Localization of \textit{Fabp6} and identification of a \textit{Fabp6} pseudogene in the rat \textit{(Rattus norvegicus)}

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\textit{submitted}
Abstract

*Fabp6* codes for ileal lipid binding protein, one of the fatty acid binding proteins (FABPs). The expression of the gene is regulated by bile acids in the ileum. A rat specific primer combination for *Fabp6* was used for linkage analysis in a radiation hybrid (RH) panel. Rat *Fabp6* was found to be located on rat chromosome 10, closely to the marker D10Got43. This location is supportive for the previously expressed view that *Fabp6* is a candidate gene for the regulation of rat liver cholesterol levels. The present study also revealed the existence of a *Fabp6* pseudogene. This pseudogene occurs in four related rat inbred strains (SHR; SHRSP; WKY; and OKA), but not in 62 other commonly used rat inbred strains. By analyzing the progeny of (SHRSPxBN)xBN backcross, the *Fabp6* pseudogene could be localized on rat chromosome 15.
Localization of Fabp6 and identification of a Fabp6 pseudogene in the rat.

Introduction

In the rat marked strain-specific differences exist in the susceptibility for dietary cholesterol. In previous studies we have found a quantitative trait locus (QTL) for postdietary liver cholesterol concentration on chromosome 10 (RNO10)\(^1\). Based on homology with mouse and human, Fabp6 was suggested as a candidate gene. Fabp6 is a member of a family of genes coding for fatty acid-binding proteins. These proteins constitute a rather conserved group of cytosolic low molecular mass proteins (14-16 kDa). They are tissue-specific and are able to bind long-chain fatty acids and their CoA derivates\(^2,3\).

Fabp6 codes for the ileal lipid binding protein. This protein occurs in ileum, ovary and adrenal gland and, unlike the other members of the family, has a low binding affinity for long-chain fatty acids, but a high affinity for bile acids\(^4,5\). Recently it has been shown in humans that bile acids regulate the expression of the gene\(^6,7\). FABP6 might function as a cytosolic receptor for bile acids\(^8\). The chromosomal location and the nucleotide sequence of the gene is known in mouse and man\(^4,9\).

In rat, Fabp6 has not been mapped yet, but the mRNA sequence of the gene is known (NM_017098, NCBI GenBank). In mouse and human the gene contains four exons. Based on sequence homology we have selected primers for the amplification of rat Fabp6 (exon2, intron2, exon3, intron3 and exon4). These primers were used for studying the chromosomal location of Fabp6 in rat.

Animals, Materials & Methods

**Animals, hybrids, DNA.**

DNA from the SHR/OlaIpcv (SHR) and the BN.lx/Cub (BN) was used to determine the sequence of the Fabp6 gene\(^10,11\). A whole genome rat/hamster (T55) Radiation Hybrid (RH) panel was purchased from Research Genetics (Huntsville, Ala) for the localization of Fabp6. This panel has been constructed by fusing irradiated cells from a Sprague Dawley fibroblast line (RatFR) with a recipient hamster line (A23). RatFR donor cells were irradiated with 3000 rad prior to fusion with A23 recipient cells. Genomic DNA of 66 commonly used rat inbred strains (see Table 3) was screened for presence of the Fabp6-ps (pseudogene). For the localization of the pseudogene a (BN x SHRSP) x BN backcross was used. The DNA from 366 backcross animals was screened.

**PCR protocol and sequencing.**

Primers for amplification of rat Fabp6 sequences were designed, based on homology with mouse Fabp6. Primers complementary to the sequence at the start of
mouse exon2, exon3 and exon4 and the end of exon2 and exon3 were used in order to amplify rat exon2, intron2, exon3, intron3 and part of exon4 (Table 1). In the 10 µl PCR reactions, 100 ng genomic rat DNA, 0.2 mM dNTPs, 1 mM primer and 1 U Taq (HT Biotechnology LTD, Cambridge, UK) were used. Standard PCR included an initial 5 min. denaturation step performed at 94ºC followed by 30 cycles of amplification beginning with a 1 min. denaturation at 94ºC, a 2 min. annealing at 55ºC, and a 2 min. extension at 72ºC. The reaction was terminated with a final 5 min. step at 55ºC followed by a 10-min extension at 72ºC. The PCR-products were separated and visualized on an 1% agarose gel. The PCR products were isolated from gel and sequenced by DNA Sequencing Core (Leiden, The Netherlands) using the Perkin-Elmer Big-Dye Terminator cycle sequencing kit.

