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# ASSIGNMENT OF A POLYMORPHIC LOCUS OF OS-4(D18S5) DNA SEGMENT TO HUMAN CHROMOSOME REGION 18q21.3→qter

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*Summary* A polymorphic human DNA fragment, OS-4, isolated from pBR322 human genomic library was mapped to chromosome 18 using a human-mouse somatic cell hybrid panel. More precise assignment of this locus to 18q21.3→qter was made by hybridization to DNA from six cell lines containing different structural abnormalities of chromosome 18.

In addition to *Taq*I polymorphism, it was proved that OS-4 could detect polymorphism in *Pst*I-digested human DNA. This probe, designated as D18S5, would be a useful marker for gene mapping as well as linkage analysis of genetic diseases.

## INTRODUCTION

Recombinant DNA technology has enabled us to obtain DNA fragments that detect restriction fragment length polymorphisms (RFLPs) from any part of the human genome. Linkage analysis using polymorphic DNA fragments has largely widened the possibility of mapping the loci of hereditary diseases to specific chromosomes (Botstein *et al.*, 1980). The loci of several hereditary diseases have been determined by this method (Gusella *et al.*, 1983; Reeders *et al.*, 1985; White *et al.*, 1985).

Recently, we have isolated nine polymorphic DNA fragments to use in linkage analysis of several hereditary diseases (Nishisho *et al.*, 1986). In this report, we determined the location of one of the DNA fragments on human chromosome 18

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using a human-mouse hybrid panel and six cell lines showing different types of structural abnormalities of chromosome 18.

## MATERIALS AND METHODS

*Cells.* Ten clones of human-mouse somatic cell hybrids were used as a mapping panel, which was originally established by M.C.Y. and provided to S.T. by the Japanese Cancer Research Program. Six human cell lines having different chromosome 18 abnormalities were used for regional mapping. GM2054 [46,XY,del(18)(pter→q21:)], GM1261 [45,XX,−13,−18, +t(13,18)(13qter→cen→18qter)] and GM2980 [46,XY,del(18)(pter→q2108:)] were obtained from the Human Genetic Mutant Cell Repository, Camden, New Jersey. 7307L [46,XX,psu dic(18)(qter→cen→p11.32::p11.32→qter)], 7953L [46,XY,rec(18)(p11.2q21.3)mat.] and 8011L [46,XX,inv(18)(p11.2q21.3)] were EBV-transformed lymphoblastoid cell lines established from peripheral blood of the patients reported elsewhere (Ikeuchi and Naito, 1982; Shinohara *et al.*, 1985).

DNA was extracted from white blood cells donated by healthy Japanese volunteers, human-mouse hybrids and cell lines containing aberrations of chromosome 18. DNA samples were digested with several restriction enzymes, electrophoresed in 0.7% agarose gels and transferred to nitrocellulose by the method of Southern (Southern, 1975). And then filters were baked under vacuum at 80°C for 3 hr. After labeled by nick-translation, OS-4 and p1E8 (D13S4) probes were hybridized to Southern blots in 1×Denhardt's solution, 1 M NaCl, 50 mM Tris-HCl, pH 7.4, 10 mM EDTA, 0.1% sodium dodecyl sulphate (SDS) and 0.1 mg/ml denatured sonicated salmon sperm DNA at 65°C for 15–20 hr. Filters were washed in 2×SSC (0.15 M NaCl, 15 mM sodium citrate) once at room temperature and twice in 0.1× SSC and 0.1% SDS at 65°C for 30 min. Hybridized filters were exposed to XRP-1 film (Kodak) at −80°C in the presence of Lightning Plus intensifying screen (DuPont) for 1–5 days.

*Densitometric analysis.* The density of bands in autoradiograms of six cell lines containing different chromosome abnormalities was analyzed by scanning densitometer (Shimadzu CS-9309). Probe p1E8 (D13S4) which locates 13q22→qter (Cavenee *et al.*, 1984) was used as an internal diploid standard in order to correct the quantity of DNA from each cell line.

## RESULTS

The OS-4 probe revealed *Taq*I polymorphism involving fragments of 8.6 and 6.7 kb (Fig. 1a), as previously reported by Tateishi *et al.* (1986), and also *Pst*I polymorphism involving two allelic fragments of 7.5 and 4.8 kb (Fig. 1b).

