

*Department of Pediatric Neurology, Hospital for Children and Adolescents and  
Department of Medical Genetics, Haartman Institute, University of Helsinki, Finland*

# **FAMILIAL DYSLEXIA**

Genetic and neuropsychological findings

*Jaanamarja Nopola-Hemmi*

*Helsinki University Biomedical Dissertations No.16*

Academic dissertation

*To be publicly discussed, with the permission of the Faculty of Medicine, University of Helsinki,  
in Jorvi Hospital Auditorium, Turuntie 150, Espoo, on the 25<sup>th</sup> of October 2002,  
at 12 o'clock noon.*

Helsinki 2002

**Supervised by:**

Juha Kere, M.D., Ph.D., Professor of Molecular Genetics  
Department of Biosciences at Novum and Clinical Research Centre  
Karolinska Institute  
Stockholm, Sweden  
and  
Haartman Institute and Finnish Genome Center  
University of Helsinki, Finland

Arja Voutilainen, M.D., Ph.D.  
Department of Pediatric Neurology  
Hospital for Children and Adolescents  
University of Helsinki, Finland

**Reviewed by:**

Professor Marit Korkman, Ph.D.  
Åbo Akademi University,  
Turku, Finland

Docent Jaakko Ignatius, M.D., Ph.D.  
Department of Clinical Genetics  
University of Oulu, Finland

**Official opponent:**

Docent Hannu Heiskala, M.D., Ph.D.  
Department of Pediatric Neurology  
Hospital for Children and Adolescents  
University of Helsinki, Finland

ISSN 1457-8433

ISBN 952-91-5105-5 (print)

ISBN 952-10-0719-2 (PDF)

Yliopistopaino

Helsinki 2002

Cover illustration: "A reading boy" by Matti Kontiola

*To Oona, Eemeli and Risto*

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## LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text by their Roman numerals.

I Nopola-Hemmi J, Myllyluoma B, Haltia T, Taipale M, Ollikainen V, Ahonen T, Voutilainen A, Kere J, Widén E. A dominant gene for developmental dyslexia on chromosome 3. *J Med Genet* 38: 658-664, 2001

II Nopola-Hemmi J, Myllyluoma B, Voutilainen A, Leinonen S, Kere J, Ahonen T. Familial dyslexia: neurocognitive and genetic correlation in a large Finnish family. *Dev Med Child Neurol* 44: 580-586, 2002.

III Nopola-Hemmi J, Taipale M, Haltia T, Lehesjoki A-E, Voutilainen A, Kere J. Two translocations of chromosome 15q associated with dyslexia. *J Med Genet* 37: 771-775, 2000.

IV Taipale M, Kaminen N\*, Nopola-Hemmi J\*, Haltia T, Myllyluoma B, Lindsberg P, Kere J. A candidate gene for developmental dyslexia encodes a novel nuclear TPR domain protein dynamically regulated in brain. Submitted.

\* Equal contribution. Publication IV will also appear in the thesis of Nina Kaminen. In addition, some unpublished data are presented.

## ABBREVIATIONS

BAC	bacterial artificial chromosome
bp	base pair
DLD	developmental language disorder
DNA	deoxyribonucleic acid
DZ	dizygotic twins
FISH	fluorescence in situ hybridization
FSIQ	full scale intelligence quotient
IQ	intelligence quotient
LD	learning disability
Lod	logarithm of odds
MEG	magnetoencephalography
MRI	magnetic resonance imaging
MZ	monozygotic twins
OMIM	online mendelian inheritance in man
PCR	polymerase chain reaction
PET	positron emission tomography
PIQ	performance IQ
QTL	quantitative trait loci
RD	reading disability
SNP	single nucleotide polymorphism
SSCP	single strand conformation polymorphism
VIQ	verbal IQ
VSTM	verbal short-term memory

## SUMMARY

Developmental dyslexia is a common neurodevelopmental disorder characterized by failure in the normal acquisition of reading and writing skills. The etiology of dyslexia is still unknown, but, according to family and twin studies, a significant heritable component exists. The genetic basis for developmental dyslexia is, however, complex involving heterogeneity, oligogenic inheritance, reduced penetrance and phenocopies.

In this study the genetic background and neurocognitive features of dyslexia were researched in Finnish families. A positional cloning strategy was used to identify the susceptibility gene(s) for developmental dyslexia. 11 multiplex families with a three-generation history of dyslexia and a pattern of autosomal dominant inheritance were selected for genetic linkage analysis, and two families with known translocation on 15q21 were selected for fluorescence in situ hybridization (FISH) analysis. All family members available were tested neuropsychologically for general cognitive ability, reading and spelling skills and reading-related neurocognitive skills in order to confirm the diagnosis and to clarify the underlying neurocognitive deficits.

As in previous studies, heterogeneity was also found in our material: families being linked to at least two different chromosomal areas. A previously unreported linkage to chromosome 3 (*DYX5*) (OMIM 606896) was found in an extended Finnish family (AB) of altogether 28 dyslexic individuals, whereas other families showed no evidence of linkage in this region. The neurocognitive subtype of dyslexia segregating in this family consisted of deficits in phonological awareness, verbal short-term memory and rapid naming.

Two independent families showed a translocation segregating with dyslexia in another chromosomal area, 15q21, a previously confirmed dyslexia locus. By performing FISH studies with two individuals from separate families we were able to refine the position of the putative dyslexia locus. The FISH results suggested that both independent translocation breakpoints on 15q map within an 6-8 Mb of each other residing in the region between markers D15S143 and D15S1029. This region overlaps the region implicated to carry the *DYX1* locus in previous linkage studies.

We were then able to clone the translocation breakpoint and characterize a candidate gene (*DYXCI*) disrupted by the translocation. Furthermore, we identified a single-nucleotide polymorphism (SNP) in *DYXCI* mRNA (1249G>T), the frequency of which was significantly

higher ( $p=0.03$ ) in dyslexic individuals ( $n=57$ ) than in controls ( $n=106$ ), supporting the role of *DYX1* as a possible candidate gene for dyslexia.

# INTRODUCTION

Learning disorders in preschool and school-aged children are today one of the main reasons of referrals to a neuropsychiatric unit. Developmental dyslexia, i.e. specific reading disability, is a common disorder and covers approximately 80% of all learning disabilities.

The basic pathophysiology of developmental dyslexia still remains largely unknown, even though developments in neuroimaging and neurofunctional studies have shed light to brain functions disturbed in dyslexia. However, there begins to be a consensus among researchers that the basic deficit in dyslexia is actually a deficiency in phonological processing, which is believed to disrupt the acquisition and automation of word recognition and other reading and writing-related skills.

In spite of the well known familiar occurrence of dyslexia the genetics of this disorder has been poorly understood. Advances in molecular genetics in the last few years have allowed researchers to identify loci segregating with developmental dyslexia on chromosomes 1, 2, 6, 15 and most recently on chromosome 18. However, the results have been inconsistent in different studies and linkage studies have mainly focused on a few chromosomal regions so far. Genome search studies have been scarce up to now.

The heterogeneity and multifactorial nature of dyslexia, as well as other neurodevelopmental disorders, make the traditional positional cloning efforts laborious, and in spite of extensive efforts none of the underlying genes have been identified so far.

At the onset of this study only little was known about the genetics of dyslexia or other neurodevelopmental disorders in Finland. However, the hereditary nature of dyslexia is familiar to all clinicians who work in the field of pediatric neurology and has long been bothering me because of the lack of a good explanation for the parents of a learning disabled child.

Before this study was started, one Finnish family segregating with dyslexia and translocation in the long arm of chromosome 15 had been identified. The aim of our study was to collect further family material in order to map the gene/genes involved in dyslexia in Finland by the positional cloning strategy.

# **REVIEW OF THE LITERATURE**

## **1. Dyslexia**

### **1.1 History**

Dyslexia (reading disability, word-blindness) has long been recognized, but the earliest descriptions have mainly focused on acquired alexia resulting from brain trauma or a vascular accident. In 1877 Kussmaul described “word-blindness” as a specific syndrome involving the inability to read in spite of normal vision as a consequence of acquired brain lesion (Kussmaul 1877). The first congenital case, a 14-year-old boy, with normal intelligence and health and the ability to calculate, but with extreme difficulties in reading and writing, was described by Morgan in 1896 (Morgan 1896). The first to introduce the term dyslexia was a Scottish ophthalmologist, James Hinshelwood, who differentiated complete word-blindness, alexia, from cases of partial impairment, dyslexia (Hinshelwood 1896). In 1905 Thomas called special attention to the fact that congenital word-blindness may assume a family type and that a hereditary tendency is probable (Thomas 1905). In 1907 Stephenson described a kindred with three affected generations (Stephenson 1907). Later on Hallgren reviewed reports with larger numbers of dyslexia families as part of his classic study of the transmission of dyslexia (Hallgren 1950).

### **1.2 Definition and diagnosis**

Dyslexia has traditionally been defined as an unexpected difficulty in learning to read despite adequate intelligence, education, and normal senses. The Orton Dyslexia Society Research Committee defined dyslexia in 1994 as a specific language-based disorder of constitutional origin characterized by difficulties in single word decoding, usually reflecting insufficient phonological processing (Lyon 1995). These difficulties in single word decoding should be unexpected in relation to age and other cognitive and academic abilities. Dyslexia usually manifests itself as a failure in learning to read in the first grades at school, and most dyslexics continue to have reading difficulties throughout their adulthood (Felton et al. 1990). Preceding, accompanying and following this reading disability, the disorder manifests itself in various difficulties in phonological coding, including problems in encoding, retrieving and using phonological codes in memory (Catts 1989). During the preschool years dyslexic individuals may have identifiable problems in language processing, i.e. word-finding and naming problems,

poor verbal short-term memory, and limited speech sound awareness (Bradley and Bryant 1983, Mann and Liberman 1984, Wolf and Bowers 1999). The spectrum of cognitive and behavioural problems in developmental dyslexia is quite broad, extending from spelling errors to serious difficulties with the reading of single words (Grigorenko 2001).

In practice, dyslexia is most often diagnosed as a discrepancy between actual reading skills and expectations based on chronological age and intelligence quotient (IQ). According to the ICD-10 research criteria the discrepancy between reading and spelling achievement and IQ should be more than two standard deviations (ICD-10 1999). ICD-10 defines dyslexia as a developmental disorder associated with abnormal neurobiological development relating to both brain structure and function (Warnke 1999).

Diagnosis is complicated by the lack of uniform, standard reading tests and by the high tendency for comorbidity of specific neurocognitive disorders.

### **1.3 Comorbidity**

Although disability to read and disability to write are usually collectively referred to as dyslexia and indeed do commonly occur together, these conditions do not necessarily overlap. Deuel (1995) describes three different dysgraphia subtypes, namely dyslexic dysgraphia, dysgraphia due to motor clumsiness and dysgraphia due to defect in understanding of space. Dysgraphia can also be due to attention deficit disorder (ADD) or movement disorders such as dystonia or essential tremor (Sandler et al. 1992).

Developmental dyscalculia (disability to calculate) is less frequently associated with dyslexia, even though dyslexics do often have difficulty with the verbal processing aspects of recalling numeric knowledge (e.g. multiplication tables) or performing basic arithmetic operations (Catts 1989). Contrary to dyslexia, dyscalculia based on spatial difficulties may be a sign of right hemisphere dysfunction and may be particularly common in Asperger syndrome (Gillberg 1995). Developmental language disorder (dysphasia) is diagnosed when a child's language skills are at least two years behind the chronological age or there is a minimum difference of 20 points between verbal and nonverbal IQ (ICD-10 1999). At school age language problems often emerge as specific learning disorder, which tends to remain for the entire school age. However, all dyslexics have not suffered from a developmental language disorder as children.

