

## How polymorphisms of the cytochrome P450 genes affect ibuprofen and diclofenac metabolism and toxicity

Valon Krasniqi<sup>1</sup>, Aleksandar Dimovski<sup>2</sup>, Iva Klarica Domjanović<sup>3</sup>, Ivan Bilić<sup>4</sup>, and Nada Božina<sup>5</sup>

University of Prishtina Faculty of Medicine, Prishtina, Kosovo<sup>1</sup>, Ss. Cyril and Methodius University of Skopje Faculty of Pharmacy, Skopje, Macedonia<sup>2</sup>, Agency for Medicinal Products and Medical Devices of Croatia<sup>3</sup>, University of Zagreb School of Medicine<sup>4</sup>, University Hospital Centre Zagreb<sup>5</sup>, Zagreb, Croatia

[Received in December 2015; CrossChecked in December 2015; Accepted in March 2016]

Interindividual variability in drug metabolism is an important cause of adverse drug reactions and variability in drug efficiency. Polymorphisms of cytochrome P450 (CYPs) genes have a significant effect on drug metabolism and toxicity. This review brings an update about how genetic polymorphisms of CYP2C8 and CYP2C9 enzymes affect the disposition and clinical outcomes of ibuprofen and diclofenac, two of the most common pain relievers. The most common side effects associated with the influence of *CYP2C8\*3* and *CYP2C9\*2\*3* variants on ibuprofen and diclofenac pharmacokinetics are hepatotoxicity and gastrointestinal bleeding. CYP genotyping may therefore identify patients at increased risk of these adverse reactions, and these patients could have their doses adjusted or start receiving another NSAID that does not share the same metabolic pathways with ibuprofen or diclofenac. However, before genotyping is introduced into regular clinical practice, more research is needed to evaluate the effectiveness of this strategy in improving treatment with ibuprofen and diclofenac.

**KEY WORDS:** *adverse effects; allelic variants; CYP2C8; CYP2C9; drug metabolism; gastrointestinal bleeding; genotyping; hepatotoxicity; pharmacogenetics; pharmacogenomics; pharmacokinetics*

Since its completion, the Human Genome Project has given a strong boost to pharmacogenetic and pharmacogenomic research of variability in drug effects based on individual genetic make-up (1). Genetic factors affect the pharmacokinetic profile of drugs and change their efficacy and toxicity properties (2). The final goal of pharmacogenomics is to make use of genetic testing to optimise pharmacotherapy and adjust it to individual needs (3).

Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most commonly prescribed medications in developed countries. According to the American Gastroenterological Association, more than 30 million Americans use NSAIDs every day (data available at <http://www.webmd.com/arthritis/features/making-decision-on-nsaids>).

NSAIDs inhibit the ability of cyclooxygenase (COX) to produce prostaglandins from arachidonic acid, which in turn suppresses inflammation in most patients (4, 5). NSAIDs are prescribed as analgesics, anti-inflammatory, and antipyretic agents for a number of indications. Ibuprofen and diclofenac are often prescribed as NSAIDs

of choice in managing rheumatoid arthritis, post-operative pain, and chronic pain associated with cancer. Both are metabolised by polymorphic phase I metabolic enzymes, predominantly cytochromes P450 (CYPs) and by phase II UDP-glucuronosyltransferases (6). Phase III polymorphic drug transporters have also been evidenced to modulate their toxicity and efficacy (7, 8).

This review brings an update about the association between genetic polymorphisms of CYP enzymes and individual differences in ibuprofen and diclofenac disposition and clinical outcomes.

### *Ibuprofen metabolism*

Ibuprofen is a racemic mixture of the (S)-(+)- and (R)-(-)- enantiomers administered through several pharmaceutical formulations. (S)-(+)-ibuprofen is the active enantiomer, both *in vitro* and *in vivo* (9). Nearly 65 % of R(-)-ibuprofen is inverted to the (S)-(+)- enantiomer in the liver and some of it is pre-systemically inverted in the gut in the presence of acylCoA thioester, with alpha-methylacyl-coenzyme A racemase (AMACR) acting as the catalyst (9, 10).

(S)-(+)-and (R)-(-)-ibuprofen are promptly metabolised by phase I detoxification enzymes in human liver (10). The metabolic pathways of its enantiomers differ significantly.

**Correspondence to:** Nada Božina, MD, PhD, University Hospital Centre Zagreb, Kišpatičeva 12, 10000 Zagreb, Croatia, e-mail: [nbozina@kbc-zagreb.hr](mailto:nbozina@kbc-zagreb.hr)

S(+)-ibuprofen is metabolised mostly by CYP2C9 while R(-)-IBU is metabolised mainly *via* CYP2C8 (11, 12).

The main primary metabolites of ibuprofen are hydroxy metabolites 1-OH-IBU, 2-OH-IBU, and 3-OH-IBU and the carboxy metabolite (carboxy-ibuprofen) (10), but none has important pharmacological activity (9, 13, 14). The phase II metabolism of ibuprofen involves glucuronidation by uridine 5'-diphospho-glucuronosyltransferase (UGT) isoenzymes 1A9, 1A3, 2B1, and 2B7, of which UGT2B7 has the highest *in vitro* activity with racemic ibuprofen (15). Most of ibuprofen is metabolised and the major route of ibuprofen excretion is through the kidney and just a small percentage of the parent drug is excreted unchanged in urine.

The risk of toxicity is related to the binding (covalent type) of ibuprofen-glucuronide to plasma proteins, which is the highest in patients with renal impairment (16). Thiolated derivatives of ibuprofen (less than 1 %) are considered reactive and may cause adverse reactions (17).

