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RESEARCH PAPER

Genetic polymorphisms of CYP2C9 and CYP2C19 are not related to drug-induced idiosyncratic liver injury (DILI)

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Background and purpose: The general view on the pathogenesis of drug-induced idiosyncratic liver injury (DILI) is that parent compounds are rendered hepatotoxic by metabolism, mainly by cytochrome (CYP) 450, although other metabolic pathways can contribute. Anecdotal reports suggest a role of CYP 450 polymorphisms in DILI. We aimed to assess in a series of Spanish DILI patients the prevalence of important allelic variants of CYP2C9 and CYP2C19, known to be involved in the metabolism of several hepatotoxic drugs.
Experimental approach: Genotyping of CYP2C9 (*2, *3) and CYP2C19 (*2 and *3), was carried out in a total of 28 and 32 patients with a well established diagnosis of DILI. CYP2C9 and CYP2C19 variants were analysed in genomic DNA by means of PCR-FRET and compared with previous findings in other Caucasian populations.
Key results: CYP2C9 and CYP2C19 allele and genotype frequencies were in agreement with Hardy-Weinberg equilibrium. Fourteen patients (50%) were heterozygous and 1(4%) found to be compound heterozygous for the CYP2C9 allele. Seven (22%) were found to carry one and 1(3%) carried two CYP2C19 mutated alleles. No patients were homozygous for *3 allele. The distribution of both CYP2C9 and CYP2C19 allelic variants in DILI patients were similar to those in other Caucasian populations. Patients with variant and those with wild-type alleles did not differ in regard to clinical presentation of DILI, type of injury and outcome.
Conclusions and Implications: We find no evidence to support CYP2C9 and CYP2C19 genetic polymorphisms as predictable potential risk factors for DILI.
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Keywords: CYP2C9; CYP2C19; hepatotoxicity; genetic predisposition

Abbreviations: ATC, anatomic therapeutic classification; A, adenine; C, cytosine; CIOMS, Council for International Organizations of Medical Science; CYP, cytochrome; DILI, drug induced idiosyncratic liver injury; G, guanine; NTCP, sodium-dependent taurocholate cotransporting polypeptide; OATP, human organic anion transporting polypeptide; SPSS, Statistical Package for Social Science; T, thymine

Introduction

Drug-induced liver injury (DILI) is a challenge in modern pharmacotherapy and remains the single leading cause of drug withdrawal despite of a rigorous preclinical and clinical review process (Temple and Himmel, 2002). DILI accounts for about one-half of the acute liver failure cases in United States (Lee, 2003) and mimics all forms of acute and chronic hepatobiliary disease (Kaplowitz, 2005).

The molecular mechanisms of DILI are still poorly understood. Acquired risk factors (such as environmental factors, age, gender, underlying diseases and associated treatments) account for only a minor percentage of the

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population-attributable risk. Thus, the drug toxic potential together with genetic factors could contribute to the development of DILI (Kaplowitz, 2000, 2005). Both *in vitro* and *in vivo* studies suggest that drug bioactivation and formation of reactive metabolites induce hepatocyte stress that leads to apoptosis, necrosis or liver failure. The general view on the pathogenesis of DILI is that parent compounds are rendered hepatotoxic during cytochrome (CYP) 450 metabolism and can exert their action within the target cell (Maddrey, 2005) although other drug-metabolizing enzymes can contribute to the activation of drugs (Tafazoli *et al*., 2005). It was recently reported that 13 of 22 (62%) of drugs withdrawn from the market for hepatotoxicity, or with black box warning for hepatotoxicity, have been shown to produce reactive metabolites (Walgren *et al*., 2005). However, data on CYP polymorphism in DILI are lacking, apart from anecdotal reports.