Radiation hybrid mapping, chromosomal mapping.

For localization of Fabp6 on the RH map, 25 ng DNA of the hybrids was used in 10 µl PCR reactions, amplifying the Fabp6 gene. Two primers (5’-TGTCAGTCTGTAGGTCTTGA-3’)FW and FA6RN4, specific for the intron between exon3 and exon4 were used. The forward primer was labeled with [γ-32P]ATP (Amersham, UK). The reaction was performed as described above. The PCR products were separated in a polyacryl-amide gel and visualized autoradiographically. The obtained results were sent to the RH mapping server of the Otsuka Gen Research Institute (http://www.otsuka.genome.ad.jp/ratmap). For the localization of the Fabp6 pseudogene, the primers FA6RN2 and FA6RN3 (Table 1) were used. The PCR products were visualized on a 3% agarose gel and were scored as present and absent. For chromosomal mapping the program MapMaker/EXP Ver. 3.0 from the Whitehead Institute was used.

Results

The primers described in Table 1 were used in different combinations for amplification of genomic DNA of the rat inbred strains BN.lx/Cub (BN) and SHR/OlaIpcv (SHR). In Table 2 the primer combinations that have been used are shown together with the sizes of PCR products that are common in BN and SHR. After sequencing these products, we found the nucleotide sequence to be identical in BN and SHR. Figure 1 illustrates the sequence as obtained for this rat Fabp6 gene. After alignment of this sequence with the sequence of rat Fabp6 mRNA and genomic mouse Fabp6, the exons and introns could be distinguished (GenBank acc. num.: 8393345). The number and size of the exons in the rat (Fig. 2.) are similar to those of mouse and human13. The sequences of the exons of the BN and SHR are completely identical to the rat mRNA sequence (GenBank acc. num.: 8393345) whereas a 98% homology is found for mouse Fabp614. In contrast, the two introns differ considerably between mouse and rat.
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### Table 1. Primers designed for amplification of rat *Fabp6* (based on data taken from references Crossman *et al.*

<table>
<thead>
<tr>
<th>Rat primer name</th>
<th>Primer sequence (5’-3’)</th>
<th>Location in mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA6RN1</td>
<td>AACTTCAAGATCATCACAGAGG</td>
<td>Start Exon 2 (FW)</td>
</tr>
<tr>
<td>FA6RN2</td>
<td>CCCAGTCTTACTCTGGGGGC</td>
<td>End Exon 2 (FW and REV)</td>
</tr>
<tr>
<td>FA6RN3</td>
<td>TTCCCCAACTATCACCAGACTTC</td>
<td>Start Exon 3 (FW and REV)</td>
</tr>
<tr>
<td>FA6RN4</td>
<td>ATCTCCACCATCGGGGATGTGA</td>
<td>Start Exon 4 (FW)</td>
</tr>
</tbody>
</table>

