

Received: 2012.10.04  
Accepted: 2012.02.16  
Published: 2012.08.01

## Lipid-lowering effect of fluvastatin in relation to cytochrome P450 2C9 variant alleles frequently distributed in the Czech population

### Authors' Contribution:

- A** Study Design
- B** Data Collection
- C** Statistical Analysis
- D** Data Interpretation
- E** Manuscript Preparation
- F** Literature Search
- G** Funds Collection

Helena Buzková<sup>1A,BCDEFG</sup>, Kristina Pechandová<sup>1BF</sup>, Vilém Danzig<sup>2B</sup>, Tomáš Vařeka<sup>3B</sup>, František Perlík<sup>1ADEG</sup>, Aleš Žák<sup>3D</sup>, Ondřej Slanar<sup>1ACDEFG</sup>

<sup>1</sup> Department of Pharmacology, 1<sup>st</sup> Faculty of Medicine, Charles University and General Teaching Hospital, Prague, Czech Republic

<sup>2</sup> 2<sup>nd</sup> Department of Medicine, 1<sup>st</sup> Faculty of Medicine, Charles University and General Teaching Hospital, Prague, Czech Republic

<sup>3</sup> 4<sup>th</sup> Department of Medicine, 1<sup>st</sup> Faculty of Medicine, Charles University and General Teaching Hospital, Prague, Czech Republic

**Source of support:** Supported by a research program of the Charles University (PRVOUK)

### Background:

*CYP2C9\*3* allele has been reported to correlate with increased plasma concentration of fluvastatin active form in healthy volunteers. We analyzed the correlation between the *CYP2C9* genotype and cholesterol-lowering effect of fluvastatin in human hypercholesterolemic patients.

### Material/Methods:

The study was prospective, without any interventions to standard procedures of hypolipidemic treatment. *CYP2C9* genotype was determined by PCR-RFLP assay in 87 patients on concomitant fluvastatin therapy, in 48 patients on monotherapy, and in a control group of 254 healthy volunteers of Czech nationality. Biochemical and clinical data were collected before the initiation of fluvastatin treatment and 12 weeks later.

### Results:

The frequency of *CYP2C9* alleles did not differ significantly among groups of patients and volunteers. The most frequently observed allele was *CYP2C9\*2*.

Treatment with 80 mg of fluvastatin daily of 48 patients on monotherapy for 12 weeks resulted in mean low-density lipoprotein cholesterol (LDL-C) reduction by 25%, mean serum total cholesterol (TC) reduction by 21%, and mean triglyceride (TG) reduction by 28%. The *CYP2C9\*1/\*3* genotype was associated with a decrease in LDL-C levels (by 40.0% for *CYP2C9\*1/\*3*, but only by 22.4% for *CYP2C9\*1/\*1*), and with the reduction of TC (by 28.6% in *CYP2C9\*1/\*3* versus 20.2% in *CYP2C9\*1/\*1*).

### Conclusions:

In hypercholesterolemic patients, LDL-C serum concentration was decreased more significantly in fluvastatin-treated subjects bearing the *CYP2C9\*1/\*3* genotype compared to *CYP2C9\*1/\*1* genotype. However, due to rare occurrence of some *CYP* genotypes, it was impossible to report a definitive positive genotype-fluvastatin effect association.

### key words:

fluvastatin • *CYP2C9* • genetic polymorphism • SNP • hypercholesterolemia

### Full-text PDF:

<http://www.medscimonit.com/fulltxt.php?ICID=883272>

### Word count:

2951

### Tables:

3

### Figures:

—

### References:

26

### Author's address:

Ondrej Slanar, Department of Pharmacology, 1<sup>st</sup> Faculty of Medicine, Charles University, Albertov 4, 128 00 Prague 2, Czech Republic, e-mail: oslan@lf1.cuni.cz

## BACKGROUND

Hypercholesterolemia plays a crucial role in the development of atherosclerotic disease, which is one of the leading causes of mortality in the Western world. Therapy with 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors – statins – substantially reduces cardiovascular morbidity and mortality in diverse populations [1]. However, there is a wide interindividual variation in response to statin therapy. The underlying causes of this phenomenon have been extensively debated, but remain uncertain. The observed variation in biological response to statins could be due to variation in patient compliance, pharmacokinetics or pharmacodynamics and drug-drug interactions, as well as interindividual genetic differences in cholesterol biosynthesis, target lipoprotein (mainly LDL) receptor uptake or metabolism of particular statins. Any predictions of biological response of individuals to statins would thus be very valuable for more efficacious, personalized treatments.

