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Chromosomal Localization and Partial Genomic Structure of the Human Peroxisome Proliferator Activated Receptor-Gamma (hPPARγ) Gene

Brock A. Beamer,* Carlo Negri,* Chung-Jen Yen,* Oksana Gavrilova,† John M. Rumberger,‡ Michael J. Durcan,‡ David P. Yarnall,‡ Anita L. Hawkins,§ Constance A. Griffin,§ Daniel K. Burns,‡ Jesse Roth,*,‡ Marc Reitman,‡ and Alan R. Shuldiner*

**Division of Geriatric Medicine and Gerontology and §Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, Maryland; †Diabetes Branch, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, Maryland; and ‡GlaxoWellcome, Research Triangle Park, North Carolina*

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We determined the chromosomal localization and partial genomic structure of the coding region of the human PPARγ gene (hPPARγ), a nuclear receptor important for adipocyte differentiation and function. Sequence analysis and long PCR of human genomic DNA with primers that span putative introns revealed that intron positions and sizes of hPPARγ are similar to those previously determined for the mouse PPARγ gene[13]. Fluorescent in situ hybridization localized hPPARγ to chromosome 3, band 3p25. Radiation hybrid mapping with two independent primer pairs was consistent with hPPARγ being within 1.5 Mb of marker D3S1263 on 3p25-p24.2. These sequences of the intron/exon junctions of the 6 coding exons shared by hPPARγ1 and hPPARγ 2 will facilitate screening for possible mutations. Furthermore, D3S1263 is a suitable polymorphic marker for linkage analysis to evaluate PPARγ's potential contribution to genetic susceptibility to obesity, lipoatrophy, insulin resistance, and diabetes.

Peroxisome proliferator activated receptor-gamma (PPARγ) is a member of the steroid hormone receptor super family. This receptor plays a pivotal role in adipocyte differentiation and fat deposition, and also may be a modulator of insulin signaling. Stimulation of PPARγ by its putative endogenous ligand, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2, results in activation of the regulatory domains of adipocyte-specific genes, including adipocyte P2 and phosphoenolpyruvate carboxykinase genes [1-6]. Thiazolidinedione drugs, some of which are under active study as agents to lower blood glucose, are high-affinity activating ligands for PPARγ and are presumed to exert their insulin sensitizing and adipogenic actions through this receptor [7-12].

Two isoforms of PPARγ (PPARγ1 and PPARγ2) are produced by the use of two discrete promoters and alternate splicing of a single gene, which in mouse (mPPARγ) contains 9 exons[13]. Human PPARγ2, compared to PPARγ1, contains an additional 28 amino acids at its amino terminus [12]. In mPPARγ1, the two most upstream exons contain the 5′ untranslated region and are spliced to the six downstream exons which encompass the common coding region and the 3′ untranslated region shared by mPPARγ1 and γ2. In mPPARγ2, the unique 5′ untranslated region and amino terminal extension are encoded by a single exon which is spliced to the same six downstream exons[13]. In order to study PPARγ as a candidate gene for human obesity, lipoatrophy, insulin resistance and diabetes mellitus, we now report the genomic structure of the common coding region of the human PPARγ (hPPARγ) gene and refinement of its chromosomal localization.

MATERIALS AND METHODS

Determination of genomic structure and estimation of intron sizes. Sense and antisense PCR primers were prepared from the published hPPARγ1 cDNA sequences [12, 14] in regions thought likely (by comparison to the mPPARγ gene [13]) to flank exon-exon junctions (Table I). Using human genomic DNA as template and primer pairs from putative adjacent exons, long PCR was performed, according to the manufacturer's directions (TaKaRa Biomedicals, Otsu, Shiga, Japan). Products were subjected to agarose gel electrophoresis and Southern blot analysis with ^{32}P-labeled internal oligonucleotide probes, and the sizes of the hybridizing products were estimated by comparison to a 1 kilobase DNA size standard[15].

Cloning and sequence analysis of exon-intron junctions. A human P1 clone containing the PPARγ gene was isolated by screening a

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TABLE I

Oligonucleotide Primers and Probes Used to Define Intron Sizes of the Human PPARγ Gene
(Also See Legend for Figure 1)

Exon → Exon	Sense primer from proximal exon (5′ → 3′)	Anti-sense primer from distal exon (5′ → 3′)	Internal oligonucleotide probe (5′ → 3′)
1 → 2*	GTGGGCCGCAGAAATGACCATGG	CATCCTTCACAAGCATGAACTCC	ATTGAATGTCGTGTCTGTGGAG
2 → 3*	GTGGAGCCTGCATCTCCACC	TGAGACATCCCCACTGCAAGGC	GGGTGATGTGTTTGAACTTGATT
3* → 4	CCGGAGAACAATCAGATTGAAGC	CGCCTTTGCTTTGGTCAGCGG	GTAGAAATAAATGTCAGTACTG
4* → 5	GGATCGCACAGGCCGAGAAGG	CTCCCTTGTCATGAAGCCTTGG	TACATAAAGTCCTTCCCGCTGA
5 → 6*	AGGAGCAGAGCAAAGAGGTGG	GTAGATCTCCTGCAGGAGCGG	AGCTCCAGGGCTTGTAGCAGGTT

