A genome scan for developmental dyslexia confirms linkage to chromosome 2p11 and suggests a new locus on 7q32

N Kaminen, K Hannula-Jouppi, M Kestilä, P Lahermo, K Muller, M Kaaranen, B Mylllyluoma, A Voutilainen, H Lytinen, J Nopola-Hemmi, J Kere

Developmental dyslexia is a distinct learning disability with unexpected difficulty in learning to read despite adequate intelligence, education, and environment, and normal senses. The genetic aetiology of dyslexia is heterogeneous and loci on chromosomes 2, 3, 6, 15, and 18 have been repeatedly linked to it. We have conducted a genome scan with 376 markers in 11 families with 38 dyslexic subjects ascertained in Finland. Linkage of dyslexia to the vicinity of DYX3 on 2p was confirmed with a non-parametric linkage (NPL) score of 2.55 and a lod score of 3.01 for a dominant model, and a novel locus on 7q32 close to the SPCH1 locus was suggested with an NPL score of 2.77. The SPCH1 locus has previously been linked with a severe speech and language disorder and autism, and a mutation in exon 14 of the FOXP2 gene on 7q32 has been identified in one large pedigree. Because the language disorder associated with the SPCH1 locus has some overlap with the language deficits observed in dyslexia, we sequenced the coding region of FOXP2 as a candidate gene for our observed linkage in six dyslexic subjects. No mutations were identified. We conclude that DYX3 appears to be important for dyslexia susceptibility in many Finnish families, and a suggested linkage of dyslexia to chromosome 7q32 will need verification in other data sets.

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As part of our ongoing study of the genetic attributes of dyslexia in Finland, we performed a genome wide scan in 11 families with 38 dyslexic subjects altogether. Linkage was observed to 2p11, corresponding to the DYX3 locus previously linked to dyslexia but, interestingly, also to a novel region at 7q31-q32 close to the SPCH1 locus. Because dyslexia is a specific form of language impairment, FOXP2 was a likely candidate gene in our linkage region. To study the possible role of FOXP2 in dyslexia, we screened the entire coding region of six dyslexic subjects for mutations, but found none.

METHODS

Subjects

Eleven families with 97 subjects, of whom 70 were available for thorough testing for dyslexia, were studied (fig 1). Nine families with 70 members altogether (28 dyslexic, 28 non-dyslexic, and 14 not tested) were recruited from the Department of Paediatric Neurology at the Hospital for Children and Adolescents (formerly Children’s Castle Hospital), University of Helsinki, Finland. These families were selected from about 140 based on their informativeness for linkage. Two families with 27 members altogether (12 dyslexic, 11 non-dyslexic, and four uncertain) were recruited from the Central Hospital of Central Finland, Jyväskylä, Finland. In these two families, dyslexia testing, with normal results, was performed in three of the non-dyslexic subjects, and a further eight subjects reporting normal reading performance were also classified as unaffected.

The diagnostic criteria for dyslexia included remarkable deviation (depending on the age, at least two years) in reading performance. The first gene associated with speech and language development, FOXP2 (forkhead box P2) on 7q31, was identified through a large pedigree, the KE family, with half of the family members affected by a severe speech and language disorder (SPCH1). They have mainly problems in articulation, expressive speech, and grammar, but also impairment in phonological processing is detected. All affected subjects have a G to A nucleotide transition in exon 14 of FOXP2. In addition, an unrelated subject with a similar phenotype has a de novo balanced reciprocal translocation t(5;7)(q22;q31.2) mapping specifically to an intron between exons 3b and 4 of FOXP2.

The FOXP2 gene belongs to the large FOX family of transcription factors, which have similar monomeric winged helix/forkhead DNA binding domains. FOXP2 consists of 25 exons, has multiple splice variants, and spans over 603 kb of genomic DNA. It is highly conserved among primates, but two amino acid changes in exon 7 separate human from chimpanzee and other ape paralogues, supporting a role for FOXP2 in language evolution.

Autism, a developmental disorder with variable speech and language abnormalities, has also been linked to 7q31. The possibility of FOXP2 contributing to autism and also to more common and milder forms of specific language impairment (SLI) was studied by mutation screening of 169 multiplex families (857 subjects) with autism and 43 families (210 subjects) with SLI. However, no evidence for frequent or common mutations was found. SLI is a broad definition for a mixture of variable problems in articulation, verbal expression, and comprehension of speech occurring occasionally with social, educational, behavioural, and psychological problems.

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skills compared to chronological age and normal performance intelligence quotient (IQ >85). The diagnosis of dyslexia was determined by Finnish reading and spelling tests designed for children under 13 years of age and adults, described elsewhere in detail. The IQ was determined by WAIS-R or WISC-R and subjects with an IQ below 85 were excluded from this study. In order to determine whether dyslexia was the result of a deficit in one or more of phonological awareness, rapid naming, or verbal short term memory, reading related neurocognitive skills were assessed by neuropsychological tests.

