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Drug-drug-gene interactions as mediators of adverse drug reactions to diclofenac and statins: a case report and literature review

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Concomitant treatment with drugs that inhibit drug metabolising enzymes and/or transporters, such as commonly prescribed statins and nonsteroidal anti-inflammatory drugs (NSAIDs), has been associated with prolonged drug exposure and increased risk of adverse drug reactions (ADRs) due to drug-drug interactions. The risk is further increased in patients with chronic diseases/comorbidities who are more susceptible because of their genetic setup or external factors. In that light, we present a case of a 46-year-old woman who had been experiencing acute renal and hepatic injury and myalgia over two years of concomitant treatment with diclofenac, atorvastatin, simvastatin/fenofibrate, and several other drugs, including pantoprazole and furosemide. Our pharmacogenomic findings supported the suspicion that ADRs, most notably the multi-organ toxicity experienced by our patient, may be owed to drug-drug-gene interactions and increased bioavailability of the prescribed drugs due to slower detoxification capacity and decreased hepatic and renal elimination. We also discuss the importance of CYP polymorphisms in the biotransformation of endogenous substrates such as arachidonic acid and their modulating role in pathophysiological processes. Yet even though the risks of ADRs related to the above mentioned drugs are substantially evidenced in literature, pre-emptive pharmacogenetic analysis has not yet found its way into common clinical practice.

KEY WORDS: drug interactions; hepatotoxicity; myotoxicity; nephrotoxicity; pharmacogenetics

Inter-individual variability in drug response is a major clinical challenge, as it can result in adverse drug reactions (ADRs) or treatment failure. It is estimated that 80 % of all ADRs depend on the dose and could therefore be prevented (1, 2).

The development of ADRs depends on a number of well-known factors, such as age, renal and liver function, and genetic predisposition. In patients receiving concomitant drug treatment, such as those with different syndromes/comorbidities, especially the elderly, this risk may further increase because of drug-drug interactions (DDIs) (3, 4). However, traditional assessment of DDI-related ADR risks needs to take into account individual genetic variations. Pharmacogenomics has made much progress in recent times, especially in the field of drug metabolism and transport, and this knowledge should be included in the assessment of clinically relevant ADRs. This particularly refers to drug-gene interactions and drug-drug-gene interactions as important triggers of ADRs (4–7).

Pharmacogenomic research has paid particular attention to phase I [cytochromes P450 (CYPs)] and phase II metabolic enzymes [UDP glucuronosyltransferases (UGTs)], as well as to drug transporters (ABC and SLC superfamilies). CYPs are especially important for variability in drug pharmacokinetics and response as they account for the metabolism of 70–80 % of all drugs (8). Moreover, by mediating the metabolism of endogenous substrates, some CYP enzymes play an important protective and physiological role (9, 10).

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Although pharmacogenomic testing can help identify patients at risk of ADRs, its wide application in clinical practice has not yet taken root due to several practical issues, most notably which patients should be tested and how to interpret and include test results in decision making that may, in turn, improve treatment efficacy and safety in patients (11). In addition, little is still known about the usefulness of pharmacogenomic testing in patients undergoing concomitant drug treatment (7).

In this combination of a case report and a review article, we argue that *pre-emptive* pharmacogenetic analysis, i.e. prior to drug administration, and assessment of drug-drug-gene interactions could improve personalised approach to drug and dose selection and minimise the risk of ADRs. Yet, even though several laboratories have adopted this approach, it has not taken root in standard clinical practice (12).

CASE PRESENTATION

On admittance to the hospital emergency department, a 46-year-old Caucasian woman presented with general weakness, difficulty breathing, oliguria and anuria, ascites, and oedema of both lower extremities.

Her medical history was without any serious acute or chronic diseases, but she reported having had regular migraine headaches for the last 10 years, which she treated with diclofenac. The migraines had become severe over the three months preceding admission to the emergency department, and the patient had upped diclofenac doses to 150–200 mg/day. She also reported having had urinary tract infections, for which she had received two 14-day courses of sulphamethoxazole/trimethoprim (400/80 mg bid) therapy.

As no emergency treatment was necessary, the patient was transferred to the nephrology department for further examination. Laboratory tests revealed dyslipidaemia, anaemia, hypokalaemia, hypocalcaemia, and elevated blood urea and creatinine, twice the upper limit of the reference interval (RI) (9.8 mmol/L and 176 μmol/L, respectively). Urinalysis revealed a high level of proteins (9 g/24-hour urine). All this pointed to renal dysfunction, but abdomen ultrasound did not reveal any kidney abnormality (size and cortical echogenicity were normal) and no evidence of urinary tract obstruction.

Patient's history of long-term high-dose diclofenac use raised suspicion of acute kidney injury (AKI) and drug-induced minimal change nephrotic syndrome, as both are associated with NSAID use.

One day following admission, a percutaneous kidney biopsy was performed, and histopathological analysis of kidney tissue samples presented complete podocyte effacement, which was accompanied by interstitial inflammation and acute tubular damage. A small segment of sclerosis was also found, as well as myelin figure and zebra corpuscles in one podocyte, raising suspicion of Fabry disease. However, genetic testing found no mutation to support it.

Diclofenac was discontinued, and the patient started receiving intravenous hydration and diuretics. However, renal parameters continued to increase (with creatinine reaching the peak of 559 μmol/L), and urine output was reduced to 100 mL, despite diuretics. The patient underwent three intermittent courses of haemodialysis and was started on methylprednisolone (at first as 250 mg/day intravenous pulse therapy and then oral doses), which gradually improved her renal function.

All the while she suffered from frequent migraine headaches. A neurologist successfully managed it with a beta-blocker propranolol (3x20 mg/day) throughout hospitalisation. Twenty days following admission, the patient's creatinine level dropped to 231 μmol/L, and daily urine output kept around 2000 mL, but nephrotic proteinuria persisted (11 g/24-hour urine). On day 21 of hospitalisation, she was discharged and prescribed the following oral therapy: furosemide 40 mg/day, prednisone 60 mg/day, pantoprazole 40 mg/day, propranolol 3x20 mg/day for migraines, atorvastatin 20 mg/day for dyslipidaemia, and calcitriol 0.25 μg every other day in combination with calcium carbonate 3x1g/day (Figure 1).

