Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical Pharmacology 65 (2003) 1521–1527
Biochemical Pharmacology
www.elsevier.com/locate/biochempharm

Frequent co-occurrence of the TATA box mutation associated with Gilbert's syndrome (*UGT*1A1*28) with other polymorphisms of the UDP-glucuronosyltransferase-1 locus (*UGT*1A6*2 and *UGT*1A7*3) in Caucasians and Egyptians

Christoph Köhle[a], Bernd Möhrle[a], Peter A. Münzel[a], Matthias Schwab[b], Dorothee Wernet[c], Osama A. Badary[d], Karl Walter Bock[a,*]

[a]*Institute of Pharmacology and Toxicology, University of Tübingen, Tübingen, Germany*
[b]*Dr. Margarete-Fischer-Bosch Institute of Clinical Pharmacology, Stuttgart, Germany*
[c]*Department of Transfusion Medicine, University Hospital, Tübingen, Germany*
[d]*Department of Pharmacology and Toxicology, College of Pharmacy, Al-Azhar University, Cairo, Egypt*

Received 24 October 2002; accepted 17 January 2003

Abstract

Polymorphisms of drug metabolizing enzymes are frequently associated with diseases and side effects of drugs. Recently, a TATA box mutation of *UGT*1A1 (*UGT*1A1*28), a common genotype leading to Gilbert's syndrome, and several missense mutations of other UDP-glucuronosyltransferase 1 (*UGT*1) family members have been described. Furthermore, co-occurrence of *UGT*1A1*28 and *UGT*1A6*2 has been observed. In order to elucidate the basis for co-occurrence of *UGT*1 mutations, fluorescence resonance energy transfer techniques were developed for rapid determination of polymorphisms of three *UGT* isoforms (*UGT*1A1*28, 1A6*2, and 1A7*2/*3). Hundred healthy Caucasians and 50 Egyptians were genotyped. All genotypes followed the Hardy–Weinberg equilibrium. Only three major haplotypes were found, including a haplotype consisting of allelic variants of all three isoforms (29% in Caucasians and 22% in Egyptians), all leading to reduced UGT activity. Frequent haplotypes containing several *UGT*1 allelic variants should be taken into account in studies on the association between diseases, abnormal drug reactions, and *UGT*1 family polymorphisms.

Keywords: UGT1 polymorphisms; Gilbert's syndrome; Genotype frequencies; Haplotype frequencies

1. Introduction

Polymorphisms of drug metabolizing enzymes are known to play important roles in disease susceptibility and clinical response to therapeutic drugs [1]. Recently, several polymorphisms of UGTs have been identified; for example, a TATA box mutation of *UGT*1A1 and missense mutations of two *UGT* isoforms of family 1 (Table 1). These polymorphisms include: (i) the TATA box mutation of bilirubin-conjugating UGT1A1 (*UGT*1A1*28) which has been shown to be a major genotype leading to Gilbert's syndrome, phenotypically presenting as a benign, non-hemolytic intermittent hyperbilirubinemia [2–4]. In addition, reduced activity of UGT1A1 was found to be related to a higher incidence of irinotecan toxicity since UGT1A1 is mostly responsible for inactivation of its major metabolite SN38 [5,6]. (ii) A missense mutation of *UGT*1A6 has also been described [7] which has been found to be associated with reduced protection against colon carcinogenesis by aspirin [8]. (iii) Recently, three missense mutations of *UGT*1A7 have been characterized [9], an isoform not expressed in liver but in the upper gastrointestinal tract [10]. Interestingly, these polymorphisms were found to be associated with hepatocellular carcinoma [11] and with orolaryngeal cancer [12].

The described *UGT* polymorphisms are present in the *UGT*1 gene locus localized on chromosome 2q37 which

* Corresponding author. Tel.: +49-7071-2972274; fax: +49-7071-292273.
E-mail address: bock@uni-tuebingen.de (K.W. Bock).
Abbreviations: UGT, UDP-glucuronosyltransferase; FRET, fluorescence resonance energy transfer.

