Glutathione *S*-Transferase M1 and T1 Null Genotypes Increase Susceptibility to Idiosyncratic Drug-Induced Liver Injury

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Individual vulnerability to drug-induced liver injury (DILI) might result from deficiencies in the detoxification process, which determines the level of exposure to the reactive metabolite. We evaluated whether a genetically determined reduction in the ability to detoxify electrophilic compounds, such as that expected among individuals with glutathione *S*-transferase (GST) null genotypes, might play a role in determining the risk for DILI and its clinical expression. Genomic DNA from 154 patients (74 men, 80 women; mean age, 53 years) with a diagnosis of DILI as assessed with the Council for International Organizations of Medical Science scale and 250 sex- and age-matched healthy controls were analyzed. A multiplex polymerase chain reaction–based method was used to detect *GSTM1* and *GSTT1* gene deletions. Carriers of double *GSTT1-M1* null genotypes had a 2.70-fold increased risk of developing DILI compared with noncarriers (odds ratio 2.70, 95% confidence interval 1.45-5.03; $P = 0.003$). The odds ratio for DILI patients receiving antibacterials, and NSAIDs were 3.52 ($P = 0.002$), and 5.61 ($P = 0.001$), respectively. Patients with amoxicillin-clavulanate hepatotoxicity (n = 32) had a 2.81-fold increased risk ($P = 0.037$). Patients classified by the combined *GSTT1* and *GSTM1* null genotypes did not differ with regard to the type of injury, clinical presentation, or outcome, except for the predominance of women in the combined null genotype ($P < 0.001$). *Conclusion:* The double-null genotype for *GSTT1* and *GSTM1* might play a role in determining the susceptibility to develop DILI, as a general mechanism that occurs regardless of the type of drug involved, and predominantly in women. (HEPATOLOGY 2008;48:588-596.)

Idiosyncratic drug-induced liver injury (DILI) is a clinical challenge due to the rarity of its diagnosis and the lack of a gold standard, which makes determination of causality difficult.[1] Efforts to enhance the identification of adverse hepatic reactions and to obtain reliable information are being made in the setting of collaborative networks.[2,3] In spite of these efforts, the genetic and environmental factors that appear

Abbreviations: CI, confidence interval; CIOMS, Council for International Organizations of Medical Science; CYP, cytochrome P450; DILI, drug-induced idiosyncratic liver injury; GST, glutathione S-transferase; NSAID, nonsteroidal anti-inflammatory drug; OR, odds ratio; PCR, polymerase chain reaction.

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to determine individual susceptibility to DILI are still poorly understood.

Cytochrome P450 (CYP) plays a prominent role in phase I metabolism representing the major pathway for drug oxidation. The general consensus on the pathogenesis of DILI is that parent compounds are rendered hepatotoxic as a consequence of CYP metabolism. However, recent data have demonstrated that genetic polymorphisms for CYP enzymes known to be involved in the metabolism of hepatotoxic drugs, such as CYP2C9 or CYP2C19, are not major risk factors for DILI,[4] suggesting that the role of CYP enzymes in DILI may be less important than initially expected. This raises the hypothesis that hepatotoxicity may be associated with the rate of biotransformation of reactive metabolites rather than the rate of parent drug metabolism.[5] Individual vulnerability to a drug might then result from deficiencies in the detoxification process, or in drug transporters which ultimately determine the level of exposure to the reactive metabolite. In this regard, glutathione *S*-transferase (GST), a major phase II family of conjugation enzymes, plays a crucial role in the detoxifying mechanisms of drugs and xenobiotics by preventing the binding of reactive metabolites to cellular proteins and modulating the by-products of oxidative stress catalyzing the conjugation of electrophilic moieties to glutathione.[6]

Several independent studies in animal models support a role for GST activity in the prevention of chemically induced hepatotoxicity.[7,8] In addition, resistance to hepatotoxicity seems to be mediated by increased expression of GST,[9] whereas increased risk for hepatotoxicity is related to decreased enzyme expression[10] or glutathione depletion.[11,12] Interestingly, such a mechanism seems to act for a variety of metabolites derived from aflatoxin, acetaminophen, benzo[a]pyrene, bromobenzene, or felbamate, among other substances,[8-10,12-14] thus underlying the role of GST as a general detoxification mechanism.