The study was approved by the appropriate ethical committee and all family members participated with informed consent.

Genotyping
Twenty ml of EDTA blood was collected from each subject and DNA was extracted by a standard non-enzymatic method. Genome wide scan was carried out at the Finnish Genome Centre, University of Helsinki, using 376 microsatellite markers from the Applied Biosystems Linkage Mapping Set MD-10. The average distance between markers was 10 cM. DNA (20 ng) was dried on microtitre plates for each PCR assay. The PCRs were performed in 5 µl volumes in conditions recommended by the reagent manufacturer (Applied Biosystems). The fluorescence labelled PCR products were pooled (10-20 markers/pool), and separated on a MegaBace 1000 capillary electrophoresis instrument (Molecular Dynamics, Sunnyvale, CA). The alleles were visualised using Genetic Profiler 1.5 software (MolecularDynamics).

Linkage analysis
The genome scan data were analysed by non-parametric and parametric linkage analysis using Genehunter and MLINK, respectively. Genehunter performs reconstruction of haplotypes and complete multipoint analysis of allele sharing identical by descent (IBD) among all affected family members at each location in the genome.

For parametric linkage analysis, a genetic model with a disease allele frequency of 0.0001, autosomal dominant inheritance (based on pedigree information, fig 1), and equal female and male recombination rates was specified. The penetrances for homozygous normal, heterozygous, and homozygous affected were 0.06, 0.95, and 0.95, respectively. In addition to dominant inheritance, parametric linkage analysis was also performed using recessive inheritance with a disease allele frequency of 0.1 and equal female and male recombination rates. The penetrances for homozygous normal, heterozygous, and homozygous affected were 0.02, 0.04, and 0.8, respectively. Both inheritance models were analysed because common recessive alleles in the population can cause inheritance patterns that are reminiscent of autosomal dominant inheritance. These particular models were chosen to reflect similar prevalence figures under both inheritance patterns. Subjects with uncertain phenotypes were not included in the analysis.

Candidate gene FOXP2
The whole coding region, consisting of 17 exons of FOXP2 and including exon 1 and alternatively spliced exons 3a, 3b, and
was sequenced from six subjects with dyslexia and three non-dyslexic subjects as controls (fig 2). One control was selected from the family members in this study and all had been tested negative for dyslexia by the neuropsychological tests (fig 1). One patient and two controls were selected from tested dyslexic families not included in this linkage analysis.

Primers flanking each exon were designed using the Primer3 program (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3). PCR amplifications were performed with an initial denaturation at 94°C for two minutes, followed by 35-40 cycles each of 35 seconds at 94°C, 35 seconds at 55°C, and 35 seconds at 72°C.

Figure 3 Linkage results of the genome scan for dyslexia with 376 markers in 11 Finnish pedigrees. The bold line shows the multipoint NPL score (scale on left) and the thin line the information content (scale on right) for each chromosome.
the previously defined region on 2p corresponding to the age of dyslexia was used. Two loci linked to dyslexia were found: FOXP2 approximately 15 Mb from marker D7S530 (fig 3) and became thus a positional candidate gene. The entire coding sequence of the candidate gene FOXP2 was sequenced from three controls and six dyslexic subjects from different families, including the two highest scoring pedigrees (10 and 5, fig 1). No mutations or SNPs were found; specifically, the G to A nucleotide transition in exon 14 was not detected in our samples.

RESULTS

A total of 88 subjects from 11 families with 38 dyslexic members were genotyped with microsatellite markers spanning the whole genome. Genome scan data were analysed by non-parametric multipoint and parametric two point linkage analysis. For non-parametric analysis, the affected only mode of analysis was used. Two loci linked to dyslexia were found: the previously defined region on 2p corresponding to DIS3 and a novel locus on 7q32 corresponding to SPCH1 (fig 3). On chromosome 2, the highest NPL score was 2.55 for marker D2S2216 on 2p11 (p=0.004), with a single high NPL 3.02 (p=0.03) in family 2 (fig 1). Marker D2S2216 is approximately 34 cM centromeric from the DIS3 locus, based on marker locus information from the Marshfield and DeCode maps. On chromosome 7, the highest NPL score was 2.77 (p=0.003) for marker D7S530 on 7q32, with one family (No 10 in fig 1) showing an NPL of 4.21 (p=0.03). Parametric analysis showed a significant two point lod score of 3.01 for marker D2S286 (2p12) in the autosomal dominant model, whereas no significant lod scores were seen for chromosome 7 (table 1). Parametric analysis with the autosomal recessive model showed no significant lod scores. We observed no evidence for linkage above background to the previously reported dyslexia loci on chromosomes 15q21, 6p21.3, 3p12-q13, and 18p11.2.

