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Genetic Polymorphism of UDP-Glucuronosyltransferase

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Abstract

Genetic polymorphism is referred to the discontinuous interspecies genetic variability among individuals having distinct alleles on a particular locus. Genetic polymorphism of genes encoding drug-metabolizing enzymes constitutes individual's susceptibility to drugs, affirmed by having discrete allelic frequencies by the individual, strengthening the concept of precision medicine. To combat with toxic consequences of drugs, the polymorphic genes associated with xenobiotic metabolism must be studied. Up to 70% xenobiotic elimination is believed to be dependent on UDP-glucuronosyltransferase (UGT), an enzyme encoded by polymorphic UGT1A and UGT2B genes. Both bimodal and trimodal distribution patterns of UGT have been reported in various human populations studied. Genetic polymorphisms of UGT may even lead to truncated and shorter gene with grossly diminished enzymatic activity. The extent of phenotypic alteration inflicted by genetic polymorphisms depends on its nature and position on gene locus. The different isoforms of UGT superfamily differ from each other regarding substrate specificity and selectivity. The incidence of genetic polymorphisms and associated altered gene functions results in inter-individual variability in metabolic clearance and elimination of drugs. Hence, the critical interaction between genetics and biotransformation of drugs has recently been the focus of pharmacology research.

Keywords: bilirubin, biotransformation, drug-metabolizing enzymes, SNP, UGT, xenobiotics

1. Introduction

Pharmacogenetics enables the personalized therapeutics based on genetic profiling and describes Patient's variation in response to therapy due to genetic factors. Pharmacogenetics

is the study of inter-individual differences in the sequence of particular genes affecting drug metabolism [1]. Genetic polymorphism is the variation in the sequence of DNA among populations and individuals. Inter-ethnic variations in drug response are connected to polymorphisms with racial populations showing discrete allele frequencies and inconsistency from each other [2–4]. Genetic polymorphisms may occur by chance and they may be caused by some agents such as chemicals, radiations or viruses. In the human genome, the most common source and simple form of genetic polymorphism are the single-nucleotide polymorphism (SNP) and it contributes to 90% of human DNA polymorphisms. Genetic polymorphisms of drug-metabolizing enzymes (DMEs) can influence the biodisposition of drugs and thus alter the concentration of drugs in plasma and in target tissues [5].

In the human population, person-to-person variations in gene expression and functional activity of drug-metabolizing enzymes have been found to be associated with the change in their responses to toxicants, carcinogens and drugs [6]. Progress in pharmacogenetics has displayed that a series of drug-metabolizing enzymes have become essential to consider the biotransformation of drugs. Allelic variants with different catalytic functions have been recognized from common and wild-type allele as the number of drug-metabolizing enzymes is increased. In individualizing drug therapy, these specific genetic variants are valuable to phenotype/genotype an individual [7, 8]. Hence, the interplay of pharmacogenetics and biotransformation of drugs has recently been a focus of research.

2. Molecular bases

Genetic polymorphism within a population arises as a result of alteration in genes encoding metabolizing enzymes with a variant allele frequency of more than 1% [9]. For such genes, an allelic site has a number of single-nucleotide polymorphisms (SNPs) which may result in increased, reduced or even no enzyme function and their disrupted biological regulations through incorporating several mechanisms. A genotype is referred to a comprehensive study of an organism's particular genetic make-up while the associated observable traits of an individual describe its phenotype. However, keeping in view that a phenotype is influenced by the combination of genetic and environmental determinants, it may not always express a complete concordance with associated genotype [10].

3. SNPs and drug-metabolizing enzymes

Single-nucleotide polymorphism in genes of drug-metabolizing enzymes affects the response of drug to body and absorption, distribution, biotransformation, and excretion of drugs. These SNPs are a source of inter-individual and inter-ethnic differences. Some mutations in the coding region cause amino acid change, which results in alterations of enzyme activity,

substrate selectivity and sometimes, protein stability. Ensuring functional differences causes different metabolizer phenotypes [11]. Pharmacogenetics analysis provides insight into mechanisms included in drug response, with the ultimate goal to achieve optimal drug efficacy and safety.

Drug-metabolizing enzymes (DMEs) have a significant role in biotransformation and final excretion of xenobiotics and drugs by increasing their hydrophilicity. Biotransformation of drugs is usually comprised of phase I (oxidation, reduction and hydrolysis) reactions carried out by cytochrome P450 enzyme, and phase II conjugation reactions involve glucuronidation by uridine diphosphate glucuronosyltransferases (UGTs), acetylation by N-acetyltransferase (NAT) and glutathione-S-transferase in the liver, with numerous proteins accountable for transportation. These phase I and phase II enzymes are highly polymorphic leading to variation in the level of enzyme expression *in vivo* [12].

The first polymorphism in drug-metabolizing enzyme was reported in patients more than 40 years ago with the incidence of side effects after administration of a recommended therapeutic dose of the drug [13]. An array of possible physiological and environmental factors has been assigned to human variability in drug response. The physiological features consist of hepatic and renal function, age, gender and bodyweight, whereas the environmental features include concomitant drug administration, contact to definite chemicals and dietary intake as well. Genetic factors have traced that individual's body respond to drug therapy in a different way as some people are well tolerated while others have harmful effects [12].