Two weeks following discharge, the patient was readmitted to the emergency department due to a two-day fever (up to 38 °C), sweating, and mild chills accompanied by a dry cough and slight pressure along the edge of the sternum. Pleuropneumonia was suspected and confirmed by X-ray along with marked elevation of blood inflammatory markers [C-reactive protein 85.5 mg/L (RI<5.0); white blood cells $10.2x10^9$/L (RI 3.4–9.7); fibrinogen 8 g/L (RI 1.8–4.1); and erythrocyte sedimentation rate 115 mm/h (RI 4–24 mm/h)]. Markedly elevated blood liver enzymes – alkaline phosphatase [ALP 203 U/L (RI 54–119)], alanine aminotransferase [ALT 154 U/L (RI 10–36)], and gamma-glutamyl transferase [GGT 205 U/L (RI 9–35)] – indicated liver injury. Total bilirubin was low (2–3 μmol/L), and prothrombin time was normal. Urinalysis pointed to possible urinary tract infection (UTI).

To manage pneumonia and possible UTI due to recent AKI and still not fully recovered renal function the patient was immediately started on IV ceftriaxone 1 g/day. Further liver tests, abdominal ultrasound, virology, autoimmune tests, and urine culture excluded biliary disease, viral infection, and autoimmune liver disease, but confirmed ascites and UTI with *Enterococcus faecalis*.

After five days of hospitalisation, chest X-ray follow-up confirmed improvement in the patient's clinical course, but high values of liver enzymes persisted.

On day seven of hospitalisation, GGT and ALP increased significantly (452 U/L and 255 U/L, respectively), while ALT dropped to 107 U/L but remained well above the RI. Physicians reviewed the patient's laboratory findings from the previous hospitalisation of two weeks earlier and

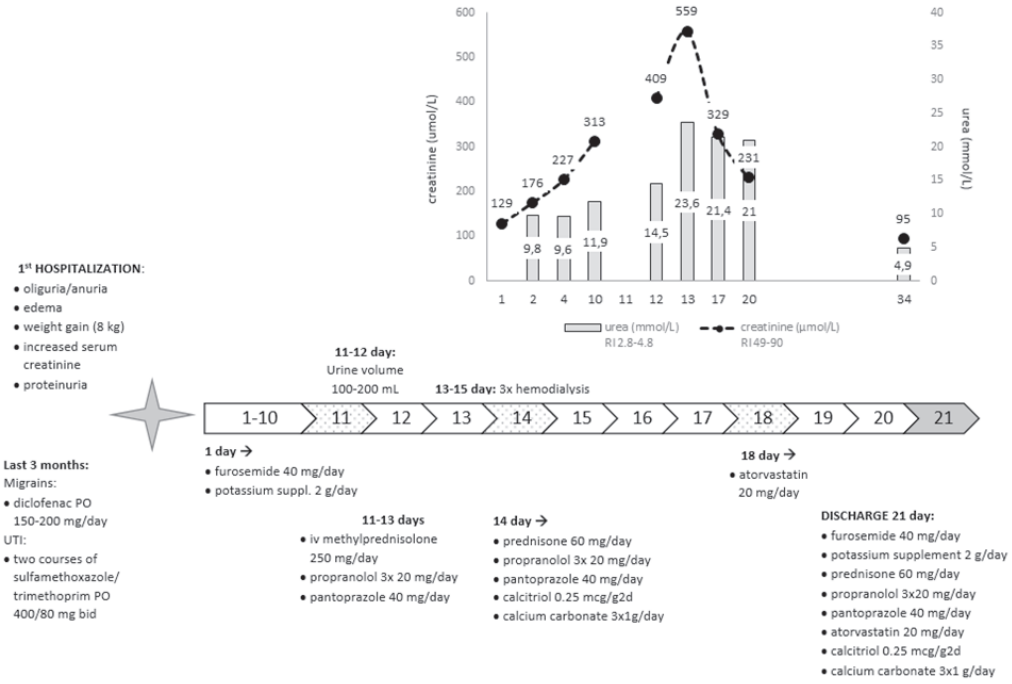

Figure 1 The timeline of the first hospitalisation with all pharmacotherapy and laboratory data

discovered that ALP had slightly increased to 134 U/L two days after the IV methylprednisolone pulse therapy and that bilirubin levels had been low (2–3 µmol/L). This is what prompted them to check all the patient's medications for potential liver toxicity. Assuming drug-induced liver injury (DILI), they replaced ceftriaxone with oral cefuroxime (1500 mg qid), and prednisone with methylprednisolone, and discontinued atorvastatin treatment.

During the second 15-day hospitalisation, blood urea and creatinine levels were normal, proteinuria decreased noticeably, liver enzyme levels normalised, and ascites withdrew (Figure 2).

On discharge, the following therapy was prescribed: methylprednisolone 40 mg/day, pantoprazole 40 mg/day, furosemide 40 mg/day, propranolol 3x20 mg/day, and calcitriol 0.25 µg every other day in combination with calcium carbonate (3x1 g/day) and potassium supplement 2 g/day (potassium citrate/potassium hydrogen carbonate).

The patient's clinical condition was followed up on a regular basis for the next two years (visits every 3–4 months). The patient was in good general health without any acute illnesses, but dyslipidaemia persisted. Two years after the second hospitalisation, the patient started taking a fixed-dose combination of fenofibrate/simvastatin (initially 145/20 mg, which was later increased to 145/40 mg). At the next follow-up visit three months later, this therapy was discontinued due to elevated creatine kinase (CK) level [607 U/L (RI<153)] and symptoms of myalgia associated with statin-induced myotoxicity. Four weeks after fenofibrate/simvastatin discontinuation, CK dropped to normal, which confirmed ADR in our patient.

Taking all these adverse drug reactions into consideration (drug-induced nephro-, hepato-, and myotoxicity), the patient underwent pharmacogenetic tests for genetic variants of the enzymes and drug transporters relevant for the metabolism and distribution of all medicines she received. The influence of possible drug-drug-gene interactions was also assessed.

