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Tolerability of statins is not linked to CYP450 polymorphisms, but reduced CYP2D6 metabolism improves cholesteraemic response to simvastatin and fluvastatin

Piergiorgio Zuccaro [a], Giuliana Mombelli [b], Laura Calabresi [b], Damiano Baldassarre [b], Ilaria Palmi [a], Cesare R. Sirtori [b,*]

[a] *Department of Drug Research and Evaluation, Italian National Institute of Health, Rome, Italy*
[b] *Center E. Grossi Paoletti, Department of Pharmacological Sciences, University of Milano, Italy*

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Abstract

Statin therapy, although generally well tolerated, leads not infrequently to significant subjective and at times objective adverse effects (AEs), mainly of a muscular nature. The genetic background of these AEs is not clear and possibly side effects and lipid lowering efficacy may be linked. Aim of the study was a detailed evaluation of CYP450 and apolipoprotein E gene polymorphisms in two large series of age-sex matched patients with and without muscular side effects to statins. In a Clinical Institution specialised in lipid-lipoprotein disorders, 50 statin treated patients were selected, with subjective or objective statin-associated myopathy, evaluated using standardized forms. These were sex and age matched with 50 statin-treated patients from the same Clinic, without any subjective or objective complaints. DNA samples for the evaluation of CYP450 genetic polymorphisms and apo E genotypes were collected in order to assess correlations with both genetic polymorphisms and AEs, as well as with therapeutic efficacy. None of the assessed CYP450 polymorphisms appeared to be related to an increased incidence of AEs. The CYP2D6 *1/*4 and *4/4* poor metabolizer (PM) status was associated to a higher efficacy of statins metabolized by this system and, in addition, the apo E2 genotype was, in this series, linked to increased HDL-C levels after therapy. Patients with statin associated myopathy are not characterized by significantly different genotypes for the CYP450s responsible for statin metabolism. On the other hand, CYP2D6 PM status is associated to an increased efficacy of statins metabolized by this system.

Keywords: Statins; Muscle toxicity; CYP450; Enzyme polymophisms; Cholesteraemic response; Apo E allele variants

1. Introduction

3-Hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statins) are very effective cholesterol lowering medications and are among the most widely used drug classes. The reason for this clinical success is a large experience witnessing effectiveness and safety as cardiovascular preventive agents [1,2]. While, in general, large scale trials support the excellent tolerability of statins, daily practice is frequently hampered by reports by the patients of, particularly, muscle symptoms during therapy [3–5]. These are mainly cramps and stiffness, at times requiring analgesic treatment and frequently leading to drug withdrawal [6]. The first report of a patient requiring hospitalization for myopathy with normal creatine phosphokinase (CPK) levels has been recently published [7].

Although very recently it was indicated [8] that the statin associated myopathy most frequently results in full resolution of muscle pain upon discontinuation of therapy, still these symptoms are particularly disturbing, also because the absence of laboratory changes may lead to physician's skepticism. Clinical studies have, on the other hand, suggested that muscle lipid accumulation may be observed in biopsies from patients with muscle symptoms without CPK rises during statin therapy [9,10]. This finding, i.e. lipid droplets inside the muscle fibers, is well complemented by a recent report indicating that respiratory chain enzyme activities in the muscle of patients taking major statins are reduced, and so are ubiquinone concentrations [11]. As a partial confirmation, Phillips et al. [12] also noted abnormal lipid

* Corresponding author at: Center E. Grossi Paoletti, Department of Pharmacological Sciences, University of Milano, Via Balzaretti 9, 20133 Milan, Italy. Tel.: +39 02 50318303; fax: +39 02 50319900.
E-mail address: cesare.sirtori@unimi.it (C.R. Sirtori).

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oxidation in patients with myotoxicity, possibly predisposing them to the symptoms.

Evaluation of statins in terms of their cytochrome P450 (CYP450) associated metabolism have clearly suggested differences in metabolic handling [13–15]. Different genotypes can influence therapeutic efficacy, so that in subjects homozygous for detrimental alleles (PM), plasma concentrations may potentially increase compared with the mean effects in the population. With the exception of pravastatin, which is transformed enzymatically in the liver cytosol, all statins undergo extensive microsomal metabolism by the CYP450 isozyme system [15].