4. SNP-mediated metabolic modulation

The uridine diphosphate glucuronosyltransferases (UGTs) exist in almost all living beings counting microorganisms (viruses, bacteria), animals, plants and humans. UDP-glucuronosyltransferase is a microsomal enzyme carrying the glucuronidation of several exogenous (different carcinogens as well as drugs) and endogenous compounds (bilirubin; breakdown product of heme, hormones). Glucuronidation is a primary reaction for the removal of countless substrates and drug compounds. The genetic variations in UGT enzyme lead to its changed regulation and expression. The activities of UDP-glucuronosyltransferase contribute to pharmacological and physiologic consequences [14].

In humans, a main drug-metabolizing reaction called glucuronidation is catalysed by uridine diphospho glucuronosyltransferase enzyme (EC 2.4.1.17). Glucuronidation is the conjugation of small lipophilic molecules with uridine diphosphate (UDP) as a sugar donor, altering them into more water-soluble metabolites and accounts for 40–70% of xenobiotic elimination approximately [15]. UDP-glucuronosyltransferases are primarily expressed in the liver but are also distributed in various organs of the body, including the heart, kidney, thymus, spleen, olfactory epithelium, brain, intestine, adrenal glands and lungs. The expression of UGT is known to be affected by genetic polymorphism, physiological and environmental factors like age, diet, disease state, induction and inhibition by chemicals [16].

5. Classification of UGT

The superfamily UGT is divided into four subfamilies called UDP-glucuronosyltransferase 1, UDP-glucuronosyltransferase 2, UDP-glucuronosyltransferase 3 and UDP-glucuronosyltransferase 8. This classification is kept on sequence similarity at the level of amino acid. The isozymes of family UGT1A have the first exon that is spliced into two to five common exons and thus producing a C-terminal and N-terminal domain. The gene-specific promoter region is possessed by each member of UGT1A family [17]. The 13 isoenzymes of UGT1A gene (9 functional and 4 pseudogenes) are all originated due to alteration in exon 1 region of this gene located on chromosome 2q37 and 6 isoforms of UGT2B subfamily emerge to be encoded by a rigid cluster of separate genes located on chromosome 4 in humans [18].

6. UDP-glucuronosyltransferases (UGTs)

The covalent conjugation of sugar with the small organic molecule is brought about by a superfamily UDP-glucuronosyltransferase (UGT). This superfamily of UDP-glucuronosyltransferase enzyme has been explored in microorganisms (bacteria), plants and animals evolutionarily conserved and adapted to combat with the dynamic interaction with lipid-soluble compounds. The UDP-glucuronosyltransferases are protein in nature that is bound to the membrane and is confined to the smooth endoplasmic reticulum (SER) and nuclear compartment of the cell. They have a significant role in glucuronidation of many antibiotics and xenobiotics [14]. UGTs are synthesized as approximately 530 residues precursor containing an N-terminal signal peptide [19].

The mammalian UGT superfamily comprises of four families, and members of each of four families can also be recognized in various lower vertebrates. The UGT superfamily comprises of all glucosyltransferases that contain the UGT signature sequence (FVA)-(LIVMF)-(TS)-(HQ)-(SGAC)-G-X(2)-(STG)-X(2)-(DE)-X(6)-P-(LIVMFA)-(LIVMFA)-X(2)-P-(LMVFIQ)-X(2)-(DE)-Q, (X is any amino acid) [20], and add sugar to small lipophilic compounds. Glucuronidation of compounds forms a range of glucuronides containing functional groups (O-, N-, S- and C-), which significantly enhance the solubility of the parent drugs and terminates its biological effect [21].

7. Distribution of UDP-glucuronosyltransferases

Approximately, the drugs from all therapeutic classes containing an extensive range of acceptor groups pass through glucuronidation process. However, non-steroidal anti-inflammatory drugs and analgesic agents are usually metabolized by this mechanism. It is well recognized that the liver has the maximum abundance and array of UDP-glucuronosyltransferases [22, 23]. The members of UGT1A and UGT2B subfamilies are also found in many other tissues and organs incorporating the epithelium, brain, nasal cavity, stomach, small intestine, colon,

kidneys, lungs, ovaries, mammary glands, testis and prostate gland, in addition to hepatic abundance [16, 23]. The kidneys and GIT (gastrointestinal tract; stomach, small intestine and colon) are the most important extra-hepatic sites in case of drug metabolism [24]. All members of the UGT1A and UGT2B families are expressed differentially in human liver with the exception of some members of both families including UGT1A5, 1A7, 1A8, 1A10 and 2A1 [25]. In contrast to the UGT1A and UGT2B, members of UGT3 family are principally expressed in thymus, testis and kidney with nearly untraceable expression in liver and GI tract [26].

8. Types of glucuronides

The UDP-glucuronosyltransferase enzyme catalyses the transfer of sugar glucuronic acid (GA) from uridine-diphospho-glucuronic acid (UDP-GA) to various exogenous as well as endogenous compounds containing hydroxyl, thiol, amine, carbonyl, carboxylic and hydroxylamine due to structural diversity of the substrates. Binding with glucuronic acid is a quantitatively most important phase II reaction and is a primary pathway in nature for detoxification of a wide range of drugs, dietary compounds, cancer causing agents and their oxidized metabolites, and a variety of environmental chemicals and thus excreting lipid-soluble waste compounds from the body in urine and bile [27, 28]. Glucuronidation of compounds

Polymorphic enzymes	Endogenous cofactor	Biochemical reactions involved	Specific substrate	Intermediate metabolites	References
Sulfotransferase (SULT)	Sulfate	Sulfation	Salbutamol	Salbutamol sulfate	[30]
UDP-glucuronosyltransferase (UGT)	UDP glucuronic acid	Glucuronidation	Morphine	Morphine-3-glucuronide	[31]
Glutathione-S-transferase (GST)	Glutathione	Glutathione conjugation	Doxorubicin	Glutathione metabolite	[32]
N-acetyltransferase (NAT)	Acetyl-CoA	Acetylation	SMX, caffeine	N-Ac SMX AFMU	[33, 34]
Methyltransferase (MT)	Methyl	Methylation	Mercaptopurine, captopril	Methyl metabolite	[35]

Table 1. Important polymorphic human DMEs, cofactors, biochemical reactions, substrates and their intermediate metabolites.

forms a range of glucuronides containing functional groups (O-, N-, S- and C-) which significantly enhance the solubility of the parent drugs and terminates its biological effect [21].

