic background.

Thorough clinical and medical examinations were performed. Childhood Autism Rating Scale (CARS), Asperger Syndrome Screening Questionnaire (ASSQ), and Asperger´s Syndrome Diagnostic Interview (ASDI) (Ehlers and Gillberg 1993) were used as screening instruments. Also, the statements of speech therapeutics and neuropsychologists available were applied in the diagnosis process. Diagnoses were carefully made by experienced child neurologists or paediatricians according to the DSM-IV, 4th Edition, or ICD-10 criteria (World Health Organization 1993). Families with associative medical conditions such as fragile-X syndrome, chromosomal aberrations, neurocutaneous syndromes and profound mental retardation were excluded. A blood sample was taken from all the available first-degree relatives of the probands. All families were Finnish, except one father who is of Turkish origin.

These studies have been approved by the ethical committees of the Hospital for Children and Adolescents of Helsinki University Central Hospital, Jyväskylä Central Hospital and National Public Health Institute, Helsinki. Informed written consent was obtained from the subjects and/or their parents.

In study I, a linkage analysis of the previously reported candidate regions on autism was performed with 17 multiplex Finnish families consisting of 14 sibling pair core families and three families (Families 22, 37 and 40) with a more remote consanguinity (Table 10). Infantile autism was aggregating in eleven, and Asperger syndrome and autism in six families. The patients with developmental dysphasia were excluded from these analyses.

For study II, some of the same families were analysed, and also patients confirmed to have developmental dysphasia were utilised. The total material consisted of 30 sib pair families, and eight extended families with altogether 87 affected individuals (Table 11). The family trees of the extended pedigrees are shown in Figure 4. For linkage analyses the patients were divided according to the phenotype into three diagnostic categories: infantile autism (criterion1; 19 families), infantile autism and AS (criterion 2; 28 families) and infantile autism, AS and developmental dysphasia (criterion 3; 38 families).

Table 10. The family material in the exclusion mapping. Aut = autism, AS = Asperger syndrome, dy = dysphasia. The numbers in the brackets refer to the number (no.) of affected individuals with a phenotype in question.

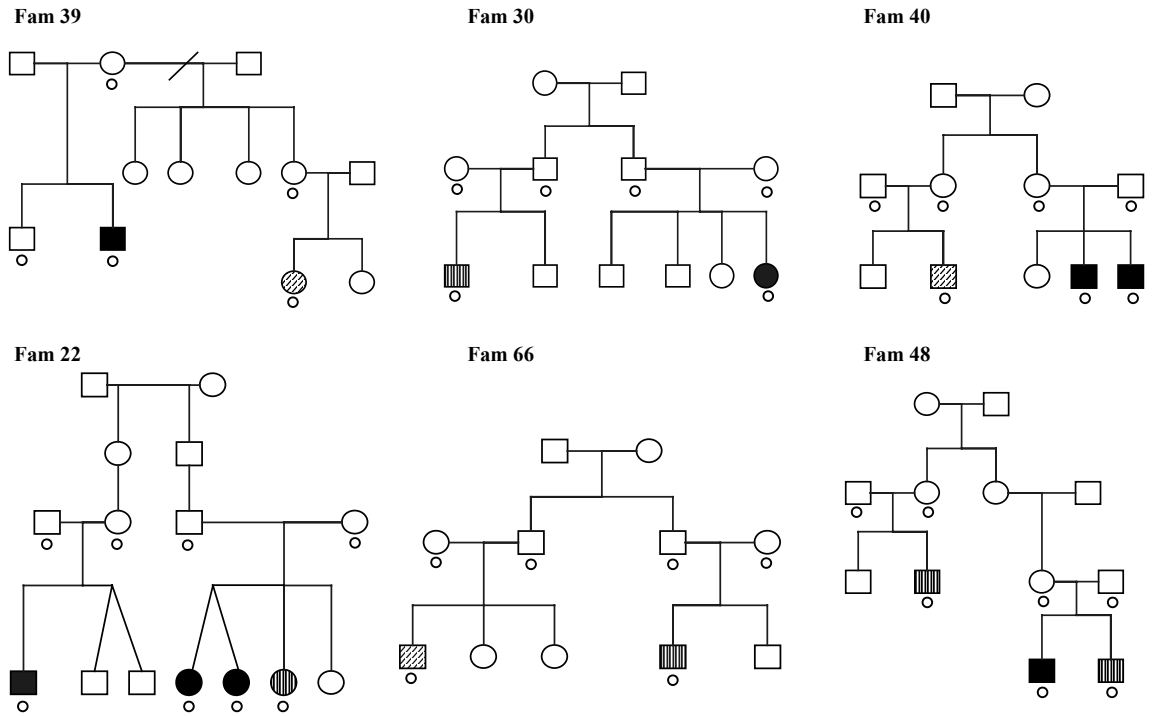
PHENOTYPES	NO. OF AFFECTED	FAMILIES			TOTAL AFFECTED
		Sib-pair	Other	Total	
Aut	2	7	–	7	14
	3	2	–	2	6
Aut+AS	2 (1+1)	3	1	4	8
	3 (2+1)	–	1	1	3
Aut+dy	3 (2+1)	1	1	2	6
Aut+AS+dy	3 (1+1+1)	1	–	1	3
	TOTAL	**14**	**3**	**17**	**40**

Table 11. Patient material in the genome-wide scan. Aut = autism, AS = Asperger syndrome, dy = dysphasia. The numbers in the brackets refer to the number (no.) of affected individuals with a phenotype in question.

PHENOTYPES	NO. OF AFFECTED	FAMILIES			TOTAL AFFECTED
		Sib-pair	Other	Total	
Aut	2	13	–	13	26
	3	1	–	1	3
AS	2	1	–	1	2
	3	1	–	1	3
Aut+AS	2 (1+1)	5	1	6	12
	3 (2+1)	–	2	2	6
Aut+dy	2 (1+1)	4	1	5	10
	3 (1+2)	–	1	1	3
	3 (2+1)	1	1	2	6
	5 (4+1)	–	1	1	5
Aut+AS+dy	3 (1+1+1)	1	–	1	3
AS+dy	2 (1+1)	3	1	4	8
	TOTAL	**30**	**8**	**38**	**87**

In order to diminish the problem of phenotypic heterogeneity, the diagnoses of the patients were reassessed during the study. We paid particular attention to the classification of the patients with developmental dysphasia. To reduce variation when making the diagnoses the reassessment was performed by one specialist in child neurology. Most of the patients (11/15) had receptive language disorder (F80.2 according to ICD-10), which is similar to the DSM-IV diagnosis for mixed receptive-expressive language disorder. Two out of 15 patients had an expressive type of dysphasia (F80.1 according to ICD-10) similar to the DSM-IV diagnosis for expressive language disorder, however one of them had the diagnosis of F80.2 in early childhood and the other was diagnosed late, the condition was first observed at the age of ten years. Further, one young male patient with an autistic sibling was first diagnosed to have the expressive type of dysphasia (F80.1), and handled as such in the analyses. Later on he fulfilled the criteria for autism. In one subject who was first considered to be dysphatic and handled as such in statistical analyses, not all the necessary diagnostic criteria for dysphasia or autism spectrum disorder were fulfilled. Problems in social interaction and language skills were observed, however, the diagnosis remained unspecified.

Figure 4. The six extended families, indivuduals participating in the genome-wide scan are marked as dots. Black symbols = infantile autism, symbols with slashed lines = Asperger syndrome, and symbols with vertical lines = developmental dysphasia.



In study III, a total of 31 core families with 48 affected individuals (37 patients with infantile autism and 11 patients with Asperger syndrome) originating from Central Finland were analysed. Autism was segregating in 23 families and AS with or without autism in eight families. Twenty-four of these families participated in our genome scan and 7 families are new. The affection status of four siblings with dysphasia and all the parents were considered as unknown in the analyses. In one family both parents, and in two families one of the parents were not available for genotyping.

5.2. RETT SYNDROME

Thirty-nine Finnish patients with classical RTT, and one patient with PSV participated in the mutation analysis of the MECP2 gene (study IV). Further 12 patients with developmental delay and with some RTT-like features were also included in the analyses. The diagnostic criteria used were as following: (I) normal pre- and perinatal development and apparently normal development for the first five to six months; (II) developmental regression with onset between six months and three years; (III) normal head circumference growth following deceleration between five months and four years; (IV) loss of purposeful hand movements and acquisition of hand movement stereotypies; and (V) marked developmental and cognitive delay. Altogether 52/80 (65%) of the Rett patients' parents were available for the study: samples from both parents were obtained in 22 families and maternal samples alone in eight families. The birthplaces of the ancestors of the patients were traced back two to six generations (see section 6.1).

6. METHODS

6.1. GENEALOGICAL STUDIES

Considering the history of isolation in the Finnish population genealogical studies were performed to locate possible founder effects in autism spectrum disorders in Finland. The names, dates and places of birth of the patient's grandparents were obtained from the participating families. To monitor for common ancestors, Teppo Varilo, MD, performed genealogical analyses using population registers (Varilo et al. 1996). Church records were also utilised for earlier periods in the Finnish National Archives.

6.2. DNA ISOLATION AND GENOTYPING

DNA was extracted from EDTA blood according to standard procedures (Blin and Stafford 1976). PCR reactions were performed in 15 μl reaction volume containing 20 ng of genomic DNA, 6 pmol of both primers, 0.2 mM of dNTP, 1.5 mM $MgCl_2$, 10 mM Tris-HCl, 50 mM KCl, 0.1% Triton X-100, and 0.23 U Dynazyme (Finnzymes Oy). The reactions were performed with the help of an MJ Research thermocycler (Cambridge, MA) using a hot-start procedure with Dynazyme added only after the first denaturation step of 5 min at 95ºC. The DNA amplification was carried out in 35 cycles as following: 30 s at 95ºC, 30 s at temperature specific for each primer (49-62ºC), and 30 s at 72ºC. An elongation step of 5 min at 72ºC terminated the reaction after the last annealing. Gel electrophoresis was performed using an ABI automated DNA sequencer (Applied Biosystems), and genotypes were assigned using the Genotype 2.0 software (Applied Biosystems) by two independent individuals.

The initial genome-wide scan (study II) was performed in 19 multiplex families with 47 affected individuals using 368 microsatellite markers from the Weber screening set 6.0 (Sheffield et al. 1995) and 28 additional markers that were selected on the basis of the previously reported positive findings (Stage I). A total of 54 new markers were included to fine map the potential candidate regions detected in Stage I locating on chromosomes 1, 3, 7, 9, 12, 14, 17, 19 and 21. In the fine mapping phase (Stage II), 19 additional families with 40 affected individuals were included in the analyses (Table 11). Markers not working were replaced by markers from the Généthon marker map (http://www.genethon.fr/genethon_en.html). The fine mapping markers were selected from the maps of The Center for Medical Genetics (http://research.marshfieldclinic.org/genetics/) and the Genetic Location Database (http://cedar.genetics.soton.ac.uk/public_html/ldb.html). All the parents were genotyped, except in one family both parents, and in three families one of the parents were not available for analyses.

6.3. LINKAGE AND ASSOCIATION ANALYSES

In the linkage and association analyses the affection status of the parents without any phenotype was considered as unknown.

In study I, two-point pairwise linkage analysis was performed in 17 families with autism and AS under recessive model of inheritance using the MLINK program of the LINKAGE package (Lathrop and Lalouel 1984; Lathrop et al. 1986). Tests of heterogeneity and calculations of the proportion of linked families (α) were performed using the HOMOG

program (Ott 1986). An affecteds-only strategy was employed to minimise the effect of penetrance assumptions allowing no phenocopies with the disease-allele frequency set at 10^{-4}. Nonparametric sib-pair analysis was performed using the SIBPAIR program (Kuokkanen et al. 1996).

In study II, the patients with developmental dysphasia were included and three different liability classes were used (1 for autism, 2 for AS and 3 for developmental dysphasia). For statistical analyses the families were classified into three categories: families with infantile autism only (diagnostic criterion 1), families with infantile autism and AS (diagnostic criterion 2), and families with all three phenotypes (diagnostic criterion 3). Because of the partially overlapping diagnostic subgroups, the obtained linkage results should be cautiously interpreted due to the problem of multiple testing. However, taking into account that the evidence of linkage observed without this correction is so significant, it is probable that this correction does not affect the conclusions made.

The maximum two point lod scores were calculated as in study I under both autosomal dominant and autosomal recessive modes of inheritance. On chromosomes 1 and 3q, non-parametric maximum MLSs were calculated using the MAPMAKER/SIBS program (Kruglyak and Lander 1995) and parametric MLS under the dominant model of inheritance utilising the SIMWALK 2.81 program (Sobel and Lange 1996) in 30 sib-pair families.

Association analyses were performed by calculating the P-values for TDT using the MENDEL 4.0 program (Lange et al. 2001) and likelihood-based haplotype relative risk (HRR-LRT) using the ANALYZE package. Gamete-competition analysis, as implemented in MENDEL 4.0 (Lange et al. 2001), was used to estimate the degree of apparent bias in the transmission of alleles from loci on chromosomes 1, 7 and 3q to the affected offspring (Sinsheimer et al. 2000).

In study III, association analyses were performed with the help of TDT, gamete-competition analysis and association sharing test (NPL option) as implemented in MENDEL 4.0 (Sinsheimer et al. 2000; Lange et al. 2001) on chromosomes 1, 3q and 7. In addition, a model-free lod score analysis in which haplotype frequencies were treated as a nuisance parameter was performed with PSEUDOMARKER (Goring and Terwilliger 2000), which uses a modified version of ILINK from the FASTLINK 4.1P package (Lathrop et al. 1984; Cottingham et al. 1993; Schäffer et al. 1994 and Alejandro Schäffer, personal communication).

For genotype error elimination the PEDCHECK, MENDEL and SIMWALK 2.81 programs (Lange 1988, Sobel and Lange 1996; O'Connell and Weeks 1998) were utilised for study II, and the former one only for studies I and III.

6.4. SEQUENCING OF THE MECP2 GENE

PCR amplification of the coding exons of MECP2 was performed as described elsewhere (Amir et al. 1999), with slight modifications. For the 5' portion of the exon four coding region a new forward primer 5'-TTCTGTACCAGGCCTGACTC-3' was used together with the published reverse primer 5'-CTTCCCAGGACTTTTCTCCA-3', at an annealing temperature of 60°C. The PCR products were purified by treatment with 0.5 U shrimp alkaline phosphatase and 2.5 U exonuclease at 37°C for 25 min, followed by inactivation for

15 min at 80°C. The purified products were sequenced directly using the ABI-PRISM dye terminator cycle sequencing ready reaction kit (Pelkin-Elmer) on an ABI 377 automatic sequencer. Sequencing results were compared with the reference human MECP2 sequence (GenBank X89430 and AF030876) using a Sequencher 4.05 (GeneCodes). Both strands were sequenced, and all the mutations were confirmed by new reactions. The clinical picture of the patients (classical vs. variant RTT) was not known at the time of analysis. In cases where a mutation was found the parental DNA was also sequenced. All mutations were screened in a panel of 50 anonymous Finnish blood donors to confirm the causative function of the mutations.

6.5. X CHROMOSOME INACTIVATION STUDIES

The androgen-receptor gene polymorphism and the methylation of *Hpa*II and *Hha*I sites at the 5' end of the trinucleotide repeat were studied in order to identify the methylation status of paternal and maternal alleles (Allen et al. 1992; Pegoraro et al. 1994). DNA was extracted from white cell nuclei from peripheral fresh blood. Samples (1 μg), were digested with 10 U *Hpa*II and *Hha*I (Amersham Life Science) in a 25 μl volume at 37°C for 4 h and heat-inactivated at 70°C for 20 minutes. The PCR-amplified alleles were electrophoresed on an ABI 377 automatic sequencer both before and after digestion, and the peak heights were analysed with Genotyper v2.0 software (Perkin Elmer). Semiquantitation of the alleles was performed by first correcting the values for unequal amplification of alleles and then by calculating the average of the two separate digestions (Pegoraro et al. 1994). The values were rendered as a percentage, and considered skewed if they were less than 20% or higher than 80%.