In humans, the activity of the cytosolic GSTs T1 (θ) and M1 (μ) are polymorphically expressed due to complete *GSTM1* and *GSTT1* gene deletions that occur in homozygosity (null genotypes) in 50% and 10% to 25% of Caucasian subjects, respectively, that cause abolished metabolizing capacity.[15] The prominent role of GSTM1 and GSTT1 enzymes as a detoxification system in humans is supported by the association of null genotypes with environmentally related cancers,[16,17] alcoholic liver disease,[18] and even facilitation of chronic hepatitis C virus infection.[19]

With regard to drug hepatotoxicity, a role for *GSTM1* or *GSTT1* null genotypes has been suggested in independent studies involving small groups of patients receiving a variety of drugs, such as antituberculosis drugs, tacrine, or troglitazone.[20-22] Taken together—and consistent with studies in animal models—these findings point to a role for these enzyme activities as a general protecting mechanism against hepatotoxicity.

To test the clinical relevance of such a hypothesis, we aimed to determine whether a genetically determined reduction in the ability to detoxify electrophilic compounds, such as that expected among individuals with *GST* null genotypes, might play a role in determining or predicting the risk for DILI and its clinical course in a large sample of patients with hepatotoxicity related to a wide variety of drugs.

Patients and Methods

Study Protocol. Cases of DILI were selected from those submitted to the Spanish Registry, which has been in use in southern Spain since 1994, and were coordinated by two of the authors (R.J.A. and M.I.L.). The operational structure of the registry, data recording, and case ascertainment have been reported elsewhere.[2]

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) outcome of liver damage.

All submitted cases were 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.[23]

The pattern of liver injury was classified according to International Consensus Meeting criteria.[24] The liver tests used for the classification of liver damage were the first blood tests available after liver injury. Alternatively, liver damage was determined on the basis of liver biopsy findings when available. Severe damage was considered if jaundice and prothrombin activity $<50\%$ were present.

The drugs responsible for hepatic reactions were classified according to the Anatomic Therapeutic Classification recommended by the World Health Organization. Cases were classified as hypersensitive if any of the following clinico-laboratory findings were present: fever, rash, serum eosinophilia, cytopenia, or pathological findings (eosinophil-rich infiltrates and/or granulomas) on biopsy specimens. Outcome was assessed by clinical, biochemical, and imaging tests and histological findings when available. Cases were classified as resolved when liver tests

had normalized within 3 months for hepatocellular damage or 6 months for a cholestatic/mixed injury; cases were classified as chronic when liver tests remained altered.[25]

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." All DILI patients were Caucasian. Excluded were cases secondary to drug overdose (acetaminophen) and occupational exposure to toxins.

A total of 154 patients (74 men, 80 women) participated in the study. The mean age was 53 years.

As a control group for GST genetic polymorphism analyses, we selected 250 unrelated Caucasian subjects who were sex-matched and age-matched within 1 year to the patients analyzed. Control subjects were selected among medical students and the staff of the University of Extremadura, Spain. Medical examination and history were obtained from each individual to exclude pre-existing disorders. To check the suitability of the healthy control population chosen, an additional group composed of 88 drug-matched controls that did not experience any adverse effect (64 individuals receiving amoxicillin clavulanate for mean duration of 10 days [range, 6-14 days] at a mean dose of 1820 mg/day, and 24 individuals receiving different classes of nonsteroidal anti-inflammatory drugs [NSAIDs] included in this study) were also included in the study. The study protocol was approved by the local ethics committee of the coordinating center at the Virgen de la Victoria University Hospital in Málaga, Spain, and all the subjects who took part in the study gave informed consent.

DNA Extraction. Venous blood was obtained in tubes containing K3-EDTA from each subject and DNA was extracted as described previously.[4]

Determination of GSTM1 and GSTT1 Genotypes. A multiplex polymerase chain reaction (PCR) assay was used to determine the GSTM1 and *GSTT1* genotypes. Because single-nucleotide polymorphisms leading to functional changes of GSTM1 and GSTT1 enzymes have not been identified in Caucasian subjects,[26,27] we analyzed deletions for both genes. PCR reactions were performed in a final volume of 12 μL. The primers used were those described by Xiong et al.[28] PCR products of 480, 215, and 280 bp revealed the presence of *GSTT1*, *GSTM1*, and *DHFR*, respectively. If none of these PCR products were present, the samples should be discarded because *DHFR* is an essential gene that should amplify in all samples. In the present study, the call rates for *DHFR* (that is, samples that were amplified) were 100% for both cases and controls.

