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Identification of a Mouse Homolog for the Human Hereditary Haemochromatosis Candidate Gene

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Recently, a novel human major histocompatibility complex (MHC) class I-like gene (HLA-H) was reported as a candidate gene for human hereditary haemochromatosis, a recessive disease of iron metabolism with a remarkably high incidence in northern Europeans. Independently we have isolated this gene in the course of a search for new human MHC class I-related genes and named it MR2. Here we report a mouse homolog of this human gene. The mouse MR2 gene is similar to the human counterpart with an overall predicted amino acid sequence similarity of ~66% and it is expressed in various tissues as in human. The extra eight amino acid residues between the α1 and the α2 domains in the mouse molecule compared to the human counterpart can be explained by the creation of the coding sequence from the intron. While the human gene is located at the site telomeric to the MHC region on human chromosome 6, our study indicated the translocation of the mouse homolog from the site telomeric to the MHC on mouse chromosome 17 to chromosome 13 along with other genes. This mouse gene should be important in clarifying a possible role in iron metabolism.

Highly polymorphic MHC class I molecules play a critical role in the immune system by presenting antigenic peptides to T cells (1). However, in addition to polymorphic human MHC class I genes (HLA-A, B and C) and closely related oligomorphic genes (HLA-E, F and G), there are other divergent class I gene families, such as CD1 family (2), Znα2gp (3), FcRn (4), MIC family (5) and MR1 (6), and some of them possess unique functions.

We have been searching for new human MHC class I-related genes and along with MR1 (6), we have isolated a new human MHC class I-related gene, MR2, which is located at chromosome 6p22 determined by fluorescence in situ hybridization (FISH) analysis. However, recently, a human MHC class I-related gene (HLA-H) located telomeric of the MHC was reported by Feder *et al.* as a candidate gene for hereditary haemochromatosis (7), and this gene turned out to be identical to our MR2 gene. Hereditary haemochromatosis is a recessive disease with a defect in iron metabolism and is very common in northern Europeans (carrier incidence: about 1 in 10). Feder *et al.* identified the candidate gene by the analysis of linkage disequilibrium and found mutations in the gene from haemochromatosis patients. Iron overload, similar to hereditary haemochromatosis, displayed by the mice deficient in β2-microglobulin (8,9), that associates with heavy chains of MHC class I and its related molecules, seems to support the conclusion of Feder *et al.* However, a mouse homolog for this human gene has not been reported yet.

In the present study, we report the mouse MR2 gene, a homolog of the human candidate gene for hereditary haemochromatosis and this mouse gene should be useful for its functional study in iron metabolism.

MATERIALS AND METHODS

Isolation of human MR2 gene. DNA fragments were amplified by polymerase chain reaction (PCR) (10) from human placenta genomic DNA with two primers that correspond to the two highly conserved regions in the MHC class I α3 domain as described (6). The two primers are: 5′-TCAGGATCCTGYCGNGCNCAYGRNTTYTAYCC (N = A, T, G or C; Y = T or C; R = G or A)(forward) and 5′-GCAGAATTCNRYYTGR(A/T)ANGTNCCRTC (reverse) with *Bam*HI site and *Eco*RI site in its 5′ region, respectively. PCR products were cloned into Bluescript vectors (STRATAGENE). In addition to three MR1 DNA fragments, another MHC class I-like fragment was revealed in the sequenced amplified products. Northern analysis with this DNA fragment using Clontech human MTN blots revealed positive signals in various tissues. A human liver cDNA library (Clontech) was screened with this DNA fragment (11), and the nucleotide sequence of a positive clone (No.49, ~1.7 kb in length) was determined and we named this gene MR2. Sequencing was performed with an automated sequencer (4000L, LI-COR) and SequiTherm long-Read Cycle Sequencing Kits (EPICENTRE TECHNOLOGIES). HLA-H gene (Gen-

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Bank accession No.U60319), recently reported by Feder *et al.* (7), turned out to be identical to our MR2 gene. Our cDNA clone was devoid of six bases at the beginning of the coding sequence. The genomic clones were isolated from a human placenta genomic library (produced by partial digestion with *Sau*3AI). A *Hin*dIII-fragment (~3.7 kb in length) prepared from a phage clone contains a part of the α1 domain and all other coding exons and a part of the 3′-untranslated exon.

