

Expert Opinion

1. Introduction
2. Cellular studies evaluating UGT pharmacogenetics
3. Utility of human tissue in UGT pharmacogenetics studies
4. Application of existing *in vitro* tools
5. Application of existing *in vivo* tools
6. Expert opinion

informa
healthcare

Update on tools for evaluation of uridine diphosphoglucuronosyltransferase polymorphisms

Upendra A Argikar, Otito F Iwuchukwu & Swati Nagar[†]

[†]Temple University School of Pharmacy, Department of Pharmaceutical Sciences, 3307 N Broad Street, Philadelphia PA 19140, USA

Background: The uridine diphosphoglucuronosyltransferase (UGT) superfamily of enzymes catalyzes conjugative metabolism of numerous endobiotics and xenobiotics. Pharmacogenetic variation has been reported in almost all UGT family members. **Objective:** To discuss tools available for evaluation of UGT polymorphisms. **Methods:** Literature search was done to include all relevant UGT polymorphism studies involving *in vitro* methods. **Results/conclusions:** Studies evaluating associations between UGT genotype and resultant phenotype are described. Mammalian cells transfected with variant UGT isoforms or variant promoters have been developed. Human liver tissue genotyped for UGT genetic polymorphisms has been successfully used. New techniques to conduct these studies include RNA inhibition and development of transgenic animal models. Challenges and opportunities in the preclinical evaluation of UGT genotype–phenotype correlations are discussed.

Keywords: genotype–phenotype correlation, pharmacogenetics, polymorphisms, uridine diphosphoglucuronosyltransferase (UGT)

Expert Opin. Drug Metab. Toxicol. (2008) 4(7):879–894

1. Introduction

1.1 Uridine diphosphoglucuronosyltransferases

The conjugation of the glucuronic acid moiety to aromatic and aliphatic alcohols, carboxylic acids, thiols and primary, secondary and tertiary amino groups in various endogenous and exogenous substrates is termed glucuronidation. This conjugation process using uridine diphosphoglucuronic acid (UDPGA) as a co-substrate is catalyzed by enzymes known as uridine diphosphoglucuronosyltransferases (UGTs or UDPGTs) [1]. Conjugation of glucuronic acid facilitates conversion of a xenobiotic substrate to a more polar and ionizable form at physiologic pH along with increasing the molecular weight by 176 amu. The resultant glucuronides are excreted through the kidney by processes such as glomerular filtration and/or active secretion, or through bile into the small intestine. Glucuronidation plays an important role in the metabolic elimination of a variety of endogenous substances including bilirubin, bile acids, hormones and biogenic amines, and serves a protective function by detoxifying and eliminating numerous carcinogens [2]. Many of these compounds are also substrates for sulfontransferases (SULTs). Whereas mono-conjugation is generally considered a terminal reaction, there is evidence that some glucuronides themselves may be substrates for oxidation as well as subsequent conjugation at further sites in the substrate. For example, cytochrome P450 (CYP) enzymes such as CYP2C9 and CYP2C8 have been reported to catalyze the oxidation of diclofenac and estradiol glucuronides, respectively [3,4]. Diglucuronides of compounds such as bilirubin [5–7] and hydroxychrysene [8,9] have been reported in the literature.

1.2 Enzyme multiplicity

Several different enzymes constitute the UGT gene families. There are three main gene families included in the UGT superfamily based on their sequence homology – UGT1, UGT2A and UGT2B. The human UGT1A family gene structure was first reported by Owens and co-workers [10]. The UGT1A family in humans uniquely comprises several enzymes that share an identical carboxyl terminal containing the UDPGA binding pocket, encoded by four conserved exons (3' exons – exons 2, 3, 4 and 5). An amino terminal region that contains the substrate binding site is encoded by twelve variable region exons or cassette exons (5' exons – exon 1), which are spliced to the constant region exons, coding for the individual enzymes. The UGT1A1 and UGT1A6 polypeptides are ~ 50% identical to those encoded by *UGT1A2P* – *UGT1A5* and *UGT1A7* – *UGT1A13P* clusters; identity for polypeptides in these clusters is 75 – 90% [11]. The entire *UGT1A* gene locus is localized on chromosome 2q37 and the individual enzymes are named as per the vicinity of their variable region exon to the constant region exons 2, 3, 4 and 5. In humans *UGT1A2*, *UGT1A11*, *UGT1A12* and *UGT1A13* are pseudogenes.

The UGT2A subfamily represents olfactory UGTs and the genes are located on chromosome 4q13. Human UGT2A enzymes have been implicated in olfactory perception and protection against airborne xenobiotics and toxins [12]. The UGT2B enzymes are encoded by separate genes on chromosome 4q12, and have been named in the order of their discovery and submission to the UGT nomenclature committee [13].

1.3 Expression and substrates

UGT2B enzymes – UGT2B4, UGT2B7, UGT2B10, UGT2B11, UGT2B15, UGT2B17 and UGT2B28 along with nine UGT1A enzymes are differentially expressed in humans. Enzymes UGT1A (chromosome 2q37) and UGT2B (chromosome 4q13) are differentially expressed in visceral organs such as liver, kidney and intestine. Most UGT enzymes are primarily expressed in the liver. UGT1A3 is expressed in bile ducts and intestine in addition to liver. UGT1A7 is expressed in stomach and esophagus. UGT1A8 and UGT1A10 are predominantly intestinal enzymes whereas UGT1A9 is expressed in the kidney, UGT1A6 is expressed in the brain and UGT2B11 in the mammary glands in addition to liver [11,14]. UGT2B28 is expressed in the liver and breast tissue [15]. Other organs with UGT expression are adrenals, spleen, lung, skin, testes, ovaries, olfactory glands and brain. The UGT2A (chromosome 4q13) family is expressed predominantly in olfactory tissues, although polymerase chain reaction (PCR) experiments have identified UGT2A1 mRNA in brain and fetal lung. UGT3A (UGT3A1, UGT3A2, chromosome 5p13.2) and UGT8A (UGT8A1, chromosome 4q26) families have also been identified by means of genome sequencing. However,

catalytic functions of enzymes from UGT3A and UGT8A families are not fully understood [11]. A large number of endogenous and exogenous compounds are reported to be conjugated by UGTs [16]. Endogenous UGT substrates include bilirubin, the primary degradation product of heme, steroids such as estrogens, androgens, progestogens, and lipids such as arachidonic acid metabolites.

1.4 UGT pharmacogenetics

Genetic polymorphisms have been reported in virtually every UGT family member. Numerous single nucleotide polymorphisms (SNPs) and promoter polymorphisms have been evaluated, with some characterized in great detail. UGT pharmacogenetics are reviewed comprehensively elsewhere [17,18]; this report focuses on techniques used to characterize key UGT genetic polymorphisms. Table 1 lists some commonly studied UGT genetic variants; the reader should note that this table is not complete by any means, and is referred to other reviews for a complete listing of UGT genetic variants [17,18]. Besides polymorphisms in coding regions, numerous intronic SNPs have additionally been reported in UGT genes [19-22]; however, the significance of this variation in intronic regions remains to be established.

Non-synonymous SNPs that alter the primary protein structure (amino acid) are thought to be the most relevant in altering the phenotype of the enzyme. It is noteworthy that synonymous SNPs, while causing no change in protein primary structure, may nevertheless alter its tertiary structure owing to variable transcription rate of a 'rare' codon compared with a 'common' codon [23]. Synonymous SNPs can also alter gene expression by altering exon splicing sites, and can affect the pool size of tRNA [24,25]. Promoter polymorphisms on the other hand are expected to alter protein expression levels, thereby possibly altering the organ clearance of a substrate. The most commonly studied UGT polymorphism is the *UGT1A1* promoter TATA box TA repeat polymorphism [26].

Clinical and epidemiologic studies are critical for translating knowledge of pharmacogenetic variability to 'bedside' therapeutic solutions such as more efficacious disease diagnosis, management and treatment, as well as prediction of disease risk and incidence. However, several human diseases are multigenic and have complex etiologies. Additionally, a single gene may have numerous variants and this confounds genotype–phenotype correlations if all possible variants are not genotyped for. Thus, multiple genes with multiple SNPs would need to be characterized with comprehensive haplotype analyses to determine the genetic make-up responsible for a phenotype (i.e., disease risk, incidence or treatment success rates). The picture is additionally complicated by complex genetics such as linkage disequilibrium among genes and variable penetrance. Finally, interethnic differences in the occurrence and frequency of UGT polymorphisms are well established,

Table 1. Examples of genetic variants among human UGT family members.

