J Hum Genet (1999) 44:414–415

BRIEF REPORT — POLYMORPHISM REPORT

Ken-ichi Morishima · Shinya Matsuura · Hiroshi Tauchi
Asako Nakamura · Kenshi Komatsu

A polymorphic CA repeat marker at the human 27-kD calbindin (*CALB1*) locus

Received: June 6, 1999 / Accepted: July 1, 1999

Abstract A polymorphic dinucleotide (CA) sequence was isolated from a BAC clone containing the human 27-kD calbindin (*CALB1*) gene at 8q21. This polymorphism will be a useful genetic marker to study genetic variations of the *CALB1* gene.

Key words 27-kD-Calbindin · CA repeat · 8q21 · Central nervous system · Polymorphism

Introduction

27-kD-Calbindin (*CALB1*) is an intracellular calcium-binding protein which is expressed in kidney, pancreas, and specific neurons in the central nervous system. The *CALB1* cDNA encodes a protein of 261 amino acids which contain four active calcium-binding domains (Parmentier et al. 1987). *CALB1* levels are reduced in the brains of patients with Hungtington's disease or Alzheimer disease (Seto-Ohshima et al. 1988; Ichimiya et al. 1988). Mice carrying a target disruption of the *CALB1* gene are severely impaired in tests of motor coordination (Airaksinen et al. 1997). These data suggested that *CALB1* may be an important regulator of neuronal degeneration in pathological processes. To facilitate genetic analysis of the *CALB1* gene in neuro-degenerative disorders, we characterized a dinucleotide repeat polymorphism in intron 3 of the *CALB1* gene.

Source and isolation of CA repeat sequence

A human BAC clone, 157K21, was isolated from the 8q21.3 genomic region (Matsuura et al. 1998). Shotgun sequencing and computer-based analyses revealed that the 77,425-bp insert contained the entire *CALB1* gene (Tauchi et al. 1999). A dinucleotide repeat sequence was identified in intron 3 of the gene. Polymerase chain reaction (PCR) primers were designed to flank this repeat sequence for polymorphism analysis.

PCR primers

Forward (CALB1CA1) 5′-TGAAAGAATGCAAAG-GTCACA-3′
Reverse (CALB1CA2) 5′-GGGGGTTTATAATGT-GCCAC-3′

PCR conditions

PCR was performed in a volume of 10 μl containing 20 ng genomic DNA, 10 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM dNTPs, 2.5 pmol of forward and reverse primer, 0.5 μM of infrared$_{770}$-9-dATP, and 0.25 units of EX *Taq* polymerase (Takara, Tokyo, Japan). Cycle conditions were 94°C for 5 min, then 30 cycles of 94°C for 45 s, 58°C for 20 s, and 72°C for 30 min, with a final extension step of 72°C for 10 min in a PC-800 thermal cycler (ASTEC, Tokyo, Japan). PCR products were electrophoresed in 0.25-mm-thick 6% polyacrylamide gels containing 8 M urea (Long Ranger gel; FMC, Rockland, ME, USA), at 2,000 V for 5–6 h, and analyzed by a DNA sequencer (model 4,000 L; LI-COR, Lincoln, NE, USA).

Polymorphism and allele frequency

Eight alleles were detected in 188 chromosomes of unrelated Japanese individuals. The observed heterozygosity was 0.78. The size and frequency of the eight alleles are shown in Table 1.

K. Morishima · S. Matsuura · H. Tauchi · A. Nakamura
K. Komatsu (✉)
Department of Radiation Biology, Research Institute for Radiation Biology and Medicine, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8553, Japan
Tel. +81-82-257-5811; Fax +81-82-256-7101
e-mail: komatsu@ipc.hiroshima-u.ac.jp

Table 1. Size and frequency of the eight alleles of the CA repeat polymorphism in the human 27-kD calbindin (*CALB1*) locus

Allele	Size (bp)	Frequency
A1	186	0.234
A2	184	0.021
A3	182	0.213
A4	180	0.122
A5	178	0.101
A6	176	0.138
A7	174	0.011
A8	172	0.160

Mendelian inheritance. Codominant inheritance was confirmed in three families.

Chromosomal localization. The human *CALB1* gene was assigned to human chromosome 8q21 (Parmentier et al. 1991).

Acknowledgments This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture of Japan.

References

Airaksinen MS, Eilers J, Garaschuk O, Thoenen H, Konnerth A, Meyer M (1997) Ataxia and altered dendritic calcium signaling in mice carrying a targeted null mutation of the calbindin 28 kD gene. Proc Natl Acad Sci USA 94:1488–1493

Ichimiya Y, Emson PC, Mountjoy CQ, Lawson DEM, Iizuka R (1988) Calbindin-immunoreactive cholinergic neurons in the nucleus basalis of Meynert in Alzheimer-type dementia. Brain Res 499:402–406

Matsuura S, Tauchi H, Nakamura A, Kondo N, Sakamoto S, Endo S, Smeets D, Solder B, Belohradsky BH, Der Kaloustian VM, Oshimura M, Isomura M, Komatsu K (1998) Positional cloning of the gene for Nijmegen breakage syndrome. Nat Genet 19:179–181

Parmentier M, Lawson DEM, Vassart G (1987) Human 27-kDa calbindin complementary DNA sequence: evolutionary and functional implications. Eur J Biochem 170:207–215

Parmentier M, Passage E, Vassart G, Mattei M-G (1991) The human calbindin 28 kD (CALB1) and calretinin (CALB2) genes are located at 8q21.3–q22.1 and 16q22–q23, respectively, suggesting a common duplication with the carbonic anhydrase isozyme loci. Cytogenet Cell Genet 57:41–43

Seto-Ohshima A, Emson PC, Lawson E, Mountjoy CQ, Carrasco LH (1988) Loss of matrix calcium-binding protein-containing neurons in Huntington's disease. Lancet I:1252–1254

Tauchi H, Matsuura S, Isomura M, Kinjo T, Nakamura A, Sakamoto S, Kondo N, Endo S, Komatsu K, Nakamura Y (1999) Sequence analysis of an 800-kb genomic DNA region on chromosome 8q21 that contains the Nijmegen breakage syndrome gene, NBS1. Genomics 55:242–247