#### *Diclofenac metabolism*

Diclofenac is rapidly and completely absorbed from human intestines after oral administration and is detoxified through hydroxylation and glucuronidation (18). Its oxidation to 4'- and 5'-hydroxylated derivatives is catalysed by CYP2C9 and CYP3A4 (19), whereas glucuronidation is catalysed by UGT2B7 (20-22). The oxidised metabolites are mainly excreted through the kidney (65 % of diclofenac), while the rest (35 %) as acyl glucuronide is excreted through the bile (23, 24). Oral bioavailability of diclofenac drops to 50-60 % of the applied dose as a result of first-pass metabolism and low enterohepatic circulation. After excretion into the intestines, diclofenac acyl glucuronide undergoes hydrolysis by intestinal bacteria enzymes called  $\beta$ -glucuronidases. As a result of its reabsorption, enterohepatic recirculation of diclofenac is high (25). Acyl glucuronides of diclofenac are chemically unstable compounds that can undergo epimerisation through acyl migration to the 2-, 3-, or 4-O-glucuronide, particularly in the alkaline medium of the bile. These isomers are believed to resist deconjugation by  $\beta$ -glucuronidases (26).

Transporters may play an important role in the drug's fate in human organism. Using a mice model, Lagas et al. (27) have shown that multidrug resistance proteins 2 (MRP2/ABCC2) and 3 (MRP3/ABCC3) as well as breast cancer resistance protein (BCRP/ABCG2) have a significant role in the pharmacokinetics of diclofenac glucuronides. In their study, MRP2/ABCC2 and BCRP significantly changed biliary excretion of diclofenac glucuronides, while MRP3/ABCC3 was the main efflux transporter from the liver to blood. Concurrent loss of function of MRP2/ABCC2, MRP3/ABCC3, and BCRP/ABCG2 resulted in significant accumulation of reactive diclofenac glucuronides in the mice liver and acute but mild toxicity.

Acquired or hereditary deficiency of ABCC2, known as Dubin-Johnson syndrome in humans, can cause an increased concentration of bilirubin glucuronides (28). As diclofenac shares the same ABCC2 transporter pathway, it should be prescribed to these individuals with caution to avoid adverse reactions to diclofenac, hepatotoxicity in particular.

#### *Genetic polymorphisms of CYP enzymes*

Interindividual variability as a consequence of polymorphisms in *CYP* genes is highly associated with the level of drug toxicity (29, 30). Among over fifty *CYP* enzymes, the human *CYP2C* family accounts for 18-30 % of the total content of *CYP450* enzymes in the human liver and is responsible for the metabolism of nearly one fifth of the commonly prescribed drugs such as angiotensin-II antagonists, NSAIDs, oral antidiabetics, antiepileptics, oral anticoagulants, psychotropic drugs, and some alkylating anticancer prodrugs (2). In addition, *CYP2C9* metabolises endogenous substrates such as arachidonic and linolenic acid. Updated information regarding the list of allelic variants of *CYP2C* isoforms is available online at <http://www.imm.ki.se/CYPalleles/>.

#### *CYP2C8*

The *CYP2C8* subfamily accounts for nearly 35 % of the total human *CYP2C*-coded enzymes in the liver and has a role in the metabolism of different drugs and endogenous compounds (31). Whereas Agúndez et al. (32) claim that *CYP2C8* is directly involved in the oxidative metabolism of NSAIDs such as diclofenac and ibuprofen, Totah et al. (33) suggest that *CYP2C8* has only a minor or intermediate contribution in their metabolism.

The *CYP2C8* gene is located on chromosome 10q24, spans 31 kb, and shares 74 % of the sequence with the *CYP2C9* gene (34). According to a recent report (35), at the locus of the *CYP2C8* gene there are 16 allelic variants. These variants are responsible for interindividual and interethnic variability in drug response (36), since their frequencies vary significantly between races and population groups. Clinically the most important variants are *CYP2C8*\*2 to \*5 (37, 38). The frequencies of the alleles \*2, \*3, and \*4 are 0.3, 10.9, and 5.9 in Caucasians and 15.9, 0.0, 0.41 in Blacks, respectively, while in the Asians these variants have not been found, or are extremely low (39, 40). Speed et al. (41) reported that 89 % of the *CYP2C8*\*3 carriers are also the carriers of the *CYP2C9*\*2 variant. These authors also suggested that due to a strong linkage disequilibrium between these variants, it would be difficult to distinguish between associations with *CYP2C8* or *CYP2C9*. Although the metabolism of most NSAIDs is associated with *CYP2C9*, *CYP2C8* variants may also define interindividual differences in the pharmacokinetics of some NSAIDs, including ibuprofen and diclofenac (42, 43).

*The relationship between the CYP2C8 genotype and the pharmacokinetics and clinical outcomes of ibuprofen and diclofenac therapy*

*Ibuprofen*

A Spanish group of scientists (43) reported results of an investigation performed with 355 healthy participants who received a single dose of 400 mg ibuprofen. Considering that the *CYP2C9* genotype could confound the association with the pharmacokinetics of ibuprofen, participants with the low-activity *CYP2C9*\*3\*3 genotype were excluded from the study. The clearance of (R)-ibuprofen was 40 % and 37.1% lower in the participants carrying the *CYP2C8*\*3\*3 and *CYP2C8*\*1\*3 alleles, respectively compared to individuals carrying the *CYP2C8*\*1\*1 genotype (wild type) ( $p=0.03$ ). This study showed that the half-life of (R)-ibuprofen was significantly longer in the *CYP2C8*\*3\*3 and *CYP2C8*\*1\*3 genotype carriers compared to wild-type carriers (9 and 4.2 h, respectively, vs. 2 h,  $p<0.025$ ). Considering that the *CYP2C8*\*3 variant was associated with the presence of one or two copies of *CYP2C9*\*2 variant in 13 of 16 participants, it is difficult to distinguish the effects of either variant on the R-ibuprofen metabolism. According to Garcia-Martin et al. (44), the clearance of both R- and S-ibuprofen clearance in *CYP2C8*\*3 homozygotes was nearly one ninth of the clearance in the wild-type homozygotes. The *CYP2C9*\*2 variant affected ibuprofen pharmacokinetics only in the participants who were also carriers of the *CYP2C8*\*3 variant. The authors observed only a limited genotype effect on the enantiospecific clearance of ibuprofen.