Aim of the present study was to investigate differences in the genetic pattern between a group of statin-treated patients with muscle complaints requiring dose reduction or outright withdrawal from therapy and a group of age–sex matched patients with comparable cholesterolaemic response [16] but free of clinical symptoms. Availability of this data also allowed to test the previously reported hypothesis [17] that statin tolerability may be linked to cholesterolaemic response.

2. Subjects and methods

2.1. Patients and sample collection

Patients were recruited among those attending the Centro Universitario per lo Studio delle Dislipidemie, examining and following patients with lipid-lipoprotein disorders, frequently associated with cardiovascular diseases. Some 120–150 patients are examined each week, among which 8–10 new cases.

One hundred patients treated with statins were recruited. Characteristics are reported in Table 1. Of these, 50 had clinical symptoms of muscle pain as assessed with a standardized medical record review form, as described previously by Hansen et al. [8]. This recorded: pain at the upper girth after limited effort; pain to the lower limbs after minimal effort, e.g. uphill or protracted walking or running; diffuse muscle pain. Of these, eight had increases of CPK, >3-fold versus normal and 1 > 10-fold versus normal. The comparative series of 50 patients was comparable for age, sex and lipid levels without any significant subjective or objective disturbances during statin treatment. Among the recruited patients, 11 reported side effects to pravastatin, known to undergo minimal metabolic handling. However, it was not desired to bias the collection of cases (the evaluating laboratory was not aware of the identity of treatments) and, further, this inclusion allowed a more complete evaluation of the influence of apo E polymorphism on response (Table 2).

Exclusion criteria were: chronic liver disease, alcoholism, renal disease, serious atherosclerotic disease limiting patient motility, malignant disease at any stage, insulin dependent diabetes as well as multiple associations with drugs and CYP3A4 inhibitors (azole antifungals, grapefruit juice, dihydropyridine calcium antagonists, others). Each patient was carefully informed and signed a consent form for the study that was approved by the Internal Review Board. From each patient 4.5 ml of blood were drawn in the presence of EDTA and kept frozen until DNA extraction.

Table 1
Baseline characteristics of patients

	Simvastatin	Fluvastatin	Pravastatin	Atorvastatin	Rosuvastatin	Total
Demographic and clinical characteristics						
Sex						
Women (%)	11 (45.8)	5 (83.3)	15 (79.0)	23 (46.9)	1 (50)	55 (55.0)
Men (%)	13 (54.2)	1 (16.7)	4 (21.0)	26 (53.1)	1 (50)	45 (45.0)
Age (mean ± S.D.), year	63.7 ± 7.1	63.1 ± 6.3	65.1 ± 6.3	58.6 ± 9.9	47.5 ± 3.5	61 ± 9.0
Age (range) year	48–73	56–70	49–72	33–75	45-50	33-75
TC (mg dl^{-1})	298.5 ± 45.4	301.6 ± 61.6	300.3 ± 46.4	328.3 ± 69.7	415.5 ± 12.0	316.2 ± 62
LDL-C (mg dl^{-1})	217.6 ± 44.4	221.2 ± 64.7	208.1 ± 44.5	241.2 ± 68.7	349.0 ± 26.8	230.4 ± 61.8
HDL-C (mg dl^{-1})	52.7 ± 13.0	54.7 ± 12.8	59.7 ± 14.7	49.9 ± 11.9	47.0 ± 8.5	52.6 ± 13.0
TG (mg dl^{-1})	144.2 ± 59.4	129.2 ± 77.2	161.2 ± 74.8	184.8 ± 108.2	98.0 ± 32.5	166.1 ± 91.7
Patients with hypercholesterolaemia (%)	19 (79.2)	5 (83.3)	12 (63.2)	34 (69.4)	2 (100)	72 (72.0)
Patients with hypercholesterolaemia and hypertriglyceridaemia (%)	5 (20.8)	1 (16.7)	7 (36.8)	15 (30.7)	0 (0)	28 (28.0)
Medical history						
Myocardial infartion (%)	3 (12.5)	1 (16.7)	0 (0)	8 (16.3)	1 (50)	13 (13.0)
Peripheral vascular disease (%)	0 (0)	1 (16.7)	1 (5.3)	5 (10.2)	0 (0)	7 (7.0)
Angina (%)	3 (12.5)	0 (0)	1 (5.3)	6 (12.2)	1 (50)	11 (11.0)
Hypertension (%)	8 (33.3)	5 (83.3)	9 (47.4)	14 (28.6)	0 (0)	35 (35.0)
Diabetes (%)	2 (8.3)	0 (0)	1 (5.3)	3 (6.1)	0 (0)	6 (6.0)
Adverse events						
Patients with AEs	13	5	11	21	0	50
Patients without AEs	11	1	8	28	2	50

