SHORT COMMUNICATION

Gene Encoding the Collagen Type I and Thrombospondin Receptor CD36 Is Located on Chromosome 7q11.2

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The human CD36 is a member of a gene family of structurally related glycoproteins and functions as a receptor for collagen type I and thrombospondin. CD36 also binds to red blood cells infected with the human malaria parasite *Plasmodium falciparum*. In the present study, the CD36 gene was assigned to chromosome 7 by using the polymerase chain reaction with DNA from human–hamster somatic cell hybrids. Furthermore, the use of a CD36 genomic probe has allowed the localization of the CD36 locus to the 7q11.2 band by fluorescence *in situ* hybridization coupled with GTG-banding. © 1993 Academic Press, Inc.

CD36 is an 88-kDa cell surface glycoprotein expressed on a wide variety of cell types, including platelets, monocytes, microvascular endothelial cells, mammary gland epithelial cells, activated keratinocytes, some melanoma cells, and erythrocytes (6). CD36 expression increases in patients with myeloproliferative disorders (3) and is regulated during cell development and differentiation (5, 7, 11). CD36 acts as a receptor for collagen type I (12) and thrombospondin (1). *In vivo,* CD36 may participate in combination with the vitronectin receptor $\alpha_v\beta_3$ in the phagocytic elimination of aged neutrophils that have migrated to inflamed tissues (10). Interestingly, CD36 also binds to red blood cells parasitized with *Plasmodium falciparum,* the most pathogenic malaria parasite (8, 9). The severity of *P. falciparum* malaria correlates with the number of parasitized erythrocytes that bind to the capillary venular endothelium within the brain and lungs, causing cerebral malaria and pulmonary edema, the main mortality causes in malaria.

Structural similarities between CD36 and the rat lysosomal integral membrane glycoprotein II (LIMPII) allowed us to consider both glycoproteins as members of a new gene family (14), whose third member has been recently identified in our laboratory (2). Therefore, in addition to the important biological significance of the CD36 molecule, it deserves special attention to determine the relative chromosomal locations of the genes that constitute the CD36 family. As a first step to address this question, we determined the chromosomal location of the CD36 gene.

Screening by standard procedures of a human λEMBL-3 genomic library (Clontech) with CD36 cDNA (9) produced two independently hybridizing clones. One of them, with an insert of approximately 14.5 kb (designated λCD36-8), contained a *Bam*HI restriction site located about 2.3 kb from one of its ends. Sequencing of both ends of the *Bam*HI restriction site confirmed that it corresponded to the *Bam*HI site present at position 759 within the CD36 cDNA (9). The nucleotide sequence obtained allowed us to identify one exon of the CD36 gene (see Fig. 1A). This exon extended between nucleotides 640 and 819 from the cDNA and encodes for amino acids 144 to 203. Canonical splicing signals (AG and GT) were found at both ends of the exon. A detailed analysis with the whole structure of the isolated genomic clones will be described elsewhere.

Primers P1 and P2 were derived from the above nucleotide sequence (see Fig. 1A). Both primers were used to screen by polymerase chain reaction (PCR) a panel of human–hamster somatic cell hybrids (Bios Blot Chromosome Test Panel I; Bios Corp., CT). PCR was carried out with 250 ng of each DNA using the following thermal cycle: 94°C for 30 s, 58°C for 30 s, and 72°C for 20 s with extension of 1 s per cycle, for 32 cycles. Gel analysis of the PCR products is shown in the upper panel of Fig. 1B. An intense band of the expected size (200 bp) was amplified from hybrid 1006. The specificity of the amplified product was confirmed by hybridization of the transferred gel with oligonucleotide P3 (see Fig. 1A). As expected, only the bands derived from hybrid 1006 and from the human positive control UP131 hybridized with oligonucleotide P3 (see lower panel of Fig. 1B). Analysis of the human chromosomes present in hybrid 1006 but absent in the other hybrids allowed us to assign the CD36 gene to chromosome 7.

