Creatine kinase elevation caused by a combination of fluvastatin and telmisartan in a patient heterozygous for the CYP2C9*3 and ABCC2 -24C > T variants: a case report

Henriette E Meyer zu Schwabedissen1, Werner Siegmund2, Heyo K Kroemer3 and Jens D Rollnik4*

Abstract

Background: Genetic factors as predictor of the individual outcome of drug therapy is one aim of personalized medicine approaches.

Case presentation: We report a drug metabolism based analysis of genetic polymorphisms in a Caucasian patient receiving fluvastatin and telmisartan experiencing myotoxicity (myalgia and moderate creatine kinase elevation).

Conclusions: The obtained findings suggest that heterocygocity of cytochrome P450 CYP2C9*3 variant in combination with multidrug resistance-associated protein MRP2 -24C > T functions as risk factor predisposing to experience drug-drug interaction combing those drugs.

Keywords: Fluvastatin, OATP1B1, CYP2C9, Cytochrome P450, Telmisartan, Genetic polymorphisms, Drug transporter, Drug-drug interaction

Background

Herein we report the case of a patient receiving fluvastatin for several years developing mild myotoxicity symptoms after co-administration of telmisartan, an AT1-receptor antagonist. Importantly, fluvastatin even if exhibiting a rather modest lipid-lowering effect has been widely recommended for patient receiving pharmacotherapy with high interaction potential due to the assumed low risk of pharmacokinetic interaction [1]. It was aim of this retrospective pharmacogenetic analysis to provide insight into genetic risk factors for the observed drug-drug interaction. In order to identify the predisposing factors the patient was assessed for frequently occurring single nucleotide polymorphisms of genes involved in the pharmacokinetic pathway of fluvastatin and telmisartan, respectively. Based on our current understanding fluvastatin is assumed to be taken up into the hepatocyte mediated by the hepatic uptake transporter organic anion-transferring polypeptide (OATP) OATP1B1, where it exerts its pharmacodynamic action by inhibiting the key enzyme of hepatic cholesterol synthesis the HMG-CoA reductase. Subsequent metabolism mediated by the microsomal cytochrome P450 enzymes especially the isoform CYP2C9 is the major step of drug elimination. In addition recent findings indicate that the efflux transporter BCRP (ABCG2) is also involved in modulating fluvastatin disposition [2,3]. For telmisartan it is assumed that OATP1B3 is predominantly involved in hepatic uptake [4], after intrahepatic metabolism mediated by UGTs the telmisartan glucuronide is eliminated via the efflux transporters ABCB1, ABCC2 and ABCG2 [5]. In addition, Weiss and co-workers reported that telmisartan is a potent inhibitor of ABCG2 and ABCC2 mediated efflux in vitro [6]. Importantly, recent findings by Cabaleiro et al suggest that pharmacokinetics of telmisartan itself is not altered by genetic variants of CYP2C9 [7], even if telmisartan is an inhibitor of this particular enzyme [8].

Every sequential step in the process of hepatic uptake, metabolism and elimination can serve as subject of drug
interaction or result in genetic variability. In this case the patient experienced myalgia accompanied by CK elevation after receiving fluvastatin and telmisartan, an angiotensin II type 1 receptor (AT1) – antagonist.

In order to elucidate the impact of common genetic variability on the observed drug interaction we determined well documented impaired function SNPs that have been associated with fluvastatin kinetics. To our knowledge this is the first case in the literature describing in vivo statin side effects in association with an AT1-receptor blocker.

Most AT1-blockers are metabolized by CYP2C9 [2]. Point mutations or single-nucleotide polymorphisms (SNPs) in the CYP2C9 genes have been identified. The most common coding mutations in CYP2C9 are CYP2C9*2, and CYP2C9*3 [2]. CYP2C9*2 and CYP2C9*3 differ from the wild-type CYP2C9*1 by a single point mutation: CYP2C9*2 is characterized by a 430C > T exchange in exon 3 resulting in an Arg144Cys amino acid substitution, while CYP2C9*3 is characterized by a 1075A > C exchange in exon 7 causing an I359L substitution in the catalytic site of the enzyme [2]. Individuals with the *2 or *3 variant alleles may have reduced enzyme activity [2].

