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Transcriptional control of human organic anion transporting polypeptide 2B1 gene

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ABSTRACT

Organic anion transporting polypeptides (OATPs) are a group of transmembrane carriers with a wide spectrum of amphipathic substrates. In particular, OATP2B1 (previously called OATP-B) can transport steroid hormone conjugates and is expressed in organs with steroidogenic activity, such as placenta, brain and skin. In this work, we have analyzed the transcription of the OATP2B1 gene (*SLCO2B1*) in 14 different human tissues by means of 5′-RACE analysis. Five promoters (only two of which were present in GenBank), associated with distinct first exons, were found to drive OATP2B1 expression, giving rise to transcripts with unique 5′-untranslated termini. Exon 1b is widely expressed and was found here in 10 tissues. It is partially coding, while the other four different first exons are untranslated. All exons are spliced to a common exon 2 that contains a putative ATG in frame with the following coding region. Sequence analysis of the 5′-flanking region of each first exon revealed a lack of TATA box, thus accounting for the use of multiple transcriptional start sites in nearly all first exons.

1. Introduction

Organic anion transporting polypeptides (rodents: Oatps; human: OATPs; gene symbol SLCO) are a superfamily of multi-specific carriers (solute transporter SLC21 family) [1]. The SLCO genes encode 12-transmembrane-domain proteins that mediate the sodium-independent transport of steroid hormone conjugates, drugs, and numerous endogenous anionic substrates across the plasma membrane of animal cells [2]. Their high conservation during evolution in the animal kingdom has been attributed to a central role in detoxification processes [3].

In the last few years, the biological significance of this group of polypeptides has been the subject of intensive research in different mammalian species, such as human, rat and mouse. Thirty-six members were identified in total and 11 members were described in human, namely: OATP1A2 (OATP-A), OATP2B1 (OATP-B), OATP1B1 (OATP-C), OATP3A1 (OATP-D), OATP4A1 (OATP-E), OATP1C1 (OATP-F), OATP4C1 (OATP-H), OATP5A1 (OATP-J), OATP6A1 (OATP-I), OATP1B3 (OATP8) and OATP2A1 (PGT) [1].

The first members of the OATP/SLCO gene superfamily were identified by expression cloning [4]; subsequently others transporters were characterized using homology screening by either hybridization experiments or *in silico* [5]. More recently, the expression profiles of OATPs in human adult and fetal tissues as well as in cancer cell lines were examined by RT-PCR [6]. Transporters such as OATP1A2, OATP1B1, OATP1B3 and OATP1C1 were found to have a limited tissue distribution, whereas OATP2B1, OATP3A1 and OATP4A1 are widely expressed [2]. The former transporters seem to function as organ-specific carriers, while the latter may be active at different sites.

In particular, OATP2B1 expression was demonstrated in the hepatocyte basolateral membrane [7,8], the basal membrane of the placental syncytiotrophoblast [9], the apical membrane of small intestinal epithelial cells [10,11], in the myoepithelial cells of the mammary gland [12] and in the kidney [6,8], brain [13,14], skin [15] and heart [16].

Steroid sulfates are substrates for several members of the OATP family, such as OATP1A2, which is mainly expressed in the brain, the liver-specific OATP1B1 and OATP1B3, as well as OATP2B1 with its wide tissue distribution [2]. The spectrum of identified substrates of OATP2B1 is restricted and mainly consists of steroid sulfate conjugates, such as dehydroepiandrosterone sulfate (DHEAS) and estrone sulfate (E1S) [17]. These conjugates are highly concentrated in human blood and function as important precursors of active androgenic and estrogenic steroids in peripheral tissues [18–20].

Because of its transport specificity and localization in tissues involved in steroid metabolism, such as placenta, mammary gland, brain and skin, OATP2B1 was implicated in the uptake of DHEAS and E1S as precursor molecules for steroid hormone biosynthesis [21]. Recently, Ugele et al. [22] confirmed a report by Pizzagalli et al. [12] about the rather low affinity of this transporter for DHEAS, which

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Table 1
Primer sequences used in the RT-PCR and 5′-RLM-RACE analyses.