According to Agúndez et al. (32), carriers of the *CYP2C8*\*3 and *CYP2C9*\*2 or *CYP2C9*\*3 variants manifested an increased risk of gastrointestinal bleeding after administration of different NSAIDs, including ibuprofen and diclofenac, but could not tell whether it was the parent drug or metabolite resulting from alternative metabolic pathways to have caused the bleeding.

There is evidence that NSAIDs lower the risk of colorectal cancer. The Colorectal Cancer Study Group tested the hypothesis that the *CYP2C8* and *CYP2C9* variants could change the protective effect of NSAIDs against colorectal cancer in 478 patients with colorectal cancer and 733 controls (45). While the use of NSAIDs, including ibuprofen and aspirin, was confirmed as beneficial in reducing the colorectal cancer risk, no variant modified their protective effects.

*Diclofenac*

Dorado et al. (46) investigated the effects of *CYP2C8* polymorphisms on diclofenac metabolism (after a single 50 mg dose) in 142 healthy Spanish volunteers. The participants were genotyped for *CYP2C8* and *2C9* variants, and the variants analysed for association with the

concentration of diclofenac and its metabolites. *CYP2C8*\*3 and *2C8*\*4 carriers had a higher urinary concentration ratio of diclofenac / 5-hydroxy-diclofenac compared to wild-type carriers. The authors pointed to the significant overlap between the *CYP2C8* and *CYP2C9* variant allele carriers and concluded that it was difficult to estimate the separate effects of *CYP2C8* polymorphisms on diclofenac metabolism. Having in mind that 65 % of diclofenac and its metabolites are excreted through the kidneys and 35 % are excreted through the liver (47), other polymorphisms (such as those of some drug transporters like *ABCC2*) and non-genetic factors (like comedication and comorbidities) may affect the metabolism of diclofenac and its excretion.

Reports of hepatotoxic effects of diclofenac (36, 48, 49) have raised the question whether the gene variants *CYP2C8*\*3, *CYP2C9*\*2,\*3 or *UGT2B7*\*2, which code for low-activity enzymes, could worsen these effects. Aithal et al. (50) found no such association with *CYP2C9*\*2 or \*3 variants, but Daily et al. (36) did. They investigated the contribution of the *CYP2C8* and *UGT2B7* gene variants coding for metabolic enzymes (responsible for the formation of reactive diclofenac metabolites) and the contribution of the variant *ABCC2-24C>T* of the *MRP2* drug transporter (responsible for the biliary excretion of the reactive metabolites). Patients who suffered diclofenac-induced hepatotoxicity were more frequent carriers of the gene variants predisposing for low-activity proteins in comparison to the patients who had not developed diclofenac-induced hepatotoxicity. They explained that increased levels of reactive metabolites may result in higher levels of protein-diclofenac adducts and consequently in hepatotoxicity. They also observed that the *CYP2C8* variants seemed to contribute less to diclofenac-induced liver injury than the allelic variants of the other two genes.

*CYP2C9*

Gene coding for the *CYP2C9* enzyme is located on the long arm of chromosome 10 in the region that also contains genes for the *CYP2C8*, *2C18*, and *2C19* enzymes (48). The enzyme contains 490 amino acids, weighs 55.6 kDa, and plays a key role in the metabolism of nearly one hundred drugs. So far, 67 allelic variants of the *CYP2C9* gene have been identified (51).

*The relationship between the CYP2C9 genotype and the pharmacokinetics and clinical outcomes of ibuprofen and diclofenac therapy*

*Ibuprofen*

Several investigations tested if the *CYP2C9* \*2, \*3, and *CYP2C8*\*3 allelic variants could reduce ibuprofen metabolism and/or clearance (40, 42, 52), predisposing the carriers of both allelic variants to a higher risk of adverse drug reaction.

Kirchheiner et al. (52) were among the first to study the kinetics of an oral 600 mg dose of ibuprofen racemate in 21 healthy carriers of all combinations of the *CYP2C9* variants \*2 and \*3. Data were evaluated using a population pharmacokinetic model that integrated pharmacogenetic information. According to their results, only the *2C9\*3* variant affected the pharmacokinetics of the racemic and S-ibuprofen. Mean S-ibuprofen clearances were 3.25 L h<sup>-1</sup>, 2.38 L h<sup>-1</sup>, and 1.52 L h<sup>-1</sup> in the carriers of the *CYP2C9* allele combinations \*1/\*1, \*1/\*3, and \*3/\*3, respectively. They did not find any significant effects of the *CYP2C9\*2* variant. In the next step, the authors tested the association between the *CYP2C9* polymorphisms and the formation of thromboxane B<sub>2</sub>, which is the stable product of thromboxane A<sub>2</sub> hydrolysis (a potent vasoconstrictor and stimulus of platelet aggregation) reflecting cyclooxygenase type 1 inhibition. The obtained results suggested that more beneficial effects, that is to say, higher inhibition of thromboxane formation was observed in the carriers of the allelic variants *CYP2C9\*1/\*3*, *\*2/\*3*, and *\*3/\*3* than in the wild-type carriers (*\*1/\*1*). A similar trend was observed for prostaglandin E<sub>2</sub> (important inflammatory mediator), reflecting cyclooxygenase type 2 inhibition. Subjects with prolonged ibuprofen availability (poor *CYP2C9*-mediated metabolism) may run a higher risk of adverse drug reactions but, on the other hand, they can have better pharmacodynamic responses, which can be clinically relevant.