Genotyping

Genomic DNA was extracted from whole blood samples collected in K_3-EDTA tubes using the FlexiGene DNA Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Single nucleotide polymorphisms (SNPs) *ABCB*1 c.3435C>T (rs1045642), *ABCC2* c.-24C>T (rs717620), *ABCC2* c.1249G>A (rs2273697), *ABCG2* c.421C>A (rs2231142), *CYP2C9**2 (rs1799853), *CYP2C9**3 (rs1057910), *CYP2C19**2 (rs4244285), *CYP2C19**17 (rs12248560), *CYP2D6**3 (rs35742686), *CYP2D6**4 (rs3892097), *CYP2D6**6 (rs5030655), CYP2D6*41 (rs28371725), *CYP3A4**22 (rs35599367), *CYP3A5**3 (rs776746), *SLCO1B1* c.521T>C (rs4149056), *UGT1A4**2 (rs6755571), *UGT1A4**3 (rs2011425), *UGT1A9* -2152C>T (rs17868320), -275T>A (rs 6714486), and *UGT2B7* -161C>T (rs7668258), were genotyped for with the TaqMan® SNP genotyping assays on a 7500 Real-Time PCR System (Applied Biosystems, Carlsbad, CA, USA) according to the manufacturer's instructions. *UGT1A1**28 was genotyped for with the LightSNiP genotyping assay (TIB Molbiol GmbH, Berlin, Germany) and *ABCB1* c.2677G>T/A (rs2032582) with real-time PCR genotyping on a LightCycler® 2.0. Instrument (Roche Diagnostics,

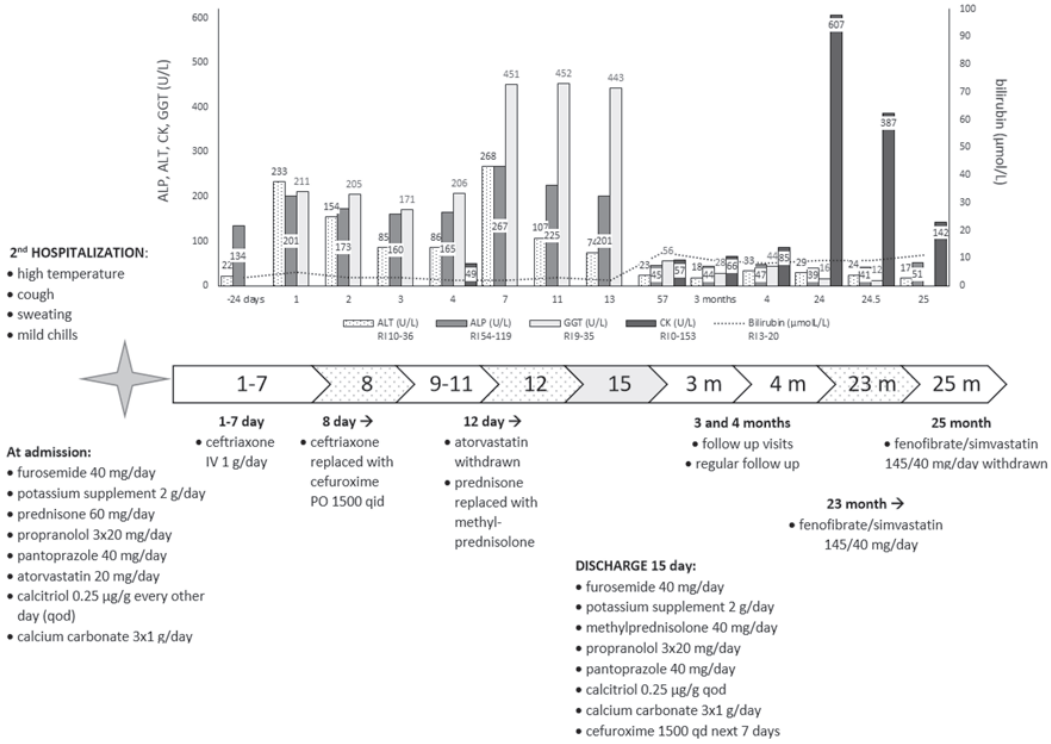

Figure 2 The timeline of the second hospitalisation with all pharmacotherapy and laboratory data

Mannheim, Germany) as described elsewhere (13). *CYP2D6**5 whole gene deletion and *CYP2D6* gene duplications were genotyped for with long-range PCR analysis on a Gene Amp PCR System 9700 (Applied Biosystems) as reported elsewhere (14, 15). All genotyping took place in a pharmacogenetic testing laboratory that regularly participates in external quality assessment schemes (RfB and EMQN).

Our findings are presented in Table 1. Based on these findings, relevant published research, and the guidelines and recommendations of pharmacogenetics consortia, including The Clinical Pharmacogenetics Implementation Consortium (CPIC) (16) and the Dutch Pharmacogenetics Working Group (DPWG) (17) for simvastatin, atorvastatin, NSAIDs, and pantoprazole, our patient was advised which drugs not to take and was prescribed alternative therapy with a lower simvastatin and atorvastatin doses in accordance with current guidelines. Creatine kinase (CK) was monitored routinely to introduce a replacement statin (pravastatin or rosuvastatin) should CK levels rise. Further treatment excluded the combination of simvastatin/atorvastatin and fenofibrate. Furthermore, the patient was advised not to take CYP2C9 substrate drugs over longer periods of time or to use the lowest effective doses when necessary, but under monitoring for signs of toxicity. This advice particularly referred to coumarin anticoagulants, some NSAIDs (celecoxib, flurbiprofen, ibuprofen, lornoxicam), and meloxicam. The recommendation for meloxicam was to start with half the lowest recommended starting dose and titrate it carefully up to clinical effect or up to half the maximum recommended dose. Monitoring for signs of toxicity was advised for short-term application, while for longer-term therapy an alternative drug, not a CYP2C9 substrate, was recommended. Treatment with propranolol was also to be monitored for signs of toxicity, and other CYP2D6 substrate drugs to be administered with caution.