Primer		Sequence	Position[a]	GeneBank no.
OATP-B2	Antisense	5′-ACATGATCCCCACCACACTCA-3′	755 → 735	AB026256
OATP-B4	Antisense	5′-GCCGAAGCGCTTCTCCAC-3′	421 → 404	AB026256
OATP-B5	Sense	5′-AGCTGTCTGTCGCTACTAC-3′	2086 → 2104	AB026256
OATP-B6	Antisense	5′-GAGATCATGAGCTGCGCCAG-3′	375 → 356	AB026256
OATP-B8	Antisense	5′-CCCAAGACAGCTCACACTC-3′	2319 → 2301	AB026256
OATP-B-R	Antisense	5′- CACTGTGGAGATGGAGCTC-3′	406 → 388	AB026256
OATP-B-1a	Sense	5′- CCTGATAAACTTCATGATGGAG-3′	194 → 215	FM209049
OATP-B-1b	Sense	5′- GGCTGGAGCTCACTGCAC-3′	294 → 311	FM209050
OATP-B-1c	Sense	5′- GCACACAGGAGGTCGGAG-3′	10 → 27	FM209051
OATP-B-1d	Sense	5′- ACTGCAGTACGGCAGGAAG-3′	121 → 140	FM209052
OATP-B-1e	Sense	5′- TGGGATTGAAGCTTCAGGGAG-3′	57 → 77	FM209053
β-Actin-1	Sense	5′-CACCAACTGGGACGACATGGAG-3′	303 → 324	BC013380
β-Actin-2	Antisense	5′-AGCCTGGATAGCAACGTACAT-3′	409 → 469	BC013380

[a] Nucleotide position in the reported sequence.

was 10-fold lower in transfected cells than that of E1S. According to these authors, OATP2B1 may not be involved in the synthesis of placental estrogens, but rather in the clearance of estrogen sulfates from the placental circulation, despite the fact that the transporter is localized in the basal membrane of the syncytiotrophoblast and that androgen sulfates are more abundant than estrogen sulfates in the fetal circulation. In other tissues, however, in combination with steroid sulfatase (STS) and 17β-hydroxysteroid dehydrogenase (17β-HSD), this transporter appears to supply cells with a basic level of active estrogens via the so-called sulfatase pathway [23].

In the present work, we focused our attention on the transcriptional regulation of the gene encoding OATP2B1 (*SLCO2B1*). Two alternatively spliced transcripts, differing in their 5′-UTRs and probably arising from two distinct promoters, could be determined analysing the deposited GenBank OATP2B1 sequences. One of these promoters was recently characterized by Maeda et al. [24]. It was found to contain a *cis*-acting GC box that binds the potential specificity protein-1 (Sp1) which controls basal gene expression in cell lines from liver and intestine. Moreover, although it includes a putative binding site for the hepatocyte-specific transcription factor HNF1α, which is involved in the expression of liver-specific OATPs [25], this was not implicated in the regulation of OATP2B1. In this work, by means of 5′-RACE analysis, we show that the regulation of *SLCO2B1* is more complex than previously assumed, because at least five alternatively spliced transcripts are expressed in different human tissues.

2. Materials and methods

2.1. Collection of human tissues and RNA extraction

Anonymous human biopsy materials were taken from surgically removed organs following national and institutional ethical guidelines and with full patient consent and review board approval. The following tissues were obtained and analyzed by 5′-RLM-RACE: adipose tissue, adrenal cortex, knee cartilage, colon, liver, lung, mammary gland, pancreas, placenta, thymus, thyroid and uterus. Dermal papillae of hair follicles were collected from scalp biopsies. Two ovarian samples were used only for RT-PCR analysis as well as 1–2 additional samples of some of the above tissues. Samples were kept at −80 °C until use. Prostate cancer adherent cell line DU145 was cultured at 37 °C under 5% CO_2 in RPMI 1640 medium (Serva-Celbio, Milan, Italy), supplemented with 5% fetal calf serum and 2 mM L-glutamine. Cells were then washed three times in PBS, counted in a Neubauer chamber, resuspended in RPMI medium and kept at −80 °C until use. Total RNA was extracted with Trizol reagent (Invitrogen, Milan, Italy).

2.2. Expression analyses

Reverse transcription (RT) of 2 μg total RNA was performed in 20 μl final volume with random hexamers and the ThermoScript RT-PCR System Kit (Invitrogen). After incubation for 10 min at 25 °C, RT was carried out for 30 min at 55 °C, followed by termination for 5 min at 85 °C. Total single-stranded cDNA (sscDNA) was amplified by PCR in a 25 μl reaction volume containing 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 1.5–2 mM $MgCl_2$, 0.2 mM of each dNTP, 0.2 μM of the respective primers, and 1.25 U of Biotherm Taq DNA polymerase (Società Italiana Chimici, Rome, Italy). Oligonucleotide primers are listed in Table 1 and amplification conditions in Table 2. The extension phase of the last cycle was prolonged by 10 min. As RNA quality control, RT-PCR of β-actin mRNA was performed for every sample with specific primers. For each PCR, negative controls were prepared by replacing the cDNA solution with sterile water.

