**Role of chemical structures and the 1331T>C bile salt export pump polymorphism in idiosyncratic drug-induced liver injury**

**Running title:** Chemical structures, *ABCC11* and DILI

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**Abstract:** 232 words

**Main text:** 3143 words

**Number of tables:** 5

**Supplementary tables:** 2

**Number of figures: 1**

***Conflict of Interest:*** None

**Financial Support:**

This study was supported, in part, by research grants from the *Agencia Española del Medicamento* and *Fondo de Investigación Sanitaria* (PS 09/01384). CIBERehd and [c]Red Genómica del Cáncer are funded by Instituto de Salud Carlos III.

**Abstract**

*Background:* Several pharmaceutical compounds have been shown to exert inhibitory effects on the bile salt export pump (BSEP) encoded by the *ABCB11* gene. *Aims:* We analysed the combined effect on drug-induced liver injury (DILI) development of the *ABCB11* 1331T>C polymorphism and the presence of specific chemical moieties, with known BSEP inhibiting properties, in the causative drug. *Methods:* Genotyping using a TaqMan 5´ allelic discrimination assay was performed in 188 Spanish DILI patients, 219 healthy controls and 91 sex, age and drug-matched controls. A chemical structure analysis was performed for each individual causative drug. *Results:* The CC genotype was significantly associated with hepatocellular damage (odds ratio (OR)= 2.1, *P*= 0.001), particularly in NSAID DILI cases (OR= 3.4, *P*= 0.007). In addition, the CC genotype was found to be significantly linked to DILI development from drugs causing <50% BSEP inhibition (OR= 1.8, *Pc*=0.011). Of the BSEP inhibitory chemical moieties 59% of the causative drugs contained a carbocyclic system with at least one aromatic ring, corresponding to 61% of the total cases. The C allele was significantly more frequent in DILI cases containing this chemical moiety, which appear to be conditioned on the *ABCB11* 1331T>C polymorphism in the absence of other BSEP inhibitory structures. *Conclusion:* Patients carrying the C allele in the *ABCB11* 1331T>C polymorphism are at increased risk of developing hepatocellular type of DILI, when taking drugs containing a carbocyclic system with aromatic rings.

**Key words:** Hepatotoxicity, *ABCB11*, canalicular transporter, pharmacogenetics, aromatic ring structure

**Introduction**

Hepatic membrane transporters play a major role in drug metabolism. Their ability to modify drug pharmacokinetics due to altered protein function, potentially influencing the level of cellular exposure to reactive metabolites, also make the transporters important toxicological targets. The bile salt export pump (BSEP), an ATP-binding cassette transporter encoded by the *ABCB11* gene, is found on the apical membrane of the hepatocyte. It is responsible for biliary

excretion of bile salts (predominantly in the form of glycocholate and taurocholate) into the bile canaliculi. Drugs or drug metabolites interacting with the BSEP transporter function could be a potential mechanism for the development of drug-induced liver injury (DILI). Indeed, drugs such as troglitazone, cyclosporin A, glibenclamide and bosentan have been shown to inhibit taurocholate transport *in vivo* as well as in canalicular membrane vesicles prepared from rat livers (1-4). Furthermore, Morgan and co-workers have suggested a correlation between the potency for BSEP vesicle transport inhibition and human hepatotoxicity after assessing more than 200 benchmark compounds (5).

Several polymorphisms in the *ABCB11* gene leading to decreased protein expression and subsequently impaired transport capacity, have been identified as the molecular basis of inherited and acquired cholestatic syndromes (6-8). A common polymorphism in exon 13 (c.1331T>C, rs2287622) is associated with reduced level of mature protein in C allele carriers, and have been linked to increased intrahepatic cholestasis of pregnancy and contraceptive-induced cholestasis susceptibility (9-11). Further insight into the role of BSEP in drug-induced cholestasis came from a study of 36 central European DILI patients caused mainly by antibiotics, hormonal therapy and proton pump inhibitors. In this study the C allele, which is the more frequent allele in Caucasians, was significantly more frequent in cases with drug-induced cholestatic type of liver injury (76%) than in those with hepatocellular type of injury (50%) and healthy controls (59%) (12).