Allele (reference SNP ID)	Type of polymorphism	Nucleotide change	Protein change	Ref.
UGT1A1				
UGT1A1*6	Coding SNP	211 G > A	G71R	[72]
UGT1A1*7	Coding SNP	1456 T > G	Y486D	[72]
UGT1A1*27	Coding SNP	686 C > A	P229Q	[73]
UGT1A1*28	Promoter	TAATA7	Altered transcription	[26]
UGT1A3				
	Coding SNP	17 A > G	Q6R	[74]
	Coding SNP	31 T > C	W11R	[74,75]
	Coding SNP	133 C > T	R45W	[74]
	Coding SNP	140 T > C	V47A	[74,75]
UGT1A4				
	Coding SNP	70 C > A	P24T	[75]
	Coding SNP	142 T > G	L48V	[75]
	Coding SNP	31 C > T	R11W	[19]
UGT1A6				
	Coding SNP	19 T > G	S7A	[76]
	Coding SNP	269 G > A	R90H	[20]
	Coding SNP	308 C > A	S103X	[20]
	Coding SNP	541 A > G	T181A	[77]
	Coding SNP	552 A > C	R184S	[77]
UGT1A7				
	Coding SNP	343 G > A	G115S	[78]
	Coding SNP	387 T > G	N129K	[79]
	Coding SNP	391 C > A	R131K	[79]
	Coding SNP	392 G > A	R131K	[79]
	Coding SNP	514 G > C	E139D	[78]
	Coding SNP	622 T > C	W208R	[79]
UGT1A8				
UGT1A8*2	Coding SNP	518 C > G	A173G	[80]
UGT1A8*3	Coding SNP	830 G > A	C277Y	[80]
UGT1A9				
	Promoter	-118T ₉ > ₁₀ ; -109 to -98 A(T) _n AT; n = 9/10	Altered transcription	[40]
	Upstream to gene	-87 G > A		[22,81]
	Coding SNP	8 G > A	C3Y	[78]
	Coding SNP	98 T > C	M33T	[78]
	Coding SNP	153 G > A	R51R (silent)	[82]
	Coding SNP	588 G > T	G196G (silent)	[82]
	Coding SNP	726 T > G	Y242X	[82]
	Coding SNP	766 G > A	D256N	[83]

Table 1. Examples of genetic variants among human UGT family members (continued).

Allele (reference SNP ID)	Type of polymorphism	Nucleotide change	Protein change	Ref.
UGT1A10				
	Coding SNP	177 G > A	M59I	[84]
	Coding SNP	415 G > A	E139K	[85]
	Coding SNP	605 C > T	T202I	[84]
	Coding SNP	730 C > A	L244I	[85]
UGT2B4				
	Coding SNP	1364 A > G	K455R	[21]
rs13119049	Coding SNP	1374 T > A	D458E	[86]
rs13142440	Coding SNP	1375 C > A	R459R (silent)	[86]
rs41298245	Coding SNP	1531 T > C	C511R	[21]
UGT2B7				
rs12233719	Coding SNP	211 G > T	A71S	[72]
rs7439366	Coding SNP	802 C > T	H268Y	[87]
	Coding SNP	1192 G > A	D398N	[21]
UGT2B15				
	Coding SNP	253 G > T	D85Y	[88]
	Coding SNP	1055 C > T	T352I	[89]
	Coding SNP	1568 A > C	K523T	[90]

and this variability is a critical area of study. As a first step towards understanding genotype–phenotype correlations, several preclinical tools have been used. These include generation of recombinant purified protein enzymes, mammalian cell lines expressing a gene variant, human tissue banks and genetically modified animal models. Preclinical models for pharmacogenetic studies either used for UGT genes or with potential application in this area include cellular expression and the use of genotyped tissue, and are discussed later. Although outside the scope of this article, it is noteworthy that numerous clinical studies have incorporated pharmacogenetic evaluations and have characterized associations between genetic variability and clinical outcome (drug disposition or disease risk) [18,22,27,28].

1.5 Clinical relevance

The glucuronidation of numerous endogenous and exogenous chemicals is catalyzed by UGTs. Genetic polymorphisms that alter the catalytic activity of UGTs become clinically important contributors in defective endogenous metabolism, altered disease risk/incidence, altered drug disposition and altered drug toxicity. A classic example is inherited unconjugated hyperbilirubinemia (Gilbert's syndrome; Crigler–Najjar type I and II) owing to decreased UGT1A1 activity because of genetic polymorphisms [29]. The role of UGT pharmacogenetics in cancer is an area of

much research [18]. Another well-studied example includes UGT1A1 pharmacogenetics in altered irinotecan disposition and toxicity [27,30]. Structural and functional aspects of the UGTs have been reviewed in the past [14,31–33]. The pharmacogenetics of UGTs have also been examined [17,18,29,34]. This review focuses on preclinical tools now used in the investigation of UGT genotype–phenotype correlations, as well as innovative models that might aid UGT pharmacogenetic studies in the future.

2. Cellular studies evaluating UGT pharmacogenetics

Expression of variant UGT enzymes has been done in transient- as well as stable-expression mammalian cell systems. Each system has its advantages and disadvantages. Transient transfection is fast and does not require a selection agent for plasmid-expressing clones but requires large amounts of plasmid DNA, achieves variable transfection in cells and yields small amounts of protein per transfection. Cells with stable expression of plasmid DNA can be passaged long-term with potentially limitless protein production; however, generating stable cell lines takes several months and is more tedious than transient transfections [35].

The commonly used mammalian cell lines engineered to express human UGTs include human embryonic kidney

(HEK) 293 cells and COS cells. Table 2 summarizes studies with transient or stable expression of variant UGT enzymes in mammalian cells. Promoter polymorphisms in several UGT gene promoters have been evaluated with luciferase assays to detect transcriptional activity. Mammalian plasmid constructs include the intronless firefly luciferase gene (as a reporter) downstream of the promoter under evaluation, leading to luciferase expression proportional to the promoter's transcriptional activity [36]. Firefly luciferase converts luciferin to oxyluciferin in the presence of ATP, with the production of yellow-green light quantifiable with a luminometer. Table 2 lists several studies on variant UGT promoters using this technique. Discussed later are two examples: promoter polymorphisms in *UGT1A1* and *UGT1A9*. Whereas promoter studies in cell systems have exhibited results similar to human tissue bank studies for *UGT1A1*, *UGT1A9* cellular versus tissue bank results do not always agree (Tables 2 and 3).

That the *UGT1A1**28 promoter polymorphism decreases promoter activity with resultant decreased enzyme activity is well established [26,29,37,38]. Several examples of genotype-phenotype studies with human tissues are discussed here. Molecular mechanisms underlying this reduced promoter activity have recently been reported with cellular studies. Cell lines transfected with either 6 or 7 TA repeats in the *UGT1A1* promoter TATA box were recently tested for promoter activity [39]. Results indicated that the *UGT1A1**28 promoter (with 7 TA repeats) exhibited decreased binding to a hepatic cell nuclear protein complex, to which the wild type promoter (with 6 TA repeats) exhibited significant binding. This was suggested as a mechanistic explanation for decreased promoter activity in patients with Gilbert's syndrome (carrying the *UGT1A1**28 promoter polymorphism). Further, the authors demonstrated decreased binding affinity of the *28 variant promoter to the TATA-binding protein [39].

Contrasting the example of the *UGT1A1**28 promoter polymorphism is that of the *UGT1A9* promoter T repeat polymorphism. With promoter luciferase assays, the *UGT1A9* T₁₀ promoter was shown to exhibit greater transcriptional activity than that of the 'wild type' T₉ promoter [40]. However, as discussed later, these results have not been unequivocally corroborated in genotype-phenotype association studies in human tissue. Some of the examples of cellular studies described in Table 2 have made significant contributions to functional studies of UGT genetic polymorphisms.

Mammalian cells obviously offer an easily accessible model that allows genetic modifications. Whereas recombinant UGT isozyme preparations in baculoviral systems are commercially available, genetic variants of each isozyme have not been thus prepared so far. Therefore, cellular tools offer the most convenient and least time-consuming model to evaluate genotype-phenotype associations. These studies have not only offered insight into the functional differences among

variants ('low' versus 'high' activity allozymes) but have also noted the importance of substrate-specific studies (a low activity allozyme for one substrate might not have markedly decreased activity towards another substrate). In the absence of human tissue banks, mammalian cells offer an excellent method to evaluate functional consequences of UGT genetic polymorphisms.

3. Utility of human tissue in UGT pharmacogenetics studies

Human tissue banks have been successfully used to conduct genotype-phenotype associations for UGT variants. As summarized in Table 3, exonic and more recently intronic SNPs [41,42] as well as promoter polymorphisms have been evaluated in human liver samples for their effect on catalytic activity towards various substrates. Advantages of human tissue studies include a system biologically similar to the *in vivo* situation, availability of normal as well as disease-state tissue to mimic various disease models, and the means to evaluate homozygous as well as heterozygous genotypes. Human organ tissue offers biologic levels of protein expression when compared against cell lines that are often generated to overexpress an allozyme. Disadvantages include the necessity for a large number of samples, especially to evaluate infrequent genotypes or alleles with appropriate statistical power. Factors such as timing of tissue procurement, tissue handling and storage, and protocols for microsome/homogenate preparation might vary among research laboratories, and might influence the enzyme activity data reported. Replication of data with the relatively small amount of microsomes from small tissue samples is often difficult. Finally, it is very difficult to evaluate enzyme induction/inhibition as a result of dietary or environmental factors in postmortem tissue. Alterations in enzyme levels owing to environmental factors can confound differences owing to genetics alone.

Although *in vitro* studies done with genotyped human tissues confirm phenotypic observations seen with the well-studied *UGT1A1* promoter TA repeat variation [43-45], a recent study by Yoder Graber and co-workers [46] illustrates the fact that results from genotype-phenotype studies done in human tissue do not necessarily correspond to cellular studies. A bank of human livers genotyped for *UGT1A1* and *UGT1A9* (two of the most-studied UGTs with regard to promoter polymorphisms) were examined for glucuronidation activity using thyroxine as a substrate. The results from their study corroborated results from previous studies involving *UGT1A1* that showed significantly lower activity in liver samples of patients with Gilbert's syndrome (homozygous for the TA 7 repeat) compared with the wild type or TA 6 repeat genotype [43,44]. No significant relationship was however observed in livers with the *UGT1A9* promoter polymorphism (-118T₉ > 10 repeat), which had been previously reported to correspond with

Table 2. Cellular studies evaluating effect of UGT genetic polymorphisms on catalytic activity of the enzyme.