2.3. Determination of the transcription start sites (TSSs) of the OATP2B1 gene in various tissues

The TSSs of *SLCO2B1* were determined with the method of RNA ligase-mediated rapid amplification of cDNA 5′-ends

Table 2
Amplification procedure of the PCR reactions.

Target gene	Primers	$[Mg^{2+}]$(mM)	Annealing (°C)	Extension time (s)
OATP-B	OATP-B5–OATP-B8	2.0	58	20
OATP-B-1a	OATP-B-1a–OATP-B-R	2.0	Touch down 60-52	20
OATP-B-1b	OATP-B-1b–OATP-B-R	2.0	Touch down 60-52	20
OATP-B-1c	OATP-B-1c–OATP-B-R	2.0	Touch down 60-52	20
OATP-B-1d	OATP-B-1d–OATP-B-R	2.0	Touch down 60-52	20
OATP-B-1e	OATP-B-1e–OATP-B-R	2.0	Touch down 60-52	20
OATP-B	5′-outer–OATP-B2	1.5	Touch down 64-54	60
OATP-B	5′-inner–OATP-B4	1.5	Touch down 64-54	50
OATP-B	5′-inner–OATP-B6	1.5	Touch down 64-54	50
β-Actin	β-Actin1–β-actin2	1.5	60	15

(5′-RLM-RACE) using the FirstChoice RLM-RACE Kit (Ambion, Celbio, Milan, Italy), as previously described [26]. OATP2B1 sscDNAs were PCR-amplified using the 5′-RACE outer primer (5′-GCTGATGGCGATGAATGAACACTG-3′) and a specific 3′-reverse primer, either OATP-B2, designed on exon 5 of the OATP2B1 gene (GenBank accession no. AB026256) [6] or OATP-B4, selected on exon 3. The amplification procedure is listed in Table 2. The diluted products were subjected to second and third rounds of amplification using the 5′-RACE inner primer (5′-CGCGGATCCGAACACTGCGTTTGCTGGCTTTGATG-3′) and a specific 3′-reverse primer, OATP-B6, selected on exon 3. The template cDNAs of the second and third rounds of PCR were diluted between 1:50 to 1:500, according to the intensity of the previous amplification. In some cases, only a second round of PCR with OATP-B4 was sufficient to obtain specific transcripts.

The cDNA from the last PCR was purified from the sliced gel band in a preparative gel, and either directly sequenced or, when more then one band was present, ligated into a pGEM-T vector using the pGEM-T Vector System I (Promega, Milan, Italy). Plasmids from positive colonies were purified and sequenced. Moreover, as it was not possible to sequence directly transcripts corresponding to exon 1b, probably due to hairpin formation, amplifications corresponding to this transcript were always cloned.

2.4. Nucleotide sequencing and bioinformatic analysis

Sequencing was performed on dsDNA, directly from PCR products or after cloning, with the ABI PRISM Dye Terminator Cycle Sequencing Core Kit (Applied Biosystems). Electrophoresis of sequencing reactions was completed on an ABI PRISM 3100 DNA Sequencer. Homology searches were carried out using the Basic Blast program, version 2.0, at http://www.ncbi.nlm.nih.gov/BLAST/.

Prediction of membrane-spanning regions and their orientation was performed with the software from http://www.ch.embnet.org/software/TMPRED_form.html. The presence of signal peptide was analyzed by the "SignalP" program (http://www.cbs.dtu.dk/services/SignalP/) and the "TargetP" program (http://www.cbs.dtu.dk/services/TargetP/).

3. Results

After extraction of total RNA, we have analyzed all samples for OATP2B1 mRNA expression by RT-PCR, in order to discard negative or barely positive samples. A few available tissues and two prostatic cell lines were excluded (not reported in Section 2). This control PCR was made with a couple of specific primers, OATP-B5 and OATP-B8, that amplify the region between exons 13 and 14. Afterwards, the first exon in each tissue was analyzed by means of 5′-RLM-RACE. A single 5′-RACE product was generated in all samples (data not shown), except in adipose tissue, placenta and uterus, in which two different products were obtained. Sequencing yielded mRNA sequences up to the cap sites, and demonstrated the occurrence of five alternative first exons differing in length and co-linear with the genomic sequence (Fig. 1). Blast search allowed the alignment of the new first exons with the clone CTD-2562J17, signed with the accession number AP001972 and containing the *Homo sapiens* chromosome 11q sequence that includes *SLCO2B1*. This gene was actually found to span approximately 105 kbp, subdivided into a 43 kbp 3′-region containing almost all the coding part (exons 2–14) and a 62 kbp 5′-flanking region encompassing the first exons and intervening sequences.