Values are numbers of each group, range or mean ± S.D.; TC, total cholesterol; LDL-C, LDL cholesterol; HDL-C, HDL cholesterol; TG, triglycerides; AEs, adverse events.

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Table 2
Baseline characteristics of patients with and without AEs

	With AEs	Without AEs
Total number for group (%)	50(50)	50(50)
Females	28	27
Males	22	23
Age (year, mean ± S.D.)	61.4 ± 8.9	61.09 ± 9.17
TC (mg dl^{-1})	305.24 ± 49.1	326.76 ± 72.69
LDL-C (mg dl^{-1})	215.87 ± 47.2	243.93 ± 72.35
HDL-C (mg dl^{-1})	51.44 ± 13.0	53.90 ± 13.11
TG (mg dl^{-1})	191.41 ± 103.7	144.22 ± 72.13
Concomitant therapy		
β-Blockers (%)	14 (28.0)	10 (20.0)
Calcium channel antagonists (%)	0 (0)	1 (2.0)
Antiarrhythmic drugs (%)	2 (4.0)	1 (2.0)
Antiplatelet drugs (%)	19 (38.0)	22 (44.0)
Coumarin anticoagulants (%)	1 (2.0)	1 (2.0)
Immunosuppressive agents (%)	1 (2.0)	0 (0)
ACE-inhibitors (%)	12 (24.0)	8 (16.0)
Nitrates (%)	2 (4.0)	7 (14.0)
Diuretics (%)	8 (16.0)	6 (12.0)
Antidepressants (%)	0 (0)	3 (6.0)
Antipsychotic agents (%)	1 (2.0)	1 (2.0)
Antiseizure agents (%)	1 (2.0)	0 (0)
Oral hypoglycemic agents (%)	0 (0)	2 (4.0)
Corticosteroids (%)	2 (4.0)	0 (0)
ATII antagonists (%)	2 (4.0)	3 (6.0)
HRT (%)	2 (4.0)	2 (4.0)
Clinical diagnosis		
Primary cardiovascular prevention (%)	35 (70.0)	33 (66.0)
Secondary cardiovascular prevention (%)	15 (30.0)	17 (34.0)

Values are numbers of each group, or mean ± S.D.; TC, total cholesterol; LDL-C, LDL cholesterol; HDL-C, HDL cholesterol; TG, triglycerides; AEs, adverse events; HRT, hormone replacement therapy.

2.2. Laboratory procedures

Genomic DNA was isolated from 200 μl of blood with the QIAmp DNA Mini Kit (Quiagen, Milan, Italy) by measuring the absorbance at 260 nm (A_{260}). PCRs were performed using published methods The PCR amplifications were done on an Applied Biosystem Gene Amp PCR System 9700.

2.2.1. CYP3A5*3

The method described by Van Schaik et al. [18] was followed, with minor modifications.

2.2.2. CYP2C9*2 and CYP2C9*3

The methods initially described by Nasu et al. [19] (CYP2C9*2) and by Sullivan-Klose et al. [20] (CYP2C9*3), and subsequently validated by Yasar et al. [21] were followed with minimal changes.

2.2.3. CYP2D6*3 and CYP2D6*4

The method described by Mulder et al. [17] was followed with some minor modifications.

2.2.4. CYP2D6*5

The method described by Hersberger et al. [22] was followed.