UGT enzyme	Genetic variants evaluated*	Cell line	Study design	Ref.
UGT1A1	Promoter TA repeat polymorphism *1 and *28 variants	Hep 3B or Huh 7 cells transfected with UGT1A1 *1 or *28 promoter plasmid	Luciferase assay done to assay promoter activity; decreased binding of the mutant *28 promoter to nuclear protein complex and TATA-binding protein reported with EMSA	[39]
	Promoter TA repeat polymorphism *1 and *2 along with -3279T > G	HepG2 cells transiently transfected with promoter plasmids, along with expression plasmids for CAR or PXR	Dual luciferase assay results indicated that the *28 mutation affects promoter transcriptional more than the -3279 mutation	[91]
UGT1A3	Variants W11R, Q6R-W11R, W11R-V47A and R45W	Transient transfection in Cos-7 cells	Estrone glucuronidation evaluated; variant activity as per cent of wild type: 121, 86, 369 and 70%, respectively	[74]
	Promoter assays as well as SNP variants *1 – *11	HepG2 cells transfected with luciferase reporter plasmids for promoter polymorphisms; stable transfection of SNP variants in HEK293 cells	Reduced transcriptional activity with luciferase assays and EMSA reported with all six variant promoters; SNPs classified as high, intermediate or low activity based on estrone-conjugating activity	[92]
UGT1A4	Variants P24T, L48V	Transient transfection in HEK293 cells	Four substrates tested: B-naphthylamine, benzidine, trans-androsterone, dihydrotestosterone; substrate-specific change in activity with the variants	[75]
UGT1A6	SNPs 541A > G and 552A > C	Transient transfection in COS-1 cells	Variant UGT had reduced activity towards 11 substrates tested	[77]
	SNPs defining variants *1 – *4	Stable transfection in HEK293 cells	Two substrates tested: <i>p</i> -nitrophenol and α -naphthol; variants exhibited greater activity than *1 wild type	[76]
UGT1A7	SNPs defining variants *1 – *4	Stable transfection in HEK293 cells	Variants exhibited different catalytic towards substrates 3-, 7- and 9-hydroxy-benzo(a)pyrene	[79]
	SNPs defining variants *1 – *4	Transient transfection in HEK293 cells	Catalytic activity towards the nine substrates studied differed among variants	[93]
	*1 – *10	Stable transfection in HEK293 cells	Catalytic activity towards SN-38 and flavopiridol evaluated, and found to be variant allozyme-dependent	[78]
UGT1A8	Variants *1 and *2	Stable transfection in HEK293 cells	Association between genetic variant and catalytic activity determined for 26 substrates	[80]
	Variants *1 – *9	Stable transfection in HEK293 cells	Activity towards mycophenolic acid evaluated, found to be associated with genetic polymorphisms	[94]
	Variants *1 – *3	Stable transfection in HEK293 cells	*3 was low activity for glucuronidation of 4-hydroxyestrone and 4-hydroxyestradiol	[95]
UGT1A9	Variant D256N	Transient transfection in COS-1 cells	SN-38 glucuronidation evaluated, found to be decreased in the variant	[96]
	Promoter T repeat polymorphism	Luciferase constructs in HepG2 cells	Transcriptional activity with dual luciferase assays revealed greater activity of the T ₁₀ repeat compared with T ₉	[40]
	Variants *1 – *3	Stable transfection in HEK293 cells	*3 was low activity for glucuronidation of 4-hydroxyestrone and 4-hydroxyestradiol	[95]
	Variants *1 – *3	Stable transfection in HEK293 cells	*3 was low activity towards SN-38 glucuronidation	[78]

*For definitions of genetic variants (nucleotide and/or amino-acid change), please refer to the corresponding references.

Table 2. Cellular studies evaluating effect of UGT genetic polymorphisms on catalytic activity of the enzyme (continued).

UGT enzyme	Genetic variants evaluated*	Cell line	Study design	Ref.
UGT1A10	Variants M59I and T202I	Transient transfection in COS-1 cells	Activity towards estradiol and 7-hydroxy-4-trifluoromethylcoumarin	[97]
	Variant I112T	Stable transfection in HEK293 cells	Several substrates including mycophenolic acid	[98]
UGT2B4	Wild type allozyme expressed	Stable transfection in HEK293 cells	Activity towards several steroid substrates established	[86]
UGT2B7	Promoter polymorphisms at -268 and -102	Transient transfection in HepG2 cells	Firefly luciferase assay revealed no significant difference in polymorphic promoter transcription activity	[99]
	Several promoter polymorphisms	Transient transfection in HepG2 and Caco-2 cells	Luciferase assay revealed significant change in transcriptional activity with the -79 variation	[100]
	Variants *1 and *2	Stable transfection in HEK293 cells	No significant differences in MPA glucuronidation	[94]
	Variants *1 and *2, and promoter -79 variant	Stable transfection in HEK293 cells, or promoter constructs in HEC-1B cells	Variant *1 had lower activity than *2 towards 4-hydroxyestrone and 4-hydroxyestradiol; -79 promoter had decreased transcriptional activity with luciferase assay	[95]

*For definitions of genetic variants (nucleotide and/or amino-acid change), please refer to the corresponding references.

higher levels of transcriptional activity [40]. The (-118T₉ > 10 repeat) polymorphism was also studied by Ramirez and colleagues [42] using flavopiridol and mycophenolic acid, known substrates for UGT1A9, and their results were in agreement with the Yoder Graber study. Other polymorphisms in UGT enzymes have been and are still being studied with varying results and some of these have been detailed (see Table 3). To the best of our knowledge, most genotype–phenotype studies done have been in human liver samples possibly because of the availability and relative ease of use of this particular tissue compared with other organ tissues where UGTs are known to be expressed such as lung, kidney, breast, intestine and colon.

4. Application of existing *in vitro* tools

So far, a variety of techniques have been developed and successfully applied to UGT pharmacogenetic studies. As discussed later, there are challenges in the use of some tools for gene regulation, whereas other emerging technologies offer promising possibilities for pharmacogenetic evaluation.

One of the most widely used systems for conditional gene expression is the Tet system [47]. Resistance to tetracycline in Gram-negative bacteria is mediated by the TetA protein. TetA is highly repressed in cells in the absence of tetracycline, and its expression is induced and controlled in a fine-tuned manner in response to tetracycline. Repression of TetA is under transcriptional control by means of Tet repressor (TetR). Tetracycline-dependent TetR has been used

for gene repression in eukaryotic cells as well as in animal models. Much research has been done to yield sophisticated applications of tetracycline-dependent gene regulation, including graded transgene expression [47]. Such models might be applied to mimic genetic polymorphisms such as the UGT promoter polymorphisms that alter gene expression, allowing characterization of the resulting phenotype. However, tetracycline-dependent gene regulation in eukaryotic systems is intricate and requires improvements and system optimization before this technique can be used appropriately in pharmacogenetic studies. To our knowledge, tetracycline-dependent gene regulation has not been used in UGT pharmacogenetic studies. It must be noted that the Tet system involves developing artificial constructs of UGT genes expressed stably or transiently in cell lines; numerous parameters therefore need to be considered in this artificial system. As discussed later, other technologies offer more promise in this field.

Among newer tools being explored in this field, small interfering RNA (siRNA) methodology offers exciting possibilities. RNA interference (RNAi) leads to post-transcriptional gene silencing and epigenetic changes such as histone modification that lead to gene transcription. Yueh and Tukey recently used siRNA to knock down *nrf2* in HepG2 cells to study the role of *nrf2* in UGT1A1 regulation [48]. RNAi was also applied towards clarification of the role of aromatic hydrocarbon receptor in induction of UGT1A1 by chrysin. siRNA constructs in this investigation were directed towards knocking down the human aromatic

Table 3. UGT genotype-phenotype association studies done in human tissue samples.