* Demarks the exon to which the internal oligonucleotide probe hybridized.

genomic library by PCR (clone DMPCHFF #1-0475-A4, Genome Systems, St. Louis, MO). To facilitate sequencing, the P1 clone was digested with each of several restriction enzymes (Hind III, Eco R1, Xba I, and Bam H1), and the fragments were subcloned into Bluescript. Clones containing each exon (and flanking intronic sequences) were selected by dot blot analysis with ^{32}P-labeled oligonucleotide probes from each putative exon. Relevant clones were subjected to dideoxy sequence analysis on an Applied Biosystems 377 automated sequencer using standard methods.

Chromosomal localization by fluorescent in situ hybridization (FISH). A Bluescript plasmid containing a 5.5 kb genomic insert encompassing exon 4 of the hPPARγ gene was nick-translated with biotin-14 dATP (Bethesda Research Laboratories, Gaithersburg, MD) and hybridized to chromosome spreads from normal male lymphocytes cultured with BrdU [16]. FISH was performed as described [17] with modifications. The biotinylated probe was detected with FITC-avidin and amplified with biotinylated anti-avidin, using standard reagents (Oncor Inc., Gaithersburg, MD).

Radiation hybrid mapping. Radiation hybrid mapping was used to refine the chromosomal localization of the hPPARγ gene. Two independent primer pairs were optimized for mapping in the Stanford G3 and Genebridge G4 radiation hybrid panels (Research Genetics, Huntsville, AL). One primer pair (5′-ATG ACC AAA GTG GTA GAC AG-3′ and 5′-CGC CTT TGC TTT GGT CAG CGG-3′) amplified a 300 bp product comprising a portion of exon 4 and its adjacent 5′ intron, and the other primer pair (5′-GAC AAC CTG CTA CAA GCC CTG G-3′ and 5′-CAG AAT AGT GCA ACT GGA AGA-3′) amplified a 240 bp product comprising portions of the coding region and the 3′ untranslated region in exon 6. Radiation hybrid map marker retention data was analyzed using RHMAP Version 2.01 for the G3 data, or the radiation hybrid mapping web server at the Whitehead Institute/MIT Center for Genome Research for the G4 data [18-20].

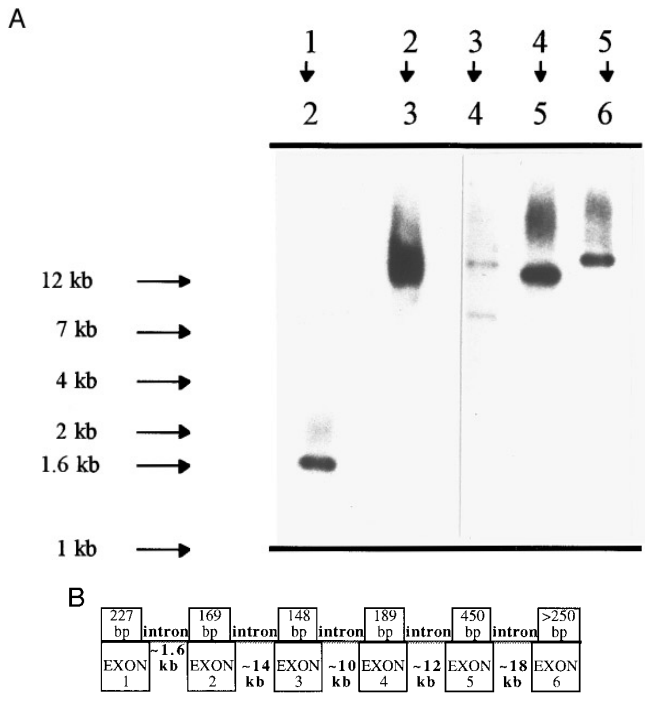

FIG. 1. Sizes of hPPAR-γ1 introns and coding exons. (A) Autoradiogram from Southern blot analysis of products of long PCR across introns of the hPPAR-γ1 gene with radiolabeled oligonucleotide probes corresponding to cDNA sequence internal to the PCR primers (also see Table I). (B) Sizes of PCR products shown in A were compared to a 1-kb ladder and intron sizes estimated.