The FOXP2 gene is located within the peak of our novel linkage region, approximately 15 Mb centromeric from marker D7S530 (fig 4) and became thus a positional candidate gene. The entire coding sequence of the candidate gene FOXP2 was sequenced from three controls and six dyslexic subjects from different families, including the two highest scoring pedigrees (10 and 5, fig 1). No mutations or SNPs were found; specifically, the G to A nucleotide transition in exon 14 was not detected in our samples.

DISCUSSION

Linkage of dyslexia to at least five chromosomal loci has been verified in previous studies and is consistent with a multifactorial phenotype. We conducted a genome wide scan in 11 families ascertained on the basis of dyslexia by commonly accepted criteria. In the genome scan, we detected novel linkage of dyslexia to 7q32 and further confirmed linkage to 2p11. No other previously linked loci showed significant NPL scores. Intriguingly, the same 7q region had previously been linked to autism and the first gene implicated in a speech and language development, FOXP2, resides in the same region. Our linkage peak at 7q32, at marker D7S530, maps approximately 15 Mb from FOXP2. FOXP2 appears to have a role in several facets of language processing and grammatical skills, which are also deficient in dyslexic subjects. Although the FOXP2 gene appears to have no role in autism or SLI, it may be a plausible candidate gene for other more specific language disorders such as dyslexia.

Our linkage peak 2p11 maps approximately 34 cM from the linkage peak reported originally by Fagerheim et al. We suggest that this difference may be caused by dissimilar sample sets, diagnostic criteria, or markers used, and the results may in fact reflect the presence of one and the same locus. Alternatively, it is possible that there are indeed two different but closely located genes for dyslexia. A similar discrepancy has previously arisen for the mapping of susceptibility genes in pre-eclampsia with three different localisations in chromosome 2. Remarkably, both the non-parametric and parametric analysis modes in our material coincided for the peak of

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Table 1 Lod score table of candidate regions

Figure 4 A genetic and physical map of the linkage region in chromosome 7q31-q32. Gene order and distances on the physical map are according to the sequence of contig NT_007933, as accessed on 1 August 2002.
linkage. It is not possible to resolve the number of loci at present, but the question will need to be addressed with additional genetic studies.

To evaluate the role of FOXP2 in dyslexia, we screened the entire coding region in six dyslexic subjects, but found no mutations or polymorphisms. Obviously, possible mutations might hide in promoter regions or introns, but our results do not support a role for FOXP2 as a dyslexia candidate gene. Recently, six novel exons have been discovered, of which only one is included in the coding region. It is possible that as yet undetected coding regions exist, with undetected mutations, but currently any such data remain beyond reach.

In addition to FOXP2, chromosome 7q31-32 contains several genes that might be considered as candidates for dyslexia. Among genes that are expressed in brain are the G protein coupled receptor 37 (GPR37) and the actin regulation protein (WASL). Other interesting candidates include TPTPRZ1, a protein tyrosine phosphatase receptor type zeta-1 that is expressed only in the central nervous system, WNT2, a putative signalling molecule involved in CNS development, and NRCAM, a neuronal cell adhesion molecule (fig 4). In the absence of a more exact genetic localisation of our linkage peak, we did not sequence any of these genes.

Although FOXP2 appears not to be a dyslexia gene, the numerous potential genes in the area make our new linkage region at 7q32 extremely interesting. Even more, the linkage of autism to the same area suggests that genes other than only FOXP2 involved in language development might reside there. The roles of the remaining candidate genes in dyslexia and also other forms of language disorders, as well as fine mapping of DX3, can now be targeted with samples from the families described here.

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Authors' affiliations

N Kaminen, K Hannula-Jouppi, M Kestilä, J Nopola-Hemmi, J Kere, Department of Medical Genetics, Biomedicum, University of Helsinki, Finland

M Kestilä, Department of Molecular Medicine, National Public Health Institute, Biomedicum, Helsinki, Finland

P Lahermo, Finnish Genome Centre, University of Helsinki, Finland

K Muller, M Koarainen, H Lyttinen, Department of Psychology and Child Research Centre, University of Jyväskylä, Finland

B Myllryinen, A Vuotilainen, J Nopola-Hemmi, Department of Paediatric Neurology, Hospital for Children and Adolescents, University of Helsinki, Finland

J Nopola-Hemmi, Department of Paediatrics, Jorvi Hospital, Espoo, Finland

J Kere, Department of Biosciences at Novum and Clinical Research Centre, Karolinska Institute, Sweden

Correspondence to: Professor J Kere, Karolinska Institute, Department of Biosciences at Novum, 14157 Huddinge, Sweden; julia.kere@ki biocentrum.ki.se

REFERENCES


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