Attention deficit-hyperactivity disorder (AD/HD) may also affect learning, and occurs commonly with dyslexia. It is, however, at present, considered to be an entirely different

disorder from dyslexia differing in proposed mechanisms, symptoms, assessments, and intervention (Shaywitz 1998). The prevalence of clinical AD/HD in seven years old Swedish boys is estimated to be 3.7% according to Kadesjö and Gillberg (1998), and further, more than 60% of children with AD/HD had reading disorder (Kadesjö and Gillberg 2001). According to Gillberg (2001), 80% of children with DAMP (deficits in attention, motor control and perception) will have dyslexia or dysgraphia by 10 years of age. Behavioural and mood disorders co-occur with LD presumably more as a consequence of the psychological and social stress caused by learning difficulties.

#### **1.4 Epidemiology**

In western populations the prevalence of developmental dyslexia varies between 3-15% according to the definition, linguistic system and stringency of criteria used (Hulme 1987, Shaywitz et al. 1990, Gilger et al. 1991, Habib 2000, Paulesu et al. 2001).

In Finland, Lyytinen et al. (1995) estimated the prevalence of dyslexia among Finnish adults to be about 6%. There are no good epidemiological studies for children. In the school year 1998-1999 there were 591 700 pupils attending the comprehensive schools, and 13.9% of them needed part-time special education, 5.6% because of reading and writing difficulties (Virtanen 2001).

Some researchers have found a slight male preponderance, the sex ratios being about 1.4-1.8 males to one female (Lefly and Pennington 1991, Wolff and Melngailis 1994). Shaywitz et al. (1990), however, found the sex ratio of one male to one female for reading disability in school-aged children, and consistent with their results Wood et al. (1991) found no evidence of gender in the subject or the predicate of the definition of dyslexia. The differences in sex ratios across different studies may result from more compensation by females, as the Shaywitz study was based on children only, and family studies are based on both children and adults (Lefly and Pennington 1991). On the other hand, dyslexia may be better noticed in boys. Shaywitz et al. (1990) have claimed that temperament differences between boys and girls cause boys' problems to be more noticeable to their teachers, and consequently more often referred.

Most dyslexics remain diagnosably dyslexic throughout their lives and their reading and/or spelling skills are deficient when compared to nondyslexic adults (Bruck 1992, Felton et al. 1990, Kitz and Tarver 1989, Pennington et al. 1990). However, there are a few adults with a clear history of dyslexia as children who are not diagnosably dyslexics as adults, so called

compensated dyslexics (Lefly and Pennington 1991). Compensated dyslexics remain slower readers possibly reflecting some subtle phonological processing deficit affecting reading speed. Compensation rates across different studies are quite similar, between 22-25 percent, females being more able to compensate than males (Scarborough 1984, Lefly and Pennington 1991, Felton et al. 1990). Clearly, compensation may abolish the disability to a great extent, which makes both epidemiological and genetic studies difficult to perform.

## **1.5 Etiology**

The etiology of developmental dyslexia is still unknown. Recent research has strongly emphasized the role of genetics (see below), but also pre- and perinatal factors as well as environmental and motivational factors may play a role. According to an American study, however, socio-economic variables (parental marital status, parental education, parental status as a welfare recipient, even the availability of books, newspapers or magazines at home) were unrelated to a child's reading scores. (Wood et al. 1991).

Moster et al. (2002) studied association between a low Apgar score combined with signs of neonatal encephalopathy (seizures, ventilator treatment, or feeding difficulties in the first week of life) and minor impairments at school age. Their study on altogether 727 Norwegian children showed that those with a five-minute Apgar score of 0-3 and with symptoms indicating encephalopathy had a significantly increased risk of developing minor motor impairment, attention deficit/hyperactivity disorder, epilepsy, need of extra resources in kindergarten or at school and reduced performance in reading and mathematics, compared with children with normal Apgar scores and lack of neonatal symptoms.

Only a few studies have been performed on Finnish children. In the study of Poussu-Olli (1993), 94 dyslexic children and 96 controls were compared in the sense of pre-, peri- and postnatal background factors. In this material the most frequent risk factors were shown to be foetal oxygen deficiency and nutritional disturbances. On the other hand, Korhonen et al. (1993) showed that only severe perinatal complications were significantly related to problems in later cognitive and motor development, and mild perinatal complications were not found to be related to increased neuropsychological problems. Taken together, these studies indicate that pre- and perinatal complications seem to increase the risk mainly of generalized neurocognitive impairments rather than specific reading disorder.

## **1.6 Underlying neurocognitive deficits**

The spectrum of developmental dyslexia includes at least two major deficits: one in phonological skills and the other in automatized lexical retrieval (rapid naming) (Cornwall 1992, Grigorenko 2001, Wolf and Bowers 1999, 2000). Neuropsychological studies have provided considerable evidence that the main mechanism leading to dyslexia is phonological in nature, namely a basic defect in segmenting and manipulating the phoneme constituents of speech (Mann and Libermann 1984, Pennington 1997, Habib 2000) and is strongly related to early reading acquisition (Schulte-Körne et al. 1999). It has been shown that a rapid naming deficit in early school years predicts persistent difficulties in reading until adolescence (Meyer et al. 1998, Korhonen 1995). The association between reading performance and verbal short-term memory is still under debate. According to some researchers poor verbal short-term memory is a consequence of reading problems, whereas others state that poor short-term memory is a good predictor of poor reading performance (Grigorenko 2001). Long Finnish words such as “mustaviinimarjamehulaatikko” may set more demands for verbal short-term memory among Finnish dyslexics (Leinonen et al. 2001). Inaccurate phonological decoding appears to be determinative of the number of errors made in text reading, while the inability to effectively utilize rapid, orthographic processes in word recognition is manifested in slow text reading speed (Leinonen et al. 2001).

The role of visual perceptual or other non linguistic processing deficits as a cause of dyslexia is still largely unknown. It has been suggested that reading problems might derive from a deficit in the functioning of the posterior parietal cortex necessary for the control of eye movements, peripheral vision and visuospatial attention (Stein and Walsh 1997). Tallal et al. (1993), on the other hand, have suggested that visual defects, instead of being causally connected to dyslexia, could be one manifestation of a deficit affecting the processing of all rapidly presented stimuli leading to reading problems via phonological and auditory impairments.

## **1.7 Neuroanatomical, neuroradiological and functional studies**

The first arguments for a neurological basis of dyslexia came from neuropathological studies of brains from dyslexic individuals (Galaburda et al. 1985). They examined the brains of four men (aged 14-32) with developmental dyslexia and found developmental anomalies of the cerebral cortex located asymmetrically and affecting inferior frontal and superior temporal regions predominantly on the left.

Neuroradiological imaging studies in dyslexia have mainly been focused on the planum temporale, a triangular region on the upper surface of the temporal lobe within the depth of the sylvian fissure (Shultz et al. 1994). Whereas earlier research showed variation in asymmetry of the planum temporale in dyslexic individuals (Galaburda et al. 1985, 1994), recent MRI studies do not support the view that anomalous planum temporale asymmetry is a predisposing factor for dyslexia (Rumsey et al. 1997, Eckert et al. 2000). The results of studies focusing on other brain structures such as the corpus callosum or the morphology of Heschl's gyrus (primary auditory cortex) are as inconsistent as those on the planum temporale (for a review, see Eckert et al. 2000).

Functional MRI (fMRI), positron emission tomography (PET), and magnetoencephalography (MEG) studies have shown differences in the reading relevant processes in the brain between dyslexic and non-impaired individuals. The common findings across different technologies appear to be a diminished left hemisphere posterior response to print stimuli and the suggestion of an anteriorized frontal lobe compensation (Pugh et al. 2000).

In fMRI studies brain activation patterns differed significantly between the groups with adult dyslexic readers showing relative underactivation in posterior regions (Wernicke's area, the angular gyrus and striate cortex) and relative overactivation in an anterior region (inferior frontal gyrus) (Shaywitz et al. 1998). Shaywitz et al. (1995) found differences in the functional organization of the brain for language between males and females. The only published study of fMRI in dyslexic children showed a failure to exhibit the left temporo-parietal activation during phonological tasks and a reduced activity in extra-striate occipital regions during an orthographic task (Temple et al. 2001). According to the authors, these results indicate that childhood dyslexia may be characterized by disruptions in the neural bases of both phonological and orthographic processes important for reading.

Paelesu et al. (2001) performed PET scans on dyslexics from three different countries (Italy, England and France) and found out that reduced activation in the left middle, inferior and superior temporal cortex and in the middle occipital gyrus was the robust universal feature of dyslexia for word reading in all three language groups. Reduced activation in the same regions have been found previously with PET (Rumsey et al. 1997) and fMRI (Shaywitz et al. 1998) studies among English-speaking dyslexics and with magnetoencephalography (MEG) in Finnish-speaking dyslexics (Salmelin et al. 1996).

In their fMRI study, Eden et al. (1996) showed a failure of phonologically impaired dyslexics to activate the visual-motion area V5 when viewing randomly moving dots. They present these observations as further evidence for a selective deficit in the magnocellular subsystem of the brain. In the visual system, neurons in the magnocellular layers of the lateral geniculate nucleus have rapid responses and are sensitive to motion, whereas neurons in the parvocellular layer have slow responses and are sensitive to form and colour. The input to V5 is dominated by the magnocellular stream (Frith et Frith 1996). Previously, evidence of abnormalities in the magnocellular layers of the lateral geniculate nucleus has been found in post-mortem studies of developmental dyslexics (Livingstone et al. 1991). According to Frith and Frith (1996) the defect in the perception of visual motion is a marker of a more general cognitive deficit in timing which affects all brain modalities corresponding to the studies of Tallal et al. (1993).

Rae et al. (1998) found biochemical differences (ratio of choline-containing compounds to A-acetylaspartate) using  $^1\text{H}$  magnetic resonance spectroscopy between dyslexic men and controls in the left temporoparietal lobe and right cerebellum reflecting changes in cell density in these areas. Richards et al. (1999) have observed greater lactate elevation in dyslexic boys compared with controls in the left anterior quadrant using Proton MR Spectroscopy. Furthermore, after a treatment period of three weeks they found improved phonological performance associated with changes in brain lactate levels (Richards et al. 2000).

According to Pihko et al. (1999) and Lyytinen et al. (2001), differences between children of dyslexic families and controls in auditory event-related potentials (ERP) to speech sounds can be seen already at the age of six months.

Kujala et al. (2001) tested the effects of a training program for dyslexic children in the first grade of school. Their results suggest that perceptual training with non-linguistic audiovisual stimuli causes plastic changes in the neural substrate of sound discrimination and an improvement in reading skills. A computer training program containing no linguistic material was used, and the effects of the training program were evaluated by measuring the subject's brain activity, behavioural stimulus discrimination and reading skills. Brain activity was measured by recording the mismatch negativity (MMN). After the training period of 7 weeks, the MMN amplitude was considerably increased in the training group but not in the control group, with a significant difference between the groups ( $p < 0.03$ ). According to the authors, the training-induced enhancement of the MMN amplitude reflects an increased accuracy of cortical auditory representations.