### Table 2. Product size (bp) found in BN and SHR progenitor inbred strains when using different primer combinations.

<table>
<thead>
<tr>
<th>Primer (REV)</th>
<th>Primer (FW)</th>
<th>\begin{tabular}{l} \text{BN} \ \text{SHR} \end{tabular}</th>
<th>\begin{tabular}{l} \text{BN} \ \text{SHR} \end{tabular}</th>
<th>\begin{tabular}{l} \text{BN} \ \text{SHR} \end{tabular}</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA6RN2</td>
<td>FA6RN3</td>
<td>FA6RN4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FA6RN1</td>
<td>72</td>
<td>72</td>
<td>1125</td>
<td>1125/206*</td>
</tr>
<tr>
<td>FA6RN2</td>
<td>1073</td>
<td>1073/154*</td>
<td>206*</td>
<td></td>
</tr>
<tr>
<td>FA6RN3</td>
<td>950</td>
<td>950/75*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*The figures in italics correspond to the product sizes found for the *Fabp6* pseudogene*
As the Fabp6 sequences of the BN and SHR progenitor inbred strains were completely identical, linkage analysis could not be performed with the use of the recombinant inbred (RI) strains. Therefore, the location of rat Fabp6 was studied by using a radiation hybrid (RH) panel (Research Genetics, Huntsville, USA). A primer, specific for rat Fabp6 was selected from the intron sequence between exon3 and exon4 (Fig. 1, bp 1538-1559). The PCR products of this primer and FA6RN4 were scored and the results were sent to the RH mapping server at the Otsuka Gen Research Institute. Fabp6 was placed on the RH map in the vicinity of D10Got43 (LOD: 16.47, 308.5 cR).

Besides the PCR products found both in BN and SHR, additional, small size, products were obtained when genomic DNA of the SHR was amplified (Table 2). No extra PCR product was detected with either the BN or SHR when the specific rat intron primer combination was used. We have sequenced the 258 bp product of FA6RN1 and FA6RN4 (Fig. 3). This sequence turned out to be completely identical to the sequence of exon2, exon3 and first part of exon4 of mouse Fabp6 and the sequences of exon2, exon3 and first part of exon4 as established in BN and SHR inbred strains (Fig. 1 and Fig. 2). This PCR product contains the sequence of the Fabp6 gene without the introns, thus indicating the existence of a Fabp6 pseudogene (Fabp6-ps), present in SHR, but not in BN.

In order to test the strain distribution pattern of Fabp6-ps, 66 commonly used rat inbred strains were screened for presence or absence of the pseudogene. Four out of these 66 strains (SHR; SHRSP; WKY; and OKA) were found to possess the pseudogene (Table 3). Linkage analysis of the Fabp6-ps was performed in a (BNxSHRSP) x BN backcross panel. From this backcross DNA of 366 animals was used for screening. Significant linkage (placement at log-likelihood threshold 7) was found on RNO15 in the vicinity of the maker D15Mit2 (Fig. 4).

Table 3. Rat inbred strains (n = 66) screened for the presence (+) or absence (-) of the Fabp6 pseudogene (Fabp6-ps). (For specifications of the rat inbred strains, see Bender et al.14).

<table>
<thead>
<tr>
<th>Fabp6-ps (+)</th>
<th>Fabp6-ps (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR; SHRSP; WKY; OKA</td>
<td>A2; ACI; AGUS; ALC; AS; AO; AUG; AVN; BBWB; BC; BDII; BDIV; BDIX; BDE; BDVII; BDX; BH; BN; BN.Ix; BP; BS; BUf; CAP; CHOC; COP; DA; DZB; E3; F344; GC; Hooded; LE; LEH; LEW; LH; LOU; MHS; MNS; MW; NAR; NEDH; OM; PAR; PD; PVG; R; RHA; RNU; SD; SDH; SDL; SPRD; SPRD-CU3; SR; SS; U; WAG; WF; WOK.1A; WOK.1W; AMORAT; ARISTORAT</td>
</tr>
</tbody>
</table>
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Fig 1. The sequence of \textit{Fabp6} as found in BN.lx/Cub and SHR/OlaIpcv (Genbank acc. nrs. : AY049763 and AY049762, respectively).

Fig. 2. Primary structure of the rat \textit{Fabp6} gene.
Exon2:
AACTTCAAGATCATCACAGAGGTCCAGCAGGATGGAGAGAACTTCACCTGGTCCCAGTCTTACTCTGGG
GGCAACATCATGAGCAACATGTTCACCATTGGCAAAGAATGTGAAATGCAGACCATGGGGGGCAAGAAG
TTCAAG

Exon3:
GCAACCGTGAAGATGGAGGGTGGCAAGGTGGTGGCAGACTTCCCCAAACTATCACCAGACTTCCGGAGGT
CGTGGGTGACAAGTTGCTGGAG

Exon4:
ATCTCCACCATCGGGGATGTGACC.