We have named the five first exons as 1a–e, from the most distant to the closest to exon 2 of 131 nt, which is common to every transcript and contains a putative ATG in frame with the following coding region (Figs. 1 and 2). All introns present the canonical splice sites consensus "GT" and "AG" (Fig. 2) [27]. When the same first exon was present in different tissues, different TSSs were

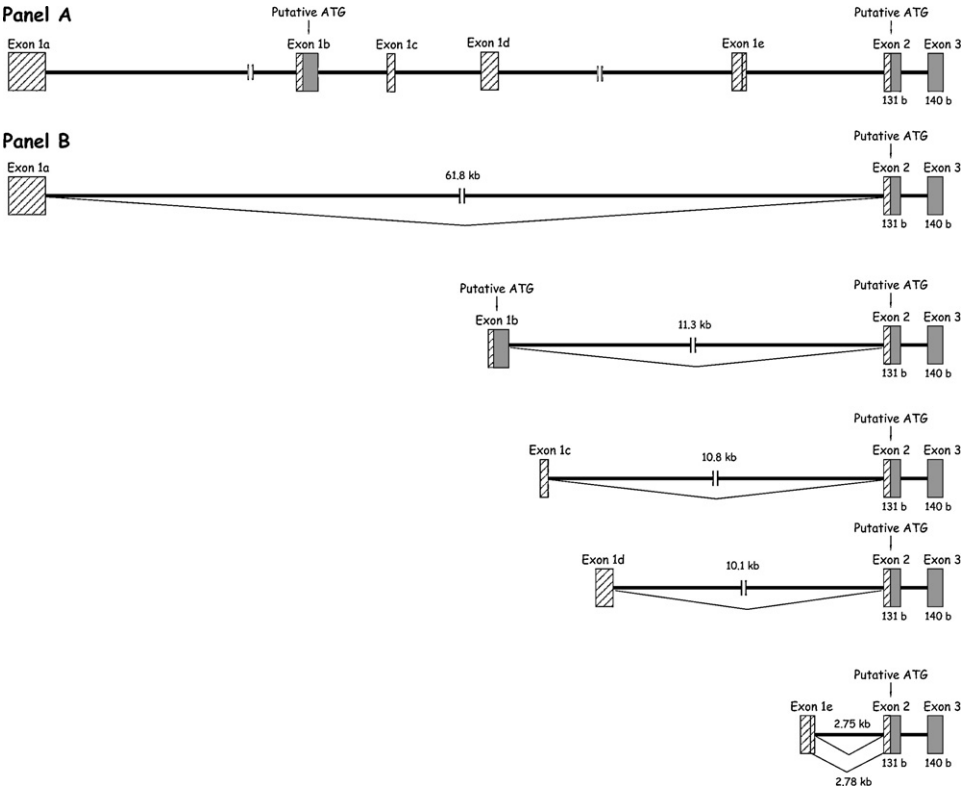

Fig. 1. Exon/intron structure of the first part of the OATP2B1 gene (panel A) and alternative transcripts with different first exons (panel B). Exons are shown as boxes and introns as lines. Filled and striped boxes indicate the translated and untranslated parts of the exons, respectively. Numbers below exons and introns indicate sizes. Vertical arrows indicate putative translation start codon positions. The angled lines connect potential splice donors and acceptors.

