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

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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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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.

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 heritance. 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
4a, 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](http://www-genome.wi.mit.edu/cgi-bin/primer/primer3)).**29** PCRs were carried out in 50 µl reactions containing 30-50 ng of genomic DNA, 1× DyNAzyme II buffer, 1.5 mmol/l MgCl₂, 160 µmol/l dNTPs, 0.6 µmol/l of each primer, 0.6 U of DNA polymerase (DyNAzyme II, Finnzymes, Espoo, Finland), and 0-4% DMSO. In case of poor amplification, the DNA polymerases AmpliTaq Gold (Perkin Elmer, Roche Molecular Systems Inc) or DyNAzyme EXT (Finnzymes) were used in similar conditions. 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, 40 seconds at 55°C for 60 seconds, and 1 minute at 72°C. **

![Figure 3](http://jmg.bmj.com/)

**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.
Two loci linked to dyslexia were found: one on chromosome 2p close to the previously defined region on 2p corresponding to FOXP2 and PTPR2, and a novel locus on 7q32 corresponding to DYX3.

**RESULTS**

A total of 88 subjects from 11 families with 38 dyslexic members were genotyped with microsatellite markers spanning the whole genome. Genotype data were analysed by non-parametric multipoint and parametric two point linkage analysis. For non-parametric analysis, the affected only mode was used. Two loci linked to dyslexia were found: the previously defined region on 2p corresponding to FOXP2 and PTPR2, and a novel locus on 7q32 corresponding to DYX3 (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 at D2S2216 on 2p11.

**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 to 7q32 and further confirmed linkage to 2p11.

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.

**Table 1**  

<table>
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<tr>
<th>Marker</th>
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<tr>
<td>D7S2465</td>
<td>2.003</td>
<td>1.731</td>
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</tbody>
</table>

**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-q32 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), the 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 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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