2.2.5. CYP2D6*6

The method described by Hersberger et al. [22] was followed with minor modifications.

2.2.6. FCYP2D6*2x

The method described by Steijns et al. [23] was followed.

2.2.7. Apo-E

We used the method described by Aozaki et al. [24], i.e. by digesting the PCR product (14 μl) with the HhaI restriction enzyme (10 U) (Amersham Pharmacia Biotech) in total volumes of 18 μl overnight at 37°.

2.3. Statistical analysis

Statistical analysis was performed using Sigma Stat 3.0 (statistical significance at $p < 0.05$). Samples were tested for homogeneity of sex, age and clinical treatment. In order to evaluate statistical dependence between allelic/genotypic frequency and presence/absence of adverse effects, Chi-square test and Fisher's exact test were performed. In order to evaluate genetic polymorphisms and lipid lowering response to statins, Student's *t*-test was performed. Groups not responding to Kolmogorov–Smirnov normality test, were analysed with the

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Mann–Whitney rank sum test. In order to evaluate CYP450 isozyme allelic variants and lipid lowering response to statin, Student's *t*-test or Mann–Whitney rank sum test (groups not responding to Kolmogorov–Smirnov normality test) were performed.

3. Results

3.1. CYP450s and adverse effects (AEs)

3.1.1. CYP3A5 genotype

In the examined clinical samples, 73 patients received simvastatin and atorvastatin, whose metabolism is handled by CYP3A5 [25,26]. Among these, 34 showed muscular symptoms following intake of the statin, whereas 39 had no disturbances. The two groups were comparable for age, sex and drug dosage. In the group of patients with AEs, 4 (11.8%) were *1/*3 versus 10 (25.6%) in the control group. The frequency of the *1/*3 genotype was 4/34 (11.8%) in the group of patients with AEs and 10/39 (25.6%) in the group without complaints (p = ns). Frequency of the *3/*3 genotype in subjects with and without AEs were 88.2% and 74.4%, respectively (p = ns). The frequency of *1/*3 was slightly lower in the group of patients with AEs but the difference did not reach statistical significance. Also allele frequencies were approximately identical in the two groups (Table 3).

3.1.2. CYP2C9 genotype

In the examined sample only 32 patients, 18 (56.2%) with AEs and 14 (43.8%) without AEs, received statins metabolized to a different degree of involvement by CYP2C9 (simvastatin, fluvastatin, rosuvastatin) [13–15,27]. In the group of patients with AEs, 6 (33.3%) had at least one mutated allele (*2 or *3), whereas in the control group the heterozygotes for the mutation were 5 (35.7%). Patients with homozygous wild-type genotype in the group with AEs and in controls, were respectively 12 (66.7%) and 9 (62-3%). By comparing genotypic as well as allele frequencies (Table 3) between the two groups of patients there appears to be no statistically significant difference.

3.1.3. CYP2D6 genotype

Thirty patients received statins whose metabolism involves CYP2D6 (simvastatin and fluvastatin) [13–15,17]. Eighteen showed AEs and 12 were free of side effects. In the group with AEs, 1 patient proved to be an ultrarapid metabolizer (UM, CYP2D6*2 × N). In the group of patients with AEs, 7 (41.2%) had at the least one mutated allele, whereas the remaining 10 (58.8%) were wild-type homozygotes. In the control group, 6 patients (50.0%) had at least one mutated allele and the remaining had genotype *1/1. Also allele frequencies were approximately identical in the two groups (Table 3).

Table 3
Adverse effects (AEs) of of the statins in relation to the different CYP genotypes and allele variants

			CYP3A5 genotype		Allele frequency (%)	
			*1/*3	*3/*3	*1	*3
Total number of patients			14 (19.2%)	59 (80.8%)		
N. pt with AEs treated with	Simvastatin	(10–20 mg)	1	12	4/68 (5.9%)	64/68 (94.1%)
N. pt with AEs treated with	Atorvastatin	(10–40 mg)	3	18		
N. pt without AEs treated with	Simvastatin	(10–20 mg)	3	8	10/78 (12.8%)	68/78 (87.2%)
N. pt without AEs treated with	Atorvastatin	(10–60 mg)	7	21		