RESULTS AND DISCUSSION

7. MAPPING OF GENETIC LOCI FOR AUTISM SPECTRUM DISORDERS IN THE FINNISH POPULATION

7.1. EXCLUSION OF THE PREVIOUSLY REPORTED AUTISM LOCI (I)

The linkage studies with Finnish families were first concentrated on the positive regions previously identified in the genome-wide screens of autism (Figure 5). None of the ten previously reported candidate regions (on chromosomes 1p, 4p, 6q, 7q, 13q, 15q, 16p, 17q, 19q and 22q) showed statistically significant evidence for linkage in the 17 multiplex Finnish families. The slightly interesting region in the analysis was on the chromosome 1p with markers D1S1675 and D1S534 that showed maximum lod scores of 0.87 and 0.76 respectively (Table 12). The affected sib-pairs shared 70.5% and 60.6% of alleles IBD with markers D1S1675 and D1S534, respectively. These sharing proportions were roughly the same as the maximal IBD sharing of 65.6% near marker D1S1361 reported by Risch et al. in 90 multiplex families (Risch et al. 1999). Multipoint analysis on chromosome 1 did not give any additional information in our material (data not shown). The results obtained in this study show that different gene loci and predisposing genes might underlie autism spectrum disorders in the Finnish population compared to more heterogeneous populations. However, taking into account the small size of our families it might be possible that we failed to replicate the previous findings due to the limited power of the data-set.

Figure 5. Overview of the strategy of Study I.

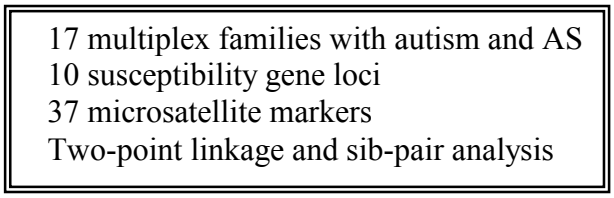


Table 12. Lod scores for two-point linkage analysis under the recessive model and sib-pair analysis on chromosome 1p. Hom = Lod scores under homogeneity, Het = Lod scores under heterogeneity, ASP = affected sib-pair analysis, All-SP = all sib-pair analysis.

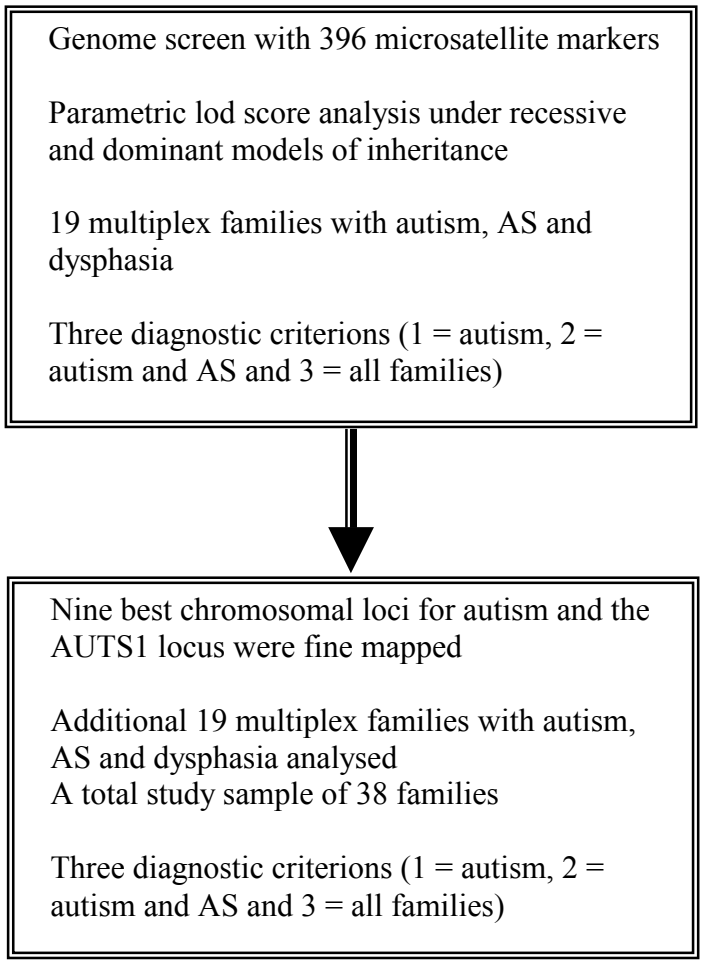
MARKER	TWO-POINT LINKAGE ANALYSIS		SIB-PAIR ANALYSIS	
	Hom	Het	ASP	All-SP
D1S1631	0.06	0.06	0.01	0.02
D1S1675	0.87	0.87	0.82	0.30
D1S534	0.76	0.76	0.33	0.12

7.2. AUTISM SUSCEPTIBILITY LOCI IN THE FINNISH POPULATION (II)

7.2.1. Localisation of a major susceptibility locus, AUTS2 for Finnish families

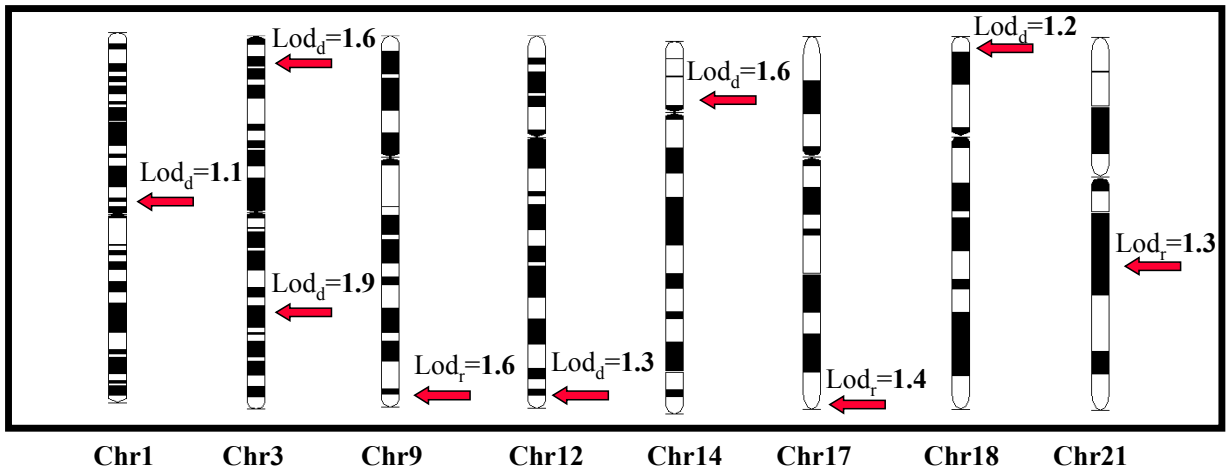
Based on the mainly negative results obtained in the analysis of the reported chromosomal loci for autism a genome-wide screen was performed (Figure 6). In Stage I we found nine chromosomal regions 1, 3p, 3q, 9, 12, 14, 17, 18 and 21, which showed a pair-wise parametric lod score >1 in criterion 1 families (12 families) (Table 13; Figure 7). When the diagnostic spectrum was broadened to include AS (criterion 2) and dysphasia (criterion 3), the number of families in the linkage analyses increased to 18 and 19, respectively. The genome regions showing some evidence for linkage (Zmax >1) for both criterion 2 and criterion 3 families were on chromosomes 1, 3p, 3q, 6, 19 and X.

Figure 6. Schematic overview of the stages of study II.



Fine mapping studies (Stage II) with additional 19 families were concentrated on the aforementioned loci detected with criterion 1 families. Based on the several positive findings on chromosome 7q region (AUTS1 locus) (IMGSAC 1998; Barrett et al. 1999; Philippe et al. 1999; Risch et al. 1999; IMGSAC 2001b; Liu et al. 2001) we fine mapped this region with five markers.

Figure 7. The Stage I susceptibility regions for autism. Lod_d = dominant lod score, Lod_r = recessive lod score



The best results were obtained on chromosomes 1, 14 and 17 with category 1 families, on chromosome 3q with category 2 families and on chromosomes 3p and X with category 3 families.

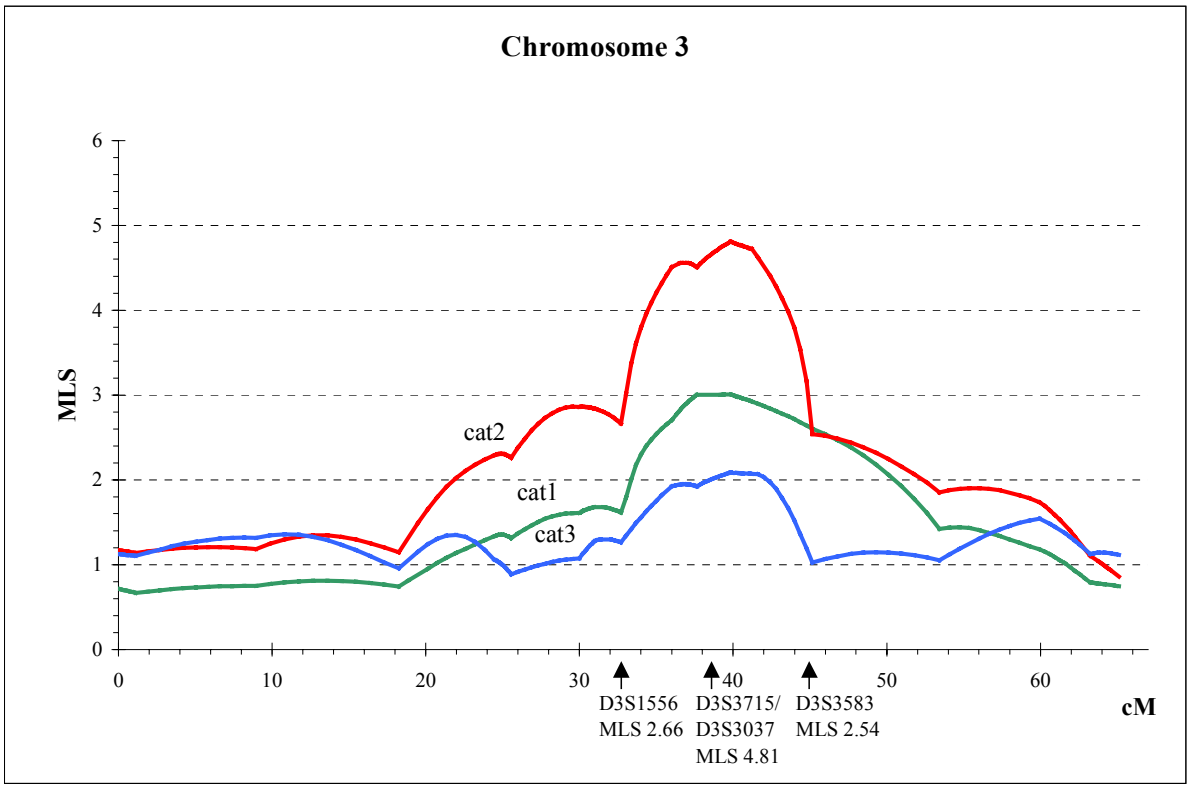
Table 13. The best two-point lod scores in autism families in Stage I. $Zmax_{rec}$ = recessive lod score, $Zmax_{dom}$ = dominant lod score, θ = recombination fraction, α = proportion of linked families.

MARKER	LOD SCORES $Zmax_{rec}$	LOD SCORES $Zmax_{dom}$
D1S1675	1.0 (θ = 0.16, α = 1.0)	1.11 (θ = 0.0, α= 1.0)
D3S3038	1.60 (θ= 0.14, α= 0.64	1.67 (θ = 0.0, α= 0.77)
D3S4009	1.60 (θ = 0.0, α = 0.59)	0.90 (θ = 0.0, α= 1.0)
D3S3554	1.64 (θ = 0.1, α = 1.0)	1.90 (θ = 0.0 α = 1.0)
D3S3053	1.10 (θ = 0.04, α = 0.65)	0.82 (θ = 0.06, α= 0.92)
D3S2427	1.45 (θ = 0.16, α = 1.0)	1.77 (θ = 0.0 α =1.0)
D3S2418	1.40 (θ = 0.12, α = 1.0)	1.30 (θ = 0.0 α =1.0)
D9S158	1.62 (θ = 0.0, α =0.64)	0.87 (θ = 0.08 α =1.0)
D12S2078	1.02 (θ = 0.14, α =1.0)	1.30 (θ = 0.0 α =1.0)
D14S297	1.53 (θ = 0.1, α =1.0)	1.55 (θ = 0.0 α =1.0)
D17S784	1.43 (θ = 0.04, α =0.51)	1.17 (θ = 0.04 α =0.75)
D18S59	1.22 (θ = 0.0, α =0.40)	1.17 (θ = 0.04 α =0.75)
D21S1440	1.29 (θ = 0.0, α =0.44)	0.72 (θ = 0.04 α =0.76)

In Stage II, supporting best evidence for linkage was observed on chromosome 3q. In this region, the highest two-point lod score of $Zmax_{dom}$ = 4.31 (θ = 0.0, α = 1.0) and ASP lod score of 4.21 (IBD sharing 82%) were observed at D3S3037 in criterion 2 families. In criterion 1 families the highest ASP lod score of 3.16 (IBD sharing 84%) was generated at the same locus. Parametric MLS analysis on the chromosome 3q region that allows for locus

heterogeneity resulted in a maximum MLS of 4.81 with an α-value of 1.0 in criterion 2 families under dominant mode of inheritance (Figure 8). The maximum MLS values of 3.01 (α-value = 1.0) and 2.08 (α-value = 0.65) were observed for criterion 1 and criterion 3 families respectively at D3S3037 (Figure 8). These results suggest a susceptibility loci for a phenotype limited to autism spectrum (autism and AS).

Figure 8. Parametric MLS on chromosome 3q in Stage II. Cat1, 2 and 3 = criterion 1, 2 and 3 families, respectively



On chromosome 3q, a statistically significant TDT result was observed under criterion 1 for marker D3S3699 locating ~1 cM distally from D3S3037 (P-value = 0.0127) (Table 14). For marker D3S3730 locating close to D3S3699 evidence for association was detected with TDT analysis under criterion 2 (P=0.0488). The most significant evidence for association was generated with marker D3S3037 under criterion 2 in families originating from Central Finland with P = 0.0077 in TDT and P = 0.0063 in gamete competition analysis (Table 14).

It is noteworthy that the highest lod scores at 3q25-27 were obtained with a broad phenotype including both infantile autism and Asperger syndrome suggesting that these two phenotypes might be caused by the same predisposing gene.

Chromosome 3q locus is a novel locus that has not been found in any of the previously reported genome scans of autism spectrum disorders. Replication studies are needed to confirm whether this locus is also found in other populations or is unique to the Finnish population.

Table 14. Chromosome 3 and 1 association and gamete-competition (GC) analyses results in Stage II. For Central Finland subset, families with autism and AS were analysed. HRR = haplotype relative risk analysis, TDT = transmission disequilibrium test.

MARKER	DIST.	CRITERION 1			CRITERION 2			CRITERION 3			CENTRAL FINLAND		
	(CM)	HRR-LRT	TDT	GC	HRR-LRT	TDT	GC	HRR-LRT	TDT	GC	HRR-LRT	TDT	GC
Chromosome 3:													
D3S2427	188.29	0.5000	0.0567	0.5649	0.5000	0.0717	0.3539	0.5000	0.1760	0.5179	0.5000	0.1582	0.3562
D3S3676	188.29	0.5000	0.3130	0.0853	0.5000	0.2550	**0.0321**	0.5000	0.1330	0.0660	0.5000	0.3606	0.1410
D3S3041	188.29	**0.0387**	0.8050	0.5676	0.0705	0.5580	0.3202	0.5000	0.2910	0.1259	0.5000	0.3532	0.3079
D3S3715	190.43	0.5000	0.4080	0.1956	0.0828	0.3140	0.2733	0.5000	0.7920	0.6542	0.4971	0.2325	0.3648
D3S3037	190.43	0.5000	0.1050	0.1330	0.5000	0.2070	0.2308	0.5000	0.4310	0.4176	0.5000	**0.0077**	**0.0063**
D3S3699	191.79	0.1252	**0.0127**	0.0524	0.5000	0.5370	0.4484	0.2347	0.6530	0.6709	0.5000	0.2180	0.0801
D3S3730	191.79	0.5000	0.1380	0.2834	0.3585	**0.0488**	0.1082	0.0952	0.0807	0.0996	0.5000	0.1900	0.2064
D3S3583	195.60	0.5000	0.6690	0.6733	0.5000	0.4850	0.4803	0.5000	0.4680	0.3546	0.5000	0.2397	0.5144
D3S2436	203.28	0.5000	0.8480	0.8816	0.5000	0.2380	0.4710	0.5000	0.1350	0.2204	0.1917	0.3716	0.7458
Chromosome 1:													
D1S1653	164.09	0.4957	**0.0069**	**0.0231**	0.5000	**0.0327**	0.13637	0.5000	0.1850	0.38575	0.5000	0.3710	0.2764
D1S2771	168.52	**0.0020**	**0.0004**	**0.0028**	**0.0075**	**0.0006**	0.13080	0.1125	**0.0232**	0.14521	**0.0418**	**0.0051**	**0.0091**

7.2.2. Haplotype analysis on chromosome 3q25-27

To further address the significance of the chromosome 3q25-27 region as a possible predisposing locus for autism in the Finnish population we constructed haplotypes in the analysed families and monitored for shared chromosomal segments.