Statistical Analysis. Genotypic frequencies of *GSTM1* and *T1* polymorphic variants were compared between DILI patients and controls using a chi-squared test.

Means were compared via Student *t* test for independent samples. Analysis of variance was used for comparison of groups. Where variables did not follow a normal distribution, a nonparametric analyses Kruskal-Wallis test was performed. The gene–dose effects were calculated using the chi-square test for trend.[29]

Odds ratios (OR) and 95% confidence intervals (CI) were calculated to assess the relative disease risk conferred by a specific genotype.

Analyses were performed using SPSS version 12.0 (SPSS Inc., Chicago, IL). $P < 0.05$ was considered significant. To account for the problem of significant associations arising by chance after multiple comparisons, the Bonferroni correction for multiple tests was applied by multiplying the probability value (p) by the number of genotypes compared (n = 2) to yield a corrected *P* value (pc).

The statistical power of the study was evaluated as with a genetic model, analyzing the frequency for carriers of the disease gene with an RR value = 2 (type I error = 0.05), as recommended for pharmacogenomic studies.[30,31] According to the sample size and genotype frequencies, the power calculated for a bilateral association is as follows: association with the *GSTM1* null polymorphism, 92.0%; association with the *GSTT1* null polymorphism, 86.5%.

Results

Clinical Characteristics of DILI Patients. Among the 154 DILI patients, hypersensitivity features were found in 39. The predominant lesion pattern was hepatocellular (n = 75). Most of the cases were classified as definitive (n = 84); 70 cases were considered probable according to the CIOMS scale. The main causative therapeutic group of drugs was anti-infectives for systemic use (n = 49), followed by drugs used in the central nervous system (n = 24), NSAIDs (n = 19), and drugs used for cardiovascular therapy (n = 17). Amoxicillin-clavulanic acid was the treatment responsible for the highest number of cases (n = 32). There was a favorable clinical outcome in 135 patients, and a worse outcome (fulminating hepatic failure, liver transplantation, death) was found in 4 patients. Fifteen patients fulfilled the criteria of chronicity.

Genetic Polymorphisms of GSTT1 and GSTM1. Table 1 shows the *GSTT1* and *GSTM1* genotype distribution among all DILI patients and healthy control subjects. The frequencies for carriers of *GSTM1* or *GSTT1* null genotypes were increased in DILI patients compared with healthy subjects. The increased frequency of *GSTM1* among patients reached statistical significance

Table 1. *GSTM1* and *GSTT1* Genotype Distribution and Number of GST Active Genotypes in DILI Patients and in Healthy Controls

	GSTM1 Genotype, n (%)		*GSTT1* Genotype, n (%)		No. of Active Genotypes, n (%)		
	Null	Active	Null	Active	Two	One	None
Cases (154)	86 (55.8)	68 (44.2)	45 (29.2)	109 (70.8)	51 (33.1)	75 (48.7)	28 (18.2)
Controls (250)	113 (45.2)	137 (54.8)	58 (23.2)	192 (76.8)	97 (38.8)	134 (53.6)	19 (7.6)
Statistics							
OR (95% CI)	1.53 (1.02–2.30)		1.37 (0.87–2.15)		0.78 (0.51–1.19)	0.82 (1.02–0.63)	2.70 (1.45–5.03)
P value	0.043		0.197		0.365	0.272	0.002
Pc*	0.085		0.394		0.544	0.730	0.003

Test for trend for null alleles: chi-square = 10.45, $P = 0.005$.
*Pc after Bonferroni's correction.