			CYP2C9 genotype		Allele frequency (%)		
			*1/*2	*1/*2 or *1/*3	*1	*2	*3
Total number of patients			21 (65.6%)	11 (34.4%)			
N. pt with AEs treated with	Simvastatin	(10–20 mg)	8	5	30/36 (83.3%)	5/36 (13.9%)	1/36 (2.8%)
N. pt with AEs treated with	Fluvastatin	(80 mg)	4	1			
N. pt with AEs treated with	Rosuvastatin	(10–20 mg)	0	0			
N. pt without AEs treated with	Simvastatin	(10–20 mg)	6	5	23/28 (82.1%)	3/28 (10.7%)	2/28 (7.2%)
N. pt without AEs treated with	Fluvastatin	(80 mg)	1	0			
N. pt without AEs treated with	Rosuvastatin	(10–20 mg)	2	0			

			CYP2D6 genotype		Allele frequency (%)		
			*1/*1	*1/*4 or*4/*4	*1	*4	2 × N
Total number of patients			16 (55.2%)	13 (44.8%)			
N. pt with AEs treated with	Simvastatin	(10–20 mg)	7	5	30/38 (78.9%)	7/38 (18.4%)	1/38 (2.7%)
N. pt with AEs treated with	Fluvastatin	(80 mg)	3	2			
N. pt without AEs treated with	Simvastatin	(10–20 mg)	5	6	17/24 (70.8%)	7/24 (29.2%)	0/24 (0%)
N. pt without AEs treated with	Fluvastatin	(80 mg)	1	0			

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3.2. CYP450 and lipid responses

3.2.1. CYP3A5 genotype

Among the 73 patients who received simvastatin and atorvastatin, both handled by CYP3A5, 14 were *1/*3, the remaining 59 were *3/*3. Statistical analysis of lipid lipoprotein and percentage responses to treatment in these patients did not show any significant differences either in baseline data or in therapeutic responses between the two groups (data not shown).

3.2.2. CYP2C9 genotype

Among the 32 patients who received statins (simvastatin, fluvastatin, rosuvastatin) partly or totally metabolized by CYP2C9, 21 (65.6%) had genotype *1/*1 and 11 (34.4%) had at least one mutated allele (*1/*1 $n = 8$ and *1/*3 $n = 3$). By comparing lipid changes in patients with the wt genotype (*1/*1) and patients with at least one mutated allele, percent variations showed essentially no difference for total, LDL-, and HDL-cholesterol as well as for triglycerides (data not shown).

3.2.3. CYP2D6 genotype

Thirty patients received statins (simvastatin, fluvastatin) at least partially metabolized by CYP2D6. Of these, 16 (53.3%) had a wt genotype (*1/*1) whereas 13 (43.3%) had at least one mutated allele (*1/*4, $n = 12$ and 4/*4, $n = 1$), and only one patient (3.4%) was an UM (*2 × N). Statistical analysis comparing subjects with wt genotype (*1/*1) and patients with at least one mutated allele (*4) (Table 3) showed homogeneity for age, sex, doses, and basal lipid levels. Fig. 1 reports the LDL-cholesterol reductions found after treatment with CYP2D6 substrates (simvastatin–fluvastatin) in patients with different CYP2D6 genotypes, i.e. from the wild type (*1/1*) to the UM (*2 × N). All fluvastatin patients received the drug in a 80 mg daily dose in the slow release formulation. In order to have comparable data for all simvastatin doses, these were brought to a unified dose of 20 mg: patients who received 40 mg had their LDL-cholesterol response reduced by 6%; those receiving 10 mg/day had their LDL-cholesterol response raised by 6%, as suggested by Roberts [28].