**Fig. 3.** The sequence of Fabp6-ps as found in SHR/OlaIpcv (GenBank acc. nr.: AY049764).

**Fig. 4.** The genetic map of RNO15 based on (BNxSHRSP) x BN backcross (n = 366) with the Fapb6-ps included.
Discussion

The fatty acid-binding proteins (FABPs) belong to a conserved group of cytosolic low mass proteins. The nine family members have 20-70% identity in their amino acid sequence in human and mouse. All members have unique tissue-specific expression patterns. It is suggested that the proteins have specialized functions in different tissues\(^3\). The human \(FABP6\) (fatty acid binding protein 6 or ileal lipid binding protein) gene encodes a protein that appears to be the cytosolic receptor for bile acids that have undergone sodium-dependent active transport into the enterocyte\(^8\). In a previous study we analyzed liver cholesterol concentration in rat recombinant inbred (RI) strains derived from the progenitors BN.lx/Cub and the SHR/OlaIpcv. A quantitative trait locus (QTL) was found on chromosome 10 in the vicinity of D10Mit\(^4\). \(Fabp6\) was suggested as a candidate gene, but the location of this gene in the rat still needed to be established.

In the search for rat \(Fabp6\) polymorphism, in the present study exon2 through exon4 were amplified and sequenced but the sequence in rat inbred strains BN and SHR were found to be identical. After alignment of the sequence with the \(Fabp6\) sequence of the mouse and the mRNA sequence of the rat\(^4,12\), the exons could be distinguished from the introns. The sequences of the three exons (144 bp, 90 bp and 24 bp) were identical to the sequence as reported by Gong et al.\(^12\). But when compared to the mouse, four bp in exon2 and two bp in exon3 were found to be different. In the mouse the introns are 995 bp and 1295 bp, whereas in rats these are 919 bp and 875 bp, respectively.

Linkage analysis revealed \(Fabp6\) to be located on chromosome 10, close to D10Got43. In the high density integrated genetic linkage map, as published by Steen et al.\(^15\), this marker is linked to D10Rat41 (279 cR; 28.4 cM) which is closely linked to the QTL marker D10Mit\(^4\). Recently, another gene involved in the hepatic control of lipid metabolism, sterol regulatory element binding transcription factor 1 (\(Srebf1\)), has been mapped on RNO10\(^16\). But \(Fabp6\) maps more closely to the QTL than \(Srebf1\).\(^1\)

We have found evidence for the existence of a \(Fabp6\) pseudogene (\(Fabp6\)-ps) on rat chromosome 15 in the vicinity of the marker D15Mit2. Prinsen and co-workers\(^17\) have reported the occurrence of a \(Fabp3\) pseudogene in humans. Pseudogenes may arise from gene duplication and increased crossing-over between homologous chromosomal regions or by reverse transcription from mRNA. The absence of introns and the location of \(Fabp6\)-ps on a different chromosome than \(Fabp6\) seem to indicate that the origin of \(Fabp6\)-ps is through reverse transcription (‘processed gene’) rather than through gene duplication and crossing-overs. It is unlikely that \(Fabp6\)-ps is coding for a physiologically active product. Most nucleotide sequences that consist of exons without introns are inactive. Also the strain distribution pattern
is not supportive for an essential role in the animal’s physiology. The pseudogene is found to occur in only four out of 66 rat inbred strains. These four strains (SHR; SHRSP; WKY and OKA) all originate from the same outbred Wistar colony at the Kyoto School of Medicine, Japan. The SHR and the SHRSP develop spontaneous hypertension but the WKY strain is frequently used as the normotensive control strain for the SHR. But, though there is no evidence for a present function, pseudogenes may play a major role in the evolution of species since these DNA sequences can readily serve as reservoirs to be changed into genes with new functions.

References


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