Cloning and stable expression of a new member of the human liver phenol/bilirubin:UDP-glucuronosyltransferase cDNA family

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A new human liver UDP-glucuronosyltransferase (HlugP4) has been cloned and expressed in cell culture. The expressed enzyme has a molecular mass of 56 kDa and preferentially catalysed the glucuronidation of halogenated and bulky alkyl phenols. The C-terminal half of the sequence (246 amino acids) is 96 % identical with the same portion of HlugP1, whereas the N-terminal half of the deduced protein sequences are only 38 % identical. These results suggest that the two isoenzymes may be derived from the same gene by differential splicing of the gene product.

INTRODUCTION

Human hepatic UDP-glucuronosyltransferases (UDP-GT; EC 2.4.1.17) are a family of microsomal enzymes that catalyse the glucuronidation of many important drugs, xenobiotics and endogenous compounds [1]. Attempts to characterize the microsomal enzymes by conventional purification techniques are often frustrated by the instability of detergent-solubilized UDP-GT and the routine availability of tissue. However, two human liver UDP-GTs have been recently purified and partially characterized [2], although problems with the instability and reconstitution of these membrane-derived lipid-dependent enzymes have caused inconsistencies in the assessment of their substrate specificities. Further, immunoblotting of human liver microsomes indicated that at least six UDP-GT isoenzymes needed to be purified [3].

Our strategy was to obtain cDNA clones encoding the human liver UDP-GTs and to stably express these enzymes in cell lines to facilitate assessment of their functional activity. Further, the availability of cDNA clones will allow the investigation of the regulation, differential expression, development and inherited defects of human UDP-GTs. The first human liver UDP-GT cDNA was cloned in 1987 [3] and subsequently transiently expressed in COS-7 cells [4] and stably expressed in V79 cells [5]. Harding *et al.* [6] reported the isolation of seven human liver UDP-GT cDNA clones which could be separated into two gene families on the basis of their sequence identities. One gene family contained five members, apparently responsible for the metabolism of steroids [7] and bile acids [4]. The second family contained two members, namely HlugP1 and HlugP2. HlugP1 was expressed in cell cultures and determined to encode a UDP-GT that catalyses the glucuronidation of phenolic compounds [8]. The other member, HlugP2, was not a full-length cDNA, but was determined to be identical with HlugP1 in the C-terminal 246-amino-acid sequence, but with only 48 % identity in the N-terminal 145 amino acids [6].

In the present paper we report the cloning and stable expression of the a new member of this UDP-GT cDNA subfamily, and we discuss the possible genetic derivation of the UDP-GT isoenzymes within this subfamily.

MATERIALS AND METHODS

A human liver cDNA library in λ UNI-ZAP was purchased

from Stratagene (La Jolla, CA, U.S.A.). XL1-Blue host cells were grown in Luria–Bertani medium [9] supplemented with 0.2 % maltose and 1 mM-MgSO₄. Plating cells were prepared by resuspending the host cells in 10 mM-MgSO₄. The library was plated out as in the manufacturer's instructions. The bacteriophage were transferred to nitrocellulose filters (Hybond-C; Amersham International). The plaques were amplified *in situ* by placing the filters on a second plate which was incubated at 37 °C for 8–10 h. The filters were processed by following the procedures recommended by the membrane manufacturers. HlugP1 [8] and HlugP2 [6] were labelled with [α -³²P]dCTP (10 mCi/ml; Amersham International) using the random primer procedure [10]. Hybridization was carried out at 65 °C with 10⁶ c.p.m./ml of probe for 12–16 h [9]. The filters were washed to a stringency of 0.1 $\times$ SSC/0.1 % SDS at 65 °C and autoradiographed at –70 °C for 16–24 h. The recombinant phage from the primary plugs were eluted into 0.5 ml of SM buffer [1 M-Tris/HCl (pH 7.2)/2 M-NaCl/330 mM-MgSO₄].

The inserts of the putative primary positives were sized by the PCR using a method adapted from that described by Asundi *et al.* [11]. Approx. 10⁶ recombinant bacteriophages were incubated at 94 °C for 5 min with 1 $\times$ PCR buffer (Perkin–Elmer/Cetus, Norwalk, CT, U.S.A.), 200 μ M of each dNTP and 0.2 μ M of each primer CAGCACTGACCCTTTTG (–40 sequencing primer) and AACAGCTATGACCATG (reverse sequencing primer). After the addition of AmpliTaq DNA polymerase (Perkin–Elmer/Cetus), the cDNA inserts were amplified by incubating for 25 cycles (94 °C for 1 min, 55 °C for 1 min and 72 °C for 2 min) in a thermal cycler [12].