The CYP2C subfamily constitutes about 18% of the CYP protein content in human liver and metabolizes around 20% of widely prescribed therapeutic drugs available in the market (Lee *et al*., 2003). Polymorphism of CYP2C enzymes are very common leading to significant heterogeneity in drug metabolism (Guengerich *et al*., 1998). Within the CYP2C subfamily, the most relevant genetic polymorphism affects CYP2C9 and CYP2C19 genes (Scordo *et al*., 2004). The nucleotide changes in the *CYP2C9*2* (430C>T), *CYP2C9*3* (1075A>C), *CYP2C19*2* (681G>A), *CYP2C19*3* (636G>A) allele could influence both the activity and substrate specificity of CYP2C9 and CYP2C19. Its polymorphisms led to severe toxic effects such as bleeding with warfarin (Aithal *et al*., 1999), fall of blood sugar levels with antidiabetics (Kidd *et al*., 1999), mental confusion (Ninomiya *et al*., 2000) and CNS toxicity with diphenylhydantoin (Bertilsson, 1995) or, conversely, to higher rates of *Helicobacter pylori* eradication with omeprazol in poor metabolizers (Furuta *et al*., 2005)

Some of the drugs that are substrates for CYP2C9 and CYP2C19 isoforms, are also known to be hepatotoxic (Larrey and Pageaux, 1997; Larrey, 2002; Kirchheiner *et al*., 2003a, b; Aithal *et al*., 2004b; Sevilla-Mantilla *et al*., 2004). However, it is not yet known whether variations in CYP2C9 and CYP2C19 enzyme activity might play a role in determining or predicting risk of hepatotoxicity. To our knowledge only single case reports suggest that CYP2C9 and CYP2C19 polymorphism is associated with DILI (Larrey and Pageaux, 1997; Kirchheiner and Brockmoller, 2005). A case of leflunomide-induced severe hepatotoxicity was associated to a rare CYP2C9*3/*3 genotype (Sevilla-Mantilla *et al*., 2004) and a case of severe tetrabamate (Larrey and Pageaux, 1997) and troglitazone hepatotoxicity (Larrey, 2002) were described in a carrier of partial or complete deficiency of CYP2C19 genotype. Unfortunately, these reports are based on single studies or on small numbers of patients and therefore need further confirmation.

We aimed to assess the prevalence of important allelic variants of CYP2C9 and CYP2C19 in DILI patients submitted to a Spanish Registry and to determine whether the susceptibility to develop DILI was associated with these genetic variants.

Methods

Subjects and study protocol

Cases of DILI were selected from those submitted to a Spanish Registry, in use in southern Spain since 1994 and coordinated by two of the authors (RJA and MIL). The operational structure of the registry, data recording and case ascertainment have been reported elsewhere (Andrade *et al*., 1999).

The report form contains full information necessary to ascertain causality: (1) the temporal relationship between start of drug intake and appearance of liver disease, and the time between discontinuation of treatment and improvement in or recovery from liver dysfunction; (2) serology and biochemical data to exclude viral hepatitis and autoimmune and metabolic liver disease, as well as appropriate imaging tests to rule out bile duct disorders; and (3) the outcome of liver damage.

All submitted cases are further evaluated for causality assessment, initially by clinical assessment and later by application of the Council for International Organizations of Medical Science (CIOMS) scale, which appears to be more accurate in attributing causality (Lucena *et al*., 2001).

The pattern of liver injury is classified according to the International Consensus Meeting Criteria (Benichou, 1990). The liver tests used for the classification of liver damage were the first blood test available after liver injury. Alternatively, liver damage was determined on the basis of liver biopsy findings when available. Cases were defined as chronic if laboratory liver tests showed persistent abnormality more than 3 months after stopping drug therapy for hepatocellular pattern of damage or 6 months for cholestatic/mixed type of injury. The drugs responsible for hepatic reactions were classified according to the Anatomic Therapeutic Classification (ATC) recommended by World Health Organization-Europe (WHO, 2005). Patients who gave informed consent and for whom a blood sample was available were considered eligible only if causality assessment score was definite or probable.

Excluded were patients with underlying liver injury or cases secondary to drug overdoses (paracetamol/acetaminophen) and occupational exposure to toxins.

The study protocol was approved by the local ethics committee of the coordination centre at the Virgen de la Victoria University Hospital in Málaga, Spain.

DNA extraction and CYP2C9, CYP2C19 polymorphism determination

Venous blood was obtained in 5-ml EDTA Vacutainer tubes and stored at −4°C for 2–4 days, the time allowed for sending the samples to the coordinating centre. Plasma and peripheral blood mononuclear cells were prepared according to standard techniques and stored at −80°C until DNA isolation and analysis. For all patients, genomic DNA was isolated from 200 μl peripheral blood cells anticoagulated with ethylene diaminetetraacetic acid using QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany) according to the guidelines of the manufacturer.