Haplotype analysis was performed in 17 criterion 1 families, 25 criterion 2 families and in 34 criterion 3 families, as well as in 18 sib-pair families originating from Central Finland using the GENEHUNTER program. The six markers were localised on the contig map of NCBI (http://www.ncbi.nlm.nih.gov/). No single shared haplotype could be identified among all families (Figure 9). Interestingly, at marker D3S3037 allele 11 was observed in 65% of affected siblings originating from the Central Finland. The frequency of this allele was 24% in the affected siblings from the entire study material. In the normal chromosomes of the entire material (nontransmitted alleles of the parents) the frequency of this allele was 19%.

The haplotype analysis shows an enrichment of certain alleles in the subisolate of Central Finland. In the χ-square analysis, significant results ($P < 0.05$) were observed with markers D3S3037 and D3S3699 in families with autism and in families originating from Central Finland, respectively (Figure 9). This finding is supported by the genealogical studies revealing a large inbred pedigree with autism in Central Finland (see section 7.3).

Figure 9. The observed chromosomal segment of ~4 cM. The P-values of the χ-square test for Category 1 and Central Finland (CF) families are shown in the bottom line.

FAMILY	Markers and contig accession number					
	D3S2427 NT_022674.3	D3S3676 NT_022458.4	D3S3041 NT_022458.4	D3S3715 NT_005503.4	D3S3037 NT_005503.4	D3S3699 NT_005950.5
Central Finland						
1	18	7	9	**8**	**11**	**7**
2	**20**	**6**	**10**	**8**	**11**	**7**
4	18	5	8	**8**	**11**	4
9	**20**	**6**	**10**	**8**	10	6
13	15	10	6	7	**11**	6
19	15	**6**	**10**	**8**	10	0
11	22	7	7	7	**11**	**7**
32	22	4	8	6	**11**	6
20	15	7	9	6	**11**	**7**
14	22	9	9	6	**11**	6
48	**20**	**6**	11	6	10	5
15	**20**	7	8	**8**	10	6
16	24	**6**	9	**8**	**11**	4
51	**20**	9	**10**	**8**	9	6
18	24	**6**	8	9	10	**7**
60	19	11	9	9	**11**	**7**
150	17	**6**	9	9	**11**	6
Outside Central Finland						
5	15	9	8	**8**	10	**7**
6	4	0	**10**	7	8	4
7	17	**6**	**10**	**8**	10	4
8	21	11	**10**	**8**	**11**	**7**
14	19	11	9	**8**	10	6
10	21	**6**	11	**8**	8	6
19	18	**6**	8	7	9	0
24	**20**	**6**	4	7	6	5
27	18	**6**	9	7	12	0
28	**20**	9	**10**	6	12	**7**
12	**20**	**6**	9	**8**	**11**	4
13	4	12	6	**8**	12	**7**
42	23	**6**	**10**	**8**	10	6
17	4	9	**10**	6	6	0
62	**20**	9	**10**	**8**	**11**	**7**
128	19	7	8	**8**	9	**7**
133	23	9	6	6	**11**	**7**
Category 1	0.561	0.610	0.704	0.287	0.547	**0.009**
CF	0.534	0.723	0.814	0.200	**0.049**	0.177

7.2.3. Other putative susceptibility loci

On chromosome 1, we obtained a $Zmax_{dom}$ = 1.98 and a $Zmax_{dom}$ = 2.63 at D1S1675 in criterion 1 and in criterion 2 families respectively. Risch et al. reported a maximum MLS = 2.15 at the same marker in 139 sibships with mixed American origin (Risch et al. 1999). The results can be interpreted as a replication in this region. The data also confirms our previous results from the analysis of ten candidate gene regions in which the region on chromosome 1p showed slight evidence for linkage in 17 multiplex families with autism spectrum disorder (study I). Some encouraging linkage evidence emerged for the long arm of chromosome 1. In criterion 1 families, we found a maximum MLS = 2.63 near D1S1653 located ~13 cM distally from the marker D1S1675.

In association analyses significant evidence for allelic association was obtained in all diagnostic classes for marker D1S2771 which was located ~4 cM telomerically from D1S1653. In TDT analysis the best P-value, 0.0004, was detected for marker D1S2771 under criterion 1 families (Table 14). Evidence of association in TDT was also observed for D1S1653 in affecteds under criterion 1 and 2 (Table 14). Interestingly, an MLS = 6.50 close to marker D1S1653 was recently reported in a study of schizophrenia (Brzustowicz et al. 2000). Earlier family studies have shown increased rates of schizoid personality traits in families with autism (Piven et al. 1994; Piven et al. 1997a; Piven et al. 1997b; Murphy et al. 2000), however it remains to be determined whether these neuropscyhiatric disorders share common susceptibility gene(s) in this particular chromosomal region.

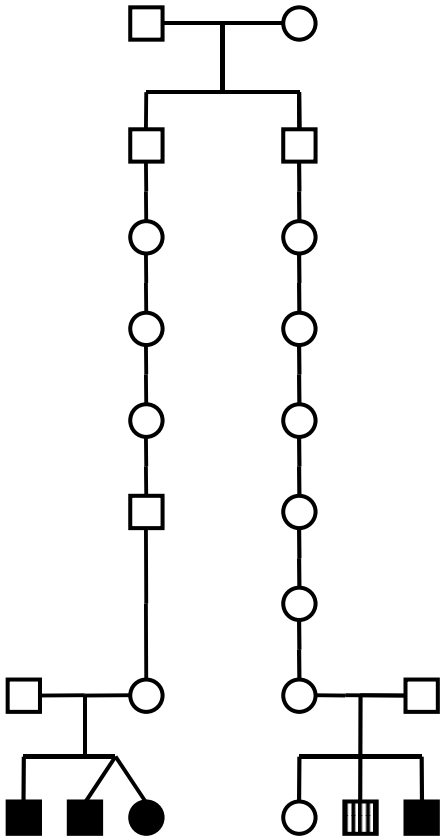
Analysis of AUTS1 locus with five markers on chromosome 7q (D7S480, D7S1804, D7S2437, D7S684 and D7S1824) did not give evidence for linkage (pair-wise lod scores <1) nor transmission disequilibrium in criterion 1 and 2 families. Interestingly, in Stage I, significant evidence for transmission disequilibrium was observed in criterion 3 families in TDT and gamete competition analysis for markers D7S2462 and D7S550 locating 8.6 cM apart (data not shown). Similarly, Liu et al. reported a maximum MLS = 2.13 with 110 sib-pair families with a broad phenotype (autism, AS or PDD) at D7S483 that resides ~4.6 cM proximal from our best marker D7S2462 (Liu et al. 2001). Taken together, our result gives further support for the localisation of a predisposing gene at 7q for a spectrum of disorders, broader than classical autism.

In Stage II analyses no evidence for the increase of linkage could be obtained for markers on chromosomes 9, 12, 18 and 21. Loci on chromosome 14q and 17q provided only suggestive evidence for linkage, but could still be interesting since some positive lod scores have previously been reported for chromosome 14 in British (IMGSAC 1998) and for chromosome 17 in American families (Risch et al. 1999). For chromosome 17, the locus was reported in a mixed American sample set (Risch et al. 1999) with markers located at a significant distance, 104 cM, proximal from our region. A Zmax = 2.19 at D17S784 was previously reported for familial schizophrenia (Brzustowicz et al. 2000). Thus, like in the case of chromosome 1q, these findings raise the possibility of shared susceptibility gene(s) underlying infantile autism and schizophrenia.

7.3. STUDIES WITH FAMILIES ORIGINATING FROM CENTRAL FINLAND (III)

Genealogical analyses of the families participating in the genome-wide scan showed that 51% of them have at least one grandparent born in Central Finland. More detailed analysis using church and population registries extending to the 18th century revealed two large pedigrees: one with two families having a common ancestor from Kuopio eight generations ago (Figure 10) and the other consisting of 18 individual families with 30 affected individuals (Figure 11). In the first pedigree, one family has three children with infantile autism and the other one child both with autism and severe developmental dysphasia (Figure 10).

Figure 10. Two core families in the genome-wide scan with a common ancestor. Black symbols = autism, symbol with vertical line = developmental dysphasia



In the second large pedigree consisting of 18 core families with autism spectrum disorders, the majority of the affected children have infantile autism whereas four families have Asperger syndrome. Interestingly, ancestors of nine families originated from the same small farm in the village of Rautalampi, where the first immigrants arrived in the 1540's. The population of Rautalampi is characterised by a relatively small founder population, which has expanded to the 4100 inhabitants of today. The ancestors of the remaining four families have lived at a distance of less than 100 km from this core area.

Given the genealogical data that shows sharing of same ancestors for the autism families in the late settlement area of Finland (Nevanlinna 1972; Norio et al. 1973), a reasonable hypothesis would be that a common susceptibility gene underlying autism is shared by multiple families in this rural subisolate.

To explore whether the common predisposing alleles IBD were present in the 31 families originating from Central Finland (including part of the large pedigree), we analysed a total of 42 markers on the previously identified susceptibility loci, 1q and 3q, and the recently reported AUTS1 locus on 7q (IMGSAC 2001a) (Figure 12).

Figure 11. The large pedigree of Central Finland. The families included in study III are marked by family numbers. The ancestors of several families originate from the same farm in Rautalampi. Black symbols = autism, grey symbols = AS.

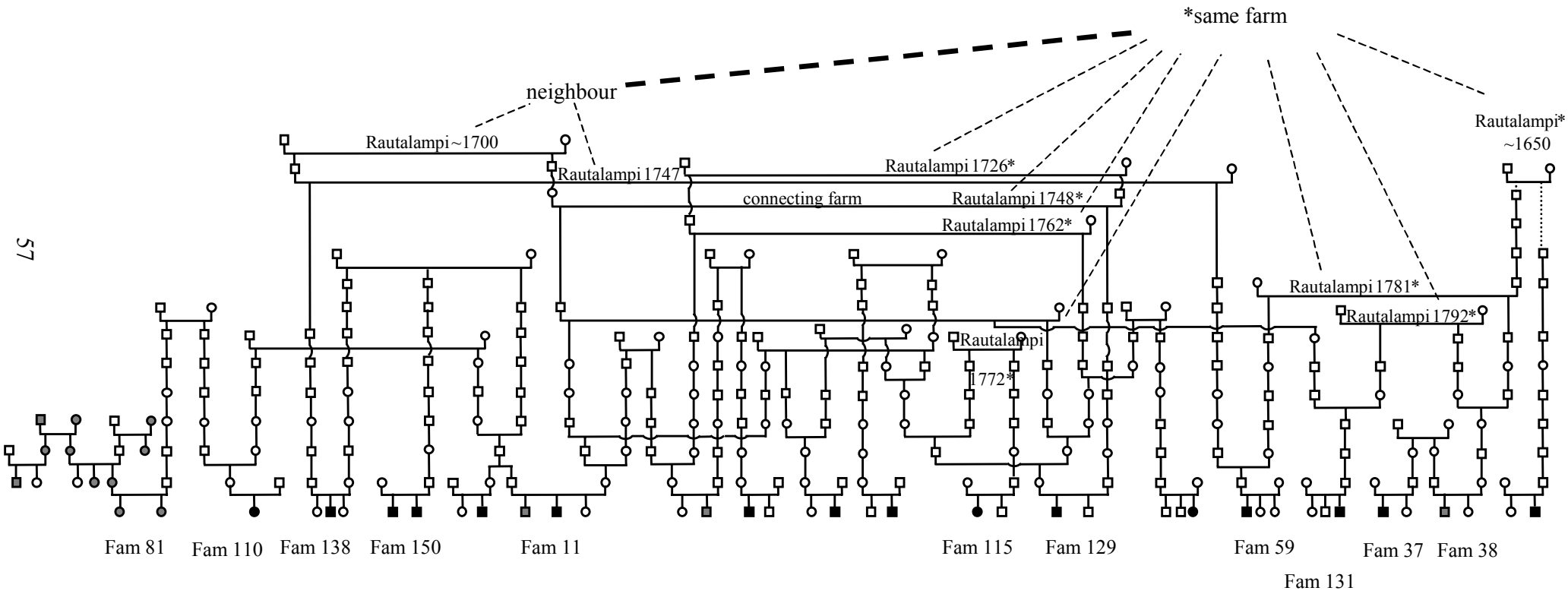
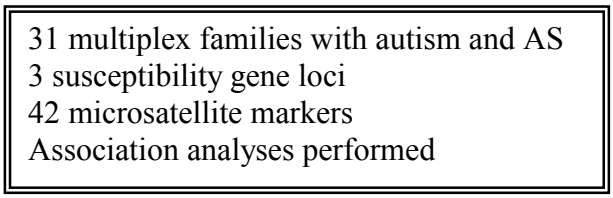


Figure 12. Overview of the strategy of study III.



On chromosome 3q25-27, a total of 14 microsatellite markers covering ~30 cM were analysed. In families with autism statistically significant results ($P < 0.05$) with allele sharing statistics were observed on a 9 cM region and in families with autism and AS on a 20 cM region. In the former families, significant P-values for markers D3S3715 and D3S3037 were observed with gamete competition (0.0138 and 0.0176), TDT (0.0288 and 0.0110) and pseudomarker analyses (dominant: 0.0026 and 0.0076) respectively, supporting the linkage evidence obtained in the genome-wide scan. However, in families originating from all of Finland in the genome scan, no association was detected with these markers in spite of the linkage finding.

For chromosome 1q a total of 18 microsatellite markers covering ~30 cM were studied. In allele sharing statistics an association was observed over an 8 cM region between markers D1S498 and D1S1653 in families with autism, however no haplotype was detected. The evidence for association was comparable to those obtained for the families from all of Finland.

The extent of LD (8-20 cM) observed in the alleles of affected individuals emerging from a subpopulation of Finland with a founder effect, isolation and rapid expansion is unique and provides further support for the localisation of a major susceptibility locus for autism spectrum disorders on 3q. Whether this association reflects a true predisposing locus for autism or only background LD due to common genetic background shown by genealogical studies remains to be confirmed by larger family material and tight microsatellite and SNP map on this region. Previous data about monogenic disorders enriched in the late settlement region of Finland have shown LD that extends 8-13 cM (Peltonen et al. 1999).

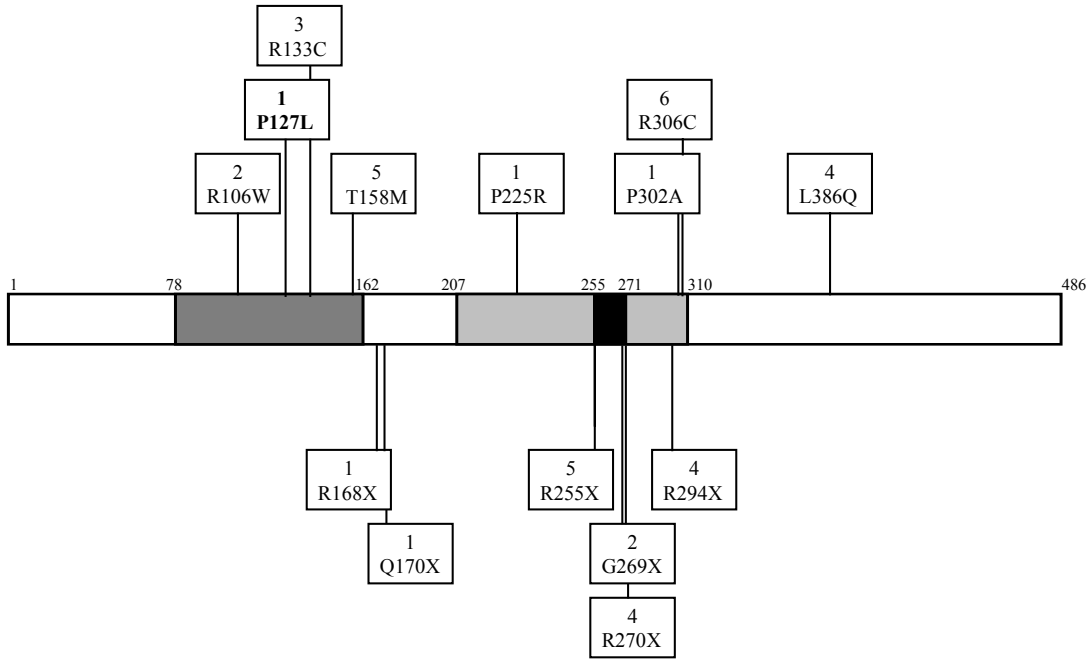
Presumably, a highly restricted number of genes that influence susceptibility to autism worldwide has been introduced into this population some 460 years ago, and became locally enriched. Interestingly, another complex disorder, hereditary nonpolypotic colon cancer (HNPCC) is also enriched in Rautalampi and results from one major mutation of the MLH1 gene that is responsible for >50% of HNPCC in the Finnish population (Nystrom-Lahti et al. 1994; Moisio et al. 1996).