0006-2952/03/$ – see front matter © 2003 Elsevier Science Inc. All rights reserved.
doi:10.1016/S0006-2952(03)00074-1

Table 1
UGT polymorphisms

Polymorphism	Allelic variation	UGT activity
*UGT*1A1*1	$(TA)_6TAA$	
*UGT*1A1*28	$(TA)_7TAA$	Reduced (ca. 3-fold) [2–4]
*UGT*1A6*1	$T^{181}R^{184}$	
*UGT*1A6*2	$A^{181}S^{184}$	Reduced (ca. 2-fold) [7]
*UGT*1A7*1	$N^{129}R^{131}W^{208}$	
*UGT*1A7*2	$K^{129}K^{131}W^{208}$	Reduced (2.6-fold) [9]
*UGT*1A7*3	$K^{129}K^{131}R^{208}$	Reduced (5.8-fold) [9]
*UGT*1A7*4	$N^{129}R^{131}R^{208}$	Reduced (2.8-fold) [9]

encodes 13 UGT isoforms, 4 of them are pseudogenes [13]. The organization of this locus appears to be unique. It consists of a large nested array of exons, preceded by their respective promoters, which are spliced to their common exons 2–5 by exon sharing. The common exons encode the part of the UGT1 isoforms which binds their common cofactor, UDP-glucuronic acid. Exons encode the part of the isoform which binds their respective substrates. Since the distance of the described polymorphic allelic variants is within ca. 90 kb, it is unlikely that it is affected by recombination events. Previously, frequent co-occurrence of the TATA box mutation of *UGT*1A1 and of the polymorphisms of *UGT*1A6 has been described [14].

In order to elucidate the basis for co-occurrence of polymorphic UGT1 isoforms, we developed rapid methods to genotype individuals for polymorphisms of the above three UGT1 isoforms using FRET technology. We analyzed in 100 healthy Caucasians and 50 Egyptians the haplotype frequency of four allelic variants of the *UGT*1 gene locus (*UGT*1A1*28, *UGT*1A6*2, *UGT*1A7*2, and *UGT*1A7*3). It was found that haplotype II was astonishingly frequent (29% for Caucasians and 22% for Egyptians), including low activity allelic variants of three UGT isoforms, e.g. the TATA box mutation of *UGT*1A1 responsible for Gilbert's syndrome.

2. Materials and methods

2.1. Study population and isolation of genomic DNA

One hundred randomly selected healthy Caucasians (50 females and 50 males), blood donors from the University Hospital, Tübingen, Germany, and 50 Egyptians (17 females and 33 males) from upper Egypt were enrolled for genetic analysis. All donors gave their written consent. Genomic DNA samples were prepared from leukocytes contained in whole blood using the QiaAmp isolation system according to the recommendations of the manufacturer.

2.2. Analysis of UGT1 polymorphisms

Wild-type and variant alleles of *UGT*1A1, *UGT*1A6, and *UGT*1A7 were determined using pairs of fluorescent probes designed to hybridize to variant regions in amplified products of genomic DNA. PCR was performed using the LightCycler-DNA Master Hybridization Probes Kit (Roche). FRET between the anchor and sensor probes was detected using the LightCycler. Melting temperature analysis was used to distinguish between the different variants. Primers and probes were synthesized by TIB MOLBIOL, with the hybridization probes containing fluorescein and LightCycler Red 640.

2.2.1. UGT1A1*28

Genotyping of the TA insertion polymorphism in the promoter region of the *UGT*1A1 gene was carried out according to Borlak *et al.* [15]. For better resolution, concentrations of the hybridization probes were increased to 75 nM.

2.2.2. UGT1A6*2

Primer sets were designed for amplification of a 231-bp fragment of exon 1 of the *UGT*1A6 gene in the complex UGT1 locus. The primers and probes used are shown in detail in Table 2. The sequence of the sensor probe was according to the mutated allele (*UGT*1A6*2). PCR was performed using 50 ng genomic DNA as template, 3 mM $MgCl_2$, 0.5 μM of each primer, 50 nM of each hybridization probe in a total volume of 20 μL. After an initial denaturation step at 95° for 3 min, amplification was performed for 55 cycles of denaturation (95°, 5 s, ramp rate 20°/s), annealing (63°, 5 s, ramp rate 20°/s), and extension (72°, 10 s, ramp rate 20°/s). The analytical melting program was 95° for 20 s and 40° for 40 s, increasing to 78° at a ramp rate of 0.1°/s, with continuous fluorescence acquisition.