when analyzed in isolation, but not after Bonferroni's correction. Because it has been proposed that hepatotoxicity is linked to the combination of *GSTM1* and *GSTT1* genotypes,[8-10,12-14] we analyzed the number of active genotypes (Table 1). Among patients with DILI, the frequencies of carriers of double-null (*GSTM1* and *GSTT1* null) genotypes is higher than in control subjects (OR = 2.70; $P = 0.002$). The test for trend with the number of null genotypes also revealed statistically significant differences among DILI patients and controls ($P = 0.005$), thus indicating that the presence of the combined *GSTT1-M1* null genotype is a risk factor for enhanced susceptibility to DILI in the drugs studied in this series. The frequencies for two, one, and no active genotypes for *GSTM1* and *GSTT1* did not differ between the drug-matched control group (38.6%, 53.4%, and 8%, respectively), and the larger control group matched for sex and age. Among patients with amoxicillin-clavulanate or NSAID-induced DILI, the frequencies of carriers of double-null (*GSTM1* and *GSTT1* Null) genotypes was higher than in the drug-matched control subjects; however, these differences did not reach statistical significance, because the power of the associations relates to the large control group (Appendix).

The distribution of the number of active genotypes of *GSTT1* and *GSTM1* in the main pharmacological group of drugs involved in drug-induced idiosyncratic hepatotoxicity is shown in Table 2. There was a predominance of carriers of null alleles in the main pharmacological group of drugs involved in DILI. The odds ratio (95% CI) for carriers of double-null alleles are as follows. Among patients receiving anti-infectives (n = 49), the OR was 3.12 (CI 1.37 to 7.11; $P = 0.006$). Among these, it is noteworthy that among the 5 patients receiving antituberculosis drugs, none was a carrier of the double-null genotype. The rest of the patients receiving antibacterials (n = 44) displayed an OR of 3.52 (CI 1.56 to 8.22; $P = 0.002$). The test for trend with the number of null genotypes in this group also show statistically significant differences compared with control subjects ($P = 0.008$). When the analysis of GST polymorphisms was confined to the 32 patients with amoxicillin–clavulanate-related hepatotoxicity (Table 2), the double-null genotype conferred a significant risk with an OR of 2.81 (CI 1.06 to 7.46; $P = 0.037$).

Among patients receiving NSAIDs (n = 19), the OR was 5.61 (CI 1.99 to 16.0; $P = 0.001$), and the test for trend with the number of null genotypes was $P = 0.002$. Among patients receiving drugs used in the central nervous system (n = 24), the OR for carriers of double-null genotypes was 1.74 (CI 0.51 to 5.99; $P = 0.400$, test for trend $P = 0.199$). Among patients receiving drugs used for cardiovascular therapy (n = 17), the OR for carriers of double-null genotypes was 3.74 (CI 1.18 to 12.08; $P = 0.024$, test for trend $P = 0.059$). Among patients receiving antineoplastic and immunosuppresive agents and endocrine therapy (n = 12), the OR for carriers of double-null genotypes was 1.11 (CI 0.18 to 7.12; $P = 0.926$, test for trend $P = 0.135$). Finally, among patients receiving the rest of the drugs (n = 33), the OR for carriers of double-null genotypes was 1.68 (CI 0.56 to 5.06; $P = 0.373$, test for trend $P = 0.670$).

A description of demographic, clinical, and biochemical findings and outcome data in DILI patients classified by the combined *GSTM1* and *GSTT1* genotypes is shown in Table 3. No differences in any of the clinical or laboratory variables evaluated affected the main findings in the present study (i.e., the increased frequency for null alleles among DILI patients). No differences in the frequencies for *GST* genotypes were found among subgroups of DILI patients except for the presence of a significantly higher number of women among patients with double-null genotype ($P < 0.001$). The time to onset and duration of treatment were independent of genotype. No differences were found in *GST* genotypes when comparing the cases

Table 2. Distribution of the Number of Active Genotypes of *GSTM1* and *GSTT1* in the Main Pharmacological Class of Drugs Involved in Idiosyncratic Hepatotoxicity