DISCUSSION AND REVIEW OF DRUG-DRUG-GENE INTERACTIONS

This brief review is focused on diclofenac and statins as the ones associated with ADRs found in our patient. What suggests that the issue may be widespread is the fact that these two drugs are among top prescriptions in Croatia (18) and many other countries.

As a NSAID, diclofenac is indicated for pain relief and inflammation in a wide range of conditions. Following oral uptake, it is mainly eliminated via hepatic biotransformation, while less than 1 % is excreted unchanged through urine (Figure 3). In the liver it is mainly metabolised through oxidation and conjugation to glucuronic acid (19). Oxidation to the major metabolite 4'-hydroxydiclofenac is mediated by CYP2C9, while oxidation to the minor metabolite 5'-hydroxydiclofenac is mediated by CYP2C8, CYP3A4, and CYP2C19 (20). Diclofenac acyl glucuronide as the product of conjugation, in turn, is mainly mediated

Table 1 Pharmacogenetic profile of our patient and related pharmacotherapy

Gene-allele	Genotype	Phenotype	Drug-substrate	Drug-inhibitor
*CYP2C9 *2, *3*	*1/***3**	intermediate metaboliser - IM	diclofenac sulphamethoxazole trimethoprim	atorvastatin fenofibrate simvastatin sulphamethoxazole
*CYP2C19 *2, *17*	*1/*1	normal metaboliser - NM	diclofenac pantoprazole propranolol	pantoprazole atorvastatin
*CYP2D6 *3, *4, *5, *6, *41, xN*	*1/***4**	intermediate metaboliser - IM	propranolol	atorvastatin propranolol
*CYP3A4 *22*	*1/*1	normal metaboliser - NM	atorvastatin diclofenac pantoprazole propranolol prednisone simvastatin	diclofenac pantoprazole
*CYP3A5 *3*	*3/*3	non-expresser	atorvastatin propranolol simvastatin	
*UGT1A1 *28*	*1/***28**	intermediate enzyme activity	atorvastatin furosemide simvastatin	atorvastatin pantoprazole
*UGT1A4 *2 (70C>A)*	*1/*1	normal enzyme activity	atorvastatin	
*UGT1A4 *3 (142T>G)*	*1/***3**	intermediate enzyme activity	atorvastatin	fenofibrate
UGT1A9 (-2152 C>T)	C/C	normal enzyme activity	fenofibrate atorvastatin	fenofibrate
UGT1A9 (-275 T>A)	T/T	normal enzyme activity	fenofibrate atorvastatin	fenofibrate
UGT2B7 -161C>T	**T/T**	substrate depending low/ high enzyme activity	atorvastatin diclofenac propranolol simvastatin	fenofibrate
ABCB1 (MDR1) 2677G>T/A *ABCB1 (MDR1) 3435C>T*	G/G **T/T**	decreased transporter function	atorvastatin pantoprazole prednisone propranolol simvastatin	atorvastatin ceftriaxone furosemide pantoprazole
ABCC2 (MRP2) -24C>T *ABCC2 (MRP2) 1249G>A*	C/C G/G	normal transporter function	atorvastatin ceftriaxone diclofenac simvastatin	furosemide
ABCG2 421C>A	C/**A**	decreased transporter function	atorvastatin ceftriaxone diclofenac fenofibrate pantoprazole	furosemide pantoprazole
*SLCO1B1 *5*	*1A/***5**	decreased transporter function	atorvastatin diclofenac simvastatin	atorvastatin diclofenac fenofibrate

by UGT2B7 (21). Approximately 65 % of diclofenac is excreted as oxidative metabolites via the kidneys, while the remaining 35 % is excreted as glucuronide metabolites in faeces via bile (22).

In vivo studies have shown that diclofenac and its glucuronide metabolites are substrates for efflux transporters: multidrug resistance protein 2 (MRP2/ABCC2), 3 (MRP3/ABCC3), and the breast cancer resistance protein (BCRP/ABCG2) (23–25).

Statins are the first-line treatment for hypercholesterolemia in both primary and secondary prevention of cardiovascular disease (26). Simvastatin is a prodrug, administered as inactive lactone and then converted to open hydroxy acid form (27). Atorvastatin is orally administered in active acid form (28). Both undergo extensive first-pass metabolism in the intestine and liver (Figure 4), mediated primarily by CYP3A4 with a minor contribution of CYP2C9 and CYP3A5 (29–32). The main enzymes involved in statin glucuronidation are UGTs (1A1, 1A3, 2B7) (27, 33–37).

Of the drug transporters, P-glycoprotein (encoded by *MDR1/ABCB1*) and BCRP/ABCG2 (encoded by *ABCG2*) mediate intestinal and biliary efflux of statins (38, 39), while OATP1B1 has a central role in hepatic uptake (40–42).

As the majority of CYPs and UGTs are polymorphic, their gene polymorphisms can affect the outcome of drug therapy (10). Transporters too have an important role in drug fate within the human body. Their interplay, along with pharmacogenetic variability, can change drug metabolism and reuptake of substances, prolonging drug bioavailability and increasing the risk of ADRs (43, 44)

There is considerable interindividual variation in susceptibility to the most common ADRs to both of these drugs, yet pre-emptive genetic testing has not yet taken root in regular clinical practice. There are several reasons for this, including insufficient training of healthcare professionals about this issue, insufficient strong evidence linking pharmacogenetic data with clinical outcomes, and a lack of cost-benefit analysis. Pharmacogenetic tests are mostly done retrospectively, as was our case, to identify and explain unexpected ADRs or therapeutic failure in a patient. In our patient the tests revealed the presence of several loss-of-function gene variants for metabolic enzymes and drug transporters (CYP2C9, UGTs, ABCs, and SLCO1B1), which pointed to drug-drug-gene interactions contributing to prolonged bioavailability of applied drugs as additional relevant factor for the observed drug-induced ADRs (nephrotoxicity, hepatotoxicity, statin-associated muscle symptoms, and elevated CK).