The first totally synthetic 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor on the market, fluvastatin [2], is not the most frequently prescribed statin nowadays, but thanks to its well-characterized metabolism, it is particularly suitable for investigations into the influence of genetic variability on interindividual variation in therapeutic effect. Statins differ in their main metabolic fates in the human body – while fluvastatin is metabolized primarily via hepatic transformation by the 2C9 isoform of the genetically polymorphic cytochrome P450 enzyme (CYP2C9) [3]; simvastatin, lovastatin and atorvastatin are metabolized by CYP3A4; and pravastatin, rosuvastatin and pitavastatin do not seem to be significantly modified by any of the CYP isoforms. Thanks to its specific metabolic route through CYP2C9, fluvastatin is only mildly susceptible to adverse drug-drug interaction effects and it is less prone to pharmacokinetic interactions compared to other HMG-CoA reductase inhibitors [4]. Importantly, chemical inhibition of the enzymatic activity of the particular CYP isoforms has been shown to elevate plasma concentrations of the active forms of the respective statins [4]. Approximately 60% of fluvastatin orally administered in its active form is metabolized via cytochrome P450 into the inactive form. An *in vivo* study calculated that the relative contribution of the CYP2C9 isoform is more than 80% [5]. The 2 main metabolites – 6-hydroxy and N-desisopropyl fluvastatin – are exclusively generated by CYP2C9, while the third known metabolite – 5-hydroxy fluvastatin – is formed by multiple pathways involving CYP2C9, CYP3A4, CYP2C8 and CYP2D6. The 2 most common single-nucleotide polymorphic (SNP) allelic variants of CYP2C9 that occur in Caucasian population are CYP2C9\*2 and CYP2C9\*3. The CYP2C9\*2 allele represents an SNP in exon 3 that leads to Arg144Cys mutation and occurs in 8%-14% of Caucasians, while the slightly less common CYP2C9\*3 allele with an SNP in exon 7 causes Ile359Leu mutation and is found in 4–11% of Caucasians [6–8]. Importantly, both allelic variants have lower enzyme activity than the wild type CYP, and both would thus be predicted to elevate or modify plasma concentrations of unmetabolized, pharmacologically active forms of CYP substrates.

Consistent with this idea, the plasma levels of 3R, 5S-fluvastatin (the active form) were found to be up to 3-fold higher in healthy volunteers of the \*3/\*3 than in

those of the \*1/\*1 genotype. This correlation between the presence of CYP2C9\*3 and fluvastatin pharmacokinetics was not reflected in the cholesterol-lowering effect of the drug [9], but that could have been due to the fact that the study was carried out on healthy volunteers, and might not have been representative of a typical treatment regime. The subjects took fluvastatin daily only for 2 weeks, and their baseline lipid levels and the daily administered dose of fluvastatin (40 mg of racemic form) [9] were lower than in patients normally requiring fluvastatin treatment. We thus hypothesized that the prospective association between CYP2C9 genotype and the hypolipidemic efficacy of fluvastatin could be more pronounced in a group of real hypercholesterolemic patients with pathologically elevated baseline lipids levels. This could be clinically relevant and have a direct impact on the preferred efficacious treatment regime in genetically defined subgroups of hypercholesterolemic patients on fluvastatin.