**Case presentation**

**DNA sample**

The patient was included in a Pharmacovigilanz Study initiated by the Department of Pharmacology of the Ernst Moritz Arndt University in Greifswald. DNA sampling and subsequent genotyping of Genes involved in pharmacokinetics and -dynamics was approved by the Ethic Committee of the University Greifswald. DNA was isolated from peripheral blood cells using a QIAcube. Purity and content of DNA was assured by NanoDrop® spectrometry.

Genotyping of CYP2C9*2 (rs1799853) and CYP2C9*3 (rs1057910) variant was performed by duplex pyrosequencing. Briefly, the fragments containing the c.430C > T (CYP2C9*2) or the c.1075A > C polymorphism were amplified by duplex PCR using the following PCR primers: 5′-GATATTTTGGGCTGAAACCATA-3′ (CYP2C9-A2-sense), 5′-biotin CACCCCTGTGTATTTTCTCAACTC-3′ (CYP2C9-A2-antisense), 5′-biotin TGCACGGATGTCACAAGAT-3′ (CYP2C9-A3-sense), and 5′-GATACTATGAATTGGGGACTTT-3′ (CYP2C9-A2-antisense). The PCR resulting in biotinylated amplicons was performed in a 50 μl reaction volume containing 1 x reaction buffer, 0.25 μl Platinum Taq DNA Polymerase (invitrogen), 0.2 mM dNTPs, 1.5 mM MgCl, 40 ng template DNA and a final concentration of 200 nM of each primer. The PCR amplification started with an initial denaturation at 95°C for 15 minutes, followed by 45 cycles of denaturation at 95°C for 15 seconds, annealing at 55°C for 45 seconds, and extension at 72°C for 45 seconds, followed by a final extension step at 72°C for 5 minutes. Subsequently the amplified fragments were purified. Briefly, 40 μl of biotinylated PCR products were incubated with 5 μl streptavidin-coated sepharose beads (GE Healthcare Bio-Sciences, Munich Germany) diluted in 35 μl of PyroMark™ Binding Buffer (Qiagen, Hilden Germany). After 5 minutes incubation biotinylated single stranded PCR products bound by the beads were isolated using the PyroMark™ Q96 Vacuum Prep Workstation (Qiagen). After treatment with 50% ethanol, denaturation in 0.2 M sodiumhydroxid-solution and washing with 10 mM TRIS-Acetat (pH 7.6) biotinylated single strands were released into designated wells containing PyroMark™ Annealing Buffer (36 μl) and 4 pmol of sequencing primers 5′-GGG AAGAGGAGCATTTGGGAAC-3′ (CYP2C9-A2 SEQ) (CYP2C9-A3 SEQ) and 5′-TGGTGGGAGAAGAAGTCC-3′, followed by a 2 minutes incubation at 80°C. Subsequently, genotyping was performed using the PSQ 96MA, the PyroMark™ Gold Q96 reagents, and the PSQ 96-Software (Qiagen). Genotyping of the frequently occurring polymorphisms of SLC01B1, SLC01B3, SLC02B1, ABCB2 and ABCG2 was performed using commercially available TaqMan® SNP Genotyping Assays for SLC01B1 c.521 T > C (rs4149056), SLC01B1 c.388A > G (rs2306283), SLC01B3 c.699A > G (rs7311358), SLC02B1 c.935G > A (rs12422149), SLC02B1 c.1457C > T (rs2306168), MRP2 (ABCC2), ABCC2 c.24C > T (rs717620) ABCB2 c.1294G > A (rs2273697), ABCB2 c.3972C > T (rs3740066) and ABCG2 c.421C > T (rs2231142) (Life Technologies GmbH, Darmstadt, Germany). After automated DNA extraction from the blood sample performed as described by the manufacturer using a QiaCube® (Qiagen, Hilden Germany) the DNA content was determined photometrically using a NanoDrop® (Peqlab, Erlangen Germany). After dilution to 10 ng/μl the DNA sample was stored at -20°C. Genotyping was performed using the pre-developed TaqMan® SNP Genotyping Assays (Applied Biosystems, Darmstadt, Germany). In detail, reactions were carried out in a 5 μl volume containing 1 μl genomic DNA, 0.25 μl Primer/Probe-Mix, 2.5 μl Genotyping Master Mix and 1.25 μl water (Applied Biosystems). Fluorescence was assessed for using the Fast Real-Time PCR system 7900 HT (Applied Biosystems) and the Sequence Detection Software SDS 2.3.