UGT polymorphisms	Tissue	Study details	Genotype-phenotype correlation	Ref.
<i>UGT1A1</i> (promoter region (TA) _n repeats)	Normal human livers (n = 60) Caucasians	Benzo(a)pyrene-trans-7R, 8R-dihydrodiol [BPD] used as substrate DNA genotyped by sequencing Western blotting used to immunoquantify UGT1A1	Significantly lower rate of glucuronidation in homozygous TA 7 repeat (*28/*28) subjects compared with heterozygous and wild type TA 6 repeat (*1/*1) subjects. No difference in glucuronidation phenotype obtained between heterozygous and wild type subjects	[44]
	Human liver total (n = 41) Normal tissue (n = 39) Gilbert's syndrome (n = 2) n = 20	Bilirubin glucuronidation DNA genotyping assay Estradiol glucuronidation	Variants showed lower activity on comparison of median bilirubin-UGT activity Significant variation seen between wild type and homozygous variants (~ 30-fold) Promoter mutation seen to alter apparent V _{max} without significantly affecting K _m	[45] [101]
	Normal human liver samples (n = 44) Caucasians = 25 Hispanic = 1 Black = 3 Unknown = 15	SN-38 used as substrate Genotyping done by PCR amplification/gel electrophoresis	Glucuronidation rates seen to be lowest in homozygous TA 7 repeats followed by heterozygous when compared with homozygous TA 6 repeats. No significant difference between heterozygous and homozygous variants	[43]
<i>UGT1A1</i> (TA repeat and PBREM polymorphisms)	Normal human liver samples (n = 83) Caucasians 68% African-Americans 18% Asians 1% Others 2% Unknown ethnicity 12%	SN-38 glucuronidation Genotyping through PCR	Significant variation observed in the TA repeat genotypes (6/6 > 6/7 > 7/7) in Caucasians and African-Americans; no significant difference seen in Asians and others; no significant variations seen with the TA repeat-PBREM haplotypes	[102]
<i>UGT1A1</i> *28 <i>UGT1A9</i> – 118T _{9 > 10} (<i>UGT1A9</i> *1b) promoter polymorphisms	Normal human liver samples (n = 53)	Thyroxine as substrate Correlation done with SN-38 and Flavopiridol UGT protein content quantification by western blotting DNA genotyping assay	Significant difference observed between the <i>UGT1A1</i> *28 wild type versus variants No significant variation in thyroxine formation seen with <i>UGT1A9</i> phenotypes	[46]
<i>UGT1A1</i> and <i>UGT1A9</i> polymorphism	Healthy unrelated Caucasian livers (n = 133) DNA samples (tissue not specified) n of 150 comprising 110 Chinese, 20 Taiwanese, 10 Japanese and 10 Southeast Asians	DNA Genotyping SN-38 Glucuronidation from previous study	Caucasian human livers stratified by <i>UGT1A1</i> - <i>1A9</i> diplotypes Threefold variation observed between 2 of the 10 diplotypes	[103]
<i>UGT1A1</i> (promoter region (TA) _n repeats) and <i>UGT1A6</i> *2	Healthy human livers (n = 39)	Bilirubin, 4-nitrophenol and 4-methylumbelliferone glucuronidation study DNA genotyping assay	No associations found between <i>UGT1A1</i> genotypes and 4-nitrophenol or 4-methylumbelliferone glucuronidation, whereas a decrease in activity was seen for bilirubin (6/6 > 6/7 > 7/7) Strong association seen between the bilirubin enzyme activity and the <i>UGT1A6</i> *2 polymorphism	[104]

PBREM: Phenobarbital-responsive enhancer module; PCR: Polymerase chain reaction; RFLP: Restriction fragment length polymorphism; SNP: Single nucleotide polymorphism.

Table 3. UGT genotype-phenotype association studies done in human tissue samples (continued).

UGT polymorphisms	Tissue	Study details	Genotype-phenotype correlation	Ref.
<i>UGT1A1</i> Promoter and <i>UGT2B7*2</i> polymorphisms	Normal human liver samples (n = 59)	Estradiol for <i>UGT1A1</i> and Zidovudine for <i>UGT2B7</i> glucuronidation assays Genotyping done using a novel high throughput method Western blotting used for immunoquantification of UGT proteins	Study showed no difference in glucuronidation activities between genotypes for <i>UGT2B7</i> allozymes For <i>UGT1A1</i> , mean activity (estradiol 3-glucuronidation) was the lowest in the 7/7 genotype (2.5 – 3.2-fold), followed by 6/7 (1.3-fold) when compared with the 6/6 genotype	[105]
<i>UGT1A4</i> (P24T polymorphism) and <i>UGT2B7</i> (H268Y polymorphism)	Human liver samples (n = 78)	HPLC assay with 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanol (NNAL) as substrate Genotyping assays	Slightly significant increase in activity seen in individuals with at least one variant <i>UGT1A4</i> 24T when compared to the wild types (<i>N</i> -glucuronides) Significant decrease observed for NNAL-O glucuronide in homozygous <i>UGT2B7</i> 268Y compared with wild type. No significant difference between heterozygous and wild type	[106]
<i>UGT1A6</i> [Exon 1 SNPs; S7A, T181A and R184S + 8 new SNPs in promoter region (to -2052 bp)]	Human liver total (n = 54) White/Caucasians (n = 48) African-American (n = 4) Hispanic (n = 2)	Two substrates (serotonin and 4-nitrophenol) mRNA quantification <i>UGT1A6</i> protein quantification (Western blotting) <i>UGT1A6</i> gene resequencing through PCR	No associations found between genotype and measured phenotypic parameters However, stratification by alcohol use found a median twofold variation in complete and closely linked sequence variants	[107]
<i>UGT1A9</i> polymorphisms (-118T ₉ > ₁₀ and I399C > T)	Normal human liver samples (n = 46)	Flavopiridol and mycophenolic acid used as substrates mRNA quantitated by real-time PCR	No significant difference seen in glucuronidation activities for the three genotypes associated with the promoter polymorphism (-118T _{9/9} , -118T _{9/10} , -118T _{10/10})	[42]
<i>UGT1A9</i> promoter polymorphisms	Healthy human livers (n = 48)	Mycophenolic acid and propofol used as substrates Genotyping and promoter resequencing studies <i>UGT1A9</i> immunoquantified through Western blot	Distribution of <i>UGT1A9</i> seen to be normal with 17-fold variation. Varying differences in glucuronidation were observed based on haplotype classification since 10 SNPs were studied. The presence of the -275/-2152 SNPs was seen to be associated with increased activity. T ₁₀ polymorphism not linked to these two SNPs	[81]
<i>UGT1A9</i> and <i>UGT1A1</i> For <i>UGT1A9</i> (2152C > T, 275T > A, -118T ₉ > 10), three new variants (5366G > T, 4549T > C, I399C > T) and <i>UGT1A1</i> (53TA6 > 7, 3156G > A, and 3279T > G)	Healthy human livers (n = 48)	7-ethyl-10-hydroxycamptothecin (SN-38), bilirubin and estradiol used as substrates <i>UGT1A1</i> and 1A9 protein content determined by semiquantitative Western blotting	Subjects with the <i>UGT1A9</i> I399T/T genotype had a significant increase in SN-38G formation. Glucuronidation of <i>UGT1A1</i> substrates, bilirubin and estradiol as well as <i>UGT1A9</i> substrates mycophenolic acid and propofol were also significantly increased. This intronic variant was not linked to any of the <i>UGT1A1</i> variants. The <i>UGT1A9</i> – 118T10 variant was also significantly associated with higher rates of SN-38G formation (p = 0.05)	[41]

PBREM: Phenobarbital-responsive enhancer module; PCR: Polymerase chain reaction; RFLP: Restriction fragment length polymorphism; SNP: Single nucleotide polymorphism.

Table 3. UGT genotype-phenotype association studies done in human tissue samples (continued).

UGT polymorphisms	Tissue	Study details	Genotype-phenotype correlation	Ref.
<i>UGT2B10</i> (codon 67 Asp > Thr polymorphism)	Human liver samples (n = 112) Gender known for 95 samples (41% female, 59% male)	Nicotine and cotinine used as substrates in glucuronidation assays Polymorphisms detected through RFLP analysis	Significant trend towards decreased glucuronidation for both substrates seen in subjects with an increasing number of the variant allele. 21% decrease in heterozygous (*1/*2) and a fivefold decrease for homozygous (*2/*2) subjects compared with wild type subjects	[108]
<i>UGT2B10*2 allele</i> <i>UGT2B10</i> (three tagging SNPs). Four common haplotypes (A – D) were identified	Human livers (n = 77) Genotyping successful for 73 subjects	NNAL and other tobacco-specific nitrosamines used as substrates. Polymorphism detected by RFLP analysis	Haplotype C found to be significantly associated (p < 0.001) with NNAL-N glucuronidation. A 1.8- and 12-fold reduction in activity seen in subjects with 1 and 2 copies of the C haplotype. This haplotype was in 100% LD with the <i>UGT2B10</i> (codon 67 Asp > Thr polymorphism)	[109]
<i>UGT2B15</i> (non-synonymous cSNPs, D85Y, T352I and K523T)	Healthy human liver samples (n = 54, 16 females and 38 males) Caucasians (n = 48) African-American (n = 4) Hispanic (n = 2)	Oxazepam was used as substrate PCR assays used to genotype for all SNPs	S-Oxazepam glucuronidation was seen to be significantly affected by gender, and the SNPs D85Y and T352I. No influence of these variables on R-Oxazepam glucuronidation	[89]

PBREM: Phenobarbital-responsive enhancer module; PCR: Polymerase chain reaction; RFLP: Restriction fragment length polymorphism; SNP: Single nucleotide polymorphism.

hydrocarbon receptor in HepG2 cells [49]. A set of siRNAs directed towards UGT1A6 resulted in significantly lower expression of the enzyme in caco-2 cells in experiments reported by Hu and co-workers [50]. Similarly, Chouinard *et al.* obtained up to 70% reduction in UGT2B15 and UGT2B17 mRNA levels in LNCaP cells [51]. Gene silencing by siRNA is shown to be robust [50,51] and hence is a dependable methodology to mimic low expression enzyme polymorphisms as well as to study the induction and regulation by various nuclear receptors. We may very well be moving closer towards the therapeutic application of RNAi [52], and the future of siRNA methodology in pharmacogenetics is promising.

