

Two translocations of chromosome 15q associated with dyslexia

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Abstract

Developmental dyslexia is characterised by difficulties in learning to read. As reading is a complex cognitive process, multiple genes are expected to contribute to the pathogenesis of dyslexia. The genetics of dyslexia has been a target of molecular studies during recent years, but so far no genes have been identified. However, a locus for dyslexia on chromosome 15q21 (*DYX1*) has been established in previous linkage studies. We have identified two families with balanced translocations involving the 15q21-q22 region. In one family, the translocation segregates with specific dyslexia in three family members. In the other family, the translocation is associated with dyslexia in one family member. We have performed fluorescence in situ hybridisation (FISH) studies to refine the position of the putative dyslexia locus further. Our results indicate that both translocation breakpoints on 15q map within an interval of approximately 6-8 Mb between markers D15S143 and D15S1029, further supporting the presence of a locus for specific dyslexia on 15q21.

(J Med Genet 2000;37:771-775)

Keywords: dyslexia; reading disability; chromosome 15; translocation

Specific reading disability or developmental dyslexia is an unexpected difficulty in learning to read despite adequate intelligence, education, and normal senses. It is regarded as a language based disorder that usually reflects insufficient phonological processing abilities.¹ The prevalence of dyslexia ranges from 3 to 15% of the population and about 4% are seriously affected.²

Developmental dyslexia is a heterogeneous clinical condition. The risk for reading impairment is greater in relatives of dyslexia probands than in the general population.^{3,4} The phenotype of dyslexia is variable and different subtypes can be distinguished. Reading is a complex cognitive process and consequently multiple genes of relatively small effects are expected to contribute to its variability.⁵

Previous linkage studies have implicated four different chromosomal areas harbouring dyslexia loci/genes. The pericentromeric region of chromosome 15 was the first locus suggested to be linked to dyslexia.⁶ These results were later questioned and linkage to chromosome 1p34-p36 was suggested.⁷ A translocation

t(1;2)(p22;q31) cosegregating with retarded speech development and dyslexia has been reported.⁸ A quantitative trait locus for dyslexia has been mapped to the HLA region at 6p21.3 by Cardon *et al*⁹ and further confirmed by Gayán *et al*.⁵ Recently, Grigorenko *et al*¹⁰ reported linkage for distinct components of dyslexia to chromosomes 6 and 15; the phonological awareness phenotype was mapped to chromosome 6p21-p22 and the single word reading phenotype was assigned to chromosome 15q21. A logarithm of odds (lod) score of 3.15 was obtained for marker D15S143 at a recombination fraction (θ) of zero, under an autosomal dominant inheritance model. Another linkage study for a different component of dyslexia, spelling disability, was conducted in seven multiplex families of German origin. The authors suggested that their positive linkage result to 15q21 (two point lod score 1.26 at $\theta=0$ with marker D15S143, maximum multipoint lod score 2.19 at marker D15S143) verified this region as an established locus for dyslexia (*DYX1*).¹¹ In an extended Norwegian pedigree, a fourth locus for dyslexia was recently identified on chromosome 2p15-p16.¹²

We describe here one family in which a translocation t(2;15)(q11;q21) cosegregates with reading problems in four translocation carriers. In another family, a translocation t(2;15)(p13;q22) associates with dyslexia in one family member but not in three other translocation carriers. Even though both translocation breakpoints were originally assigned to different chromosomal bands but close to the *DYX1* locus on chromosome 15, we performed a FISH analysis to refine the chromosome 15 breakpoints further and to allow more precise localisation of *DYX1*. Our results indicate that both breakpoints map within a narrow interval between markers D15S143 and D15S1029, further supporting a locus for specific dyslexia in 15q21.

Materials and methods

ASCERTAINMENT AND TESTING OF CASES

The pedigrees of the two families (A and B) are shown in fig 1A and B, respectively. All the children except II.4 from family A have been examined by a child neurologist at a neuropaediatric unit. II.1, II.2, and II.3 from family A and II.1 from family B have previously been tested also by a neuropsychologist for clinical purposes. II.1, II.2, and II.3 from family A received remedial education and attended special classes, and II.1 from family B received remedial teaching because of dyslexia. The

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Revised version received
11 May 2000
Accepted for publication 20
June 2000