The determination of two variant allele in the CYP2C9 gene (C→T at position 430 (CYP2C9*2) A→C at position 1075 (CYP2C9*3)) and in the CYP2C19 gene (G→A at position 636 (CYP2C19*3) and G→A at position 681(CYP2C19*2)), was assessed by analysis of the melting temperature of the hybrids formed between the polymerase chain reaction (PCR) products and specific fluorochrome labelled oligonucleotides. Detection of hybridization signal was based on fluorescence resonance energy transfer on Light Cycler (Roche Light Cycler, Software Version 5.32) real-time PCR system. For each amplification, 50–100 ng of genomic DNA was subjected to PCR in a total reaction volume of 20 μl. The thermocycling reactions for CYP2C9 and CYP2C19 polymorphism were carried out using the LightCycler-CYP2C9 Mutation Detection kit (CYP2C9*2 and CYP2C9*3) and LightCycler-CYP2C19 Mutation Detection Kit (CYP2C19*2 and CYP2C19*3) (Roche Molecular Biochemicals) according to the manufacturer's instructions.

Data analysis

Data were compiled according to the genotype and allele frequencies estimated from the observed number of each specific allele. Data were analysed with the Statistical Package for Social Science (SPSS, version 12.0; SPSS Inc., Chicago, IL, USA) for Windows software. Hardy–Weinberg equilibrium was determined by comparing the genotype frequencies with the expected values using a contingency table χ^2 statistics with Yates's correction. Analysis of variance (ANOVA) was used for comparison of groups. Where variables did not follow a normal distribution, nonparametric analyses (Kruskal–Wallis test) were performed. A P-value <0.05 was considered statistically significant.

Results

Overall description of DILI patients

A total of 28 DILI patients exposed to drugs biotransformed by CYP2C9 (17 females; 17–82 years old; mean 53 years) and 32 DILI patients exposed to drugs biotransformed by CYP2C19 (18 females; 14–70 years old; mean, 48 years,) were analysed for CYP2C9 and CYP2C19 polymorphism analysis (Table 1). The frequency of variant CYP2C9 was higher (15/28; 54%) than that of CYP2C19 (8/32; 25%) ($\chi^2=5$; $P=0.03$).

The search for drugs which are substrates for CYP2C9 and CYP2C19 was carried out at http://medicine.iupui.edu/flockhart/table.htm. In 11 DILI patients, drugs were substrates for both CYP2C9 and CYP2C19. Among the DILI group exhibiting CYP2C9 and CYP2C19 genotypes, the type of liver damage was classified as hepatocellular (18 and 21 cases) or cholestatic and mixed (10 and 11 cases, respectively). Hypersensitivity features were found in 12% of the patients (3/28 and 4/32, respectively). Most of the cases were classified as definite (80%) and 12 cases were considered as probable according to the CIOMS scale.

The main class of therapeutic activity of the drugs biotransformed by CYP2C9 was central nervous system (CNS) (7/28; 25%), followed by cardiovascular (6/28; 21%) and musculoskeletal (6/28; 21%). The main therapeutic class of drugs known to be substrates for CYP2C19 was CNS (12/32; 38%), followed by proton-pump inhibitors (3/32; 9%) and cardiovascular (2/32; 6%)

Distribution of CYP2C9 and CYP2C19 allele in DILI patients

In the 28 DILI patients, CYP2C9 allele frequencies were 0.71 for CYP2C9*1, 0.13 for CYP2C9*2 and 0.16 for CYP2C9*3 (Table 2). Of the patients, 14 (50%) were heterozygous and 1(4%) was found to be compound heterozygous for the CYP2C9 allele, respectively. In the 32 DILI patients, CYP2C19 allele frequencies were 0.86 for CYP2C19*1, 0.14 for CYP2C19*2 and 0 for CYP2C19*3. Seven of the patients (22%) were found to carry one and only one patient (3%) carried two (homozygous) CYP2C19 mutated alleles, respectively.

CYP2C19*3, which is considered an Asian mutation, was not detected in DILI patients. No individuals homozygous for CYP2C9*3 and CYP2C19*3 were identified in the study.