**Case report**

A 39 year old male patient of Caucasian ethnicity, was treated for dyslipidemia with 40 mg/d fluvastatin, starting in January 2006. The patient had only moderately elevated cholesterol serum levels (total cholesterol 270 mg/dl; LDL cholesterol 179.8 mg/dl) but had a positive family history of cardiovascular diseases. Until June 2008, fluvastatin was combined with the cholesterol absorption inhibitor...
ezetimibe (10 mg/d). Although this medication was well tolerated (lipid levels as well as liver enzymes and creatine kinase (CK) are displayed in Table 1), the combination with ezetimibe was discontinued due to gastrointestinal side effects reported by the patient. During combination of ezetimibe and fluvastatin, the patient had CK levels (185-234 U/L) in the upper normal range (CK norm levels 60-175 U/L). In September 2008, treatment with the AT1 blocker telmisartan (20 mg/day) was started due to borderline hypertension. After 4 weeks of treatment, the patient complained of mild myalgia, cramps und fasciculations in the legs. Subsequent testing for CK revealed an approximately two-fold increase of plasma levels (229 U/L to 439 U/L) compared to baseline. Myalgia and cramps were no longer reported by the patient. Anti-hypertensive therapy was changed to candesartan. Retrospective genotyping of the patient for frequently occurring single nucleotide polymorphisms (SNPs) in genes previously described to be involved in fluvastatin and telmisartan kinetics, was performed respectively (illustrated in Figure 1). As displayed in Table 2, the patient did not show genetic variability for the hepatic uptake transporter OATP1B1 (SLCO1B1 c.388G > A (p.N130D), c.521 T > C (p.V174A)), OATP1B3 (SLCO1B3 c.699G > A (p.M233I)), OATP2B1 (SLCO2B1 c.935G > A (p.R312Q), c.1457C > T (p.S486F)) and the efflux transporter ABCG2 (c.421C > A (p.Gln141Lys; rs2231142). However, genotyping of frequently occurring polymorphisms of the major metabolizing enzyme of fluvastatin namely CYP2C9 revealed that the patient is heterozygote carrier of the less frequent occurring SNP located in exon 7 (CYP2C9*3; c.1075A > C p.Ile359Leu, rs1057910), Figure 2. In addition, the patient was heterozygote for the -24C > T polymorphism located in the 5′ UTR of the hepatic efflux transporter ABC2.

Conclusions
In this report we describe the retrospective genetic analysis of a patient who experienced mild myalgia and CK increase after co-administration of fluvastatin with the AT1-receptor antagonist telmisartan. Findings suggest that that genetic variability of CYP2C9 in addition to the ABC2 (-24C > T) variant might be a risk factor for developing drug-drug interactions combining fluvastatin and telmisartan.

Like other exogenous compounds, statin therapy harbours the risk of rare but significant adverse reactions targeting organs such as muscle (myotoxicity) or liver (transaminase elevation). In particular, the risk of rhabdomyolysis which can lead to renal failure and death has to be addressed. The finding of a recent report showing that a functional polymorphism in the hepatic uptake transporter OATP1B1 (SLCO1B1) is the only predictor of simvastatin induced myotoxicity [9] has given rise to several studies focussing on effects of this particular polymorphism on statin disposition [10].

In general, fluvastatin undergoes extensive first pass metabolism resulting in a bioavailability of only 19-29%. In addition, the majority of fluvastatin is eliminated as metabolites into the bile, and it has been suggested that CYP2C9 accounts for 50-80% of fluvastatin metabolism, whereas CYP2C8 and CYP3A4 play only a minor role. In line with this finding it has been shown that impaired function alleles of CYP2C9 are associated with changes in fluvastatin disposition and efficacy in healthy volunteers [11]. In particular, CYP2C9 exhibits commonly occurring SNPs, namely the CYP2C9*2 (c.430 C > T; p.Arg144Cys; rs1799853) variant, which is frequent among Caucasians with approximately 1% of the population being homozygous carriers and 22% heterozygous, whereas the corresponding figures for the CYP2C9*3 (c.1075A > C p.Ile359Leu, rs1057910) allele in a Caucasian population are much lower with only 0.4% and 15%, respectively [6]. Especially the CYP2C9*3 allele, which is