## MATERIAL AND METHODS

### Subjects and data collection

All subjects were of Czech nationality and gave their written informed consent prior to participating in the trial. The study protocol was approved by the Ethics Committee of the General University Hospital in Prague. Fluvastatin-treated patients were recruited by the internal medicine ward of the General University Hospital in Prague under exclusion and inclusion criteria. The exclusion criteria were: history of diabetes mellitus, any liver disease, any other disease causing modification of metabolic functions, previous treatment with fluvastatin, concomitant therapy with strong CYP2C9 inducers or inhibitors, history of stomach or gut surgery influencing drug absorption, any known or suspected cancers, immunosuppressive treatment, pregnancy or ongoing breastfeeding, and alcoholism. The inclusion criterion for enrolment in the trial was the initiation of treatment of hypercholesterolemia by fluvastatin in 80 mg daily per oral dose in compliance with the standard therapeutic approach in the hospital. All patients were treated by Lescol XL fluvastatin (Novartis Pharmaceuticals). Concomitant use of other medications was documented and possible drug-drug interactions were recorded. No interventions to standard therapeutic procedures have been done during the study, except for 1 extra blood sampling for DNA isolation. The untreated control group of unrelated healthy Czech Caucasian volunteers was recruited as a control population for CYP2C9 genotype distribution. Biochemical data, including plasma creatine kinase, ALT, AST, GMT, and ALP, electrolyte balance and plasma lipid levels before and 12 weeks after fluvastatin treatment, were determined by standard methods in the Institute for Clinical Biochemistry and Laboratory Diagnostics of the General University Hospital.

### CYP2C9 genotyping

Genomic DNA was isolated from peripheral leukocytes by QIAmp Blood mini Kit (Qiagen). Purified DNA was stored at 4°C until polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP) analysis was carried out using previously described methods and primer sets [10]. Taq DNA polymerase, other PCR reagents and restriction enzymes were from Fermentas (Lithuania). The PCR

**Table 1.** Demographic and allelic frequency data for the subject groups involved in the study. All patients were fluvastatin naïve, and fluvastatin monotherapy patients group did not undergo any concomitant therapy.

	Fluvastatin all	Fluvastatin monotherapy	Healthy volunteers
Total number	87	48	254
Male	62	36	94
Female	25	12	160
Age span (years)	25–89	25–84	22–28
Average age (years) (mean ± SD)	59.8±11.8	58.3±11	24.6±1.4
Allelic frequency <i>CYP2C9</i> *2	9.8	11.4	12.2
Allelic frequency <i>CYP2C9</i> *3	5.7	6.2	5.9
Average number of concomitant drugs	4.1	3.0	–

products of the 2 used amplicons were separated by electrophoresis on 3% agarose gel and identified by ethidium bromide staining. An allele was assigned as *CYP2C9*\*2 when the 372 base pair (bp) PCR product of the first amplicon contained a Cfr 13I restriction site that yielded fragments of 253 and 119 bp upon cleavage by Cfr 13I. Similarly, an allele was assigned as *CYP2C9*\*3 when the 137 bp product of the second amplicon was digested into fragments of 104 and 33 bp by Sty I. Whenever the cleavage of an amplicon produced the expected cleavage fragments, but also left the original PCR product intact, the subject was assigned as heterozygous in the respective allele. The subjects without any of these 2 variants were assumed to be homozygous wild-type carriers of *CYP2C9*\*1.

### Statistical analysis

The evaluation of fluvastatin's hypolipidemic effect and its genotype-dependency was done by Student's t test. Throughout the study, arithmetic mean and standard deviation were used as central tendency and dispersion measures, respectively. Statistical significance was considered at  $P < 0.05$  or  $P < 0.001$ , as indicated. The expected genotype frequencies were calculated from the observed allelic frequencies using Hardy-Weinberg equilibrium ( $p^2 + 2pq + q^2 = 1$ ). Prevalence was compared by the chi-square test, and 95% confidence interval (95% CI) of genotype frequencies was calculated. Data were processed using Microsoft Excel 8.0 (Microsoft, USA) and Statgraphics Plus 3.1 (StatPoint, Inc., USA).