López-Rodríguez et al. (42) studied the effects of the *CYP2C9* variants on the metabolism of both ibuprofen enantiomers in healthy volunteers and found lower metabolism of racemic ibuprofen in the *2C9\*3* variant carriers, resulting in significantly higher area under the curve (AUC) and lower clearance than in the *2C9\*1* allele carriers ( $p < 0.05$ ). As for S-ibuprofen, the clearance was 45 % lower in the *2C9\*3* allele carriers, AUC 87 % higher, and half-life 47 % longer than in the *2C9\*1* allele carriers. R-ibuprofen clearance was also lower in the *2C9\*3* variant carriers by 30 %. In terms of safety, the *CYP2C8\*3* carriers had fewer adverse events, which was explained with lower expression of inducible nitric oxide synthase.

García-Martín et al. (44) analysed genetic factors responsible for interindividual differences in the pharmacokinetics of ibuprofen and its enantiomers in subjects who received a single 400 mg oral dose of racemic ibuprofen. The *CYP2C9\*3* and *CYP2C8\*3* variants lowered the clearance of S-(+)-ibuprofen, whereas the *2C8\*3* allele was responsible for lower clearance of the R-(-) enantiomer. The lowering effect of the *CYP2C9\*2* variant on ibuprofen clearance was observed only in combination with *2C8\*3*. Compared to the wild-type carriers, participants with the *2C9\*1/\*2* and *2C8\*1/\*3* variant combinations (19 % of the participants) showed a 65 % clearance whereas the *2C9\*3* and *2C8\*3* homozygotes and double heterozygotes had only 7-27 % of the wild-type ibuprofen clearance ( $p < 0.001$ ). Karażniewicz-Lada et al. (53) looked for correlations between the *CYP2C9* and *CYP2C8* variants and the

concentrations of ibuprofen and its metabolites in the plasma and urine of healthy volunteers who received a single 400 mg dose of racemic ibuprofen. The *2C9\*2* and *\*3* and *2C8\*3* variant carriers showed lower ibuprofen metabolism than other genotypes, and the lowest metabolism was observed in a carrier of the *CYP2C9\*1/\*2* and *CYP2C8\*1/\*3* variant combination. Variant allele carriers maintained S-ibuprofen plasma levels for much longer, which led to a 40 % higher AUC. They also differed from the wild-type carriers in the plasma levels of ibuprofen metabolites ( $C_{\max} = 1.53$  vs 2.71 mg L<sup>-1</sup> vs for IBP-OH and 1.66 vs 4.52 mg L<sup>-1</sup> for IBP-COOH, respectively). Reduced clearance and longer half-life were also observed.

Patent ductus arteriosus (PDA) affects as many as 31 % of infants whose birth weight is between 501 and 1500 g (54). Ibuprofen is the first-line therapy for PDA with the aim to close the ducts through a mechanism that is most likely based on prostaglandin inhibition. Durrmeyer et al. (55) studied the association between *CYP2C9* and *CYP2C8* polymorphisms and response to ibuprofen therapy (ductus closure) in extremely preterm neonates diagnosed with haemodynamically significant PDA. In multivariate analysis the only two factors significantly associated with the response to ibuprofen were higher gestational age and non Caucasian ethnicity but not *CYP2C* polymorphism. This can be explained by the developmental influence: *CYP* enzymes in the liver of neonates, especially preterm neonates, are still maturing, and polymorphisms at that age do not have the same impact as in adults. Besides, *CYP* variant frequencies differ significantly between races and populations, which can also explain why ethnicity was found to be a significant predictor of ibuprofen efficacy. Other factors like heterogeneity of studied population and sample size could also have affected response to ibuprofen and could have masked the effects of *CYP* polymorphisms.

#### Diclofenac

Zi et al. (56) investigated *in vitro* the effects of the *CYP2C9\*2* and *CYP2C9\*13* variants expressed in yeast (and corresponding to the most common gene variants in the Chinese population) on the kinetics of diclofenac 4'-hydroxylation. The *in vitro* data suggest that these variants could lower the clearance of oral diclofenac. They also tested the effects of these enzymes on diclofenac interactions with nine drugs that inhibit diclofenac 4'-hydroxylation. The *CYP2C9\*13* enzyme significantly weakened the inhibitory potencies of sulphaphenazole, fluvastatin, fluvoxamine, and tranlycypromine. These findings can help in co-administration of diclofenac with other drugs in individuals carrying the *CYP2C9* low-activity alleles.

Another group (57) investigated the impact of *CYP2C9\*2* and *\*3* variants on diclofenac metabolism *in vitro* and *in vivo* but did not find significant differences in 4'-hydroxylation of diclofenac between the genotypes.

Moreover, the absence of correlation between diclofenac hydroxylation and losartan (also a substrate of CYP2C9) oxidation *in vivo* fuelled the scepticism regarding the benefits of using diclofenac as a predictor of CYP2C9 metabolic activity.

Pilotto et al. (58) investigated the effects of the *CYP2C9* genotypes on gastroduodenal toxicity related to diclofenac. They studied several NSAIDs, including diclofenac, ibuprofen, celecoxib, and naproxen in 26 patients with gastroduodenal bleeding who were using NSAIDs and 52 matched controls. Gastroduodenal bleeding was strongly associated with *CYP2C9*\*3 carriers as opposed to non-carriers (adjusted odds ratio 7.3).

Exploring the risk factors of NSAID-induced small intestinal injuries, including diaphragm disease, Ishihara et al. (59) tested the role of the *CYP2C9*\*2, \*3, and \*13 alleles. Multivariate analysis indicated that the use of oxicams or diclofenac and the presence of comorbidities were associated with an increased risk of NSAID-induced small intestinal injury (adjusted odds ratio 2.97,  $p=0.041$ ), but other factors including age, sex, concomitant use of proton pump inhibitors, indications for NSAID use, duration of NSAID use, and the *CYP2C9*\*2, \*3 and \*13 single nucleotide polymorphisms were unrelated. However, the use of meloxicam and the *CYP2C9*\*3 variant were significantly associated with an increased risk of diaphragm disease.