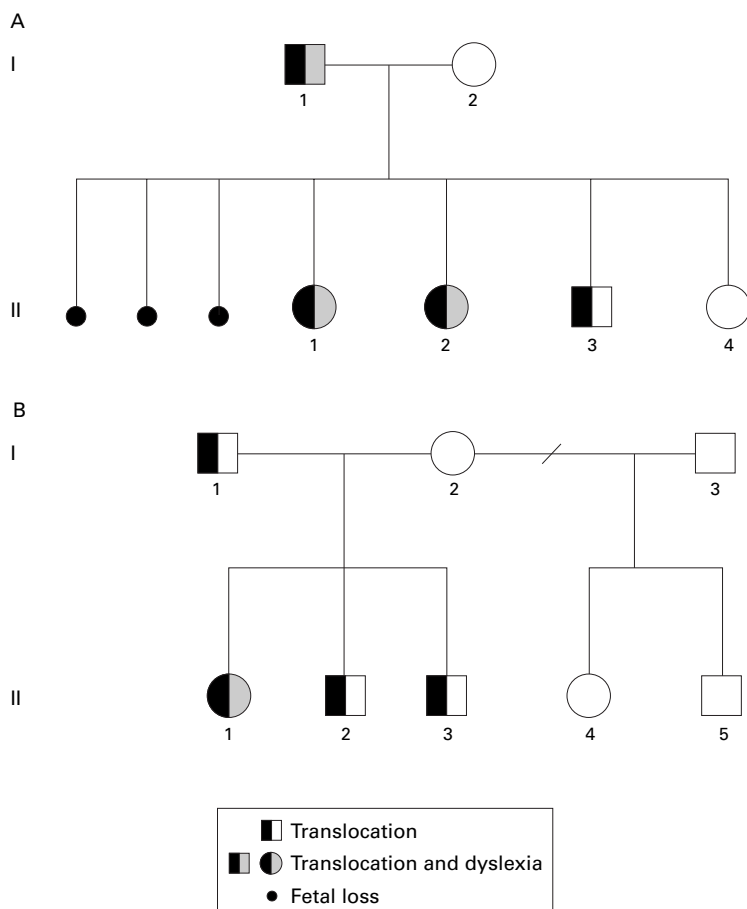


Figure 1 Pedigrees of the families studied. (A) In family A, cosegregation between $t(2;15)(q11;q21)$ and reading disability is observed. (B) In family B, the father and all three children carry $t(2;15)(p13;q22)$, but only II.1 has dyslexia.

diagnostic tests included a Finnish reading and writing test, an intelligence test (WISC-R or Wechsler Intelligence Scale for Children¹³), and a battery of neuropsychological tests, including phonological processing, phonological retrieval (rapid naming), verbal short term memory, and reading comprehension tasks (Nepsu¹⁴). The diagnostic criteria for dyslexia include normal performance intelligence quotient (IQ>85) and remarkable deviation (depending on the age, at least two years) in reading skills compared to chronological age. Subjects with normal reading ability and no history of school problems were not tested. This study has been approved by the Research Ethics Committee of the Children's Castle Hospital, University of Helsinki.

FAMILY A

I.1 is an otherwise unimpaired 44 year old male who has a history of profound reading and writing difficulties at school. Blood lymphocytes show the karyotype $46,XY,t(2;15)(q11;q21)$.

I.2 is a 43 year old female with a normal karyotype ($46,XX$) and normal ability in reading and writing. She suffered three fetal losses in gestational weeks 7-12 and subsequently had four normal pregnancies.

II.1 is a 19 year old female, who was referred to the Children's Castle Hospital, University of Helsinki, because of learning difficulties at the

age of 8 years. Her psychomotor development was normal, but in the first grade of school at the age of 7 she had difficulty in learning to read and write. Neurological and physical examinations were normal, as was CT scan of the brain. Audiometry did not indicate any hearing deficit. Psychological assessment¹³ at the age of 8 indicated normal intelligence. However, neuropsychological tests¹⁴ showed dyslexia based on deficits in the verbal short term memory and in phonological retrieval. Her karyotype is $46,XX,t(2;15)(q11;q21)$.

II.2 is a 17 year old female with the karyotype $46,XX,t(2;15)(q11;q21)$ and dyslexia. The pregnancy and delivery were uneventful and her psychomotor development was normal. She was admitted to a neuropaediatric unit at the Children's Castle Hospital, University of Helsinki, at the age of 9 because of learning difficulties at school. Neurological and physical examination did not show any abnormalities and electroencephalography was normal. In the psychological and neuropsychological tests,^{13,14} her non-verbal intelligence quotient was within the normal range, but she had deficits in verbal short term memory and phonological retrieval, and milder difficulties in visuomotor coordination and visual perception.