Table 1 Frequencies of CYP2C9 and CYP2C19 genotypes in patients with DILI recorded in a registry of hepatotoxicity

Genotype	*Activity*	*DILI patients*	*Observed frequency (%)*	*Predicted frequency by Hardy–Weinberg law*
CYP2C9		*N=28*		
1/1	Normal	13	46	51
1/2	Minor Reduction	6	21	18
1/3	Moderately reduced	8	29	23
2/2	Moderately reduced	0	0	1.5
2/3	Moderately reduced	1	4	4
3/3	Very low	0	0	2.5
CYP2C19		*N=32*		
1/1	Normal	24	75	74
1/2	Minor Reduction	7	22	24
1/3	Moderately reduced	0	0	0
2/2	Moderately reduced	1	3	2
2/3	Moderately reduced	0	0	0
3/3	Very low	0	0	0

Abbreviations: DILI, drug-induced liver injury.

Genotyping frequencies of the common allelic variants of CYP2C9 and CYP2C19 in DILI group are shown in Table 1. The distribution of allelic and genotype frequencies for CYP2C9 and CYP2C19 were in agreement with Hardy–Weinberg equilibrium.

Allelic frequencies of CYP2C9 and CYP2C19 genotype of the DILI group were compared with published data for other populations studied. CYP2C9 and CYP2C19 allele frequencies were similar to that in Caucasians (Table 2).

Based on the frequency of the 2C9*3 and 2C19*3 alleles, the incidence of poor metabolizers (2C9 or 2C19 *2/*3, *2/*2, *3/*3 individuals) among the various populations compared were determined (Table 2). In our DILI group the CYP2C9 frequency of poor metabolizers was 4% and the corresponding figures for CYP2C19 being 3%, similar to Caucasians, however, there were no patients exhibiting very low enzyme activity for CYP2C9 *3/*3 and CYP2C19 *3/*3 alleles.

We also classified patients according to sex, age, type of liver damage (hepatocellular, cholestatic/mixed), presence or absence of hypersensitivity features, mean duration of treatment and disease outcome. However, no differences were found when compared to CYP2C9 and CYP2C19 genotype.

Because both the CYP2C9 (*2,*3) and CYP2C19 (*2,*3) allele have been shown to lead to decreased enzyme activity, the genotype frequencies were assembled into two groups for analysis: homozygous carriers of wild-type allele (CYP2C9*1/*1 and CYP2C19*1/*1) and individuals carrying one or two variant alleles for CYP2C9 and CYP2C19. Demographic and clinical–biochemical parameters and outcome in these groups are shown in Table 3. No differences in any of the variables evaluated were found between groups.

Individual characteristics of the total DILI patients, CYP2C9 and CYP2C19 genotype together with causative drugs, demographic and clinical–biochemical parameters are shown in Table 4.

Carriers for CYP2C9*3 (*1/*3) were recorded in patients with DILI due to nonsteroidal anti-inflammatory drugs (2/5; 40%), anti-tuberculous drugs (2/3; 67%), fluoxetine, phenytoin, irbesartan and ticlopidine. The only carrier for CYP2C9*3 (*2/*3) was a case of lovastatin/gemfibrozil related hepatotoxicity. Homozygote for CYP2C19 *2 was recorded in a patient with DILI due to isoniazid and rifampicin.

Table 2 CYP2C9 and CYP2C19 allelic and PM genotypic frequencies in the DILI group compared with other populations studied

Population study	*Number of subjects*	**1*	**2*	**3*	*PM*	*Reference*
CYP2C9						
DILI group	28	71	13	16	4	Our study
Caucasian	157	69	14	16	7	Garcia-Martin *et al.* (2001)
Asian	102	95	0	5	0	Gaedigk *et al.* (2001)
North American	325	78	15	7	4	Gaedigk *et al.* (2001)
CYP2C19						
DILI group	32	86	14	0	3	Our study
Caucasian	360	89	11	0	2	Scordo *et al.* (2004)
Asian	121	50	45.5	4.5	24	Yamada *et al.* (2001)
African American	108	75	25	0	7	Goldstein *et al.* (1997)

Abbreviations: DILI, drug-induced liver injury; PM, poor metabolizer.