## CONCLUSION

Examples provided in this review article suggest that knowing which enzymes are involved in ibuprofen and diclofenac metabolism may help to predict their bioavailability and behaviour.

In this respect, *CYP2C9* and *CYP2C8* genotyping may identify subpopulations of patients who run a higher risk of overexposure to the two NSAIDs with adverse consequences. Besides CYP enzymes, relevant are phase II metabolic pathways, especially UGT enzymes (mainly UGT2B7) and drug transporters. Some of the ABC members (like ABCC2) can modulate the hepatobiliary as well as renal transport/excretion. Since both pathways are coded by polymorphic genes, the need to apply polygenic approach in future studies is highly recommended.

Other genetic factors and ethnicity could also mitigate the impact of *CYP2C* genotype on response to ibuprofen and diclofenac.

In addition, further research should address factors that have received poor coverage so far but that can shed new light on the associations between *CYP2C* polymorphisms and NSAID efficacy and toxicity. CYP2C enzymes are involved in the metabolism of arachidonic acid to biologically active epoxyeicosatrienoic acids (EETs) (60), which have potent vasodilator and anti-inflammatory properties (61). CYP2C enzymes have also been recognised

as physiologically relevant in the generation of reactive oxygen species (ROS) in vascular endothelial cells, affecting thus the vascular tone and homeostasis (62).

Once the relevant genotypes are established, the dose can be better predicted and adjusted or another NSAID chosen that does not share the same metabolic pathways. However, before genotyping is introduced into regular clinical practice, more research is needed to answer how effective would genotyping be for adjusting doses to the individual needs of NSAID users. Current guidelines on translating pharmacogenomics into clinical practice seem to bring some promise (3). It is important, however, to bear in mind that pharmacogenetics/pharmacogenomics is just a tool that has to be assessed with other relevant factors that may affect drug behaviour, including age, gender, comorbidities, and other concomitant drugs (63). This approach will improve treatment with NSAIDs while avoiding serious adverse effects.

## Competing interests

All authors have completed the Unified Competing Interest form available at [www.icmje.org/coi\\_disclosure.pdf](http://www.icmje.org/coi_disclosure.pdf) and declare that they have received no support from any organisation for the submitted work; have no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; and have no other relationships or activities that could appear to have influenced the submitted work.

## REFERENCES

1. Ma Q, Lu AY. Pharmacogenetics, pharmacogenomics, and individualized medicine. *Pharmacol Rev* 2011;63:437-59. doi: 10.1124/pr.110.003533
2. Božina N, Bradamante V, Lovrić M. Genetic polymorphism of metabolic enzymes P450 (CYP) as a susceptibility factor for drug response, toxicity, and cancer risk. *Arh Hig Rada Toksikol* 2009;60:217-42. doi: 10.2478/10004-1254-60-2009-1885
3. Swen JJ, Nijenhuis M, de Boer A, Grandia L, Maitland-van der Zee AH, Mulder H, Rongen GA, van Schaik RH, Schalekamp T, Touw DJ, van der Weide J, Wilffert B, Deneer VH, Guchelaar HJ. Pharmacogenetics: from bench to byte--an update of guidelines. *Clin Pharmacol Ther* 2011;89:662-73. doi: 10.1038/clpt.2011.34
4. Warner TD, Giuliano F, Vojnovic I, Bukasa A, Mitchell JA, Vane JR. Nonsteroid drug selectivities for cyclo-oxygenase-1 rather than cyclo-oxygenase-2 are associated with human gastrointestinal toxicity: a full *in vitro* analysis. *Proc Natl Acad Sci USA* 1999;96:7563-8. PMID: 10377455
5. Bleumink GS, Feenstra J, Sturkenboom MC, Stricker BH. Nonsteroidal antiinflammatory drugs and heart failure. *Drugs* 2003;63:525-34. PMID: 12656651
6. Jinno N, Tagashira M, Tsurui K, Yamada S. Contribution of cytochrome P450 and UDP-glucuronosyltransferase to the metabolism of drugs containing carboxylic acid groups: risk assessment of acylglucuronides using human hepatocytes.