2.2.3. UGT1A7

The PCR was employed to amplify a 425-bp fragment of exon 1 of the *UGT*1A7 gene in the *UGT*1 locus. Primer sequences are shown in Table 2. Sensor 1 was designed to cover the polymorphic sites at codons 129/131 and to be complementary to the N129K and R131K mutations. Sensor 2 covered the codon 208 and was complementary to the wild-type allele. PCR amplifications on the LightCycler were performed using 50 ng genomic DNA, 3.0 mM $MgCl_2$, 0.6 μM forward primer, and 0.2 μM reverse primer in 20 μL reactions. Hybridization probes concentrations were 50 and 100 nM of the anchor 1/sensor 1 and anchor 2/sensor 2 pairs, respectively. The cycling program consisted of 3 min of initial denaturation at 95° and 45 cycles of 95° for 10 s, 57° for 7 s, and 72° for 20 s, with maximum ramp rate. The program for analytical melting of anchor 1/sensor 1 pair was 95° for 10 s, 40° for 40 s, and an increase to 70° at a 0.1°/s ramp rate. The program for analytical melting of anchor 2/sensor 2 pair was 95° for 10 s, 40° for 20 s, and an increase to 86° at a 0.2°/s ramp rate.

Table 2
Primers and labeled probes

Primers/probes	Sequences	GenBank accession number/mapping positions
*UGT*1A1		D87674
Forward	5′-AAGTGAACTCCCTGCTACCTT-3′	3045–3065
Reverse	5′-CCACTGGGATCAACAGTATCT-3′	3297–3277
Anchor	5′-CTTTGCTCCTGCCAGAGGTTCGCCCT-F	3186–3161
Sensor	5′-LC-CCTACTTATATATATATATATATGGCAAAAACC-p	3158–3126
*UGT*1A6		M84130
Forward	5′-GGCCTGTACTTCATCAACTGCC-3′	571–592
Reverse	5′-GTAGCACCTGGGAATGTAGGAC-3′	797–780
Anchor	5′-ACCTCTTCAGGGGTTTTCCGTGTTCCC-F	719–745
Sensor	5′-LC-AGCATGCATTCAGCAGCAICCC-p	749–770
*UGT*1A7		U39570
Forward	5′-TGCCGATGCTCGCTGGACG-3′	443–461
Reverse	5′-AATTGATGTGTGGCTGTAGAGAT-3′	914–892
Anchor 1	5′-CGAGAAACACTGCATCAAAACAACTCTCC-F	606–578
Sensor 1	5′-LC-AGTATTCTACTAATTTTTTGTCCTTAAAC-p	573–545
Anchor 2	5′-LC-AAAGTCATGGCGTCTGAGAACCCTAAG-p	772–746
Sensor 2	5′-TGATGTGGTTCCATACTCTCTCCTTG-F	798–773

Variant position is underlined. I, inosine; F, carboxyfluorescin; LC, LightCycler Red 640, p, 3′-phosphate.

2.2.4. Cloning of PCR standards

To identify and discriminate *UGT*1A polymorphisms, we constructed control plasmids containing sequences homologous to either the wild-type or the polymorphic alleles to use as assay standards. Wild-type and polymorphic sequences were generated by PCR with the use of primers listed in Table 2 and the genomic DNA from subjects identified by dideoxy sequencing as homozygous for wild-type alleles or mutant alleles as template. PCR reactions were carried out in a final volume of 20 μL on a GeneAmp 2400 (Perkin-Elmer). The reaction mixture consisted of 100 ng genomic DNA, 1.5 mM $MgCl_2$, 0.2 mM each dATP, dCTP, dGTP, and dTTP, 0.5 μM of each forward and reverse primer, and 2 U AmpliTaq Gold (Applied Biosystems) with recommended buffer. The cycling conditions were as follows: initial denaturation for 10 min at 95°; 30 cycles of 95° for 30 s, 60° for 30 s, and 72° for 1 min. The resulting PCR products were cloned into plasmid pCR2.1 using the TA Cloning Kit (Invitrogen). Sequences were confirmed by custom sequencing (MWG Biotech).

2.3. Statistical analysis

Allele frequencies for each polymorphic allele were determined by gene counting. The significance of deviations from Hardy–Weinberg equilibrium was tested using the random-permutation procedure implemented in the ARLEQUIN package [16], which is available on the ARLEQUIN web page.

The population samples studied consisted of unrelated individuals; therefore, family data could not be used to determine haplotypes in multiply heterozygous individuals. Instead, maximum-likelihood estimates of haplotype frequencies and the standard errors were calculated from the multi-site genotyping data using the ARLEQUIN software, which implements the expectation-maximization (EM) algorithm [17].