## RESULTS

### Demographic characterization of the participants

The effect-observing pharmacodynamic part of our study involved 87 hypercholesterolemic participants treated for the first time in their treatment regime with fluvastatin (fluvastatin all patients, median age 59 years). From within this group, 48 subjects on fluvastatin monotherapy (75% males, median age 57 years) were selected by excluding patients treated with *CYP2C9* inducers or inhibitors, patients taking any other hypolipidemic medications in the 8 weeks prior to our study, and patients that were on concomitant therapy with any potentially lipid-lowering agent, including over-the-counter medicines. All hypercholesterolemic subjects

and control group of 254 healthy volunteers were genotyped for *CYP2C9* alleles as described in Methods. The demography and allelic frequency data of the analyzed groups of subjects are summarized in Table 1.

### Frequency of *CYP2C9* alleles was similar among fluvastatin-treated patients and a control group of healthy volunteers

As summarized in Table 1, the *CYP2C9*\*2 allele occurred in 9.8% of fluvastatin-treated patients and 12.2% of healthy volunteers, while the less common *CYP2C9*\*3 variant was found in 5.9% of healthy volunteers and 5.7% of fluvastatin-treated patients. Approximately 9.2%, and 2.3% of the fluvastatin-treated subjects and 9.8%, and 0.4% of healthy volunteers were \*1/\*3 and \*2/\*3 heterozygotes, respectively. While the \*3/\*3 and \*2/\*2 genotypes were identified in 0.8% and 2.0% of healthy volunteers, no fluvastatin-treated subjects were homozygous for these alleles, likely reflecting their smaller group size (Table 2). Overall, demographic characteristics of the patients in different genotype groups were comparable, genotype frequency distribution did not show a significant deviation from the Hardy-Weinberg equilibrium, and genotype frequencies were comparable to those published for other Caucasian populations (see Discussion). The observed allelic frequencies and genotype distribution did not differ among healthy volunteers and patients on concomitant treatment or fluvastatin monotherapy ( $P = 0.001$ ), indicating that *CYP2C9* polymorphism is not a predicting factor for hypercholesterolemia, assuming that genotype expression is age-independent. In our further analysis of genotype dependency of fluvastatin treatment we focused on the group of patients on fluvastatin monotherapy to exclude any possible effects of drug-drug interactions.

### Fluvastatin treatment caused no significant adverse effects and had a positive hypolipidemic effect

All related adverse effects were recorded during the course of the study. To facilitate detection of any previously undiagnosed diseases potentially complicating interpretation of the data, patients' clinical biochemical indicators for liver function (ALT, AST, GMT, ALP), electrolyte balance and general metabolism were examined before, during the study, and after at least 12 weeks of treatment with 80 mg dose of

**Table 2.** Prevalence of the *CYP2C9* genotypes among fluvastatin patients, patients on fluvastatin monotherapy and healthy volunteers in comparison to the published frequency data for Caucasians [8].

<i>CYP2C9</i> genotype	*1/*1	*1/*2	*1/*3	*2/*2	*2/*3	*3/*3
<b>Cytochrome P450 enzyme activity</b>	<b>Normal</b>	<b>Minor reduction</b>	<b>Moderate reduction</b>	<b>Moderate reduction</b>	<b>Moderate reduction</b>	<b>Very low</b>
Healthy subjects (n=254)	No. 170	51	25	5	1	2
	% 66.9	20.1	9.8	2.0	0.4	0.8
	95% CI 61.14–72.71	15.15–25.00	6.18–13.51	0.26–3.67	0.00–1.16	0.00–1.87
All patients on fluvastatin (n=87)	No. 62	15	8	0	2	0
	% 71.3	17.2	9.2	0	2.3	0
	95% CI 61.75–80.77	9.30–25.18	3.12–15.27	0.00	0.00–5.45	0.00
Patients on fluvastatin monotherapy (n=48)	No. 33	9	4	0	2	0
	% 68.7	18.8	8.3	0	4.1	0
	95% CI 55.64–81.86	7.71–29.79	0.51–16.15	0.00	0.00–9.82	0.00
Allele frequency in Caucasians <sup>#</sup>	% 65.3	20.4	11.6	0.9	1.4	0.4