5. Application of existing *in vivo* tools

Noteworthy methodologies summarized so far in this review have been *in vitro* or *ex vivo*. Correlation between *in vitro* and *in vivo* results or prediction of the *in vivo* outcome based on *in vitro* information remains one of the most challenging fields in the pharmaceutical arena. Although several models have been applied for studying the role of nuclear receptors and their role in regulation of drug metabolizing enzymes [53], *in vivo* models for evaluation of UGT pharmacogenetics are limited. In 2004, Yoshizato and co-workers reported a chimeric mouse model with almost completely humanized livers [54]. The replacement of mouse hepatocytes by those of the human donor was achieved up to 96% in

mice with hepatic failure and immunodeficiency. This model was applied rapidly towards studying the expression of CYPs as well as UGTs and other conjugative enzymes [55,56]. Not only fresh but also cryopreserved human hepatocytes from several donors can be injected and propagated in this model, making the chimeric mice with humanized liver an interesting prospect towards evaluation of UGT pharmacogenetics. Expression of mRNA of human drug metabolizing enzymes has been shown to be robust in this model [57]. Human hepatocytes proliferate easily and rapidly on injection in these mice and if cells can be obtained from individual donors with known genetic polymorphisms, the effect of an allelic variant can be studied *in vitro* and *in vivo* [58]. Because chimeric mice maintain the same inter-individual variability as the donor population [56,59], this *in vivo* system would better predict the ADME properties. Recent studies have demonstrated that cefmetazole was excreted primarily in urine in the chimeric mice (an excretory profile similar to humans) as compared to fecal excretion in the control mice [60]. The human transporters obtained from chimeric mouse hepatocytes have been well characterized [57]. However, given that 20% mouse mRNA was also detected in these mice, this model may be less effective in the subset of xenobiotics that are substrates for mouse and human transporters, especially with differential affinities. In addition, whether parameters such as bile flow, liver blood flow, gastrointestinal transit time, intestinal absorption/reabsorption, blood partitioning and

factors such as degree of replacement of mouse hepatocytes and the timelines for generation of chimeric mice can be optimized remains to be examined.

Another landmark approach that could be applied towards pharmacogenetic studies of UGTs in the near future is the transgenic mouse model developed by Tukey and co-workers. A bacterial artificial chromosome encoding the entire human *UGT1A* gene locus (*UGT1A*) was microinjected into mouse eggs, followed by transplantation of these eggs in pseudo-pregnant mice [61]. Established founder lines of mice were bred to further produce the transgenic *Ugt1* mice. Studies with specific *UGT1A* antibodies and mRNA analysis along with *in vitro* phenotyping confirmed the establishment of the transgenic mouse model [61]. *In vitro* induction studies with various inducers of different human *UGT1A* enzymes such as pregnenolone-16 α -carbonitrile and 2,3,7,8-tetrachloro-dibenzo-para-dioxin revealed that these *UGT1A* enzymes could be both differentially expressed and regulated in various tissues [61]. This model has since been applied to studying the induction and regulation by ligands of aromatic hydrocarbon receptor, peroxisome proliferator-activated receptor ligands, liver X receptor and *nrf2* keap signaling pathway [48,62,63]. This model could provide a useful platform for studying UGT pharmacogenetics, if a version of the transgenic mouse bearing relevant mutations in the gene locus can be produced. The effect of *UGT1* polymorphisms as well as the effect of regulation and induction of these variants could be then studied *in vitro* and *in vivo* using specific probe substrates. The transgenic mouse was also shown to possess a basal level albeit minimal enzyme activity owing to the mouse *Ugt1a* enzymes [61]. Knocking out the mouse *Ugt1a* gene locus would generate a transgenic mouse with human *UGT1* genes only. But it is also not known if knocking out the mouse *Ugt1a* gene locus would result in a change in the constitutive activity of the knocked-in *UGT1* enzymes, similar to the observation by Ariyoshi *et al.* [64]. The difficulties and efforts in the generation of such models cannot be underestimated and therefore the chimeric humanized as well as the transgenic mice models remain elusive for day-to-day research activities.

6. Expert opinion

Evaluation of UGT pharmacogenetics with preclinical studies as well as population studies is expected to discover extra genetic variability and clarify the phenotypic result of this genetic variance. Complex variability in human genetics is continually being discovered, and will need to be incorporated in pharmacogenetic studies to clearly understand a 'genotype'; recent genetic studies with UGTs highlight this point [65,66]. Pharmacogenetic studies need to move from 'monogenic' to complex disease models, and now there is technology to support genome-wide association studies. Population studies need to include large subjects and offer

strong statistical power; they also need to include multi-ethnic subpopulations to get a better handle on inter-ethnic variability owing to issues such as variable linkage disequilibrium among geographically different subgroups. A recent review underlines these and other critical issues to be taken into account for meaningful pharmacogenetic analyzes [24].

The UGT superfamily of enzymes plays an important role in endo- and xenobiotic metabolism. Pharmacogenetic variability in virtually every UGT family member has been discovered (Table 1). Whereas many advances have been made in our understanding of the role of UGT pharmacogenetics in variable drug disposition and toxicity, the importance of numerous UGT genetic polymorphisms remains to be clarified. It is noteworthy, for example, that whereas high-throughput SNP discovery has led to the identification of several intronic SNPs in UGT genetic sequences, we still do not understand the role this variability plays in human health and disease. The importance of characterizing the phenotype of a genetic polymorphism cannot be stressed enough. The overall goal of pharmacogenetics – 'personalized medicine' – is to predict clinical outcome for a 'genotyped' individual even before he/she is administered a drug. For this to be possible, we need to clearly understand the physiologic role of variability in the human genome.

Whereas clinical trials have begun to evaluate pharmacogenetics of drug metabolizing enzymes, transporters and receptors in a prospective manner, preclinical tools offer critical insight into genotype–phenotype associations. Specific to UGTs, several mammalian cell lines expressing UGT genetic variants have been developed (Table 2). Variable transcriptional activity of polymorphic UGT promoters has been evaluated with luciferase promoter assays. Together, these studies have greatly enhanced our understanding of functional differences in UGT catalytic activity/expression attributable to genetic polymorphisms. Similarly, the availability of human liver banks has made possible the study of associations between UGT genotype and phenotype for numerous drug substrates (Table 3). Human organ tissue offers biologic levels of protein expression when compared to cell lines overexpressing a UGT variant. However, evaluation of infrequent genotypes or alleles is difficult with a small number of samples. Additionally, as discussed earlier, various procedural as well as environmental factors might confound studies done in human tissue banks. Finally, extrahepatic glucuronidation cannot be discounted, and there is a need to evaluate appropriate extrahepatic tissue for genetic variability in UGT enzymes expressed in these tissues. The lack of availability of non-hepatic tissue samples often precludes such genotype–phenotype studies, although extrahepatic wild type glucuronidation has nevertheless been evaluated in several different human tissues [67,68].

As discussed in detail earlier, there are newer tools to conduct preclinical pharmacogenetic studies and these are

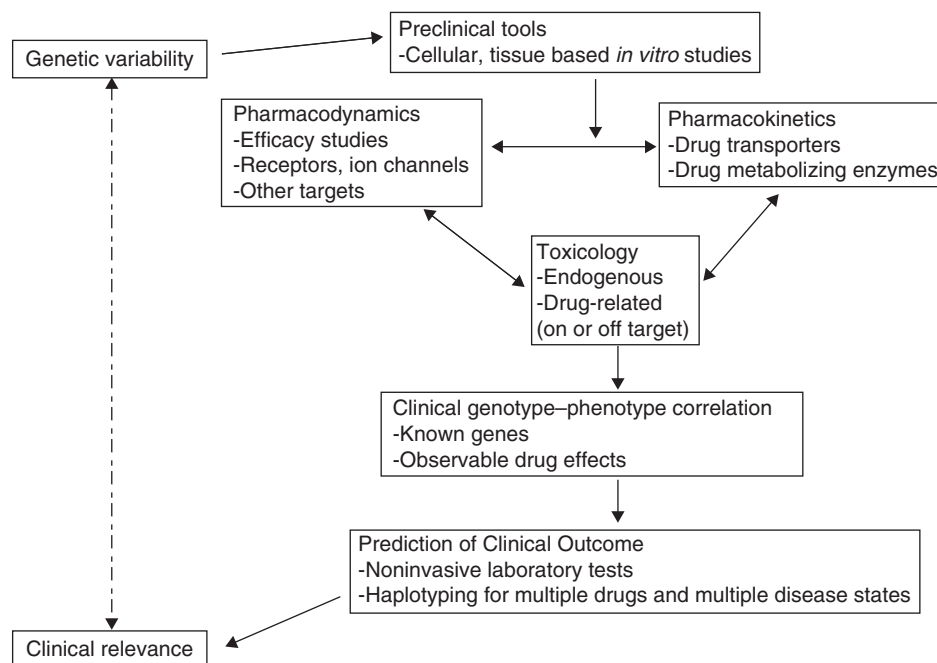


Figure 1. Translation of basic pharmacogenetics research into clinical practice.

beginning to be used. Promising techniques include RNAi and the development of chimeric and transgenic mouse models. These tools might aid immensely in an as yet understudied yet critical area – haplotype analysis of UGT polymorphisms. It is well appreciated that most human diseases are multigenic in nature, and single SNP evaluations do not address the complexity of multiple genes with multiple polymorphisms affecting drug disposition and toxicity. Human population studies inform us of frequent haplotypes detected from among the numerous possible combinations; animal models that mimic the detected human haplotypes might be useful to study drug pharmacokinetics, dynamics and toxicity in relation to UGT pharmacogenetics.

Once we completely understand an important phenotype in the lab, it will become necessary to translate this information to the bedside. Thus, tools will need to be developed to ‘phenotype’ an individual with an inexpensive, non-invasive and high-throughput test. There are such tools for the cytochrome P450 enzymes, and include the erythromycin breath test to detect *in vivo* CYP3A4 activity, and urine analysis of drug:metabolite ratio on dextromethorphan administration for CYP2D6 activity. There are no similar tools for UGTs but it is hoped that the identification of key polymorphisms will eventually lead to their development. Probe substrates have been identified for many UGT enzymes (e.g., bilirubin and estradiol for UGT1A1) [69-71] and can potentially be used to develop phenotyping assays.