To localize in more detail the chromosomal region in which the CD36 gene maps, we performed *in situ* hybridization followed by GTG-banding. The whole λCD36-8 phage DNA labeled by nick-translation with digoxigenin-11-dUTP (Boehringer Mannheim, Mannheim, Germany) was hybridized to denatured human chromosomes as described (4). Of a total 79 metaphase spreads analyzed for the presence of fluorescent spots, 62 were

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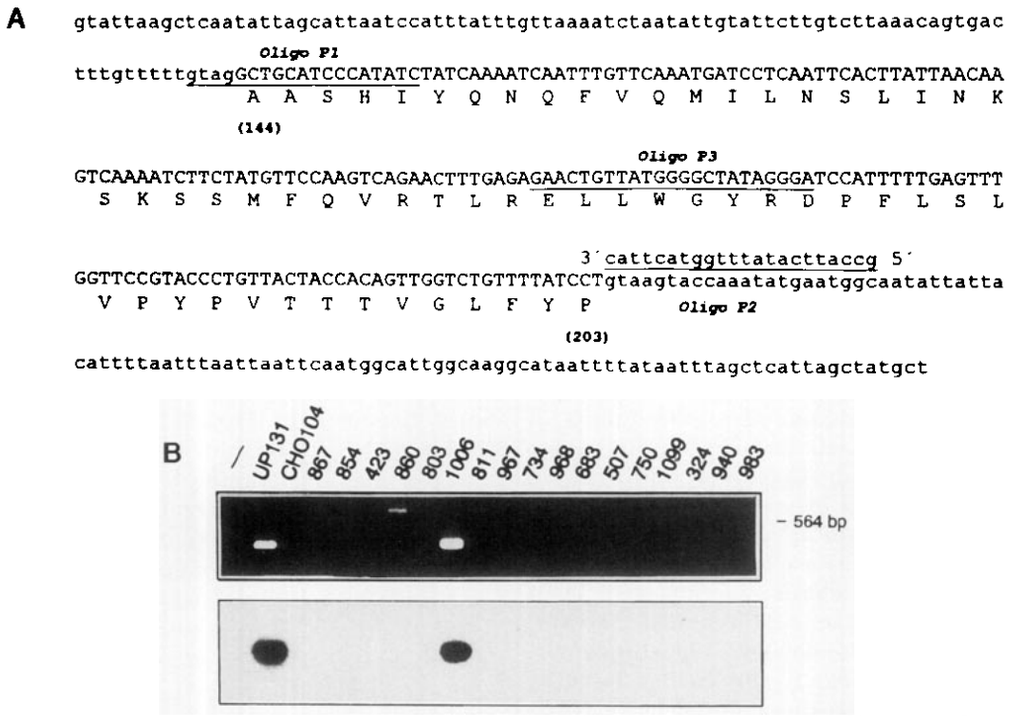

FIG. 1. (**A**) Nucleotide and deduced amino acid sequences of one exon and its flanking regions of the CD36 gene. Capital letters designate the nucleotides of the exon, lowercase letters the nucleotides of the introns. Oligonucleotides used for PCR and hybridization are underlined. Numbers in parentheses indicate the residue number within the amino acid sequence of CD36 (9). (**B**) PCR with DNA from somatic human–hamster cell hybrids. **Upper panel:** Agarose gel electrophoresis of the resulting PCR products. Names of the different hybrids used are denoted above, with the exception of UP131 and CHO104, which correspond to genomic DNAs from human (used as positive control) and hamster (used as negative control), respectively. Location of the 564-bp λ *Hin*dIII marker is indicated at the right. **Lower panel:** Autoradiography of the transferred gel shown in the upper panel and hybridized with the CD36-specific oligonucleotide P3.