8. RETT SYNDROME (IV)

8.1. THE MUTATION SPECTRUM IN FINLAND

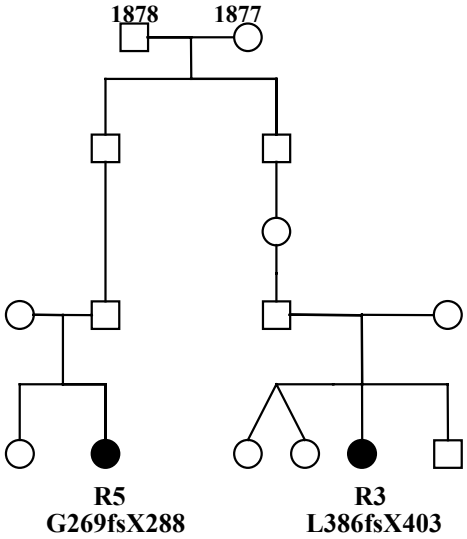
Out of 52 Finnish patients who were diagnosed as classical or atypical RTT, an MECP2 mutation was found in all classical RTT patients (39/39; 100%) and in one PSV patient. However, no mutations were found in 12 patients with developmental delay and some RTT-like features. The clinical course of the PSV patient fulfilled the diagnostic criteria for classical RTT in other respects apart from the longer preservation of speech. There were altogether 19 RTT patients with missense and 21 patients with frameshift or nonsense mutations that were scattered fairly evenly along the cDNA of the MECP2 gene. Eleven mutations (25%) were clustered in the NLS domain of 17 amino acid residues (Nan et al. 1996). The mutational hot spot, R168X previously found in 83 RTT families was detected only once among this patient material. The most prevalent mutations were transitions R306C with six, and T158M and R255X with five occurrences (Figure 13). The PSV patient was found to carry a novel missense mutation P127L in the middle of the MBD region, which changes a conserved proline to a leucine, (Figure 13). The mutation affects the first codon of exon four and was not detected in the parents and in 75 normal non-RTT X chromosomes. In all, no mutations were detected in the parental DNA available from 52/80 (65%) of the parents.

Figure 13. MECP2 mutations in the Finnish RTT patients. The mutation in the PSV patient is shown in bold type. The exons 3 and 4 are shown in grey, the NLS domain in black.



The genealogical studies revealed one family in which two RTT patients (R3 and R5) were shown to be second and half-cousins. However, they carried different mutations: L386fsX403 and G269fsX288 (Figure 14), which further supports the sporadic nature of the MECP2 mutations in RTT. No other consanguinities could be detected even within the group carrying the same mutation.

Figure 14. The consanguineous RTT patients have different MECP2 gene mutations.



8.2. XCI STUDIES

Dosage compensation in humans is achieved through random inactivation of one of the two X chromosomes in the cells of normal females (Lyon 1989) who are essentially cellular mosaics with either maternal or paternal X chromosome inactivated (Lyon 1972). A skewed XCI pattern analysed from the peripheral cell leucocytes was reported to associate with non-penetrant obligate carriers of the disease (Amir et al. 1999; Wan et al. 1999; Amir et al. 2000; Bienvenu et al. 2000), and random XCI in the patients. This led to a proposal that regardless of the type of mutation in the MECP2 gene, the XCI pattern would be the major determinant of the phenotype ranging between ratios 50/50-80/20 in the RTT patients (Schanen et al. 1997; Sirianni et al. 1998; Wan et al. 1999).

To determine whether the XCI pattern differs among the classical and atypical cases and to find out its contribution to the clinical phenotype, we performed XCI studies in 35 classical RTT, one PSV and in eight patients with RTT-like features, who were informative for the AR loci. All analysed cases with RTT-like features (8/12) had random XCI (Table 16). In the 25 classical RTT patients (72%) and in the PSV patient the XCI pattern was random consistent with the previous reports. The methylated and subsequently inactive X chromosome was found in six cases of paternal origin and in one case of maternal origin.

Recently, the parental origin of the MECP2 mutations was systematically studied by linkage analysis between the mutation of the MECP2 gene and intronic polymorphisms in 27 families (Trappe et al. 2001). In 26/27 families with a sporadic RTT patient the mutation was of paternal origin. Girard et al. found a paternal origin of the mutation in 5/7 sporadic RTT patients (Girard et al. 2001), and also in single familial cases the origin of the mutation is suggested to be in the grand paternal germline (Imessaoudene et al. 2001; Villard et al. 2001). These findings suggest that the excess of females in RTT is not explained by lethality of male embryos, but the fact that they are naturally protected from the mutations since they do not inherit the mutation-prone X chromosome (Thomas 1996). The explanation for the increased male mutation rate is not known, but it might be explained by the hypermethylated X chromosomes in sperm cells due to chromatin inactivation this contrasts with the oocytes where X chromosomes are undermethylated (Goto and Monk 1998).

8.3. THE CLINICAL PHENOTYPE OF THE FINNISH RTT PATIENTS

8.3.1. Classical patients

No significant correlation between the clinical course and mutation type was identified among Finnish patients with the same or different mutations (Table 15). This is in agreement with previous findings, although some indication for a difference in the phenotype between missense and truncating mutations has been reported (Amir et al. 2000; Cheadle et al. 2000; Huppke et al. 2000). In our patients the clinical picture associated with the R294X mutation was characterised by a mild clinical outcome: all learned to walk independently and if epilepsy was present, the seizures were infrequent. Two patients from this group (R10 and R42) have the best preserved gross motor skills of the entire patient material. The XCI pattern, which was skewed in R10 and R20 subjects carrying this mutation does not seem to contribute to the clinical outcome. Random XCI was also associated with two missense mutations (R133C and R306C), both with overall mild clinical phenotype (all patients learned to walk, and developed normally before regression set in at ≥ 10 months). The patients with nonsense mutations R255X, G269fsX288 or R270X have on the whole a more severe outcome than other patients. Seven out of 11 (64%) of them never learned to walk, compared with 5/29 (17%) of the patients with other mutations (Table 15).

The relatives of RTT probands were monitored for any neurological features. The mother of one patient (R17) had epilepsy. However, she was not a carrier of the R270X mutation (data not shown). The second cousin of patient R1 presented with features from the autism spectrum disorders. This prompted us to investigate the whole coding region of MECP2 for mutations in the cousin, but no sequence alterations were detected (data not shown). The sister of patient R32 (DNA not available) has mild learning disabilities, and her mother dystony in the neck (torticollis), strabismus and myopia. However, no mutation could be detected in the DNA isolated from the peripheral blood of the mother (data not shown).

To explain the clinical variability several reports have been published in favor of a more complex model consisting partly of an XCI pattern, the MECP2 mutation type and some modifier gene effects (De Bona et al. 2000). Healthy or mildly affected carrier females with random XCI patterns and A140V or G406X mutations in MECP2 gene have been reported (Meloni et al. 2000; Couvert et al. 2001). However, the current analysis has been limited to the peripheral tissue and X chromosome skewing in the CNS tissue cells cannot be excluded. Also, the A140V mutation has been reported in a sporadic male patient with language disorder and schizophrenia (Cohen et al. 2002), and in addition in patients with PPM-X syndrome characterised by psychosis, pyramidal signs and macro-orchidism (Klauck et al. 2002).

Our data suggest that an MECP2 mutation can be found in almost every classical Rett patient. Patients with a classical clinical picture without identifiable MECP2 mutation in the peripheral cell leucocytes may represent somatic mosacism for the mutation, as has been described (Bourdon et al. 2001). Alternatively, genetic heterogeneity exists, and mutations in other genes than in MECP2 might underlie RTT.

The large number of mutations identified so far in the MECP2 gene makes the application of the mutation test laborious in clinical practice. However, if sequencing is considered, one should bear in mind that 85% (altogether 66) of all MECP2 mutations so far described are located in exon four (1084bp). Only twelve mutations are so far found in exon three. Two

mutations involve the splicing site between exons three and four. Considering the Rett variants, PSV patients have often an MECP2 mutation, so far 12 cases with a mutation out of 14 have been reported. Only one Rett patient with MECP2 mutation has been reported in the congenital onset group so far. More patients need to be analysed in order to clarify the mutation prevalence in these groups. Today no Rett variants of late regression or early onset seizures with a MECP2 mutation have to our knowledge been characterised.

8.3.2. Atypical RTT

We have screened the coding region of MECP2 in 12 patients with developmental delay and one or two features of Rett syndrome (Table 16) and found no mutations in this small sample. The patients with developmental delay had one or two features of Rett, but criteria III, partial or total loss of purposeful hand use, was not seen (Table 16). Similarly, patients with significantly milder impairment of hand use in whom an MECP2 gene mutation was not found have been reported (Cheadle et al. 2000). It has also been found that patients with an MECP2 mutation more frequently lose acquired purposeful hand movements than the patients without the mutation, although no significant P-value was obtained (Bienvenu et al. 2000).

All but one patient (R25) had EEG abnormalities and/or epilepsy: early onset seizures (R30, R39, R202), petit mal seizures (R205) and infantile spasms (R41). Scoliosis and breathing disorders considered as supportive criteria for Rett occurred in only 1/11 (9%) and 2/11 (18%) of the patients, which was significantly less than in classical RTT (Table 16). In atypical patients R30, R39 and R202 developmental regression was associated with the onset of seizures. However, in RTT the regression often occurs clearly earlier than epileptic seizures. In patients R29 and R43 the regression was of an autistic type mainly involving the use of language.

The spectrum of clinical features associated with an MECP2 mutation is wide ranging from male patients with early onset lethal encephalopathy to adult cases with severe mental retardation, and in female cases from asymptomatic or mild mental retardation to the severe variant of RTT with congenital onset (Wan et al. 1999; Meloni et al. 2000; Orrico et al. 2000; Couvert et al. 2001).

Table 15. The MECP2 mutation, XCI status and clinical characteristics of the classical RTT patients. N.d. = no data.

PATIENT	MUTATION	XCI (INACTIVE CHR.)	YEAR OF BIRTH	NORMAL DEVELOPMENT (M)	WALKING AGE (M)	EPILEPSY	BREATHING DISORDER	SCOLIOSIS	SPECIAL REMARKS
R9	R106W	Noninf.	-88	15	14	+	+	+	sister has epilepsy
R40		Random	-71	<12	~24	+	n.d.	+	walked until 26 years
R33	P127L	Random	-93	~12	32	+	-	-	PSV, premature puberty
R24	R133C	Random	-95	~18	18	-	-	-	uses hands purposefully
R26		Random	-79	~24	16	+	+	+	walked until 9-10 years
R47		Random	-75	~24	13	+	+	+	obese
R8	T158M	Random	-94	~12	20	-	-	+	probably can read
R200		100% skewed	-68	18	15	+	+	n.d.	
R37		Random	-89	<12	-	+	+	n.d.	weakness in contact
R48		Random	-82	6	-	+	+	+	
R206		Random	-88	~6	-	+	+	+	floppy infant
R15	R168X	85% paternal	-81	12	~12	+	+	+	small and underweight
R203	Q170X	Random	-71	12	24	+	+	+	
R14	P225R	Random	-69	<24	15	+	+	+	uses hands purposefully
R4	R255X	Random	-76	~12	18	+	+	+	walked until 12 years
R12		82% paternal	-96	~12	20	-	+	-	
R13		90% skewed	-67	6	-	+	+	+	
R19		Random	-90	<12	-	+	+	+	weakness in contact
R36		90% paternal	-79	<12	-	+	n.d.	n.d.	

PATIENT	MUTATION	XCI (INACTIVE CHR.)	YEAR OF BIRTH	NORMAL DEVELOPMENT (M)	WALKING AGE (M)	EPILEPSY	BREATHING DISORDER	SCOLIOSIS	SPECIAL REMARKS
R1	G269fsX288	83% maternal	-86	<12	-	+	+	+	mild hypotonia
R5		85% paternal	-80	<12	19	+	n.d.	n.d.	
R17	R270X	Random	-67	<12	-	+	+	+	mother has epilepsy
R32		Noninf.	-68	6	-	+	+	+	mother has torticollis
R38		Random	-69	~12	22	+	+	+	spasticity
R44		Noninf.	-86	7	-	+	+	+	
R20	R294X	82% skewed	-71	n.d.	21	+	+	+	walked until 28 years
R21		Random	-79	18	16	+	+	+	
R10		90% paternal	-88	~12	12	+	-	+	
R42		Random	-93	~24	~14	-	+	-	
R23	P302A	90% paternal	-79	~12	20	+	+	+	
R11	R306C	Random	-81	~10	21	+	+	+	walked until 10 years
R22		Random	-86	<24	20	+	+	+	premature delivery
R34		Random	-78	~12	25	-	+	+	still walks
R35		Random	-83	~18	16	+	+	+	walked until 10 years
R7		Random	-88	~12	20	+	+	-	
R16		Random	-60	~12	24	+	+	+	walked until 20 years
R3	L386fsX403	Noninf.	-77	6	-	+	+	+	
R6		Random	-91	~12	18	+	+	+	
R27		Random	-85	7	-	+	+	+	brother died at 2 months
R46		Random	-85	~12	24	+	+	+	

Table 16. The clinical features of the atypical patients.

PATIENT	XCI	YEAR OF BIRTH	MAJOR CRITERIA MET	WALKING AGE (M)	HAND STEREO-TYPIES	EPILEPSY	BREATHING DISORDER	SCOL	SPECIAL REMARKS
R25	Random	-98	III, V	-	-	-, EEG normal	-	-	MRI normal, hypotonic at birth, later hypertonic.
R28		-92	V	26	+ (stoking of hand)	+, before 24 mths	-	-	Weak eye contact and slow development from birth
R29		-94	I, II, III, V	12	+, flapping	+	- (during seizures)	-	Retardation and partial loss of speech at 24 mths, AF
R30	Random	-93	I, II, V	12	-	+, since 6 mths	-	-	Retardation and loss of speech at 24 mths, ataxy
R39		-71	I, II, V	-	+	+, infantile spasms since 7 mths	+	-	Regression since infantile spasms
R41		-97	III, V	?	+, twisting	+, since 2 mths	-	-	MRI normal, no loss of hand skills
R43		-91	I, II, III, V	21	+	-, EEG abnormalities	-	-	AF, brother has unknown mental retardation without AF
R45		-97	I, V	-	+, active hand use	-, minor EEG abnormalities	-	?	Does not speak, febrile seizures, normal development till 7 mths, hypotonic
R50		-62	I, V	16	-, fingers a robe	+	-	-	AF, developmental delay since 6 mths
R201	Random	-46	V	20	+	+	-	+	Abnormal development and hypotonia from birth, breast tumor,
R202				15		+, since 14 mths	+	-	Febrile seizures since 4 mths, autistic behaviour, dysmorphic features, does not speak.
R205	Random	-93	I, V	36	-, flapping while excited	+,Petit mal, since 75 mths	?	-	Hypotonic, does not speak

(I) normal pre- and perinatal development and apparently normal development for the first 5-6 months; (II) developmental regression with onset between 6 months and 3 years; (III) deceleration of head circumference growth; (IV) loss of purposeful hand movements and acquisition of hand movement stereotypies; and (V) marked developmental and cognitive delay. AF = autistic features; mths = months; scol = scoliosis

8.3.3. Impact on clinical practice

To explain the sex-limited expression of RTT it has been suggested that the *de novo* mutations occur only in male germ cells resulting therefore in affected daughters. Males would be protected from the disease because they do not inherit the mutation-prone paternal X chromosome. Indeed, studies of the MECP2 gene mutations in sporadic cases of RTT have demonstrated an almost exclusive paternal origin for the mutations (Girard et al. 2001; Trappe et al. 2001). In all familial cases of RTT so far the mother is either a carrier or has a germline mosaicism for the mutation. This suggests that mutations appearing on the maternal alleles have a higher possibility for causing familial RTT. An analysis of the origin of the mutation would be beneficial for families with more than one RTT patient. This has a great impact on genetic counselling since the recurrence risk of a maternal germline mutation can be as high as 50%.