fluvastatin; they did not reveal any idiosyncrasy or abnormal elevation of any markers or biochemical parameters. Fluvastatin was well tolerated by all participants – patients did not complain spontaneously, and fluvastatin treatment had no effect on the activities of creatine kinase at the administered dose of 80 mg daily. To assess the efficacy of hypolipidemic therapy, the threshold limits were defined as follows: low-density lipoprotein cholesterol (LDL-C) levels between 2.2–3.4 mmol/l, serum total cholesterol (TC) levels between 3.83–5.2 mmol/l, and triglyceride (TG) levels between 0.68–1.69 mmol/l. After 12 weeks of fluvastatin monotherapy of hypercholesterolemic patients, their plasma lipid levels became significantly reduced ( $P < 0.001$ , Table 3), demonstrating the efficacy of the treatment. Triglyceride levels fell on average by 28.1% (ranging between 5.0–72.9%), TC by 21.5% (6.2–49.4%) and LDL-C by 25.0% (2.6–82.4%). Eighty-nine percent of patients reached more than 10% reduction in TG levels, 84% of patients reached more than 10% reduction in TC, and 92% of patients reached more than 10% reduction in LDL-C.

#### *CYP2C9* genotype and the hypolipidemic effect of fluvastatin treatment

We next investigated the correlation between the lipid-regulating effect of fluvastatin monotherapy and *CYP2C9* genotype. Subjects carrying the \*1/\*3 genotype achieved a greater reduction in plasma levels of LDL-C than subjects with \*1/\*1 or \*1/\*2 genotypes (39.95% vs. 22.35% or 29.92%, respectively) with statistical significance of  $P < 0.05$  (Table 3). In addition, subjects bearing the *CYP2C9*\*1/\*3 genotype had

slightly greater reductions in TC than \*1/\*2 or \*1/\*1 carriers (28.56% vs. 20.16% or 25.00%, respectively). In contrast, the reduction in plasma levels of TG did not show any correlation with *CYP2C9* genotype, fluctuating around 28% in all genotype subgroups (see Table 3). No genotype-related dependency was observed for high-density lipoprotein levels (data not shown).

The upper threshold for clinically normal levels of LDL-C (3.4 mmol/l) was reached by 87.5% of all fluvastatin monotherapy patients, and among these the allelic frequencies of *CYP2C9*\*3 was 6.0% (5 heterozygous subjects) and the allelic frequencies of *CYP2C9*\*2 was 10.7% (9 heterozygous subjects). The threshold for TC level (5.2 mmol/l) was reached by 60.4% of patients which the *CYP2C9*\*3 was present in 8.6% cases (5 heterozygous subjects), and *CYP2C9*\*2 was present in 15.5% cases (9 heterozygous subjects). Plasma concentration of TG reached the threshold point (1.69 mmol/l) in 58.3% patients, of whom 7.1% carried the *CYP2C9*\*3 allele (4 heterozygous subjects) and 10.7% were carriers of *CYP2C9*\*2 (6 heterozygous subjects). The number of patients that met the clinical threshold concentrations after the 12 weeks of treatment is detailed in Table 3. Despite the clear effects of *CYP2C9*\*3 presence on the treatment-induced reduction in LDL-C and TC levels, there was no statistically significant correlation between the *CYP2C9* genotype and the overall final clinical outcome of fluvastatin treatment, as judged by the treatment adjustment of plasma lipid levels to threshold ranges mentioned above. The differences in the distribution of *CYP2C9* genotypes between the groups of patients reaching and not reaching normal

**Table 3.** Plasma levels of low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC), and triglycerides (TG) before (BL -baseline) and 12 weeks of follow-up after (FU) fluvastatin treatment, in relation to *CYP2C9* genotype in fluvastatin naïve and monotherapy patient group (48 subjects at all). All lipid level values are in mmol/l and are represented as mean  $\pm$  SD. P-value of significance in reduction from baseline to final follow up was \* P<0.05, or \*\* P<0.001. The% reduction in levels (hypolipidemic effect) was calculated as follows:

$$\% = 100 - \left( \frac{\sum \frac{FU}{n}}{\sum \frac{BL}{n}} * 100 \right) ; \text{ where } n = \text{number of patients in a subgroup.}$$