- Xenobiotica 2014;44:677-86. doi: 10.3109/00498254.2014.894219
7. Zhang Y, Han YH, Putluru SP, Matta MK, Kole P, Mandlekar S, Furlong MT, Liu T, Iyer RA, Marathe P, Yang Z, Lai Y, Rodrigues AD. Diclofenac and its acyl glucuronide: determination of *in vivo* exposure in human subjects and characterization as human drug transporter substrates *in vitro*. Drug Metab Dispos 2016;44:320-8. doi: 10.1124/dmd.115.066944
  8. Kindla J, Müller F, Mieth M, Fromm MF, König J. Influence of non-steroidal anti-inflammatory drugs on organic anion transporting polypeptide (OATP) 1B1- and OATP1B3-mediated drug transport. Drug Metab Dispos 2011;39:1047-53. doi: 10.1124/dmd.110.037622
  9. Rudy AC, Knight PM, Brater DC, Hall SD. Stereoselective metabolism of ibuprofen in humans: administration of R-, S- and racemic ibuprofen. J Pharmacol Exp Ther 1991;259:1133-9. PMID: 1762067
  10. Woodman TJ, Wood PJ, Thompson AS, Hutchings TJ, Steel GR, Jiao P, Threadgill MD, Lloyd MD. Chiral inversion of 2-arylpropionyl-CoA esters by human  $\alpha$ -methylacyl-CoA racemase 1A (P504S): a potential mechanism for the anti-cancer effects of ibuprofen. Chem Commun (Camb) 2011;47:7332-4. doi: 10.1039/c1cc10763a
  11. Davies NM. Clinical pharmacokinetics of ibuprofen. The first 30 years. Clin Pharmacokinet 1998;34:101-54. doi: 10.2165/00003088-199834020-00002
  12. Neunzig I, Göhring A, Drăgan CA, Zapp J, Peters FT, Maurer HH, Bureik M. Production and NMR analysis of the human ibuprofen metabolite 3-hydroxyibuprofen. J Biotechnol 2012;157:417-20. doi: 10.1016/j.jbiotec.2011.12.016
  13. Hamman MA, Thompson GA, Hall SD. Regioselective and stereoselective metabolism of ibuprofen by human Cytochrome P450 2C. Biochem Pharmacol 1997;54:33-41. doi: 10.1016/S0006-2952(97)00143-3
  14. Chang SY, Li W, Traeger SC, Wang B, Cui D, Zhang H, Wen B, Rodrigues AD. Confirmation that Cytochrome P450 2C8 (CYP2C8) plays a minor role in (S)-(+)- and (R)-(-)-ibuprofen hydroxylation *in vitro*. Drug Metab Dispos 2008;36:2513-22. doi: 10.1124/dmd.108.022970
  15. Sakaguchi K, Green M, Stock N, Reger TS, Zunic J, King C. Glucuronidation of carboxylic acid containing compounds by UDP-glucuronosyltransferase isoforms. Arch Biochem Biophys. 2004;424(2):219-25. PMID: 15047194.
  16. Castillo M, Lam YW, Dooley MA, Stahl E, Smith PC. Disposition and covalent binding of ibuprofen and its acyl glucuronide in the elderly. Clin Pharmacol Ther 1995;57:636-44. doi: 10.1016/0009-9236(95)90226-0
  17. Grillo MP, Lohr MT, Khera S. Interaction of  $\gamma$ -glutamyltranspeptidase with ibuprofen-S-acyl-glutathione *in vitro* and *in vivo* in human. Drug Metab Dispos 2013;41:111-21. doi: 10.1124/dmd.112.048645
  18. Tang W. The metabolism of diclofenac - enzymology and toxicology perspectives. Curr Drug Metab 2003;4:319-29. doi: 10.2174/1389200033489398
  19. Tang W, Stearns RA, Bandiera SM, Zhang Y, Raab C, Braun MP, Dean DC, Pang J, Leung KH, Doss GA, Strauss JR, Kwei GY, Rushmore TH, Chiu SH, Baillie TA. Studies on Cytochrome P-450-mediated bioactivation of diclofenac in rats and in human hepatocytes: identification of glutathione conjugated metabolites. Drug Metab Dispos 1999;27:365-72. PMID: 10064567
  20. King C, Tang W, Ngui J, Tephly T, Braun M. Characterization of rat and human UDP-glucuronosyltransferases responsible for the *in vitro* glucuronidation of diclofenac. Toxicol Sci 2001;61:49-53. doi: 10.1093/toxsci/61.1.49
  21. Shipkova M, Armstrong VW, Oellerich M, Wieland E. Acyl glucuronide drug metabolites: toxicological and analytical implications. Ther Drug Monit 2003;25:1-16. PMID: 12548138
  22. Bailey MJ, Dickinson RG. Acyl glucuronide reactivity in perspective: biological consequences. Chem Biol Interact 2003;145:117-37. doi: 10.1016/S0009-2797(03)00020-6
  23. Deer TR, Leong MS, Buvanendran A. Comprehensive treatment of chronic pain by medical, interventional, and integrative approaches: the American Academy Of Pain Medicine textbook on patient management. New York (NY): Springer; 2013
  24. King C, Tang W, Ngui J, Tephly T, Braun M. Characterization of rat and human UDP-glucuronosyltransferases responsible for the *in vitro* glucuronidation of diclofenac. Toxicol Sci 2001;61(1):49-53. PMID: 11294973.
  25. Aithal GP, Ramsay L, Daly AK, Sonchit N, Leathart JB, Alexander G, Kenna JG, Caldwell J, Day CP. Hepatic adducts, circulating antibodies, and cytokine polymorphisms in patients with diclofenac hepatotoxicity. Hepatology 2004;39:1430-40. doi: 10.1002/hep.20205
  26. Sallustio BC, Sabordo L, Evans AM, Nation RL. Hepatic disposition of electrophilic acyl glucuronide conjugates. Curr Drug Metab 2000;1:163-80. PMID: 11465081
  27. Lagas JS, Sparidans RW, Wagenaar E, Beijnen JH, Schinkel AH. Hepatic clearance of reactive glucuronide metabolites of diclofenac in the mouse is dependent on multiple ATP-binding cassette efflux transporters. Mol Pharmacol 2010;77:687-94. doi: 10.1124/mol.109.062364
  28. Nies AT, Keppler D. The apical conjugate efflux pump ABCC2 (MRP2). Pflugers Arch 2007;453:643-59. doi: 10.1007/s00424-006-0109-y
  29. Johansson I, Ingelman-Sundberg M. Genetic polymorphism and toxicology - with emphasis on Cytochrome p450. Toxicol Sci 2011;120:1-13. doi: 10.1093/toxsci/kfq374
  30. Sim SC, Kacevska M, Ingelman-Sundberg M. Pharmacogenomics of drug-metabolizing enzymes: a recent update on clinical implications and endogenous effects. Pharmacogenomics J 2013;13:1-11. doi: 10.1038/tj.2012.45
  31. Zhang D, Surapaneni S, editors. ADME-enabling technologies in drug design and development. Hoboken (NJ): Wiley; 2012.
  32. Agúndez JA, García-Martín E, Martínez C. Genetically based impairment in CYP2C8- and CYP2C9-dependent NSAID metabolism as a risk factor for gastrointestinal bleeding: is a combination of pharmacogenomics and metabolomics required to improve personalized medicine? Expert Opin Drug Metab Toxicol 2009;5:607-20. doi: 10.1517/17425250902970998
  33. Totah RA, Rettie AE. Cytochrome P450 2C8: substrates, inhibitors, pharmacogenetics, and clinical relevance. Clin Pharmacol Ther 2005;77:341-52. doi: 10.1016/j.cpt.2004.12.267
  34. Klose TS, Blaisdell JA, Goldstein JA. Gene structure of CYP2C8 and extrahepatic distribution of the human CYP2Cs. J Biochem Mol Toxicol 1999;13:289-95. PMID: 10487415
  35. The Human Cytochrome P450 (CYP) Allele Nomenclature Database [displayed 20 October 2015]. Available at <http://www.cypalleles.ki.se/>