Theoretically, subjects with a genetically determined decrease in BSEP expression might be more susceptible to developing hepatotoxicity when exposed to drugs containing chemical structures with BSEP inhibiting properties. Recently, Hirano and co-workers developed a quantitative structure-activity relationship (QSAR) analysis method to investigate the interaction of BSEP with a variety of drugs using chemical fragmentation codes (CFCs), representing specific fragments or moieties in the drug's chemical structure. This method estimates *ABCB11*-mediated taurocholate transport inhibition as a linear equation of the

combined CFCs present in a specific drug (13). Hence, this method may be used to assess the BSEP inhibition level caused by drugs with known chemical structures. In this study, we aimed to analyse the influence of the *ABCB11* c.1331T>C polymorphism on DILI development and its clinical expression, and to examine whether drugs with specific chemical moieties enhance the effect of allelic variations and subsequently the susceptibility to DILI.

## Patients and Methods

### *Subjects and Study Protocol*

Cases of DILI were selected from those submitted to the Spanish DILI Registry, a collaborative network established in 1994 to prospectively identify cases of DILI in a standardized manner. The criteria for DILI were: an increase in alanine aminotransferase (ALT) ≥3 times the upper limit of normal (ULN) or ≥2 times the ULN of alkaline phosphatise (ALP) or total bilirubin (TB) ≥2 times the ULN if associated with any elevation of ALT or ALP. The pattern of liver injury was classified based on R value calculations as previously described (14). A detailed description of the operational structure of the registry, data recording and case ascertainment has been reported elsewhere (15).

As a control group for the *ABCB11* c.1331T>C polymorphism analyses, we selected 219 healthy Spanish subjects, unrelated to the DILI patients. These controls were recruited from blood donors in the Spanish Bone Marrow Donor Registry from the same geographic region. A second group of 91 sex, age and drug-matched controls were also analysed to justify the use of the larger non-drug matched control group. The genotype distribution and allele frequency of the non-drug-matched control group did not differ significantly from that of the smaller drug-matched control group when tested. In addition the significant association between the CC genotype/C allele (of the studied polymorphism) and risk of DILI development was found using the two control groups independently (Supplementary table 1). Hence, the two control groups were therefore combined and used as a single control group to enhance statistical power in this study. The study protocol was approved by the local Ethics Committee of the coordinating

centre at the Virgen de la Victoria University Hospital in Málaga, Spain. All the subjects who took part in the study gave informed consent.

### *DNA Extraction and Determination of ABCB11 Genotypes*

Venous blood was obtained from each subject and DNA was extracted as described previously (16). Samples and controls were genotyped for the *ABCB11* c.1331T>C polymorphism (rs2287622) using a validated 5' nuclease PCR based assay with allele specific fluorescent probes (TaqMan SNP Genotyping Assays, Applied Biosystems, Foster City, CA, USA) as previously described (17). In short, 10 ng of sample DNA in 25 μL of reaction solution containing 12.5 μL of the 2x Taqman® Universal PCR Mix (Applied Biosystems), and 1.25 μL of pre-developed assay reagent from the SNP genotyping product (Applied Biosystem), containing two primers and two MGB-Taqman probes. Reaction conditions consisted of pre-incubation at 50ºC for 2 min and 95ºC for 10 min followed by 40 cycles of 95ºC for 15 s and 60ºC for 1min.

### *Statistical Analysis*

The rs2287622 genotype distribution and allelic frequency were analysed in DILI patients and controls using the PLINK program (http://pngu.mgh.harvard.edu/purcell/plink/) (18). Data were adjusted to dominant, recessive and allelic models. Means were compared by Student's t test for independent sample. Analysis of variance (ANOVA) was used for comparison of groups. Where variables did not follow a normal distribution, a nonparametric Kruskal-Wallis analysis was performed. Odds ratios (OR) and 95% confidence intervals (CI) were calculated to assess the relative disease risk conferred by a specific genotype. Genotype distribution in population subgroups were analysed by comparison of proportions, a derivative of the Fisher's exact test, which is more valid for smaller sample sizes. Analyses were performed using the SPSS 19.0 statistical software package program (SPSS Inc, Chicago, IL, USA) and $P<0.05$ was considered to be statistically significant.

Bonferroni's correction for multiple tests was used with comparison of proportions, whereby the probability value (*P*) was multiplied by the number of genotypes compared (CC vs CT/TT, n=2) to give a corrected *P* value (*Pc*) in order to account for problems of significant associations arising by chance after multiple comparisons.