Testing for linkage disequilibrium was performed using the maximum-likelihood haplotype frequencies obtained from EM algorithm and then applying a likelihood ratio test in order to determine whether the resolution of haplotypes are significantly non-random, which is equivalent to testing whether there is statistically significant linkage disequilibrium between loci [18]. The calculations were done using ARLEQUIN software.

3. Results

3.1. FRET analysis of UGT1 polymorphisms

PCR product identification was carried out by DNA melting curves (Fig. 1). Melting curves clearly distinguished homozygous and heterozygous individuals of polymorphisms of the three *UGT*1 family members. In addition to identification of the linked double mutation of the *UGT*1A6*2 allele (Table 1), a rare subtype [7] could also be identified in which only codon 184 was mutated: DNA from individuals harboring this subtype showed an atypical melting curve with a peak at 60° (not shown). Direct DNA sequencing revealed that the DNA contained a single mutation in one allele (R184S). The wild-type sequence and the more common double mutation melted at 51 and 65°, respectively. For *UGT*1A7 analysis, a 425-bp fragment was amplified in the presence of hybridization probes corresponding to either codons 129/131 or codon 208. Melting temperatures for 129/131 probes were 48 and

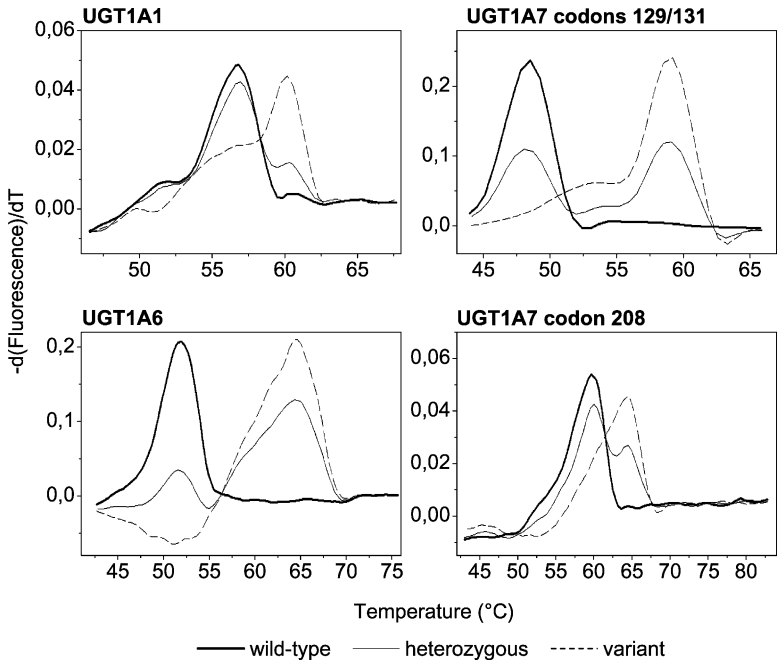

Fig. 1. DNA melting curves for homozygous and heterozygous genotypes of polymorphic *UGT*1 genes. Each peak represents the melting of the fluorescence-labeled sensor probe/target duplex.

59° for the wild-type and mutant allele, respectively. At codon 208, homozygous donors displayed melting peaks at 60° (wild-type) and at 64° (mutated sequence). All melting curves were verified by comparison with cloned standards (not shown).

3.2. Genotype and allele frequencies of UGT1 polymorphisms and Hardy–Weinberg equilibrium

Genotype frequencies of the three *UGT*1A polymorphisms investigated in the two populations are reported in Table 3. In both population samples, each of the three *UGT*1A sites was separately tested for agreement with the assumption of the Hardy–Weinberg equilibrium. No systematic departures were found. Allele frequencies for each polymorphic site determined by simple gene counting are shown in Table 4. Three TATA box genotypes, with 6/6, 6/7, and 7/7 TA repeats, were found in the promoter region of the *UGT*1A1 gene. They were present at frequencies of 50:42:8 (%) for Caucasians and of 56:36:8 (%) for Egyptians, in agreement with previous results [2,3]. *UGT*1A1 alleles containing five and eight TA repeats were not found.

Similarly, three major genotypes were determined for the *UGT*1A6 in both populations, in agreement with Ciotti *et al.* [7]. Three (of 100) Caucasians and three (of 50) Egyptians carried the rare *UGT*1A6 R184S allele, discussed in the previous section. We did not uncover an individual with only the T181A mutation.