36. Daily EB, Aquilante CL. Cytochrome P450 2C8 pharmacogenetics: a review of clinical studies. *Pharmacogenomics* 2009;10:1489-510. doi: 10.2217/pgs.09.82
37. Gao Y, Liu D, Wang H, Zhu J, Chen C. Functional characterization of five CYP2C8 variants and prediction of CYP2C8 genotype-dependent effects on *in vitro* and *in vivo* drug-drug interactions. *Xenobiotica* 2010;40:467-75. doi: 10.3109/00498254.2010.487163
38. Paganotti GM, Gramolelli S, Tabacchi F, Russo G, Modiano D, Coluzzi M, Romano R. Distribution of human CYP2C8\*2 allele in three different African populations. *Malar J* 2012;11:125. doi: 10.1186/1475-2875-11-125
39. Daly AK, Aithal GP, Leathart JB, Swainsbury RA, Dang TS, Day CP. Genetic susceptibility to diclofenac-induced hepatotoxicity: contribution of UGT2B7, CYP2C8, and ABCB2 genotypes. *Gastroenterology* 2007;132:272-81. doi: 10.1053/j.gastro.2006.11.023
40. Wu X, Zuo J, Guo T, Yuan L. CYP2C8 polymorphism frequencies among Han, Uighur, Hui, and Mongolian Chinese populations. *Genet Test Mol Biomarkers* 2013;17:104-8. doi: 10.1089/gtmb.2012.0256
41. Speed WC, Kang SP, Tuck DP, Harris LN, Kidd KK. Global variation in CYP2C8-CYP2C9 functional haplotypes. *Pharmacogenomics J* 2009;9:283-90. doi: 10.1038/tpj.2009.10
42. López-Rodríguez R, Novalbos J, Gallego-Sandín S, Román-Martínez M, Torrado J, Gisbert JP, Abad-Santos F. Influence of CYP2C8 and CYP2C9 polymorphisms on pharmacokinetic and pharmacodynamic parameters of racemic and enantiomeric forms of ibuprofen in healthy volunteers. *Pharmacol Res* 2008;58:77-84. doi: 10.1016/j.phrs.2008.07.004
43. Martínez C, García-Martín E, Blanco G, Gamito FJ, Ladero JM, Agúndez JA. The effect of the Cytochrome P450 CYP2C8 polymorphism on the disposition of (R)-ibuprofen enantiomer in healthy subjects. *Br J Clin Pharmacol* 2005;59:62-9. doi: 10.1111/j.1365-2125.2004.02183.x
44. Garcia-Martin E, Martínez C, Tabarés B, Frias J, Agúndez JA. Interindividual variability in ibuprofen pharmacokinetics is related to interaction of Cytochrome P450 2C8 and 2C9 amino acid polymorphisms. *Clin Pharmacol Ther* 2004;76:119-27. doi: 10.1016/j.clpt.2004.04.006
45. McCreavey LE, Turner F, Smith G, Boylan K, Timothy Bishop D, Forman D, Roland Wolf C, Barrett JH; Colorectal Cancer Study Group. No evidence that polymorphisms in CYP2C8, CYP2C9, UGT1A6, PPARdelta and PPARgamma act as modifiers of the protective effect of regular NSAID use on the risk of colorectal carcinoma. *Pharmacogenet Genomics* 2005;15:713-21. PMID: 16141797
46. Dorado P, Cavaco I, Cáceres MC, Piedade R, Ribeiro V, Llerena A. Relationship between CYP2C8 genotypes and diclofenac 5-hydroxylation in healthy Spanish volunteers. *Eur J Clin Pharmacol* 2008;64:967-70. doi: 10.1007/s00228-008-0508-4
47. Benzon H, Raja SN, Fishman SE, Liu S, Cohen SP eds. *Essentials of Pain Medicine*. 3rd ed. Elsevier Health Sciences; 2011.
48. Aithal GP, Day CP. Nonsteroidal anti-inflammatory drug-induced hepatotoxicity. *Clin Liver Dis* 2007;11:563-75. doi: 10.1016/j.cld.2007.06.004
49. O'Connor N, Dargan PI, Jones AL. Hepatocellular damage from non-steroidal anti-inflammatory drugs. *QJM* 2003;96:787-91. PMID: 14566034
50. Aithal GP, Day CP, Leathart JB, Daly AK. Relationship of polymorphism in CYP2C9 to genetic susceptibility to diclofenac-induced hepatitis. *Pharmacogenetics* 2000;10:511-8. PMID: 10975605
51. CYP2C9 allele nomenclature [displayed 20 October 2015]. Available at <http://www.cypalleles.ki.se/cyp2c9.htm>
52. Kirchheiner J, Meineke I, Freytag G, Meisel C, Roots I, Brockmüller J. Enantiospecific effects of cytochrome P450 2C9 amino acid variants on ibuprofen pharmacokinetics and on the inhibition of cyclooxygenases 1 and 2. *Clin Pharmacol Ther* 2002;72:62-75. doi: 10.1067/mcp.2002.125726
53. Karażniewicz-Łada M, Luczak M, Głowska F. Pharmacokinetic studies of enantiomers of ibuprofen and its chiral metabolites in humans with different variants of genes coding CYP2C8 and CYP2C9 isoenzymes. *Xenobiotica* 2009;39:476-85. doi: 10.1080/00498250902862705
54. Poon G. Ibuprofen lysine (NeoProfen) for the treatment of patent ductus arteriosus. *Proc (Bayl Univ Med Cent)* 2007;20(1):83-5.
55. Durrmeyer X, Hovhannisyan S, Médard Y, Jacqz-Aigrain E, Decobert F, Barre J, Alberti C, Aujard Y, Danan C, Baud O. Are cytochrome P450 CYP2C8 and CYP2C9 polymorphisms associated with ibuprofen response in very preterm infants? *PLoS One* 2010;5(8):e12329. doi: 10.1371/journal.pone.0012329
56. Zi J, Liu D, Ma P, Huang H, Zhu J, Wei D, Yang J, Chen C. Effects of CYP2C9\*3 and CYP2C9\*13 on diclofenac metabolism and inhibition-based drug-drug interactions. *Drug Metab Pharmacokinet* 2010;25:343-50. doi: 10.2133/dmpk.DMPK-10-RG-009
57. Yasar U, Eliasson E, Forslund-Bergengren C, Tybring G, Gadd M, Sjöqvist F, Dahl ML. The role of CYP2C9 genotype in the metabolism of diclofenac *in vivo* and *in vitro*. *Eur J Clin Pharmacol* 2001;57:729-35. doi: 10.1007/s00228-001-0376-7
58. Pilotto A, Seripa D, Franceschi M, Scarcelli C, Colaizzo D, Grandone E, Niro V, Andriulli A, Leandro G, Di Mario F, Dallapiccola B. Genetic susceptibility to nonsteroidal anti-inflammatory drug-related gastroduodenal bleeding: role of Cytochrome P450 2C9 polymorphisms. *Gastroenterology* 2007;133:465-71. doi: 10.1053/j.gastro.2007.05.025
59. Ishihara M, Ohmiya N, Nakamura M, Funasaka K, Miyahara R, Ohno E, Kawashima H, Itoh A, Hirooka Y, Watanabe O, Ando T, Goto H. Risk factors of symptomatic NSAID-induced small intestinal injury and diaphragm disease. *Aliment Pharmacol Ther* 2014;40:538-47. doi: 10.1111/apt.12858
60. Node K, Huo Y, Ruan X, Yang B, Spiecker M, Ley K, Zeldin DC, Liao JK. Anti-inflammatory properties of cytochrome P450 epoxygenase-derived eicosanoids. *Science* 1999;285(5431):1276-9. PMID: 10455056;
61. Bellien J, Joannides R. Epoxyeicosatrienoic acid pathway in human health and diseases. *J Cardiovasc Pharmacol* 2013;61(3):188-96. doi: 10.1097/FJC.0b013e318273b007
62. Fleming I, Michaelis UR, Bredenkötter D, Fisslthaler B, Dehghani F, Brandes RP, Busse R. Endothelium-derived hyperpolarizing factor synthase (Cytochrome P450 2C9) is a functionally significant source of reactive oxygen species in coronary arteries. *Circ Res* 2001;88(1):44-51. PMID: 11139472.
63. Stamer UM, Zhang L, Stuber F. Personalized therapy in pain management: where do we stand? *Pharmacogenomics* 2010;11:843-64. doi: 10.2217/pgs.10.47

