Jpn J Human Genet 39, 441–443, 1994

RFLP Report

DINUCLEOTIDE REPEAT POLYMORPHISM AT THE D8S1055

Lin YE,[1] Koichi KIHARA,[1] Jun NAKURA,[1] Kouzin KAMINO,[1] Noriaki MITSUDA,[1] Asako TAKAHASHI-FUJII,[2] Yoshizumi ISHINO,[2] Tetsuro MIKI,[1] and Toshio OGIHARA[1]

[1]*Department of Geriatric Medicine, Osaka University Medical School, 2–2 Yamadaoka, Suita, Osaka 565, Japan*
[2]*Biotechnology Research Laboratories, Takara Shuzo Co., Ltd., 3–4–1 Seta, Ohtsu, Shiga 520–21, Japan*

A polymorphic dinucleotide (CA) repeat clone isolated from a chromosome microdissection library was mapped to chromosome 8p11.2-p12 using human-mouse cell hybrids and linkage analysis of 5 CEPH families.
Key Words microsatellite, chromosome microdissection, linkage map

A microdissected library of human chromosome 8p11.2-p22 was prepared and clones were screened for long dinucleotide repeats (Nagano *et al.*, 1993; Kamino *et al.*, 1993). A clone, pMS8-134, was sequenced and mapped on chromosome 8 using human-rodent cell hybrids. Complex dinucleotide repeats $(CA)_{18}$ was detected (GDB accession No. G00-341-901).

PCR primers. PCR primers were designed from sequences flanking repeat as MS8-134-F, 5′-CTTCTCCCGGTCATTTTTG-3′, and MS8-134-R, 5′-GGTAGGAGTAAGCATGGTAATTT-3′.

Polymorphism. Allele fragments were resolved on DNA sequencing gel. Allele frequencies were calculated from the genotypes of 50 unrelated Japanese individuals. Heterozygosity was 0.86. Allele frequencies were also calculated from the genotypes of 55 unrelated CEPH parents with heterozygosity of 0.82. Mendelian inheritance was observed in all cases.

Reference CEPH genotypes. 1424-01 113/99; 1424-02 115/99.

PCR condition. The reaction was carried out in a volume of 10 μl containing 20 ng genomic DNA, 2 pmol of rhodamine-labeled primers, 200 μM dNTP, 10 mM Tris-HCl pH 8.3 (25°C), 0.001% (w/v) gelatin, 1% deionized formamide, 1.5 mM $MgCl_2$, 50 mM KCl and 0.25 U Taq polymerase (Perkin-Elmer) for 35 cycles as

Received June 27, 1994; Accepted August 1, 1994.

Allele sizes and frequencies.

Allele	Sizes (bp)	Frequencies	
		Japanese	Caucasian
A1	125	0.02	0.00
A2	123	0.04	0.04
A3	121	0.03	0.04
A4	119	0.02	0.04
A5	117	0.07	0.07
A6	115	0.06	0.07
A7	113	0.01	0.20
A8	111	0.07	0.04
A9	109	0.36	0.20
A10	107	0.01	0.01
A11	105	0.02	0.00
A12	103	0.00	0.01
A13	101	0.04	0.00
A14	99	0.22	0.24
A15	97	0.02	0.00
A16	95	0.01	0.04

follows: 94°C for 30 sec, 55°C for 30 sec, and 72°C for 30 sec in a Gene Amp PCR system 9600 (Perkin-Elmer) and the images were obtained by scanning the gels with a fluorescent image analyzer FMBIO (Ishino *et al.*, 1992). The amplified product was fractionated in 6% polyacrylamide gel. Allele size were determined by comparison of each amplified band with DNA sequencing ladders of M13mp18 DNA.

Chromosomal localization. Localized to chromosome 8 using human-rodent cell hybrids. Linkage analysis with 5 CEPH families using data from the CEPH database v5 gave a maximum LOD score of 3.28 at theta=0.18 with D8S298 (AFM234yh10), 7.53 at theta=0.00 with D8S259 (AFM107yb2), 12.04 at theta=0.00 with D8S283 (AFM238yh12), 6.32 at theta=0.00 with D8S278 (AFM200ye1), and 11.73 at theta=0.02 with D8S255 (AFM023xc1), which indicated the location of D8S1055 at 8p11.2-p12.

Comments. A highly polymorphic clone, pMS8-134 (D8S1055), was mapped to 8p11.2-p12. Though there was no obligate recombination among D8S1055, D8S259, D8S283, and D8S278, flanking sequences from 4 microsatellites were different from each other. This result suggests that the D8S1055 is a new microsatellite.

References

Nagano K, Nakura J, Kihara K, Ye L, Kamino K, Mitsuda N, Ohta T, Jinno Y, Niikawa N, Miki T, Ogihara T (1993): Isolation and mapping of microsatellites from a library microdissected from the Werner syndrome region, 8p11.2-p22. Jpn J Human Genet **38**: 391–397

Kamino K, Nakura J, Kihara K, Ye L, Nagano K, Ohta T, Jinno Y, Niikawa N, Miki T, Ogihara T (1993): Population variation in the dinucleotide repeat polymorphism at the D8S360 locus. Hum Mol Genet **2**: 1751

Ishino Y, Mineno J, Inoue T, Fujimiya H, Yamamoto K, Tamura T, Homma M, Tanaka K, Kato I (1992): Practical application in molecular biology of sensitive fluorescence detection by a laser-excited fluorescence image analyzer. BioTechniques **13**: 936–943