A 4 Mb cryptic deletion associated with inv(8)(q13.1q24.11) in a patient with trichorhinophalangeal syndrome type I

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Abstract

We report on an 11 year old girl with trichorhinophalangeal syndrome type I (TRPS1), postaxial polydactyly of the fingers, and a de novo paracentric inversion on the long arm of chromosome 8 involving bands q13.1 and q24.11. Molecular analysis using FISH and polymorphic DNA markers detected an approximately 4 Mb, cytogenetically unidentified deletion occurring between two STSs markers, AFMB331YA9 and D8S1200, around the region of the distal inversion breakpoint. Although the deletion is large, mental retardation was not present in the patient. This is the first report of a cryptic deletion in a TRPS1 patient, both ends of which were analysed at the molecular level. The data obtained are useful for defining the location of the putative mental retardation gene(s) in TRPS1 and Langer-Giedion syndrome (TRPS2), as well as a locus for postaxial polydactyly.

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Trichorhinophalangeal syndrome type I (TRPS1, MIM 190350¹) is characterised by sparse and slowly growing scalp hair, pear shaped nose, long philtrum, short stature, short fingers, and clinodactyly with cone shaped epiphyses of the middle phalanges. Autosomal dominant inheritance has been seen in many familial cases.² Clinical manifestations of TRPS1 are similar to those of Langer-Giedion syndrome (LGS, trichorhinophalangeal syndrome type II, MIM 150230¹), with the exception that LGS patients have multiple exostoses and mental retardation. The majority of LGS patients have cytogenetically detectable deletions of the long arm of chromosome 8, while a chromosome abnormality is less common in TRPS1 patients. The loci for the two disorders have been assigned to the same region of chromosome 8q24.1, based mainly on cytogenetically defined chromosome aberrations in patients.³⁻⁸ The gene (EXT1) responsible for hereditary multiple cartilaginous exostoses (MIM 133700¹) has recently been isolated from this region.⁹ Thus, it is most likely that LGS is a contiguous gene syndrome caused by a deletion of both the TRPS gene and EXT1.

A Japanese girl with clinical manifestations of TRPS1 was referred to us. She also had bilateral postaxial polydactyly of the fingers. Chromosome analysis on this patient showed a de novo paracentric inversion involving 8q13.1 and 8q24.11. This report describes the cytogenetic and molecular analysis of the inversion.

Materials and methods

CLINICAL REPORT

The patient was an 11 year old Japanese girl who was born after an uneventful 41 week pregnancy to unrelated, healthy parents; birth weight was 3250 g. At birth, the mother and father were 32 and 35 years old, respectively. The patient had bilateral postaxial polydactyly of the fingers with nails, which were surgically removed at 1 year of age. By the age of 5 years, she developed deformities of the middle phalangeal joints of the middle fingers and right fourth finger. Her growth was retarded; height was 106 cm (-1.6 SD) at 6 years and 123 cm (-2.3 SD) at 10 years 7 months, and weight was 19 kg at 10 years. When first seen by us aged 10 years 7 months, she had the following abnormalities: sparse hair; a peculiar facies with a pear shaped nose, a long philtrum, thin lips, micrognathia, and large prominent ears; shortening of all fingers but the right third finger; short big toes and right little toe; broadening of the middle phalangeal joints; bilateral clinodactyly of fingers 3 and 4. She showed no signs of developmental delay in childhood and her intelligence is normal. Endocrinological examinations showed normal levels of serum T3, T4, TSH, LH, FSH, and somatomedin C. A radiographic survey of the bones showed cone shaped or premature fusion of the epiphyses in left metacarpals 1-5, right metacarpals 1-2/4-5 and all middle phalanges, bilateral vestiges of fifth finger polydactyly (fig 1A), and short metatarsals of both big toes. No evidence for exostosis was observed. Her bone age at 10 years 7 months was estimated to be 9 years, being somewhat retarded. Her older and younger sisters and the parents were all phenotypically normal.

Peripheral blood lymphocyte cultures of the patient, younger sister, and parents were synchronised with the fluorouracil/thymidine/ethidium bromide method¹⁰ to obtain 850 band stage high resolution GTG bands. An apparent paracentric inversion in the long arm of chromosome 8 (inv(8)(q13.1q24.11)) was found in the patient (fig 1B). No other abnor-

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Figure 1 Clinical data of the patient. (A) X ray of the right hand, (B) normal and inverted chromosomes 8 (*inv*(8)(q13.1q24.11)) and ideogram. Arrows indicate breakpoints on the inverted chromosome.

ality was observed. Karyotypes of the younger sister and parents were normal.