	GSTM1/GSTT1		
	W/W (51)	W/N + N/W (75)	N/N (28)
Anti-infectives for systemic use			
Antibacterials (n = 44)			
Amoxicillin-clavulanate (n = 32)	12	14	6
Macrolides (n = 4)	1	3	0
Quinolones (n = 3)	1	0	2
Other* (n = 5)	1	2	2
Drugs for treatment of tuberculosis (n = 5)	1	4	0
NSAIDs			
Causative drugs			
Acetylsalicylic acid (n = 1)	0	0	1
Diclofenac (n = 4)	0	2	2
Ibuprofen (n = 5)	3	2	0
Indomethacin (n = 1)	1	0	0
Naproxen (n = 1)	0	1	0
Nimesulide (n = 5)	1	1	3
Ketorolac (n = 1)	0	1	0
Rofecoxib (n = 1)	1	0	0
Central nervous system			
Antiepileptics (n = 4)	0	3	1
Anxiolytics (n = 6)	1	4	1
Antidepressants (n = 6)	1	4	1
Other† (n = 8)	3	5	0
Cardiovascular system			
ACE inhibitors + ARAII (n = 6)	1	3	2
Serum lipid reducing agents (n = 10)	2	6	2
Other‡ (n = 1)	1	0	0
Drugs for peptic ulcer (n = 8)	3	5	0
Antineoplastic agents, immunosupressive agents, and endocrine therapy			
Causative drugs			
Asparaginase (n = 1)	1	0	0
Azathioprine (n = 4)	3	0	1
Leflunomide (n = 2)	0	2	0
Flutamide (n = 5)	4	1	0
Herbal plants (n = 4)	1	3	0
Other§ (n = 21)	8	9	4

Herbal plants: *Camellia sinensis,* kava, valerian, *Cassia angustifolia.* Drugs for peptic ulcer: ebrotidine, omeprazol, ranitidina.

Abbreviations: ACE, angiotensin-converting enzyme; ARAII, angiotensin receptor antagonist II; N/N (null/null); W/N + N/W (wild/null + null/wild); W/W (wild/wild).

*Amoxicillin, cefaclor, ceftriaxone, cefuroxime, and minocycline.

†Chlorpromazine, ciclobenzaprina, clomethiazole, metamizole, paracetamol, risperidone, and tetrabamate.

‡Propafenone.

§Alendronic acid, carbimazole, cinitapride, clomifene, clopidogrel, danazol, ethinylestradiol, extasis, finasteride, montelukast, repaglinide, stanozolol, sulfasalazine, tibolone, ticlopidine, transilat, zafirlukast.

according to the presence or absence of any of the classic hypersensitivity features.

It is noteworthy that 15 out of the 21 women with DILI that were carriers of a double-null genotype were ≥45 years of age. However, 40 out of the 59 women with DILI that were carriers of other genotypes were aged >45 years of age. These differences were not statistically significant ($P = 0.759$). Five of the seven men with a double-null genotype were also in the same age category. The associated conditions in these patients with double-null genotype were dyslipidemia (two patients), arterial hypertension (six patients), hypothyroidism (one patient), osteoarthritis (one patient), and diabetes mellitus (one patient). No underlying disease was noticeable in the remaining patients.

Discussion

Available data support a crucial role of genetic factors in determining the susceptibility to DILI. Currently, the cooperative efforts of several groups in recruiting clinical data with a systematic collection of genomic DNA from patients with well-defined diagnosis of DILI is clearly the best way to progress in understanding the underlying mechanisms and the key to predicting and preventing DILI. Previous studies performed in patients with DILI related to single agents such as tacrine and troglitazone[20-22] have identified a statistically significant prevalence of the combined *GSTM1-T1* double-null genotype in these patients. However, considering the large body of evidence obtained *in vitro* that indicates that GST enzymes are likely to play a prominent role as a general detoxification mechanism preventing hepatotoxicity,[8-10,12-14] the lack of clinical studies addressed to analyze *GST* genotypes in DILI patients receiving diverse types of drugs is surprising. This is the first study to demonstrate that the combined *GSTM1-T1* double-null genotype is an independent risk factor for the development of DILI as a general mechanism that occurs for several types of drugs. These findings support the hypothesis for a role of GST enzymes as a general mechanism involved in protection against hepatotoxicity. Moreover, when the analysis was restricted to cases of hepatotoxicity associated with amoxicillin–clavulanate, the drug solely responsible for the highest number of cases in most of the prospective large case series published in Western countries,[2,3,32,33] the results mirrored those found in the entire DILI population, suggesting that the presence of combined alleles *M1* and *T1* deficiency in GST genes is also a risk factor for the susceptibility to amoxicillin–clavulanate hepatotoxicity. In addition, among patients receiving NSAIDs, the effect of the double-null genotype was consistent in cases of diclofenac-induced and nimesulide-induced hepatotoxicity.