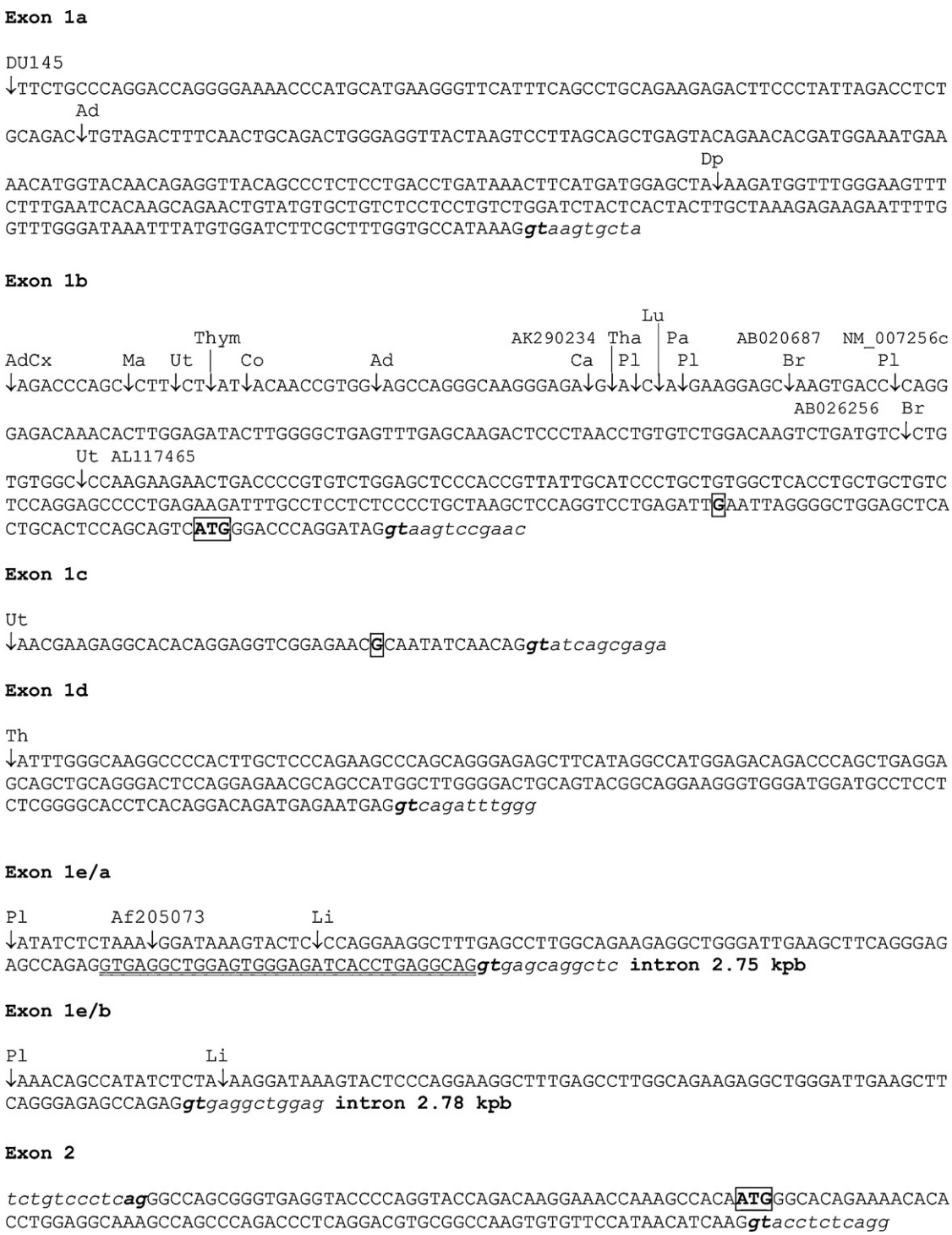

Fig. 2. Sequences of the different first exons and exon 2 of the OATP2B1 gene with determined transcription start site (TSS) positions. In italics: initial 12 nt of the first introns; in bold: 5′ and 3′ splice signals. Putative translation start codons are in bold and boxed, like the nucleotides found to be different (G instead of A) in the cDNA sequences of exons 1b and 1c. Arrows show the TSS positions of each first exon in different tissues. The underlined sequence in exon 1e/a corresponds to intron in exon 1e/b. Ad, adipose tissue; AdCx, adrenal cortex; Br, brain; Ca, cartilage; Co, colon; Dp, dermal papillae of hair follicles; Du145, prostatic cell line; Lu, lung; Ma, mammary gland; Li, liver, Pa, pancreas; Pl, placenta, Th, thyroid; Thal, thalamus; Thym, thymus; Ut, uterus. The cDNA sequences of the new OATP2B1 transcripts have been submitted to the EMBL Nucleotide Sequence Database under the accession nos. FM209049 for exon 1a (sequenced from Du145 cell line); FM209050 for exon 1b (adrenal cortex); FM209051 for exon 1c (uterus); FM209052 for exon 1d (thyroid); FM209053 for exon 1e/a (placenta); FM209054 for exon 1e/b (placenta).

found. The presence of multiple TSSs is normally due to the lack of TATA box. Our analyses on the proximal promoters by Cister program (http://zlab.bu.edu/~mfrith/cister.shtml) do not show such a region upstream of the five first exons of the human OATP2B1 gene.

The first transcriptional variant was found in the prostatic cell line DU145, adipose tissue and dermal papillae of hair follicles. It includes a novel untranslated first exon (exon 1a) which is 361, 276 and 143 nt long, respectively, and is spliced to exon 2 after an intron of 61.8 kb (Figs. 1 and 2).