The UGT superfamily is just one set of enzymes included in the large variety of polymorphic drug metabolizing enzymes. Drug transporters and target receptors are additionally polymorphic, adding to the vast number of genetic variations that can cause changes in drug absorption, distribution, metabolism and elimination, and pharmacologic and toxicity profiles (Figure 1). Predicting clinical outcome on drug therapy on the basis of pharmacogenetics therefore becomes extremely complicated, especially in the realm of multigenic diseases, multiple drug therapy and multiple disease states in a patient. Interethnic variability in occurrence and frequency of genetic polymorphisms needs to be evaluated in large numbers of populations before ‘personalized’ medicine can become reality. Further, the role of environmental variability such as diet, smoking and exercise cannot be discounted. Despite these challenges, there are examples of real-life advances in therapy owing to pharmacogenetics. One such example is the recent FDA revision of the irinotecan label, with a warning for individuals carrying low-activity UGT1A1 variants and increased risk of drug-related toxicity [30]. UGT pharmacogenetics will play an important role in understanding inter-individual variability and designing safer and more effective therapeutic regimens.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Dutton GJ. Glucuronic acid – free and combined. Academic press: New York and London; 1966
2. Rimmel RP, Nagar S, Argikar U. Conjugative metabolism of drugs in Drug metabolism in drug design and development: Wiley Interscience, John Wiley and Sons, Inc. 2008
3. Delaforge M, Pruvost A, Perrin L, Andre F. Cytochrome P450-mediated oxidation of glucuronide derivatives: example of estradiol-17beta-glucuronide oxidation to 2-hydroxy-estradiol-17beta-glucuronide by CYP 2C8. *Drug Metab Dispos* 2005;33(3):466-73
4. Mohan J, Gandhi AA, Bhavya BC, et al. Caspase-2 triggers Bax-Bak-dependent and -independent cell death in colon cancer cells treated with resveratrol. *J Biol Chem* 2006;281(26):17599-611
5. Burchell B, Blanckaert N. Bilirubin mono- and di-glucuronide formation by purified rat liver microsomal bilirubin UDP-glucuronyltransferase. *Biochem J* 1984;223(2):461-5
6. Peters WH, Jansen PL, Nauta H. The molecular weights of UDP-glucuronyltransferase determined with radiation-inactivation analysis. A molecular model of bilirubin UDP-glucuronyltransferase. *J Biol Chem* 1984;259(19):11701-05
7. Ritter JK, Crawford JM, Owens IS. Cloning of two human liver bilirubin UDP-glucuronosyltransferase cDNAs with expression in COS-1 cells. *J Biol Chem* 1991;266(2):1043-7
8. Bock KW, Gschaidmeier H, Seidel A, et al. Mono- and diglucuronide formation from chrysene and benzo(a)pyrene phenols by 3-methylcholanthrene-inducible phenol UDP-glucuronosyltransferase (UGT1A1). *Mol Pharmacol* 1992;42(4):613-8
9. Gschaidmeier H, Bock KW. Radiation inactivation analysis of microsomal UDP-glucuronosyltransferases catalysing mono- and diglucuronide formation of 3,6-dihydroxybenzo(a)pyrene and 3,6-dihydroxychrysene. *Biochem Pharmacol* 1994;48(8):1545-9
10. Ritter JK, Chen F, Sheen YY, et al. A novel complex locus UGT1 encodes human bilirubin, phenol, and other UDP-glucuronosyltransferase isozymes with identical carboxyl termini. *J Biol Chem* 1992;267(5):3257-61
- **Description of new UGT locus.**
11. Mackenzie PI, Walter Bock K, Burchell B, et al. Nomenclature update for the mammalian UDP glycosyltransferase (UGT) gene superfamily. *Pharmacogenet Genomics* 2005;15(10):677-85
- **Updated UGT nomenclature.**
12. Jedlitschky G, Cassidy AJ, Sales M, et al. Cloning and characterization of a novel human olfactory UDP-glucuronosyltransferase. *Biochem J* 1999;340(Pt 3):837-43
13. Available from: <http://som.flinders.edu.au/FUSA/ClinPharm/UGT/>
14. Tukey RH, Strassburg CP. Human UDP-glucuronosyltransferases: metabolism, expression, and disease. *Ann Rev Pharmacol Toxicol* 2000;40:581-616
- **Good review of UGTs, including tissue expression.**
15. Levesque E, Turgeon D, Carrier JS, et al. Isolation and characterization of the UGT2B28 cDNA encoding a novel human steroid conjugating UDP-glucuronosyltransferase. *Biochemistry* 2001;40(13):3869-81
16. Ung D, Nagar S. Variable sulfation of dietary polyphenols by recombinant human sulfotransferase (SULT) 1A1 genetic variants and SULT1E1. *Drug Metab Dispos* 2007;35(5):740-6
17. Guillemette C. Pharmacogenomics of human UDP-glucuronosyltransferase enzymes. *Pharmacogenomics J* 2003;3:136-58
- **Comprehensive review of UGT pharmacogenetics.**
18. Nagar S, Rimmel RP. Uridine diphosphoglucuronosyltransferase pharmacogenetics and cancer. *Oncogene* 2006;25(11):1659-72
- **Reviews all UGT polymorphisms until 2006 and their role in cancer risk and incidence.**
19. Saeki M, Saito Y, Jinno H, et al. Genetic variations and haplotypes of UGT1A4 in a Japanese population. *Drug Metab Pharmacokinet* 2005;20(2):144-51
20. Saeki M, Saito Y, Jinno H, et al. Genetic polymorphisms of UGT1A6 in a Japanese population. *Drug Metab Pharmacokinet* 2005;20(1):85-90
21. Saeki M, Saito Y, Jinno H, et al. Single nucleotide polymorphisms and haplotype frequencies of UGT2B4 and UGT2B7 in a Japanese population. *Drug Metab Dispos* 2004;32(9):1048-54
22. Carlini LE, Meropol NJ, Bever J, et al. UGT1A7 and UGT1A9 polymorphisms predict response and toxicity in colorectal cancer patients treated with capecitabine/irinotecan. *Clin Cancer Res* 2005;11(3):1226-36
- **Functional impact of UGT1A polymorphisms on a common substrate (SN-38) in colorectal cancer patients.**
23. Kimchi-Sarfaty C, Oh JM, Kim IW, et al. A “silent” polymorphism in the MDR1 gene changes substrate specificity. *Science (New York NY)* 2007;315(5811):525-8
- **Research explaining mechanism of variable phenotype due to silent polymorphisms.**
24. Nebert DW, Zhang G, Vesell ES. From human genetics and genomics to pharmacogenetics and pharmacogenomics: past lessons, future directions. *Drug Metab Rev* 2008;40(2):187-224
- **An excellent review encompassing past, present, and future directions for genetics and pharmacogenetics, and personalized medicine.**
25. Cartegni L, Chew SL, Krainer AR. Listening to silence and understanding nonsense: exonic mutations that affect splicing. *Nat Rev Genet* 2002;3(4):285-98
- **Review explaining how silent polymorphisms can affect phenotype.**
26. Bosma PJ, Chowdhury JR, Bakker C, et al. The genetic basis of the reduced expression of bilirubin UDP-glucuronosyltransferase 1 in Gilbert's syndrome. *N Engl J Med* 1995;333(18):1171-5
- **Among the first reports describing the UGT1A1 TA repeat promoter polymorphism.**
27. Innocenti F, Undevia SD, Iyer L, et al. Genetic variants in the UDP-glucuronosyltransferase 1A1 gene predict the risk of severe neutropenia of irinotecan. *J Clin Oncol* 2004;22(8):1382-8
28. Guillemette C, Millikan RC, Newman B, Housman DE. Genetic polymorphisms in uridine diphospho-glucuronosyltransferase 1A1 and association with breast cancer among African Americans. *Cancer Res* 2000;60(4):950-6
29. Burchell B. Genetic variation of human UDP-glucuronosyltransferase. Implications