Substrates containing different functional groups chemically form different glucuronides like aliphatic alcohols and phenols form ether glucuronide, while those containing a COOH group form ester glucuronides (acyl glucuronides). The compounds, which possess both

phenolic and COOH groups, can be transformed into both ether and ester glucuronides such as mycophenolic acid (MPA). Glucuronidation of amines (primary, secondary and tertiary) and sulfhydryl compounds result in the formation of N-glucuronides and S-glucuronides, respectively, whereas C-glucuronides are obtained from compounds containing carbonyl group. The most common and rare drug glucuronides in humans are O-glucuronides and C-glucuronides, respectively [29].

There are many enzymes in humans that are polymorphic in nature and metabolize a variety of drugs through biotransformation reactions presented in **Table 1**.

9. UGT isozymes

The large substrate specificity of each UGT isoform makes possible the glucuronidation of structurally isolated molecules. In humans, roughly (40–70%) drugs administered clinically undergo glucuronidation [36]. In humans, 19 UGT isoforms have been recognized, expressed and differentiated: 9 UGT1A members encoded by the UGT1A gene locus are positioned on chromosome number 2 and nine members of UGT2B family (7 members of UGT2B and 2 members of the UGT2A subfamily) are located on chromosome 4. The isoforms of the UGT1A and UGT2B families have a key role in dispensing lipophilic compounds because of their ability to glucuronidate an extensive array of structurally different substrates. Different UGT isoforms are involved in the formation of drug glucuronides but they reveal overlapping and unusual substrate selectivity and specificity. Individual UGTs differ from each other in sense of regulation and expression. UGT activity is known to be affected by physiological and environmental factors like age, diet, disease state, induction and inhibition of UDP-glucuronosyltransferase by chemicals, ethnicity, genetic polymorphism and hormonal level [37]. Some general conjugation reactions of glucuronidation carried by UGT are expressed through chemical equations A–F, given below [38].

- A. $\text{R-OH} + \text{UDP-GA} \rightarrow \text{UDP} + \text{R-O-GA}$
- B. $\text{R-SH} + \text{UDP-GA} \rightarrow \text{UDP} + \text{R-S-GA}$
- C. $\text{R-NH}_2 + \text{UDP-GA} \rightarrow \text{UDP} + \text{R-NH-GA}$
- D. $\text{R-CHO} + \text{UDP-GA} \rightarrow \text{UDP} + \text{R-CO-GA}$
- E. $\text{R-COOH} + \text{UDP-GA} \rightarrow \text{UDP} + \text{R-CO-(OGA)}$
- F. $\text{R-NHOH} + \text{UDP-GA} \rightarrow \text{UDP} + \text{R-N-(OH)GA}$

10. Structural organization of UDP-glucuronosyltransferase 1

The UGT1A isoforms are encoded by a single intricate and complex locus that is organized similarly in all mammals. The nine functional proteins are encoded by UGT1A gene locus

(1A1, 1A3, 1A4, 1A5, 1A6, 1A7, 1A8, 1A9 and 1A10). In humans, the UGT1A gene size is about 200 kb located on chromosome 2q37 and encodes 13 isozymes. Each isoform has a distinctive promoter region, unique exon-1, and shares (2–5) four common exons [39]. Transcription is commenced at unique promoter. Consequently, each of the 13 transcripts has a unique 5' end and a similar 3' end, which is engaged in identification of the UDP-sugar molecule. The human UGT1A isoforms can be divided into four groups on the basis of sequence resemblance, amplification and divergence of gene: UGT1A1, UGT1A6 and UGT1A2P to 1A5 cluster and UGT1A7 to 1A13P cluster. Exons of UGT1A1 and UGT1A6 made proteins that are nearly 50% alike; conversely, within exons of 1A2P–1A5 and 1A7–1A13P clusters, the resulting proteins are more than 90% identical [17]. Four members (1A2P, 1A11P, 1A12P and 1A13P) of UGT2A subfamily have been recognized as pseudogenes since they have mutations that would avert their translation into functional proteins. It has been observed that complexity to the UGT1A locus produces another form of the common exon 5 so-called as 5b exon that can be merged into the human UGT1 proteins. The alternative forms produce mRNAs that encode smaller polypeptides of each UGT1 protein. These smaller proteins do not have a transmembrane part but keep their ability to confine themselves to smooth endoplasmic reticulum. These small UGT1 forms are non-functional and can heterodimerize with whole UGT1 form and lack their activity [40]. In human tissues, short-form UGT1 transcripts are largely distributed and expressed at considerable levels [41].