For chromosomal assignment of the OS-4, a total of ten human-mouse hybrid cell DNA were analyzed for presence of the OS-4 fragment by Southern blot analysis

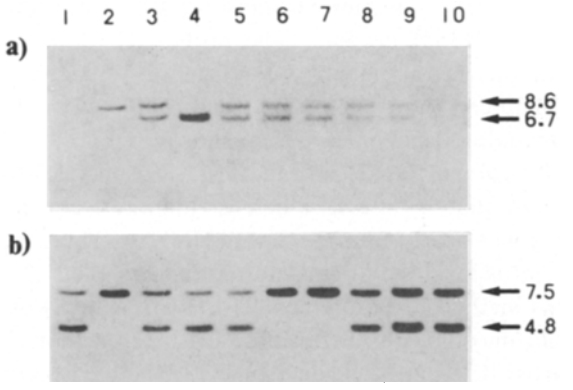


Fig. 1. Restriction fragment length polymorphisms detected by the OS-4 probe in *Taq*I-digested DNA from ten unrelated Japanese (a) and in *Pst*I-digested DNA (b); numbers to the right indicate length of DNA in kb.

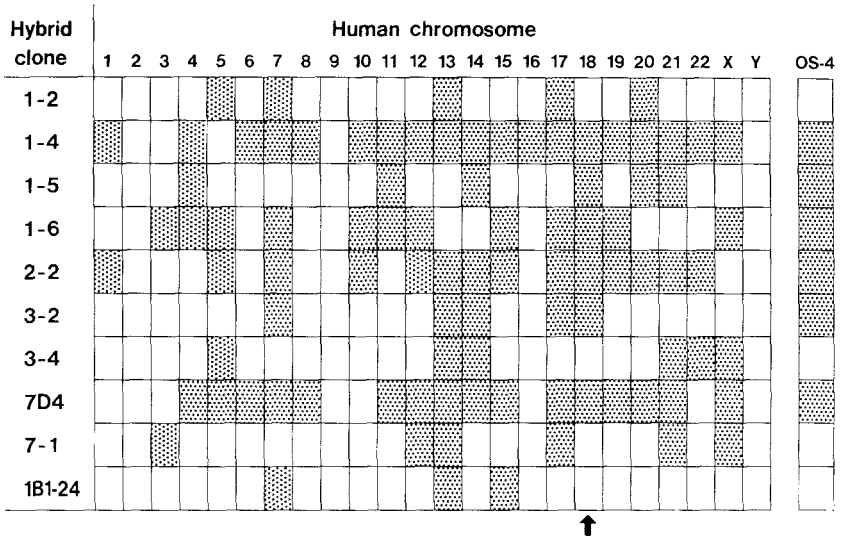


Fig. 2. Chromosomal assignment of the OS-4 probe using ten human-mouse somatic cell hybrids. Complete concordance was obtained with chromosome 18 as shown by an arrow.

after cleavage with *Hin*dIII endonuclease. No cross hybridization with mouse DNA was observed under the stringent condition of hybridization. Human DNA exhibited to segregate within the hybrid panel. As summarized in Fig. 2, the results were completely concordant only with the presence of chromosome 18, indicating that the OS-4 is located on chromosome 18.

To determine more precise location of the OS-4 segment on chromosome 18,

DNA from six cell lines with different chromosome 18 constitutions were hybridized with OS-4 and p1E8 (D13S4) probes. The probe p1E8 was used as an internal diploid standard for the measurement of band density. As shown in Fig. 3, each cell line exhibited different band intensity to both OS-4 and p1E8 probes and the data obtained by scanning densitometry were shown in Fig. 5. The density of the bands was about 0.5-fold in GM2054 and GM2980, and 1.5-fold in 7307L and 7953L compared with that in 8011L which is quantitatively normal diploid. In GM2980, the density was nearly equal to that in 8011L. Thus, these results indicate that the OS-4 segment is localized at chromosome region 18q21.3→qter.

Apart from this method, we examined RFLPs at the locus of OS-4 in the same six cell lines with chromosome 18 aberrations. If heterozygosity is observed in cell lines showing a partial deletion of chromosome 18, the locus can be excluded from

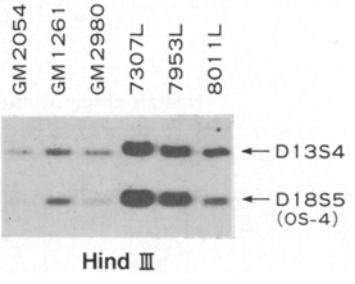


Fig. 3. Autoradiograms of quantitative hybridization to DNA from six cell lines with chromosome 18 abnormalities. Probe p1E8(D13S4) was used here as an internal diploid standard. Density of each band was measured by scanning densitometry.