<i>CYP2C9</i> genotype		*1/*1	*1/*2	*1/*3	*2/*3	All
Number of subjects		(n=33)	(n=9)	(n=4)	(n=2)	(n=48)
LDL-C	BL	3.83 $\pm$ 0.98	4.09 $\pm$ 0.89	3.17 $\pm$ 0.55	4.51 $\pm$ 0.06	3.86 $\pm$ 0.95
	FU	2.98 $\pm$ 0.73	2.87 $\pm$ 0.63	1.91 $\pm$ 1.10	3.60 $\pm$ 0.90	2.89 $\pm$ 0.82
	%	22.35**	29.92**	39.95*	20.29	24.96**
Number of subject below/above threshold 3.4 mmol/l		29/4	8/1	4/0	1/1	42/6
TC	BL	6.60 $\pm$ 1.23	6.53 $\pm$ 1.04	6.13 $\pm$ 1.51	6.89 $\pm$ 0.33	6.56 $\pm$ 1.21
	FU	5.27 $\pm$ 0.95	4.89 $\pm$ 0.81	4.38 $\pm$ 0.86	5.87 $\pm$ 0.64	5.15 $\pm$ 0.96
	%	20.16**	25.00**	28.56	14.80	21.48**
Number of subjects below/above threshold 5.2 mmol/l		16/17	8/1	4/0	1/1	29/19
TG	BL	2.43 $\pm$ 1.97	2.08 $\pm$ 0.53	2.23 $\pm$ 0.89	2.16 $\pm$ 0.41	2.34 $\pm$ 1.68
	FU	1.73 $\pm$ 1.48	1.48 $\pm$ 0.47	1.60 $\pm$ 0.82	1.77 $\pm$ 0.53	1.68 $\pm$ 1.28
	%	28.79**	28.53*	28.25	18.10	28.12**
Number of subjects below/above threshold 1.69 mmol/l		19/14	5/4	3/1	1/1	28/20

plasma lipid levels were not statistically significant. In conclusion, *CYP2C9* polymorphism indeed seems to have an impact on the lipid-lowering efficacy of fluvastatin in hypercholesterolemic patients, but this effect does not directly translate into clinically significant differences in individuals heterozygous for the \*3 allele.

## DISCUSSION

Our study suggests that the *CYP2C9*\*3 allele does partly influence the response to fluvastatin treatment in hypercholesterolemic patients. We found that heterozygous *CYP2C9*\*1/\*3 carriers had a greater reduction in plasma LDL-C levels than wild-type subjects. They also showed a greater reduction in TC, but this effect lacks statistical significance due to low allelic frequencies of *CYP2C9*\*3. Both of these effects were specific to the *CYP2C9*\*1/\*3 carriers and were not found in *CYP2C9*\*3/\*2 patients (2 subjects). This finding may just reflect the small sample size, or patient compliance, or an interaction between \*2 and \*3. The *CYP2C9*\*3 allele-specific effects would be more pronounced in homozygous *CYP2C9*\*3/\*3 subjects, but those were not found in our fluvastatin monotherapy patients due to their low frequency and limited sample size in our study.

*CYP2C9* polymorphisms occur at high frequency in most of the ethnic populations and the *CYP2C9*\*3 allele is more frequent in Caucasian populations than in Asians (11.6% vs. 3.5%) and African Americans (4.3%). Among European

nations, the frequency of *CYP2C9*\*3 allele varies from 6% to 10%: 10% in the Spanish [11], 9.5% in Croats [12], 9% in Italians [13], 8.5% in the British [14], 8.0% in French [15], 6.7% in Russians [16], and 6.6% in Swedes [17]. Allelic frequency of *CYP2C9*\*3 in the Czech population (5.9%) falls at the lower margin of this range and does not differ between the fluvastatin-treated and the control groups [18]. The latter suggested that *CYP2C9*\*3 itself is not a susceptibility factor for hypercholesterolemic disease.

The lipid-lowering effect of fluvastatin among all 87 patients (irrespective of *CYP2C9* genotype) in our study was compared to the data presented by Novartis Pharma in Leskol XL prescribing information for physicians. Lipid-lowering data for Leskol XL (80 mg per tablet) from three 24-week controlled trials are as follows: LDL-C reduction by 35%, TC reduction by 25% and TG reduction by 19%, which corresponds reasonably well to the data obtained in our study: 25%, 21% and 28%, respectively.