Transcript number 2 with first exon 1b was identified in 10 organs, namely: adipose tissue, adrenal cortex, cartilage, colon, lung, mammary gland, pancreas, placenta, thymus and uterus

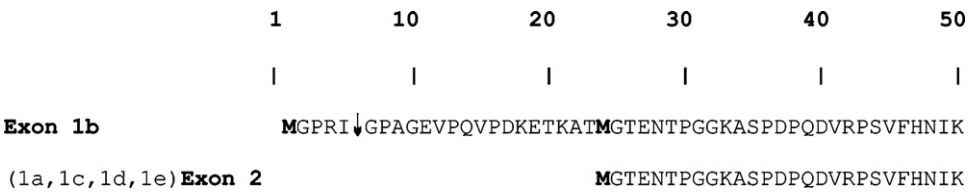

Fig. 3. N-terminal regions of the OATP2B1 protein encoded using different putative translation start codons located in exons 1b and 2. The arrow indicates the intron position between exons 1b and 2.

(Fig. 1). It also shows different TSS (Fig. 2). Exon 1b is partially coding, as it contains a putative ATG 13 nt upstream of its 3′-end. It is spliced to exon 2 after an intron of 11.26 kb. This transcriptional form corresponds to that deposited in GenBank and isolated from thalamus (AK290234), brain (AB020687 and AB026256), uterus (AL117465) and placenta (NM_007256c). A nucleotide was found to be different (G instead of A) in the cDNA sequences of exon 1b in all samples analyzed with respect to the genomic sequence (AP001972) (Fig. 2).

The transcriptional variant 3 contains a novel untranslated first exon (exon 1c) of 43 nt, that is spliced to exon 2 after an intron of 10.8 kb (Figs. 1 and 2). This variant was found only in the uterus. The cDNA presents a G instead of A with respect to the genomic sequence (Fig. 2).

The transcriptional variant 4 was found only in the thyroid and presents a novel untranslated first exon (exon 1d) of 192 nt (Figs. 1 and 2), which is spliced to exon 2 with an intervening intron of 10.14 kb (Fig. 1).

The transcriptional variant 5 with the first exon 1e was identified in two tissues, liver and placenta. The single band obtained in liver was cloned as the sequencing reaction showed overlapping pecks due to two different PCR templates. In this case, the exon presents two variants (exon 1e/a and 1e/b), 1e/a corresponding to that previously deposited in GenBank (AF205073), without specification of the tissue expressing this transcript. The sequence AF205073 presents a first exon of 106 nt that splices directly to exon 2 with an intervening intron of 2.75 kb. We found the exon 1e/a in single sequenced clones from placenta and liver with lengths of 117 and 93 nt, respectively. Moreover, other single clones from placenta and liver contained also the first exon 1e/b, that uses a different 32 nt-upstream splice signal leading to a longer intervening intron of 2.78 kb (Figs. 1 and 2).

While exon 1b is partially coding, the other four first exons are all untranslated. Exon 1b contains a putative ATG, only partially matching the proposed consensus sequence for the initiation of translation [28] in frame with the following coding region. With the other transcripts, translation must start from the ATG in exon 2, 50 nt downstream of its 5′-end. This putative initiation codon matches the Kozak consensus sequence for translation [28]. Thus, if the ATG present in exon 1b is used, the N-terminal region of the protein would be 22 aa longer (Fig. 3). No sequence that conforms to a signal peptide consensus was found in both the resulting deduced proteins, as previously found by Lee et al. [29] for the OATP2B1 protein deriving from exon 1b and by Hsiang et al. [30] for other members of the OATP family. According to the TMPRED program, both proteins have 12-transmembrane segments with both the C- and N-termini projecting into the cytoplasm.

Finally, to verify our results, we used the sequences of the new first exons of OATP2B1 mRNAs and the protein as baits to search the expressed sequence tag (EST) libraries available in GenBank by means of the Blast and TBlastN programs. As expected, a high number of different tissues use the promoter associated with exon 1b (data not shown). However, we found also transcripts corresponding to exon 1a (uterus, DC423326; macrophage BP299608) and exon 1e (hippocampus DC334108, testis DC400360, liver DC362630 and DC362722, amygdala DC317920). No transcripts corresponding to exons 1c and 1d were reported. Interestingly, in a liver EST library (NEDO human cDNA project) no transcript with exon 1b was listed.

