Mitotic Checkpoint Locus *MAD1L1* Maps to Human Chromosome 7p22 and Mouse Chromosome 5

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Functional gene description: The human mitotic arrest deficient 1 (MAD1L1) protein has been characterized as an essential component of the mitotic spindle-assembly checkpoint that prevents the onset of anaphase until all chromosomes are properly aligned at the metaphase plate (3). MAD1L1 functions as a homodimer and interacts with MAD2L1. The induction of *MAD1L1* transcripts by p53 (5) and the abrogation of MAD1L1 function by human T-cell leukemia virus type 1 oncoprotein Tax (3) suggest that MAD1L1 has critical roles in cell cycle control and tumor suppression.

Name and description of clones or DNA: HsMAD1/TXBP181 cDNA has previously been described (3). HsMAD1a is a full-length cDNA encoding an isoform of human MAD1L1 protein. MmMAD1a and MmMAD1b are full-length cDNAs encoding two isoforms of mouse Mad1l1 protein.

DJ0826E18 is a human chromosome 7-specific PAC clone obtained from Roswell Park Cancer Institute, New York (URL: http://bacpac.med.buffalo.edu).

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Methods used to validate gene identity: The complete nucleotide sequences of cDNA clones HsMAD1/TXBP181, HsMAD1a, MmMAD1a, and MmMAD1b were determined (GenBank Accession Nos. U33822, AF083811, AF083812, and AF083813). These four cDNA clones share more than 75% identity in their nucleotide sequences.

Approximately 120 kb of assembled sequence from PAC clone DJ0826E18 was also obtained (Genome Sequencing Center, Washington University, St Louis, MO; URL: http://genome.wustl.edu/gsc). The *MAD1L1, FJH1,* and *MTH1* loci in DJ0826E18 were confirmed by specific PCR amplification using two separate pairs of oligonucleotide primers for each gene. Primers used for amplification of *MAD1L1* are 5′-CTGGAGGAAAGAGCAGAGCAGATCC-3′ and 5′-TGGTGCTGGCTGCTCTCTCCA-3′, and 5′-CGGGAATTCATGGAAGACCTGGGGGAA-3′ and 5′-ACCTGGGGCCGAGGTAGAA-3′. Both amplification products are 120 bp. Products of the same size were also obtained in the PCR amplification of total human or mouse genomic DNA using these two pairs of primers.

Flanking markers used: Human marker SHCG-33698 (used for radiation hybrid mapping; see below) was previously reported to be within 7 cM of human chromosome 7pter (URL: http://www.ncbi.nlm.nih.gov/genemap). Human markers for physical mapping were *FJH1* (an unidentified human homolog of bacterial cell-division protein FtsJ; GenBank Accession Nos. AA151905 and AA149768) and *MTH1* (2). Mouse markers *Zp3, Ccnb1rs1,* and *Atrc1* were typed as described previously (C. E. Buckler, and C. A. Kozak, Mouse Genome Informatics Resource, Mouse Genome Informatics, The Jackson Laboratory, Bar Harbor, ME. URL: http://www.informatics.jax.org/crossdata).

Methods of mapping: For RH mapping, the Stanford G3 panel (Research Genetics, Huntsville, AL) and PCR were used. Primers for the amplification of *MAD1L1* are 5′-CCCTCGAACTGAGAATTAC-3′ and 5′-CAGGTCTTCCATGGTTGC-3′. The amplification product is 565 bp. For physical mapping, human chromosome 7-specific PAC clone DJ0826E18 from the RPCI human PAC library (Roswell Park Cancer Institute, New York) was used. Relative positions of human *MAD1L1, FJH1,* and *MTH1* within DJ0826E18 were mapped by PCR and verified by DNA sequence data. For genetic mapping, MmMAD1b was used as a hybridization probe to follow inheritance of restriction enzyme size differences in the cross (NFS/N × *Mus spretus*) × *M. spretus* or C58/J.