Approx. 500 ng of PCR product was electrophoresed through a 0.7 % agarose gel. The DNA was transferred to a nylon membrane [13]. The blot was probed with the same ³²P-labelled cDNA fragment that was used in the primary screen [9]. The clones with the longest inserts were purified by successive (usually four) rounds of screening.

Bacteriophage R408 (Stratagene, La Jolla, CA, U.S.A.) was used to superinfect XL1-Blue cells containing λ UNI-ZAP clones and excisions were performed *in vivo* according to the manufacturers' instructions. The cDNA inserts were subcloned into M13 (mp18 or mp19) and sequenced on both DNA strands and across all restriction sites by the dideoxy-chain-termination method [14] using fluorescently labelled primers. The termination

Abbreviations used: UDP-GT, UDP-glucuronosyltransferase; 1 $\times$ SSC, 0.15 M-NaCl/0.015 M-sodium citrate.

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-34 AGTCATCTTCCAGTTCCTCCCACTCACCTCTGGC

ATG GCT TGC ACA GGG TGG ACC AGC CCC CTT CCT CTA TGT GTG TGT CTG CTG CTG ACC TGT GGC TTT GCC GAG GCA GGG AAG CTA GTG GTA	90
Met Ala Cys Thr Gly Trp Thr Ser Pro Leu Pro Leu Cys Val Cys Leu Leu Thr Cys Gly Phe Ala Glu Ala Gly Lys Leu Val Val	
GTG CCC ATG GAT GGG AGC CAC TGG TTC ACC ATG AGG TCG GTG GTG GAG AAA CTC ATT CTC AGG GGG CAT GAG GTG GTT GTA GTC AGT GCC	180
Val Pro Met Asp Gly Ser His Trp Phe Thr Met Arg Ser Val Val Glu Lys Leu Ile Leu Arg Gly His Glu Val Val Val Val Ser Ala	
AGA GGT GAG TTG GCA ACT GGG AAG ATC AAT GAA TTG CAC AGT GAA GAC TTA TTC AAC TTC ATA TAC CTG GAG GAT CTG GAC CGG GAG TTC	270
Arg Gly Glu Leu Ala Thr Gly Lys Ile Asn Glu Leu His Ser Glu Asp Leu Phe Asn Phe Ile Tyr Leu Glu Asp Leu Asp Arg Glu Phe	
AAG GCT TTT GCC CAT GCT CAA TGG AAA GCA CAA GTA CGA AGT ATA TAT TCT CTA TTA ATG GGT TCA TAC AAT GAC ATT TTT GAC TTA TTT	360
Lys Ala Phe Ala His Ala Gln Trp Lys Ala Gln Val Arg Ser Ile Tyr Ser Leu Leu Met Gly Ser Tyr Asn Asp Ile Phe Asp Leu Phe	
TTT TCA AAT TGC AGG AGT TTG TTT AAA GAC AAA AAA TTA GTA GAA TAC TTA AAG GAG AGT TCT TTT GAT GCA GTG TTT CTC GAT CCT TTT	450
Phe Ser Asn Cys Thr Arg Ser Leu Phe Lys Asp Lys Lys Leu Val Glu Ser Ser Phe Asp Ala Val Met Phe Leu Asp Pro Phe	
GAT AAC TGT GGC TTA ATT GTT GCC AAA TAT TTC TCC CTC CCC TCC GTG GTC TTC GCC AGG GGA ATA CTT TGC CAC TAT CTT GAA GAA GGT	540
Asp Asn Cys Gly Leu Ile Val Ala Lys Tyr Phe Ser Leu Pro Ser Val Val Phe Ala Arg Gly Ile Leu Cys His Tyr Leu Glu Glu Gly	
GCA CAG TGC CCT GCT CCT CTT TCC TAT GTC CCC AGA ATT CTC TTA GGG TTC TCA GAT GAC ATG ACT TTC AAG GAG AGA GTA CGG AAC CAC	630
Ala Gln Cys Pro Ala Pro Lys Ser Tyr Val Pro Arg Ile Leu Glu Gly Phe Ser Asp Met Thr Phe Lys Glu Val Met Phe Arg Asn His	
ATC ATG CAC TTG GAG GAA CAT TTA TTA TGC CAC CGT TTT TTC AAA AAT GCC CTA GAA ATA GCC TCT GAA ATT CTC CAA ACA CTT GTT ACG	720
Ile Met His Leu Glu Glu His Leu Leu Cys His Arg Phe Phe Lys Asn Ala Leu Glu Ile Ala Ser Glu Ile Leu Gln Thr Pro Val Thr	
GAG TAT GAT CTC TAC AGC CAC ACA TCA ATT TGG TTG TTG CGA ACG GAC TTT GTT TTG GAC TAT CCC AAA CCC GTG ATG CCC AAC ATG ATC	810