*UGT*1A7 genotyping results showed that only 11% of the Caucasians (16% of the Egyptians) were homozygous for the *UGT*1A7 wild-type allele. Individuals carrying the *UGT*1A7 genotypes *1/*4, *3/*4, and *4/*4 were not found. In the case of individuals who were heterozygous at codons 129/131 and 208, theoretically two genotypes are possible, *UGT*1A7*1/*3 or *2/*4. Because

Table 3
Observed frequencies of *UGT*1A1 promoter, *UGT*1A6 and *UGT*1A7 genotypes compared to expected frequencies calculated from respective single-allele frequencies according to the Hardy–Weinberg equilibrium (in parenthesis)

Gene (genotype)	Frequency (%)	
	Caucasians (N = 100)	Egyptians (N = 50)
*UGT*1A1		
*1/*1	50 (50.4)	56 (54.8)
*1/*28	42 (41.2)	36 (38.4)
*28/*28	8 (8.4)	8 (6.8)
P values	1.000	0.711
*UGT*1A6		
*1/*1	44 (43.6)	40 (39.7)
*1/*2	42 (42.9)	42 (42.8)
*2/*2	11 (10.6)	12 (11.6)
*1/R184S	2 (2.0)	4 (3.8)
*2/R184S	1 (1.0)	2 (2.0)
P values	0.920	1.000
*UGT*1A7		
*1/*1	11 (12.6)	16 (17.6)
*1/*2	24 (19.9)	20 (16.8)
*2/*2	6 (7.8)	4 (4.0)
*1/*3	25 (25.9)	32 (32.0)
*2/*3	20 (20.4)	12 (15.2)
*3/*3	14 (13.3)	16 (14.4)
P values	0.713	0.837

P values resulting from an exact test using a Markov chain as implemented by the ARLEQUIN software are also given.

Table 4
Allele frequencies calculated from the observed genotype frequencies

Gene (allele)	Frequency (%)	
	Caucasians (N = 200)	Egyptians (N = 100)
*UGT*1A1		
*1	71.0 ± 3.2	74.0 ± 4.0
*28	29.0 ± 3.2	26.0 ± 4.0
*UGT*1A6		
*1	66.0 ± 3.5	63.0 ± 4.8
*2	32.5 ± 3.4	34.0 ± 4.9
R184S	1.5 ± 0.7	3.0 ± 2.0
*UGT*1A7		
*1	35.5 ± 3.2	42.0 ± 5.5
*2	28.0 ± 3.0	20.0 ± 4.2
*3	36.5 ± 3.3	38.0 ± 4.8

Standard deviations were calculated by the EM algorithm with 100 restarts.

the *UGT*1A7*4 allele was not found in both population samples and its frequency is very low in Caucasians [9], all ambiguous subjects were assumed to represent the genotype *UGT*1A7*1/*3.

In general, allele frequencies did not differ significantly between Caucasians and Egyptians. However, individuals of Egyptian origin tended to have a higher frequency of *UGT*1A7*1 allele and a lower frequency of the *UGT*1A7*2 allele.

3.3. Linkage disequilibrium and multi-site haplotype inference

A likelihood ratio test detected in the Caucasian population highly significant linkage disequilibrium between *UGT*1A1 and 1A6, between *UGT*1A1 and 1A7, and between *UGT*1A6 and 1A7 ($P < 0.00001$). In Egyptians, pairwise linkage disequilibrium was also detected between the three *UGT*1A sites ($P < 0.00001$).

Table 5 lists the haplotypes and their frequencies, as estimated by the EM algorithm. With little evidence for a departure from Hardy–Weinberg equilibrium, use of EM algorithm for the construction of haplotypes is justified [17]. Of the 18 possible haplotypes (the *UGT*1A1, *UGT*1A6, and *UGT*1A7 genes harbored two, three, and three alleles, respectively (Table 4)), only 12 were found, 9 were present in the Caucasian population and 9 in the Egyptian population. Interestingly, only three major haplotypes with frequencies higher than 10% were observed in the two populations: (i) haplotype I, including the wild-type alleles of the three UGTs, (ii) interestingly, haplotype II with a frequency of 29% in Caucasians and 22% in Egyptians in which all three UGT isoforms were low function allelic variants, and (iii) haplotype III in which *UGT*1A1 and *UGT*1A6 were wild-type linked to *UGT*1A7*2.