Diclofenac nephrotoxicity

There are several possible mechanisms of genetic influence on diclofenac nephrotoxicity. Diclofenac inhibits prostaglandin biosynthesis from arachidonic acid in the kidney by inhibiting cyclooxygenase enzymes (45). The vasodilating effect of prostaglandins increases renal blood flow and glomerular filtration rate. Their inhibition, in turn, reduces renal blood flow, which may lead to peripheral oedema, increased pressure, body weight, and acute renal failure (46). All these symptoms have been observed in our patient.

NSAIDs are known to cause kidney failure even at therapeutic doses, as they interfere with the vasodilation response of renal prostaglandins to vasoconstrictor hormones released by the body (47). However, this effect is often overlooked, because the symptoms are usually moderate and transitory or even absent, like with the absence of anuria (48). ADRs to diclofenac can be potentiated further by pharmacogenetic variants affecting absorption, distribution, metabolism, and excretion (ADME), and our patient had several that could have contributed to weaker diclofenac metabolism (*CYP2C9**3, *UGT2B7 -161TT*, and *UGT1A1**28) and transport (*ABCB1 3435TT* and *ABCG2 421CA*).

In vitro studies have shown that *CYP2C9**3 and other *CYP2C9* alleles *5, *8, *13, and *35 significantly decrease diclofenac metabolism (49–51) but not *CYP2C9**2 (51). Conflicting results have been obtained in clinical studies. While some indicate that *CYP2C9*3* is associated with decreased diclofenac metabolism (higher diclofenac to 4-'hydroxy-diclofenac metabolic ratio in urine) (52), other more convincing data show no association between *CYP2C9*3* polymorphism and increased oral diclofenac plasma concentration or lower clearance (53). The CPIC guideline (54) states that the pharmacokinetics of diclofenac is not affected by the *CYP2C9* genotype and there is not enough evidence to provide recommendation for clinical practice.

It is important that, besides diclofenac metabolism, the *CYP2C9*3* variant may have had an additional effect on the development of nephrotoxicity. One of the physiological roles of some CYP enzymes is to mediate metabolism of arachidonic acid (AA) (55). Since the knowledge about this third AA metabolism pathway emerged (in addition to lipoxygenase and cyclooxygenase), subsequent research has revealed that its products, epoxyeicosatrienoic acids (EETs) (56) and 20-hydroxyeicosatetraenoic acid (20-HETE), have essential roles in regulating renal tubular and vascular function, such as lowering pressure and protecting against renal and vascular injury by reducing inflammation, oxidative stress, and endothelial dysfunction (57, 58).

Furthermore, some studies suggest that CYP variants mediating weaker AA metabolism can contribute to kidney damage (59) and that carriers of loss-of-function alleles *CYP2C9**2 and *CYP2C9**3 have reduced EET production (60). Furthermore, loss-of-function *CYP2C8**3, *CYP2C9**2, *CYP2C9**3, and *CYP2J2**7 variants have been associated with endothelial dysfunction, myocardial infarction, and stroke (61–64).

As our patient is the carrier of the loss-of-function allele *CYP2C9**3, we can assume that reduced EET production

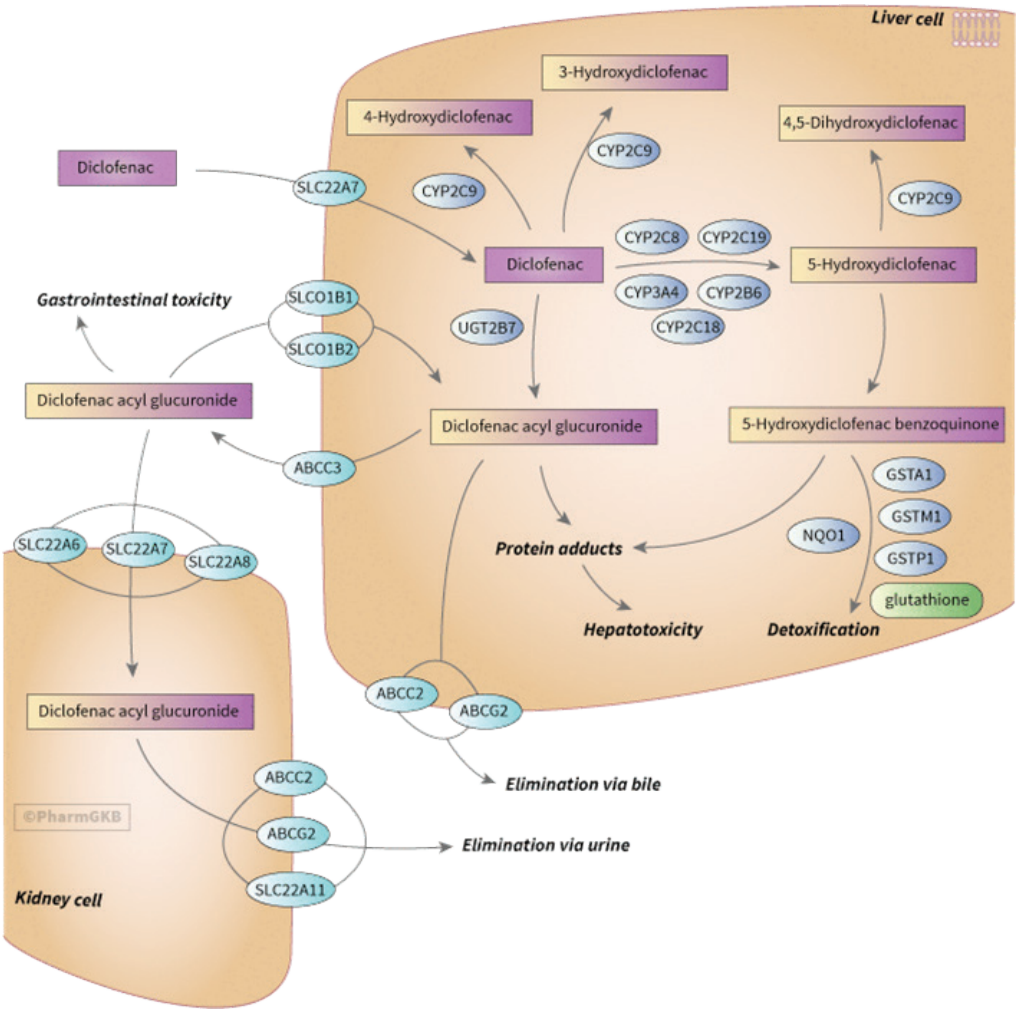

Figure 3 Diclofenac transport and metabolism (adopted from PharmGKB pathway images at https://www.pharmgkb.org/pathway/PA166163705 under the Creative Commons BY-SA 4.0 license)

increased her susceptibility to the nephrotoxic effect of diclofenac.