II.3, the proband of family A, is a 12 year old male who was born at term after a normal pregnancy. His motor and language development was normal but problems in verbal and visual memory were noticed at the age of 6. He was referred to the neuropaediatric unit of the Jorvi Hospital, Finland, at the age of 6 and a translocation, $46,XY,t(2;15)(q11;q21)$, was detected. His neurological and physical examination were normal. Differing from the other children in family A, his overall cognitive achievement was below the normal range in both verbal and non-verbal performance according to the psychological tests,^{13,14} though specific difficulty was seen in verbal short term memory, phonological awareness, and phonological retrieval. Despite starting school in a special class, he had difficulties in learning to read.

II.4 is an 8 year old girl born after an uncomplicated pregnancy and delivery with normal development. Blood lymphocytes show the normal karyotype, $46,XX$. She has no history of learning difficulties and attends a normal class.

FAMILY B

I.1 is a 39 year old male with the autosomal dominant form of cornea plana (an eye disease with microphthalmia and cataract¹⁵ that is not known to be associated with learning disability). He has no history of reading and writing difficulties, but he has not been available for psychological testing. His karyotype is $46,XY,t(2;15)(p13;q22)$.

I.2 is a 42 year old female with the normal karyotype $46,XX$. She is healthy and has not had any problems in reading or writing. She has five children, the two oldest of whom are from a previous marriage. They have no history of learning difficulties.

II.1, the proband of family B, is an 11 year old female with the karyotype 46,XX,t(2;15)(p13;q22), dyslexia, and cornea plana. The pregnancy and delivery were uncomplicated and her psychomotor development was normal, although she has been somewhat clumsy. She was admitted to a paediatric unit of Seinäjoki Hospital, Finland at the age of 8 because of difficulties in learning to read. Neurological and physical examinations were normal and the EEG did not show any abnormality. In psychological tests,^{13 14} her non-verbal performance was normal. However, in verbal skills, she had problems with phonological awareness, resulting in a severe impairment in reading and writing.

II.2 is a 10 year old male with the karyotype 46,XY,t(2;15)(p13;q22). He has had two epileptic seizures and his EEG shows spike wave complexes over the right parietotemporal region. His neurological and ophthalmological examinations did not yield any abnormal findings. At school he has had no problems in learning to read or write.

II.3 is an 8 year old male with the karyotype 46,XY,t(2;15)(p13;q22). The pregnancy and delivery were uneventful and his psychomotor development was normal. His ophthalmological examination did not show cornea plana. His neurological examination and language skills were normal.

CYTOGENETICS AND FISH

Karyotypes were obtained from phytohaemagglutinin stimulated blood lymphocytes with G banding using standard procedures. For fluorescence in situ hybridisation studies, metaphase spreads were obtained from EBV transformed lymphoblastoid cell lines derived from II.1 (family A) and II.1 (family B). Genomic YAC clones were obtained from CEPH (Paris, France) and BAC clones from Genome Systems (St Louis, Missouri). YAC DNA was extracted with the glass bead phenol-chloroform extraction method and BAC DNA by a standard alkaline lysis method.¹⁶ Approximately 1 µg of YAC or BAC DNA was labelled with biotin-14-dATP using nick translation. Labelled DNA was ethanol precipitated with 50 µg of Cot-1 DNA and 50 µg of herring sperm DNA, and dissolved in hybridisation buffer (50% formamide, 10% dextran sulphate, 1 × SSC). Before hybridisation, the slides were denatured for two minutes in 70% formamide, 2 × SSC (pH 7.0) at 65°C and the probe for five minutes at 75°C. Hybridisation was performed in a humid chamber at 37°C for two days.

The slides were washed at 45°C with 4 × SSC, 0.2% Tween 20. BSA (5%) was used as a blocking agent before detection with avidin conjugated fluorescein isothiocyanate (FITC). The signal was amplified with biotinylated anti-avidin and avidin-FITC. Chromosomes were counterstained with 4',6'-diamidino-2-phenylindole (DAPI) and propidium iodide.

Signals were visualised with a Zeiss Axioplan 2 fluorescent microscope (Carl Zeiss, Oberkochen, Germany). Images were captured with Hamamatsu CCD camera (Hamamatsu Phot-

onics, Hamamatsu, Japan) and pseudocoloured with ISIS software (Metasystems GmbH, Altlußheim, Germany).

Results

Partial karyotypes of G banded metaphase chromosomes from two translocation carriers are shown in fig 2. The karyotype shows the presence of a balanced translocation between chromosomes 2 and 15 in both cases. The breakpoint on chromosome 15 occurs approximately in the same region, whereas different chromosome arms of chromosome 2 are involved in the translocations. To define molecularly the translocation breakpoints, we used FISH with chromosome 15 specific YAC and BAC clones as probes. First, BAC clones located distally and proximally to the D15S143 region were used to establish borderlines of both translocations. The YAC clones were then used to narrow down the interval between the markers flanking the translocation breakpoints.