- in disease and drug glucuronidation. *Am J Pharmacogenomics* 2003;3:37-52
- **UGT review, includes disease implications.**
- Nagar S, Blanchard RL. Pharmacogenetics of uridine diphosphoglucuronosyltransferase (UGT) 1A family members and its role in patient response to irinotecan. *Drug Metab Rev* 2006;38(3):393-409
 - Burchell B, Brierley CH, Rance D. Specificity of human UDP-glucuronosyltransferases and xenobiotic glucuronidation. *Life Sci* 1995;57(20):1819-31
 - Burchell B, Coughtrie MW. Genetic and environmental factors associated with variation of human xenobiotic glucuronidation and sulfation. *Environ Health Perspect* 1997;105(Suppl 4):739-47
 - Mackenzie PI, Owens IS, Burchell B, et al. The UDP glycosyltransferase gene superfamily: recommended nomenclature update based on evolutionary divergence. *Pharmacogenetics* 1997;7(4):255-69
 - de Wildt SN, Kearns GL, Leeder JS, van den Anker JN. Glucuronidation in humans. Pharmacogenetic and developmental aspects. *Clin Pharmacokinet* 1999;36(6):439-52
 - Walker J, Rapley R. *Molecular biology and biotechnology*. 4th edition. Royal Society of Chemistry; 2001
 - de Wet JR, Wood KV, DeLuca M, et al. Firefly luciferase gene: structure and expression in mammalian cells. *Mol Cell Biol* 1987;7(2):725-37
 - Bosma PJ, Chowdhury JR, Huang TJ, et al. Mechanisms of inherited deficiencies of multiple UDP-glucuronosyltransferase isoforms in two patients with Crigler-Najjar syndrome, type I. *FASEB J* 1992;6(10):2859-63
 - 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(14):8170-4
 - Hsieh TY, Shiu TY, Huang SM, et al. Molecular pathogenesis of Gilbert's syndrome: decreased TATA-binding protein binding affinity of UGT1A1 gene promoter. *Pharmacogenet Genomics* 2007;17(4):229-36
 - **Explains molecular basis for low expression of the well-studied UGT1A1 TATA repeat promoter polymorphism.**
 - Yamanaka H, Nakajima M, Katoh M, et al. A novel polymorphism in the promoter region of human UGT1A9 gene (UGT1A9*22) and its effects on the transcriptional activity. *Pharmacogenetics* 2004;14(5):329-32
 - Girard H, Villeneuve L, Court MH, et al. The novel UGT1A9 intronic I399 polymorphism appears as a predictor of 7-ethyl-10-hydroxycamptothecin glucuronidation levels in the liver. *Drug Metab Dispos* 2006;34(7):1220-8
 - Ramirez J, Liu W, Mirkov S, et al. Lack of association between common polymorphisms in UGT1A9 and gene expression and activity. *Drug Metab Dispos* 2007;35(12):2149-53
 - Iyer L, Hall D, Das S, et al. Phenotype-genotype correlation of in vitro SN-38 (active metabolite of irinotecan) and bilirubin glucuronidation in human liver tissue with UGT1A1 promoter polymorphism. *Clin Pharmacol Ther* 1999;65(5):576-82
 - Fang JL, Lazarus P. Correlation between the UDP-glucuronosyltransferase (UGT1A1) TATAA box polymorphism and carcinogen detoxification phenotype: significantly decreased glucuronidating activity against benzo(a)pyrene-7,8-dihydrodiol(-) in liver microsomes from subjects with the UGT1A1*28 variant. *Cancer Epidemiol Biomarkers Prev* 2004;13(1):102-9
 - Raijmakers MT, Jansen PL, Steegers EA, Peters WH. Association of human liver bilirubin UDP-glucuronosyltransferase activity with a polymorphism in the promoter region of the UGT1A1 gene. *J Hepatol* 2000;33(3):348-51
 - Yoder Graber AL, Ramirez J, Innocenti F, Ratain MJ. UGT1A1*28 genotype affects the in vitro glucuronidation of thyroxine in human livers. *Pharmacogenet Genomics* 2007;17(8):619-27
 - Berens C, Hillen W. Gene regulation by tetracyclines. Constraints of resistance regulation in bacteria shape TetR for application in eukaryotes. *Eur J Biochem* 2003;270(15):3109-21
 - **Nice review on the Tet system of gene regulation.**
 - Yueh MF, Tukey RH. Nrf2-Keap1 signaling pathway regulates human UGT1A1 expression in vitro and in transgenic UGT1 mice. *J Biol Chem* 2007;282(12):8749-58
 - Bonzo JA, Belanger A, Tukey RH. The role of chrysin and the ah receptor in induction of the human UGT1A1 gene in vitro and in transgenic UGT1 mice. *Hepatology* 2007;45(2):349-60
 - Liu X, Tam VH, Hu M. Disposition of flavonoids via enteric recycling: determination of the UDP-glucuronosyltransferase isoforms responsible for the metabolism of flavonoids in intact Caco-2 TC7 cells using siRNA. *Mol Pharm* 2007; 4(6):873-82
 - Chouinard S, Barbier O, Belanger A. UDP-glucuronosyltransferase 2B15 (UGT2B15) and UGT2B17 enzymes are major determinants of the androgen response in prostate cancer LNCaP cells. *J Biol Chem* 2007;282(46):33466-74
 - Soutschek J, Akinc A, Bramlage B, et al. Therapeutic silencing of an endogenous gene by systemic administration of modified siRNAs. *Nature* 2004;432(7014):173-8
 - Gong H, Sinz MW, Feng Y, et al. Animal models of xenobiotic receptors in drug metabolism and diseases. *Methods Enzymol* 2005;400:598-618
 - Tateno C, Yoshizane Y, Saito N, et al. Near completely humanized liver in mice shows human-type metabolic responses to drugs. *Am J Pathol* 2004;165(3):901-12
 - Katoh M, Matsui T, Nakajima M, et al. Expression of human cytochromes P450 in chimeric mice with humanized liver. *Drug Metab Dispos* 2004;32(12):1402-10
 - **On chimeric mice and their potential in vitro and in vivo in ADME studies.**
 - Katoh M, Watanabe M, Tabata T, et al. In vivo induction of human cytochrome P450 3A4 by rifabutin in chimeric mice with humanized liver. *Xenobiotica* 2005;35(9):863-75
 - Nishimura M, Yoshitsugu H, Yokoi T, et al. Evaluation of mRNA expression of human drug-metabolizing enzymes and transporters in chimeric mouse with humanized liver. *Xenobiotica* 2005;35(9):877-90
 - Katoh M, Yokoi T. Application of chimeric mice with humanized liver for predictive

- ADME. *Drug Metab Rev* 2007;39(1):145-57
59. Katoh M, Matsui T, Okumura H, et al. Expression of human Phase II enzymes in chimeric mice with humanized liver. *Drug Metab Dispos* 2005;33(9):1333-40
 - **On chimeric mice and their potential *in vitro* and *in vivo* in ADME studies.**
 60. Okumura H, Katoh M, Sawada T, et al. Humanization of excretory pathway in chimeric mice with humanized liver. *Toxicol Sci* 2007;97(2):533-8
 61. Chen S, Beaton D, Nguyen N, et al. Tissue-specific, inducible, and hormonal control of the human UDP-glucuronosyltransferase-1 (UGT1) locus. *J Biol Chem* 2005;280(45):37547-57
 - **Characterization of transgenic UGT mouse model.**
 62. Senekoe-Effenberger K, Chen S, Brace-Sinnokrak E, et al. Expression of the human UGT1 locus in transgenic mice by 4-chloro-6-(2,3-xylylidino)-2-pyrimidinylthioacetic acid (WY-14643) and implications on drug metabolism through peroxisome proliferator-activated receptor alpha activation. *Drug Metab Dispos* 2007;35(3):419-27
 63. Verreault M, Senekoe-Effenberger K, Trottier J, et al. The liver X-receptor alpha controls hepatic expression of the human bile acid-glucuronidating UGT1A3 enzyme in human cells and transgenic mice. *Hepatology* 2006;44(2):368-78
 64. Ariyoshi N, Imaoka S, Nakayama K, et al. Comparison of the levels of enzymes involved in drug metabolism between transgenic or gene-knockout and the parental mice. *Toxicol Pathol* 2001;29(Suppl):161-72
 65. Girard H, Levesque E, Bellemare J, et al. Genetic diversity at the UGT1 locus is amplified by a novel 3' alternative splicing mechanism leading to nine additional UGT1A proteins that act as regulators of glucuronidation activity. *Pharmacogenet Genomics* 2007;17(12):1077-89
 66. Levesque E, Girard H, Journault K, et al. Regulation of the UGT1A1 bilirubin-conjugating pathway: role of a new splicing event at the UGT1A locus. *Hepatology* 2007;45(1):128-38
 67. Vogel A, Ockenga J, Ehmer U, et al. Polymorphisms of the carcinogen detoxifying UDP-glucuronosyltransferase UGT1A7 in proximal digestive tract cancer. *Z Gastroenterol* 2002;40(7):497-502
 68. Strassburg CP, Kneip S, Topp J, et al. Polymorphic gene regulation and interindividual variation of UDP-glucuronosyltransferase activity in human small intestine. *J Biol Chem* 2000;275(46):36164-71
 69. Patten C. New technologies for assessing UDP-glucuronosyltransferase (UGT) metabolism in drug discovery and development. *Drug Discov Today Technol* 2006;3(1):73-8
 70. Miners JO, Knights KM, Houston JB, Mackenzie PI. In vitro-in vivo correlation for drugs and other compounds eliminated by glucuronidation in humans: pitfalls and promises. *Biochem Pharmacol* 2006;71(11):1531-9
 71. Court MH. Isoform-selective probe substrates for in vitro studies of human UDP-glucuronosyltransferases. *Methods Enzymol* 2005;400:104-16
 - **Details some selective substrates for various human UGTs.**
 72. Hirota T, Ieiri I, Takane H, et al. Sequence variability and candidate gene analysis in two cancer patients with complex clinical outcomes during morphine therapy. *Drug Metab Dispos* 2003;31(5):677-80
 73. Koiwai O, Nishizawa M, Hasada K, et al. Gilbert's syndrome is caused by a heterozygous missense mutation in the gene for bilirubin UDP-glucuronosyltransferase. *Hum Mol Genet* 1995;4(7):1183-6
 74. Iwai M, Maruo Y, Ito M, et al. Six novel UDP-glucuronosyltransferase (UGT1A3) polymorphisms with varying activity. *J Hum Genet* 2004;49(3):123-8
 75. Ehmer U, Vogel A, Schutte JK, et al. Variation of hepatic glucuronidation: Novel functional polymorphisms of the UDP-glucuronosyltransferase UGT1A4. *Hepatology* 2004;39(4):970-7
 76. Nagar S, Zalatoris JJ, Blanchard RL. Human UGT1A6 pharmacogenetics: identification of a novel SNP, characterization of allele frequencies and functional analysis of recombinant allozymes in human liver tissue and in cultured cells. *Pharmacogenetics* 2004;14(8):487-99
 77. Ciotti M, Marrone A, Potter C, Owens IS. Genetic polymorphism in the human UGT1A6 (planar phenol) UDP-glucuronosyltransferase: pharmacological implications. *Pharmacogenetics* 1997;7(6):485-95
 78. Villeneuve L, Girard H, Fortier LC, et al. Novel functional polymorphisms in the UGT1A7 and UGT1A9 glucuronidating enzymes in Caucasian and African-American subjects and their impact on the metabolism of 7-ethyl-10-hydroxycamptothecin and flavopiridol anticancer drugs. *J Pharmacol Exp Ther* 2003;307(1):117-28
 79. Guillemette C, Ritter JK, Auyeung DJ, et al. Structural heterogeneity at the UDP-glucuronosyltransferase 1 locus: functional consequences of three novel missense mutations in the human UGT1A7 gene. *Pharmacogenetics* 2000;10(7):629-44
 80. Huang YH, Galijatovic A, Nguyen N, et al. Identification and functional characterization of UDP-glucuronosyltransferases UGT1A8*1, UGT1A8*2 and UGT1A8*3. *Pharmacogenetics* 2002;12(4):287-97
 81. Girard H, Court MH, Bernard O, et al. Identification of common polymorphisms in the promoter of the UGT1A9 gene: evidence that UGT1A9 protein and activity levels are strongly genetically controlled in the liver. *Pharmacogenetics* 2004;14(8):501-15
 82. Saeki M, Saito Y, Jinno H, et al. Three novel single nucleotide polymorphisms in UGT1A9. *Drug Metab Pharmacokinet* 2003;18(2):146-9
 83. Jinno H, Hanioka N, Tanaka-Kagawa T, et al. Transfection assays with allele-specific constructs: functional analysis of UDP-glucuronosyltransferase variants. *Methods Mol Biol* 2005;311:19-29
 84. Saeki M, Ozawa S, Saito Y, et al. Three novel single nucleotide polymorphisms in UGT1A10. *Drug Metab Pharmacokinet* 2002;17(5):488-90
 85. Elahi A, Bendaly J, Zheng Z, et al. Detection of UGT1A10 polymorphisms and their association with orolaryngeal carcinoma risk. *Cancer* 2003;98(4):872-80
 86. Levesque E, Beaulieu M, Hum DW, Belanger A. Characterization and substrate specificity of UGT2B4 (E458): a UDP-glucuronosyltransferase encoded by a polymorphic gene. *Pharmacogenetics* 1999;9(2):207-16
 87. Bhasker CR, McKinnon W, Stone A, et al. Genetic polymorphism of