***QSAR Analysis Using Chemical Fragmentation Codes***

To analyze potential relationships between chemical drug structures and the studied *ABCB11* polymorphism we applied a quantitative structure-activity relationship (QSAR) analysis, developed by Hirano *et al* to calculate predicted level of *ABCB11*-mediated taurocholate transport inhibition (13). The QSAR analysis was developed using chemical fragmentation codes (CFC) derived from the Markush TOPFRAG program (Derwent Information, Ltd., London, U.K), representing particular chemical moieties present in the test compound. The percentage of BSEP inhibition for each causative drug was calculated using the following formula generated by Hirano *et al* (13):

$$\text{BSEP inhibition (\%)} = \sum C(\text{i}) \times \text{score(i)} + \text{constant}$$

where the symbol (i) designates a specific CFC and the "score" refers to the presence (≥1) or absence (0) of the corresponding CFC (i) in the causative drug. The symbol *C* designates a specific chemical fragmentation coefficient obtained by multiple linear regression of calculated and observed *in vitro* transport inhibition.

Causative agents of herbal origin were excluded from the chemical structure analysis due to uncertainty over the active ingredient. Similarly, drugs in which the CFCs could not be easily identified were also excluded. In total 20 causative agents corresponding to 30 DILI cases in the cohort were omitted from this analysis.

**Results**

***DILI patient characteristics***

A total of 188 DILI patients were included in the study (99 males), mean age 54 years (range 14-83 years). Hypersensitivity features were found in 26% of the patients. The predominant pattern of injury was hepatocellular (n=89) followed by cholestatic (n=51) and mixed (n=48).

The main causative therapeutic drug group was antiinfectives (30%), followed by nervous system, NS (16%), cardiovascular (11%) and musculo-skeletal system drugs (13%) including non-steroidal anti-inflammatory drugs, NSAIDs (11%). There was a favourable clinical outcome in 187 patients, while one patient developed fulminant hepatic failure.

***Genetic Polymorphism of ABCB11 c.1331T>C***

Table 1 shows the *ABCB11* c.1331T>C genotype distribution in overall DILI patients and control subjects. Carriers of the CC genotype were more frequently found in DILI patients following a recessive genetic model for the major allele (OR= 1.6 (95% CI= 1.1-2.4); *P*= 0.01). Statistical significance was also observed in the allelic model (OR= 1.4 (1.1-1.9); *P*= 0.006). These results were confirmed using a comparison of proportions test (CC: *Pc*=0.023) and Armitage's test for trends (C: *P*= 0.007), respectively. The CC genotype was also more prevalent in patients with hepatocellular injury (OR= 2.1 (1.4-3.6); *P*= 0.001, including in the allelic model (OR=1.7 (1.2-2.5); *P*= 0.002. No statistical differences were found in patients with cholestatic or mixed damage, either alone or combined. Comparisons of demographic data and laboratory findings did not show any significant differences between the three genotypes (CC, TC and TT) neither in the overall DILI cohort (Table 2), nor when distributed according to type of liver damage (data not shown).

When analysing genotype distribution of the *ABCB11* c.1331T>C polymorphism classified by main pharmacological drug groups of the causative agent a significant difference was found for the CC genotype in NSAID-induced DILI cases (60% cases vs 32% controls), following a recessive genetic model for the major allele (OR= 3.4 (1.3-8.6); *P*= 0.007). A similar trend was

seen for antiinfetives (*P*= 0.027) and alimentary tract drugs (P= 0-019), in particular drugs for acid related disorders (*P*=0.022) (Table 3). However, the statistical significance for the three latter drug groups did not remain after comparison of proportions with Bonferoni's correction, which is better suited for smaller sample sizes, suggesting that these potential associations are too weak to be reliable. In contrast the significance for NSAID-induced cases remained independent of the statistical method used (comparison of proportions test *Pc*=0.019).

***Interaction between chemical structures and genetic variability in ABCB11 c.1331T>C***

To examine the effect of structural drug components on DILI development we searched the causative drugs for the presence of specific chemical moieties with BSEP-mediated taurocholate transport inhibiting properties, as described by Hirano and co-workers (13), in order to calculate the level of BSEP inhibition. Only eleven of all the examined drugs were found to cause ≥50% BSEP inhibition at a concentration of 100 μM. The CC genotype was only seen in 23% of the corresponding cases and subsequently did not have a significant impact on the DILI risk. On the contrary, in the DILI cases induced by drugs causing <50% BSEP inhibition the CC genotype was significantly more frequent than in the controls and subsequently associated with DILI development (45% vs 31, *Pc*=0.011) (Table 4).