## **2 Genetics of dyslexia**

### **2.1 Heritability**

Familial aggregation of developmental dyslexia has been well documented for nearly a century and numerous segregation and twin studies have consistently supported a significant role for genetic factors in the etiology of this disorder (Thomas 1905, Hallgren 1950, DeFries and Fulker 1985, DeFries et al. 1987, Olson et al. 1989, Pennington et al. 1991, Pennington 1995). Results of a large Colorado-based study suggest probandwise concordance rates of 68% in monozygotic (MZ) twins, versus 38% in dizygotic (DZ) twins (DeFries and Alarcón 1996). The median relative increase in risk for a child having an affected parent is about eight times the population risk, which is about 5% (Gilger et al. 1991).

Pennington (1995) has found that in 20-30% of families with dyslexic children, the inheritance pattern is consistent with autosomal dominant inheritance. An additive or dominant autosomal major locus is suggested because of the high and similar recurrence rates in parents and siblings (Pennington et al. 1991, Pennington 1995).

The reading skill, like dyslexia, seems to be familial. According to the Colorado Family Reading Study sample, primary relatives have a correlation for reading skill of about 0.40 (Pennington 1995).

Byring (1984) has studied Finnish dyslexic adolescents. He found a hereditary disposition for reading and spelling problems in 77% of the cases in patients of an outpatient department. Among these, a significantly weaker short-term memory for digits was observed in cases of inheritance from the paternal side.

### **2.2 Previous molecular genetic studies**

Several genetic linkage studies have been performed since the 1980s. In these studies regions likely to contain genes contributing to developmental dyslexia have been found in the short and long arms of chromosome 15, in the short arm of chromosome 6 near the human leukocyte antigen region, and on chromosomes 1, 2 and 18 (Table 1). A translocation  $t(1;2)(p22;q31)$  segregating with retarded speech development and dyslexia has been reported by Froster et al. (1993). Lubs et al. (1991) have identified a family in which six out of seven dyslexic family members have a translocation with a centric fusion of chromosomes 13 and 14, so called

Robertsonian balanced translocation. As Robertsonian translocation is a relatively common finding, this is most probably a coincidence.

**Table 1.** Previously reported dyslexia loci

<b>Locus</b>	<b>Markers</b>	<b>Authors</b>	<b>Method</b>	<b>Material</b>	<b>Pheno-type</b>	<b>Psychomet-ric tests</b>	<b>Dg criteria</b>
1p34-p36	Rh marker, FUCA1, DIS165	Rabin et al. (1993)	Linkage	9 families	Dyslexia	No information	No information
1p22	Trans-location 1p22;2q31	Froster et al. (1993)	Cytogenetic analysis	1 family: 3 individuals	Dyslexia DS	German writing test, IT	Writing standard score <2SD, IQ>85
Distal to 1p22	DIS199	Grigorenko et al. (1998)	Linkage	8 families	PD	No information	No information
2p15-p16 ( <b>DYX3</b> )	D2S2183, D2S393, D2S378	Fagerheim et al. (1999)	Linkage Genome scan	1 large family	Dyslexia	KOAS, KOAP (Norwegian RT)	Reading performance discrepancy to age
6p	BF, 2C5	Smith et al (1991)	Linkage Sib pair analysis	18 US families	Dyslexia	GORT, PIAT WRAT (spelling subtest)	Children at least 2 years below expected grade level, FSIQ>90
6p21.3-p23 ( <b>DYX2</b> )	D6S105, TNFB	Cardon et al. (1994)	Linkage Sibpair analysis	114 sib pairs, 50 DZ twins US patients	Dyslexia	PIAT, WISC-R	VIQ or PIQ>90 reading performance 2 years below grade
6p21.3-p23	D6S109, D6S461, D6S299, D6S464, D6S306	Grigorenko et al. (1997)	Linkage	6 families (N=94), US patients	PA	PA, PD, RN, SWR, NWR, WISC-R	Discrepancy from IQ, > 20 score points
6p21.3-p23	D6S461, D6S276, D6S105, D6S306, D6S258	Gayan et al. (1999)	Confirmation of linkage	126 sib pairs US patients	PA, PD, O	PIAT, WISC-R, WR, OC, PD, PA	Performances< 2SD
6p21.3	D6S422, D6S1660, D6S276, D6S105	Fisher et al. (1999)	Confirmation of linkage	181 sib pairs UK patients	PD, O	WR, OC, PC, NW, WISC-R	IQ/ reading discrepancy
6p21.3-	D6S464-D6S273	Grigorenko et al. (2000)	Extension of study Grigorenko et al. 1997	8 families (N=171) US patients	PA, PD, RN, SWR, D, V, S	PA, PD, RN, WRAT, PIAT, WISC-R	As in Grigorenko et al. 1997
6q13-q16.2	D6S254, D6S965, D6S286, D6S251	Petryshen et al. (1999)	Linkage Sibpair analysis	96 Canadian families, at least 2 affected	PC, S Spelling	No information	No information
15p hetero-morphism		Smith et al. (1983)	Linkage analysis	9 families 84 individuals US		GORT, PIAT WRAT (spelling subtest)	Children at least 2 years below expected grade level, FSIQ>90

15q15- qter	ynz90	Smith et al. (1991)	Linkage Sib pair analysis	18 families	Dyslexia	As in Smith et al. 1983	As in Smith et al. 1983
15q21 <b>(DYX1)</b>	D15S143	Grigorenko et al. (1997)	Linkage	6 families N=94	SWR	L, R, W-J, D-R, WISC-R	Discrepancy from IQ (>20 standard score points)
15q21	D15S143, D15S132	Schulte- Körne et al. (1998)	Linkage	7 families N=67 German	Spelling		Discrepancy between achievement and IQ (>1SD) or positive history (adults)
15q21	D15S994, D15S214, D15S146	Morris et al. (2000)	Family- based association mapping	178 parent- proband trios UK	Dyslexia	NARA, WISC III	Discrepancy between age and reading skills (>2.5 years), IQ>85
18p11.2	D18S53	Fisher et al. (2002)	QTL based genome scan	195 sib pairs UK 180 sib pairs US	Dyslexia	SWR, PA	Discrepancy between achievement and IQ >2SD

O	orthographic skills	D&R	Denckla and Rudel test for rapid naming
PA	phonological awareness	GORT	Gray Oral Reading Test
PD	phonological coding	L	Lindamood auditory conceptualization test
PC	phonological decoding	NARA	Neale Analysis of Reading Ability
QTL	quantitative trait locus	PIAT	Peabody Individual Achievement Test
RN	rapid naming	R	Rosner auditory analysis test
S	spelling	W- J	Woodcock-Johnson test
SWR	single word recognition	WAIS-R	Wechsler intelligence test for adults
V	vocabulary	WISC-R	Wechsler intelligence test for children
		WRAT	Wide Range Achievement Test

### Chromosome 15 (DYX1, OMIM 127700)

Smith et al. (1983) reported first linkage to the short arm of chromosome 15 in about 20% of dyslexia families studied. Subsequent studies, some of which included a proportion of the original Smith's pedigrees failed to replicate this finding in a Danish material, however (Bisgaard et al. 1987, Lubs 1991).

In further studies of Smith et al. (1991), another locus was found in the long arm of chromosome 15. In addition, possible susceptibility locus was found on 6p indicating heterogeneity among patients. Later, a lod score >3 was obtained in 15q21 in six independent families by Grigorenko et al. (1997) and the results were further confirmed by Schulte-Körne et al. (1998) supporting linkage between chromosome 15q21 markers and a putative dyslexia locus, called (*DYX1*, OMIM 127700). Further evidence was obtained from a family-based

association mapping study (Morris et al. 2000), where a highly significant association was detected between RD and a three-marker haplotype (D15S994/D15S214/D15S146) on chromosome 15q in 178 proband-parent trios.

#### Chromosome 6 (*DYX2*, OMIM 600202)

After the findings of Smith et al. (1991), the human leukocyte antigen (HLA) region was targeted for the study of Cardon et al. (1994, 1995) due to putative association between autoimmune disorders and dyslexia. In their studies a QTL was defined to a 2 cM region within HLA complex. Grigorenko et al. (1997) reported significant evidence for linkage of the phonological awareness phenotype to 6p21.3, thus providing partial replication of the results of Smith et al. (1991) and Cardon et al. (1994). These findings were further confirmed by Gayán et al. (1999). The results of Fisher et al. (1999a) indicate that this QTL may affect both phonological and orthographic skills and is not thus specific to phoneme awareness as previously suggested. An extension study of Grigorenko et al. (2000) contributes to the converging evidence from three other independent studies that a region on 6p21.3 influences various dyslexia-spectrum processes and is now called *DYX2* (OMIM 600202).

In contrast with these findings, Field and Kaplan (1998) failed to replicate linkage to the 6p23-6p21.3 region in a sample of 79 Canadian families with at least two affected siblings. Instead, they reported a new locus on 6q13-q16.2 for phonological coding dyslexia.

#### Chromosome 2 (*DYX3*, OMIM 604254)

Fagerheim et al. (1999) studied a large Norwegian family with 36 dyslexic family members by a genome scan and were able to map a locus on 2p15-p16 cosegregating with dyslexia. Their result has been replicated by Fisher et al. (2002) and this locus has been named *DYX3* (OMIM 604254).

#### Chromosome 18 (OMIM 606616)

Recently, Fisher et al. (2002) presented two complete QTL-based genome-wide scans in large samples of families from the United Kingdom and United States. Using single-point analysis, linkage to marker D18S53 was independently identified as being one of the most significant results of the genome in each scan ( $p < 0.0004$  for single-word reading ability in each family sample). Multipoint analysis gave increased evidence of 18p11.2 linkage for single-word reading, yielding top empirical  $p$  values of 0.00001 (UK) and 0.0004 (US). Measures related to orthographic and phonological processing also showed linkage at this locus. Linkage was

replicated to 18p11.2 in a third independent sample of families (from the UK), in which the strongest evidence came from a phoneme-awareness measure. A combined analysis of all UK families confirmed that this newly discovered 18p QTL (OMIM 606616) is probably a general risk factor for dyslexia, influencing several reading-related processes.

### Chromosome 1

A locus on 1p has also been suggested because of a suggestive lod score of 1.95 at 1p34-p36 (Rabin et al. 1993) and identification of a translocation 46,XY;t(1;2)(p22;q22) cosegregating with dyslexia in three family members in a family (Froster et al. 1993).

### X-chromosome

Multipoint QTL analyses of X-linked markers also suggested a locus on Xq26 in the UK sample, which is notable given the possibility that there may be a higher incidence of dyslexia in males than in females (Fisher et al. 2002).

## **2.3 Previous candidate gene studies**

At present, no genes influencing dyslexia have been cloned and no obvious candidate genes have emerged. A report excluding possible candidate genes for dyslexia has been written by Franck's et al. (2002), who characterized the exon/intron borders of two positional candidate genes, *SEMA4F* and *OTX1*, within the region on 2p12-p16, reported previously by Fagerheim et al. (1999), and screened the exons for polymorphisms. *SEMA4F* encodes a protein involved in axonal growth cone guidance, and *OTX1* encodes a homeodomain transcription factor involved in forebrain development. However, they were not able to find significant association, suggesting that linkage with reading disability at 2p12-p16 is not caused by coding variants of *SEMA4F* or *OTX1*.

