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Assignment of Fragile Site 8E (FRA8E) to Human Chromosome Band 8q24.11 Adjacent to the Hereditary Multiple Exostoses 1 Gene and Two Overlapping Langer-Giedion Syndrome Deletion Endpoints

April L. Hill
University of Richmond, ahill2@richmond.edu

Y. Harada

E. Takahashi

J. Hou

M. J. Wagner

See next page for additional authors

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Authors
April L. Hill, Y. Harada, E. Takahashi, J. Hou, M. J. Wagner, and D. E. Wells

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Assignment[a] of fragile site 8E (FRA8E) to human chromosome band 8q24.11 adjacent to the hereditary multiple exostoses 1 gene and two overlapping Langer-Giedion syndrome deletion endpoints

A. Hill,[1] Y. Harada,[2] E. Takahashi,[2] J. Hou,[1] M.J. Wagner,[1] and D.E. Wells[1]

[1] Department of Biology, and Institute for Molecular Biology, University of Houston, Houston TX (USA);
[2] Division of Genetics, National Institute of Radiological Sciences, Chiba (Japan)

[a] This is a more precise localization of the fragile site than has been previously reported.

Rationale and significance

The distamycin A inducible fragile site, FRA8E, Fra(8)(q24.11) has been previously mapped to 8q24.11, proximal to the MYC gene, by fluorescent in situ hybridization (Takahasi et al., 1991). This fragile site has been shown to be present in about 1 out of every 140 healthy Japanese individuals (Takahasi et al., 1988). To more precisely map FRA8E, we have used cosmids isolated from the Langer-Giedion chromosomal region (LGCR) for FISH analysis. Langer-Giedion syndrome (LGS) is a contiguous gene syndrome characterized by chromosome deletions in 8q24.11 (Lüdecke et al., 1991). One of the genes known to be involved in the etiology of LGS is hereditary multiple exostosis type 1 (EXT1). In addition to being associated with LGS, hereditary multiple exostosis is an independent disorder characterized by cartilage capped exostoses on the juxtaepiphyseal regions of the enchondral bones (Hennekam, 1991).

Materials and methods

The fragile site was expressed in a lymphoblastoid cell line established from an affected individual using Hoechst 33258 (25 μg/ml, 24 h treatment). In situ hybridization was done as per Takahashi et al. (1991). PCR reactions and polymorphism analysis were carried out under conditions described in Cook et al. (1993).

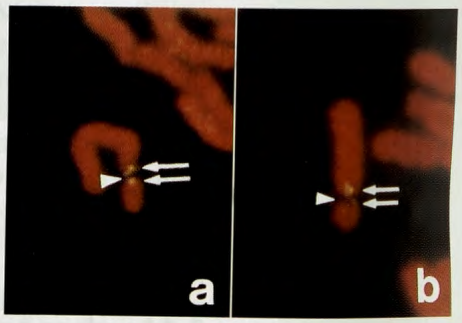

Fig. 1 . (**a** and **b**) Partial chromosome plates with fra(8)(q24.11) hybridized with cosmid c44G12. Arrowheads indicate the fragile site and arrows indicate the split signals from c44G12 on either side of fra(8)(q24.11). Although the intensity of the distal/proximal signal was variable, dependent on hybridization conditions, they were clearly observed in over 100 cells.

Results

Using FISH analysis, cosmid c44G12 was determined to span the fragile site (Fig. 1). This cosmid has been mapped to the overlapping region between YACs 236G8 and 10G12 in the distal portion of the LGCR (Hou et al., 1995).

The EXT1 gene has been cloned and maps to the distal portion of the LGCR (Ahn et al., 1995). The 5′ end of the EXT1 gene is also present on YAC 236G8 (Fig. 2). The distal end points of interstitial chromosome deletions in 2 patients with LGS (NH and GM09888, Hou et al., 1995), overlap with c44G12.

Sequence analysis of c44G12 revealed a highly polymorphic pentanucleotide repeat, $(TAAAA)_{16}$ TAA $(TAAAA)_7$ located in the middle of the cosmid that has been shown to be expanded

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Request reprints from Dr. Dan Wells, Department of Biology, University of Houston, Houston TX 77204 (USA); telephone: 713-743-2671; fax: 713-743-2626; email: dwells@uh.edu

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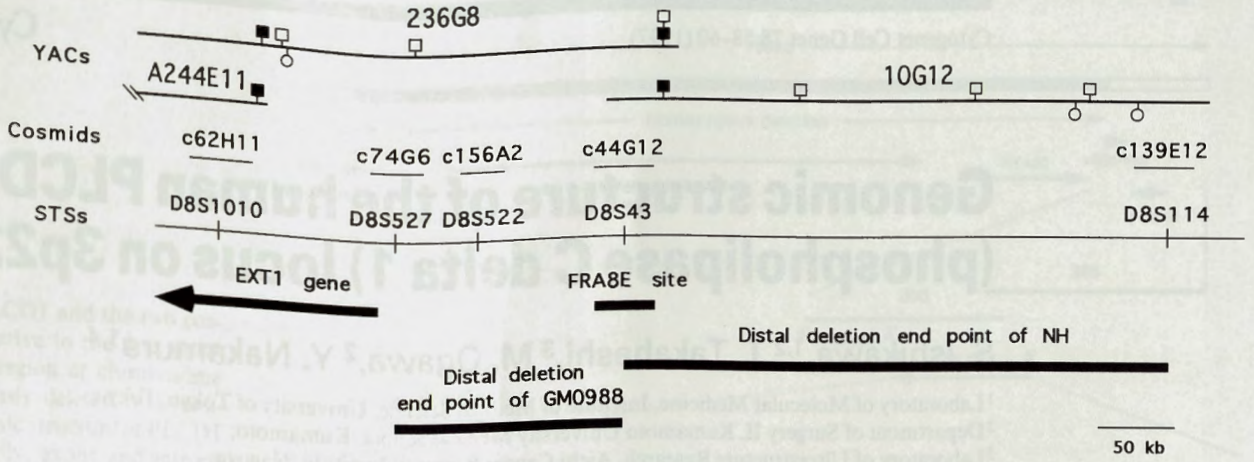

Fig. 2. Physical map of the region of 8q24.11 surrounding the FRA8E site. Two YACs, 238G8 and 10G12 are shown in their entirety. Only the distal portion of YAC A244E11 is shown. Restriction sites for the YACs are as follows: *Bss*HII (○); *Sfi*I (■); *Eag*I (□). Cosmids shown were mapped by hybridization to Southern blots of digested YACs. In addition STSs, corresponding to each cosmid were mapped to a larger overlapping set of YACs to confirm their location. The location of the 5′ end of the EXT1 gene (~350 kb total) is indicated. The distal end points of 2 LGS derived cell lines (NH and GM09888) have been mapped to the intervals shown by thick black lines.

in affected individuals. PCR primers flanking the repeat were designed to amplify a 540-bp fragment of this sequence. Nine different alleles, from 490 to 540 bp, were seen and the observed heterozygosity of 87% was estimated from 46 chromosomes of 23 unrelated Caucasian individuals. Computer analyses of the nucleotide sequence immediately surrounding the repeat give no indication of a gene structure.

Further analysis is needed to determine whether or not there is a correlation between this fragile site and chromosome deletions which are known to occur in LGS and EXT patients. However, since there are few highly polymorphic STSs in the region surrounding the EXT1 gene and since the STS polymorphism described here is easily scoreable it should be very useful in linkage studies for families segregating genes for EXT on human chromosome 8.

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