Increased levels of 20-HETE pose the risk of cardiovascular diseases (65) and glomerular injury (57). 20-HETE elimination in humans mainly follows the glucuronidation pathway by UGTs and can vary 10 times between individuals (66). 20-HETE glucuronidation extensively correlates with the UGT2B7 and UGT1A9 protein expression (65) and is under considerable control of their genetic polymorphisms (*UGT2B7* 802C>T, *UGT1A9* –118T9>T10, and *UGT1A9* 1399C>T) in the liver. The *UGT2B7* 802TT genotype significantly decreases 20-HETE glucuronidation (65). As the *UGT2B7* 802C>T polymorphism is in complete linkage disequilibrium with the *–UGT2B7* -161C>T polymorphism (67, 68) we can assume that the *UGT2B7 -161TT* genotype in our patient was responsible for slower glucuronidation and 20-HETE elimination and may have contributed to kidney failure. In addition to the genetic *UGT2B7* 802C>T (*2) variant, recent data point to a significant role of NSAID, above all diclofenac, in the inhibition of 20-HETE glucuronidation, which may have further potentiated nephrotoxicity (69).

Drug-induced hepatotoxicity

As the *CYP2C9**3 variant can lower diclofenac oxidation to the major metabolite, 4'-hydroxydiclofenac, this could lead to increased production of the minor metabolite 5'-hydroxydiclofenac via CYP2C8, CYP3A4, and CYP2C19 and the formation of hepatotoxic benzoquinones such as diclofenac-2, 5-quinone imines (70). Along with diclofenac acyl glucuronide, these protein-reactive diclofenac-2,5-quinone imines have been suggested to play an important role in diclofenac hepatotoxicity (70).

UGT2B7 has the main role in diclofenac glucuronidation. *UGT2B7* polymorphisms were shown to have substrate-dependent effects on catalytic activity, and its variants can be associated with no effect (71, 72), decreased (73, 74), or even increased enzyme activity (75, 76). Some studies indicate that *UGT2B7**2 (802T/-161T) is more frequent in patients with diclofenac-induced hepatotoxicity (68, 77), which is associated with reduced diclofenac acyl glucuronidation and increased bioactivation to quinonimines, resulting in increased risk of diclofenac-induced liver damage (70).

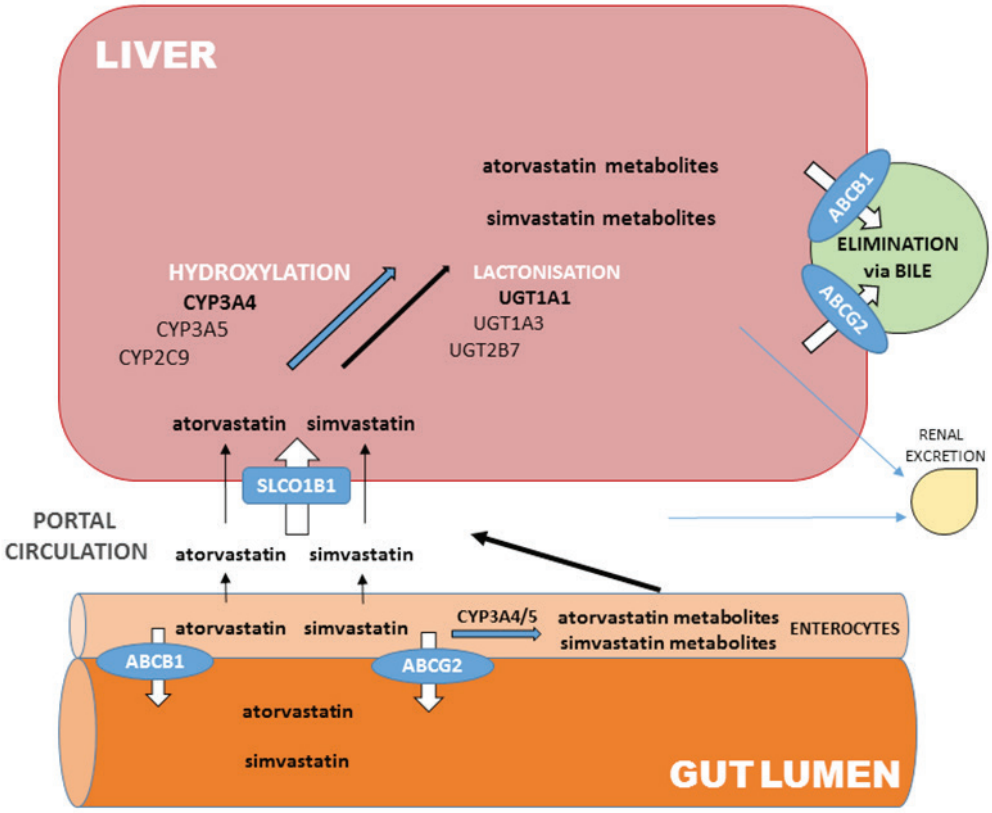

Figure 4 Major statins transport and metabolism pathways (own illustration)

The bioavailability of diclofenac, furthermore, depends on the function of ABCC2 (MRP2) and ABCG2 (BCRP) transporters, which are involved in its absorption, distribution, and excretion (78, 79). In our patient the *ABCG2* gene variant may have therefore decreased the function of this transporter and contributed to drug accumulation and nephrotoxicity due to delayed excretion. We believe that due to the above described mechanisms of diclofenac metabolism, our patient's liver function may have already been sensitised and more prone to hepatotoxicity induced by other drugs, which developed two weeks later. This conclusion stems from the fact that the signs of hepatotoxicity developed in our patient two weeks after hospital discharge, during which time she was receiving atorvastatin, propranolol, furosemide, pantoprazole, prednisone, and calcitriol. This is a rather short time for atorvastatin to induce liver injury, were it not for previous sensitisation. Admittedly, liver injury could also have been exacerbated by ceftriaxone prescribed in the hospital, which points to other factors, including polypharmacy, as additional risk of DILI.