Table 3 Demographics, clinical and laboratory parameters of DILI patients grouped according to pattern of CYP2C9 and CYP2C19 genotype

Variables	*Wild type for CYP2C9 (N = 13)*	*Mutant for CYP2C9 (N = 15)*	*Wild type for CYP2C19 (N = 24)*	*Mutant for CYP2C19 (N = 8)*
Mean age (range), years	51 (17–76)	54 (27–82)	49 (27–70)	43 (14–62)
Gender (male/female)	6/7	5/10	10/14	4/4
Clinical presentation, n (%)				
Jaundice, *n* (%)	10 (76%)	13 (87%)	15 (63%)	5 (62%)
Hypersensitivity features, *n* (%)	0	3 (20%)	2 (8%)	2 (25%)
Type of damage				
Hepatocellular damage, *n* (%)	9 (69%)	9 (60%)	15 (62%)	6 (75%)
Cholestatic and mixed damage, *n* (%)	4 (31%)	6 (40%)	10 (38%)	2 (25%)
Laboratory parameters, mean value (range)				
Total bilirubin (μM)	141.9 (5.1–465.1)	75.3 (8.6–189.8)	100.9 (5.1–658.4)	83.8 (10.3–376.2)
ALT ($\times$ ULN)	21 (1.2–64)	23 (1.5–102.5)	18.3 (1.1–102.5)	20.5 (3.8–63.8)
Alkaline phosphatase ($\times$ ULN)	1.7 (0.2–3.5)	2.5 (0.5–11.1)	1.5 (0.5–3.1)	1.7 (0.2–3.7)

Abbreviation: ALT, alanine transaminase; DILI, drug-induced liver injury.
The ALT and alkaline phosphatase values are those at presentation whereas bilirubin values are the peak values recorded. Values are expressed as multiples of the upper limit of the normal range (ULN). Hypersensitivity features refers to the presence of fever, rash, eosinophilia and/or positive titres of autoantibodies.

Table 4 Demographic characteristics, clinical and biochemical parameters of DILI patients exposed to drugs biotransformed by CYP2C9 and/or CYP2C19