The FISH studies show that both breakpoints are located between markers D15S143 and D15S1029. CEPH YACs 965E5 and 956E3, both of which are positive for marker D15S143, are located proximal to the translocation breakpoints in families A and B (fig 3A, C). BAC clone 134E18 hybridised distal to the translocation breakpoint in family A (fig 3B), and CEPH YAC clone 770D11 mapped distal to the breakpoint in family B (fig 3D). Both clones are positive for marker D15S1029. A summary of the FISH data as well as regions implicated in previous linkage studies are presented in fig 3E.

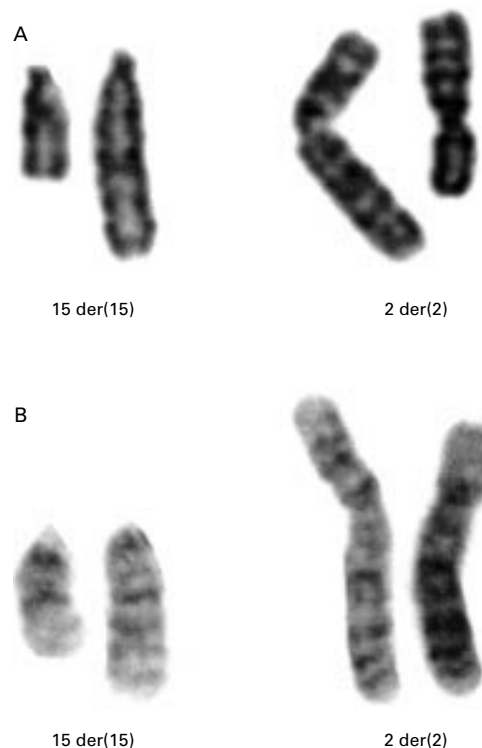


Figure 2 Partial karyotypes of G banded metaphase chromosome pairs 2 and 15 from II.3 with t(2;15)(q11;q21) in family A and II.1 with t(2;15)(p13;q22) in family B.