4. Discussion

FRET technology on the LightCycler was developed to rapidly genotype polymorphisms of three *UGT*1 family members, including the TATA box mutation of *UGT*1A1 responsible for Gilbert's syndrome as well as the *UGT*1A6*2 and *UGT*1A7*2/*3 polymorphisms. The technology was used to genotype 100 healthy Caucasians and 50 Egyptians. The rare allelic variant *UGT*1A7*4 was not found. The remaining polymorphic allelic variants followed the Hardy–Weinberg equilibrium, suggesting that the genotypes developed in the absence of evolutionary forces.

Haplotype frequencies were estimated for the first time to determine to what extent polymorphic variants of three *UGT*1 isoforms were present on the same chromosome 2q37. Although 18 haplotypes are theoretically possible with two, three, and three allelic variants at three loci, only three major haplotypes (>10%) were found. Interestingly, the haplotype II, including low activity allelic variants of three *UGT* isoforms, was frequent (29% in Caucasians and 22% in Egyptians). These findings provide hints why all eight individuals in the Caucasian population and three of four individuals in the Egyptian population who were

Table 5
Haplotypes and their estimated frequencies in each population

Number	Haplotype			Frequency (%)	
	*UGT*1A1	*UGT*1A6	*UGT*1A7	Caucasians	Egyptians
I	*1	*1	*1	35.0 ± 3.2[a]	40.9 ± 4.6[a]
II	*28	*2	*3	28.5 ± 3.1[a]	21.8 ± 4.1[a]
III	*1	*1	*2	26.3 ± 2.4[a]	14.7 ± 3.5[a]
IV	*1	*2	*3	3.5 ± 1.3[a]	11.1 ± 3.5[a]
V	*1	*1	*3	4.2 ± 1.4[a]	5.1 ± 2.2[a]
VI	*1	R184S	*2	1.2 ± 0.8	1.1 ± 1.1
VII	*28	*1	*2	–	2.3 ± 1.8
VIII	*28	R184S	*2	–	1.9 ± 1.5
IX	*1	*2	*1	–	1.1 ± 1.0[a]
X	*28	*1	*1	0.5 ± 0.5[a]	–
XI	*1	*2	*2	0.5 ± 0.5	–
XII	*1	R184S	*3	0.3 ± 0.4	–

[a] The presence of this haplotype could be unambiguously inferred from genotypes in which only zero or one allele varied.

homozygous for the *UGT*1A1*28 promoter mutant were also homozygous for the *UGT*1A6*2 and the *UGT*1A7*3 allelic variants. It may be of interest, to study a Japanese population, where the *UGT*1A1*28 genotype is rare [19]. Using the FRET technology it was found that the individual from which the widely studied Caco-2 cells (frequently used in studies of drug absorption and intestinal metabolism) are derived, also had a haplotype II pair (not shown). In general, major genotypes and allele frequencies were similar in Caucasians and Egyptians. But differences between these two populations were also observed (see haplotypes III, IV, and other minor haplotypes).

The reason for the frequently occurring haplotype II is intriguing. Since the studied *UGT*1 allelic variants are present within a short distance of ca. 90 kb on chromosome 2q37 [13], recombination events appear to be unlikely. While the proximity may explain co-inheritance, evolutionary persistence in two different populations of the unusually frequent haplotype II, including many low activity allelic variants, remains to be explained. Generation of this intriguing haplotype II by sequential mutations appears to be unlikely. However, we cannot exclude a hot spot of mutation or of recombination in this evolutionary assembly of cassette-like elements. Generation of haplotype II must have occurred before the separation of Caucasian and Egyptian populations. In this context, it may be relevant that variability in the *UGT*1A1 promoter may be a 'balanced polymorphism' (i.e. persistence of genetic form by selection), since it may fine-tune bilirubin levels: hyperbilirubinemia in newborns may be neurocytotoxic and lead to kernicterus, a possibly fatal neurologic disorder. On the other hand, low bilirubin is cytoprotective and serves together with biliverdin reductase as a powerful antioxidant [19–22].