FLUORESCENCE IN SITU HYBRIDISATION (FISH)

Seven CEPH-YACs (767E8, 804C6, 883B10, 919G10, 930C8, 950F4, and 956E8), a St Louis YAC (A33F4), and nine cosmid clones (c206G4 (D8S1200), c4G6, c59B5 (D8S51), c103C8, c178C4 (D8S1216), c81D8, c176C9, c33F9, and c96D4, all located on the YAC 767E8) were obtained from Research Genetics Inc (USA) and kindly provided by Dr Craig Chinault at Baylor College of Medicine and by

Professor D E Wells at the University of Houston, respectively. These YAC/cosmid clones were previously mapped to the 8q24 region, especially around YAC-A33F4.^{11,12} A cosmid library was constructed from A33F4 DNA that was isolated from yeast spheroplasts using the sucrose gradient procedure.¹³ Cosmid clones with human DNA inserts were then selected by colony hybridisation using total human genomic DNA as a pool of probes. DNA was extracted from these cosmids by means of an automatic plasmid isolation system (PI-100, Kurabo, Japan). Alu sequence based polymerase chain reaction (Alu-PCR) was performed using primers as described by Kuwano *et al.*¹⁴

The total DNA of YAC/cosmid subclones, a cosmid (cW143), and Alu-PCR products were labelled with biotin-16-dUTP (Boehringer-Mannheim, Germany) with a nick translation labelling kit (Boehringer-Mannheim) and used as FISH probes. Chromosome in situ suppression hybridisation was performed on metaphase chromosomes from the patient and a normal control, using each labelled DNA (500 ng/chromosome slide) as a probe. The labelled cW143 cosmid that was previously mapped to 8q11,¹⁵ and human Cot-1 DNA (1 µg/slide) (GIBCO-BRL, USA) were simultaneously used as control probes for the detection of chromosomes 8 and as a competitor, respectively, according to the methods of Takahashi *et al.*¹⁶ Chromosomes were then counterstained with propidium iodide (PI). Hybridisation signals were detected with FITC conjugated avidin (Vector Laboratories, USA), and chromosomes were photographed under a fluorescence microscope (Nikon, Japan) equipped with a B-2A filter for PI/FITC or a B-2E filter for FITC.

POLYMORPHIC MARKER ANALYSIS

Polymorphic DNA markers used in this study included a restriction fragment length polymorphism (RFLP) marker, D8S49,¹⁷ and the following four microsatellite (CA repeat) mark-

Figure 2 FISH on metaphase chromosomes from the patient using YACs A33F4 (A), 919G10 (B), and 804C6 (C), and cosmid c206G4 (D) as probes. Arrow and arrowhead indicate a twin signal at a distal one-third region and at a proximal one-third region on the long arm of chromosome 8, respectively.

Figure 3 Parent-child transmissions of microsatellite markers (D8S555, D8S539, D8S588, and AFMB331YA9) and an RFLP marker (D8S49). Deletion of the paternal allele was observed at the D8S555, D8S539, D8S588, and D8S49 loci, and biparental alleles were present at the AFMB331YA9 locus. Numbers on the right of autoradiograms for microsatellite markers denote variable alleles. Lanes F, P, M, and S indicate father, patient, mother, and younger sister, respectively.

ers with sequence tagged sites (STSs): AFMB331YA9, D8S588, D8S539, and D8S555. These CA repeat markers were all assigned to the CEPH-YAC, 804C6, and ordered as cen - AFMB331YA9 - D8S588 - D8S539 - D8S555 - tel. Sequence information of PCR primers for the markers was obtained from the Genome Database (GDB, Baltimore, USA), the Online Database of the Whitehead Institute for Biomedical Research/MIT Center for Genome Research (Cambridge, USA), or from published data by Chen *et al.*¹⁸ The relative locations of the AFMB331YA9 and D8S49 to D8S588 loci were determined from the results of the present study.

Genomic DNA was extracted directly from peripheral blood leucocytes of the patient and from Epstein-Barr virus transformed lymphoblastoid cell lines of the patient, younger sister, and parents and used for the polymorphism analysis. The method for the CA repeat marker analysis was a modification of one previously described,¹⁹ using a 5% Long Ranger (FMC BioProducts, USDA)/7 mol/l urea gel. The procedure for the RFLP analysis was as previously described.²⁰ A PCR product at D8S49, a locus more centromeric to D8S588, was used as a Southern hybridisation probe for RFLP analysis. DNA from the family members was digested with *Bcl*I, and the blot was hybridised with the probe.

Results

DETECTION OF INVERSION ASSOCIATED DELETION
The A33F4-YAC, which covered the 8q breakpoint of t(8;18)(q24.1;q23) in a previous TRPS1 patient (KS2166),⁸ was first used as a

FISH probe to find out whether the distal breakpoint of the inverted chromosome 8 (inv(8)) in our patient corresponded to the breakpoint in KS2166. Both Alu-PCR products and the whole A33F4 derived DNA gave only one twin signal in all the patient's metaphase cells analysed (fig 2A). The results suggested that the sequence corresponding to A33F4 is deleted in the inv(8) of our patient. Then, the eight CEPH-YACs that had been localised around A33F4^{11 12} were used as FISH probes. When using 956E8, a twin signal appeared on one chromosome 8, while all of 767E8, 883B10, 930C8, and 919G10 (fig 2B) gave signals at a distal one-third 8q region of both homologues 8. The YAC 804C6 produced a signal at the distal 8q region of one chromosome 8 and a signal at a proximal one-third 8q region of the other chromosome 8 (fig 2C). Since these proximal and distal regions most likely corresponded to the proximal and distal breakpoints of the inv(8), respectively, the results indicated that 804C6 covers the inversion breakpoint 8q24.1. All these seven probes gave signals at the 8q24 region on both chromosomes 8 in the control (data not shown). The other YAC, 950F4, did not produce any signals on the metaphase chromosomes of the patient or the control, probably because it may be chimeric or deleted.