## **3. Mapping genes in complex diseases**

### **3.1 General**

A complex genetic disease refers to a trait phenotype with an unknown mode of inheritance but having moderate to high evidence of genetic involvement and exhibiting familial aggregation of cases. Typically, this term denotes common disorders that are considered to be either polygenic or multifactorial (multiple genes interacting with the environment) in nature, as for example asthma, diabetes and psychiatric disorders (Haines and Pericak-Vance 1998). Genes involved in

a multifactorial or polygenic disorder are referred to as susceptibility genes in order to distinguish them from highly penetrant causative genes. However, even simple Mendelian disease may result from mutations in different genes. On the other hand, in a complex trait, a subset of families could be due to a single Mendelian locus. Often the subset attributable to a monogenic effect in common diseases is small, for example in Alzheimer disease the Mendelian forms due to mutations in amyloid precursor protein and the presenilin 1 and 2 genes represent less than 5% of all cases (Pericak-Vance 1998).

The lack of a perfect correspondence between genotype and phenotype in complex traits complicates genetic mapping (Kruglyak and Lander 1995, Ott and Bhat 1999). Individuals carrying a susceptibility allele may have a higher risk of disease, but some carriers may be unaffected (incomplete penetrance) whereas non-carriers may get the disease as a result of environmental or random causes (phenocopies). Moreover, mutations in any one of several genes may result in identical phenotypes (Lander and Schork 1994). Careful selection of study subjects, restricting the phenotype as similar as possible between cases, collecting those individuals that possess a strong genetic determinant to their phenotype and representing the most severe forms of a trait relieves the task of mapping complex traits (Lander and Schork 1994).

### **3.2 Positional cloning**

Positional cloning is an approach used to identify a gene based on its chromosomal location and is to be chosen when the basic biochemical defect of a disease is unknown (Collins 1992, 1995). The process of positional cloning begins with phenotype definition, identification of families and collecting blood samples for genotyping purposes. The next steps include genotyping markers, performing linkage analyses for initial localization, followed by fine genetic mapping, physical mapping, characterization of candidate genes and, finally, identification of mutations (Haines and Pericak-Vance 1998, Collins 1992, 1995).

#### Ascertainment of Families

Family ascertainment is perhaps one of the most time-consuming and laborious step in the process of positional cloning. The types of families to be collected (e.g. sib pairs, cousin pairs, extended families) have to be evaluated on the basis of the knowledge of phenotype and possible genetic model (Haines and Pericak-Vance 1998). Large extended families are chosen when a single major gene defect is suspected, but for a more complex genetic model, small

families, such as sib pairs, may prove to be most efficient. However, the number of sib pairs needed is quite large. Thus, for example localising a gene to 1 cM requires a median of 200 sib pairs for a locus causing a fivefold increased risk to an offspring and 700 sib pairs for a locus causing a twofold increased risk (Kruglyak and Lander 1995).

### Genetic mapping

Genetic mapping aims at finding those chromosomal regions that tend to be shared among affected relatives and differ between affected and unaffected individuals. A genome-wide scan is used when there is no a priori information of the disease pathogenesis - as is often the case in complex diseases - or when a study focuses on finding novel loci contributing to a disease. The entire genome is genotyped with a dense collection of polymorphic markers spaced evenly on the chromosomes and an appropriate linkage statistics is calculated at each position along the genome (Lander and Kruglyak 1995). The next step is to fine-map the regions showing the strongest evidence of linkage in order to narrow down the linked chromosomal region.

Instead of identifying large multigenerational families one can focus on the ascertainment of a large number of sib-pairs using a nonparametric method within small nuclear families (Fisher et al. 1999b, Ziegler 1999). In contrast to linkage studies which look for co-inheritance of chromosomal regions within the families, association studies look for differences in the frequency of genetic variants between controls and unrelated affected individuals (Kruglyak 1999).

### Linkage analysis

Linkage analysis aims at localizing a disease gene with respect to its chromosomal region. Two genetic loci are considered to be linked, when they are inherited together more often than expected by chance. The closer the two loci are to each other, the more likely it is that they are not separated by a crossing-over event or recombination during meiosis. The genetic unit for measuring distances between loci is centimorgan (cM), which corresponds to a genetic length in which recombination occurs in 1% of meioses. In terms of physical distance, 1 cM corresponds to 1 Mb of DNA on average (Ott 1991, Terwilliger and Ott 1994). The human genetic map spans on the average 3690 cM, being 2644 cM in the male and 4481 cM in the female. Thus, the map lengths differ considerably between sexes indicating that recombination events are much more common in female meioses (Gyapay et al. 1994).

The standard lod score analysis is called parametric linkage analysis since it requires a precise mode of inheritance, gene frequencies, and penetrance for each genotype. Parametric linkage analysis is suitable for localising disease genes for Mendelian disorders. In two-point linkage analysis phenotype data and allele data are used at one marker locus for calculating lod scores, whereas in multipoint linkage analysis allele data from several loci are simultaneously considered improving the efficiency of analysis. The likelihood that two loci, e.g. a marker and a disease locus, are linked at a certain recombination fraction ( $\theta$ ) is compared to the likelihood that they are not linked ( $\theta=0.50$ ). The ratio of these likelihoods, an odds ratio, is transformed to a  $\log_{10}$  ratio and presented as a logarithm of odds, lod score (Ott 1991, Terwilliger and Ott 1994). By agreement, a lod score  $>3$  (odds ratio 1:1000) indicates significant evidence for linkage, whereas a lod score  $<-2$  (odds ratio 1:100) is considered as proof against linkage. The most likely distance between the loci tested is the recombination fraction at which the lod score is highest (Ott 1991). Lod scores can be summed from multiple families or in multipoint analysis from several marker loci to give the final lod score. The statistical significance of the cosegregation of two loci is evaluated by means of specific linkage computer software designed for the purpose, the most commonly used being the LINKAGE package (Lathrop et al. 1984).

For complex diseases, the use of parametric Lod score analysis is more complicated because the precise inheritance pattern is often unknown. Non-parametric linkage (NPL) analysis is a model-free method which does not require knowledge about the inheritance pattern of the disease and is predominantly used for searching genes predisposing to multifactorial diseases. Non-parametric methods ignore unaffected people and look for alleles that are shared by those affected. In the case of extended pedigrees, IBD (identical by descent) sharing can be assessed among affected relatives using the program package of GENEHUNTER (Kruglyak et al. 1996).

### Chromosomal translocations

When identifying a gene for a hereditary disorder, different types of chromosomal aberrations are useful. The chromosomal breakpoints are characterized to pinpoint the critical gene region, which has been interrupted by the translocation. A number of genes have been mapped by finding balanced translocations, for example neurofibromatosis type I in 17q11.2 (OMIM 162200), Duchenne muscular dystrophy in Xp21 (OMIM 310200) (Aylsworth 1998), and more recently, *FOXP2* (OMIM 605317) gene in developmental speech and language disorder in chromosome 7q31 (Lai et al. 2000, 2001, Fisher 1998).

### Mutation identification

After a candidate gene or genes have been found for example by positional cloning strategy, they need to be analyzed for the presence of putative disease causing mutations. A candidate gene or a part of it is tested for sequence variations in a set of patients and controls. Commonly used mutation screening methods are direct sequencing, Single-strand conformational polymorphism analysis (SSCP) (Orita et al. 1989), Heteroduplex analysis (Gray et al. 2000), Chemical (CCM) (Cotton et al. 1988) and enzymatic (ECM) (Youil et al. 1995) cleavage of mismatches and Denaturing gradient gel electrophoresis (DGGE) (Fisher and Lerman 1979). Often direct sequencing is used as a secondary method to confirm and characterize the mutations detected by one of the methods mentioned above. Mutation screening usually starts from the coding region of the gene, although some mutations affecting the function of the gene are located outside the coding region. After a sequence change in the coding region of a gene has been found, its possible pathogenicity is to be verified by screening a large number of normal control chromosomes for the presence of the change and other patients with the same disease. (Haila 2001).

## AIMS OF THE STUDY

This study was carried out to elucidate genetic loci predisposing to dyslexia in Finnish families. The study was stimulated by the emergence of a family where chromosomal translocation cosegregated with dyslexia in 1995. The specific aims of the study were as follows:

- To collect family material where several family members had dyslexia to search for extended pedigrees suitable for linkage studies.
- To search for new genetic loci predisposing to dyslexia by performing genome-wide scan studies in multiplex families.
- To characterize the neuropsychological profiles of the dyslexic individuals in the pedigrees and to compare the type and severity of dyslexia within the families.
- To further refine the position of the known dyslexia locus (*DYX1*) on chromosome 15 by examining translocations involving 15q21.

# SUBJECTS AND METHODS

## 1. Family material

The majority of families were ascertained through probands from the Hospital for Children and Adolescents, Department of Pediatric Neurology, University of Helsinki (I, II, IV).

In the first phase of the study, all medical records of patients examined at the Department of Pediatric Neurology during the 1990-1995 and diagnosed as having dyslexia +/- dysgraphia, a specific language deficit, or other learning disorder were collected and evaluated.

The inclusion criteria for the probands included normal performance intelligence quotient (PIQ>85) and remarkable deviation in reading skills compared to chronological age. Children with low general intelligence, developmental language disorder (dysfacia), a major neurological handicap such as cerebral palsy, minimal brain dysfunction (MDB), fetal alcohol syndrome (FAS), psychiatric disorder or children with severe psychosocial problems in the family were excluded.

Altogether 147 patients born in 1976-1987 were found (116 boys, 31 girls), aged 8-14. These patients had previously been examined by a child neurologist and tested by a child neuropsychologist for intelligence using WISC-R (Wechsler 1984) and for neuropsychological functions using a Finnish version of the NEPSY (Korkman 1988; corresponding version in English: Korkman et al. 1998). After careful evaluation of their medical records and family histories, 128 patients (101 boys, 27 girls) from 108 families were selected for further analysis. 19 patients (15 boys and 4 girls) were excluded from the study for the following reasons: low general achievement (n=6), MBD (n=2), history of a psychiatric disorder (n=3), dysphasia (n=1), FAS (n=2), or severe psychosocial problems in the family (n=4). In addition, one individual was excluded due to sex-chromosomal aberration; karyotype XYY.

126 out of 128 subjects included for further evaluation had dyslexia due to a deficit in one or more of the following functions: phonological awareness, verbal short-term memory and rapid naming. Two additional male subjects (1.56%) had dyslexia due to a visuospatial deficit. One of them also had long QT-syndrome.

In the second phase, 108 families were sent a detailed questionnaire regarding reading and writing difficulties, school history and attendance to remedial education in all first and second

grade relatives. 68 out of 128, i.e. 53% of the children had either one or two dyslexic parents (48% one and 5% two) according to the history.

11 most informative families (at least four affected individuals, apparent dominant inheritance and willingness to participate in further studies) were selected for genetic analysis and psychological assessment (I, II). In addition, all these families were personally interviewed by the author.

Also, two dyslexia families with known translocations on chromosome 15 (III, IV) were selected for FISH-studies from the departments of pediatrics of Jorvi Hospital and Seinäjoki District Hospital.

23 additional patients were collected from HERO (Association of Learning Disabled Individuals of Helsinki) (IV).

The control group for adult neuropsychological assessment consisted of 15 non-dyslexic Finnish individuals (12 males and 3 females) matched for age, educational and economical background.