11. Function/effect of UGT1A isoforms

UGT1A1 is the most important isoform of UGT1A gene that brings about the glucuronidation of bilirubin (a breakdown product of haemoglobin) which required to be eliminated from the body. Genetic alterations in the UGT1A gene are presumed to have an intense effect on the health of affected individuals, particularly those that modify UGT1A1 activities and correspond to rare mutations. Sixty rare mutations have been well known as point mutations, deletions and insertions in UGT1A1 gene. Only a small number of these mutations are reported in common population with high frequency (up to 41%). These mutations called polymorphisms in TATA box region of UGT1A1 promoter produce variant alleles that lead to decrease in activity and rate of glucuronidation by UDP-glucuronosyltransferase [42–44]. These mutations in the UGT1 gene are correlated with two forms of the unconjugated hyperbilirubinemia syndromes. The wild type and common variant allele include six and seven repeats, respectively [45]. The UGT1A isoforms appeared to be expressed at a lower level in different populations of the world: approximately 0–3% in the Asian population, 2–13% in Caucasian population and up to 16–19% in Africans [44].

Paracetamol is being used as a probe drug to study slow and fast acetylation capacity of human [46], which is mainly cleared by hepatic glucuronidation from the body. Inter-individual variability in capacity to glucuronidate paracetamol and possible risk for liver damage could be explained by polymorphisms in genes encoding the paracetamol glucuronidation [47].

Court et al. used human liver samples for accessing paracetamol glucuronidation activity by UGT isoforms (1A1, 1A6, 1A9 and 2B15) that chiefly glucuronidate paracetamol.

Three single-nucleotide polymorphisms (rs10929303, rs1042640 and rs8330) positioned in 3' untranslated region of UGT1A1 were found to be associated with paracetamol glucuronidation activity. Consistently the highest glucuronidation activity is observed with SNP rs8330. This single-nucleotide polymorphism did not modify stability of mRNA and translation capacity [48].

In the pharmacokinetic studies, acetaminophen and SN-38 were used as phenotyping probes *in vivo* to check glucuronidation activity. Study subjects were given irinotecan and acetaminophen separately after a specific wash out period, and no association was observed between irinotecan and acetaminophen glucuronidation activity, proposing affinity for many different UGT1 isoforms [49, 50].

12. UDP-glucuronosyltransferase 2

The UGT2 gene family is further divided into two subfamilies on the basis of sequence resemblance: UGT2A and UGT2B. The genes in each subfamily usually have more than 70% sequence resemblance. The great level of resemblance makes it complex to establish orthologous associations between species in each subfamily. Therefore, a sequential numbering system based on their chronological order of discovery has been used for UGT2 genes [20]. In the human genome, the UGT2 family comprises of three UGT2A and six UGT2B members. The members of UGT2A subfamily are called pseudogenes as they do not form functional proteins. The members of UGT2B subfamily are encoded by separate and independent genes, including UGT2B4, UGT2B7, UGT2B10, UGT2B11, UGT2B15 and UGT2B17. The UGT2B genes emerge to be encoded by a firm group of genes located on chromosome number four [18].

The members of UGT2 family are supposed to be developed by replication of whole UGT2 gene. UGT2A3 gene has six distinct exons. However, UGT2A1 and UGT2A2 genes have a variable and shared exon structure similar to that of the UGT1A family. The UGT2 members also have first unique exon and a set of five shared (2–6) exons. The first 2 exons of each UGT2 gene encode a region of 241 amino acids similar to that of UGT1A gene locus [51].

13. Function and effect of UGT2B genes

UGT2B4 gene of UGT2 family plays an important role in biotransformation and glucuronidation of bile acids allowing their glucuronide efflux from hepatocytes *via* transporters such as multi-drug resistance-associated protein (MRP) 2 or MRP3 [52] and detoxification of many phenols [18, 53].

UGT2B4 gene is expressed in the chief metabolizing organ: liver and in several extrahepatic tissues [18, 54]. Polymorphism in the co-substrate binding region of UGT2B4 gene resulted in mutant allele, which causes change in position of amino acid from aspartate to glutamate at 458 codons [54]. The single change in position of amino acid alters the rate

of glucuronidation. This variant allele is present in the Caucasian, African and Hispanic populations at a frequency varying between 0.17 and 0.38, whereas it is absent from the Asian population [54–56]. Lampe et al. used human liver samples to study polymorphisms in UGT2B4 gene. These polymorphisms cause a change in position of amino acids: from phenylalanine to leucine at codons 109 and 396. It was found that these polymorphisms are rare because they were absent in a big group of 272 individuals [56].

UGT2B15 gene showed glucuronidation activity towards steroid hormones, a number of classes of drugs and their metabolites [57–60]. A guanine to thymine replacement at codon 85 causes an amino acid change from aspartate to tyrosine in substrate binding site of UGT2B15 gene. The homozygous wild-type allele is not common in Caucasians as compared to the Asian, Hispanic and African populations. On the whole, 19–32% of the studied population have been found to bear homozygous variant allele. Levesque et al. studied the effect of UGT2B15 gene polymorphisms on function and enzyme activity *in vitro* using liver microsomes. The homozygous variant allele presented same reaction constant (K_m) values but the rate of reaction (V_{max}) is two times different for steroid hormones [58]. Court et al. determined the functional impact of UGT2B15 variants towards catalytic activities in HEK 293 cellular fractions by using S-oxazepam as probe drug. The glucuronidation activity of homozygous wild-type allele for S-oxazepam was five times more than homozygous variant allele UGT2B15*2 [59].