| Table 1 Lab results and medication of the patient from January 2006 until November 2009 |
|-----------------------------------------------|
| **Date** | **01/17/06** | **02/26/07** | **05/21/07** | **11/22/07** | **03/07/08** | **06/27/08** | **10/17/08** | **10/22/08** | **11/18/09** |
| **Lab parameters:** | | | | | | | | | |
| LDL [mg/dl] | 179.8 | 74.2 | 71.4 | 74.4 | - | 87.6 | 117.8 | - | 137.6 |
| HDL [mg/dl] | 47.0 | 31.0 | 49.0 | 51.0 | - | 52.0 | 48.0 | - | 46.0 |
| CK [U/l] | n.d. | 215 | 191 | 223 | 234 | 229 | 439 | 145 | 212 |
| GGT [U/l] | n.d. | 29 | 27 | 25 | 22 | 20 | 25 | - | 36 |
| **Medication:** | | | | | | | | | |
| Fluvastatin | - | 40 mg/d | 40 mg/d | 40 mg/d | 40 mg/d | 40 mg/d | 40 mg/d | 40 mg/d | 40 mg/d |
| Ezetimibe | - | 10 mg/d | 10 mg/d | 10 mg/d | 10 mg/d | - | - | - | - |
| Telmisartan | - | - | - | - | - | - | 20 mg/d | - | - |
| Candesartan | - | - | - | - | - | - | - | 8 mg/d | 8 mg/d |

(CK norm level 60-175U/L).
associated with the nucleotide exchange (c.1075A > C) in exon 7 and results in an amino acid exchange in the catalytic site of the enzyme has been previously shown to result in impaired metabolism of substrate drugs [12]. However, the effect of genetic variants on catalytic activity and the clinical impact seems to be substrate specific.

Genotyping for the above described polymorphisms in CYP2C9 revealed that the patient was heterozygous for CYP2C9*3 allele, suggesting that the patient carries a predisposition for higher fluvastatin plasma levels, which could contribute to increased susceptibility for adverse side effects. However, the history of the patient shows that the CYP2C9*3 variant alone was not the only factor inducing myalgia and CK elevation, which began after co-administration of telmisartan. This is in accordance with the previously described lack of association of genetic variants of CYP2C9 variants with fluvastatin induced myotoxicity. However, it should be noted that only one patient was carrier of the CYP2C9*3 variant in this report [13].

Based on the current understanding of telmisartan pharmacokinetics, the drug-drug interaction described in our patient could involve additional mechanisms especially as telmisartan is only a moderate CYP2C9 inhibitor [7,8]. However, in individuals harbouring a low function allele of CYP2C9 in heterocycocity, the inhibitory capacity might be of clinical significance. Taken together, the importance of otherwise non-significant pharmacokinetic drug-drug interaction in the presence of pharmacokinetic variant alleles like CYP2C9*3 merits further discussion.

However, the majority of telmisartan is assumed to be eliminated hepatically in a glucuronidated form mediated associated with the nucleotide exchange (c.1075A > C) in exon 7 and results in an amino acid exchange in the catalytic site of the enzyme has been previously shown to result in impaired metabolism of substrate drugs [12]. However, the effect of genetic variants on catalytic activity and the clinical impact seems to be substrate specific.

Genotyping for the above described polymorphisms in CYP2C9 revealed that the patient was heterozygous for CYP2C9*3 allele, suggesting that the patient carries a predisposition for higher fluvastatin plasma levels, which could contribute to increased susceptibility for adverse side effects. However, the history of the patient shows that the CYP2C9*3 variant alone was not the only factor inducing myalgia and CK elevation, which began after co-administration of telmisartan. This is in accordance with the previously described lack of association of genetic variants of CYP2C9 variants with fluvastatin induced myotoxicity. However, it should be noted that only one patient was carrier of the CYP2C9*3 variant in this report [13].