The study design was approved by the ethical committee of the Children's Castle Hospital, University of Helsinki, Finland.

## **2. Psychological assessment**

All individuals included in the linkage study (I, II) who had not previously been tested at the Children's Castle Hospital were assessed by the same neuropsychologist. The presence and degree of dyslexia was evaluated by Finnish reading and spelling tests; children under 13 years of age were assessed by age- and grade normed Finnish reading and spelling tests (Häyrinen et al. 1999) and subjects aged 13 or older were assessed by the procedure and criteria based on the Jyväskylä longitudinal study of dyslexia (Lyytinen et al. 1995, Leinonen et al. 2001).

The adult (aged 13 or over) reading assessment included:

1. oral text reading (two passages of 218 and 127 Finnish words, accuracy and speed were recorded)
2. pseudo- and nonword reading (30 pseudowords and 30 nonwords with 4-8 letters and 2-4 syllables, the number of reading errors was counted)

3. spelling to dictation (10 meaningless words and 10 actual words, some of them of foreign origin and consisting of 6-14 letters and 2-7 syllables; the number of writing errors was counted and the type of error classified).

Pseudo-words are meaningless letter strings and non-words are experimentally designed letter strings that are still orthographically acceptable. Reading samples were recorded on digital audio tape and analysed for speed and accuracy.

Reading comprehension was evaluated by a Finnish text intended for pupils who have passed the sixth grade and consisted of 252 words, from which 20 irrelevant words should be found; correct performances were counted (Häyrynen et al. 1999).

A person's subjective experience regarding dyslexia at present and during the school years according to the questionnaire and personal interview was classified as present or absent.

Intelligence quotient (IQ) was estimated by using Wechsler Intelligence Scale Children-revised (WISC-R) (Wechsler 1984) or Wechsler Adult Intelligence Scale-revised (WAIS-R) (Wechsler 1992). Eight subtests covering verbal and visual skills were used: Information, Digit Span, Vocabulary, Similarities, Picture Completion, Picture Arrangement, Block Design and Coding.

Reading-related neurocognitive skills were evaluated by the following tests: Phonological awareness was tested using Phonological processing subtest of NEPSY (Korkman et al. 1997), rapid automatized naming using six different measures (Denckla and Rudel 1976, Wolf et al. 1986, Ahonen et al. 1999), and verbal short-term memory using digit span, forwards and backwards (WAIS-R or WISC-R), and Lurian word series (Christensen 1982). NEPSY is a neuropsychological investigation for children consisting of 37-40 subtests that represent the developmental areas of attention, language, sensory-motor functions, visual-spatial functions, memory and learning. It was developed to analyse disturbances of complex, cognitive functions, such as speech and language, by assessing their subcomponents. The subtests of NEPSY are sensitive to age and to specific developmental disorders, but not to socio-economic background or different kindergarten experience (Korkman and Häkkinen-Rihu 1994).

Subjects in study III had previously been assessed neuropsychologically at the Children's Castle Hospital and at the pediatric departments of Jorvi Hospital and Seinäjoki District Hospital (WISC-R, NEPSY subtests). Additional subjects in study IV have been assessed by a local psychologist (WISC-R, WAIS-R, NEPSY, Denckla).

### 3. Statistics

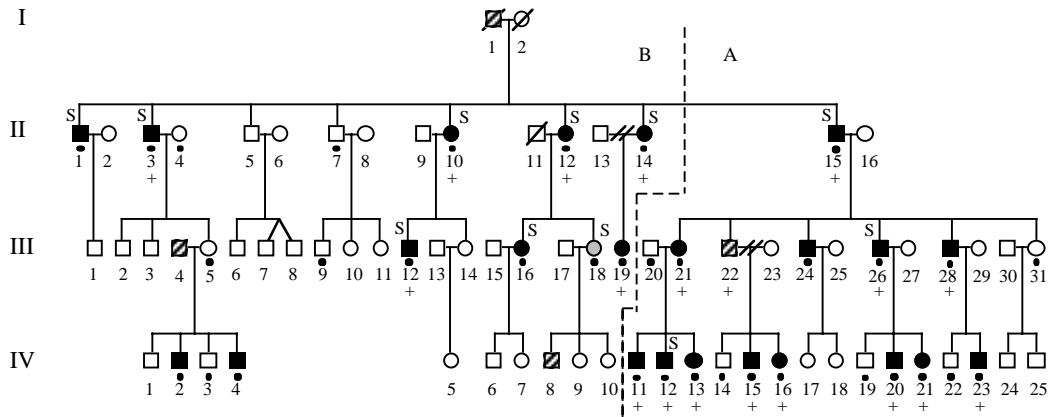
The test scores illustrating neurocognitive skills of affected persons aged 13 or older (n=18) were converted to standardized scores, i.e. z-scores (SD), by using the means and standard deviations of the control group (n=15). The means of z-scores were counted in groups according to the severity of dyslexia. To analyze group differences for rapid naming, phonological awareness, verbal short-term memory and reading comprehension, one-way ANOVA's were performed. The groups with mild and compensated dyslexia were combined for statistical analysis. Polynomial contrasts and Tukey's post hoc t-tests were applied for further analysis.

### 4. Genotyping

In the first phase of the study, the two most informative families AB (Figure 1.) and C (Figure 2.), which were estimated to have enough power to show linkage by the simulation programme SLINK (Weeks et al. 1980, Ott 1989), were chosen for the linkage study (Study I). A set of 320 highly informative microsatellite markers (derived from Weber set 6, <http://www.chlc.org>) was used for genotyping, the average intermarker distance being 11.8 cM. For fine-mapping purposes, additional markers on chromosome 3 were genotyped manually using silver staining method.

In the second phase of the study, a genome wide scan with 371 markers with an approximately 10 cM intermarker distance was performed on 9 additional families (3005-3013) at the Finnish Genome Center. All microsatellite markers and the genetic distances used in the study are shown at <http://www.genome.helsinki.fi>.

Genomic DNA was extracted from blood leucocytes using a standard non-enzymatic method. PCR was carried out in 15 µl reactions containing 50 ng of genomic DNA and fluorescently labelled primers. The amplified PCR products were separated using 4.25% polyacrylamide gels run on a ABI 377 sequencer (PE Biosystems, Foster City, CA). Lane tracking and allele calling were carried out using Genescan and Genotyper software (PE Biosystems). All gels were double-scored by two investigators and any discrepancies between the two scorings were re-evaluated and resolved.

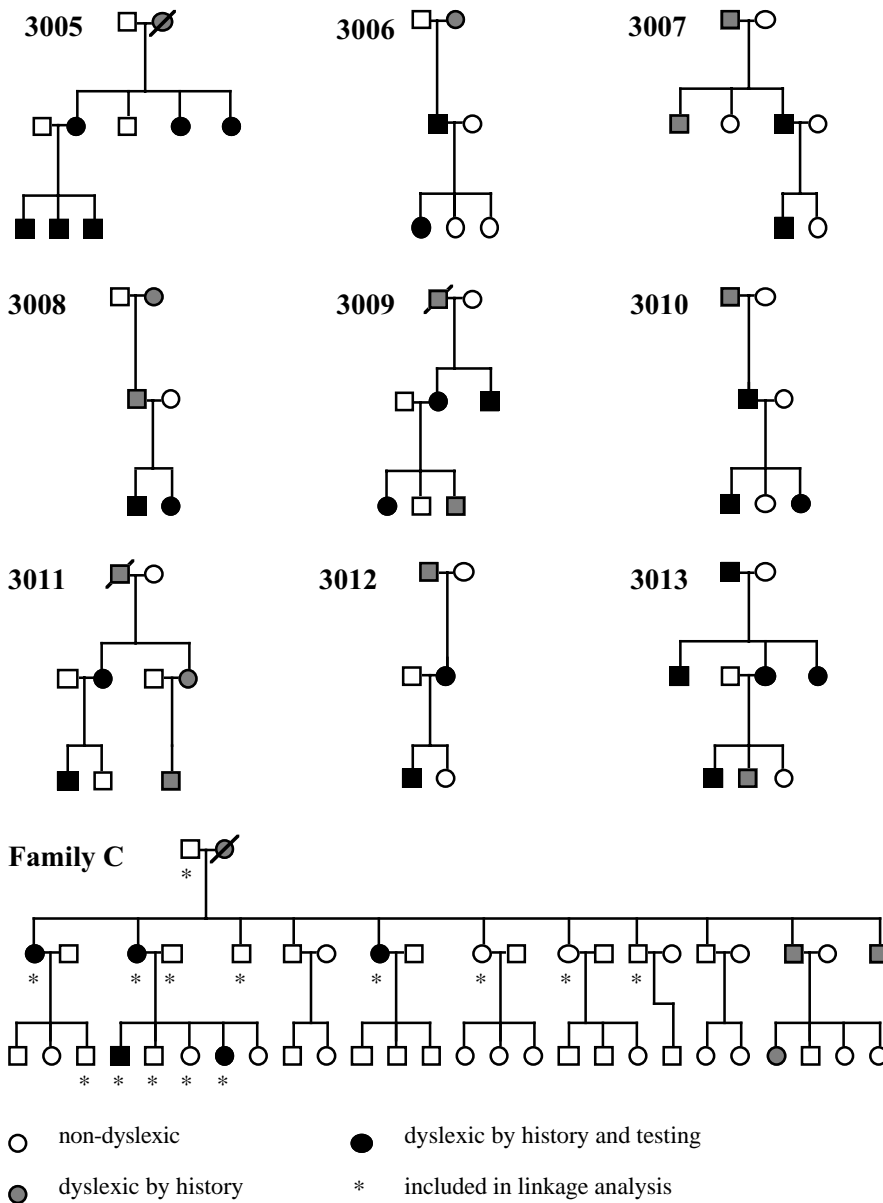


- Normal
- Dyslexic by history and testing
- ▨ Dyslexic by history
- Dyslexic by testing

**Figure 1.** Pedigree of the extended family AB linked to chromosome 3. Squares denote males and circles females. Roman numerals indicate the generation and Arabic numerals the subject within a generation. S indicates severe dyslexia. Individuals marked with a dot were tested neuropsychologically. + denotes the affected haplotype.

### 5. Linkage analysis

As the exact mode of inheritance of dyslexia is unknown, the genome scan data was first analyzed by a non-parametric multipoint linkage analysis using “Genehunter” (Kruglyak et al. 1996). This software performs the reconstruction of haplotypes and complete multipoint analysis of identical-by-descent (IBD) allele sharing among all affected family members at each location in the genome. In addition to nonparametric analysis, a parametric linkage analysis was performed; two-point linkage analysis was performed using MLINK and five-point linkage analysis using LINKMAP (Lathrop et al. 1984). A genetic model with the disease allele frequency of 0.0001, autosomal dominant inheritance, and equal female and male recombination rates was used in Study I. The penetrances for homozygous normal, heterozygous, and homozygous affected were set at 0.06, 0.80, and 1.00, respectively.