Isolation of mouse MR2 gene. A DNA fragment corresponding to the α1 domain of a mouse MR2 homolog was amplified by PCR from genomic DNA of the BALB/c mouse liver with two primers synthesized based on the human MR2 sequence as follows: 5′-GCAGAATTCTNCAYTAYCTNTTYATG (forward) and 5′-GCAGAATTCAT(R/T)ATNGTCCARAARTC (reverse) with *Eco*RI sites in their 5′ regions. ALB/C mouse lung cDNA library (Clontech) was screened with this mouse MR2 fragment that gave a single positive band in Southern blots with each of several restriction enzymes (such as *Eco*RI, *Hin*dIII and *Bam*HI) (data not shown). The nucleotide sequence of a cDNA clone (No.35, ~1.5 kb in length, GenBank accession No.U66849) containing all coding region was determined. A mouse genomic DNA fragment containing the intron between the α1 and α2 domains was obtained from genomic DNA of the BALB/c mouse liver by PCR with two primers as follows: 5′-GCAGGATCCTCACATTCTCTAAGATAC (forward, 5′ region of the α1 domain) and 5′-GCAGGATCCTTGCTGTCCCAGAACGCC (reverse, 3′ region of the α2 domain) with *Bam*HI sites in their 5′ regions. PCR reaction mixture (100 μl) contained 5 units of LA Taq polymerase (Takara), LA Taq buffer, 200 μM dNTP, 125 nM each primer and 1 μg of the template. PCR conditions were: 30 cycles of 94 °C 1 min, 55 °C 2 min, 72 °C 5 min. Nucleotide sequences of independently amplified fragments were analyzed to exclude PCR errors.

Northern analysis. ALB/C mouse MTN blot membrane was obtained from Clontech. Polyadenylated RNA (2 μg) from various tissues was probed with the α1 domain fragment of the mouse MR2 homolog described above.

FISH analysis. The probe for the human MR2 gene was a genomic *Hin*dIII fragment (~3.7 kb in length) described above. The probe for the human myelin/oligodendrocyte glycoprotein (MOG) gene (GenBank accession No.Z48051), which maps to the distal end of the MHC (12), was a genomic DNA fragment sandwiched between exon 4 and exon 7 (~3 kb in length) that had been amplified by PCR with two primers: 5′-GACAAGCTTGAAAACTTCGAGCAGAGATAG (forward) and 5′-CAGAAGCTTGTAGCTCTTCAAGGAATTGCC (reverse) with *Hin*dIII sites in their 5′ regions. The probe for the mouse MR2 gene was cDNA clone No.35 described above. FISH analysis was performed by the method described previously (13). The biotinylated human genomic DNA probes (MR2 and MOG) were hybridized to R-banded chromosomes from phytohemagglutinin-stimulated lymphocytes of normal donors. The biotinylated mouse cDNA probe (~1.5 kb) was hybridized to R-banded chromosomes from cultured splenocytes of male mice (BALB/c) prepared as described (14). After overnight hybridization, the slides were washed in 50 % formamide/2 × SSC at 42 °C for 10 min, followed by a wash in 1 × SSC at room temperature for 15 min. The slides were then blocked with 4 % bovine serum albumin/4 × SSC at 37 °C for 30 min. Signal amplification was achieved using rabbit anti-biotin (ENZO), fluorescein-labeled goat anti-rabbit IgG (ENZO) and Cy2-labeled donkey anti-goat IgG (Amersham). Chromosomes were counterstained with propidium iodide. Only twin-spot hybridization signals were scored.

RESULTS AND DISCUSSION

We isolated a human MHC class I-related gene, MR2, in the course of a search for new human MHC class I-related genes and this gene turned out to be identical to HLA-H gene recently reported by Feder *et al.* (7) as a candidate gene for human hereditary haemochromatosis (Fig.1). Feder *et al.* found mutations (cysteine-to-tyrosine substitution at amino acid 282) in haemochromatosis patients (7), that seem to disrupt the conformation of the molecule. The observation of iron overload displayed by the mice deficient in β2-microglobulin (8,9) appears to support that MHC class I-related molecules may play a role in iron metabolism.