Glu Tyr Asp Leu Tyr Ser His Thr Ser Ile Trp Leu Leu Asp Thr Asp Phe Val Leu Asp Tyr Pro Lys Pro Val Met Pro Asn Met Ile	
TTC ATT GGT GGT ATC AAC GCG CAT GAG AGG AAA GCG TTG CCT ATG <u>GAA TTT GAA GCC TAC ATT AAT GCT TCT GGA GAA CAT GGA ATT GTG</u>	900
Phe Ile Gly Gly Ile Asn Cys His Glu Arg Lys Ala Glu Pro Met <u>Glu Phe Glu Ala Tyr Ile <u>Asn Ala Ser</u> Gly Glu His Gly Ile Val</u>	
GGT TTC TCT TTG GGA TCA ATG GTC TCA GAA ATT CCA GAG AAG AAA GCT ATG GCA ATT GCT GAT GCT TTG GGC AAA ATC CCT CAG ACA GTC	990
Gly Phe Ser Leu Gly Ser Met Val Ser Glu Ile Pro Glu Lys Ala Met Ala Ile Ala Asp Ala Leu Gly Lys Ile Pro Gln Thr Val	
CTG TGG CGG TAC ACT GGA ACC CGA CCA CCA AAT CTT GCG AAC AAC ACG ATA CTT GTT AAG TGG CTA CCC GAA Asn Asp CTG CTT GGT CAC	1080
Leu Trp Arg Tyr Thr Gly Thr Arg Pro Cys Asn Leu Ala <u>Asn Asn Thr</u> Ile Leu Val Lys Trp Leu Pro Gln Asn Asp Leu Leu Gly His	
CCG ATG ACC AGT GCC TTT ATC ACC CAT GCT GGT TCC CAT GGT GTT TAT GAA AGC ATA TGC AAT GGC GTT CCC ATG GTG ATG ATG CCC TTG	1170
Pro Met Thr Arg Ala Phe Ile Thr His Ala Gly Ser His Gly Val Tyr Glu Ser Ile Cys Asn Gly Val Pro Met Val Met Met Pro Leu	
TTT GGT GAT CAG ATG GAC AAT GCA AAG CGC ATG GAG ACT AAG GGA GCT GGA GTG ACC ATG AAT GTT CTG GAA ATG ACT TCT GAA GAA TTA	1260
Phe Gly Ser Gln Met Asp Asn Ala Lys Arg Met Glu Thr Lys Gly Ala Gly Val Thr Met Asn Val Leu Glu Met Thr Ser Glu Asp Leu	
GAA AAT GCT CTA AAA GCA GTC ATC AAT GAC AAA AGT TAC AAG GAG AAC ATC ATG CGC CTC TCC AGC CTT CAC AAG GAC CGC CCG GTG GAG	1350
Glu Asn Ala Leu Lys Ala Val Ile Asn Asp Lys Ser Tyr Lys Glu Asn Ile Met Arg Leu Ser Ser Leu His Lys Asp Arg Pro Val Glu	
CCG CTG GAC CTG GCC GTG TTC TGG GTG GAG TTT GTT ATG AGG CAC AAG GGC GCG ACA CAC CTG CGC CCC GCA GCC CAC GAC CTC ACC TGG	1440
Pro Leu Asp Leu Ala Val Phe Trp Val Glu Phe Val Met Arg His Lys Gly Ala Thr His Leu Arg Pro Ala Ala His Asp Leu Thr Trp	
TAC CAG TAC CAT TCC TTG GAC GTG ATT GGT TTC CTC TTG GCC GCC GTG CTG ACA GTG GCC TTC ATC ACC TGT AAA TGT TGT GCT TAT GGC	1530
Tyr Gln Tyr His Ser Leu Asp Val Ile Gly Phe Leu Leu Ala Ala Val Leu Thr Val Ala Phe <u>Ile Thr Cys Lys Cys Cys Ala Tyr Gly</u>	
TAC CCG AAA TGC TTG GGG AAA AAA GGG CGA GTT AAG AAA GCC CAC AAA TCC AAG ACC CAT TGA GAGGTGGGTGGGAATAAGGTAATAATTTTGAACCA	1626
Tyr Arg Lys Cys Leu Gly Lys Gly Arg Val Lys Lys Ala His Lys Ser Lys Thr His Stop	
TCCTAGTCATTTCACAACTTGAACACAGAATCAGTGTTAAATTCATTATTCTTTAATGAAGAAATCTTTGCATAAATTAATCAGCCCGAGGTGCTTTAAAAATTCCTTAAT	1745
AAAAATAATAGACTCGTAGTCAGTAAGATATTTGAATATGTATCGTGCCCTCCGGAGTCTTTGATCAGGATGACATGTGCCATTTTCAAGAGCGTGAGCAGAGCTGGCATT	1864
TTAGATTACTTTTCTACTCTGAACATGCGCTGTGTTGGGAGTGCGGATTCAAGGTGGTCCCGGCTGCCCTACTGCAAAATGGGAGTTTAAATCCTATCTTTTGCTCTCGAGA	1983
TGGTTGCATTGATCTTAACCAATAATGGTCAGTCCTCATCTGCTGCTGACTTCATAGGTGCCAGGTGTGTGTTTAAAGAAGGGAAGCTTTGTACCTTTAGAGTGTAGGTGAATGAA	2102
TGAATGGCTTGGAGTGCAGTGAAGACGATATGATTTCTTGTCTTTGGGAAAAAAGATGATGCTATGAAATTTGGTGGTGGTTTATTTGAGAAGATAATCATTGCTATGTCAAAATGG	2221
AGGTTAATTTGATAAAAAACCAAAATACAGCTATGAAGTCTGGGCAAGTTTACTTTTTTTCTGTATGTTCTTACAAATAAAAAATAAATAAATTTATATAAATCTATTAAAAA	2340
AAAAAAAAAAAAAAAA	2356