Frequent co-occurrence of *UGT*1 mutations, such as *UGT*1A7*3, leading to reduced detoxification of tobacco smoke carcinogens may have important implications in studies on the association of particular polymorphisms with diseases, such as hepatocellular carcinoma [11], orolaryngeal cancer [12], gastrointestinal cancer [23], and pancreatitis [24]. *UGT*1 polymorphisms are also associated with unwanted side effects of drugs, such as bone marrow and intestinal toxicity in colon carcinoma patients treated with irinotecan [5,6,25–27]. All allelic *UGT*1 variants studied reduce their detoxification capacity. *UGT*1A7*3 combines the allelic variation present in *UGT*1A7*2 and *4 and leads to the strongest reduction of enzyme activity towards benzo[*a*]pyrene phenols (Table 1). It is intriguing that allelic variants of *UGT*1A7 were associated with hepatocellular carcinoma [9] although UGT1A7 is not expressed in liver but in the upper gastrointestinal tract [10]. Due to the described frequent co-occurrence of allelic variants of *UGT*1A7 with polymorphisms of two other *UGT*1 isoforms (and more allelic variants of *UGT*1 isoforms are likely to be identified), it remains to be established which *UGT*1 polymorphism is responsible for the association with hepatocellular carcinoma. In conclusion, frequent co-occurrence of multiple allelic variants in the *UGT*1 gene locus was observed in the present study in both Caucasian and Egyptian populations. This co-occurrence of *UGT* allelic variants has to be taken into account in future studies on the association with diseases and abnormal drug reactions.

Acknowledgments

The authors thank Prof. Dr. K. Dietz (Institute of Medical Biometry, University of Tübingen), Prof. Dr. J. Kömpf and Prof. Dr. J. Tomiuk (Institute of Anthropology and Human Genetics, University of Tübingen), Dr. O. Landt (TIB MOLBIOL, Berlin, Germany) for their help and the Deutsche Forschungsgemeinschaft (DFG) for financial support. O.A. Badary appreciates a fellowship from the Alexander-von-Humboldt foundation and M. Schwab support from the Robert-Bosch foundation, Stuttgart.

References

[1] Nebert DW, Ingelman-Sundberg M, Daly AK. Genetic epidemiology of environmental toxicity and cancer susceptibility: human allelic polymorphisms in drug metabolizing enzyme genes, their functional importance, and nomenclature issues. Drug Metab Rev 1999;31:467–87.

[2] Bosma PJ, Roy Chowdhury J, Bakker C, Gantla S, de Boer A, Oostra BA, Lindhout D, Tytgat GN, Jansen PL, Oude Elferink RP, Roy Chowdhury N. The genetic basis of the reduced expression of bilirubin UDP glucuronosyltransferase 1 in Gilbert's syndrome. N Engl J Med 1995;333:1171–5.

[3] Monaghan G, Ryan M, Seddon R, Hume R, Burchell B. Genetic variation in bilirubin UPD glucuronosyltransferase gene promoter and Gilbert's syndrome. Lancet 1996;347:578–81.

[4] Raijmakers MT, Jansen PL, Steegers EA, Peters WH. Association of human liver bilirubin UDP-glucuronyltransferase activity with a polymorphism in the promoter region of the UGT1A1 gene. J Hepatol 2000;33:348–51.

[5] Ando Y, Saka H, Asai G, Sugiura S, Shimokata K, Kamataki T. UGT1A1 genotypes and glucuronidation of SN 38 the active metabolite of irinotecan. Ann Oncol 1998;9:845–7.

[6] Innocenti F, Iyer L, Ratain MJ. Pharmacogenetics of anticancer agents: lessons from amonafide and irinotecan. Drug Metab Dispos 2001;29:596–600.

[7] Ciotti M, Marrone A, Potter C, Owens IS. Genetic polymorphism in the human UGT1A6 (planar phenol) UDP-glucuronosyltransferase: pharmacological implications. Pharmacogenetics 1997;7:485–95.

[8] Bigler J, Whitton J, Lampe JW, Fosdick L, Bostick RM, Potter JD. CYP2C9 and UGT1A6 genotypes modulate the protective effect of aspirin on colon adenoma risk. Cancer Res 2001;61:3566–9.

[9] Guillemette C, Ritter JK, Auyeung DJ, Kessler FK, Housman DE. Structural heterogeneity at the UDP-glucuronosyltransferase 1 locus: functional consequences of three novel missense mutations in the human UGT1A7 gene. Pharmacogenetics 2000;10:629–44.

[10] Tukey RH, Strassburg CP. Genetic multiplicity of the human UDP-glucuronosyltransferases and regulation in the gastrointestinal tract. Mol Pharmacol 2001;59:405–14.