Sex/Age (years)	*CYP2C9 Substrates*	*Duration of therapy (days)*	*Time to onset (days)*	*Time to resolution (days)*	*Bilirubin (μM)*	*ALT (× ULN)*	*Alk Phos (× ULN)*	*Pattern of liver damage**	*Comments*	*CYP2C9 genotype*	*CYP2C19 genotype*
F/76	Diclofenac	93	30	120	50.5	18.3	2.38	HC		*1/*1	
M/21	Diclofenac	55	30	45	15.4	2.3	0.16	HC		*1/*1	
M/31	Tamoxifen/Stanozolol	64	62	85	465.1	4.9	3.32	Chol		*1/*1	
M/74	Losartan/Clarithromycin	7	20	65	68.4	43.0	0.78	HC		*1/*1	
M/82	Rofecoxib	60	59	90	44.5	6.1	5.13	Chol		*1/*3	
F/46	Irbesartan	274	234	60	58.0	44.4	1.44	HC	Rechallenge	*1/*3	
F/63	Gemfibrozil/Lovastatin	16	21		112.9	11,6	11,10	Chol	Chronicity	*2/*3	
F/49	Isoniazid	46	46	40	47.9	46,8	1,07	HC		*1/*1	
F/70	Diclofenac/Amox-Clavulanic	16	7	240	283.9	1,2	3,54	Chol	Chronicity	*1/*1	
F/56	Montelukast	15	15	28	126.6	41.3	1.62	HC		*1/*1	
F/80	Diclofenac	171	171	95	83.8	37.9	1.92	HC		*1/*2	
M/52	Ibuprofen/Amox-Clavulanic	6	12	41	128.3	4.6	3.00	Chol		*1/*1	
M/31	Naproxen/Cefaclor	3	3	83	68.4	3.3	1.54	Mixed		*1/*3	
F/61	Fluvastatin	1	1	30	17.8	4.2	1.00	Mixed	HPS, Rechallenge	*1/*2	
F/60	Fenofibrate/Raloxifene	15	14		189.8	6.7	1.70	Chol	Chronicity, rash	*1/*2	
M/48	Fluvastatin	1	1		188.1	4.9	2.46	Chol	HPS, Rechallenge	*1/*2	
F/53	Leflunomide	8	10	57	35.9	15.4	1.1	HC		*1/*1	
CYP2C9 and CYP2C19 Substrates											
F/44	Amitriptyline/ Clotiazepam	63	30	75	8.6	2.9	0.53	HC		*1/*2	*1/*1
F/57	Rip + Inh + Piz	78	30	180	5.1	3.5	1.57	Mixed		*1/*1	*1/*1
F/59	Ticlopidine	62	15	336	54.7	17.6	2.47	HC		*1/*3	*1/*1
M/17	Rip + Inh + Piz	49	45	53	376.2	63.8	0.74	HC		*1/*1	*2/*2
M/27	Phenytoin	18	13	50	8.6	102.5	1.24	HC	HPS	*1/*3	*1/*1
F/43	Fluoxetine	185	180	70	17.1	1.6	0.90	HC	Rechallenge	*1/*3	*1/*1
F/62	Rip + Inh	9	2	18	59.9	26.6	1.73	HC		*1/*3	*1/*2
M/52	Rip + Inh + Piz	62	60	32	95.8	56.8	0.95	HC		*1/*3	*1/*1
F/55	Sertraline/Amoxicillin	77	40	51	18.8	21.4	1.98	HC		*1/*2	*1/*1
F/49	Amitriptyline	82	82	90	10.3	5.9	0.80	HC		*1/*1	*1/*2
M/56	Ticlopidine	29	25	90	136.3	11.3	1.61	HC		*1/*1	*1/*1
CYP2C19 drug substrates											
M/65	Bentazepam	428	120	120	188.1	3.6	0.85	Chol	Chronicity		*1/*1
F/14	Carbamazepine	46	21	30	71.8	17.2	3.61	Mixed	HPS		*1/*2
F/47	Bentazepam	90	92	110	8.5	2.9	1.24	Mixed			*1/*1
F/34	Omeprazole	12	11	26	177.9	26.8	1.71	HC			*1/*1
M/60	Bentazepam	152	120	120	22.2	3.8	0.16	HC			*1/*2
F/33	Potassium Chloracepate	305	180	—	5.1	1.4	1.49	Chol	Chronicity		*1/*1
F/63	Tetrabamate	98	86	93	306.1	25.4	2.97	HC			*1/*1
M/44	Tetrabamate	21	15	—	254.8	2.7	3.06	Chol	Alcohol 100 g/d		*1/*1
M/35	Tetrabamate	215	120	90	17.1	12.6	2.13	HC			*1/*2
F/54	Indomethacin	11	8	43	17.1	16.2	3.65	HC			*1/*2
M/65	Tetracepam/Naproxen	44	4	135	76.9	7.5	3.07	Mixed			*1/*1
F/41	Paroxetine	617	617	60	6.9	5.2	0.69	HC	Chronicity		*1/*1
M/63	Omeprazole	8	5	27	6.9	4.9	0.50	HC			*1/*1
F/29	Carbamazepine	105	11	–	8.6	1.1	0.52	Mixed			*1/*1
F/38	Paroxetine/Alprazolam	1.096	1.096	150	658.4	51.5	1.16	Chol			*1/*1
M/54	Omeprazole	6	3	49	104.3	18.0	1.09	Chol	HPS		*1/*2
F/46	Propafenone	2	0	19	59.9	38.9	0.98	HC	HPS, Rechallenge		*1/*1
M/44	Citalopram	62	62	60	15.4	18.9	0.79	HC			*1/*1
M/70	Tetrabamate	994	0	50	10.3	4.2	0.57	HC			*1/*1
F/42	Tetrabamate	116	116	72	198.4	25.8	1.63	HC			*1/*1
M/60	Atenolol	1.432	1.432	180	54.7	1.2	3.12	Chol			*1/*1

Abbreviations: ALT, alanine transaminase; Alk Phos, alkaline phosphatase; Chol, cholestatic; DILI, Drug-induced liver injury; F, female; HC, hepatocellular; M, male. Values are expressed as multiples of the upper limit of normal (ULN). The bilirubin, ALT and Alk Phos values are those at presentation; INH + RIF, isoniazid plus rifampicin; INH + RIF + PIZ, isoniazid, rifampicin and pyrazinamide; CYP2C9 inhibitors: isonizid, ticlopidine; sertraline, fluvastatin. CYP2C9 inducer: Rifampin. CYP2C19 inhibitors: fluoxetine, omeprazol, ticlopidine, fluoxetine, indometacin; CYP2C19 inducers: carbamazepine, rifampin.

*Liver biopsy was performed. HPS = hypersensitivity features (refers to the presence of fever, rash, eosinophilia and/or detectable titres of autoantibodies). The pattern of liver damage is classified according to the criteria of the International Consensus Meeting for the drug-induced liver injury into hepatocellular or cholestatic and mixed types.