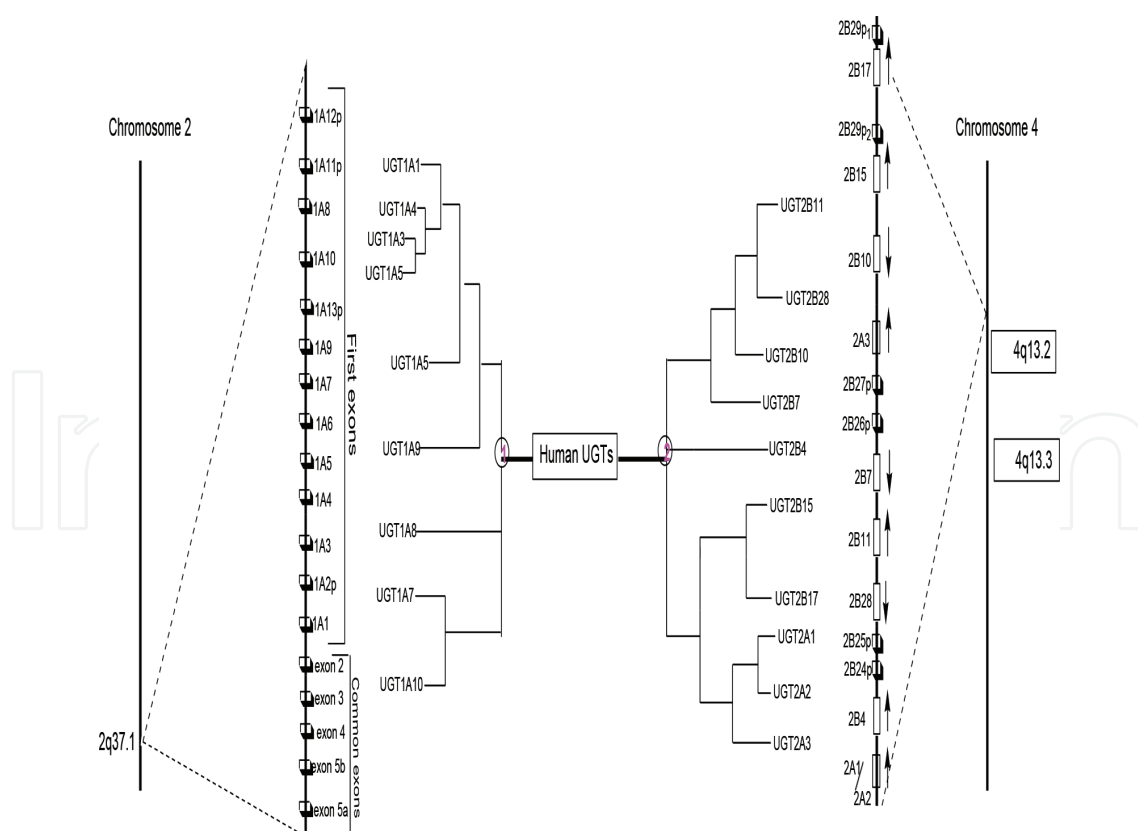


Figure 1. Schema representing the genetic polymorphic pattern of human UGT1A & UGT2B genes.

Lampe et al. evaluated the rate of recurrence of the UGT2B4, UGT2B7 and UGT2B15 polymorphisms in Caucasian and Asian individuals. For all polymorphisms, the genotype and allele frequencies were considerably different in both populations. The distribution of the genotypes also differed by ethnic group. All Asians were homozygous for common allele, and incidence of wild-type allele is two times higher in them as compared to Caucasians [56].

Navarro et al. investigated the effect of UGT1A6 and UGT2B15 genotypes on paracetamol glucuronidation in a controlled feeding trial. 1000 mg paracetamol was administered to healthy volunteers orally, and saliva and urine samples were collected for determining the concentration of paracetamol and its metabolites. The concentration of unchanged paracetamol is higher in men as compared to women who had more paracetamol glucuronide. The percentage of unchanged paracetamol is higher in individuals with homozygous common allele relative to heterozygote and homozygous variants. The UGT2B15 gene considerably affected the paracetamol glucuronidation while the involvement of UGT1A6 in paracetamol glucuronidation was moderate [61].

Mutlib et al. demonstrated the importance of UGT1A and UGT2B15 genotypes: 1A1, 1A6, 1A9 and 2B15 on paracetamol glucuronidation. It was observed that UGT1A1 and UGT2B15 contribution was highest in conjugating paracetamol [62]. The polymorphic variations in UGT1A and UGT2B genes are presented in **Figure 1**.

14. UDP-glucuronosyltransferase 3

The third family of UGT superfamily is UGT3A that consists of two genes: UGT3A1 and UGT3A2. It is situated in a 115 kb piece of chromosome number 5p13. Each gene of this family includes seven unique exons that are just about 30 kb size and encodes 523 amino acids of protein. At protein level, both genes are 80% similar which shows larger conservation of sequence in UGT3A than that found in UGT1A and UGT2B families [63].

15. UDP-glucuronosyltransferase 8

The fourth family of UGT superfamily is UGT8 gene family. This family is composed of a single gene which contains five exons and is situated on chromosome number four (4q26) together with genes that encode other phase II enzymes. The UGT8 gene is greatly conserved and does not involve in the biotransformation of drugs. This is due to its partial expression in the brain where exposure to xenobiotics is restricted [51].