To confirm the results of 5′-RLM-RACE, RT-PCR analyses were performed with five different sets of primers, in which a common reverse primer was designed on exon 3 (OATP-B-R) and the sense primers on each of the five alternative first exons. The same RNAs were used as for the 5′-RLM-RACE (signed by * in Fig. 4), except for placenta, mammary gland, uterus, liver, lung, cartilage and adipose tissue, for which one or two additional samples were utilized. Analysis of dermal papilla and the original sample of placenta, mammary gland, cartilage and adipose tissue were omitted, because their RNA were no longer available. PCR was always performed at 40 cycles and thus results are only qualitative. Moreover, amplification efficiency probably differed among the sets of primers, because sense primers had to be designed on a limited region of the 3′-end of each first exon in order to obtain products also from tissues with shorter TSSs. PCR products for each first exons were sequenced to confirm amplificate identity.

As shown in Fig. 4, a low level of transcripts corresponding to the first exon 1a were found in the cell line DU145 and in the adipose

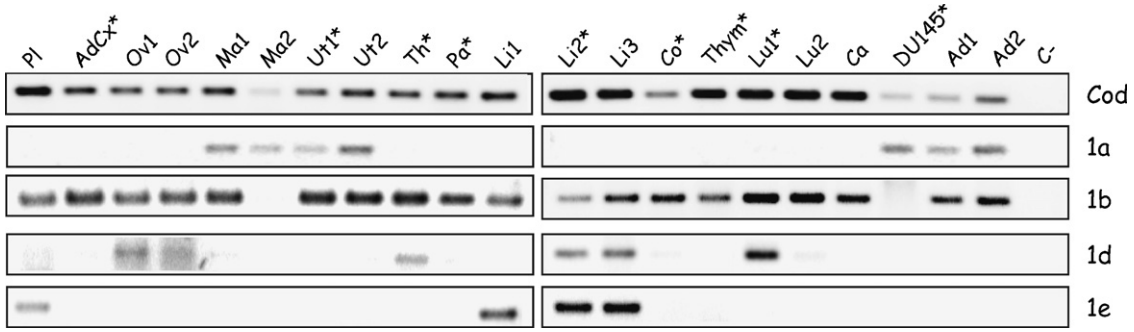

Fig. 4. Tissue-specific expression of the alternative OATP2B1 first exons 1a, 1b, 1d, 1e and for the coding region (cod). RT-PCR was carried out with sense primers for the four first exons and an antisense primer for exon 3. Samples marked by an asterisk were used for 5′-RACE analysis. Ad, adipose tissue; AdCx, adrenal cortex; Br, brain; Ca, cartilage; Co, colon; Dp, dermal papillae of hair follicles; Du145, prostatic cell line; Lu, lung; Ma, mammary gland; Li, liver, Pa, pancreas; Pl, placenta, Th, thyroid; Thal, thalamus; Thym, thymus; Ut, uterus; Ov, Ovary; C−, negative control.

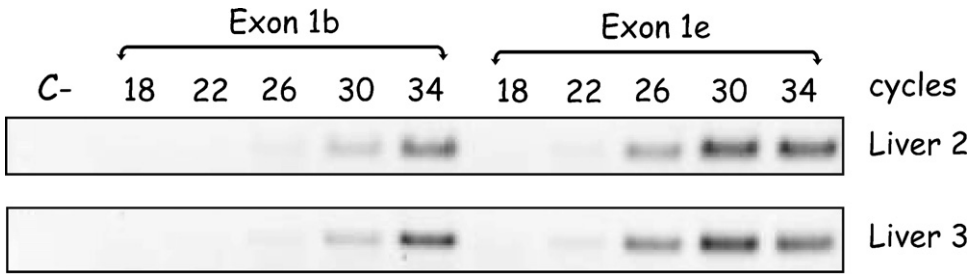

Fig. 5. Representative expression analysis of exon 1b and 1e in two liver samples as determined by semi-quantitative RT-PCR (cycles from 18 to 34). C–, negative control (water).

tissues, thus confirming the results obtained by 5′-RACE analysis. Moreover, both samples of mammary gland and uterus express low levels of exon 1a. The promoter linked to exon 1b was used by all tissues, except the cell line DU145. In mammary gland sample 2 presents a very faint signal that is not visible in the picture. Transcripts with exon 1c were found only in the sample of uterus previously analyzed by 5′-RLM-RACE and at low levels (this amplification was not shown in Fig. 4). Exon 1d was found in the thyroid (as shown by 5′-RACE), but also at low levels in ovary, liver, and lung. The expression of exon 1e was instead restricted to placenta (at low levels) and liver.