[11] Vogel A, Kneip S, Barut A, Ehmer U, Tukey RH, Manns MP, Strassburg CP. Genetic link of hepatocellular carcinoma with polymorphisms of

the UDP glucuronosyltransferase UGT1A7 gene. Gastroenterology 2001;121:1136–44.
[12] Zheng Z, Park JY, Guillemette C, Schantz SP, Lazarus P. Tobacco carcinogen-detoxifying enzyme UGT1A7 and its association with orolaryngeal cancer risk. J Natl Cancer Inst 2001;93:1411–8.
[13] Gong QH, Cho JW, Huang T, Potter C, Gholami N, Basu NK, Kubota S, Carvalho S, Pennington MW, Owens IS, Popescu NC. Thirteen UDPglucuronosyltransferase genes are encoded at the human UGT1 gene complex locus. Pharmacogenetics 2001;11:357–68.
[14] Lampe JW, Bigler J, Horner NK, Potter JD. UDP-glucuronosyltransferase (UGT1A1*28 and UGT1A6*2) polymorphisms in Caucasians and Asians: relationships to serum bilirubin concentrations. Pharmacogenetics 1999;9:341–9.
[15] Borlak J, Thum T, Landt O, Erb K, Hermann R. Molecular diagnosis of a familial nonhemolytic hyperbilirubinemia (Gilbert's Syndrome) in healthy subjects. Hepatology 2000;32:792–5.
[16] Schneider S, Roessli D, Excoffier L. ARLEQUIN ver. 2.000: a software for population genetics data analysis. University of Geneva, Switzerland: Genetics and Biometry Laboratory.
[17] Excoffier L, Slatkin M. Maximum-likelihood estimation of molecular haplotype frequencies in a diploid population. Mol Biol Evol 1995;12:921–7.
[18] Slatkin M, Excoffier L. Testing for linkage disequilibrium in genotypic data using expectation-maximization algorithm. Heredity 1996;76:377–83.
[19] Hall D, Ybazeta D, Destro-Bisol G, Petzl-Erler ML, Di Rienzo A. Variability at the uridine diphosphate glucuronosyltransferase 1A1 promoter in human populations and primates. Pharmacogenetics 1999;9:591–9.
[20] Beutler E, Gelbart T, Demina A. Racial variability in the UDP-glucuronosyltransferase 1 (UGT1A1) promoter: a balanced polymorphism for regulation of bilirubin metabolism? Proc Natl Acad Sci USA 1998;95:8170–4.
[21] Stocker R, Yamamoto Y, McDonagh AF, Glazer AN, Ames BN. Bilirubin is an antioxidant of possible physiological importance. Science 1987;235:1043–6.
[22] Baranano DE, Rao M, Ferris CD, Snyder SH. Biliverdin reductase: a major physiologic cytoprotectant. Proc Natl Acad Sci USA 2002;99:16093–8.
[23] Vogel A, Ehmer U, Barut A, Kneip S, Manns MP, Strassburg CP. Gene–environmental interaction: polymorphisms of the UDP-glucuronosyltransferase UGT1A7 gene in gastrointestinal cancer. Gastroenterology 2002;122(Suppl):W1248.
[24] Ockenga J, Vogel A, Teich N, Keim V, Manns MP, Strassburg CP. Polymorphisms of the UDP-glucuronosyltransferase UGT1A7 gene as a risk factor in chronic pancreatitis and pancreatitic cancer. Gastroenterology 2002;122(Suppl):W1088.
[25] Ando Y, Saka H, Ando M, Sawa T, Muro K, Ueoka H, Yokoyama A, Saitoh S, Shimokata K, Hasegawa Y. Polymorphisms of UDP-glucuronosyltransferase gene and irinotecan toxicity: a pharmacogenetic analysis. Cancer Res 2000;60:6921–6.
[26] Tukey RH, Strassburg CP, Mackenzie PI. Pharmacogenomics of human UDP-glucuronosyltransferases and irinotecan toxicity. Mol Pharmacol 2002;62:446–50.
[27] Gagne JF, Montminy V, Belanger P, Journault K, Gaucher G, Guillemette C. Common human UGT1A polymorphisms and the altered metabolism of irinotecan active metabolite 7-ethyl-10-hydroxycamptothecin (SN-38). Mol Pharmacol 2002;62:608–17.