EXTENT OF DELETION AND ENDPOINTS

As the FISH experiments detected a cryptic deletion associated with the inversion in our patient, the presence or absence of parental alleles was analysed at four microsatellite and one RFLP loci, which are within YAC-804C6,

Figure 4 The extent of the deletion in our patient (NS) (bottom) on an integrated map around the TRPS and LGS chromosomal regions, modified from that by Chen *et al.*¹⁸ Bold letters show STSs, YACs, and cosmids used in this study, and the dotted line a deletion of undetermined endpoint at the molecular level. Two YACs, 883B10 and 930C8, do not appear in this figure, because they are mapped far from this region.

in order to determine the proximal endpoint of the deletion. Since the paternal allele was absent in D8S49 but present in the AFMB331YA9 locus (fig 3), the proximal endpoint lies between these two loci and the deletion is of paternal origin. Although the locus order information for AFMB331YA9 had been provided from the Online Database, only as being located more centromeric to D8S539, our data may confine the AFMB331YA9 locus to a segment encompassing D8S65 and D8S1202 (fig 4).

Then, we tried to narrow the distal end of the deletion by FISH using the nine cosmid clones mapped on YAC-767E8 as probes. All these cosmids, especially c206G4 which is located in the most proximal part of YAC-30C3,¹² produced signals on both homologues 8 of the patient, indicating that the c206G4 corresponding sequence of YACs (30C3 and 767E8) was not deleted in the inv(8) of our patient (fig 2D). Furthermore, the 50 cosmid subclones derived from the YAC-A33F4 were divided into 10 groups on the basis of their *Eco*RI digestion patterns, and a representative clone from each group was used as a FISH probe. All these cosmids gave FISH signals only on one chromosome 8 of the patient (data not shown). Thus, the distal endpoint of the deletion lies between D8S1200 and D8S98 (fig 4). Finally, from an integrated map of the 8q23-q24.1 region,¹⁸ the extent of the deletion in our patient was estimated to be approximately 4 Mb in size, between the AFMB331YA9 and D8S1200 loci, although other flanking markers were not exhaustively analysed.

Discussion

Chromosome abnormalities occurring in patients with a single gene defect may often become a clue for isolating the disease gene. Since both TRPS1 and the chromosome inversion observed in our patient were of de novo

origin, we first presumed that the putative TRPS gene would have been disrupted in this patient by the inversion and therefore began to try to isolate breakpoint DNA. However, unexpectedly, an approximately 4 Mb deletion was identified by molecular analysis. The distal deletion endpoint of our patient lies between D8S98 (A33F4) and D8S1200 within an interval of about 400 kb. If this endpoint is compared with a breakpoint map made from chromosome rearrangements in four previous TRPS1 patients (KS2166, JS1380, EA, and JL1386),¹² it was located between the most proximal and the most distal translocation breakpoints in three of the four patients: the former corresponds to the breakpoint of KS2166 and the latter to that of EA (fig 4). Thus, our finding is not inconsistent with the previous mapping of the TRPS gene at the proximal end of the minimal region of overlap of LGS deletions (LGS MRO).¹² If we assume that all the rearrangements in the five patients including ours had disrupted the TRPS gene, the gene may be relatively large, encompassing more than 800 kb and extending across the proximal boundary of LGS MRO.¹²

Mapping of the putative gene(s) responsible for mental retardation involved in LGS or TRPS1 or both merits discussion. Bühler and Malik⁵ stated that mental retardation (MR) as a non-specific symptom of LGS is expected when a large 8q segment is deleted. Lüdecke *et al.*⁶ tentatively mapped the MR gene outside a TRPS-EXT1 interval, because LGS patients with minute deletions were mentally normal,²¹ whereas TRPS1 patients with large deletions extending more centromerically were mentally retarded.²² However, although the deletion in our TRPS1 patient is large and extends centromerically, no MR is present. Lüdecke *et al.*⁶ also showed with quantitative Southern hybridisation that the distal endpoint of a deletion in a TRPS1 patient with MR (JL1386, fig 4) lay between clones YL2.2 and YL2.4, which

were proximal end fragments of YACs 340B11 and 30C3, respectively. The endpoint in this patient almost corresponds to that in our patient. Thus, the proximal deletion endpoint of JL1386 is of great interest.

Bilateral postaxial polydactyly of the fingers present in our patient has not previously been described in TRPS1 patients. It is unlikely that the abnormality is a forme fruste of exostosis, because the extra digits were said to have nails and the remaining fingers were not involved in exostosis or had its vestiges. Therefore, it is plausible that one polydactyly locus is present within a segment that was deleted in our patient or around a region (8q13.1) at which the proximal inversion breakpoint is situated. Alternatively, the polydactyly in our patient was just coincidental.

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