Even so, atorvastatin-induced liver injury (AILI) has amply been evidenced following atorvastatin treatment (81, 82). Post-marketing surveillance revealed that 1.5 % of patients who received atorvastatin treatment suffered from liver injury.

Possible hepatotoxicity predispositions in our patient included prolonged exposure to diclofenac, atorvastatin-related hepatotoxicity, and pharmacogenetics of *ABCB1* and *ABCG2*. Our patient is a carrier of the *ABCB1 3435TT* and *2677GG* genotype. The effects of ABCB1 (MDR1) transporter on the pharmacokinetics of statins have been reported in several studies (38, 83, 84), and variant-weakened transport activity could lead to lower biliary clearance and hepatic accumulation of atorvastatin. *ABCB1* polymorphisms rs1128503 (1236C>T), rs2032582 (2677G>T/A), and rs1045642 (3435C>T) have been shown to markedly affect atorvastatin area under the plasma concentration-time curve (AUC) (38). However, a recently published study did not establish an association between *ABCB1* polymorphisms and the bioavailability of atorvastatin (85).

One study (86) pointed to the association between AILI and the *ABCB1* 2677G>T/A variant (rs2032582) in a Japanese population. Carriers of the *ABCB1* 2677G variant (like our patient) were more vulnerable to AILI, which was also confirmed by a cytotoxicity test *in vitro* in the same study. Since no differences were observed in atorvastatin bioavailability between Asian and Caucasian populations (87), the increased risk of AILI associated with the *ABCB1* rs2032582 allele might therefore also apply for the Caucasian population and our patient. Atorvastatin accumulation in the liver of our patient might have been exacerbated by the interaction with concomitantly administered pantoprazole, a known substrate and inhibitor of MDR1/ABCB1 (88).

Polymorphic ABCG2 is an efflux transporter with significant function in numerous tissues, as it modulates the bioavailability of many drugs, including statins (89). Our patient is a carrier of the *ABCG2* 421C>A variant, associated with reduced transporter activity, which suggests that she could have been exposed to higher systemic and hepatic atorvastatin levels (39, 90). The Keskitalo group (39) found that carriers of the c.*421AA* genotype had a 72 % larger mean atorvastatin AUC than those with the c.*421CC* genotype. Another study (91) in a Japanese population revealed that patients carrying the rs2622604 *ABCG2* allele variant had a 55 % increase in oral atorvastatin bioavailability vs non-carriers.

In line with these increased bioavailability findings, our previous research (92) showed that patients with *ABCG2 421CA* or *AA* genotypes had 2.9 times higher odds of developing atorvastatin dose-dependent ADRs. Even after adjustments for clinical and other genetic risk factors, ABCG2 remained statistically significant for ADRs. The relevance of the ABCG drug transporter has also been recognised by regulatory authorities (93, 94), which recommend that the development of new medicinal products should take into account whether they are substrates or inhibitors of ABCG2. The incidence of *ABCG2* gene variants varies greatly among populations and races. It is significantly higher in the Asian (30 %) than Caucasian (10–15 %) and black (2 %) populations (39, 95).

Although the views on the role of UGTs as predictors of atorvastatin pharmacokinetics and toxicity still diverge (35, 96, 97), we believe that the *UGT1A1*, *UGT1A4,* and *UGT2B7* gene variants in our patient could have had some influence on prolonged atorvastatin systemic and hepatic exposure and susceptibility to ADRs, including liver damage.

Some authors suggest that these inconsistencies considering *UGT* gene variants are due to extensive linkage disequilibrium in the *UGT1A* locus (29, 35).

Statin-associated muscle symptoms

Myopathy is one of the most serious ADRs to statins (98, 99). Pharmacogenetic testing has shown that our patient is a heterozygous carrier of the decreased function allele *SLCO1B1* 521T>C, which is associated with elevated systemic exposure to several statins (100) and an increased risk of myotoxicity. However, short-term (two-week) administration of atorvastatin did not produce this effect in our patient. Instead, she developed signs of myotoxicity, i.e. myalgia and elevated CK only when simvastatin was administered in combination with fenofibrate and then she had to discontinue simvastatin.

The *SLCO1B1**5 (c.*521T*>C, p.V174A, rs4149056) variant has been associated with a 221 % higher systemic exposure to simvastatin acid in carriers of the *521CC* genotypes than in the wild-type carriers (*521TT*). Although to a lesser extent, the relevance of this polymorphism was confirmed for other statins (85) as well, except for fluvastatin (100). Atorvastatin AUC increased 145 % in *521CC* carriers (100). The prevalence of rs4149056 is estimated to be 1 %, 12 %, and 16 % in Africans, East Asians, and Europeans, respectively (101). We therefore assume that the *SLCO1B1* polymorphism in our patient slowed down hepatic uptake of simvastatin and increased its bioavailability in systemic circulation, making her more prone to myotoxicity and interactions with other drugs, including fenofibrate.

Fibrates can increase the risk of statin ADRs due to pharmacodynamic and pharmacokinetic interactions (102). Although this ADR risk for simvastatin and fenofibrate combination is low in general population, it increases in patients with a pharmacogenetic predisposition, like in carriers of the ADME gene variants that prolong drug exposure. We believe this to have been the case with our patient as a carrier of several polymorphisms (*UGT1**28, *UGT2B7* -161T, *ABCB1* 3435T, *ABCG2* 421A and *SLCO1B1* 521C), as she developed signs of myotoxicity and elevated CK after taking the simvastatin/fenofibrate combination.

In vitro, fenofibric acid is a mild-to-moderate inhibitor of CYP2C9, weak inhibitor of CYP2C8, CYP2C19, and CYP2A6 (103,104), and moderate inhibitor of MDR1 and to a minor degree of OATP1B1 (105). *In vivo*, it inhibits hepatic MDR1 (106). Most of active fenofibric acid undergoes glucuronidation by the UGT isoforms (1A9 and 2B7), forming glucuronides which are excreted in the urine and bile (26).