Couvert et al. found an MECP2 mutation in 2/30 families with X-linked MR (Couvert et al. 2001). In 185 sporadic mentally retarded males a mutation was detected in ~2% (4 cases) suggesting that the proportion of MECP2 mutations in X-linked MR is comparable with the 3-4% rate of CGG expansions associated with fragile X syndrome. A systematic screening of the MECP2 gene should thus be considered in male patients with unexplained MR (Couvert et al. 2001).

The finding of mutations in 12.5-16% of female and in 2.6-4.5% of male patients with AnS (Imessaoudene et al. 2001; Watson et al. 2001), indicates that the mutation analysis should be considered in AnS patients without a demonstrable molecular or cytogenetic abnormality of 15q11-13 (Watson et al. 2001). In the families where AnS is diagnosed on clinical grounds only, the recurrence risk can be as high as 50%, however if an MECP2 mutation was detected in the proband this risk would be much lower. Also, screening should be indicated for females not fulfilling the classical diagnostic criteria for RTT who show delayed motor development regardless of age, and in patients with PPM-X syndrome.

Before an MECP2 sequence variation is considered to be disease causing in male patients, the analysis of parental and grandparental DNA was recently strongly emphasised (Laccone et al. 2002). Also, the frequency of the variation should be studied in a suitable population. Indeed, Laccone et al. showed that an MECP2 gene mutation (G428S), previously described to be responsible for the disease phenotype in a male patient, is actually a rare genetic variant. They suggested that the novel amino acid changes reported should be defined as "unclassified" until they are definitely confirmed as disease causing mutations (Laccone et al. 2002).

The current guidelines for MECP2 mutation screening is advised in girls with developmental delay, hypotonia, tremulous movement, poor feeding, poor mobility, fall-off in the growth of the head circumference and onset of epileptic seizures or nonepileptic vacant spells (Kerr et al. 2001). Also the screening of infants fulfilling at least five of the necessary diagnostic criteria for RTT has been recommended (Inui et al. 2001).

Finally, the role of MECP2 mutations in developmental delay of male patients needs further studies and careful characterisation of the affected patients before screening criteria can be formulated.

CONCLUSIONS

Molecular genetic studies performed to date on autism spectrum disorders have resulted in the identification of numerous genetic loci. Of the positive findings the chromosome 2q and 7q loci have been repeatedly found in independent family materials. The majority of the studies have been performed in families with infantile autism only. In this study it has become increasingly evident that the phenotypic spectrum in families with autism is wider including siblings not only with Asperger syndrome but also developmental language disorders currently classified as a separate disease entity. Also others have reported positive linkage findings in patients belonging to the autism spectrum (IMGSAC 1998; IMGSAC 2001a; Buxbaum et al. 2001; Liu et al. 2001), however developmental dysphasia has not been included in these studies.

The above findings reflect the inadequacy of our understanding of the phenotypic diversity in neuropsychiatric disorders. Currently, the diagnosis, which depends only on a certain behavioural phenotype as no biochemical markers are available, contains the risk for phenotypic heterogeneity. Development of new diagnostic instruments is needed to distinguish between various endophenotypes within similar genetic backgrounds in the affected children and their family members (Shao et al. 2002a). The failure to replicate the previous positive linkage findings may also be explained by different ethnicity, inadequate statistical approaches used or by the relatively small family material. New statistical methods have already been developed for quantitative trait analysis and models for several interacting genes (Daniels et al. 1996; Liu et al. 2001). Development of statistical methods in this field will further help us to understand the interaction between disease traits and environmental factors.

A novel locus for autism spectrum disorders, AUTS2, was found on chromosome 3q25-27 with a strong allelic association in the Finnish families. The region of significant LD was even larger in families originating from a subisolate of Central Finland. Whether these results reflect a closely locating trait for autism spectrum disorders or only background LD due to the isolation needs further studies. Indeed, significant background LD was detected in a subisolate of Sardinia in two regions of the X chromosome (Zavattari et al. 2000). The role of founder populations in the mapping of genes for complex disease traits has not yet been established. However, the unique population history may have enriched rare mutations specific to certain consanguineous families. It might also be easier to identify the environmental factors in these families who originate from the same rural area once the predisposing gene alterations have been identified. The localisation of a putative disease-predisposing variation for autism spectrum disorders on chromosome 3q will be further studied by concentrating on the families originating from the subisolate. Additional association studies will be performed with the help of non-autistic control group from this particular region of Finland.

The increasing amount of the human genome sequence data will add to our knowledge about new candidate genes for autism spectrum disorders. Furthermore, the increasing number of SNPs, currently over 4 million found (Gabriel et al. 2002), will facilitate the construction of haplotypes in different chromosomal regions and different populations. The data about background haplotype structures will help in utilising LD-mapping to evaluate the association

of a genetic marker and a disease trait. Also, it will help to sort out which type of populations would be ideal for the identification of complex disease traits.

Rett syndrome was the first human disease characterised by mutations in an X-linked gene involved in DNA methylation. Recently, at least 20 genes with causative mutations have been detected for syndromes with severe MR on X chromosome, some of which are also involved in chromatin remodelling (Chelly and Mandel 2001). The gene defective in RTT, MECP2, belongs to a gene family with a functional MBD-domain and abilities to repress transcription (Hendrich and Bird 1998). The MECP2 gene undergoes X chromosome inactivation, and consequently the affected patients are functionally mosaics of the MECP2 gene that is expressed ubiquitously in humans. However, on the basis of the mouse models loss of function only in the central nervous system is enough to cause the disease symptoms (Chen et al. 2001; Guy et al. 2001). The cell environment in neurons most probably demands tightly controlled genomic methylation (Tucker 2001). The genes silenced by MECP2 gene and the pathogenetic mechanisms leading to the alterations observed –shrunken brain size and loss of tissue morphology and structure– are currently being studied.

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REFERENCES

Allen RC, Zoghbi HY, Moseley AB, Rosenblatt HM, Belmont JW (1992) Methylation of HpaII and HhaI sites near the polymorphic CAG repeat in the human androgen-receptor gene correlates with X chromosome inactivation. Am J Hum Genet 51:1229-39.

Amano K, Nomura Y, Segawa M, Yamakawa K (2000) Mutational analysis of the MECP2 gene in Japanese patients with Rett syndrome. J Hum Genet 45:231-6.

Amir RE, Van den Veyver IB, Schultz R, Malicki DM, Tran CQ, Dahle EJ, Philippi A, Timar L, Percy AK, Motil KJ, Lichtarge O, Smith EO, Glaze DG, Zoghbi HY (2000) Influence of mutation type and X chromosome inactivation on Rett syndrome phenotypes. Ann Neurol 47:670-9.

Amir RE, Van den Veyver IB, Wan M, Tran CQ, Francke U, Zoghbi HY (1999) Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl- CpG-binding protein 2. Nat Genet 23:185-8.

Anneren G, Dahl N, Uddenfeldt U, Janols LO (1995) Asperger syndrome in a boy with a balanced de novo translocation: t(17;19)(p13.3;p11). Am J Med Genet 56:330-1.

Archidiacono N, Lerone M, Rocchi M, Anvret M, Ozcelik T, Francke U, Romeo G (1991) Rett syndrome: exclusion mapping following the hypothesis of germinal mosaicism for new X-linked mutations. Hum Genet 86:604-6.

Ardlie KG, Kruglyak L, Seielstad M (2002) Patterns of linkage disequilibrium in the human genome. Nat Rev Genet 3:299-309.

Armstrong D, Dunn JK, Antalffy B, Trivedi R (1995) Selective dendritic alterations in the cortex of Rett syndrome. J Neuropathol Exp Neurol 54:195-201

Ashley-Koch A, Wolpert CM, Menold MM, Zaeem L, Basu S, Donnelly SL, Ravan SA, Powell CM, Qumsiyeh MB, Aylsworth AS, Vance JM, Gilbert JR, Wright HH, Abramson RK, DeLong GR, Cuccaro ML, Pericak-Vance MA (1999) Genetic studies of autistic disorder and chromosome 7. Genomics 61:227-36.

Aylward EH, Minshew NJ, Goldstein G, Honeycutt NA, Augustine AM, Yates KO, Barta PE, Pearlson GD (1999) MRI volumes of amygdala and hippocampus in non-mentally retarded autistic adolescents and adults. Neurology 53:2145-50.

Bailey A, Le Couteur A, Gottesman I, Bolton P, Simonoff E, Yuzda E, Rutter M (1995) Autism as a strongly genetic disorder: evidence from a British twin study. Psychol Med 25:63-77.

Bailey A, Luthert P, Dean A, Harding B, Janota I, Montgomery M, Rutter M, Lantos P (1998a) A clinicopathological study of autism. Brain 121:889-905.

Bailey A, Palferman S, Heavey L, Le Couteur A (1998b) Autism: the phenotype in relatives. Journal of Autism & Developmental Disorders 28:369-92.

Bailey A, Phillips W, Rutter M (1996) Autism: towards an integration of clinical, genetic, neuropsychological, and neurobiological perspectives. Journal of Child Psychology & Psychiatry & Allied Disciplines 37:89-126.

Barrett S, Beck JC, Bernier R, Bisson E, Braun TA, Casavant TL, Childress D, et al. (1999) An autosomal genomic screen for autism. Am J Med Genet 88:609-15.

Bartak L, Rutter M, Cox A (1977) A comparative study of infantile autism and specific developmental receptive language disorders. III. Discriminant function analysis. J Autism Child Schizophr 7:383-96.

Bauman M, Kemper TL (1985) Histoanatomic observations of the brain in early infantile autism. Neurology 35:866-74.

Bauman ML, Kemper TL, Arin DM (1995) Microscopic observations of the brain in Rett syndrome. Neuropediatrics 26:105-8.

Belichenko PV, Dahlstrom A (1995) Studies on the 3-dimensional architecture of dendritic spines and varicosities in human cortex by confocal laser scanning microscopy and Lucifer yellow microinjections. J Neurosci Methods 57:55-61.

Belichenko PV, Oldfors A, Hagberg B, Dahlstrom A (1994) Rett syndrome: 3-D confocal microscopy of cortical pyramidal dendrites and afferents. Neuroreport 5:1509-13.

Betancur C, Leboyer M, Gillberg C (2002) Increased Rate of Twins among Affected Sibling Pairs with Autism. Am J Hum Genet 70:1381-3.

Bienvenu T, Carrie A, de Roux N, Vinet MC, Jonveaux P, Couvert P, Villard L, Arzimanoglou A, Beldjord C, Fontes M, Tardieu M, Chelly J (2000) MECP2 mutations account for most cases of typical forms of Rett syndrome. Hum Mol Genet 9:1377-1384.

Bishop DV (1989) Autism, Asperger's syndrome and semantic-pragmatic disorder: where are the boundaries? Br J Disord Commun 24:107-21.

Bishop DV, North T, Donlan C (1995) Genetic basis of specific language impairment: evidence from a twin study. Dev Med Child Neurol 37:56-71.

Blin N, Stafford DW (1976) A general method for isolation of high molecular weight DNA from eukaryotes. Nucleic Acids Res 3:2303-8

Bolton P, Macdonald H, Pickles A, Rios P, Goode S, Crowson M, Bailey A, Rutter M (1994) A case-control family history study of autism. J Child Psychol Psychiatry 35:877-900

Bourdon V, Philippe C, Bienvenu T, Koenig B, Tardieu M, Chelly J, Jonveaux P (2001) Evidence of somatic mosaicism for a MECP2 mutation in females with Rett syndrome: diagnostic implications. J Med Genet 38:867-71.

Brzustowicz LM, Hodgkinson KA, Chow EW, Honer WG, Bassett AS (2000) Location of a major susceptibility locus for familial schizophrenia on chromosome 1q21-q22. Science 288:678-82

Buxbaum JD, Silverman JM, Smith CJ, Kilifarski M, Reichert J, Hollander E, Lawlor BA, Fitzgerald M, Greenberg DA, Davis KL (2001) Evidence for a susceptibility gene for autism on chromosome 2 and for genetic heterogeneity. Am J Hum Genet 68:1514-20.

Casanova MF, BuxhoevedenDP, Switala AE, Roy E (2002) Minicolumnar pathology in autism. Neurology 58:428-32.

Chakrabarti S, Fombonne E (2001) Pervasive developmental disorders in preschool children. Jama 285:3093-9.

Cheadle JP, Gill H, Fleming N, Maynard J, Kerr A, Leonard H, Krawczak M, Cooper DN, Lynch S, Thomas N, Hughes H, Hulten M, Ravine D, Sampson JR, Clarke A (2000) Long-read sequence analysis of the MECP2 gene in Rett syndrome patients: correlation of disease severity with mutation type and location. Hum Mol Genet 9:1119-1129.

Chelly J, Mandel JL (2001) Monogenic causes of X-linked mental retardation. Nat Rev Genet 2:669-80.

Chen RZ, Akbarian S, Tudor M, Jaenisch R (2001) Deficiency of methyl-CpG binding protein-2 in CNS neurons results in a Rett-like phenotype in mice. Nat Genet 27:327-31.

Christen HJ, Hanefeld F (1995) Male Rett variant. Neuropediatrics 26:81-2.

Clayton-Smith J, Watson P, Ramsden S, Black GC (2000) Somatic mutation in MECP2 as a non-fatal neurodevelopmental disorder in males. Lancet 356:830-2.

Cohen D, Lazar G, Couvert P, Desportes V, Lippe D, Mazet P, Heron D (2002) MECP2 mutation in a boy with language disorder and schizophrenia. Am J Psychiatry 159:148-9.

Coleman M (1990) Is classical Rett syndrome ever present in males? Brain Dev 12:31-2

Comings DE, Wu S, Chiu C, Muhleman D, Sverd J (1996) Studies of the c-Harvey-Ras gene in psychiatric disorders. Psychiatry Res 63:25-32.

Cook EH (1990) Autism: review of neurochemical investigation. Synapse 6:292-308

Cook EH, Jr., Courchesne R, Lord C, Cox NJ, Yan S, Lincoln A, Haas R, Courchesne E, Leventhal BL (1997) Evidence of linkage between the serotonin transporter and autistic disorder. Molecular Psychiatry 2:247-50.

Cook EH, Jr., Courchesne RY, Cox NJ, Lord C, Gonen D, Guter SJ, Lincoln A, Nix K, Haas R, Leventhal BL, Courchesne E (1998) Linkage-disequilibrium mapping of autistic disorder, with 15q11-13 markers. American Journal of Human Genetics 62:1077-83

Cottingham RW, Jr., Idury RM, Schaffer AA (1993) Faster sequential genetic linkage computations. Am J Hum Genet 53:252-63.

Courchesne E, Karns CM, Davis HR, Ziccardi R, Carper RA, Tigue ZD, Chisum HJ, Moses P, Pierce K, Lord C, Lincoln AJ, Pizzo S, Schreibman L, Haas RH, Akshoomoff NA, Courchesne RY (2001) Unusual brain growth patterns in early life in patients with autistic disorder: An MRI study. Neurology 57:245-54.

Courchesne E, Muller RA, Saitoh O (1999) Brain weight in autism: normal in the majority of cases, megalencephalic in rare cases. Neurology 52:1057-9.

Courchesne E, Townsend J, Saitoh O (1994) The brain in infantile autism: posterior fossa structures are abnormal. Neurology 44:214-23.

Couvert P, Bienvenu T, Aquaviva C, Poirier K, Moraine C, Gendrot C, Verloes A, Andres C, Le Fevre AC, Souville I, Steffann J, des Portes V, Ropers HH, Yntema HG, Fryns JP, Briault S, Chelly J, Cherif B (2001) MECP2 is highly mutated in X-linked mental retardation. Hum Mol Genet 10:941-946.

Curtis AR, Headland S, Lindsay S, Thomas NS, Boye E, Kamakari S, Roustan P, Anvret M, Wahlstrom J, McCarthy G, et al. (1993) X chromosome linkage studies in familial Rett syndrome. Hum Genet 90:551-5.

Daly MJ, Rioux JD, Schaffner SF, Hudson TJ, Lander ES (2001) High-resolution haplotype structure in the human genome. Nat Genet 29:229-32.