We did not detect any genotype-related increase in observed adverse events nor any abnormalities in creatine kinase activities, indicative of negligible adverse-effects of fluvastatin at the doses administered (80 mg daily). Fluvastatin is generally known for its high safety and low potential for interactions, and thanks to these qualities it is frequently and preferably administered to patients with medical history of transplantations [19]. Because of the non-intervention design of our study, patients did not undergo any pharmacokinetics

testing, such as measurement of plasma concentrations of fluvastatin metabolites, to validate previous findings that the mean plasma levels of the active enantiomer of fluvastatin (single dose of 40mg) were 3-fold higher in the *CYP2C9*\*3 carriers than in non-carriers [9].

The metabolism of fluvastatin is well documented by *in vitro* studies. The racemic mixture of the active 3R, 5S-fluvastatin and its inactive 3S, 5R enantiomer is primarily metabolized to 5-hydroxy-, 6-hydroxy- and N-desisopropyl-fluvastatin by CYP2C9. This route accounts for about 50% to 80% of the total clearance, although alternative metabolic enzymes such as CYP3A4, CYP2C8 and CYP2D6 are also involved in formation of 5-hydroxy fluvastatin. However, the *CYP3A4* may be a genetic determinant of interindividual differences in response to certain statins (simvastatin, lovastatin, and atorvastatin), and hypolipidemic efficacy of fluvastatin is not dependent on *CYP3A4* [20]. The possible effect of SNPs of these CYP enzymes is likely to be concealed by the variation derived from compliance, diet, living condition of the patients, concomitant therapy and disease and other environmental variables, and the central position of CYP2C9 enzyme and its polymorphism in fluvastatin metabolism remains relevant.

Genetic variations in other genes can provide theoretical explanations for the interindividual variability of fluvastatin treatment – from the genetic variation in the cholesterol/lipid pathways and spatial arrangement of the receptors to the transporters and other metabolizing enzymes. Fluvastatin transport in the human body is more complex than that of other statins because of its relatively high lipophilicity, which allows, at least partly, passive diffusion via the hepatocyte plasma membrane and increased absorption in the gut by transcellular passive diffusion [21]. In addition, numerous studies claimed that fluvastatin is not transported by the P-glycoprotein drug transporter [21–23], unlike some other statins, but it rather seems to be a substrate for organic anion transporters OATP1B1, OATP1B3, and OATP2B1, which are rich in SNPs [24,25]. Some of these, especially OATP2B1, have been reported to play an important role in statin uptake into hepatocytes and were implicated in modulating the pharmacological action and efficacy of fluvastatin [26]. Furthermore, it was reported that genetic polymorphism in cholesteryl-ester transfer protein could also be associated with variable lipid response to fluvastatin [22], and there are SNPs in other genes not explicitly associated with fluvastatin transport, metabolism or receptors that impact the efficacy of the treatment.

## CONCLUSIONS

In conclusion, although the limited size of patient cohorts and the consequent absence of rare genotypes including *CYP2C9*\*3/\*3 homozygous subjects make our results preliminary in nature, the study nevertheless suggests that the *CYP2C9*\*3 allele might correlate with a better LDL-C lowering efficacy of fluvastatin.