Results: By RH mapping, the human *MAD1L1* locus was found to be linked (lod score = 11.5) to the chromosome 7 marker SHCG-33698 at a distance of 9.3 cR. The identified human PAC clone DJ0826E18 is positive for *MAD1L1, MTH1* (2), STS marker sWSS2995 (1), and *FJH1.* DJ0826E18 maps to 7p22. The human *MAD1L1* locus at 7p22 is <9 kb centromeric of the previously mapped *MTH1* locus (2), which contains the sWSS2995

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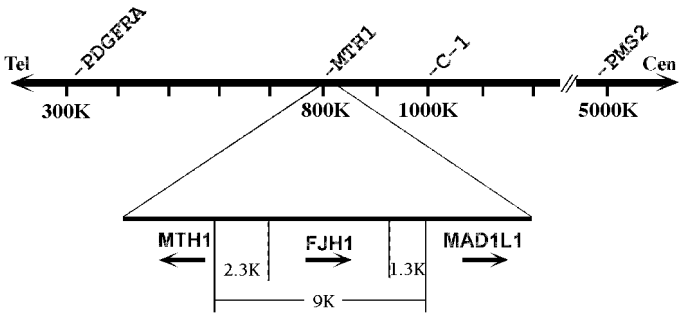

FIG. 1. Physical map of the human *MAD1L1* chromosome region at 7p22. Positional numbering (in kb) is anchored at the p telomere. PDGFRA, platelet-derived growth factor receptor α; MTH1, *mutT* homolog 1 (8-oxo-dGTPase); C-1, cell cycle-specific helix-loop-helix protein; PMS2, DNA mismatch repair gene (*mutL* homolog); FJH1, *ftsJ* homolog 1; Tel, telomeric; Cen, centromeric.

marker (1). *FJH1* is <1.3 kb telomeric of *MAD1L1* and <2.3 kb centromeric of *MTH1* (Fig.1). The orientation of the *MTH1* locus has been previously determined (1). This polarity is opposite to the tandem *MAD1L1* and *FJH1* genes. Thus *MAD1L1* is transcribed toward the centromere. Interestingly, all three genes (*MAD1L1, FJH1,* and *MTH1*) are involved in cell cycle control and DNA repair.

For the genetic mapping of mouse *Mad1l1,* MmMAD1b identified *Sca*I fragments of 16.5 and 6.8 kb in *M. spretus* and 9.0 and 6.5 kb in NFS/N. The variant fragments were mapped to distal chromosome 5 with the following gene order and distances: *Zp3*–2.3 ± 1.6–*Mad1l1*–2.6 ± 1.7–*Ccnb1rs1*–6.5 ± 2.6–*Atrc1.*

Additional comment: The human *MAD2L1* locus maps to 4q27 (4).

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Human *cerberus* Related Gene *CER1* Maps to Chromosome 9

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Functional description of gene: *CER1* encodes a cytokine member of the cystine knot superfamily which is related to the *Xenopus* head-inducing factor, cerberus (2, 3). Sequence comparisons suggested that *CER1* is the human homologue of the recently identified murine cerberus-related gene *Cer1/Cerl/Cerr1* (1, 2, 7) (Fig. 1A). The cerberus-related cytokines, together with the structurally similar proteins Dan and DRM/Gremlin, represent a distinct group of bone morphogenetic protein (BMP) antagonists that can bind directly to BMPs and inhibit their activity (1, 2, 6, 7, 9).

Name of clone: *CER1* was the clone studied.

Description of DNA: Human genomic DNA cloned in Lambda Dash II was used.

Source: A human genomic library in Lambda DASH II (Stratagene) was screened with a radiolabeled mouse *Cer1* cDNA fragment (2; GenBank Accession No. AF035579). Filters were hybridized at 65°C washed in 2× SSC/0.1% SDS at 65°C. Inserts from positively hybridizing phage were subcloned into pBluescript II SK(+/−) (Stratagene) and subjected to restriction analysis. The sequence of the two exons and the single intron of one clone was determined using an ABI automated sequencer. The sequence of this genomic fragment is described in GenBank Accession No. AF090189.

Method used to validate gene identity: Southern blot analysis of human genomic DNA showed that under strin-

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