Assignment of the Human Adipocyte Fatty Acid-Binding Protein Gene (FABP4) to Chromosome 8q21 Using Somatic Cell Hybrid and Fluorescence in Situ Hybridization Techniques

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Adipocyte fatty acid-binding protein (A-FABP), also known as aP2 and adipocyte lipid-binding protein (ALBP), is a member of a well-characterized conserved family of intracellular lipid-binding proteins, constituting the so-called fatty acid-binding proteins (FABPs) and the retinoid binding proteins. FABPs are thought to bind fatty acids after their translocation to the cytoplasmic side of the plasma membranes and transport these ligands to cellular organelles for β-oxidation or for triacylglycerol or phospholipid synthesis (10). Currently, eight FABP types have been identified and are named by the tissue in which they were first detected (10). No structural mutants of FABPs have been identified except, recently, an alanine to threonine substitution in the human intestinal FABP, which is associated with increased fatty acid binding and transport, increased fat oxidation, and insulin resistance (1, 2). A similar dispersed location of FABP genes is found in the mouse. We report here that FABP4 (nomenclature according to the Genome DataBase), the gene coding for human A-FABP, maps to 8q21 on the basis of PCR screening of a panel of human–rodent somatic cell hybrids and fluorescence in situ hybridization on normal human metaphase spreads.

Human A-FABP was purified and its cDNA cloned and sequenced previously (3). To establish the chromosomal localization of FABP4, two primers (5'-AGC ACC ATA AGC TTA GAT GG-5'; 3'-ATA CTC TCT CTT ATT CCG TTC-5') were developed to PCR amplify FABP4 intron 3 and flanking parts of exon 3 and exon 4 from human genomic DNA. Sequence analysis of the amplified 650-bp fragment using the Sequenase kit (USB) revealed identity to FABP4 intron 3. This fragment was used as a probe to screen a human genomic library in λ EMBL3. One positive clone (A181) of approximately 14 kb was obtained. Direct sequencing on its DNA using the fmol DNA Sequencing system (Promega) and the primers mentioned before revealed the sequences of intron 3 and flanking parts of exon 3 and exon 4 of FABP4. Clone A181 DNA was subjected to Southern blot analysis using three different human FABP4-specific probes, the FABP4 intron 3 fragment, the entire FABP4 cDNA, and an oligonucleotide that contains codons 7–13 (exon 1) of the FABP4 coding region. All three probes hybridized to the A181 clone DNA.

A somatic cell hybrid panel, which consists of monochromosomal hybrid cell lines of the NIGMS Mapping Panel 2 (Coriell Cell Repository), was analyzed by PCR using the FABP4 intron 3-specific primers. A strong signal of the expected size was detected in the monochromosomal cell line containing human chromosome 8 and a minor but reproducible signal in the DNA of a monochromosomal cell line containing human chromosome 20 (not shown). This minor signal could be due to a low-frequency presence of (parts of) other human chromosomes in this hybrid line. No background signal due to endogenous FABP4 gene amplification was detected in the mouse and hamster DNAs. To confirm the chromosomal location, we used fluorescence in situ hybridization (FISH), performed essentially according to published procedures (8, 9). Briefly, λ EMBL3 DNA (1 µg) was labeled with biotin-14—dATP (Gibco, Life Technologies) and the chromosome 8 centromere probe (D8Z2) with dig-11—dUTP (Boehringer) using a nick-translation kit (Gibco, Life Technologies). A total of 250 ng λ EMBL3 DNA and a 50-fold excess of Cot-1 DNA (Gibco, Life Technologies) were dissolved in 6 µl of a hybridization mixture (2× SSC/10% dextran sulfate/1% Tween-20/60% formamide). Prior to hybridization, the probe was heat-denatured and then incubated at 37°C for 30 min allowing...
preannealing. For D8Z2 20 ng DNA in 6 μl hybridization solution was used per reaction, and no competitor DNA was added. Metaphase spreads were prepared according to standard procedures. After denaturation of the slides, probe incubations were carried out under a 20 × 20-min coverslip in a moist chamber for 48 h. Immunocytochemical detection of the EMBL3 probe was achieved using fluorescein isothiocyanate (FITC)-conjugated avidin (Vector Laboratories), followed by two amplification steps using rabbit-anti-FITC (DAKO) and mouse-anti-rabbit-FITC (Jackson Immunoresearch Lab.). The D8Z2 probe was detected using anti-digoxigenin-rhodamine Fab fragments (Boehringer) and donkey-anti-sheep-Texas Red (Jackson Immunoresearch Lab.). Counterstaining of the chromosomes was done by 4',6-diamidine-2'-phenylindole dihydrochloride (DAPI). A Zeiss epifluorescence microscope equipped with appropriate filters for visualization of Texas Red, DAPI, and FITC fluorescence was used. Digital images were acquired using a high-performance cooled CCD camera (Photometrics) coupled to a Macintosh Quadra 950 computer and further processed using the BDS-Image FISH software package (Oncor). Twenty normal metaphases were analyzed, and over 75% showed a signal for the centromeric probe of chromosome 8 and an additional FABP4 signal in the region 8q21 (Fig. 1). No signal was observed on chromosome 20 in any of the chromosome preparations studied.

Interestingly, the PMP2 (peripheral myelin protein 2, myelin FABP) gene maps to the same chromosomal region (8q21.3–q22.1) (4). Phylogenetic studies have revealed a high degree of similarity between the human myelin and adipocyte FABPs (7), and comparison of the coding sequences showed a 73% homology (10). Using PMP2 cDNA as a template, the primer set did not yield any amplification product. Furthermore, the amplified FABP4 intron 3 fragment was 650 bp while intron 3 of the PMP2 gene was reported to be approximately 1200 bp (4). From this, we conclude that the coinciding chromosomal locations do not result from cross-hybridization of FABP4 and PMP2 gene sequences.