RESULTS AND DISCUSSION

Long PCR from human genomic DNA across putative exon-intron junctions as predicted from the mouse genomic structure revealed that the coding region exons that are shared by hPPARγ1 and hPPARγ2 are encoded by six exons. The intron sizes of the human PPARγ gene are similar to the mouse PPARγ gene (Figure 1)[13]. Isolation and characterization of a P1 clone containing the human PPARγ gene confirmed the positions and sequences of the exon-intron junctions (Table II). All junctions obeyed the gt . . . ag rule. The positions of the introns in the human PPARγ gene were similar to the mouse PPARγ gene [13]. Comparison of our exon sequences to the published cDNA sequence of hPPARγ1 [14] revealed three differences: a GC to CG transversion at nucleotides 108-109, a GC to CG transversion at nucleotides 639-640, and an ATG insertion following nucleotide 719. None of these differences were adjacent to exon-intron splice junctions. These sequence differences (and the nucleo-

TABLE II

Sequences of Exon–Intron Boundaries and Approximate Intron Sizes of the Human PPARγ Gene

Donor DNA sequence and translation product	Intron (3′)	Intron size	Intron (5′)	Acceptor DNA sequence and translation product	Exon size (nt #)*
			. . . cta gtc tat ttt tcc ttt cag	AA ATG ACC . . .	1) 227 bp
. . . TAC CAA A				*2/3E M T*	(83–310)
Y Q 1/3S	gta tga tgt tta ttt tca ctt . . .	~1.6 kb	. . . ctc aca tgt ctc cat aca cag	GT GCA ATC . . .	2) 169 bp
. . . GGA TGC AAG				*2/3S A I*	(311–480)
G C K	gta att aaa aaa aaa gtc ttc . . .	~14 kb	. . . ttc ttt ttt atc cct ttg cag	GGT TTC TTC . . .	3) 148 bp
				G F F	(481–619)
. . . CAT AAT G				CC ATC AGG . . .	4) 189 bp
H N 1/3A	gta agt aaa cag tca tca cca . . .	~10 kb	. . . gtc att cct ctt cct cta tag	*2/3A I R*	(620–819)
. . . GAC AAA TCA				CCA TTC GTT . . .	5) 450 bp
D K S	gtt agt tct ctt ctg ctg tct . . .	~12 kb	. . . ttt tcc ctg ttt tat ttg cag	*P F V*	(820–1270)
				AC CGC CCA . . .	6) 247 bp
. . . AGT GGA G				*2/3D R P*	(1271–1518)
S G 1/3D	gta aga ttt gtc ttt tga tct . . .	~18 kb	. . . ttt cca tat gtg ctt ccc cag		

* Nucleotide numbers (nt #) are based on that of Elbrecht, et al. (12).

FIG. 2. Ideogram of human chromosome 3 p-arm, showing localization of human PPARγ gene to chromosome 3p25. Each dot represents a paired signal clearly located on a single band on metaphase chromosomes. Four other signals were bracketed (on p25-26 and p24.3-25) and left off ideogram per CAG.

tide numbers used here) are identical to those recently described by Elbrecht et al [12]. Differences between sequences found by Greene et al [14] from cDNA and those found by Elbrecht et al from cDNA and again by us from genomic DNA, may be explained by sequence polymorphism, RNA editing or, more likely, sequencing errors.

FISH analysis with G-banding by fluorescence plus Giemsa revealed 26 paired signals. Of these, 25 were on chromosome 3 on bands 3p24.3-26, with the majority (19/25 = 76%) on band 3p25, consistent with previous findings [14] (Figure 2). The remaining signal was on chromosome 6q. Radiation hybrid mapping confirmed localization of the hPPARγ gene to chromosome 3p24.2-p25 [14, 20]. Breakage probabilities and distance estimates in two independent panels from RH vector analysis of sequence tagged sites separated by 40 kilobases within the gene are consistent with hPPARγ being located within 1.5 megabases of marker D3S1263 [Het. 0.87] (Research Genetics, Huntsville, AL)[Table III].

In conclusion, the coding regions shared by hPPARγ1 and hPPARγ2 are encoded by six exons. Additional studies will be required to define the exons encoding the unique γ2 coding region (28 amino acids) and the 5′ noncoding γ1 region, as well as the regulatory/promoter regions. Here we present the approximate intron sizes and the sequences of the exon/intron junctions of those exons shared by hPPARγ1 and hPPARγ 2, which includes the entire coding region of hPPARγ1. The genomic structure of the hPPARγ gene, including the intron positions and sizes, is similar to that of the mPPARγ gene. This information will facilitate screening for possible mutations. Furthermore, D3S1263 on chromosome 3p25 is in linkage disequilibrium with the hPPARγ gene and is a suitable informative marker for linkage analysis to evaluate the potential contribution of hPPARγ to genetic susceptibility to obesity, lipoatro-

TABLE III

Radiation Hybrid Map Linkage Data

Locus 1	Locus 2	Both typed	−/−	−/+	+/−	+/+	P(BR)	DIST*	LOD** score
PPAR-γ	D3S1263	83	68	6	1	8	0.353	43.6	5.37
PPAR-γ	WICGR EST207274	82	71	2	2	7	0.250	28.7	6.27
D3S1263	WICGR EST207274	82	69	0	4	9	0.210	23.6	7.82

* DIST is the distance measurement in centirays (cR), for the Stanford Hybrid panel 1 $cR_{10,000} \approx 30$ Kb.
** Maximum LOD scores and breakage probability and distance estimates derived from G3 data using RH2PT routine from RHMAP Version 2.01.

phy, insulin resistance, diabetes mellitus, and other diseases of humans.

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