Daniels SE, Bhattacharrya S, James A, Leaves NI, Young A, Hill MR, Faux JA, Ryan GF, le Souef PN, Lathrop GM, Musk AW, Cookson WO (1996) A genome-wide search for quantitative trait loci underlying asthma. Nature 383:247-50.

Daniels WW, Warren RP, Odell JD, Maciulis A, Burger RA, Warren WL, Torres AR (1995) Increased frequency of the extended or ancestral haplotype B44-SC30-DR4 in autism. Neuropsychobiology 32:120-3.

De Bona C, Zappella M, Hayek G, Meloni I, Vitelli F, Bruttini M, Cusano R, Loffredo P, Longo I, Renieri A (2000) Preserved speech variant is allelic of classic Rett syndrome. Eur J Hum Genet 8:325-30.

Eaves IA, Merriman TR, Barber RA, Nutland S, Tuomilehto-Wolf E, Tuomilehto J, Cucca F, Todd JA (2000) The genetically isolated populations of Finland and sardinia may not be a panacea for linkage disequilibrium mapping of common disease genes. Nat Genet 25:320-3.

Eden S, Hashimshony T, Keshet I, Cedar H, Thorne AW (1998) DNA methylation models histone acetylation. Nature 394:842.

Eeg-Olofsson O, al-Zuhair AG, Teebi AS, Zaki M, Daoud AS (1990) A boy with the Rett syndrome? Brain Dev 12:529-32.

Ehlers S, Gillberg C (1993) The epidemiology of Asperger syndrome. A total population study. Journal of Child Psychology & Psychiatry & Allied Disciplines 34:1327-50

Ekelund J, Lichtermann D, Hovatta I, Ellonen P, Suvisaari J, Terwilliger JD, Juvonen H, Varilo T, Arajarvi R, Kokko-Sahin ML, Lonnqvist J, Peltonen L (2000) Genome-wide scan for schizophrenia in the Finnish population: evidence for a locus on chromosome 7q22. Hum Mol Genet 9:1049-57.

Ellison KA, Fill CP, Terwilliger J, DeGennaro LJ, Martin-Gallardo A, Anvret M, Percy AK, Ott J, Zoghbi H (1992) Examination of X chromosome markers in Rett syndrome: exclusion mapping with a novel variation on multilocus linkage analysis. Am J Hum Genet 50:278-87.

Ferrari M (1982) Childhood autism: deficits of communication and symbolic development. I. Distinctions from language disorders. J Commun Disord 15:191-208.

Fisher SE, Vargha-Khadem F, Watkins KE, Monaco AP, Pembrey ME (1998) Localisation of a gene implicated in a severe speech and language disorder. Nat Genet 18:168-70.

Folstein S, Rutter M (1977) Infantile autism: a genetic study of 21 twin pairs. J Child Psychol Psychiatry 18:297-321.

Folstein SE, Mankoski RE (2000) Chromosome 7q: where autism meets language disorder? Am J Hum Genet 67:278-81.

Folstein SE, Rosen-Sheidley B (2001) Genetics of autism: complex aetiology for a heterogeneous disorder. Nat Rev Genet 2:943-55.

Fombonne E (1999) The epidemiology of autism: a review. Psychol Med 29:769-86.

Freimer NB, Reus VI, Escamilla MA, McInnes LA, Spesny M, Leon P, Service SK, Smith LB, Silva S, Rojas E, Gallegos A, Meza L, Fournier E, Baharloo S, Blankenship K, Tyler DJ, Batki S, Vinogradov S, Weissenbach J, Barondes SH, Sandkuijl LA (1996) Genetic mapping using haplotype, association and linkage methods suggests a locus for severe bipolar disorder (BPI) at 18q22-q23. Nat Genet 12:436-41.

Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, Higgins J, DeFelice M, Lochner A, Faggart M, Liu-Cordero SN, Rotimi C, Adeyemo A, Cooper R, Ward R, Lander ES, Daly MJ, Altshuler D (2002) The structure of haplotype blocks in the human genome. Science 296:2225-9.

Geerdink N, Rotteveel JJ, Lammens M, Sistermans EA, Heikens GT, Gabreels FJ, Mullaart RA, Hamel BC (2002) MECP2 Mutation in a Boy with Severe Neonatal Encephalopathy: Clinical, Neuropathological and Molecular Findings. Neuropediatrics 33:33-6.

Gillberg C (1989) Asperger syndrome in 23 Swedish children. Developmental Medicine & Child Neurology 31:520-31.

Gillberg C (1998a) Asperger syndrome and high-functioning autism. British Journal of Psychiatry 172:200-9.

Gillberg C (1998b) Chromosomal disorders and autism. J Autism Dev Disord 28:415-25

Gillberg C, Coleman M (1996) Autism and medical disorders: a review of the literature. Dev Med Child Neurol 38:191-202.

Gillberg IC, Gillberg C (1989) Asperger syndrome--some epidemiological considerations: a research note. Journal of Child Psychology & Psychiatry & Allied Disciplines 30:631-8.

Ginns EI, Ott J, Egeland JA, Allen CR, Fann CS, Pauls DL, Weissenbachoff J, Carulli JP, Falls KM, Keith TP, Paul SM (1996) A genome-wide search for chromosomal loci linked to bipolar affective disorder in the Old Order Amish. Nat Genet 12:431-5.

Girard M, Couvert P, Carrie A, Tardieu M, Chelly J, Beldjord C, Bienvenu T (2001) Parental origin of de novo MECP2 mutations in Rett syndrome. Eur J Hum Genet 9:231-6.

Goring HH, Terwilliger JD (2000) Linkage analysis in the presence of errors IV: joint pseudomarker analysis of linkage and/or linkage disequilibrium on a mixture of pedigrees and singletons when the mode of inheritance cannot be accurately specified. Am J Hum Genet 66:1310-27.

Goto T, Monk M (1998) Regulation of X chromosome inactivation in development in mice and humans. Microbiol Mol Biol Rev 62:362-78.

Greenberg DA, Hodge SE, Sowinski J, Nicoll D (2001) Excess of Twins among Affected Sibling Pairs with Autism: Implications for the Etiology of Autism. Am J Hum Genet 69:1062-7.

Guy J, Hendrich B, Holmes M, Martin JE, Bird A (2001) A mouse Mecp2-null mutation causes neurological symptoms that mimic Rett syndrome. Nat Genet 27:322-6.

Haas RH, Nasirian F, Hua X, Nakano K, Hennessy M (1995) Oxidative metabolism in Rett syndrome: 2. Biochemical and molecular studies. Neuropediatrics 26:95-9.

Hagberg B (1985) Rett's syndrome: prevalence and impact on progressive severe mental retardation in girls. Acta Paediatr Scand 74:405-8.

Hagberg B (1993b) Clinical criteria, stages and natural history. In: Hagberg B (ed) Rett syndrome - clinical & biological aspects. London, Mac Keith Press, pp 4-20

Hagberg B (1995) Clinical delineation of Rett syndrome variants. Neuropediatrics 26:62

Hagberg B, Aicardi J, Dias K, Ramos O (1983) A progressive syndrome of autism, dementia, ataxia, and loss of purposeful hand use in girls: Rett's syndrome: report of 35 cases. Ann Neurol 14:471-9.

Hagberg B, Gillberg C (1993c) Rett variants - Rettoid phenotypes. In: Hagberg B (ed) Rett syndrome - clinical & biological aspects. London, Mac Keith Press, pp 40-60

Hagberg B, Witt-Engerström I (1990) Swedish Rett syndrome basic data register 1960-1990. Acta Paediatrica Scandinavica. Supplement 369:43-54.

Haines JL, PerIcak-Vance MA (1998) Approaches to gene mapping in complex human diseases. USA, Wiley-Liss Inc.

Hallmayer J, Hebert JM, Spiker D, Lotspeich L, McMahon WM, Petersen PB, Nicholas P, Pingree C, Lin AA, Cavalli-Sforza LL, Risch N, Ciaranello RD (1996) Autism and the X chromosome. Multipoint sib-pair analysis. Psychiatry Res 65:33-43.

Hallmayer J, Glasson EJ, Bower C, Petterson B, Croen L, Grether J, Risch N (2002) On the twin risk in autism. Am J Hum Genet 71:941-46.

Hardan AY, Minshew NJ, Harenski K, Keshavan MS (2001) Posterior fossa magnetic resonance imaging in autism. J Am Acad Child Adolesc Psychiatry 40:666-72.

Hearne CM, Ghosh S, Todd JA (1992) Microsatellites for linkage analysis of genetic traits. Trends Genet 8:288-94.

Heils A, Teufel A, Petri S, Stober G, Riederer P, Bengel D, Lesch KP (1996) Allelic variation of human serotonin transporter gene expression. J Neurochem 66:2621-4.

Hendrich B, Bird A (1998) Identification and characterization of a family of mammalian methyl-CpG binding proteins. Mol Cell Biol 18:6538-47

Herault J, Petit E, Martineau J, Cherpi C, Perrot A, Barthelemy C, Lelord G, Muh JP (1996) Serotonin and autism: biochemical and molecular biology features. Psychiatry Res 65:33-43

Herault J, Petit E, Martineau J, Perrot A, Lenoir P, Cherpi C, Barthelemy C, Sauvage D, Mallet J, Muh JP (1995) Autism and genetics: clinical approach and association study with two markers of HRAS gene. Am J Med Genet 60:276-81

Hoffbuhr K, Devaney JM, LaFleur B, Sirianni N, Scacheri C, Giron J, Schuette J, Innis J, Marino M, Philippart M, Narayanan V, Umansky R, Kronn D, Hoffman EP, Naidu S (2001) MeCP2 mutations in children with and without the phenotype of Rett syndrome. Neurology 56:1486-95.

Holttum JR, Minshew NJ, Sanders RS, Phillips NE (1992) Magnetic resonance imaging of the posterior fossa in autism. Biol Psychiatry 32:1091-101.

Honda H, Shimizu Y, Misumi K, Niimi M, Ohashi Y (1996) Cumulative incidence and prevalence of childhood autism in children in Japan. Br J Psychiatry 169:228-35.

Horikawa Y, Oda N, Cox NJ, Li X, Orho-Melander M, Hara M, Hinokio Y, Lindner TH, Mashima H, Schwarz PE, del Bosque-Plata L, Oda Y, Yoshiuchi I, Colilla S, Polonsky KS, Wei S, Concannon P, Iwasaki N, Schulze J, Baier LJ, Bogardus C, Groop L, Boerwinkle E, Hanis CL, Bell GI (2000) Genetic variation in the gene encoding calpain-10 is associated with type 2 diabetes mellitus. Nat Genet 26:163-75.

Hovatta I, Varilo T, Suvisaari J, Terwilliger JD, Ollikainen V, Arajarvi R, Juvonen H, Kokko-Sahin ML, Vaisanen L, Mannila H, Lonnqvist J, Peltonen L (1999) A genomewide screen for schizophrenia genes in an isolated Finnish subpopulation, suggesting multiple susceptibility loci. Am J Hum Genet 65:1114-24.

Howlin P, Mawhood L, Rutter M (2000) Autism and developmental receptive language disorder--a follow-up comparison in early adult life. II: Social, behavioural, and psychiatric outcomes. J Child Psychol Psychiatry 41:561-78.

Huppke P, Laccone F, Kramer N, Engel W, Hanefeld F (2000) Rett syndrome: analysis of MECP2 and clinical characterization of 31 patients. Hum Mol Genet 9:1369-1375

Huttenlocher PR (1979) Synaptic density in human frontal cortex - developmental changes and effects of aging. Brain Res 163:195-205.

Imessaoudene B, Bonnefont JP, Royer G, Cormier-Daire V, Lyonnet S, Lyon G, Munnich A, Amiel J (2001) MECP2 mutation in non-fatal, non-progressive encephalopathy in a male. J Med Genet 38:171-4.

Hästbacka J, de la Chapelle A, Mahtani MM, Clines G, Reeve-Daly MP, Daly M, Hamilton BA, Kusumi K, Trivedi B, Weaver A, et al. (1994) The diastrophic dysplasia gene encodes a novel sulfate transporter: positional cloning by fine-structure linkage disequilibrium mapping. Cell 78:1073-87.

IMGSAC (1998) A full genome screen for autism with evidence for linkage to a region on chromosome 7q. International Molecular Genetic Study of Autism Consortium. Hum Mol Genet 7:571-8.

IMGSAC (2001a) Further characterization of the autism susceptibility locus AUTS1 on chromosome 7q. Hum Mol Genet 10:973-82.

IMGSAC (2001b) A Genomewide Screen for Autism: Strong Evidence for Linkage to Chromosomes 2q, 7q, and 16p. Am J Hum Genet 69:570-581.

Ingram TTS (1959) Specific developmental disorders of speech in childhood. Brain 82:450-454

Ingram JL, Stodgell CJ, Hyman SL, Figlewicz DA, Weitkamp LR, Rodier PM (2000) Discovery of allelic variants of HOXA1 and HOXB1: genetic susceptibility to autism spectrum disorders. Teratology 62:393-405.

Inui K, Akagi M, Ono J, Tsukamoto H, Shimono K, Mano T, Imai K, Yamada M, Muramatsu T, Sakai N, Okada S (2001) Mutational analysis of MECP2 in Japanese patients with atypical Rett syndrome. Brain Dev 23:212-5.

Jamain S, Quach H, Quintana-Murci L, Betancur C, Philippe A, Gillberg C, Sponheim E, Skjeldal OH, Fellous M, Leboyer M, Bourgeron T (2002) Y chromosome haplogroups in autistic subjects. Mol Psychiatry 7:217-9

Jellinger K, Seitelberger F (1986) Neuropathology of Rett syndrome. Am J Med Genet Suppl 1:259-88

Jeppesen P, Turner BM (1993) The inactive X chromosome in female mammals is distinguished by a lack of histone H4 acetylation, a cytogenetic marker for gene expression. Cell 74:281-9.

Jones PA, Takai D (2001) The role of DNA methylation in mammalian epigenetics. Science 293:1068-70.
Jones PL, Veenstra GJ, Wade PA, Vermaak D, Kass SU, Landsberger N, Strouboulis J, Wolffe AP (1998) Methylated DNA and MeCP2 recruit histone deacetylase to repress transcription. Nat Genet 19:187-91
Jorde LB, Watkins WS, Kere J, Nyman D, Eriksson AW (2000) Gene mapping in isolated populations: new roles for old friends? Hum Hered 50:57-65.
Kadesjo B, Gillberg C (1999) Developmental coordination disorder in Swedish 7-year-old children. J Am Acad Child Adolesc Psychiatry 38:820-8.
Kaludov NK, Wolffe AP (2000) MeCP2 driven transcriptional repression in vitro: selectivity for methylated DNA, action at a distance and contacts with the basal transcription machinery. Nucleic Acids Res 28:1921-8.
Kanner L (1943) Autistic disturbances of affective contact. J Nerv Child 2:217-150
Kates WR, Mostofsky SH, Zimmerman AW, Mazzocco MM, Landa R, Warsofsky IS, Kaufmann WE, Reiss AL (1998) Neuroanatomical and neurocognitive differences in a pair of monozygous twins discordant for strictly defined autism. Ann Neurol 43:782-91.
Kaufmann WE, Moser HW (2000) Dendritic anomalies in disorders associated with mental retardation. Cereb Cortex 10:981-91.
Kaufmann WE, Naidu S, Budden S (1995) Abnormal expression of microtubule-associated protein 2 (MAP-2) in neocortex in Rett syndrome. Neuropediatrics 26:109-13.
Kemper TL, Bauman M (1998) Neuropathology of infantile autism. J Neuropathol Exp Neurol 57:645-52.
Kere J (2001) Human population genetics: Lessons from Finland. Ann Rev Genomics Hum Genet 2:103-28.
Kerr AM, Nomura Y, Armstrong D, Anvret M, Belichenko PV, Budden S, Cass H, Christodoulou J, Clarke A, Ellaway C, d'Esposito M, Francke U, Hulten M, Julu P, Leonard H, Naidu S, Schanen C, Webb T, Engerstrom IW, Yamashita Y, Segawa M (2001) Guidelines for reporting clinical features in cases with MECP2 mutations. Brain Dev 23:208-11.
Kielinen M, Linna SL, Moilanen I (2000) Autism in Northern Finland. Eur Child Adolesc Psychiatry 9:162-7.
Kishino T, Lalande M, Wagstaff J (1997) UBE3A/E6-AP mutations cause Angelman syndrome. Nat Genet 15:70-3.
Klauck SM, Lindsay S, Beyer KS, Splitt M, Burn J, Poustka A (2002) A mutation hot spot for nonspecific X-linked mental retardation in the MECP2 gene causes the PPM-X syndrome. Am J Hum Genet 70:1034-7.
Klauck SM, Poustka F, Benner A, Lesch KP, Poustka A (1997) Serotonin transporter (5-HTT) gene variants associated with autism? Human Molecular Genetics 6:2233-8
Kleiman MD, Neff S, Rosman NP (1992) The brain in infantile autism: are posterior fossa structures abnormal? Neurology 42:753-60.
Klin A, Volkmar FR, Sparrow SS, Cicchetti DV, Rourke BP (1995) Validity and neuropsychological characterization of Asperger syndrome: convergence with nonverbal learning disabilities syndrome. J Child Psychol Psychiatry 36:1127-40.
Knoll JH, Nicholls RD, Magenis RE, Graham JM, Jr., Lalande M, Latt SA (1989) Angelman and Prader-Willi syndromes share a common chromosome 15 deletion but differ in parental origin of the deletion. Am J Med Genet 32:285-90.
Kruglyak L (1999) Prospects for whole-genome linkage disequilibrium mapping of common disease genes. Nat Genet 22:139-44.