There exists an extended synteny between human chromosome 8 and mouse chromosome 3, and the localization of FABP4 on human chromosome 8 is consistent with the localization of the mouse FABP4 (Ap2) gene on chromosome 3 (5). In both cases, the FABP4 gene has been found in close proximity (2.2 cM in mouse) to the carbonic anhydrase II gene. The latter gene is located on human chromosome 8q13–q22.1 (11). These data confirm the syntenic origin of this chromosomal segment.

We localized the FABP4 gene to human chromosome 8q21 using somatic cell hybrid and fluorescence in situ hybridization techniques. More detailed mapping of the FABP4 and PMP2 genes should shed further light on their genomic organization and their possible common origin.

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Fine Chromosome Mapping of the Genes for Human Liver and Muscle Carnitine Palmitoyltransferase I (CPT1A and CPT1B)

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Long-chain fatty acyl-CoAs are transported into mitochondria for oxidation via the carnitine palmitoyltransferase system, which consists of three components: the outer mitochondrial membrane protein, carnitine palmitoyltransferase I (CPT I), and the two inner membrane proteins, carnitine palmitoyltransferase II (CPT II) and a carnitine-acyl-carnitine translocase (14). Through its inhibition by malonyl-CoA, the first committed intermediate in fatty acid biosynthesis, CPT I subserves a key regulatory function for the overall system (14). In diabetes mellitus, this regulation is disrupted, leading to excessive fatty acid oxidation, which, in addition to causing ketoacidosis, impacts negatively on glucose homeostasis, raising the possibility that pharmacological inhibition of CPT I might be of therapeutic benefit for such conditions (1, 13). In addition, genetic defects in the CPT system can lead to serious, and sometimes fatal, disturbances in fatty acid oxidation (4, 17, 18). Following the isolation of a cDNA for rat liver mitochondrial CPT II (19) and its human counterpart (7), the human liver CPT II gene has been characterized in detail (18). While there seems to be a single isoform of CPT II, it has been suspected for some time that CPT I exists in at least two forms, the liver (L-CPT I) and muscle (M-CPT I) variants, which exhibit very different kinetic properties and sensitivity to malonyl-CoA (15). cDNAs encoding rat and human L-CPT I (~88 kDa) have now been cloned (2, 5), as has the cDNA for rat M-CPT I (6, 20). Curiously, the muscle protein (also predicted to be ~88 kDa) was found to be expressed in rat brown and white adipocytes as well (6).

Using the cDNA for rat muscle mitochondrial CPT I as a probe, we isolated its counterpart from a human heart cDNA library using standard methodology (16). Both the nucleotide sequence of the human cDNA and the predicted primary structure of the protein (GenBank, Accession No. U62733) proved to be very similar to those of the rat enzyme (84.6 and 85.9% identity, respectively). Screening of a human genomic library with the newly obtained cDNA yielded a positive clone from which a 2.3-kb fragment was analyzed. Partial sequencing revealed the presence of two complete exons, three complete introns, and fragments of at least two additional exons.

To determine the chromosomal locus of the M-CPT I gene, fluorescence in situ hybridization (FISH) was employed (10, 11). Briefly, the 2.3-kb human M-CPT I fragment was subcloned into a plasmid vector and labeled with biotin–dATP using the BRL BioNick labeling kit. After overnight hybridization in a mixture that contained 50% formamide, 10% dextran sulfate, and labeled probe, slides of fixed human lymphocytes (blocked at metaphase) were washed and subjected to the procedures described previously for detection of FISH signals and the DAPI (4', 6-diamidino-2-phenylindole) banding pattern (12). Under the conditions used, the hybridization efficiency of the human muscle gene fragment probe was approximately 95% (among 100 checked mitotic figures, 95 showed signals on one pair of the chromosomes). Since the DAPI banding pattern was used to identify the specific chromosome, assignment of the signal from the probe to the long arm of chromosome 22 was possible. No additional locus was detected. Analysis of 10 independent photographs localized the gene to chromosome 22q13.3 (Fig. 1). Further refinement of the gene's position was obtained by radiation hybrid mapping (RHM) using the Stanford G3 (3) and Genebridge G4 (9) hybrid panels together with oligonucleotide primers specific for an exon and its contiguous downstream intron within the M-CPT I gene. The G3 vector placed the M-CPT I gene 8.5 G3 cR (1 G3 cR = 30 kb) from the arylsulfatase gene that maps to 22q13.31 on the Location Data Base (LDB) integrated map. The G4 vector localized M-CPT I at 0.1 G4 cR (1 G4 cR = 270 kb) from the Whitehead framework marker WI-8917 (D22S1254), indicating that it resides within regions 22q13.31 and 22q13.32 on the LDB map.

We had previously mapped the gene for human L-CPT I to chromosome 11q, based on somatic cell hybridization studies (2). Applying the FISH procedure as described above and using a fragment of the L-CPT I genomic clone (2) as a probe, the gene was readily located in the region of 13.1 to 13.5 of chromosome 11q (Fig. 1). This conclusion is supported by RHM studies that placed the gene within 1 Mb of the D11S987 marker on the LDB map of chromosome 11 (studies conducted by D. K. Burns and D. P. Yarnall in another context and to be published elsewhere).

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2 The HGMW-approved symbols for the genes described in this paper are CPT1A and CPT1B.