We then focused on individual chemical moieties in the causative agents. Fifty nine percent (44/75) of the examined drugs contained carbocyclic systems with at least one aromatic ring (R-CC). These drugs included NSAIDs (8/8), cardiovascular system drugs (10/13) and nervous system drugs (10/19) and corresponded to 61% (115 /188) of the total cohort, suggesting that this moiety might be related to DILI development (Supplementary table 2). Table 5 outlines the effect of the C allele on DILI susceptibility classified by chemical moieties present in the causative drug. The CC genotype was found to be significantly more frequent in the 115 cases containing at least one R-CC moiety with or without any other moieties (Pc= *Pc*= 0.031). When classifying these cases into those which causative agents containing R-CC and other moieties and those with only R-CCs, the presence of a C allele was not associated with enhanced risk in

the former group (which included the 13 cases with an BSEP inhibitory activity of ≥50%), while being so in the latter one. The CC genotype distribution in the latter group was substantially higher than in the control group (50% vs 31%), but did not reach significance after Bonferon's correction (*Pc*=0.057), However, the NSAID cases, making up a large proportion of this subgroup, displayed a significant proportion of C allele carriers (*Pc*=0.01), with homozygotes being more susceptible than heterozygotes (3.9 vs 3.1 times relative risk). When separating the group of only R-CC containing drugs into those with one (M531) and two (M532) R-CCs no significance was detected on the genotype level, though the C allele remained significant in the M531 group (*Pc*=0.031). Neither the C allele nor the CC genotype was found to be associated with increased DILI risk in conjunction with drugs containing only moieties other than R-CCs.

**Discussion**

Strict regulation of intrahepatic bile acid concentration is critical to maintain and optimise hepatocyte functions. Chemical compounds, such as drugs or derived metabolites selectively impairing the canalicular bile secretary processes may lead to cholestasis or other forms of liver damage, whereby the detergent-like effects of accumulated bile acids may lead to cell death by apoptosis and/or necrosis (19). We set out to investigate potential associations between the *ABCB11* c.1331T>C polymorphism and the risk of developing hepatotoxicity in 188 Spanish DILI patients. We found a significant association between the C allele as well as the CC genotype and the development of hepatocellular damage. The C allele has previously been demonstrated to reduce BSEP expression compared to the T allele (10, 12, 20). The association between the CC genotype and hepatocellular injury differ from earlier findings where this genotype has been associated with specific cholestatic liver diseases (11). Furthermore, the CC genotype has also been associated with cholestatic type of drug-induced liver injury in a Swiss study by Lang and co-workers (12). The discrepant findings in the Swiss and our study might be due to a) our study cohort being considerably larger than that studied by Lang and coworkers (188 cases vs 36 cases) b) a broader spectrum of causative agents in our cohort and c) Lang's

cohort having a predominance of cholestatic cases (64%), while only 27% of the cases studied in our cohort were cholestatic.

Stratification according to liver injury type in DILI pharmacogenetic studies is, however, disputable. Classification of DILI based on the activity of alanine aminotransferase relative to that of alkaline phospatase is often used to determine the type of liver injury, particularly in cases where no biopsy is performed, and can provide a prognostic value. However, this classification may be too simplistic to actually take into account the complexity of the underlying molecular mechanism. This becomes evident for the mixed type of injury where some cases behave more like hepatocellular while others behave clearly cholestatic. Early signs of BSEP-mediated hepatotoxicity can indeed manifest in elevated transaminases as a consequence of bile acid–related hepatocyte injury instead of an increase in prototypical cholestatic liver enzymes, such as alkaline phosphatase and gamma glutamyl transpeptidase (5). Thus, inhibition of the BSEP function leading to retention of bile acids can result in a biochemical appearance of hepatocellular damage. Furthermore, recent studies using membrane vesicles to measure BSEP inhibition have demonstrated associations between pharmacological interference with BSEP function and human hepatotoxicity (5, 21). Interestingly, among the drugs with BSEP inhibitory properties were troglitazone and other peroxisome proliferator-activated receptor gamma inhibitors, nefazodone, ketoconazole, telithromycin, bosentan, antiretroviral drugs and tyrosine kinase inhibitors, which are all known to cause mainly hepatocellular damage.