- UDP-glucuronosyltransferase 2B7 (UGT2B7) at amino acid 268: ethnic diversity of alleles and potential clinical significance. *Pharmacogenetics* 2000;10(8):679-85
88. Levesque E, Beaulieu M, Green MD, et al. Isolation and characterization of UGT2B15(Y85): a UDP-glucuronosyltransferase encoded by a polymorphic gene. *Pharmacogenetics* 1997;7(4):317-25
89. Court MH, Hao Q, Krishnaswamy S, et al. UDP-glucuronosyltransferase (UGT) 2B15 pharmacogenetics: UGT2B15 D85Y genotype and gender are major determinants of oxazepam glucuronidation by human liver. *J Pharmacol Exp Ther* 2004;310(2):656-65
90. Iida A, Saito S, Sekine A, et al. Catalog of 86 single-nucleotide polymorphisms (SNPs) in three uridine diphosphate glycosyltransferase genes: UGT2A1, UGT2B15, and UGT8. *J Hum Genet* 2002;47(10):505-10
91. Sugatani J, Mizushima K, Osabe M, et al. Transcriptional regulation of human UGT1A1 gene expression through distal and proximal promoter motifs: implication of defects in the UGT1A1 gene promoter. *Naunyn Schmiedebergs Arch Pharmacol* 2008
92. Caillier B, Lepine J, Tojic J, et al. A pharmacogenomics study of the human estrogen glucuronosyltransferase UGT1A3. *Pharmacogenet Genomics* 2007;17(7):481-95
93. Strassburg CP, Vogel A, Kneip S, et al. Polymorphisms of the human UDP-glucuronosyltransferase (UGT) 1A7 gene in colorectal cancer. *Gut* 2002;50(6):851-6
94. Bernard O, Tojic J, Journault K, et al. Influence of nonsynonymous polymorphisms of UGT1A8 and UGT2B7 metabolizing enzymes on the formation of phenolic and acyl glucuronides of mycophenolic acid. *Drug Metab Dispos* 2006;34(9):1539-45
95. Thibaudeau J, Lepine J, Tojic J, et al. Characterization of common UGT1A8, UGT1A9, and UGT2B7 variants with different capacities to inactivate mutagenic 4-hydroxylated metabolites of estradiol and estrone. *Cancer Res* 2006;66(1):125-33
96. Jinno H, Saeki M, Saito Y, et al. Functional characterization of human UDP-glucuronosyltransferase 1A9 variant, D256N, found in Japanese cancer patients. *J Pharmacol Exp Ther* 2003;306(2):688-93
97. Jinno H, Saeki M, Tanaka-Kagawa T, et al. Functional characterization of wild-type and variant (T202I and M59I) human UDP-glucuronosyltransferase 1A10. *Drug Metab Dispos* 2003;31(5):528-32
98. Martineau I, Tchernof A, Belanger A. Amino acid residue ILE211 is essential for the enzymatic activity of human UDP-glucuronosyltransferase 1A10 (UGT1A10). *Drug Metab Dispos* 2004;32(4):455-9
99. Holthe M, Rakvag TN, Klepstad P, et al. Sequence variations in the UDP-glucuronosyltransferase 2B7 (UGT2B7) gene: identification of 10 novel single nucleotide polymorphisms (SNPs) and analysis of their relevance to morphine glucuronidation in cancer patients. *Pharmacogenomics J* 2003;3(1):17-26
100. Duguay Y, Baar C, Skorpen F, Guillemette C. A novel functional polymorphism in the uridine diphosphate-glucuronosyltransferase 2B7 promoter with significant impact on promoter activity. *Clin Pharmacol Ther* 2004;75(3):223-33
- **Characterized UGT2B7 promoter variant activity *in vivo* and correlated effects of variants in clinical setting.**
101. Fisher MB, Vandenbranden M, Findlay K, et al. Tissue distribution and interindividual variation in human UDP-glucuronosyltransferase activity: relationship between UGT1A1 promoter genotype and variability in a liver bank. *Pharmacogenetics* 2000;10(8):727-39
- **UGT1A1 genotype-phenotype study in a tissue bank.**
102. Innocenti F, Grimsley C, Das S, et al. Haplotype structure of the UDP-glucuronosyltransferase 1A1 promoter in different ethnic groups. *Pharmacogenetics* 2002;12(9):725-33
103. Innocenti F, Liu W, Chen P, et al. Haplotypes of variants in the UDP-glucuronosyltransferase 1A9 and 1A1 genes. *Pharmacogenet Genomics* 2005;15(5):295-301
104. Peters WH, te Morsche RH, Roelofs HM. Combined polymorphisms in UDP-glucuronosyltransferases 1A1 and 1A6: implications for patients with Gilbert's syndrome. *J Hepatol* 2003;38(1):3-8
105. Peterkin VC, Bauman JN, Goosen TC, et al. Limited influence of UGT1A1*28 and no effect of UGT2B7*2 polymorphisms on UGT1A1 or UGT2B7 activities and protein expression in human liver microsomes. *Br J Clin Pharmacol* 2007;64(4):458-68
106. Wiener D, Fang JL, Dossett N, Lazarus P. Correlation between UDP-glucuronosyltransferase genotypes and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone glucuronidation phenotype in human liver microsomes. *Cancer Res* 2004;64(3):1190-6
107. Krishnaswamy S, Hao Q, Al-Rohaimi A, et al. UDP glucuronosyltransferase (UGT) 1A6 pharmacogenetics: II. Functional impact of the three most common nonsynonymous UGT1A6 polymorphisms (S7A, T181A, and R184S). *J Pharmacol Exp Ther* 2005;313(3):1340-6
108. Chen G, Blevins-Primeau AS, et al. Glucuronidation of nicotine and cotinine by UGT2B10: loss of function by the UGT2B10 Codon 67 (Asp>Tyr) polymorphism. *Cancer Res* 2007;67(19):9024-9
109. Chen G, Dellinger RW, Gallagher CJ, et al. Identification of a prevalent functional missense polymorphism in the UGT2B10 gene and its association with UGT2B10 inactivation against tobacco-specific nitrosamines. *Pharmacogenet Genomics* 2008;18(3):181-91

Affiliation

Upendra A Argikar¹ PhD,
 Otito F Iwuchukwu² & Swati Nagar^{†3} PhD
[†]Author for correspondence
¹Research Investigator
 Novartis Institutes for BioMedical Research, Inc.,
 250 Massachusetts Avenue,
 Cambridge, MA 02139, USA
²Graduate student
 Temple University School of Pharmacy,
 Department of Pharmaceutical Sciences,
 3307 N Broad Street,
 Philadelphia PA 19140, USA
³Assistant Professor
 Temple University School of Pharmacy,
 Department of Pharmaceutical Sciences,
 3307 N Broad Street,
 Philadelphia PA 19140, USA
 Tel: +1 001 215 707 9110;
 Fax: +1 001 215 707 3678;
 E-mail: swati.nagar@temple.edu