Fig. 1. Nucleotide sequence of human phenol UDP-GT cDNA (HlugP4) and derived amino acid sequence

A bold line divides the variable 5' portion of the cDNA from the 3' conserved region. The putative polyadenylation and *N*-linked glycosylation sites are underlined with hatched boxes. The proposed *N*-terminal signal sequence and transmembrane sequences are underlined (continuous and broken lines respectively).

products were resolved and detected on an Applied Biosystems 370A automated DNA sequencer (ABI, Foster City, CA, U.S.A.).

HlugP4 was subcloned into the expression vector pKCRH2 [15], kindly provided by Dr. S. Shibahara (Friedrich Meischer Institute, Basel, Switzerland). V79 Chinese-hamster lung fibroblast cells were co-transfected with Semiliki Forest virus and recombinant plasmids containing HlugP4 in the correct orientation with respect to the simian-virus-40 promoter. Geneticin-resistant colonies were isolated and cultured as described by Fournel-Gigleux *et al.* [5]. UDP-GT assays were performed with 0.1 mg of protein from transfected cells or 0.05 mg of microsomal protein as previously described [16]. The following aglycone substrates were used: 1-naphthol, 4-fluorophenol, 4-bromophenol, 4-iodophenol, 4-methylphenol, 4-ethylphenol, 4-propylphenol, 4-*t*-butylphenol (Aldrich, Gillingham, Dorset, U.K.), 4-chlorophenol (Merck, Poole, Dorset, U.K.), oestriol, androsterone (Sigma, Poole, Dorset, U.K.) and (–)-morphine (Allardye Chemists, Dundee, Scotland, U.K.).

Protein concentrations were determined by the method of Lowry *et al.* [17], with BSA as standard. Immunoblot analysis was performed as described by Coughtrie *et al.* [18] using the alkaline phosphatase detection system.