**Figure 2.** Families 3005-3013 and family C.

## 6. Candidate gene studies on chromosome 3

Two genes were chosen for candidate gene studies based on their location at chromosome 3. The 5-hydroxytryptamine receptor 1F gene (5HT1F) is located within the linked region

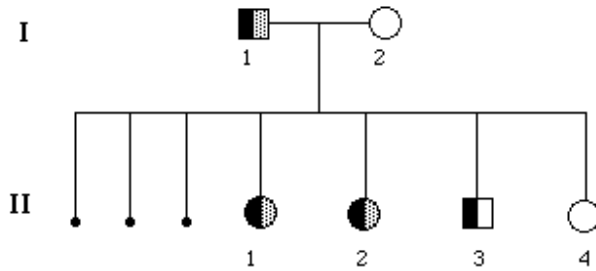
(DeLoukas et al. 1998). The entire coding region of 5HT1F was sequenced from two individuals from family AB (Figure 1.) sharing the common haplotype (II-12 and III-12, Fig1) and two individuals not sharing it (II-2 and III-1, Fig1) using direct sequencing of PCR products with an ABI377 automated sequencer (PE Biosystems, Foster City, CA, USA).

The physical location of another nearby candidate gene, the dopamine D3 receptor gene (DRD3), was determined by radiation hybrid mapping using the Genebridge 4 panel (Research Genetics, Huntsville, AL, USA). A fragment of DRD3 was amplified by PCR (Griffon et al. 1996) and its location was compared to the markers D3S2406, D3S2459, D3S3045 and D3S2465. The data were analysed as described at <http://carbon.wi.mit.edu:8000/cgi-bin/contig/rhmapper.pl/>.

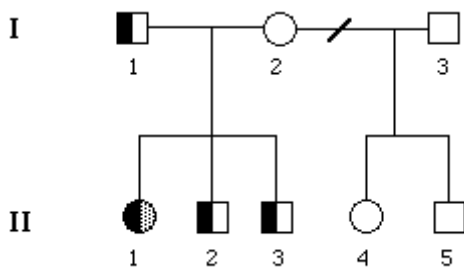
## **7. FISH and Southern hybridization**

Two families with known translocations in chromosome 15 (Figure 3.) segregating with dyslexia were selected for fluorescence in situ hybridization (FISH) studies (Study III). Karyotypes were determined from phytohemagglutinin stimulated blood lymphocytes with G-banding using standard procedures. For fluorescence in situ hybridization studies, metaphase spreads were obtained from EBV-transformed lymphoblastoid cell lines derived from individuals II-1 (family A, Figure 3.) and II-1 (family B, Figure 3.).





In Study IV, RPCI-11 BAC clone 178D12 (Genbank accession number AC013355) was used as a probe in FISH. 15 µg of total genomic DNA from an individual carrying the translocation and from an unrelated control person was digested with BamHI, EcoRI, HindIII, BsaAI, PstI, or SphI, and run in 0.7% agarose gel. DNA was transferred to Hybond N+ membrane (Amersham Pharmacia Biotech) with a standard alkaline blotting method. PCR fragments derived from human genomic DNA were TA-cloned into pCR2.1 TOPO-TA vector (Invitrogen, Carlsbad, CA), and insert was removed with EcoRI digestion and gel-purified (Qiagen, Venlo, The Netherlands).  $\alpha^{32}\text{P}$ -labeled insert was used as a probe in Southern hybridization (Southern 1975). The hybridization was performed overnight at 65°C in Church buffer (0.5 M NaHPO<sub>4</sub>, 1 mM EDTA, 7% SDS, 1% BSA), and the filter was washed in 2 x SSC, 0.05% SDS at 65°C for 1 hour. The filters were autoradiographed with a phosphoimager plate.



**Family A**



**Family B**

-  Translocation
-   Translocation and dyslexia
-  Fetal loss

**Figure 3.** Pedigrees of the families studied. In family A, cosegregation between  $t(2;15)(q11;q21)$  and reading disability is observed. In family B, the father and all three children carry  $t(2;15)(p13;q22)$ , but only II.1 has dyslexia.

## 8. Cloning of *DYXC1* and sequence analysis

Novel genes in the sequence of clone 178D12 were predicted *in silico* with Genscan and Fgenes software. Predicted genes were confirmed by sequencing RT-PCR products. *DYXC1* cDNA has been deposited in GenBank with accession number AF337549. Mouse *mDYXC1* was constructed from two overlapping EST sequences (accession numbers BG242087 and AK005832) and verified by comparing it to all available mouse *mDYXC1* EST sequences. cDNA sequences of *mDYXC1* and *hDYXC1* were aligned with ClustalX. The alignment was

improved manually and shaded with BOXSHADE. The secondary structure of the TA rich region was predicted with MFOLD with default parameters. (available at <http://bioinfo.math.rpi.edu/~mfold/dna/form1.cgi>) The expression of *DYXCI* was analyzed by RT-PCR from Clontech's multiple tissue cDNA panels 1 and 2. RT-PCR was performed in 25 µl volume in the following conditions: 94°C 2' (94°C 1', 68°C 2') x 30, 1 x DyNAzyme buffer with MgCl<sub>2</sub> (Finnzymes, Espoo, Finland), 0.2 u DyNAzyme II polymerase (Finnzymes), 15 pmol forward primer GTTGACAGAATGCTGTTCCACGTCG, 15 pmol reverse primer CAAGCTGAGGCACGAAGAGCAATGA. Promoter region of *DYXCI* was predicted with TSSG and TSSW software at Baylor College of Medicine, available at <http://searchlauncher.bcm.tmc.edu/seq-search/gene-search.html>, and neural network promoter prediction (NNPP) software at University of California, Berkeley, available at [http://www.fruitfly.org/seq\\_tools/promoter.html](http://www.fruitfly.org/seq_tools/promoter.html).

## **9. SSCP analysis**

*DYXCI* exons were amplified with PCR and digested with suitable enzymes to obtain 100-300 bp fragments. Denaturing gel was run for 16 hours at room temperature with 5 W constant power. Gels were stained with silver according to standard protocols.

More detailed information about the methods are given in the original articles (I-IV).

## RESULTS AND DISCUSSION

### 1. Mapping a novel dyslexia locus (*DYX5*, OMIM 606896) on chromosome 3 (Study I)

The linkage analysis was started by the exclusion of previously known loci in chromosomes 1, 6 and 15 in two large, most informative families A and C (Table 2., Figure 1. and Figure 2.). After this a genome wide scan was performed in the two families with 320 polymorphic markers spanning the whole genome to assess excess allele sharing among affected pedigree members. As the exact mode of inheritance of dyslexia is unknown, the genome scan data was first analyzed by non-parametric multipoint linkage analysis using Genehunter (Kruglyak et al. 1996). Because the families did not share a common origin and might segregate different loci, they were analyzed separately for linkage. The results for family C did not reach statistical significance for linkage, but family A showed linkage to chromosome 3 with a locus near the centromere (3p12-q13) co-segregating with dyslexia (non-parametric  $Z=5.8$ ,  $p=0.0017$ ). At this point additional family members (family B, Figure 1.) consented to the study. Seven microsatellite markers included in the genome scan and spanning a region of 60 cM of the linked region on chromosome 3 were genotyped in family B. In addition, for fine mapping purposes, a set of 11 microsatellite markers on chromosome 3 were genotyped in the whole pedigree AB, resulting in an average intermarker distance of about 2 cM in the center of the linked haplotype. The total number of individuals in pedigree AB was 74 (43 males and 31 females), aged 6-66 years. 35 individuals were included in genotyping, of whom 21 were affected and 14 non-affected.

A nonparametric linkage analysis of families A and B yielded a combined p-value of  $6 \times 10^{-5}$ . A multipoint parametric linkage analysis using a dominant model was performed with the most informative markers D3S2454, D3S3039, D3S1595, and D3S3655 and resulted in a maximum LOD score of 3.84.

The haplotype analysis showed that 19 out of 21 dyslexic individuals of family AB shared identical copies of chromosome 3 in this region. Recombinations observed in individual III-19 limited the haplotype to a maximum region of 20 cM between markers D3S3039 and D3S3045. A representation of the haplotype is shown in Figure 2. in Study I. Two dyslexic individuals, II-1 and III-16 (Figure 1.), did not share any part of the haplotype and were considered as phenocopies or dyslexic due to another gene.

62 individuals from nine additional families (3005-3013) (Figure 3.) were genotyped and analysed by Genehunter with 14 fluorescent microsatellite markers in chromosome 3 flanking the region between markers D3S1311 and D3S1050. The linkage to chromosome 3 was excluded by lod scores between  $-1.9$  and  $-12.0$ . In addition, triplets (two parents and one child, with at least one affected) were genotyped by silver staining methods for markers D3S3049, D3S2446 and D3S1538, and 1.6 cM haplotypes were constructed from altogether 15 families (including the nine families 3005-3013). No haplotype association was shared with the AB family (unpublished data).

Later a whole genome scan was performed with the same families 3005-3013 (62 individuals; 34 affected and 28 non-affected) and suggestive linkages were found at two other locations in the genome (unpublished data).

This locus co-segregating with dyslexia is the fifth dyslexia locus (*DYX5*, OMIM 606896). So far this linkage to chromosome 3 has been found in only one Finnish family analyzed, and these results further confirm the earlier observations of the heterogeneity of dyslexia. After our study, Fisher et al. (2002) obtained results that can be viewed as a replication of linkage to chromosome 3.

**Table 2. Exclusion of known loci**

chromosome	markers	lod score	p-value
1p34-p36	D1S552	-0.2- - 2.5	0.22-0.84
6p21.31-p21.1	D6S1280, D6S1017, D6S1019, D6S1281	-0.38- - 0.68	-0.46- -0.82
15q21.1	D15S659	-0.89-2.1	0.20-0.24

## 2. Search for possible candidate genes on chromosome 3 (Study I)

The centromeric region of chromosome 3 contains an interesting candidate gene, the 5-hydroxytryptamine receptor 1F (5HT1F) which is expressed at low levels in the brain, with greatest expression in the cortex, hippocampus and striatum (Lovenberg et al. 1993). Extensive evidence suggests that 5-hydroxytryptamine receptors have a role in learning and memory (Meneses 1998). We sequenced the entire coding part of 5HT1F, but found no sequence variation in two dyslexic individuals sharing the susceptibility haplotype.

Another promising candidate gene near the linked region is the dopamine D3 receptor gene (DRD3) (Le Coniat et al. 1991). This gene has previously been suggested to have a role in schizophrenia (Griffon et al. 1996). However, radiation hybrid mapping placed DRD3 telomeric of marker D3S3045, outside the linked region, thus formally excluding it as a candidate gene.