### **Kako polimorfizmi gena citokroma P450 utječu na metabolizam i toksičnost ibuprofena i diklofenaka**

Interindividualne razlike u metabolizmu mogu biti važan čimbenik nastanka nuspojava te varijabilnosti u učinkovitosti lijeka. Polimorfizmi gena koji kodiraju metaboličke enzime citokrome P450 (CYP) mogu imati značajan učinak na metabolizam lijeka i toksičnost. Ovaj pregled donosi spoznaje o tome kako polimorfizam enzima CYP2C8 i CYP2C9 utječe na bioraspoloživost i kliničke ishode liječenja ibuprofenom i diklofenakom, koji se svrstavaju među najčešće propisivane nesteroidne protuupalne lijekove. Hepatotoksičnost i gastrointestinalno krvarenje najčešće su nuspojave povezane s utjecajem varijanti *CYP2C8\*3* i *CYP2C9\*2\*3* na farmakokinetiku ibuprofena i diklofenaka. Na osnovi rezultata genotipizacije CYP-a mogu biti prepoznati pacijenti koji imaju povećani rizik od razvoja nuspojava te im je nužno prilagoditi dozu lijeka ili odabrati drugi lijek koji ne dijeli isti metabolički put. Osim enzima CYP, značajan utjecaj imaju i polimorfizmi gena koji kodiraju fazu II metabolizma, osobito enzimi UGT, te transporter, poput ABCC2, koji mogu modulirati ne samo transport na barijeri jetre i žuči nego i izlučivanje bubrezima. Stoga je u budućim istraživanjima nužan poligenski pristup. Prije uvođenja genotipizacije u redovitu kliničku praksu potrebno je provesti daljnja istraživanja koja će uključivati veće fenotipski dobro definirane skupine ispitanika za procjenu učinkovitosti ove strategije u poboljšanju liječenja ibuprofenom i diklofenakom. Zbog značajne međuetničke razlike u učestalosti polimorfizama gena CYP istraživanja treba provesti među različitim rasama i populacijama.

**KLJUČNE RIJEČI:** CYP2C8; CYP2C9; *genotipizacija; hepatotoksičnost; farmakogenetika; farmakogenomika; farmakokinetika; nuspojave lijeka*