Kruglyak L, Daly MJ, Reeve-Daly MP, Lander ES (1996) Parametric and nonparametric linkage analysis: a unified multipoint approach. Am J Hum Genet 58:1347-63.
Kruglyak L, Lander ES (1995) Complete multipoint sib-pair analysis of qualitative and quantitative traits. Am J Hum Genet 57:439-54.
Kuokkanen S, Gschwend M, Rioux JD, Daly MJ, Terwilliger JD, Tienari PJ, Wikstrom J, Palo J, Stein LD, Hudson TJ, Lander ES, Peltonen L (1997) Genomewide scan of multiple sclerosis in Finnish multiplex families. Am J Hum Genet 61:1379-87
Kuokkanen S, Sundvall M, Terwilliger JD, Tienari PJ, Wikstrom J, Holmdahl R, Pettersson U, Peltonen L (1996) A putative vulnerability locus to multiple sclerosis maps to 5p14-p12 in a region syntenic to the murine locus Eae2. Nat Genet 13:477-80.
Laccone F, Zoll B, Huppke P, Hanefeld F, Pepinski W, Trappe R (2002) MECP2 gene nucleotide changes and their pathogenicity in males: proceed with caution. J Med Genet 39:586-8.
Laan LA, v Haeringen A, Brouwer OF (1999) Angelman syndrome: a review of clinical and genetic aspects. Clin Neurol Neurosurg 101:161-70.
Lai CS, Fisher SE, Hurst JA, Vargha-Khadem F, Monaco AP (2001) A forkhead-domain gene is mutated in a severe speech and language disorder. Nature 413:519-23.
Lainhart JE, Piven J (1995) Diagnosis, treatment, and neurobiology of autism in children. Curr Opin Pediatr 7:392-400.
Laitinen T, Daly MJ, Rioux JD, Kauppi P, Laprise C, Petays T, Green T, Cargill M, Haahtela T, Lander ES, Laitinen LA, Hudson TJ, Kere J (2001) A susceptibility locus for asthma-related traits on chromosome 7 revealed by genome-wide scan in a founder population. Nat Genet 28:87-91.
Landa R, Piven J, Wzorek MM, Gayle JO, Chase GA, Folstein SE (1992) Social language use in parents of autistic individuals. Psychol Med 22:245-54.
Lander E, Kruglyak L (1995) Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. Nat Genet 11:241-7.
Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, Baldwin J, Devon K, et al. (2001) Initial sequencing and analysis of the human genome. Nature 409:860-921.
Lange K, Weeks D, Boehnke M (1988) Programs for Pedigree Analysis: MENDEL, FISHER, and dGENE. Genet Epidemiol 5:471-2.
Lange K, Cantor R, Horvath S, Perola M, Sabatti C, Sinsheimer JS, Sobel E (2001) MENDEL version 4.0: A conputer package for the exact genetic analysis of discrete traits in pedigree and populaiton data sets. Am J Hum Genet 69 (suppl 4):505.
Lappalainen R, Riikonen RS (1994) Elevated CSF lactate in the Rett syndrome: cause or consequence? Brain Dev 16:399-401.
Lassig JP, Vachirasomtoon K, Hartzell K, Leventhal M, Courchesne E, Courchesne R, Lord C, Leventhal BL, Cook EH, Jr. (1999) Physical mapping of the serotonin 5-HT(7) receptor gene (HTR7) to chromosome 10 and pseudogene (HTR7P) to chromosome 12, and testing of linkage disequilibrium between HTR7 and autistic disorder. Am J Med Genet 88:472-5.
Lathrop GM, Lalouel JM (1984) Easy calculations of lod scores and genetic risks on small computers. Am J Hum Genet 36:460-5.
Lathrop GM, Lalouel JM, Julier C, Ott J (1984) Strategies for multilocus linkage analysis in humans. Proc Natl Acad Sci U S A 81:3443-6.
Lathrop GM, Lalouel JM, White RL (1986) Construction of human linkage maps: likelihood calculations for multilocus linkage analysis. Genet Epidemiol 3:39-52.
Leonard H, Silberstein J, Falk R, Houwink-Manville I, Ellaway C, Raffaele LS, Engerstrom IW, Schanen C (2001) Occurrence of Rett syndrome in boys. J Child Neurol 16:333-8.

Lewis BA, Thompson LA (1992) A study of developmental speech and language disorders in twins. J Speech Hear Res 35:1086-94.

Lewis JD, Meehan RR, Henzel WJ, Maurer-Fogy I, Jeppesen P, Klein F, Bird A (1992) Purification, sequence, and cellular localization of a novel chromosomal protein that binds to methylated DNA. Cell 69:905-14.

Li J, Tabor HK, Nguyen L, Gleason C, Lotspeich LJ, Spiker D, Risch N, Myers RM (2002) Lack of association between HoxA1 and HoxB1 gene variants and autism in 110 multiplex families. Am J Med Genet 114:24-30.

Liu J, Nyholt DR, Magnussen P, Parano E, Pavone P, Geschwind D, Lord C, Iversen P, Hoh J, Autism Genetic Resource Exchange Consortium t, Ott J, Gilliam TC (2001) A genomewide screen for autism susceptibility loci. Am J Hum Genet 69:327-40.

Lotspeich LJ, Ciaranello RD (1993) The neurobiology and genetics of infantile autism. International Review of Neurobiology 35:87-129.

Lyon MF (1972) X-chromosome inactivation and developmental patterns in mammals. Biol Rev Camb Philos Soc 47:1-35.

Lyon MF (1989) X-chromosome inactivation as a system of gene dosage compensation to regulate gene expression. Prog Nucleic Acid Res Mol Biol 36:119-30

Maestrini E, Lai C, Marlow A, Matthews N, Wallace S, Bailey A, Cook EH, Weeks DE, Monaco AP (1999) Serotonin transporter (5-HTT) and gamma-aminobutyric acid receptor subunit beta3 (GABRB3) gene polymorphisms are not associated with autism in the IMGSA families. The International Molecular Genetic Study of Autism Consortium. Am J Med Genet 88:492-6.

Magenis RE, Toth-Fejel S, Allen LJ, Black M, Brown MG, Budden S, Cohen R, Friedman JM, Kalousek D, Zonana J, et al. (1990) Comparison of the 15q deletions in Prader-Willi and Angelman syndromes: specific regions, extent of deletions, parental origin, and clinical consequences. Am J Med Genet 35:333-49.

Mahtani MM, Widen E, Lehto M, Thomas J, McCarthy M, Brayer J, Bryant B, Chan G, Daly M, Forsblom C, Kanninen T, Kirby A, Kruglyak L, Munnelly K, Parkkonen M, Reeve-Daly MP, Weaver A, Brettin T, Duyk G, Lander ES, Groop LC (1996) Mapping of a gene for type 2 diabetes associated with an insulin secretion defect by a genome scan in Finnish families. Nat Genet 14:90-4.

Matsuishi T, Urabe F, Percy AK, Komori H, Yamashita Y, Schultz RS, Ohtani Y, Kuriya N, Kato H (1994) Abnormal carbohydrate metabolism in cerebrospinal fluid in Rett syndrome. J Child Neurol 9:26-30.

Matus A (1988) Microtubule-associated proteins: their potential role in determining neuronal morphology. Annu Rev Neurosci 11:29-44

Mawhood L, Howlin P, Rutter M (2000) Autism and developmental receptive language disorder--a comparative follow-up in early adult life. I: Cognitive and language outcomes. J Child Psychol Psychiatry 41:547-59.

Meehan R, Lewis J, Cross S, Nan X, Jeppesen P, Bird A (1992a) Transcriptional repression by methylation of CpG. J Cell Sci Suppl 16:9-14

Meehan RR, Lewis JD, Bird AP (1992b) Characterization of MeCP2, a vertebrate DNA binding protein with affinity for methylated DNA. Nucleic Acids Res 20:5085-92

Meloni I, Bruttini M, Longo I, Mari F, Rizzolio F, P DA, Denvriendt K, Fryns JP, Toniolo D, Renieri A (2000) A Mutation in the Rett Syndrome Gene, MECP2, Causes X-Linked Mental Retardation and Progressive Spasticity in Males. Am J Hum Genet 67:982-985

Miller RD, Kwok PY (2001) The birth and death of human single-nucleotide polymorphisms: new experimental evidence and implications for human history and medicine. Hum Mol Genet 10:2195-8.

Moisio AL, Sistonen P, Weissenbach J, de la Chapelle A, Peltomaki P (1996) Age and origin of two common MLH1 mutations predisposing to hereditary colon cancer. Am J Hum Genet 59:1243-51.

Morton NE (1995) LODs past and presence. Genetics 140: 7-12.

Murphy M, Bolton PF, Pickles A, Fombonne E, Piven J, Rutter M (2000) Personality traits of the relatives of autistic probands. Psychol Med 30:1411-24.

Nan X, Campoy FJ, Bird A (1997) MeCP2 is a transcriptional repressor with abundant binding sites in genomic chromatin. Cell 88:471-81

Nan X, Meehan RR, Bird A (1993) Dissection of the methyl-CpG binding domain from the chromosomal protein MeCP2. Nucleic Acids Res 21:4886-92

Nan X, Ng HH, Johnson CA, Laherty CD, Turner BM, Eisenman RN, Bird A (1998) Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex. Nature 393:386-9

Nan X, Tate P, Li E, Bird A (1996) DNA methylation specifies chromosomal localization of MeCP2. Mol Cell Biol 16:414-21

Narayan M, Srinath S, Anderson GM, Meundi DB (1993) Cerebrospinal fluid levels of homovanillic acid and 5- hydroxyindoleacetic acid in autism. Biol Psychiatry 33:630-5.

Naruse Y, Aoki T, Kojima T, Mori N (1999) Neural restrictive silencer factor recruits mSin3 and histone deacetylase complex to repress neuron-specific target genes. Proc Natl Acad Sci U S A 96:13691-6

Nevanlinna HR (1972) The Finnish population structure. A genetic and genealogical study. Hereditas 71:195-236

Newbury DF, Bonora E, Lamb JA, Fisher SE, Lai CS, Baird G, Jannoun L, Slonims V, Stott CM, Merricks MJ, Bolton PF, Bailey AJ, Monaco AP (2002) FOXP2 Is Not a Major Susceptibility Gene for Autism or Specific Language Impairment. Am J Hum Genet 70:1318-27.

Nielsen JB, Henriksen KF, Hansen C, Silahtaroglu A, Schwartz M, Tommerup N (2001) MECP2 mutations in Danish patients with Rett syndrome: High frequency of mutations but no consistent correlations with clinical severity or with the X chromosome inactivation pattern. Eur J Hum Genet 9:178-84.

Norio R, Nevanlinna HR, Perheentupa J (1973) Hereditary diseases in Finland; rare flora in rare soul. Ann Clin Res 5:109-41.

Nystrom-Lahti M, Sistonen P, Mecklin JP, Pylkkanen L, Aaltonen LA, Jarvinen H, Weissenbach J, de la Chapelle A, Peltomaki P (1994) Close linkage to chromosome 3p and conservation of ancestral founding haplotype in hereditary nonpolyposis colorectal cancer families. Proc Natl Acad Sci U S A 91:6054-8.

Obata K, Matsuishi T, Yamashita Y, Fukuda T, Kuwajima K, Horiuchi I, Nagamitsu S, Iwanaga R, Kimura A, Omori I, Endo S, Mori K, Kondo I (2000) Mutation analysis of the methyl-CpG binding protein 2 gene (MECP2) in patients with Rett syndrome. J Med Genet 37:608-10

O' Connell JR, Weeks DE (1998) PedCheck: a program for identification of genotype incompatibilities in linkage analysis. American Journal of Human Genetics 63:259-66

Orrico A, Lam C, Galli L, Dotti MT, Hayek G, Tong S, Poon PM, Zappella M, Federico A, Sorrentino V (2000) MECP2 mutation in male patients with non-specific X-linked mental retardation. Febs Lett 481:285-8

Ott J (1976) A computer program for linkage analysis of general human pedigrees. Am J Hum Genet 28:528-9.

Ott J (1986) Linkage probability and its approximate confidence interval under possible heterogeneity. Genet Epidemiol Suppl 1:251-7.

Paunio T, Ekelund J, Varilo T, Parker A, Hovatta I, Turunen JA, Rinard K, Foti A, Terwilliger JD, Juvonen H, Suvisaari J, Arajarvi R, Suokas J, Partonen T, Lonnqvist J, Meyer J, Peltonen L (2001) Genome-wide scan in a nationwide study sample of schizophrenia families in Finland reveals susceptibility loci on chromosomes 2q and 5q. Hum Mol Genet 10:3037-48.

Pedone PV, Pikaart MJ, Cerrato F, Vernucci M, Ungaro P, Bruni CB, Riccio A (1999) Role of histone acetylation and DNA methylation in the maintenance of the imprinted expression of the H19 and Igf2 genes. FEBS Lett 458:45-50.

Pegoraro E, Schimke RN, Arahata K, Hayashi Y, Stern H, Marks H, Glasberg MR, Carroll JE, Taber JW, Wessel HB, et al. (1994) Detection of new paternal dystrophin gene mutations in isolated cases of dystrophinopathy in females. Am J Hum Genet 54:989-1003

Peltomaki P, Aaltonen LA, Sistonen P, Pylkkanen L, Mecklin JP, Jarvinen H, Green JS, Jass JR, Weber JL, Leach FS, et al. (1993) Genetic mapping of a locus predisposing to human colorectal cancer. Science 260:810-2.

Peltonen L, Jalanko A, Varilo T (1999) Molecular genetics of the Finnish disease heritage. Hum Mol Genet 8:1913-23

Peltonen L, Palotie A, Lange K (2000) Use of population isolates for mapping complex traits. Nat Rev Genet 1:182-90.

Perola M, Sajantila A, Sarti C, Stengard J, Tamminen M, Puska P, Huttunen J, Tuomilehto J, Peltonen L (1995) Angiotensin-converting enzyme genotypes in the high- and low-risk area for coronary heart disease in Finland. Genet Epidemiol 12:391-9

Philippart M (1990) The Rett syndrome in males. Brain Dev 12:33-6

Philippe A, Martinez M, Guilloud-Bataille M, Gillberg C, Rastam M, Sponheim E, Coleman M, Zappella M, Aschauer H, van Malldergerme L, Penet C, Feingold J, Brice A, Leboyer M, tP (1999) Genome-wide scan for autism susceptibility genes [In Process Citation]. Hum Mol Genet 8:805-12

Piven J, Palmer P, Jacobi D, Childress D, Arndt S (1997a) Broader autism phenotype: evidence from a family history study of multiple-incidence autism families. Am J Psychiatry 154:185-90

Piven J, Palmer P, Landa R, Santangelo S, Jacobi D, Childress D (1997b) Personality and language characteristics in parents from multiple- incidence autism families. Am J Med Genet 74:398-411.

Piven J, Wzorek M, Landa R, Lainhart J, Bolton P, Chase GA, Folstein S (1994) Personality characteristics of the parents of autistic individuals. Psychol Med 24:783-95.

Puffenberger EG, Hosoda K, Washington SS, Nakao K, deWit D, Yanagisawa M, Chakravart A (1994) A missense mutation of the endothelin-B receptor gene in multigenic Hirschsprung's disease. Cell 79:1257-66.

Rapin I (1996) Practitioner review: developmental language disorders: a clinical update. J Child Psychol Psychiatry 37:643-55.