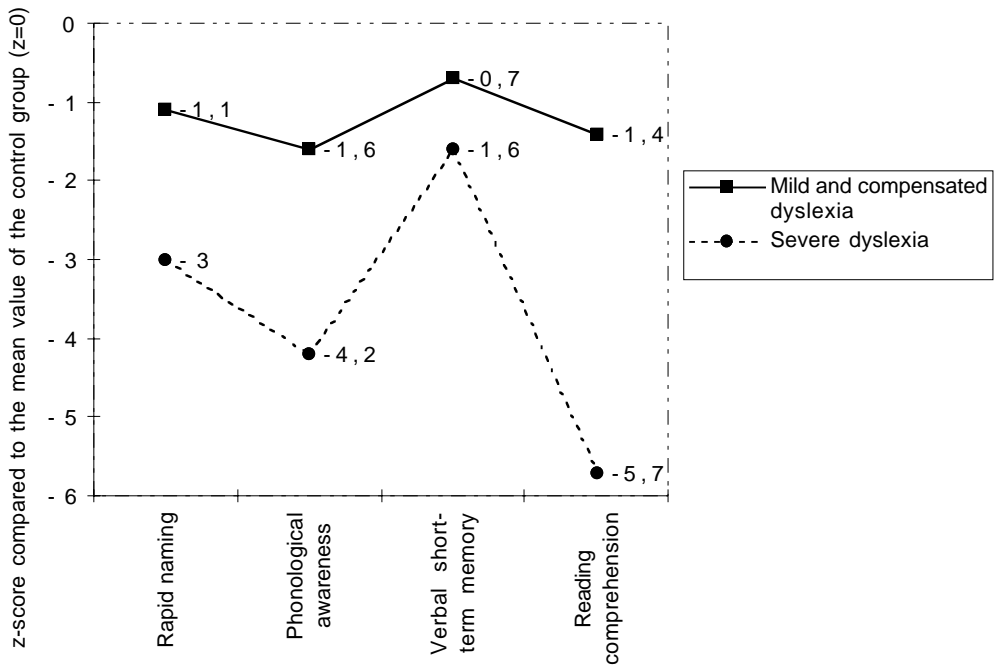
### **3. Clinical findings of *DYX5* (chromosome 3 linked dyslexia) (Study II)**

Study II was undertaken to compare individuals with dyslexia in the same families in terms of the type and severity of dyslexia and to characterize the neuropsychological features typical for chromosome 3 linked dyslexia. Dyslexic individuals were divided into three groups according to the severity of dyslexia and the groups were compared in the sense of different neuropsychological functions and reading comprehension.

The most extensively evaluated family was family AB, in which altogether 34 individuals were neuropsychologically assessed. This is so far the only family in our material where linkage to chromosome 3 has been confirmed. Based on the test results, altogether 24 out of the 74 members in family AB (Figure 1.) were diagnosed as dyslexic by testing: 18 individuals (eight females, 10 males) over 13 years of age and six children (4 males, 2 females) aged 8 to 12. In addition, three males, III-4, III-22 and IV-8, were classified as affected on the basis of a history of dyslexia; they, however, were not available for testing and were excluded from further analyses. Individual I/I was deceased but had a history of reading and writing impairment. Individual III-18 had a discrepancy in the phenotype; in the tests she performed dyslexic, but she had never subjectively experienced any reading or writing problems. Thus, the number of dyslexic individuals in this extended pedigree is 28.

On the basis of the reading and writing tests, the subjects were divided into four groups: those without dyslexia, and those with *compensated dyslexia*, *mild dyslexia*, or *severe dyslexia*. The groups with mild and compensated dyslexia were combined for statistical analyses. The classification was based on the standardized scores, i.e. z-scores, when compared to the normative group as follows: in compensated dyslexia the z-score of the subject was -1.0 or lower regarding either the reading speed or any other selection criterion. In mild dyslexia the z-score of the subject was -1.0 or lower regarding the reading speed and in addition -0.7 - -0.9 regarding any other selection criterion. In severe dyslexia the z-scores of the subject were -1.0 or lower regarding both the reading speed and any other selection criterion. In addition, a positive history of reading problems was required for all dyslexia groups.

To further refine the cognitive phenotype of each participant, reading-related neurocognitive skills (phonological awareness, rapid naming and verbal short-term memory) were assessed. Only individuals over 13 years of age (N=18) were included for further analyses in order to be able to use uniform criteria in neuropsychological tests. Out of them, 11 persons had severe, 2 mild and 5 compensated dyslexia.



**Figure 4.** Group comparison of the z-score of neuropsychological background features of dyslexia and reading comprehension.

Figure 4. shows a comparison of the neuropsychological functions and reading comprehension between the different dyslexia groups. In the task of rapid naming slowness seemed to be connected with the grade of dyslexia: at group level the performance was the slower the more severe dyslexia was. In the word segmentation task which measured phonological awareness the difficulties were also greater with more severe dyslexia. The difference between severe dyslexics and mild or compensated dyslexics was quite notable (z-score -4.2 vs. -1.6). None of the severe dyslexics and only two of the group of mild or compensated dyslexics were able to carry out the word segmentation task within the normal range. This corresponds to the earlier findings of other research groups that phonological awareness deficit seems to be very

persistent (Pennington 1987, Kitz and Tarver 1989). Kinsbourne et al. (1991) compared neuropsychological deficits in adult dyslexics and found, that the RAN (rapid automatized naming) test was the most discriminating between dyslexic, compensated dyslexic and controls. In the present study naming difficulty was connected with the grade of dyslexia. Also reading comprehension difficulties were common in our material: 61% of all dyslexics and 82% of severe dyslexics had difficulties in reading comprehension.

The dyslexia phenotype segregating in this family linked to chromosome 3 was based on a phonological impairment and consisted of deficits in all the measured reading-related neurocognitive skills: phonological awareness, rapid naming and verbal short-term memory. The individual results of neurocognitive tests of affected family members over 13 years of age are shown in Table 3.

**Table 3.** Neuropsychological test results of individuals 13 years or older using the z-score comparison to the mean values of the control group. Person numbers correlate to the pedigree in Figure 1.

<b>Severe dyslexia</b>						
<b>Person number</b>	<b>Sex</b>	<b>Age</b>	<b>Rapid naming</b>	<b>Phonological awareness</b>	<b>Short-term memory</b>	<b>Reading comprehension</b>
II-1	M	66	**	***	*	**
II-3	M	64	***	*	*	**
II-10	F	48	***	***	**	***
II-12	F	61	**	**	*	**
II-14	F	55	**	***	***	***
II-15	M	66	**	***	*	***
III-12	M	15	***	***	*	***
III-16	F	38	0	**	*	***
III-18	F	27	*	*	**	0
III-26	M	38	**	***	*	0
IV-12	M	16	***	***	**	***
<b>Percentages in the group</b>						
$z < -3,0$	***		36,4%	63,6%	9,1%	54,5%
$-3,0 \leq z < -2,0$	**		45,5%	18,2%	27,3%	27,3%
$-2,0 \leq z < -1,0$	*		9,1%	18,2%	63,6%	0,0%
$z \geq -1,0$	0		9,1%	0,0%	0,0%	18,2%

**Mild or compensated dyslexia**

Person number	Sex	Age	Rapid naming	Phonological awareness	Short-term memory	Reading comprehension
III-19	F	24	0	*	*	0
III-21	F	41	*	**	*	0
III-24	M	43	**	**	*	0
III-28	M	36	0	0	*	0
IV-11	M	15	**	0	0	***
IV-15	M	18	*	**	0	0
IV-16	F	13	***	*	**	***

**Percentages in the group**

$z < -3,0$	***	14,3%	0,0%	0,0%	28,6%
$-3,0 \leq z < -2,0$	**	28,6%	42,9%	14,3%	0,0%
$-2,0 \leq z < -1,0$	*	28,6%	28,6%	57,1%	0,0%
$z \geq -1,0$	0	28,6%	28,6%	28,6%	71,4%

\*\*\*  $z < -3,0$  , \*\*  $-3,0 \leq z < -2,0$  , \*  $-2,0 \leq z < -1,0$  , 0  $z \geq -1,0$

As shown in Figure 1., all affected individuals, except II-1 and III- 16, have inherited the 16 marker long haplotype or a part of it on the pericentromeric region of chromosome 3. None of the unaffected individuals has this haplotype. Individual II-1 is a 66-year old male, classified as severely dyslexic having deficits in the tasks of phonological awareness, rapid naming, verbal short-term memory and reading comprehension. Individual III-16 is a severely dyslexic woman of age 38, who performed well in the tasks of rapid naming but had difficulties in all other reading-related tasks. She is the only one from the group of severely dyslexic persons who did not have naming difficulties and differs in this sense from the other members of the group.

The severity of dyslexia seems to be connected with age in this pedigree; all dyslexics, (6/6), from the oldest (II) generation, 4/8 from the III generation and only 1/10 from the IV generation were classified as severely dyslexic. This is probably due to better education and possibilities for remedial education and rehabilitation as well as more extensive exposure to written language in the younger generations. None of the dyslexics in generations II and III have obtained special education at their school time, but all dyslexics in generation IV have had regular remedial education (part-time special education at school).

Children under 13 years of age were not included in further analyses since there are no uniform diagnostic tests for children and adults. However, they were carefully tested for IQ, reading and

spelling skills and for reading-related neurocognitive skills prior to the linkage analysis. Only one dyslexic child (IV-4) had a history of speech delay and when tested at the age of six, a developmental language disorder was diagnosed. He had also graphomotor difficulty. He was not included in the linkage study due to bilinear heritability (father had a history of developmental language disorder and mother is an unaffected member of family AB). Two dyslexic individuals (III-12 and III-18) reported stuttering during childhood, two (III-18 and IV-12) had misarticulation and two (II-15 and III-16) had suffered from word finding difficulties in speech. Dyslexic individual IV-15 has had attention problems, impulsivity and emotional difficulties. Non-dyslexic individual IV-25 has had attention problems at school. Motor development of subjects in the IV generation has been normal by history. Individuals IV-11, IV-12 and IV-13 have been examined by a child neurologist and neurological examination has not revealed any abnormalities. According to the data available, this family does not show evidence of comorbidity, such as attentional or motor problems; on the other hand, no medical records exist for the older generation comparable with, for example, the present 5-year examination of health care centers.

To summarize, chromosome 3- linked dyslexia is characterized by a phonological deficit with difficulties in phonological awareness, rapid naming and verbal short-term memory. Phenotypically, this corresponds to the most common type of dyslexia among adult Finnish speakers.

#### **4. Further evidence for a dyslexia locus at chromosome 15q (Study III)**

Two Finnish families ( A and B, Figure 3.) with apparently balanced translocation involving the 15q21-22 region were identified. In family A, a translocation 46,XY,t(2;15)(q11;q21) segregates with specific dyslexia based on phonological difficulties in three family members. In addition, one child with translocation had an overall cognitive achievement (FSIQ) below the normal range differing thus from other children of this family. Specific difficulty was seen in verbal short-term memory, phonological awareness, and rapid naming, however. In family B translocation 46,XY,t(2;15)(p13;q22) segregates with dyslexia in only one family member having dyslexia on the basis of difficulties in phonological awareness. Three other translocation carriers lacked the history of reading or spelling problems; unfortunately they have not been available for testing. Individuals I/1 and II/1 in family B have the autosomal dominant form of cornea plana; an eye disease with microftalmia and cataract (Tahvanainen et al. 1996) which is not known to be associated with learning difficulties. We performed fluorescence in situ

hybridization (FISH) studies with individuals 1-II from family A and 1-II from family B to further refine the position of the putative dyslexia locus. The FISH results suggested that both independent translocation breakpoints on 15q map within an 6-8 Mb of each other residing in the region between markers D15S143 and D15S1029. This region overlaps the region implicated to carry the *DYX1* locus in previous linkage studies, further strengthening the conclusion that at least one locus for developmental dyslexia resides within 15q21 (Figure 3., Study III). It is possible that there is more than one locus for dyslexia at 15q21, suggested by the fact that genetic linkage results are in part inconsistent (Table 1), ( Figure 3., Study III). In both families dyslexia is based on a phonological deficit. However, previous clinical descriptions are all too scanty to make any comparison between phenotypes possible, and to evaluate whether or not 15q linked dyslexia has some specific features remains to be evaluated in further studies.