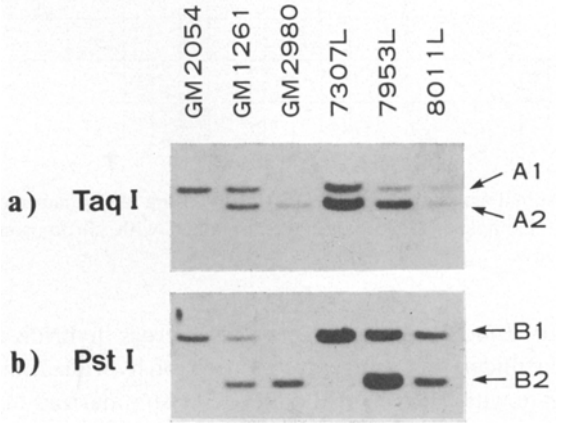


Fig. 4. Autoradiograms indicating maintenance of heterozygosity in the six cell lines containing chromosome 18 abnormalities. The density of the two polymorphic bands in 7953L was measured. a) *Taq*I-digested DNA, b) *Pst*I-digested DNA.

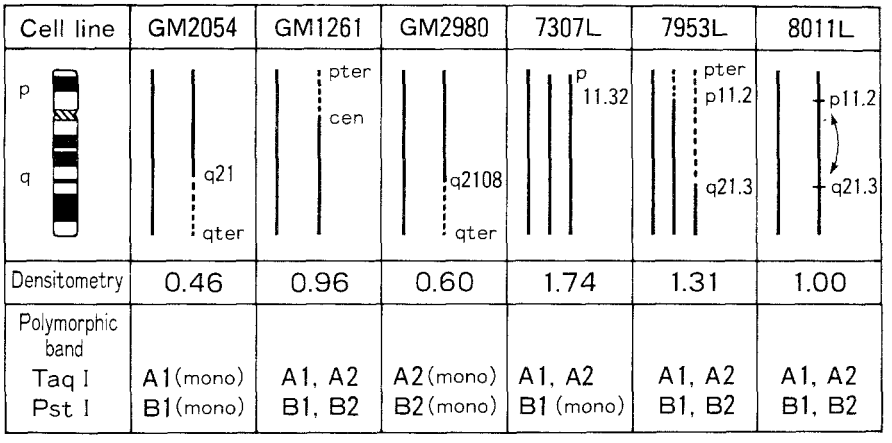


Fig. 5. Data of densitometric analysis and maintenance of heterozygosity in the six cell lines with indicated chromosome 18 constitutions. In schematic diagram, the solid and dotted lines indicate the presence and absence of the regions of chromosome 18, respectively. The arrow in 8011L indicates the pericentric inversion between the two break points.

Table 1. Genetic characteristics of OS-4 locus.

| Region | Enzyme | Allele | Length(kb) | Frequency | PIC |
|---|---|---|---|---|---|
| 18q21.3-qter | *Taq*I | A1 | 8.6 | 0.42 | 0.37 |
| | | A2 | 6.7 | 0.58 | |
| | *Pst*I | B1 | 7.5 | 0.65 | 0.35 |
| | | B2 | 4.8 | 0.35 | |

the deleted region. As shown in Fig. 4, heterozygosity was not observed in GM2054 and GM2980. Moreover, while the two polymorphic bands in 8011L were of equal density, one of the two polymorphic bands in 7953L, 6.7 kb band in *Taq*I-digested DNA (A2 in Fig. 4) and 4.8 kb band in *Pst*I-digested DNA (B2 in Fig. 4), showed approximately 2-fold density. This result indicates the presence of three copies of the locus homologous to OS-4 in the 7953L, confirming cytogenetical analysis of trisomic constitution of chromosome 18 at region q21.3→qter in this line.

These findings indicate that the locus homologous to OS-4 must lie in the region of 18q21.3→qter.

Genetic characteristics of OS-4 locus are listed in Table 1. The polymorphism information content (PIC) is 0.37 in *Taq*I and 0.35 in *Pst*I-digested human DNA.

## DISCUSSION

The polymorphic locus specified by the OS-4 DNA segment has been localized on chromosome 18q21.3→qter by the use of cell lines containing different con-

stitutions of chromosome 18. This regional mapping is based on the concurrence of chromosomal dosage associated with a partial deletion or duplication and DNA dosage in these lines, although DNA dosage was estimated from densitometric analysis of the autoradiographic bands. Heterozygosity for the OS-4 polymorphism in these lines is equally useful for the regional mapping.

OS-4, designated as D18S5, shows polymorphisms not only in *Taq*I but also in *Pst*I-digested human DNA. Although the PIC value is not so high in each restriction enzyme, frequent heterozygosity can be obtained when the two enzymes are combined.

Four DNA segments have already been assigned to chromosome 18 (HGM8, 1985). However, very few DNA segments that detect RFLPs have been regionally mapped to the long arm of chromosome 18. Numerous genes have been mapped to chromosome 18, including familial amyloid neuropathy and diastrophic dysplasia. We believe that the OS-4 RFLPs described here are useful in linkage studies with these and other genes located on chromosome 18.

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