16. Genotype-dependent functional polymorphism

In the human population, individual-to-individual variations exist genetically in UGT activity and are phenotypically divided as rapid or slow glucuronidator. On the basis of

UDP-glucuronosyltransferase activity, humans are divided into bimodal and trimodal distributions. In bimodal distribution, humans are categorized as fast and slow glucuronidators or fast, intermediate and slow glucuronidators in trimodal distribution. UGT1A and UGT2B subfamilies exhibit overlapping but different substrate specificities and variable levels of expression in different organs [16, 36, 64, 65].

An increasing integer of functional single-nucleotide polymorphisms (SNPs) is identified in humans at UGT1A gene locus with a potential relevance for drug therapy. A single-nucleotide polymorphism or a combination of multiple nucleotide substitutions and insertion or deletion is responsible for allelic variants of UGT (UGT1A and UGT2B) [66]. The most common isoforms of UGT associated with drug metabolism are UGT1A1, 1A6, 1A9 and UGT2B4, UGT2B15. Many studies have described associations between specific phenotypes for UGT polymorphic enzyme and susceptibility to cancer, particularly lung and bladder, breast cancer, Parkinson's disease and the autoimmune disease systemic lupus erythematosus. These associations may be due to differences in the ability of enzyme phenotypes to activate or detoxify chemical toxins or, alternatively, to linkage disequilibrium where a particular allele coding for another gene with a direct role in determining disease susceptibility shows genetic linkage with an allelic variant of the xenobiotic-metabolizing enzyme [67].

Genetic polymorphisms lead to truncated and shorter gene which efficiently diminishes enzymatic activity and even a single mutation alone can account for decrease or increase in enzymatic regulation. On the other hand, increased and elevated enzymatic activity may be due to elevated mRNA stability [59]. The expression and activity of UGT increase during childhood and adolescence. Many cis factors (polymorphism) and trans-factors including transcription factors (TFs) and nuclear receptors (NRs), miRNA targeting and other epigenetic regulating factors play an important role in the regulation of UGT enzyme expression. In the liver, transcription levels of UGTs determine their activity. The tissue specific and ligand-activated TFs and NRs play a chief role in the expression of UGTs by binding to cis-regulatory elements. The ontogeny of drug-metabolizing enzymes is analogous by the maturation of organ systems among children and has intense effects on drug disposition [68].

In the beginning, UGT1A and UGT2B families assumed to be evolved as consequences of their neutralizing function against noxious chemical at the hepatic and gastrointestinal barrier. Regulation of UGTs may have developed along with some other drug-metabolizing enzymes (DMEs) in the course of animal and plant competition [69]. The families expanded in different ways on different chromosomes, but similar exon-intron structures encode a protein family with similar functional architecture despite the amino acid sequence diversity [70]. UGTs performed their functions at pre-systemic, systemic level and locally in cells.

Glucuronidation by UGT provides a critical detoxification pathway for exogenous compounds and drugs from several therapeutic classes, including analgesic and non-steroidal anti-inflammatory agents, anticonvulsants, antipsychotics, anti-viral agents and benzodiazepines [27, 28, 71].

17. Role of UGT isoforms in metabolism

Some substrates and drugs are fairly selective for a particular UGT enzyme as the endogenous molecule; bilirubin is particularly glucuronidated by UGT1A1 enzyme. However, the bulk of substrates (small and hydrophobic molecules) are often metabolized by several UGT isoforms [72], thus making it complex to recognize which UGT enzyme is primarily accountable for the glucuronidation of compounds. The accessibility of individual cDNA has made it possible to study the function of each UGT enzyme, even though the quantitative variations in the rate of reaction might be present based on their expression [28]. Moreover, there are facts and figures which designate that UDP-glucuronosyltransferase enzymes are linked as homo and hetero-oligomers *in vitro* and *in vivo*. Oligomerization may be necessary for the activity of the UGT enzymes and sometimes it may even vary the rate of the biotransformation [65, 72]. Formation and disposition of glucuronides in cells and even in the whole organisms are under control of several factors. However, the activity of isoform-specific substrate *in vitro* associates very well with activity *in vivo* and is appropriate for the anticipation of metabolic detoxification *in vivo* [73, 74]. The functional significance of numerous UGT isoforms is ambiguous for many reasons: substrate specificity of isoform is weak displaying overlapping substrate specificity and substrate conjugating regions of UGT enzymes have not been recognized [75].

Lampe et al. found that individuals possessing homozygous variant allele of UGT1A1 and UGT1A6 isoform presented abnormality in the conjugation of a variety of drugs. In Tankaniltler's study, the combined effect of UGT1A1 and UGT1A6 genes was investigated in thalassemic patients. Thalassemic patients were grouped into three categories. It was figured out that there is no difference in glucuronidation capacity of patients with homozygous wild-type allele and those who were heterozygous, but rate of glucuronidation is lower in patients with variant alleles signifying that variant alleles are a potent modifier of acetaminophen glucuronidation [76].

In the human population, wide variations are found in their glucuronidation capacity of drugs. The extent of variations can range from 3-fold to more than 100-fold, considering the studied drug. Many genetic and environmental factors act discretely and in combination to produce broad inter-individual differences. Phenotype-like glucuronidation capacity of UGT is not separated from genotype but somewhat displays continuous and overlapping changes due to multiple interacting environmental and genetic factors [77].