RESULTS

More than 1500 recombinant plaques that hybridized to ³²P-labelled HlugP1 were identified after screening approx. 500 000 recombinants of the human liver cDNA library in λUNI-ZAP. The cDNA insert of each putative primary positive was amplified using the PCR, and eight cDNA clones that were longer than 2.3 kb were plaque-purified. Initial sequence data suggested that one cDNA was similar to HlugP1 [8], whereas two were similar to HlugP2 [6], and a further two exhibited a strong identity with HlugP3 [19]. The remaining three clones were similar to one another. The longest of these, HlugP4, appeared to encode a novel UDP-GT.

Fig. 2. Immunoblot analysis of human microsomes and V79 stable cell lines expressing HlugP1 and HlugP4

V79 cells expressing HlugP1 [(20 μ g) lane 1], HlugP4 [(10 μ g) lane 2], control (pKCRH2 only) transfected V79 cells [(10 μ g) lane 3] and human liver microsomes [10 μ g) lane 4] were analysed by SDS/7% PAGE. The proteins were transferred to a nitrocellulose filter that was incubated with an anti-(rat liver UDP-GT) IgG. Chromogenic detection was as described in [18]. The molecular-mass marker proteins were BSA (68 kDa), pyruvate kinase (57 kDa), fumarase (49 kDa) and aldolase (40 kDa).

HlugP4 has 34bp and 764bp of 5' and 3' non-coding sequence respectively. These flank an open reading frame of 1593bp (Fig. 1). The sequence from base 856 to 2356 of HlugP4 had a high identity (99%) with three other human UDP-GT cDNAs, HlugP1, HlugP2 and HlugP3 [19]. In this region there were 20 nucleotide differences, and 13 of these fall within the open reading frame of the cDNA. There are two silent point mutations in the coding region; five of the codon changes are for similar amino acids, and the remaining six codon changes are for different amino acids. The differences between the conserved regions of HlugP1 and HlugP4 are probably due to inter-individual variation, as both clones were isolated from different human liver cDNA libraries.

Translation from base 1 of HlugP4 predicts a protein of 531 amino acid residues. The initiation methionine residue is analogous to the start site in other members of the phenol:UDP-GT cDNA subfamily and conforms to the consensus sequence described by Kozak [20]. The derived amino acid sequence from HlugP4 shows an overall identity of 67% with that obtained from HlugP1. Moreover, when the *N*-terminal 285 amino acid residues are compared, this identity decreases to 38%, the *C*-terminal 246 amino acids being 96% identical with one another (Fig. 1).

A stable cell line that expresses HlugP4 was constructed in V79 Chinese-hamster lung fibroblasts as described in the Materials and methods section. A cell line that stably expresses HlugP1 was described previously [5]. Samples of both cell lines were subjected to SDS/PAGE and transferred to nitrocellulose (as described in [5]). The blots were probed with anti-(rat liver UDP-GT) polyclonal antibodies which have previously been shown to cross-react with human UDP-GTs [3]. Fig. 2 shows that the expressed UDP-GTs HlugP4 and HlugP1 have molecular masses of 56 and 55 kDa respectively.

The catalytic activity of the HlugP4 cell line was assessed using a large number of aglycones as putative substrates. The results in Fig. 3 show the data from the expression of HlugP4 and

Fig. 3. UDP-GT activity of human microsomes and V79 stable cell lines expressing HlugP1 and HlugP4

UDP-GT activity was measured as described in the Materials and methods section. ■, Human microsomes; ▨ and ▩, V79 cells expressing HlugP1 and HlugP4 respectively. The UDP-GT activities present in V79 cells towards the following substrates were all less than 5 pmol/min per mg of protein. The substrates were: 1, 4-methylphenol; 2, 4-ethylphenol; 3, 4-propylphenol; 4, 4-*t*-butylphenol; 5, 4-fluorophenol; 6, 4-chlorophenol; 7, 4-bromophenol; 8, 4-iodophenol; and 9, 1-naphthol. The activities (expressed as pmol of glucuronide produced/min per mg of protein) are the averages of two independent results.

HlugP1 compared with the UDP-GT activity in human microsomes. There is a striking similarity in the substrate specificities of the two cloned UDP-GTs towards the halogenated phenols. This was despite the dissimilarity of the variable *N*-terminal sequence (38% identity), which is thought to be responsible for aglycone binding [21,22]. The difference between the two expressed UDP-GTs was their ability to glucuronidate phenols substituted with increasingly bulky alkyl groups. The transferase encoded by HlugP1 exhibited a high activity towards planar phenols such as 1-naphthol, 4-methylphenol and 4-ethylphenol. However, expressed HlugP4 showed a higher activity towards 4-propylphenol and 4-*t*-butylphenol.