The study included 44 (88%) self-limited DILI cases and six patients with a chronic outcome. Among those with chronic outcome, two cases exhibited the variant CYP2C9*2 allele (Table 4).

Discussions and conclusions

The present study was undertaken to determine the potential genetic contribution of CYP2C9 and CYP2C19 polymorphisms in *bona fide* DILI cases and to explore if differences in incidence of variant alleles in this population might determine the susceptibility to develop DILI. This is the first study to establish the CYP2C9 and CYP2C19 allele frequencies in a large cohort of prospectively identified series of patients with a well-characterised diagnosis of hepatotoxicty caused by drugs or herbal medications. Watkins and Seeff (2006) stated that a successful hunt for genetic basis of susceptibility to drug toxicity would require at least 100 individuals clearly suffering from the same event. Undoubtedly, the creation and maintenance of a collaborative network of specialists into the Spanish Registry were essential in carrying out this project. Although we were able to collect DNA samples only from a subset of patients, the average demographic and clinical characteristics, type of liver injury, and causative drugs in our group of patients analysed did not differ from those seen in the cohort of patients recorded in the registry at the time of the analysis (Andrade *et al*., 2005)

Contrary to expectations, no significant differences in the distribution of variant CYP2C9 and CYP2C19 genotypes were found when results from the Spanish DILI group were compared with data from Caucasian subjects (García-Martin *et al*., 2001; Scordo *et al*., 2004). Overall, there was no significant difference for homozygotes, heterozygotes and compound heterozygotes for CYP2C9 and CYP2C19 isoforms, which are related to abnormal drug metabolism. The study group of DILI patients recorded in the Spanish Registry was at Hardy–Weinberg's equilibrium for overall defective alleles. In addition, no individuals homozygous for CYP2C9*3 and CYP2C19*3 (known to exhibit slow metabolic genotype) were identified in the study (Larrey and Pageaux, 1997; Kirchheiner and Brockmoller, 2005).

There were no sex differences among CYP2C9 and CYP2C19 genotypes. An association between specific genotypes and conventional risk factors such as age, duration of treatment, drug dosage, type of liver damage, liver biochemical parameters and disease outcome and severity (hospitalisation and chronic liver damage) could not be established.

We found no support for the hypothesis that the presence of CYP2C9 and CYP2C19 variant alleles might lead to increased risk of developing DILI. The distributions of the polymorphically expressed 2C9 and 2C19 enzymes have been suggested to have important clinical implications on hepatotoxicity for some individual drugs.

In our study, and contrary to the report of a single case, leflunomide-induced hepatotoxicity was described in a carrier of a wild-type CYP2C9*1/*1 genotype. Besides, in cases of tetrabamate related hepatotoxicity, all patients ($n=5$) except one (CYP2C19 *1/*2) exhibited a normally functioning CYP2C19 genotype, therefore not supporting the view that complete or partial deficiency of the CYP2C19 allele might influence its pathogenetic mechanism (Larrey, 2002). With regard to proton-pump inhibitors ($n=3$) studied, only one case of omeprazole induced hepatitis carrying the CYP219 *1/*2 genotype was detected.

CYP2C9 is known to mediate biotransformation of the majority of non-steroidal anti-inflammatory drugs (NSAIDs) such as diclofenac, ibuprofen, indomethacin, naproxen and COX-2 inhibitors by methyl-hydroxylation. Based on clinical reports and experimental studies, diclofenac has developed into a paradigm of possible NSAID hepatotoxicity and CYP2C9 was thought to be a promising probe candidate (Morin *et al*., 2001). However, the pharmacokinetics of diclofenac were shown to be independent of CYP2C9 polymorphism (Aithal *et al*., 2000; Yasar *et al*., 2001; Kirchheiner *et al*., 2003a, b). Moreover, when patients with hepatotoxicity induced by diclofenac and healthy controls were compared, the genotype frequency of variant CYP2C9 allele did not differ among groups. In the present study, when cases related to diclofenac liver damage were considered, only one patient out of three was heterozygous for the CYP2C9*2 allele, which is known to have no, or only moderate, functional effect (Miners and Birkett, 1996; Kirchheiner and Brockmoller, 2005), while the remaining subjects exhibited a normally functioning CYP2C9*1 allele. These data further support the view that CYP2C9 genotype does not contribute in the development of diclofenac-induced hepatotoxicity. However, a possible implication of other toxification/detoxification and immunological mechanisms should also be considered (Aithal *et al*., 2004b). Indeed, individual susceptibility to idiosyncratic hepatotoxicity is determined by the interaction of multiple metabolic pathways and immunological factors that might influence immune responsiveness and tissue injury. With regard to diclofenac hepatotoxicity, other multistep processes with overlapping polymorphisms in the activation (CYP2C8) and detoxification (UGT2B7) pathways along with immunological mechanisms (IL-10, IL-4 poylmorphisms) have been proposed (Aithal, 2004a; Aithal *et al*., 2004b).