The oxidative damage in the liver of DILI patients could be a consequence of cytosolic oxidant stress generated from drug metabolism or could arise from oxidant stress directly generated in mitochondria. Indeed, reactive

Table 3. Comparison of Demographics and Clinical and Laboratory Findings in DILI Patients Classified by Combined *GSTM1* and *GSTT1*

Characteristics of Patients	*GSTM1/GSTT1*		
	W/W (n = 51)	W/N + N/W (n = 75)	N/N (n = 28)
Mean age (range), years	55 (15–82)	53 (14–83)	52 (21–83)
Sex (male/female)	28/23	39/36	7/21*
Time to onset, mean ± SD, days	39 ± 59	81 ± 175	108 ± 180
Duration of treatment, mean ± SD, days	48.4 ± 84.5	99.0 ± 193.0	114.9 ± 172.2
BMI, mean ± SD, kg/m^2	26.6 ± 3.3 (n = 26)	25.6 ± 3.8 (n = 44)	28.9 ± 4.9 (n = 18)
Clinical presentation, n (%)			
Jaundice	34 (67)	50 (67)	16 (57)
Hospitalization	28 (55)	38 (51)	11 (39)
Hypersensitivity features	10 (20)	21 (28)	8 (29)
Type of damage, n (%)			
Hepatocelular damage	23 (45)	39 (52)	13 (46)
Cholestatic damage	16 (31)	16 (21)	9 (32)
Mixed damage	12 (24)	20 (27)	6 (21)
Laboratory parameters, mean ± SD			
Total bilirubin, mg/dL	7.4 ± 6.5	7.9 ± 8.8	8.6 ± 11.2
ALT, × ULN	15.0 ± 15.9	18.1 ± 21.5	11.0 ± 13.4
AP, × ULN	2.5 ± 2.2	3.2 ± 8.3	3.0 ± 4.7
Severity, n (%)			
Death/fulminant damage/transplantation	0 (0)	3 (4)	1 (4)
Severe damage	1 (2)	4 (5)	0 (0)
Clinical course			
Recovery, mean ± SD, days	66.6 ± 47.0	100.7 ± 114.9	71.2 ± 51.5
Chronic outcome, n (%)	3 (6)	11 (15)	1 (4)

Abbreviations: ALT, alanine aminotransferase; AP, alkaline phosphatase; BMI, body mass index; N/N (null/null); SD, standard deviation; ULN, upper limit of normal; W/N + N/W (wild/null + null/wild); W/W (wild/wild).

*OR 3.41 (95% CI 1.38–8.38; $P = 0.007$) compared with men with DILI; OR 4.39 (95% CI 1.91–9.70; $P < 0.001$) compared with healthy control women.

oxygen species could be metabolism derived, and important examples could be provided.[34] The metabolism of troglitazone at the thiazolidinedione ring leads to the formation of glutathione adducts.[34] The benzoquinone metabolite that results from nefazodone metabolism is conjugated with glutathione. The cytosolic GSTs interact with an oxidative microsomal metabolite of iproniazid to enzymatically produce a glutathione conjugate that inhibits its covalent binding.[34] Flutamide decreased glutathione and protein thiols in hepatocytes suspensions *in vivo*. Collectively, these data suggest that in patients with an underlying genetic impairment in GST enzyme activity—those with combined *GSTT1/GSTM1* genotypes—an endogenous antioxidant deficiency may occur, leading to idiosyncratic liver damage. If the antioxidant defense is compromised, this might shift the pro-oxidant/antioxidant balance toward an increased oxidant stress that sensitizes these individuals to the protoxicant effects of drugs leading to critical sulfhydryl oxidation or the activation of cell death signaling pathways.

Putative confounders in the present study were evaluated. It could be argued that using serum alanine aminotransferase levels > 2 times the upper limit of normal to define hepatic injury according to CIOMS may allow for the inclusion of cases with minor and nonspecific alterations in liver tests not representative of DILI (i.e., nonalcoholic fatty liver disease). Recently, a more stringent threshold using serum aminotransferases levels > 5 times the upper limit of normal as the enrollment criterion of DILI is being used by the Drug-Induced Liver Injury Network.[35] Indeed, DILI cases included in this study were rather severe, and only seven cases had increases in serum alanine aminotransferase levels < 5 times the upper limit of normal. An association between at-risk genotypes and conventional risk factors such as age, duration of treatment, time to onset, drug dosage, type of liver damage, liver biochemical parameters, and disease outcome and severity (hospitalization and chronic liver damage) could not be identified, except for a very significant predominance of women in the restricted group of patients with the *GSTM1* and *GSTT1* double-null genotype. This sex-specific susceptibility to oxidative stress in idiosyncratic DILI is an interesting finding. Indeed, most of the women with a combined *GST* null genotype were older than 45 years of age. Estrogens have been found to exert protective effects against oxidative stress in some tissues[36] and, in addition, *GSTM1* null smokers women may have higher risk than men for the development of lung cancer.[37] It could therefore be speculated that postmenopausal women with the double-null genotype are more susceptible to DILI than age-matched men. Independent studies indicating that GST