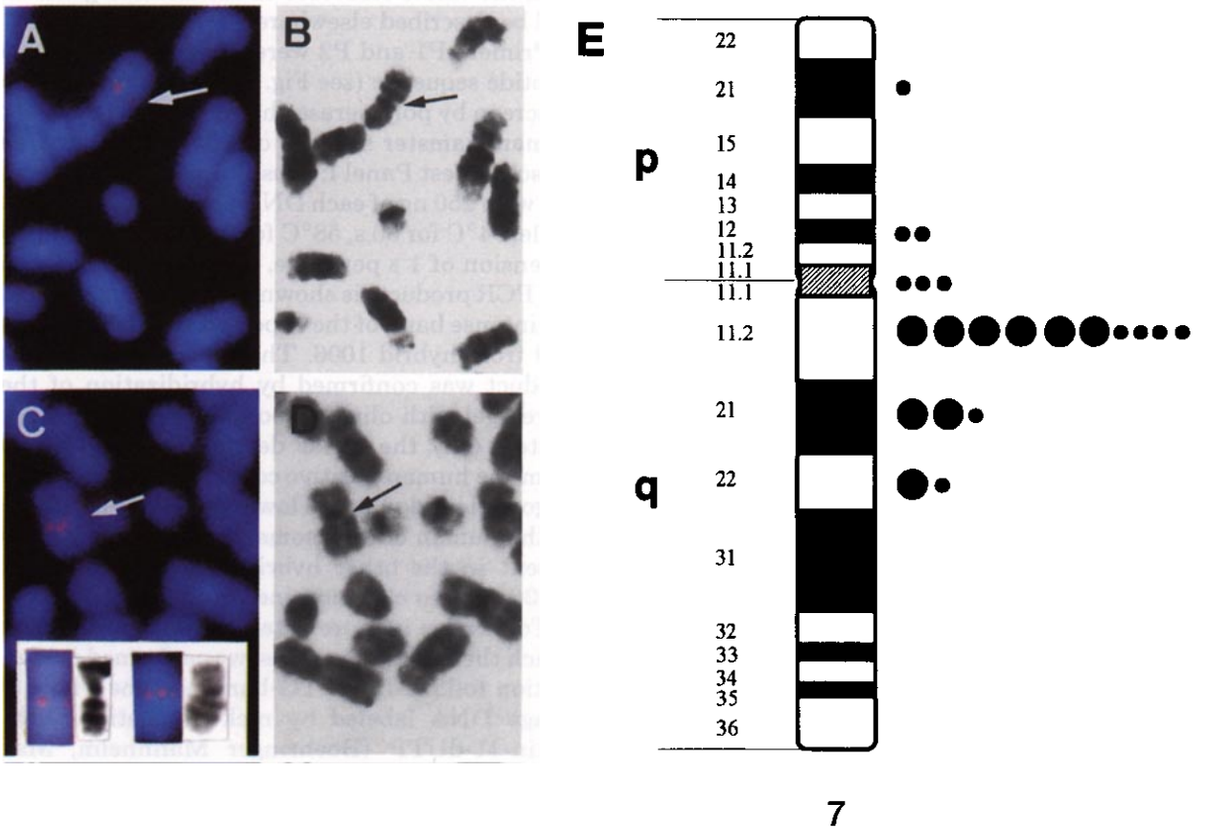

FIG. 2. Assignment of the CD36 gene to 7q11.2 by fluorescence *in situ* hybridization on human metaphases. Arrows indicate the specific sites of hybridization of the CD36 probe. (**A, C**) Digoxigenin-labeled probe hybridized to chromosomes was detected with anti-digoxigenin antibodies coupled to rhodamine. Chromosomes were counterstained with 4′,6-diamino-2-phenylindole (DAPI). (**B, D**) GTG-banding of the mitotic cell shown in **A** and **C**. The inset shows examples of DAPI- and GTG-banding of chromosome 7 from other cells. (**E**) The idiogram of human G-banded chromosome 7 illustrates the distribution of fluorescent hybridization signals for the CD36 locus. The large dots represent five fluorescent spots.

positive. The majority (78%) showed fluorescent signals on one or both chromatids of chromosome 7 (Figs. 2A and 2C). Twenty-eight metaphases were analyzed in detail, yielding a total of 92 fluorescent signals with an average of three signals per mitotic cell. Of the total fluorescent spots recorded, 59 (64%) were located on chromosome 7. To determine more precisely the regional localization of the fluorescent spots, GTG-banding was performed (Figs. 2B and 2D and insert). As summarized in Fig. 2E, 58% of the total chromosome 7 fluorescent spots appeared clustered on 7q11.2. From these data, we conclude that the CD36 gene maps to 7q11.2.

CD36 may be a helpful marker given the reduced number of genes so far mapped within region 7q11.2 (13). To our knowledge only the peroxisomal disease known as Zellweber syndrome maps to the 7q11 region. With respect to the gene localization of the characterized CD36 ligands, it is worth noting that the $\alpha 2$ chain of collagen type I also maps to the long arm of chromosome 7, at the adjacent telomeric G-band q21.

Chromosomal localization of the CD36 gene will acquire special relevance when the genes of the other CD36 structurally related proteins are mapped. That information will provide some insights into their evolutionary, and perhaps functional, relationships.

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