When analyzing the *ABCB11* 1331T>C polymorphism according to main pharmacological groups we found that carriers homozygous for the C allele had a 3.4 times higher risk of developing DILI induced by NSAIDs compared to the controls. NSAIDs are widely consumed and, together with antimicrobial agents, the most frequent cause of DILI in Spain (22). Evidence that NSAIDs can affect transporter protein activity has been shown with recent *in vitro* data demonstrating an interaction between NSAIDs and the MRP4 transporter in human peripheral

blood lymphocytes. This transporter is responsible for the efflux of nucleoside monophosphate analogs, and NSAIDs can improve antiretroviral activity of nucleoside reverse transcriptase inhibitors (NRTIs) by blocking MRP4 activity and consequently increase the intracellular NRTI concentration (23). One might speculate that NSAIDs could exert a similar inhibitory effect on the activity of other ABC transporters. Hence, the association between *ABCB11* 1331CC carriers and DILI development may be enhanced in NSAID DILI patients due to a putative inhibitory effect on BSEP activity by this group of drugs. However, further studies are required to confirm this and to elucidate the underlying mechanism. Nevertheless, BSEP transporter could potentially represent a novel pharmacological site of interaction for NSAIDs.

The association between CC carriers with NSAID treatments and DILI development prompted us to search for additional common denominators that could enhance DILI susceptibility. In a previous pharmacogenetic DILI study we raised the idea of focusing on chemical structures of the culprit drug (24). Associations between chemical drug structures and biological effects in the form of quantitative structure activity relationship (QSAR) analyses have indeed shown to be a promising tool for predicting potential toxicity (25, 26). Based on the presence of specific chemical moieties the majority of the culprit drugs revealed <50% BSEP activity inhibition. The CC genotype was also found to be significantly associated with this group, but not with the >50% BSEP inhibition group. This suggests that the studied polymorphism does not have any greater impact on DILI when drugs with high BSEP inhibition are involved. On the other hand, drugs which affect BSEP activity to a lesser extent may need further contributing factors to reach a threshold inhibition level.

In terms of specific moieties, drugs containing a carbocyclic system with at least one aromatic ring (R-CC) constituted a greater proportion of the causative DILI agents and were in conjunction with the CC genotype significantly associated with DILI susceptibility, suggesting that this genotype exerts a greater DILI risk with drugs containing an R-CC moiety. In accordance, CC carriers were not seen to be overly represented among the DILI cases caused by drugs lacking the R-CC moiety. Furthermore, the effect of the CC genotype is less relevant with

drugs containing R-CC and additional moieties. This is probably due to the additional inhibition contributed by these moieties. In fact, all the causative drugs with a calculated inhibition of >50% contained R-CC and other moieties. The combined genetic and structural effect was particularly apparent in the NSAID group, where the R-CC moiety was the only BSEP inhibiting structure. Hence the structure rather than the pharmacological effect may be the underlying factor for the DILI risk association of this group.

In conclusion, our data support a role for the *ABCB11* c.1331 CC genotype in enhancing the risk of developing hepatocellular type of DILI. In addition, specific chemical moieties, such as a carbocyclic system with at least one aromatic ring, as exemplified by the NSAIDs studied, further enhance DILI susceptibility in homozygous c.1331C carries. This study highlights the need to combine chemical structure information related to functional responses, with genetic background to more accurately predict individual susceptibility to DILI.

**Acknowledgements**

This study was supported, in part, by research grants from the *Agencia Española del Medicamento* and *Fondo de Investigación Sanitaria* (PS 09/01384). The funding sources had no involvement in the study design; in the collection, analysis, and interpretation of data; in the writing of the report and in the decision to submit the manuscript for publication. CIBERehd and Red Genómica del Cáncer are funded by Instituto de Salud Carlos III.

We wish to thank the Spanish DILI Registry collaborators for their help with case recruitment.