## **5. A candidate gene for dyslexia on chromosome 15 (*DYXCI*) (Study IV)**

Further FISH-studies restricted the translocation breakpoint within the BAC clone RP-11-178D12 containing two known genes, cell-cycle restoration protein 8 (CPR8) and complementation class B phosphoinositol glycan (PIG-B), in addition to the genes described here. To further localize the breakpoint, we used amplified non-repetitive genomic DNA fragments from BAC clone RPCI-11-178D12 as probes in Southern hybridization. A probe corresponding to nucleotides 102317-102837 of the complete sequence of 178D12 revealed a genomic rearrangement with 6 different restriction enzymes (Figure 1 C, Study IV). Thus, we could pinpoint the breakpoint to a 3229 bp region, limited by PstI and HindIII restriction sites (Figure 1 D, Study IV). This interval includes exons 8 and 9 of a novel gene called *DYXCI*. *DYXCI* consists of 10 exons spanning approximately 78 kb of genomic DNA.

In order to study the possible role of *DYXCI* in other individuals with dyslexia, we screened the *DYXCI* cDNA for polymorphism in 57 dyslexic individuals from 22 unrelated families with single-stranded conformation polymorphism (SSCP) analysis and direct sequencing of altered fragments. As controls, we screened DNA from 91 anonymous blood donors and 15 non-dyslexic subjects from the 22 dyslexia families. Three SNPs were found: two of the SNPs (4C->T, 572G->A) were in the coding region and resulted in amino acid substitutions, whereas the third one (-164C->T) resided in the 5' untranslated region.

The -164T allele was found in 6 dyslexic individuals from three families and in 5 control subjects. In one three-generation family, the T allele segregated with dyslexia (Fig 2. Study IV).

In the other two families, its segregation was not compatible with linkage to dyslexia. 4C->T, a nonconservative substitution of proline-2 to serine-2, was found in two dyslexic individuals (a father and a son), but in no control. Further examination showed, however, that there were other relatives with dyslexia who did not carry that allele. Therefore, the role of this sequence change is unknown. The frequency of the common polymorphism 572G->A did not differ between dyslexic and control individuals.

After this, we sequenced the whole coding region of *DYXCI* from an individual carrying the -164T allele in the family presented in Figure 2. (Study IV). A G to T transversion at position 1249 of the *DYXCI* mRNA was found which results in the substitution of a glutamic acid for an ochre stop codon at amino acid position 417 and the deletion of the C-terminal tetrapeptide Glu-Leu-Lys-Ser. In this family, 1249T transmitted in the same chromosome as -164T, which therefore cosegregate with dyslexia. Screening of controls showed that 1249T is relatively common with a frequency of 0.055 (10/174 chromosomes in blood donor samples, 1/28 chromosomes in control subjects from dyslexia families). However, among 57 dyslexic subjects the frequency of 1249T was elevated to 0.123 (14/114 chromosomes), yielding a relative risk of 2.3 (95% confidence interval 1.1-3.4, p=0.03). All the control subjects were heterozygous for the SNP, whereas there was one dyslexic subject homozygous for the T allele.

The position of the *DYXI* locus is somewhat uncertain (Morris et al. 2000, Grigorenko et al. 1997, Schulte-Körne et al. 1998). The peak of two linkage studies maps about 7 Mb or 2.2 cM proximally from the breakpoint defined in our study. *DYXCI* might correspond to *DYXI*, because linkage mapping is somewhat imprecise. Alternatively, it is possible that there might be more than one locus for dyslexia on chromosome 15. In the family cosegregating 1249T with dyslexia, a recombination event between the marker D15S1028 and *DYXCI* in one individual excluded the peak region of *DYXI* as a candidate locus in this family (Figure 2., Study IV).

The cellular function of *DYXCI* is unknown. The amino acid sequence offers very little information about the function. It is possible that the C-terminal part of the *DYXCI* protein (the last four amino acids) is functionally important. TPR motifs are found in a wide variety of proteins in many different species. They are general protein-protein interaction modules and are thought to be of ancient origin. Computational analysis of the human genome has revealed a total of 72 genes encoding proteins with at least one TPR motif (Venter et al. 2001). Most of the TPR-domain containing proteins are associated with multiprotein complexes (Blatch and Lässle 1999).

## CONCLUSIONS

The aim of this study was to elucidate genetic loci predisposing to dyslexia in Finland by examining families with multiple affected individuals and to characterize the type of dyslexia linked to known chromosomal regions.

Two different chromosomal regions showed to be of interest, namely 3p12-q13 and 15q21.

A new, previously unreported linkage to the pericentromeric area of chromosome 3 (*DYX5*, OMIM 606896) was found in a single large family AB with 28 dyslexic individuals (Study I). The other ten families examined did not show any evidence of linkage in this region. Later a whole genome scan was performed with the same families and suggestive linkages were found at two other locations in the genome, but further studies are needed to confirm these findings.

Recently, evidence supporting a locus for chromosome 3 has been obtained by Fisher et al. (2002) in their study of QTL-based genome-wide scans in two large independent sets of nuclear families from the UK and the US, suggesting that the 3p12-q13 locus may have a more general role in developmental dyslexia.

In our material the underlying neurocognitive deficit leading to chromosome 3 linked dyslexia seemed to be of a language type and associated with impairment of different aspects of phonological processing. The more severe the degree of dyslexia, the more evident were impairments involving all three main functions considered most crucial for reading acquisition: phonological awareness, rapid naming and verbal short-term memory. The performance profiles of children with dyslexia under 13 years of age were qualitatively similar to those of the older dyslexic individuals from the same kindred, but the difficulties were milder on average. They performed better in the phonological tasks, which could be due to the fact that more attention is now paid to phonological awareness in preschool and school education on the whole. In addition, with the younger individuals standardized tests were used, which may have had stricter norms than the tests used with the adult dyslexics.

Our study (IV) is the first one to identify a specific gene, *DYX1*, on chromosome 15q as a possible susceptibility gene for developmental dyslexia and provides a starting point for prospective population studies and further biochemical and functional research. The neuropsychological deficit underlying dyslexia linked to chromosome 15 was phonological in nature.

There was no clear difference between the phenotypes of the families linked to chromosome 3 or chromosome 15 in the sense of the type or severity of dyslexia: this is in line with the fact that in multifactorial traits different genes may cause the same disease phenotype.

Many people still think that the prognosis of genetic disorders is poor, as is often the case when neurodegenerative disorders are in question. However, we have an example here that the consequences of a genetic defect may be reduced or even abolished to some extent when recognized early enough.

Thus, in the case of a child with a family history of dyslexia, early identification of underlying neurocognitive deficits, rehabilitation before school age, and remedial education at school is of major importance. Further, knowing the persistence of the deficits underlying dyslexia and low compensation rate, continuous remedial education is essential and should be available for all dyslexic children and adolescents.

## ACKNOWLEDGEMENTS

This study was carried out at the Department of Pediatric Neurology, Hospital for Children and Adolescents, and the Department of Medical Genetics, University of Helsinki, between 1996-2002. I express my sincere gratitude to former and present heads of the Department of Pediatric Neurology; Professors Matti Iivanainen, Helena Pihko and Lennart von Wendt and of the Department of Medical Genetics; Professors Juha Kere, Pertti Aula, Anna-Elina Lehesjoki and Leena Palotie, for the excellent research facilities.

Since I started this study in 1996 from scratch with material consisting of a single dyslexia family and no knowledge or experience in modern genetics, many people have been needed to collaborate and help me to accomplish this study.

My warmest thanks go to my supervisors Dr. Arja Voutilainen and Professor Juha Kere, for their guidance during these years; Arja for her great knowledge in learning difficulties and Juha for his continuous support and endless optimism and interest towards my work. Without Juha's enthusiasm and encouragement I would probably have given up ages ago.

I am very grateful to Professor Marit Korkman and Docent Jaakko Ignatius, the official reviewers of this study, for their valuable advice and comments. I have always admired Professor Marit Korkman for her huge knowledge in child neuropsychology and valuable work in developing the neuropsychological assessment for children. Docent Jaakko Ignatius is a great personality with vast experience in various fields of medicine, and I greatly enjoyed the scientific conversations with him this summer.

I sincerely thank all my collaborators for their invaluable contribution: Birgitta Myllyluoma for performing the neuropsychological examinations of children and adults; Elisabeth Widén and Ralf Krahe for patiently introducing me to medical genetics and to the laboratory work; Mikko Taipale for his young and brilliant brains; Docent Tuomas Haltia for his deep interest towards dyslexia; Professor Timo Ahonen and Seija Leinonen from the Niilo Mäki Institute for their contribution to the neuropsychological part of the study; Vesa Ollikainen for performing the simulation studies and Nina Kaminen for continuing the project. Riitta Lehtinen and Siv Knaapila are thanked for their skillful assistance in the laboratory, for teaching me laboratory techniques from the very beginning, and for their friendship. I thank all my colleagues in the Department of Medical Genetics for their help and interest, especially Kati Asumalahti,

Hannele Koillinen and Sari Koskenmies, for sharing the room and problems with me. I truly find their friendship as one of the most valuable outcomes of these past years.

I warmly thank Sinikka Lindh for her help with the sample collection, Minna Maunula and Eila Karonen for their kind help in many practical matters.

I owe my warm thanks to the Department of Pediatrics in Jorvi Hospital, where I have had the privilege to work with skilled and sympathetic colleagues since 1998. Dr Marketta Mutttilainen, Dr Pekka Ojajärvi and Docent Jukka Rajantie are warmly thanked for their interest, patience and practical help in finishing this project. I thank all pediatricians for sharing everyday (and night) work, and for many nice off-work activities such as singing and ornithology sessions. I thank Dr. Kaija-Leena Komsa for the yearly picnics under blooming apple-trees. I also want to thank all the personnel in my own department, L4, for creating a warm atmosphere. I also express my thanks to all the personnel in the Children's Castle Hospital, where I enjoyed working for many years. My warm thanks go to my many friends among child neurologist, pediatricians and neurologists. I thank Dr. Tarja Linnankivi for her friendship, good sense of humour and for being my stand-in when needed.

I wish to thank all my friends for support; Carmela Kantor-Aaltonen and Heikki Aaltonen for their friendship since school times; my friends from the medical school; "ryhmä rämä", especially Helena Lanki and Marja-Leena Hauhia for being such good friends during good and bad times.

I owe my warm thanks to my godfather Matti Kontiola for the illustration of the cover and to my cousin Susanna Lundén for the layout of the cover.

I acknowledge Selja Saarialho for revising the language of my thesis.

I want to thank my father for his love and help with the language of the manuscripts. He has always been ready to help me in spite of having been twice resuscitated during the course of this thesis. I thank my late mother for her enormous love and caring, and for encouraging me to start this research before her death in 1995. My three sisters Reetta, Kukka and Ulla and their families are warmly thanked for sharing joys and sorrows. My family-in-law, especially Leena and Lauri Hemmi, deserve my cordial thanks for the support and never-failing interest towards my work.

I am deeply grateful to all families, children and adults, who participated in this study.

Finally, with all my heart, I want to thank my husband Risto and our children Oona and Eemeli for their love, patience and willingness to help in every step of this study during the past years.

This study was financially supported by the Arvo and Lea Ylppö Foundation, the Helsingin Sanomat Centenary Foundation, the Sigrid Juselius Foundation, the Märta Donner Fund, the Helsinki University Central Hospital research funds, the Jorvi Hospital research funds, and the Academy of Finland.

*Janna Nykäs-Huusi*

Helsinki, September 2002

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