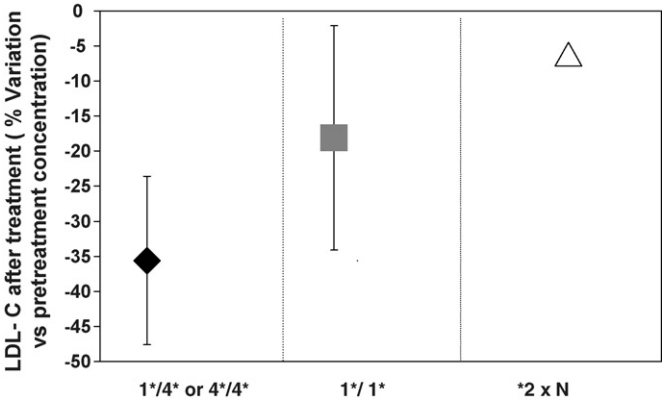

Fig. 1. LDL cholesterol reductions following simvastatin/fluvastatin, CYP2D6 substrates, regulated by the CYP2D6 polymorphisms. The best reduction was found in individuals with impaired CYP2D6 function (*1/*4) and the least reduction in the individual with the UM genotype (CYP*2 × N).

3.2.4. Apo E genotype

Carriers of the apo E2 genotype, as expectable [29], had lower total and LDL cholesterol levels versus the other three genotypes. The difference, however, was not statistically significant. They also experienced the most marked reduction of both total

Table 4
Effectiveness of the statins in relation to the Apo-E genotype and allele frequencies

	ApoE-genotype			Apo-E allele frequency (%)		
	E2/E3	E3/E3	E3/E4 or E4/E4	ε2	ε 3	ε 4
Number patients for phenotype	3	74	23	3/200 (1.5%)	174/200 (87%)	25/200 (12.5%)
Women	2	1	15			
Men	1	36	8			
Mean age (Years)	52.7 ± 17.2	60.8 ± 9.0	62.9 ± 7.7			
	ApoE-genotype			Apo-E allele frequency (%)		
	E2/E3	E3/E3	E3/E4 or E4/E4	P E2 vs. E3	P E3 vs. E4	P E2 vs. E4
Basal Total C (mg dl^{-1})	284.7 ± 56.8	320.8 ± 66.1	305.9 ± 47.5	0.421	0.125	0.133
Total C post-treatment (mg dl^{-1})	184.7 ± 30.7	248.9 ± 46.0	239.8 ± 40.2			
Mean Total-C change (%)	−34.0 ± 14.3	−21.4 ± 14.1	−26.6 ± 14.8			
Basal LDL-C (mg dl^{-1})	172.0 ± 23.1	236.1 ± 65.6	220.0 ± 47.7	0.209	0.407	0.193
LDL-C post-treatment (mg dl^{-1})	100.4 ± 19.4	166.0 ± 45.4	156.2 ± 36.1			
Mean LDL-C change (%)	−41.6 ± 8.3	−28.2 ± 17.5	−27.0 ± 19.1			
Basal HDL-C (mg dl^{-1})	42.0 ± 7.2	50.8 ± 13.0	59.7 ± 11.2	0.07	0.10	0.03
HDL-C post-treatmen (mg dl^{-1})t	54.7 ± 11.0	52.6 ± 12.6	57.1 ± 14.4			
Mean HDL-C change (%)	30.3 ± 12.6	5.5 ± 19.3	−3.8 ± 19.2			
Basal TG (mg dl^{-1})	353.3 ± 232.6	168.8 ± 83.2	132.8 ± 63.9	0.041	0.084	0.029
TG post-treatment (mg dl^{-1})	148.0 ± 88.4	153.3 ± 81.8	116.9 ± 52.0			
Mean TG change (%)	−49.1 ± 30.9	−5.4 ± 33.4	−3.4 ± 34.9			

Data are expressed as mean ± S.D.

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and LDL-C levels (respectively −34 and −41.6%) versus lesser reductions in the other two genotypes. Again, none of these differences turned out to be statistically significant. Nonertheless, triglyceride reduction in the E2/E3 carriers reached close to a mean of 50%, versus minimal reductions in the other two groups. Exclusion of one patient with the highest triglyceride level in the E2/E3 genotype group led to the loss of statistical significance. In view of the very high mean triglyceridaemia in the E2/E3 genotype carriers, basal HDL-C levels were also lowest in this group and rose about 30% after drug treatment. The increase was maximal for the E2/E3 carriers, with no changes in HDL in the other two groups (Table 4).