Neither of the cloned expressed UDP-GTs catalysed the glucuronidation of (–)-morphine, oestriol or androsterone,

although the most suitable endogenous or xenobiotic substrate(s) may remain to be identified.

DISCUSSION

Kinetic studies of human liver microsomal phenol glucuronidation have suggested the existence of more than one UDP-GT catalysing the glucuronidation of phenols [23,24]. Previously we have described the isolation of a UDP-GT cDNA (HlugP1) that encodes a phenol-glucuronidating transferase [8]. Here we report the cloning and expression of a second UDP-GT (HlugP4) that has a similar functional activity.

Analysis of the substrate specificity of the expressed HlugP4 indicates that this UDP-GT preferentially catalysed the glucuronidation of bulky phenols (see Fig. 3). In contrast, the transferase encoded by HlugP1 poorly glucuronidated bulky phenols whilst exhibiting a higher activity towards planar phenols (for example 1-naphthol). Further, the expressed clones do not appear to accept some steroids or bile acids as substrates.

Interestingly, two other UDP-GT cDNAs (HlugP2 [6] and HlugP3 [19]) were isolated previously in this laboratory that share an identical C-terminal half with HlugP4 and HlugP1. HlugP2 and HlugP3 correspond to two human bilirubin UDP-GT cDNAs that were reported by Ritter *et al.* [25]. Therefore four cDNAs encoding UDP-GTs have been isolated with identical C-terminal 246-amino-acid-residue sequences, but variable N-terminal 285-amino-acid sequences. This supports the previous evidence that the N-terminal portion determines the substrate specificity of the transferase [21,22].

Evidence from Southern-blot analysis indicates that there is only one copy of the conserved 3' region in the human genome (N. Moghrabi, M. Boxer & B. Burchell, unpublished work). Chromosomal mapping of the sequences encoding the four variable regions using specific probes indicated that all of these sequences were located on human chromosome 2 [26]; N. Moghrabi, M. Boxer, S. Povey, R. Wooster, L. Sutherland & B. Burchell, unpublished work). These results suggest that at least four isoenzymes may be derived from variable exons and conserved exons within the same gene. The above studies have been authenticated by the work of Ritter *et al.* [27]. They had reported the isolation of four human cosmid clones that span 110 kb and contain the four constant 3' exons of the phenol/bilirubin UDP-GTs. Moreover, these genomic clones contain six variable 5' exons that include the coding sequence for HlugP1, HUGBr1 and HUGBr2 and three other UDP-GTs. It has been proposed that these UDP-GT mRNAs result from alternative splicing from this gene complex. Other examples of differential splicing of a single gene, to obtain functionally different G-proteins and fibronectins, has been previously reported [28,29].

The variable 5' exon(s) of HlugP4 had not been identified within the phenol/bilirubin:UDP-GT gene complex. Therefore the complete phenol/bilirubin:UDP-GT gene may cover more than 110 kb and contain more as-yet-unidentified variable 5' exons. Studies of the structure and function of the cloned gene encoding all of these exons is required to explain the derivation and differentially regulated expression of the different isoenzymes. The above gene organization correlates with the structural domains of proteins, as has been suggested for the NADPH:cytochrome P-450 oxidoreductase [30] and low-density-lipoprotein-receptor genes [31].

The differential expression of gene products encoded by this phenol/bilirubin:UDP-GT gene complex has been illustrated in Crigler-Najjar-type-1-disease patient livers, where phenol UDP-GT activity was normal but bilirubin UDP-GT was completely abolished [32]. The existence of a similar gene has also been

identified in rats ([33,34]; L. Sutherland, D. Clarke, L. Gordon & B. Burchell, unpublished work), where it is known that the phenol- and bilirubin-metabolizing isoforms are regulated differentially during development [18] and by xenobiotic inducers [35].

Therefore further studies of this intriguing gene structure should resolve these unanswered questions about the differential regulation of expression of this subfamily of UDP-GTs and provide an insight to the genetic lesions in inherited hyperbilirubinaemias.

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