Based on the current understanding of telmisartan pharmacokinetics, the drug-drug interaction described in our patient could involve additional mechanisms especially as telmisartan is only a moderate CYP2C9 inhibitor [7,8]. However, in individuals harbouring a low function allele of CYP2C9 in heterocycocity, the inhibitory capacity might be of clinical significance. Taken together, the importance of otherwise non-significant pharmacokinetic drug-drug interaction in the presence of pharmacokinetic variant alleles like CYP2C9*3 merits further discussion.

However, the majority of telmisartan is assumed to be eliminated hepatically in a glucuronidated form mediated
by the efflux transporters MRP2 (ABCC2), BCRP (ABCG2) and MDR1 (ABCB1) [8]. Testing the influence of frequently occurring SNPs in ABCB1 and ABCC2 revealed an impact of the ABCC2 -24C > T variant on telmisartan disposition [9,10]. Considering the inhibitory capacity of telmisartan on CYP2C9 mediated catalysis, which had been identified in vitro by Kamiyama and co-workers (IC$_{50}$ 41.9 ± 15.1 μM), the identified heterozygocity of the patient for the MRP2 -24C > T allele gives reason for the speculation of this polymorphism being involved in the susceptibility of the herein described drug-drug interaction. However, Kamiyama and co-workers who conducted the in vitro study, suggested that due to the high Ki in association with relatively low free plasma levels of telmisartan would be unlikely to exert clinically relevant inhibition of CYP2C9 [8]. In accordance with those findings is results from an in vivo study showing that telmisartan did not influence the plasma levels and efficacy (INR) of warfarin another CYP2C9 substrate [14].

However, we assume that the prevalence of both predisposing factors reduced hepatic elimination of telmisartan in association with reduced hepatic metabolism of fluvastatin, resulted in the herein described drug-drug interaction. This report further strengthens the hypothesis that the success of “personalized medicine” in terms of genotype driven risk stratifications are not sufficiently supported by monogenetic pharmacological analyses, which are commonly conducted [15]. This case illustrates that a combination of both – pharmacogenomics and the knowledge of drug-drug interactions – could help to achieve better clinical outcomes by personalized medicine.

Consent
Written informed consent was obtained from the patient for publication of this Case Report and any accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

Abbreviations
AT1: Angiotensin II receptor type 1; BCRP: Breast cancer resistance protein; CK: Creatine kinase; CYP: Cytochrome P450; DNA: Deoxyribonucleic acid; HMG-CoA: 3-hydroxy-3-methylglutaryl-coenzyme A; INR: International normalized ratio; MRP: Multi-resistance protein; DATP: Organic anion-transporting polypeptide; PCR: Polymerase chain reaction; SNP: Single-nucleotide polymorphism.

![Figure 2 Result of cytochrome P450 CYP2C9 genotyping of the patient in comparison to a control sample.](image-url)
Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
HMS carried out the molecular genetic studies and drafted the manuscript. JDR contributed the case presentation. WS and HKK authors also helped to draft the manuscript. All authors read and approved the final manuscript.

Acknowledgments
This work was supported by the GANI_MED-project (Greifswald Approach to Individualized Medicine), funded by the Federal Ministry of Science and Technology, Berlin, Germany and a project of the BfArM.

Author details
1Biopharmacy, Department of Pharmaceutical Sciences, University of Basel, Basel, Switzerland. 2Department of Pharmacology, Ernst Moritz Arndt University of Greifswald, Greifswald, Germany. 3Medical Faculty, University of Göttingen, Göttingen, Germany. 4Institute for Neurorehabilitational Research (InFo), BDH-Clinic, Hessisch Oldendorf, Germany, Teaching Hospital of Hannover Medical School (MHH), Germany.