Based on our human MR2 cDNA sequence, we isolated a mouse MR2 homolog (Fig.1) and the comaprison between human and mouse MR2 amino acid sequences revealed ~66 % of overall conservation between the two species (amino acid identity percentages are: 34 %, 78 %, 70 %, 70 % and 51 % for leader peptide, α1, α2, α3 and transmembrane/cytoplasmic domains, respectively). The mouse MR2 gene is expressed in various tissues (Fig.2) as observed in human (unpublished results and Feder *et al.* (7)).

As shown in Fig.1, the most structurally distinct region between the human and mouse MR2 molecules is that between the α1 and α2 domains. In this region, compared to the human molecule, extra eight amino acid residues are present in the mouse molecule. The comparison of the genomic DNA sequences between the human and mouse genes revealed that these extra amino acids-coding DNA sequences could be derived from the intron sequence because the DNA sequences at and aroud the beginning of the α2 domains from the human and mouse genes could be aligned (Fig.3) and other MHC class I and their related molecules resemble the human MR2 molecule in the beginning of their α2

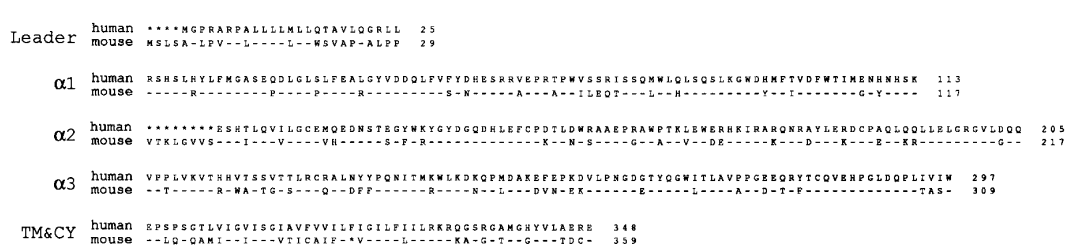

FIG. 1. Comparison of the predicted amino acid sequences between human MR2 (HLA-H by Feder *et al.* (7)) and mouse MR2 molecules. TM/CY denotes transmembrane/cytoplasmic domains. Dashes in the mouse sequence indicate the same amino acids as those in the human sequence. Amino acid numbers are shown on the right.

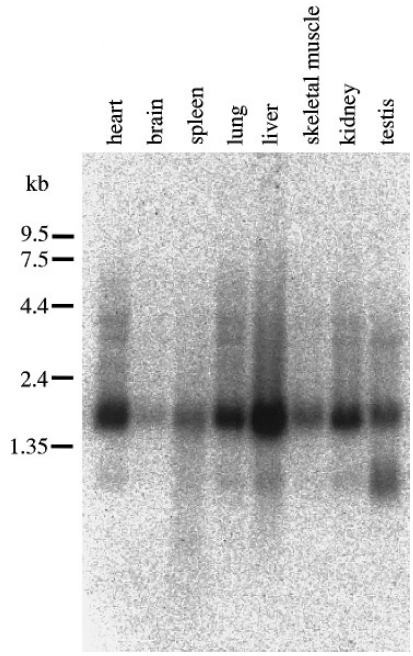

FIG. 2. Northern analysis of mouse MR2 expression. The mouse MR2 probe was hybridized to a membrane containing polyadenylated RNA from various mouse tissues indicated. Size markers are indicated on the left.

domains suggesting the human gene being the ancestral. A possible mutation at the splicing sinal preceding the α2 domain probably led to the use of another potential splicing site in mice. The region between the α1 and α2 domains in the mouse MR2 mature protein would form a long loop between the α-helix of the α1 domain and the β-sheet of the α2 domain. This portion of the molecule may not interfere with the function of this molecule.

The proline residue (amino acid position 188 in the human molecule) that is shared with FcRn molecule (4) and discussed by Feder *et al.* (7) is conserved in the mouse MR2 molecule. A break in the helix is introduced in FcRn by the presence of this proline residue resulting in its conformational rearrangement narrowing the cleft between the α1 and α2 helices compared to the classical class I molecules and FcRn does not bind a peptide (15). The MR2 molecule also may not bind a peptide and its possible function in iron metabolism may be carried out, as observed with FcRn (16), by the surfaces of the molecule but excluding the region between the α1 and α2 domains.

