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

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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 hPPARγ2 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. © 1997 Academic Press

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 32P-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...
TABLE I
Oligonucleotide Primers and Probes Used to Define Intron Sizes of the Human PPARγ Gene
(Also See Legend for Figure 1)

<table>
<thead>
<tr>
<th>Exon → Exon</th>
<th>Sense primer from proximal exon (5' → 3')</th>
<th>Anti-sense primer from distal exon (5' → 3')</th>
<th>Internal oligonucleotide probe (5' → 3')</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 → 2*</td>
<td>GTGGGCCGCCGACAAATGACCCTTGG</td>
<td>CATCCTTCACAAAGCATGAACTCC</td>
<td>ATTGAATCTGGTTCAGTTGGAG</td>
</tr>
<tr>
<td>2 → 3*</td>
<td>GTGGGACCTGCCATCTCCACC</td>
<td>TGAGACATCCCCACCTGCAAGGC</td>
<td>GGGTGATGTGGTTGAAACCTGATT</td>
</tr>
<tr>
<td>3* → 4</td>
<td>CCGGAGAAACATCAGATTGCC</td>
<td>CTCCTTTCGTTTGTCTCAGGGG</td>
<td>GTGAAATTTGTCCAGTACTG</td>
</tr>
<tr>
<td>4* → 5</td>
<td>GATGCCGACAGGCGAGAGAGG</td>
<td>CTCCCCTTGCTAGAAGCTTGG</td>
<td>TACATAAAGTCTCCTTCGCCGTTA</td>
</tr>
<tr>
<td>5 → 6*</td>
<td>AGGAGCAGAGAAAGAGG</td>
<td>GTAGACTCCCTGCAGAGGG</td>
<td>AGGTCGGAGGCTTTGAGCAGG</td>
</tr>
</tbody>
</table>

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

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 I). 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-
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 hPPARγ2, 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

<table>
<thead>
<tr>
<th>Locus 1</th>
<th>Locus 2</th>
<th>Both typed</th>
<th>(-/-)</th>
<th>(-/+)</th>
<th>(+/-)</th>
<th>(+/+)</th>
<th>P(BR)</th>
<th>DIST*</th>
<th>LOD** score</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPAR-(\gamma)</td>
<td>D3S1263</td>
<td>83</td>
<td>66</td>
<td>6</td>
<td>1</td>
<td>8</td>
<td>0.353</td>
<td>43.6</td>
<td>5.37</td>
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<td></td>
<td>WICGR</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPAR-(\gamma)</td>
<td>EST207274</td>
<td>82</td>
<td>71</td>
<td>2</td>
<td>2</td>
<td>7</td>
<td>0.250</td>
<td>28.7</td>
<td>6.27</td>
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<tr>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D3S1263</td>
<td>EST207274</td>
<td>82</td>
<td>69</td>
<td>0</td>
<td>4</td>
<td>9</td>
<td>0.210</td>
<td>23.6</td>
<td>7.82</td>
</tr>
</tbody>
</table>

* DIST is the distance measurement in centirays (cR), for the Stanford Hybrid panel 1 cR_{10,000} = 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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**REFERENCES**