4. Discussion

Very wide use of statins and their unquestionable efficacy in lipid lowering and in preventing coronary disease has led to an approximately 3% use, e.g. in the adult UK population [30]. They are generally well tolerated, but side effects, in particular of the muscle type, are frequently reported by patients and may result in reduced compliance or interruption of therapy. This study attempted to elucidate an important and controversial aspect of statin muscle toxicity. If indeed this toxicity is due to elevated statin levels rather than to idiosyncratic vulnerability in certain patients, then patients unable to metabolize statins by way of the appropriate CYP450 isoform should be predisposed to muscle toxicity [31].

AEs to statins are in general associated to two major types of events: increased hepatic transaminase levels and myopathy. While increased transaminase is a relatively rare phenomenon and not of major importance in daily practice [32], myopathy is particularly disturbing both for patients and physicians [6] leading, not infrequently, to drug withdrawal with the accompanying loss of benefit from this important drug treatment [7,33], although the long term severity of muscular changes [8] may be relatively modest.

The mechanism of myopathy due to statins is still overall unclear. A number of potential risk factors has been listed, e.g. advanced age (particularly above 80 years and women more than men) and interaction with a number of inhibitors of CYP3A4, e.g. amiodarone, verapamil, diltiazem, nefazodone, grapefruit juice, clarithromycin, azole antifungals [34], or also inhibitors of CYP2C9 such as diclofenac, tolbutamide and others [27] as well as lipid lowering agents such as fibrates, preexisting disease (renal or liver, complicated diabetes, renal infection), excessive ethanol intake (apparently predisposing to myopathy) [35] and intensive physical exercise, this last controindicating statin use in professional athletes [36].

The molecular mechanism of statin myopathy has been directly related to chloride (Cl^-) channel antagonism. Statins antagonize this channel to a concentration related extent, thus leading to potentially raised muscle contractility; the effect is maximal with lipophilic statins and of a lesser degree for hydrophilic molecules, such as pravastatin [37]. Additional mechanisms of toxicity may be the downregulation of the Na^+/Ca^{2+} exchanger described with fluvastatin in the rat cardiomyoblast H9C2 cells [38] and the induction of apoptosis due to sustained muscle cell Ca^{2+} transients, leading to calpain activation [39].

It is frequently assumed that statin muscle toxicity should be dose-related and consequently linked to plasma drug concentrations [11]. Thus, if significant interindividual statin metabolic handling does occur, a reduced expression of drug metabolizing enzymes, resulting in higher plasma drug levels should lead to enhanced toxicity. Plasma statin levels are generally not very informative, since most statins have a very short elimination half life; the case may be different for statins with a longer elimination half life (atorvastatin, rosuvastatin). While preliminary findings from our group attempting to correlate drug levels of atorvastatin/rosuvastatin with reported toxicity have not been particularly informative, in a very recent report, myopathy was related to an increased systemic exposure to the metabolites atorvastatin lactone and p-hydroxyatorvastatin [40]. In the present series of atorvastatin treated patients there was no evidence of a difference in CYP3A5 carrier status.

From the present investigation, it appears that the incidence of myopathy, both CPK associated and non associated, does not significantly differ in the carriers of the major CYP450 genotypes, in particular as relates to CYP3A5, 2D6, and 2C9. Some borderline higher incidence of toxicity was found with specific genotypes e.g. *3/*3 for CYP3A5. These last findings contrast with those of Fiegenbaum et al. [25], suggesting that this allele is significantly associated with a higher incidence of side effects to simvastatin. Their patient series was, however, small and statistical significance might be a chance finding. The apparently increased incidence of AEs in carriers of the *1/*1 genotypes for both CYP2D6 and CYP2C9, did not instead reach statistical significance. Even if a larger patients database could possibly lead to the identification of an increased risk of myopathy associated with these genotypes, the finding would probably not be of major clinical interest.