Rapin I (1998) Understanding childhood language disorders. Curr Opin Pediatr 10:561-6.

Rapin I, Dunn M (1997) Language disorders in children with autism. Semin Pediatr Neurol 4:86-92

Reich DE, Cargill M, Bolk S, Ireland J, Sabeti PC, Richter DJ, Lavery T, Kouyoumjian R, Farhadian SF, Ward R, Lander ES (2001) Linkage disequilibrium in the human genome. Nature 411:199-204.

Rett A (1966a) Uber ein zerebral-atrophisches Syndrome bei Hyperammonemie. In. Bruder Hollinek, Vienna

Rett A (1966b) Über ein eigenartiges hirnatrophisches Syndrom bei Hyperammonämie im Kindesalter. Wiener Medizinische Wochenschrift 116:723-739

Rioux JD, Daly MJ, Silverberg MS, Lindblad K, Steinhart H, Cohen Z, Delmonte T, et al. (2001) Genetic variation in the 5q31 cytokine gene cluster confers susceptibility to Crohn disease. Nat Genet 29:223-8.

Risch N, Merikangas K (1996) The future of genetic studies of complex human diseases. Science 273:1516-7.

Risch N, Spiker D, Lotspeich L, Nouri N, Hinds D, Hallmayer J, Kalaydjieva L, et al. (1999) A genomic screen of autism: evidence for a multilocus etiology. Am J Hum Genet 65:493-507.

Risch N (2000) Searching for genetic determinants in the new millennium. Nature 405:847-56.

Riva D, Giorgi C (2000) The cerebellum contributes to higher functions during development: evidence from a series of children surgically treated for posterior fossa tumours. Brain 123:1051-61.

Rolando S (1985) Rett syndrome: report of eight cases. Brain Dev 7:290-6

Rosenbloom S, Campbell M, George AE, Kricheff, II, Taleporos E, Anderson L, Reuben RN, Korein J (1984) High resolution CT scanning in infantile autism: a quantitative approach. J Am Acad Child Psychiatry 23:72-7.

Rutter M (1978) Diagnosis and definition of childhood autism. Autism Child Schizophr 8: 139-61.

Rutter M (1979) Language, cognition, and autism. Res Publ Assoc Res Nerv Ment Dis 57:247-64

Rutter M, Bailey A, Bolton P, Le Couteur A (1994) Autism and known medical conditions: myth and substance. Journal of Child Psychology & Psychiatry & Allied Disciplines 35:311-22

Sawa A, Snyder SH (2002) Schizophrenia: diverse approaches to a complex disease. Science 296:692-5.

Schaffer AA, Gupta SK, Shriram K, Cottingham RW, Jr. (1994) Avoiding recomputation in linkage analysis. Hum Hered 44:225-37.

Schanen C, Francke U (1998) A severely affected male born into a Rett syndrome kindred supports X- linked inheritance and allows extension of the exclusion map [letter]. Am J Hum Genet 63:267-9

Schanen NC, Dahle EJ, Capozzoli F, Holm VA, Zoghbi HY, Francke U (1997) A new Rett syndrome family consistent with X-linked inheritance expands the X chromosome exclusion map. Am J Hum Genet 61:634-41

Schanen NC, Kurczynski TW, Brunelle D, Woodcock MM, Dure LSt, Percy AK (1998) Neonatal encephalopathy in two boys in families with recurrent Rett syndrome. J Child Neurol 13:229-31

Schutz CK, Polley D, Robinson PD, Chalifoux M, Macciardi F, White BN, Holden JJ (2002) Autism and the X chromosome: No linkage to microsatellite loci detected using the affected sibling pair method. Am J Med Genet 109:36-41.

Shao Y, Raiford KL, Wolpert CM, Cope HA, Ravan SA, Ashley-Koch AA, Abramson RK, Wright HH, DeLong RG, Gilbert JR, Cuccaro ML, Pericak-Vance MA (2002a) Phenotypic homogeneity provides increased support for linkage on chromosome 2 in autistic disorder. Am J Hum Genet 70:1058-61.

Shao Y, Wolpert CM, Raiford KL, Menold MM, Donnelly SL, Ravan SA, Bass MP, McClain C, von Wendt L, Vance JM, Abramson RH, Wright HH, Ashley-Koch A, Gilbert JR, DeLong RG, Cuccaro ML, Pericak-Vance MA (2002b) Genomic screen and follow-up analysis for autistic disorder. Am J Med Genet 114:99-105.

Sheffield VC, Weber JL, Buetow KH, Murray JC, Even DA, Wiles K, Gastier JM, Pulido JC, Yandava C, Sunden SL, et al. (1995) A collection of tri- and tetranucleotide repeat

markers used to generate high quality, high resolution human genome-wide linkage maps. Hum Mol Genet 4:1837-44.
Shifman S, Darvasi A (2001) The value of isolated populations. Nat Genet 28:309-10.
Siegfried Z, Eden S, Mendelsohn M, Feng X, Tsuberi BZ, Cedar H (1999) DNA methylation represses transcription in vivo. Nat Genet 22:203-6
Sinsheimer JS, Blangero J, Lange K (2000) Gamete-competition models. Am J Hum Genet 66:1168-72.
Sirianni N, Naidu S, Pereira J, Pillotto RF, Hoffman EP (1998) Rett syndrome: confirmation of X-linked dominant inheritance, and localization of the gene to Xq28. Am J Hum Genet 63:1552-8
Skjeldal OH, von Tetzchner S, Aspelund F, Herder GA, Lofterld B (1997) Rett syndrome: geographic variation in prevalence in Norway. Brain Dev 19:258-61
Skuse DH (2000) Imprinting, the X-chromosome, and the male brain: explaining sex differences in the liability to autism. Pediatr Res 47:9-16.
Smalley SL (1997) Genetic influences in childhood-onset psychiatric disorders: autism and attention-deficit/hyperactivity disorder. Am J Hum Genet 60:1276-82
Smalley SL, Tanguay PE, Smith M, Gutierrez G (1992) Autism and tuberous sclerosis. J Autism Dev Disord 22:339-55.
Sobel E, Lange K (1996) Descent graphs in pedigree analysis: applications to haplotyping, location scores, and marker-sharing statistics. American Journal of Human Genetics 58:1323-37
Steffenburg S, Gillberg C, Hellgren L, Andersson L, Gillberg IC, Jakobsson G, Bohman M (1989) A twin study of autism in Denmark, Finland, Iceland, Norway and Sweden. J Child Psychol Psychiatry 30:405-16
Stone EM, Fingert JH, Alward WL, Nguyen TD, Polansky JR, Sunden SL, Nishimura D, Clark AF, Nystuen A, Nichols BE, Mackey DA, Ritch R, Kalenak JW, Craven ER, Sheffield VC (1997) Identification of a gene that causes primary open angle glaucoma. Science 275:668-70.
Szatmari P (1992) The validity of autistic spectrum disorders: a literature review. J Autism Dev Disord 22:583-600
Szatmari P (2000) The classification of autism, Asperger's syndrome, and pervasive developmental disorder. Can J Psychiatry 45:731-8.
Szatmari P, Bartolucci G, Bremner R (1989) Asperger's syndrome and autism: comparison of early history and outcome. Developmental Medicine & Child Neurology 31:709-20
Szatmari P, Jones MB, Zwaigenbaum L, MacLean JE (1998) Genetics of autism: overview and new directions. J Autism Dev Disord 28:351-68.
Taillon-Miller P, Bauer-Sardina I, Saccone NL, Putzel J, Laitinen T, Cao A, Kere J, Pilia G, Rice JP, Kwok PY (2000) Juxtaposed regions of extensive and minimal linkage disequilibrium in human Xq25 and Xq28. Nat Genet 25:324-8.
Tanguay PE, Robertson J, Derrick A (1998) A dimensional classification of autism spectrum disorder by social communication domains. J Am Acad Child Adolesc Psychiatry 37:271-7.
Tate P, Skarnes W, Bird A (1996) The methyl-CpG binding protein MeCP2 is essential for embryonic development in the mouse. Nat Genet 12:205-8
Tentler D, Brandberg G, Betancur C, Gillberg C, Annere´n G, Orsmark C, Green ED, Carlsson B, Dahl N (2001) A balanced reciprocal translocation t(5;7)(q14;q32) associated with autistic disorder: Molecular analysis of the chromosome 7 breakpoint. Am J Med Genetics 105: 729-36.
The Rett Syndrome Diagnostic Criteria Work Group (1988) Diagnostic criteria for Rett syndrome. Ann Neurol 23:425-8.

Thomas GH (1996) High male:female ratio of germ-line mutations: an alternative explanation for postulated gestational lethality in males in X-linked dominant disorders. Am J Hum Genet 58:1364-8.

Tomblin JB, Buckwalter PR (1998) Heritability of poor language achievement among twins. J Speech Lang Hear Res 41:188-99.

Tomblin JB, Records NL, Buckwalter P, Zhang X, Smith E, M OB (1997) Prevalence of specific language impairment in kindergarten children. J Speech Lang Hear Res 40:1245-60.

Topcu M, Akyerli C, Sayi A, Toruner GA, Kocoglu SR, Cimbis M, Ozcelik T (2002) Somatic mosaicism for a MECP2 mutation associated with classic Rett syndrome in a boy. Eur J Hum Genet 10:77-81.

Topcu M, Topaloglu H, Renda Y, Berker M, Turanli G (1991) The Rett syndrome in males. Brain Dev 13:62

Trappe R, Laccone F, Cobilanschi J, Meins M, Huppke P, Hanefeld F, Engel W (2001) Mecp2 mutations in sporadic cases of rett syndrome are almost exclusively of paternal origin. Am J Hum Genet 68:1093-101.

Tucker KL (2001) Methylated cytosine and the brain: a new base for neuroscience. Neuron 30:649-52.

Vacca M, Filippini F, Budillon A, Rossi V, Mercadante G, Manzati E, Gualandi F, Bigoni S, Trabanelli C, Pini G, Calzolari E, Ferlini A, Meloni I, Hayek G, Zappella M, Renieri A, M DU, M DE, MacDonald F, Kerr A, Dhanjal S, Hulten M (2001) Mutation analysis of the MECP2 gene in British and Italian Rett syndrome females. J Mol Med 78:648-55.

Wade PA, Gegonne A, Jones PL, Ballestar E, Aubry F, Wolffe AP (1999) Mi-2 complex couples DNA methylation to chromatin remodelling and histone deacetylation. Nat Genet 23:62-6

Wan M, Lee SS, Zhang X, Houwink-Manville I, Song HR, Amir RE, Budden S, Naidu S, Pereira JL, Lo IF, Zoghbi HY, Schanen NC, Francke U (1999) Rett Syndrome and Beyond: Recurrent Spontaneous and Familial MECP2 Mutations at CpG Hotspots. Am J Hum Genet 65:1520-1529

Varilo T, Savukoski M, Norio R, Santavuori P, Peltonen L, Jarvela I (1996) The age of human mutation: genealogical and linkage disequilibrium analysis of the CLN5 mutation in the Finnish population. Am J Hum Genet 58:506-12.

Warburton P, Baird G, Chen W, Morris K, Jacobs BW, Hodgson S, Docherty Z (2000) Support for linkage of autism and specific language impairment to 7q3 from two chromosome rearrangements involving band 7q31. Am J Med Genet 96:228-34.

Warren RP, Singh VK, Averett RE, Odell JD, Maciulis A, Burger RA, Daniels WW, Warren WL (1996) Immunogenetic studies in autism and related disorders. Molecular & Chemical Neuropathology 28:77-81

Watson P, Black G, Ramsden S, Barrow M, Super M, Kerr B, Clayton-Smith J (2001) Angelman syndrome phenotype associated with mutations in MECP2, a gene encoding a methyl CpG binding protein. J Med Genet 38:224-8.

Weber JL (1990) Human DNA polymorphisms and methods of analysis. Curr Opin Biotechnol 1:166-71.

Veenstra-VanderWeele J, Kim SJ, Lord C, Courchesne R, Akshoomoff N, Leventhal BL, Courchesne E, Cook EH Jr. (2002) Transmission disequilibrium studies of the serotonin 5-HT2A receptor gene (HTR2A) in autism. Am J Med Genet. 114:277-83.

Wenk GL, Hauss-Wegrzyniak B (1999) Altered cholinergic function in the basal forebrain of girls with Rett syndrome. Neuropediatrics 30:125-9.

Wenk GL, O'Leary M, Nemeroff CB, Bissette G, Moser H, Naidu S (1993) Neurochemical alterations in Rett syndrome. Brain Res Dev Brain Res 74:67-72.
Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GG, Smith HO, et al. (2001) The sequence of the human genome. Science 291:1304-51.
Villard L, Kpebe A, Cardoso C, Chelly PJ, Tardieu PM, Fontes M (2000) Two affected boys in a Rett syndrome family: clinical and molecular findings. Neurology 55:1188-93.
Villard L, Levy N, Xiang F, Kpebe A, Labelle V, Chevillard C, Zhang Z, Schwartz CE, Tardieu M, Chelly J, Anvret M, Fontes M (2001) Segregation of a totally skewed pattern of X chromosome inactivation in four familial cases of Rett syndrome without MECP2 mutation: implications for the disease. J Med Genet 38:435-42.
Williams CA, Angelman H, Clayton-Smith J, Driscoll DJ, Hendrickson JE, Knoll JH, Magenis RE, Schinzel A, Wagstaff J, Whidden EM, et al. (1995) Angelman syndrome: consensus for diagnostic criteria.Angelman Syndrome Foundation. Am J Med Genet 56:237-8.
Vincent JB, Herbrick JA, Gurling HM, Bolton PF, Roberts W, Scherer SW (2000) Identification of a novel gene on chromosome 7q31 that is interrupted by a translocation breakpoint in an autistic individual. Am J Hum Genet 67:510-4.
Wing L (1981) Asperger's syndrome: a clinical account. Psychol Med 11:115-29.
Wing L, Gould J (1979) Severe impairments of social interaction and associated abnormalities in children: epidemiology and classification. J Autism Dev Disord 9:11-29.
Volkmar FR, Klin A, Schultz RT, Rubin E, Bronen R (2000) Asperger's disorder. Am J Psychiatry 157:262-7.
Volkmar FR, Nelson DS (1990) Seizure disorders in autism. J Am Acad Child Adolesc Psychiatry 29:127-9.
World Health Organization (1993) International classification of diseases (10th ed., chap. 5). Mental and behavioural disorders Diagnostic criteria for research. Geneva:Author.
Vorsanova SG, Demidova IA, Ulas V, Soloviev IV, Kazantzeva LZ, Yurov Yu B (1996) Cytogenetic and molecular-cytogenetic investigation of Rett syndrome: analysis of 31 cases. Neuroreport 8:187-9.
Vourc'h P, Bienvenu T, Beldjord C, Chelly J, Barthelemy C, Muh JP, Andres C (2001) No mutations in the coding region of the Rett syndrome gene MECP2 in 59 autistic patients. Eur J Hum Genet 9:556-8.
Yan WL, Guan XY, Green ED, Nicolson R, Yap TK, Zhang J, Jacobsen LK, Krasnewich DM, Kumra S, Lenane MC, Gochman P, Damschroder-Williams PJ, Esterling LE, Long RT, Martin BM, Sidransky E, Rapoport JL, Ginns EI (2000) Childhood-onset schizophrenia/autistic disorder and t(1;7) reciprocal translocation: identification of a BAC contig spanning the translocation breakpoint at 7q21. Am J Med Genet 96:749-53.
Yirmiya N, Pilowsky T, Nemanov L, Arbelle S, Feinsilver T, Fried I, Ebstein RP (2001) Evidence for an association with the serotonin transporter promoter region polymorphism and autism. Am J Med Genet 105:381-6.
Zappella M, Gillberg C, Ehlers S (1998) The preserved speech variant: a subgroup of the Rett complex: a clinical report of 30 cases. J Autism Dev Disord 28:519-26.
Zavattari P, Deidda E, Whalen M, Lampis R, Mulargia A, Loddo M, Eaves I, Mastio G, Todd JA, Cucca F (2000) Major factors influencing linkage disequilibrium by analysis of different chromosome regions in distinct populations: demography, chromosome recombination frequency and selection. Hum Mol Genet 9:2947-57.
Zoghbi H (1988) Genetic aspects of Rett syndrome. J Child Neurol 3:S76-8

Zwick ME, Cutler DJ, Chakravarti A (2000) Patterns of genetic variation in Mendelian and complex traits. Annu Rev Genomics Hum Genet 1:387-407.