In general population, polymorphisms in phase I and phase II drug-metabolizing enzymes lead to differences in enzyme expression level. This variability in enzyme expression is due to the presence of genetic polymorphisms and mutations in the wild-type gene, resulting in impaired or reduced activity of the enzyme. Individuals having mutated enzymes may differ from normal individuals in their vulnerability to certain diseases. Some specific phenotypes for these polymorphic enzymes are related to increased vulnerability to cancer, predominantly lung, bladder cancer and Parkinson's disease. This altered enzyme activation or certain chemicals including constituents of tobacco, xenobiotics and neurotoxin initiate these diseases [78].

Biotransformation is a key process in the body that finds out the pharmacokinetics of an administered drug. Several factors, which control the level of biotransformation, include physiological state of the patient, genetics and co-administered drugs, and these may cause the toxic or sub-therapeutic concentration of drugs. The large inter-individual variability is gradually accumulating in glucuronidation due to underlying genetic mechanisms but a few polymorphisms have been described in UGTs with probable clinical relevance. The polymorphisms can lead to altered drug clearance with a clinically relevant phenotype. However, the proof *in vivo* for such a link is very weak [36].

18. UGT SNPs and disease

Human UDP-glucuronosyltransferase enzyme was appeared to be polymorphic genetically. Genetic polymorphisms in this enzyme are unlikely to participate in toxicity of drugs since the isozymes illustrate broad overlapping specificity and tissue distribution [79]. Polymorphisms lead to varying degree of transcriptional as well as functional variations that might reduce the activity of UGT enzyme and consequently affected individuals present some sort of pathology. The polymorphisms in UGT1A and UGT2B gene families were also suggested to change the risk of diseases either due to reduced inactivation of hormone or as a result of decreased detoxification of carcinogenic chemicals and production of their reactive conjugates [64].

The variant alleles of UGT1A1 gene result in some syndromes associated with the diminishing bilirubin conjugation capacity of UGT1A1 isoform. Kadakol et al. summarized the data of approximately 50 polymorphisms of UGT1A1 gene contributing to Crigler-Najjar syndrome type I and II. Crigler-Najjar syndrome type 1, also known as non-hemolytic jaundice, is entirely deficient of UGT1A1 activity due to which bilirubin exerts toxic effects on brain [80]. One of the widespread diseases caused by inactivity of UGT1A1 gene is Gilbert's syndrome. More than 10% of the population suffered from this localized chaos was differentiated by sporadic unconjugated hyperbilirubinemia. This syndrome is associated with polymorphism in the promoter region of UGT1A1 enzyme [45]. In patients with Gilbert's syndrome, when irinotecan drug was administered, it resulted in increased toxicity of an active metabolite of SN-38 because in these patients, UGT1A1 gene is not functional as it is responsible for the formation of inactive glucuronide of irinotecan [81].

Several studies assessed the function and differential expression of the UGT1A isoforms in the colon [82], liver [83], pancreas [84] and kidney cancers [85]. The inquiry of a case-controlled study exposed that individuals carrying variant alleles of UGT1A gene were at increased risk of having colorectal cancer [86]. In two independent studies, it was investigated that polymorphisms in promoter and coding regions of UGT1A isoforms were correlated with the toxicity of irinotecan drug in Japanese cancer patients and with neutropenia [66, 87].

Yilmaz et al. examined the association between mRNA expression of UGT (1A3 and 1A7) isoforms and pancreatic cancer. Healthy and tumour samples were collected from pancreatic patients. The mRNA expression of both isoforms of UGT1A gene was notably higher

in pancreatic cancer tissue than normal healthy tissue, and this high expression was also linked with tumour size and its progression [88].

Cengiz et al. reported that expression of UGT1A genes was different in healthy normal and tumour cells of patients suffering from stomach cancer. This differential expression might affect the growth and progression of a variety of cancers [89]. Several authors studied the role of UGTs in breast cancer risk and incidence in different populations. They focused on the expression of isoforms of UGT1A gene by using cell lines of breast cancer and concluded that women with mutations in UGT1A1 enzyme were at higher risk of developing breast cancer due to its reduced activity but no relationship was observed between estrogen receptor status and UGT1A1 genotype [44, 90].

Bigler et al. examined the function of UGT1A6 polymorphisms in healthy controls and patients of colon adenoma in the context of aspirin use. A conflicting association was found between aspirin users' colon adenoma patients and the UGT1A6 variants [82]. Several studies have explored the relationship between polymorphisms of UGT1A7 gene and colorectal, hepatocellular carcinoma and lung cancer in Japanese [91, 92], Chinese [83], Caucasian [93], French [94] and Koreans [95] with reduced incidence of common wild-type allele [96].

19. Risk assessment with SNPs

Uridine diphospho glucuronosyltransferase (UGT1A and UGT2B) enzyme contribute to the removal of miscellaneous drugs, environmental chemicals and endogenous compounds [27, 28]. The majority of human uridine diphospho glucuronosyltransferase enzymes metabolize aliphatic alcohol and phenol compounds of low molecular mass. Most of UGT genes contain single-nucleotide polymorphisms (SNPs) those altered drug metabolism, excretion and drug function and have been found to be associated with Crigler-Najjar syndrome type 1 and 2, hyperbilirubinemia and Gilbert's syndrome. It is believed that SNPs in regulatory regions have the highest impact on phenotype [97].