This study allowed thus to evaluate a large population of statin treated patients with well documented subjective or objective muscular AEs. While the number of patients treated with each statin may seem relatively small, it should be noted that statins must be grouped into molecules handled by specific CYPs; it is thus evident that a significant number of patients were treated with, e.g. CYP2D6 or CYP3A5 substrates. The study also allowed to test in parallel the influence of CYP450 genotypes on lipid responses to statins. This topic is widely debated, since initially Kivistö et al. [26] in a study on 46 hypercholesterolaemic patients noted a higher mean percent reduction of total and LDL-C in the CYP3A5 *1/*3 genotype carriers versus non expressors. This finding was not confirmed in our series of 100 individuals; we found, however, that the CYP2D6 genotype is significantly correlated with lipid responses to statins handled by this cytochrome. A significant correlation between LDL-C reduction and reduced expression of CYP2D6 had been earlier reported by Nordin et al. [41] and similar findings were suggested by Mulder et al. [17], who noted a higher response in patients carrying at least one mutated allele. In this latter study [17], a significant difference in the hypolipidemic efficacy was in fact noted particularly among homozygotes for the inactivating genotype, compared with the wt *1/*1

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carriers, whereas we also found some differences by comparing heterozygotes and wt patients; finally the patient with the ultrarapid phenotype had the worst cholesteraemic response [17,41]. These findings support the conclusion that metabolic handling is not significantly related to AEs, since a minimally higher incidence of AEs was noted among carriers of the mutated genotype, who should have higher drug levels. The influence of CYP2D6 on the hypolipidaemic activity of simvastatin/fluvastatin appears to be more significant than that reported for pravastatin (not a CYP2D6 substrate) in a study investigating mainly SNPs within candidate genes related to lipid metabolism [42].

In the case of apo E genotypes, correlation studies investigating hypolipidaemic drug responses have provided in general quite consistent findings, as reviewed by Kajinami et al. [43], allowing to conclude that the apo E genotype is a major determinant of statin response. This was clearly shown in clinical studies with atorvastatin in carriers of the $\varepsilon 2$ and $\varepsilon 4$ alleles with, respectively, significantly higher and reduced cholesteraemic responses [44,45]. These findings can be explained by the higher affinity of apo E4 for the LDL receptor, resulting in lower cholesterol synthesis and increased absorption; conversely, E2 carriers with lower absorption and higher biosynthesis are generally more sensitive to statin treatment [28]. In the present series, apo E2 carriers showed both a non significantly higher LDL-C reduction versus carriers of the other alleles (not reaching statistical significance because of the small number of these individuals) and, more so, an increase of HDL-C levels. This last finding fits well with data from a study on patients with combined hyperlipoproteinaemia [46]. The marked HDL rises of E2/E3 heterozygotes is likely to be attributable to the higher incidence of hypertriglyceridaemia, associated with low HDL-C; drug treatment led both to reduced triglycerides and to increased HDL-C levels [47].

The lack of conclusive data on the genetic associations between skeletal muscle toxicity and CYP450 polymorphisms probably indicates that this subjective and at times objective phenomenon is not significantly regulated by the CYP450 handling of statins. There is instead definite evidence that most likely CYP2D6 isotypes influence the cholesteraemic response. Myotoxicity would thus be an idiopathic phenomenon possibly associated with individual cell changes resulting in an increased risk of apoptosis [9,12,39]. Muscle from subjects with subjective myopathy indicated extensive lipid-filled vacuoles and lipid droplet accumulation after statins, most likely associated to lower respiratory activity and, as a consequence, muscular lipid metabolism.

A limitation in the reported study is the lack of evaluation of the organic anion transporter protein (OATP) polymorphisms, recently indicated as potentially regulating blood levels of pravastatin, rosuvastatin and pitavastatin [48–50]. We only saw limited muscle toxicity with both pravastatin and rosuvastatin (this latter has entered the Italian market only recently) and pitavastatin has not been as yet marketed in this part of the world. Of course, participating individuals are all of Italian origin and it cannot be excluded that metabolic differences may be found in other parts of the world.

5. Conclusion

This large scale evaluation of CYP450 polymorphism versus myotoxic effects and lipid lowering activity provides, therefore, extensive evidence indicating the need for careful assessment of the patients without the need for monitoring of genetic risk factors. It also underlines the potential significance of evaluating CYP450 and apo E polymorphisms versus lipid lowering activity with the different statins

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