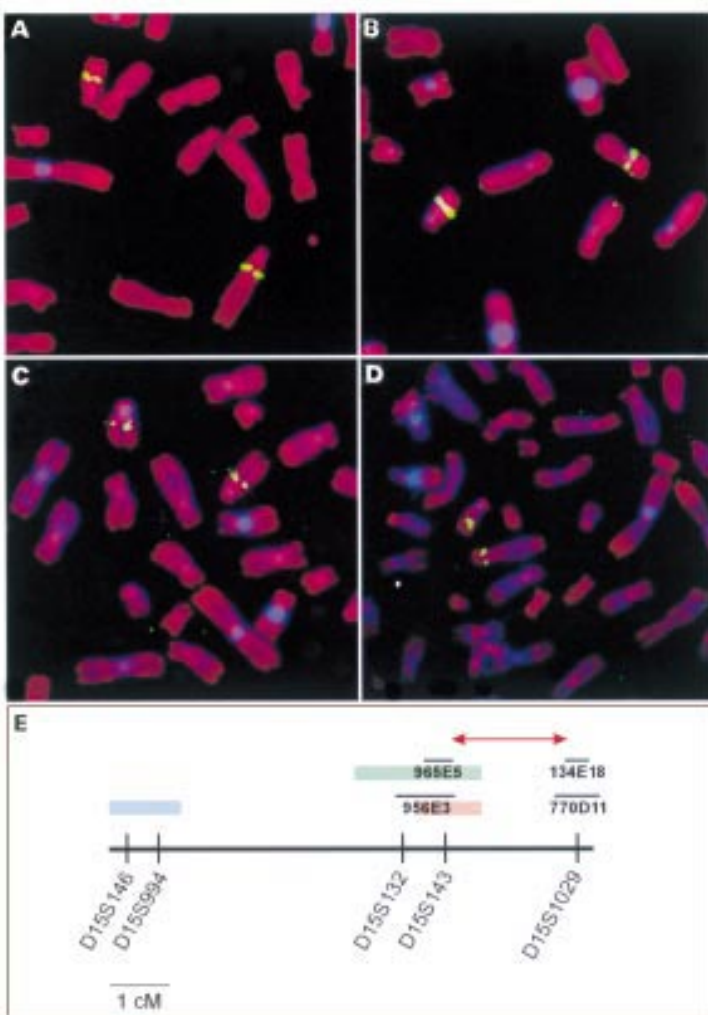


Figure 3 FISH analysis of translocations in families A and B. The probes were labelled with FITC (green) and chromosomes counterstained with DAPI (blue) and propidium iodide (red). In family A (II.1), YAC 965E5 hybridises to normal chromosome 15 and der(15) (A), and BAC clone 134E18 to normal 15 and der(2) (B). In family B (II.1), YAC 956E3 shows hybridisation signals in normal chromosome 15 and der(15) (C), whereas YAC 770D11 hybridises to normal 15 and der(2) (D). (E) A map of chromosome 15q markers. The FISH mapping clones are shown as black bars, bracketing the interval of the two translocation breakpoints identified in this study (red arrow). Markers showing peak significance in previous studies are highlighted with coloured bars (Grigorenko *et al.*,¹⁰ red; Schulte-Körne *et al.*,¹¹ green; Morris *et al.*,¹⁹ blue).

Markers D15S143 and D15S1029 are separated by some 2.2 cM, or 6–8 Mb, according to the Marshfield genetic linkage map¹⁷ and the CEPH YAC contig map of the human genome,¹⁸ respectively. We conclude that both independent translocation breakpoints map in a region corresponding to approximately 2% of the whole of chromosome 15.

Discussion

We have studied two families in which balanced translocations involve different arms of chromosome 2 but the same narrow region on chromosome 15q. In family A, t(2;15)(q11;q21) is present in the father and two children who all have dyslexia (II.3 has low general achievement and thus his diagnosis with respect to dyslexia remains uncertain), whereas the mother and one child have normal karyotypes as well as normal reading ability.

In family B, the mother has a normal karyotype, whereas the father and all three children are carriers of t(2;15)(p13;q22). In this family, the phenotype pattern is complex; the father and the oldest child have cornea plana, and there is a history of cornea plana in the father's mother and one sister. In addition, the oldest child has a severe reading disability, whereas the two younger translocation carrier sibs have no symptoms of dyslexia. There is no history of learning difficulties in the father who unfortunately has not been available for testing. Even though the association of dyslexia with t(2;15) involves only one person in family B and is inconsistent with genetic linkage, we decided to map the chromosome 15 translocation breakpoint in this family also.

The FISH results suggest that both independent translocation breakpoints on chromosome 15q map within 6–8 Mb of each other, residing in the region limited by the markers D15S143 and D15S1029. This region overlaps the region implicated in carrying the *DYX1* locus in previous linkage studies, and thus further strengthens the conclusion that at least one locus for dyslexia resides within 15q21. Cell lines from the translocation patients provide an opportunity for the physical fine mapping and the identification of a dyslexia gene(s) in 15q21.

It is conceivable that there is more than one locus for dyslexia at 15q21. Such a working hypothesis might be suggested by the fact that genetic linkage results are in part inconsistent. Grigorenko *et al.*,¹⁰ studying single word reading, obtained a significant lod score of 3.15 at $\theta=0$ for marker D15S143, but the nearby markers D15S132 (mapping just 1 cM proximal of D15S143) and D15S209 (mapping 2 cM distal to D15S143) yielded exclusion or highly negative lod scores at $\theta=0$ to 0.05 under the dominant model. A recent study by Morris *et al.*¹⁹ identified a significant association of dyslexia with markers slightly proximal of our translocation breakpoints (fig 3E). An example of the clustering of chromosomal abnormalities and the probable presence of several genes is provided by the split hand/split foot anomaly gene region in chromosome 7q21.3–q22.1.²⁰ In the patients with this syndrome, independent deletions and translocations associated with the anomaly span a region of approximately 2.5 Mb. However, the hypothesis of more than one dyslexia associated genes at 15q21 can be more directly assessed by the simultaneous fine mapping of multiple translocation breakpoints.

In conclusion, we describe two families in which two different translocation breakpoints at 15q21, mapping less than 6–8 Mb apart, are associated with dyslexia in one or several family members. Our results support the existence of at least one dyslexia locus at 15q21 and may prove valuable in the identification of dyslexia genes within 15q21.

We would like to thank Drs Marketta Muttilainen and Timo Talvitie for their help with patient ascertainment, and Drs Johanna Aaltonen, Ralf Krahe, and Mauri Keinänen for advice and instruction in the initial phases of this study. We are grateful to Ms Ying Zhu and Professor Sakari Knuutila for their help with the cytogenetic part of this study. We thank families A and B for their cooperation. This research was supported by the

- Arvo and Lea Ylppö Foundation, the Helsingin Sanomat Centenary Foundation, the Sigrid Juselius Foundation, Helsinki University Hospital research funds, and the Academy of Finland.
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