Received: 10 May 2014 Accepted: 26 September 2014
Published: 3 October 2014

References
1. Sadoni S, Kaczmarek I, Delgado O, Schmockel M, Reichart B, Meiser B: Fluvatran as co-medication in heart transplant recipients with elevated creatine-kinase. Transplant Proc 2007, 39:556–559.
2. Keskitalo JE, Pasanen MK, Neuvonen PJ, Niemi M: Different effects of the ABCG2 c.421C>A SNP on the pharmacokinetics of fluvatran, pravastatin and simvastatin. Pharmacogenomics 2009, 10:1617–1624.
3. Mirosevic Skvrce N, Bozina N, Zibar L, Barisic I, Pejnovic L, Macolic Sarinic V: Different effects of the angiotensin receptor blockers. Between sex, polymorphisms in CYP2C8 and CYP2C9, and pharmacokinetics of angiotensin receptor blockers. Drug Metab Dispos 2006, 34:109–1115.
4. Ishiguro N, Maeda K, Kishimoto W, Saito A, Harada A, Ebner T, Roth W, Igarashi T, Sugiyama Y: Predominant contribution of OATP1B3 to the hepatic uptake of telmisartan, an angiotensin II receptor antagonist, in humans. Drug Metab Dispos 2008, 36:1107–1115.
5. Ishiguro N, Maeda K, Saito A, Kishimoto W, Mitsushita S, Ebner T, Roth W, Igarashi T, Sugiyama Y: Establishment of a set of double transfectants coexpressing organic anion transporting polypeptide 1B3 and hepatic efflux transporters for the characterization of the hepatobiliary transport of telmisartan acylglucuronide. Drug Metab Dispos 2008, 36:796–805.
6. Weiss J, Sauer A, Divac N, Herzog M, Schwedhelm E, Boger RH, Haefliger WE, Benndorf RA: Interaction of angiotensin receptor type 1 blockers with ATP-binding cassette transporters. Biopharm Drug Dispos 2010, 31:150–161.
7. Cabaleiro T, Roman M, Ochoa D, Talegon M, Prieto-Perez R, Wojnicz A, Lopez-Rodriguez R, Novalllos J, Abad-Santos F: Evaluation of the relationship between sex, polymorphisms in CYP2C9 and CYP2C9, and pharmacokinetics of angiotensin receptor blockers. Drug Metab Dispos 2013, 41:224–229.
8. Kamiyama E, Yoshigae Y, Kasuya A, Takei M, Kunihara A, Ikeda T: Inhibitory effects of angiotensin receptor blockers on CYP2C9 activity in human liver microsomes. Drug Metab Pharmacokinet 2007, 22:267–275.
9. Link E, Parish S, Armitage J, Bowman L, Heath S, Matsuda F, Gut I, Lathrop M, Collins R: SLCO1B1 variants and statin-induced myopathy—a genomewide study. N Engl J Med 2008, 359:789–799.
10. Romaine SP, Bailey KA, Hall AS, Balmforth AJ: The influence of SLCO1B1 (OATP1B1) gene polymorphisms on response to statin therapy. Pharmacogenomics J 2010, 10:3–11.
11. Kircheiner J, Kudlitz D, Meisel C, Bauer S, Meineke I, Roots I, Brockmoller J: Influence of CYP2C9 polymorphisms on the pharmacokinetics and cholesterol-lowering activity of (-)-3S,5R-fluvastatin and (+)-3R,5S-fluvastatin in healthy volunteers. Clin Pharmacol Ther 2003, 74:186–194.
12. Zhou SF, Zhou ZW, Huang M: Polymorphisms of human cytochrome P450 2C9 and the functional relevance. Toxicology 2010, 278:165–188.
13. Zuccaro P, Mombelli G, Calabresi L, Baldassarre D, Palmi I, Sirtori CR: Tolerability of statins is not linked to CYP450 polymorphisms, but reduced CYP2D6 metabolism improves cholesteraemic response to simvastatin and fluvastatin. Pharmacol Res 2007, 55:310–317.
14. Stangier J, Su CA, Hendriks MG, van Lier JJ, Sollie FA, Oosterhuis B, Jonkman JH: Steady-state pharmacodynamic and pharmacokinetics of warfarin in the presence and absence of telmisartan in healthy male volunteers. J Clin Pharmacol 2000, 40:1331–1337.
15. Kroemer HK, Meyer zu Schwabedissen HE: A piece in the puzzle of personalized medicine. Clin Pharmacol Ther 2010, 87:19–20.

doi:10.1186/1756-0500-7-688
cite this article as: Meyer zu Schwabedissen et al.: Creatine kinase elevation caused by a combination of fluvastatin and telmisartan in a patient heterozygous for the CYP2C9*3 and ABCC2 -24C > T variants: a case report. BMC Research Notes 2014 7:688.