As shown in Fig.4, the chromosomal localizations of the human and mouse MR2 genes were determined by FISH analysis. For the localization of the probe for human MR2, 110 metaphase cells were examined. A total of 27 specific hybridization signals were located on chromosome 6. These signals were observed at the middle portion of band p22 (Fig.4(A)). Since it was difficult to define R-positive subband 6p22.2, the MR2 was assigned to 6p22. No other consistent hybridization sites were found. As for the human myelin/oligodendrocyte glycoprotein (MOG) gene, which maps to the distal end of the MHC (12), 73 metaphase cells were examined. Twelve twin-spot signals were located on chromosome 6 at the boundary region between band p21.3 and band p22.1 (Fig.4 (B)). From these results, the human MR2 locus on chromosome 6 was confirmed to be telomeric of the human MOG locus and these are consistent with the results by Feder *et al.* (7) who reported a physical map made by yeast artificial chromosome (YAC) clones and localized HLA-H gene (equivalent to MR2) 4 Mb telomeric of MOG. For the cDNA probe for mouse MR2, 120 metaphases were examined. Of a total of 35 twin-spot signals observed, 32 were detected on chromosome 13A2-A4 (Fig.4(C)). Three other nonspecific signals did not show any other consistent hybridization sites. Thus, the mouse MR2 gene is not on chromosome 17 on which the mouse MHC, H-2 region is present, although some investigators assumed that a mouse homolog for the hereditary haemochromatosis gene, like in human, also exists at the site close to the MHC region (9). Amadou *et al.* (17) reported that the region telomeric of the MHC in human is found on mouse chromosome 13 and they suggested that the human chromosomal organization would be the putative human/mouse ancestral one because structually homologous genes can be found around the breakpoint. In the mouse lineage, MR2 gene appears to have trans-

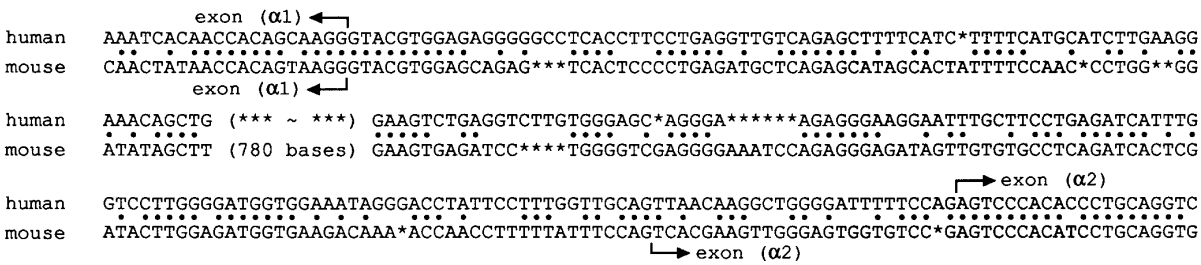

FIG. 3. Comparison of the MR2 genomic DNA sequences between the α1 and α2 domains from human (GenBank Accession No. U8O9I4) and mouse (GenBank Accession No. U8O6O4). Dots indicate the amino acids shared by the two species. Exon/intron boundaries are indicated by arrows.