genotypes may be linked to hepatocellular liver injury were conducted in patients who used drugs such as tacrine, antituberculous drugs, carbamazepine, and troglitazone, which are almost exclusively linked to hepatocellular liver damage.[5,21,22,38] In the present study, we could not establish an association between type of liver damage and the combined genetic polymorphism exhibited by *GSTM1* and *GSTT1*, suggesting that this genetic factor may not be relevant to the mechanism leading to either hepatocellular or cholestatic mixed type of injury.

Another putative confounder may be related with the incidence of diabetes mellitus. Diabetes mellitus is associated with an increased production of reactive oxygen species and a reduction in antioxidant defenses. In this regard, it has been shown that the combined wild *GSTM1-T1* genotypes conferred a significant reduction in risk of diabetes.[39] In addition to diabetes, obesity and fatty liver have been shown to result in chronic oxidant stress and mitochondrial dysfunction.[40] However, the mean body mass index in our population was within normal limits across different GST genotype groups, and the prevalence of diseases typically associated with diabetes in the DILI patients with the combined null GST genotype was close to that expected in an adult Caucasian population. Therefore, it is unlikely that these diseases could account for the differences observed.

In summary, the double-null *GSTM1* and *GSTT1* genotype might play a role in determining the susceptibility to develop DILI regardless of the type of drug involved and predominantly in women. However, determination of *GST* genotypes do not explain all DILI cases, because a mulfactorial and multigenic processes seems to be involved in complex DILI course including those involved in cellular signaling, adaptation, and regeneration/repair processes. The mitochondrial enzyme manganese superoxide dismutase (*MnSOD*, *Sod2*) is the major scavenger of mitochondrial superoxide. Interestingly, Ong and colleauges[41,42] have recently demonstrated using a genetic mitochondrial alteration to manipulate the mitochondrial redox—the heterozygous *Sod 2* knockout mice—that these mice were sensitive to the mitochondria-damaging effects of prolonged administration of the nitroaromatid drug nimesulide[41] and of the thiazolidinedione troglitazone,[42] developing delayed oxidative mitochondrial injury and hepatic necrosis, resembling the clinical picture that these drugs produce in susceptible patients. Recently, the *MnSOD* mutant C allele has been shown to increase the susceptibility to DILI in Chinese patients.[20] Future studies are needed to evaluate the relevance of *MnSOD* polymorphisms in DILI development.

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Appendix

Distribution of *GSTM1* and *GSTT1* Genotypes Among Amoxicillin–Clavulanate Hepatotoxicity Cases and Drug-Matched Controls

	No. of Active Genotypes, n (%)		
	Two	One	None
Amoxicillin–clavulanate cases (32)	12 (37.5)	14 (43.8)	6 (18.8)
Amoxicillin–clavulanate controls (64)	25 (39.1)	33 (51.6)	6 (9.4)
Statistics			
OR (95% CI)	0.94 (0.39–2.24)	0.73 (1.04–0.42)	2.23 (1.43–3.03)
P value	0.970	0.540	0.252

Distribution of *GSTM1* and *GSTT1* Genotypes Among NSAID Hepatotoxicity Cases and Drug-Matched Controls

	No. of Active Genotypes, n (%)		
	Two	One	None
NSAID cases (18)	6 (33.3)	7 (38.9)	5 (27.8)
NSAID controls (24)	9 (37.5)	14 (58.3)	1 (4.2)
Statistics			
OR (95% CI)	0.83 (0.23–3.00)	0.46 (1.24 to −0.33)	8.85 (6.67–11.03)
P value	0.907	0.275	0.052

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