MacLeod et al. observed that the individuals with elevated level of androgens might be at higher risk of prostate cancer due to presence of lower activity allele [98]. An independent case-control study verified that the patients of prostate cancer with homozygous variant allele had lower enzymatic activity [99]. A positive association has been reported between prostate cancer risk and low-activity UGT2B15 allele or a complete UGT2B17 deleted gene [99, 100]. The UGT2B15 gene is recognized to be greatly expressed in cell lines of prostate cancer [58, 101], and a noteworthy involvement was found between the UGT2B15 homozygous variant genotype and cancer recurrence [102]. The UGT2B15*2 variant allele appeared to be a risk factor for the incidence and poor subsistence of patients of breast cancer. It was reported in breast cancer patients who had mutations in more than one drug-metabolizing enzymes as one UGT2B15*2 allele and also the SULT1A1*2 allele had mainly reduced survival rates of five years.

In humans, uridine diphospho glucuronosyltransferase 2B (UGT2B) proteins facilitate the elimination of testosterone hormone in form of glucuronide metabolites. Grant et

al. evaluated the association between UGT2B15 and UGT2B17 genes of UGT2B family relative to plasma levels of androgens and development of cancer in Whites and Blacks. An association was determined between copy number variant of the UGT2B17 gene and plasma androgen levels in Whites, but surprisingly, no association was found in Blacks [103].

A number of glucuronides are known to acquire pharmacologically useful and harmful activities. Morphine is mainly cleared from the body by glucuronidation pathway and possessed two binding domains [38]. The morphine-3-glucuronide (M-3-G) is the main glucuronide and is a powerful inhibitor of morphine [31, 104]. The deposition of M-3-G reduces the effectiveness of morphine and exerts excitatory effects on the central nervous system [105]. Decreased glucuronidation of morphine to morphine-6-glucuronide *in vivo* was associated with polymorphisms in UGT2B7 gene [106].

Navarro et al. [61] evaluated the impact of UGT1A6 gene and dietary inducers on urinary excretion of aspirin in Caucasians and Asians. They undertook this study on healthy 264 men and 264 women aged between 21 and 45 years. These healthy volunteers were administered 650 mg aspirin, and their urine samples were collected at predetermined time intervals. Considerable variation was found in excretion pattern of aspirin between sexes and ethnicities. Asians excreted more aspirin, salicylic acid acyl-glucuronide and salicylic acid phenolic glucuronide as compared to Caucasians. Diet might manipulate the excretion of aspirin but effects were due to binding of glycine endogenous molecule rather than glucuronic acid. In another study, the glucuronidation of aspirin was undertaken in relevance to UGT isoforms: 1A1, 1A3, 1A4, 1A6, 1A7, 1A8, 1A9, 1A10, 2B4, 2B7, 2B15 and 2B17. It was analysed that all UGT isoforms conjugated aspirin except UGT1A4 and UGT2B17 [107].

Two polymorphisms in exon 1 region of UGT1A6 gene resulted in amino acid substitutions, and these substitutions were likely to be related to reduce enzyme activity towards numerous phenolic compounds [108]. UGT2B15 gene catalyses the metabolism of multiple substrates, thus enhancing their clearance from the body [60].

20. Conclusion

Many polymorphisms have been reported for UGT1A and UGT2B genes and new polymorphisms are continuously evolving. These polymorphisms affect the phenotype and extent of variation depends on the nature of the polymorphisms and their position in the gene. Knowledge about genetic polymorphisms underlying the extensive inter-individual differences in glucuronidation process is progressively gathering but only a small number of polymorphisms in UDP-glucuronosyltransferase enzyme have been reported with possible clinical significance. Patients possessing different genetic polymorphisms illustrated lower or even diminished glucuronidation of a wide range of drugs from almost all therapeutic classes. The incidence of polymorphisms is variable in different populations of the world for various isoforms of UGT1A and UGT2B.

Classification of UGT implicated in phenotyping reaction of a compound depends upon the activity of UGT, the expression and the incidence of glucuronidation rate. But the ever-increasing accessibility of agonists and antagonists probes for individual UGTs provides the dependable prospect for glucuronidation phenotyping. Moreover, many factors affect the functions and activities of UGT proteins *in vivo* such as age (i.e. neonatal period), consumption of alcohol, smoking of cigarette, diet and nutritional habits, pathological condition, ethnicity, hormonal factors and polymorphisms of genes (i.e. occurrence or frequency). The techniques required for glucuronidation phenotype of UGT enzymes have been developed extensively with the discovery and differentiation of specific and selective probes (substrates and inhibitors), accessibility of recombinant UGT enzymes and optimization of incubation states necessary for measuring glucuronidation of drugs.

The tissue-specific expression of each UGT1A and UGT2B isoforms is the subject of genetic polymorphisms, and these individual isoforms can be induced and inhibited by drugs. The individual isoforms differed from each other in sense of substrate specificity and selectivity, the incidence of genetic polymorphisms and gene functions that resulted in broad inter-individual variability in metabolic clearance. It is apparent that polymorphisms in the coding and regulatory segments of all UGT enzymes are likely to be altered their functions and expression. In addition, induction of metabolism may increase the clearance of therapeutic agents, leading to sub-therapeutic exposures and lack of pharmacological effect. Alternatively, inhibition of metabolism may reduce the clearance of drugs and lead to supratherapeutic exposures, resulting in undesired side effects or toxicity.

Variation in DNA sequence results in SNPs, and analysis of SNPs in diseases allows investigation of the influence of genetic polymorphisms on disease vulnerability, drug resistance and eventually the health care. The study of SNPs provides a potent resource for establishing a relationship between a phenotype and regions of the DNA. Genetic polymorphism are sources of variations at all levels. Genetic polymorphism of genes encoding phase I and phase II enzymes describes inter-individual variability in the biotransformation of xenobiotics.

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