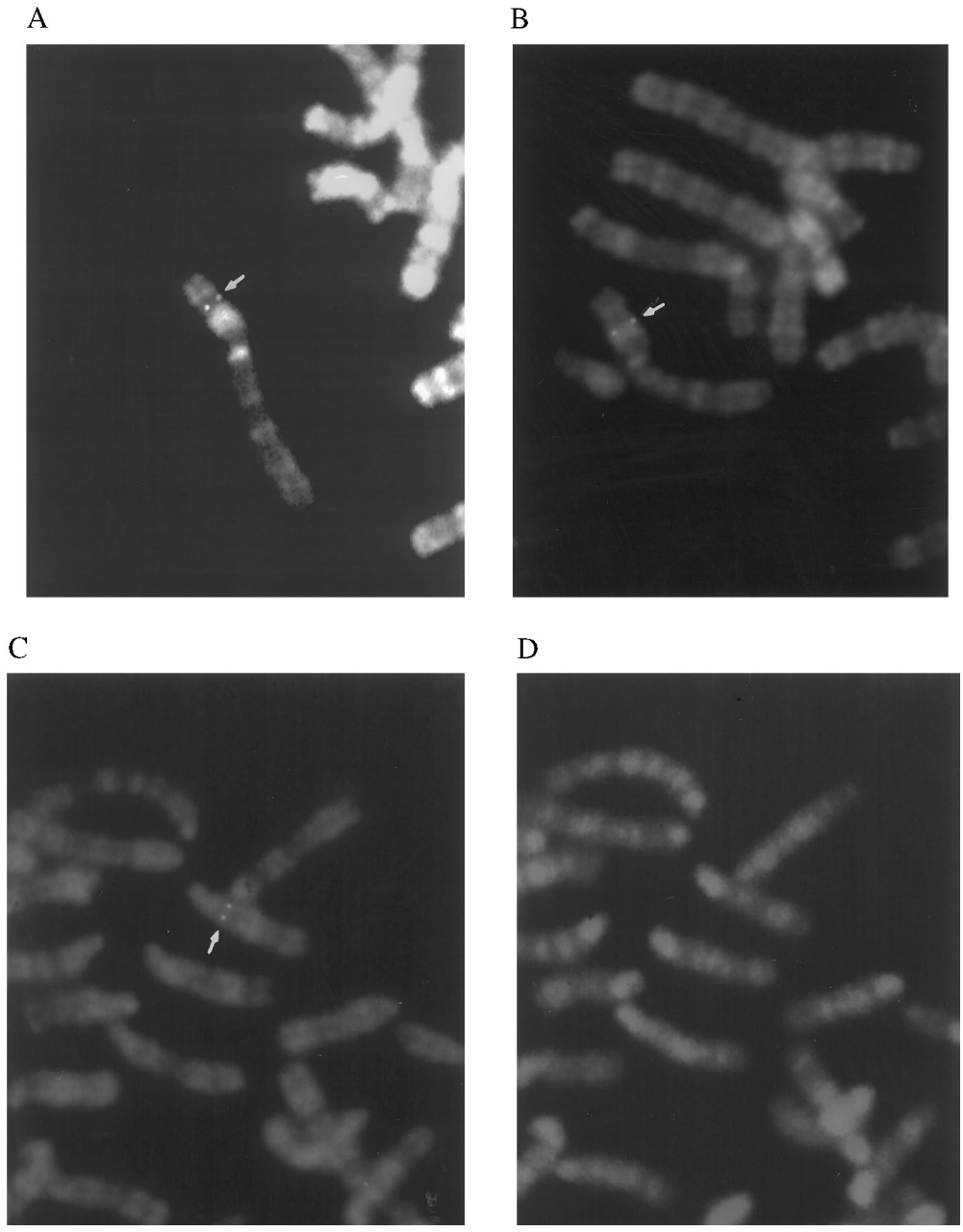

FIG. 4. Chromosomal localization of the genes for human MR2, human MOG, and mouse MR2 by FISH analysis. (A) Partial metaphase showing the hybridization site of human MR2 probe on chromosome 6 at region p22 (arrow). (B) Partial metaphase showing the hybridization site of human MOG probe on chromosome 6 at region p21.33-p22.1 (arrow). (C) Partial metaphase showing the hybridization site of mouse MR2 probe on chromosome 13 at region A2-A4 (arrow). (D) The same metaphase as (C) viewed with a UV filter for G-band analysis.

located to chromosome 13 along with other genes, such as Rfp, Btn and Fim1 as a conserved synteny unit (17).

In the present study, we identified the mouse MR2 homolog, a homolog for the human hereditary haemochromatosis candidate gene. Although mice deficient in β2-microglobulin exhibit similar symptoms of iron overload as hereditary haemochromatosis, a definitive answer for a role of this gene product in iron metabolism should be obtained with mice deficient in mouse MR2 homolog described in this study along with the investigation of its normal function.

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