## REFERENCES:

- Zuber RE: Anzenbacherova, and P. Anzenbacher, Cytochromes P450 and experimental models of drug metabolism. *J Cell Mol Med*, 2002; 6(2): 189–98
- Asberg A, Holdaas H: Fluvastatin and fluvastatin extended release: a clinical and safety profile. *Expert Rev Cardiovasc Ther*, 2004; 2(5): 641–52
- Scripture CD, Pieper JA: Clinical pharmacokinetics of fluvastatin. *Clin Pharmacokinet*, 2001; 40(4): 263–81
- Neuvonen PJ: Drug interactions with HMG-CoA reductase inhibitors (statins): the importance of CYP enzymes, transporters and pharmacogenetics. *Curr Opin Investig Drugs*, 2010; 11(3): 323–32
- Toda T et al: Roles of different CYP enzymes in the formation of specific fluvastatin metabolites by human liver microsomes. *Basic Clin Pharmacol Toxicol*, 2009; 105(5): 327–32
- Scordo MG et al: Genetic polymorphism of cytochrome P450 2C9 in a Caucasian and a black African population. *Br J Clin Pharmacol*, 2001; 52(4): 447–50
- Schwarz UI: Clinical relevance of genetic polymorphisms in the human CYP2C9 gene. *Eur J Clin Invest*, 2003; 33(Suppl.2): 23–30
- Goldstein JA: Clinical relevance of genetic polymorphisms in the human CYP2C subfamily. *Br J Clin Pharmacol*, 2001; 52(4): 349–55
- Kirchheiner J et al: 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(2): 186–94
- Aynacioglu AS et al: Frequency of cytochrome P450 CYP2C9 variants in a Turkish population and functional relevance for phenytoin. *Br J Clin Pharmacol*, 1999; 48(3): 409–15
- Dorado P et al: CYP2C9 genotypes and diclofenac metabolism in Spanish healthy volunteers. *Eur J Clin Pharmacol*, 2003; 59(3): 221–25
- Bozina N et al: Genetic polymorphisms of cytochromes P450: CYP2C9, CYP2C19, and CYP2D6 in Croatian population. *Croat Med J*, 2003; 44(4): 425–28
- Scordo MG et al: Allele and genotype frequencies of CYP2C9, CYP2C19 and CYP2D6 in an Italian population. *Pharmacol Res*, 2004; 50(2): 195–200
- Stubbins MJ et al: Genetic analysis of the human cytochrome P450 CYP2C9 locus. *Pharmacogenetics*, 1996; 6(5): 429–39
- Yang JQ et al: Frequency of cytochrome P450 2C9 allelic variants in the Chinese and French populations. *Fundam Clin Pharmacol*, 2003; 17(3): 373–76
- Gaikovitch EA et al: Polymorphisms of drug-metabolizing enzymes CYP2C9, CYP2C19, CYP2D6, CYP1A1, NAT2 and of P-glycoprotein in a Russian population. *Eur J Clin Pharmacol*, 2003; 59(4): 303–12
- Yasar U et al: Validation of methods for CYP2C9 genotyping: frequencies of mutant alleles in a Swedish population. *Biochem Biophys Res Commun*, 1999; 254(3): 628–31
- Buzkova H, Pechandova K, Slanar O, Perlik F: Geneticky polymorfismus cytochromu CYP2C9 v ceske populaci. *Klinicka Biochemie a Metabolismus*, 2007; 15(36): 102–5
- Kashani A et al: Risks associated with statin therapy: a systematic overview of randomized clinical trials. *Circulation*, 2006; 114(25): 2788–97
- Kivisto KT et al: Lipid-lowering response to statins is affected by CYP3A5 polymorphism. *Pharmacogenetics*, 2004; 14(8): 523–25
- Lindahl A et al: Concentration- and region-dependent intestinal permeability of fluvastatin in the rat. *J Pharm Pharmacol*, 1998; 50(7): 737–44
- Bercovich D et al: The association of common SNPs and haplotypes in the CETP and MDR1 genes with lipids response to fluvastatin in familial hypercholesterolemia. *Atherosclerosis*, 2006; 185(1): 97–107
- Bogman K et al: HMG-CoA reductase inhibitors and P-glycoprotein modulation. *Br J Pharmacol*, 2001; 132(6): 1183–92
- Kopplow K et al: Human hepatobiliary transport of organic anions analyzed by quadruple-transfected cells. *Mol Pharmacol*, 2005; 68(4): 1031–38
- Konig J: Uptake transporters of the human OATP family: molecular characteristics, substrates, their role in drug-drug interactions, and functional consequences of polymorphisms. *Handb Exp Pharmacol*, 2011; (201): 1–28
- Couvert P et al: Association between a frequent allele of the gene encoding OATP1B1 and enhanced LDL-lowering response to fluvastatin therapy. *Pharmacogenomics*, 2008; 9(9): 1217–27