In addition, when analysing cases of fluvastatin induced hepatotoxicity, these were found to be heterozygous for CYP2C9*2 allele ($n=2$). However, the CYP2C9*2 genotype has not been shown to affect significantly the pharmacokinetics of fluvastatin (Kirchheiner *et al*., 2003a, b). Indeed, these patients with fluvastatin hepatotoxicity received a high dose of fluvastatine (80 mg), and a dose-related risk of adverse drug effects has already been pointed out for fluvastatin (Siekmeier *et al*., 2001).

Recently, the importance of the expression of hepatic transporters (OATP and NTCP) involved in the hepatic uptake and clearance of statins has been outlined (Ho *et al*., 2006). This evidence emphaisizes the role of transport proteins as a key factor in the elimination of statins. The presence of marked interindividual variability in the expression of transport proteins through genetic polymorphisms and drug–drug interactions (Schneck *et al*., 2004) might be a contributing factor to the appearance of hepatic toxicity. The implications of transport proteins to drug disposition should be addressed during drug development.

It should be noted that the present study includes two cases of hepatotoxicity that could be ascribed to a drug–drug interaction such as with gemfibrozil/lovastatin (Schneck *et al*., 2004) and raloxifene/fenofibrate (Lucena *et al*, 2006).

The possible role of CYP2C family polymorphism on DILI outcome has not yet been described. It is worth noting that out of six chronic cases, one was a heterozygote for CYP2C9*2 (*1/*2) and another heterozygote for the CYP2C9 (*2/*3) allele.

In summary, genetic polymorphism of the CYP2C9 and CYP2C19 alleles do not enhance the risk of DILI overall. Indeed, determination of single-nucleotide polymorphisms (SNPs) in particular CYP enzymes may not be sufficient to predict risk of hepatotoxicity, as very many mulfactorial and multigenic processes seem to be involved in the complex causation of DILI. Further studies are needed to evaluate relevance of other markers in DILI development such as polymorphism of other toxifiying and detoxification pathways and to identify currently unknown factor(s) responsible for the occurrence of DILI, a condition likely to be multifactorial and multigenic.

In conclusion, the current study has provided preliminary evidence that genetic polymorphisms in CYP2C9 and CYP2C19 are not related to DILI, a conclusion that needs to be substantiated in a larger number of patients.

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Conflict of interest

The authors state no conflict of interest

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Appendix A

On behalf of the Spanish group for the Study of Drug-Induced Liver Disease (GEHAM)

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Hospital de Puerto Real, Cádiz: JM Pérez-Moreno, M Puertas.

Hospital Universitario San Cecilio, Granada: J Salmerón, A Gila.

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H Sant Pau, Barcelona: C Guarner, D Monfort.

Hospital Carlos Haya, Málaga: M Jiménez-Pérez.

Hospital Xeral-Calde, Lugo: S Avila-Nasi.

Hospital de Donosti, San Sebastián: M García-Bengoechea.

Hospital de Mendaro, Guipuzcuoa: A Castiella.

Hospital de Basurto, Vizcaya: S Blanco.

Hospital Clinic, Barcelona: M Bruguera

Hospital Morales Messeguer: H Hallaf

Hospital Clínico Universitario Miguel Servet, Zaragoza: MA Simón.

Hospital Juan Ramón Jiménez, Huelva: M Ramos, T Ferrer.

Hospital Ciudad de Jaén: E Baeyens.

Hospital de Osuna, Sevilla: J Pérez-Martínez.

Hospital General Básico de Vélez, Málaga: F Santalla, C Sánchez-Robles.

Hospital Gregorio Marañon, Madrid: R Bañares.

Hospital General de Valencia: M Diago.

Hospital Sagunto, Valencia: J Primo, JR Molés.