As the 5′-RACE and the expressed sequence tag (EST) libraries analyses showed no exon 1b utilization in liver samples, we studied alternative expression in two samples of liver by means of semi-quantitative RT-PCR analysis. As it is possible to see in Fig. 5, the expression was higher for exon 1e in both samples: the amplification is visible in agarose gel at 22 cycles for exon 1e, whereas a positive fragment could be seen only at 26 cycles for exon 1b.

4. Discussion

In the last few years, the OATP family has attracted intensive research, because of its important role in detoxification processes, whereas the OATP2B1 member has been especially studied for its implication in drug and steroid conjugate uptake by several organs. However, limited information was available regarding the regulation of OATP2B1 transcription and expression. By 5′-RACE analysis, we demonstrate that at least five splice variants are used in *SLCO2B1* transcription. The different transcriptional forms are based on five distinct first exons, three of which (1a, 1c and 1d) were so far unknown, while exon 1b had been previously reported in thalamus, brain, uterus and placenta and exon 1e in an unspecified tissue. The 5′-flanking region of the gene, therefore, extends for 62 kbp, a much longer distance than previously surmised.

All alternative first exons, except exons 1c and 1d that were found only in uterus and thyroid, respectively, vary in length in different tissues owing to the occurrence of multiple TSSs, as previously reported for TATA box-less genes in which transcriptional constraints are normally relaxed [26,31,32].

The broadest tissue distribution was found for transcripts containing exon 1b, that occurred in 10 out of the 14 tissues investigated. This result was confirmed analysing the EST libraries available in GenBank (8 out of 10 of the tissues positive for exon 1b in our analysis were present in EST libraries with the same first exon). Moreover, this result was further validated by RT-PCR analysis with specific primers performed on the same samples subjected to 5′-RLM-RACE plus duplicates or triplicates of some tissues. The other first exons have shown a more restricted utilization: three tissues/cell line by 5′-RACE and four tissues by RT-PCR (exons 1a), one tissue by 5′-RACE and four tissues by RT-PCR (exon 1d), two tissues with both 5′-RACE and RT-PCR (exon 1e), or only one tissue (exons 1c). Although according to the analyses of EST libraries and 5′-RLM-RACE, hepatic OATP2B1 transcription seems regulated by the promoter associated with exon 1e, the results obtained with RT-PCR showed, as in placenta, an overlapping use of the first exons 1b and 1e. However, semi-quantitative RT-PCR established the prevalence of the promoter associated with exon 1e.

Only exon 1b is partially coding with a putative ATG in frame with the translation codon located in exon 2, which is common to every transcripts. Thereby, the N-terminal region of the encoded protein is 22 aa longer than when translation starts in exon 2. Actually, the ATG located in exon 1b corresponds to the right start of translation for this transcript, as demonstrated by N-terminal sequencing of the OTP2B1 protein expressed in *Sf9* insect cells [33]. The same prediction of membrane-spanning regions and orientation was obtained with the analysis of both proteins, suggesting that the addition of the first 22 aa does not interfere with these features.

In 2006, the promoter region associated with exon 1b was characterized in intestinal and hepatic cell lines [24]. The transcription factor Sp1 was required for constitutive expression of OATP2B1 in liver and small intestine, whereas HNF1α, which is involved in the expression of liver-specific OATPs [25], did not seem to play a role in OATP2B1 expression. However, the transcriptional regulation of OATP2B1 in intestinal and hepatic cell lines was investigated using the promoter associated with exon 1b. Instead, a very intense band corresponding to exon 1e, either 1e/a or 1e/b, was found in the liver, where it is the prevalent transcript expressed also according to the analysis of the EST libraries and the semi-quantitative RT-PCR. Thus, understanding OATP2B1 transcription regulation in this organ requires the analysis of the promoter associated with exon 1e as well. In the light of our results, also the promoters of the other transcripts must be determined in tissues or cell lines.

OATP2B1 has been suggested to be one of the membrane transporter proteins implicated in the uptake of steroid sulfates through the cell membrane. Lately, progesterone was shown to enhance E1S uptake in a concentration-dependent manner, whereas unconjugated androgens are potent inhibitors of OATP2B1 [34]. Moreover, a recent report has focused on the correlation between STS, the enzyme responsible for the hydrolysis of inactive steroid conjugates into active hormones [35], and OATP2B1 expression levels in patients with breast cancer in order to investigate the potential prognostic value of the transporter [36]. This study established that OATP2B1 expression levels correlate with the grade and stage of the disease, but not with the clinical outcome.

In conclusion, the present work demonstrates that the regulation of *SLCO2B1* transcription is more complex than earlier supposed, involving a longer 5′-flanking region and multiple promoters for the specificity and fine tuning of its tissue expression.

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