Since simvastatin also uses UGT enzymes for its biotransformation (Figure 2), interactions through UGTs can be expected, especially if the enzyme activity is reduced due to genetic predisposition.

Drug-drug, drug-gene, and drug-drug-gene interactions

The concomitant use of medicinal products should consider all three types of interactions (drug-drug, drug-gene, and drug-drug-gene) as relevant factors in ADRs. In our patient, diclofenac with sulphamethoxazole/trimethoprim resulted in nephrotoxicity, atorvastatin with furosemide, pantoprazole, and ceftriaxone resulted in hepatotoxicity, while the simvastatin/fenofibrate fixed-dose combination resulted in myotoxicity. Sulphamethoxazole and trimethoprim are both substrates of CYP2C9 and can inhibit CYP2C9 and CYP2C8 activities, respectively (107), which in the case of our patient as a carrier of the loss-of-function *CYP2C9**3 variant could be of even greater significance.

Proton pump inhibitors (PPIs) and loop diuretics have recently been associated with modest increases in the levels of atorvastatin and its metabolites (14 % and 38 %, respectively) (108). PPIs inhibit the CYP2C9, 2C19, 2D6, and 3A4 enzymes, which can result in interactions with other drugs which are the substrates of these enzymes (109).

Furthermore, PPIs have been reported to interact with drug efflux transporters ABCB1 and ABCG2 both as inhibitors and substrates (110–112).

Since ceftriaxone, which was replaced in our patient over the risk of increased hepatotoxicity (113), is a substrate of ABCC2 and ABCG2 transporters, drug-drug interactions at this level should also be taken into consideration.

As for furosemide, it has been identified as a substrate for OAT1, OAT3, BCRP/ABCG2, OATP1B1, and OATP1B3 and a potent inhibitor of BCRP *in vitro* (114,115). The *ABCG2* rs2231142 (141K) variant, however, may attenuate BCRP-mediated loop diuretic-atorvastatin interaction, yet Turner et al. (108) did not establish any interaction between atorvastatin and furosemide in relation to the *ABCG2* rs2231142 (*421C*>A, Q141K) variant, most probably because their study included too few carriers. They concluded that the magnitude of the identified PPI and loop diuretic interactions at the population level were modest and of questionable clinical relevance. However, they also added that these newly discovered drug interactions could contribute to the risk of ADRs in specific patients – such as ours – who already have other risk factors for prolonged drug exposure, including comorbidities and polypharmacy. This consideration was confirmed by Klarica Domjanović et al. (116), who showed that the *ABCG2* 421C>A polymorphism significantly modulated drug-drug interactions between valproate and lamotrigine.

CONCLUSIONS

In our patient, ADRs were the consequence of interactions between drugs and ADME-affecting gene variants encoding for metabolic enzymes (CYPs and UGTs) and drug transporters ABCB1, ABCG2, and SLCO1B1. ADME pharmacogenetic variants can significantly modulate/increase the range of drug-drug interactions, prolonging their bioavailability and leading to ADRs as a result. In addition, variations in CYP and UGT enzyme function may reflect on the biotransformation of endogenous substrates such as arachidonic acid. Through inflammation, oxidative stress, and endothelial dysfunction, this may add to the risk of organ damage.

A number of ADRs could have been prevented by pharmacogenetic testing in advance of treatment with diclofenac, atorvastatin, and simvastatin/fenofibrate. This testing could also have provided information about possible drug-drug-gene interactions in concomitant therapy, especially with sulphamethoxazole/trimethoprim, pantoprazole, and furosemide. This is why we believe that drug-drug-gene interactions deserve further comprehensive studies and that pharmacogenetic testing should find its rightful place in managing patients with polypharmacy.

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Conflicts of interest

None to declare.

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Interakcije lijek-lijek-gen kao posrednici nuspojava diklofenaka i statina – prikaz slučaja i pregled literature

Statini i nesteroidni protuupalni lijekovi (NSAID) učestalo se propisuju, pa i kao konkomitantna terapija. Postoji značajna interindividualna razlika u osjetljivosti na njihove najčešće nuspojave. Rizični čimbenici za razvoj nuspojava tih lijekova mogu biti povezani s lijekom i pacijentom ili s vanjskim čimbenicima. Polifarmacija je česta u kroničnih bolesnika i povećava rizik od razvoja interakcija lijekova. Istodobna primjena lijekovima koji inhibiraju CYP, UGT, ABC i / ili SLC prijenosnike lijekova (ABCB1, ABCG2 i OATP1B1) povezana je s produljenjem bioraspoloživosti lijekova, što rezultira povećanim rizikom od razvoja nuspojava. S tim u vezi, predstavljamo slučaj 46-godišnje žene koja je tijekom dvije godine doživjela akutno oštećenje bubrega i jetre, kao i mialgiju, dok je uzimala diklofenak, atorvastatin, fiksnu kombinaciju simvastatina / fenofibrata istovremeno s nekoliko drugih lijekova, uključujući pantoprazol i furosemid. Analiza dobivenih farmakogenetičkih rezultata te pregled dosadašnjeg znanja u tom području upućuju na zaključke da interakcije lijek-lijek-gen mogu produljiti bioraspoloživost primijenjenih lijekova. Mehanizam se argumentirano temelji na sporijoj sposobnosti detoksikacije i smanjenoj eliminaciji putem jetre i bubrega, što rezultira toksičnošću za više organa. Jednako tako, prikazuje se važnost polimorfizama CYP u biotransformaciji endogenih supstrata poput arahidonske kiseline i u njihovoj modulacijskoj ulozi u patofiziološkim procesima. Danas postoje prilično značajni znanstveni dokazi o određenim farmakogenetičkim spoznajama koji rezultiraju povećanim rizikom od razvoja nuspojava za spomenute lijekove, no unatoč tomu, farmakogenetička analiza prije uvođenja lijekova u terapiju još nije uvedena u redovitu kliničku praksu.

KLJUČNE RIJEČI: farmakogenetika; hepatotoksičnost; interakcije lijekova; miotoksičnost; nefrotoksičnost