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**Table 1.** Genotype distribution of the *ABCB11* c.1331T>C polymorphism in 188 drug-induced liver injury (DILI) patients and 310 controls.

| ***ABCB11* 1331T>C** | **Controls n=310** | **DILI n=188** | **HC n=89** |
|---|---|---|---|
| **CC, n (%)** | 95 (31) | 79 (42) | 44 (49) |
| **OR (95% CI)** | | 1.6 (1.1-2.4) | 2.1 (1.4-3.6) |
| ***P**** | | **0.01** | **0.001** |
| **TC, n (%)** | 151 (49) | 82 (44) | 33 (37) |
| **TT, n (%)** | 64(21) | 27 (14) | 12 (14) |
| **Frequency C allele, %** | 55 | 64 | 68 |
| **OR (95% CI)** | | 1.4 (1.1-1.9) | 1.7 (1.2-2.5) |
| ***P*** | | **0.006** | **0.002** |
| **Frequency T allele, %** | 45 | 36 | 32 |

HC: hepatocellular type of damage, CI: confidence intervals, OR: odds ratio
**P* after recessive genetic model for the major allele.
Comparison of proportions (CC genotype): DILI *Pc*=0.023; HC *Pc*=0.003
Armitage's test for trend (C allele): DILI *P*= 0.007; HC *P*= 0.003.

**Table 2**. Comparison of demographic, clinical and laboratory findings in 188 DILI patients classified by *ABCB11* c.1331T>C genotypes.

| | DILI n= 188 | | | |
|---|---|---|---|---|
| | **CC n=79** | **TC n=82** | **TT n=27** | ***P*** |
| **Mean age (range), years** | 53 (14-83) | 54 (17-83) | 57 (17-78) | 0.551 |
| **Gender (male/female)** | 36/43 | 44/38 | 10/17 | 0.281 |
| **Time to onset, mean ±SD, days** | 76±275 | 92±222 | 94±192 | 0.902 |
| **Duration of treatment, mean ±SD, days** | 88±280 | 114±234 | 104±187 | 0.790 |
| ***Clinical presentation, n (%)*** | | | | |
| **Jaundice** | 56 (71) | 46 (56) | 18 (67) | 0.141 |
| **Hospitalization** | 42 (53) | 40 (49) | 12 (44) | 0.705 |
| **Hypersensitivity features** | 20 (25) | 21 (26) | 7 (26) | 0.917 |
| ***Laboratory parameters, mean ± SD*** | | | | |
| **Total bilirubin (mg/dL)** | 8±8 | 7±8 | 8±8 | 0.864 |
| **ALT (xULN)** | 19±23 | 15±19 | 13±17 | 0.404 |
| **ALP (xULN)** | 3±8 | 3±4 | 3±2 | 0.910 |

ALT: alanine aminotransferase, ALP: alkaline phosphatase, SD: standard deviation, ULN: upper limit of normal,

**Table 3**. Distribution of the *ABCB11* (c.1331T>C) CC genotype in 188 Spanish DILI patients classified according to main pharmacological groups of the causative agents.

| | ***ABCB11* c.1331T>C Genotype distribution** | | |
|---|---|---|---|
| **Pharmacological Drug Groups** | **CC (%)** | **OR (95% CI)** | ***P**** |
| **Antiinfectives, J** (n=62) | 28 (45) | 1.9 (1.1-3.2) | **0.027** |
| **Nervous system, N** (n=28) | 11 (39) | (0.7-3.2) | 0.345 |
| **Musculoskeletal system, M** (n=25) | 12 (48) | (0.9-4.7) | 0.073 |
| *NSAIDs*, M01A (n=20) | 12 (60) | 3.4 (1.3-8.6) | **0.007** |
| **Cardiovascular system, C** (n=21) | 7 (33) | (0.4-2.8) | 0.796 |
| **Antineoplastics, L** (n=14) | 4 (29) | (0.3-2.9) | 0.818 |
| **Alimentary tract, A** (n=13) | 8 (61) | 3.6 (1.1-11.4) | **0.019** |
| *Acid related disorders*, A02 (n=9) | 6 (67) | 4.5 (1.1-18.5) | **0.022** |

CI: confidence intervals, OR: odds ratio

**P* after recessive genetic model for the major allele

Comparison of proportions test: antiinfectives *Pc*=0.064, NSAIDs *Pc*=0.019, alimentary tract drugs *Pc*=0.057, Acid related disorder drugs *Pc*=0.070

**Table 4.** Genotype distribution of the *ABCB11* c.1331T>C polymorphism according to calculated level of BSEP inhibition induced by the causative agent in 150 Spanish DILI patients

| **BSEP inhibition** | | **CC (%)** | **OR (95% CI)** | ***Pc*** |
|---|---|---|---|---|
| **≥ 50%** | DILI, n= 13 | 3 (23) | 0.6 (0.2-2.4) | 1 |
| **< 50%** | DILI, n= 137 | 61 (45) | 1.8 (1.2-2.8) | 0.011 |

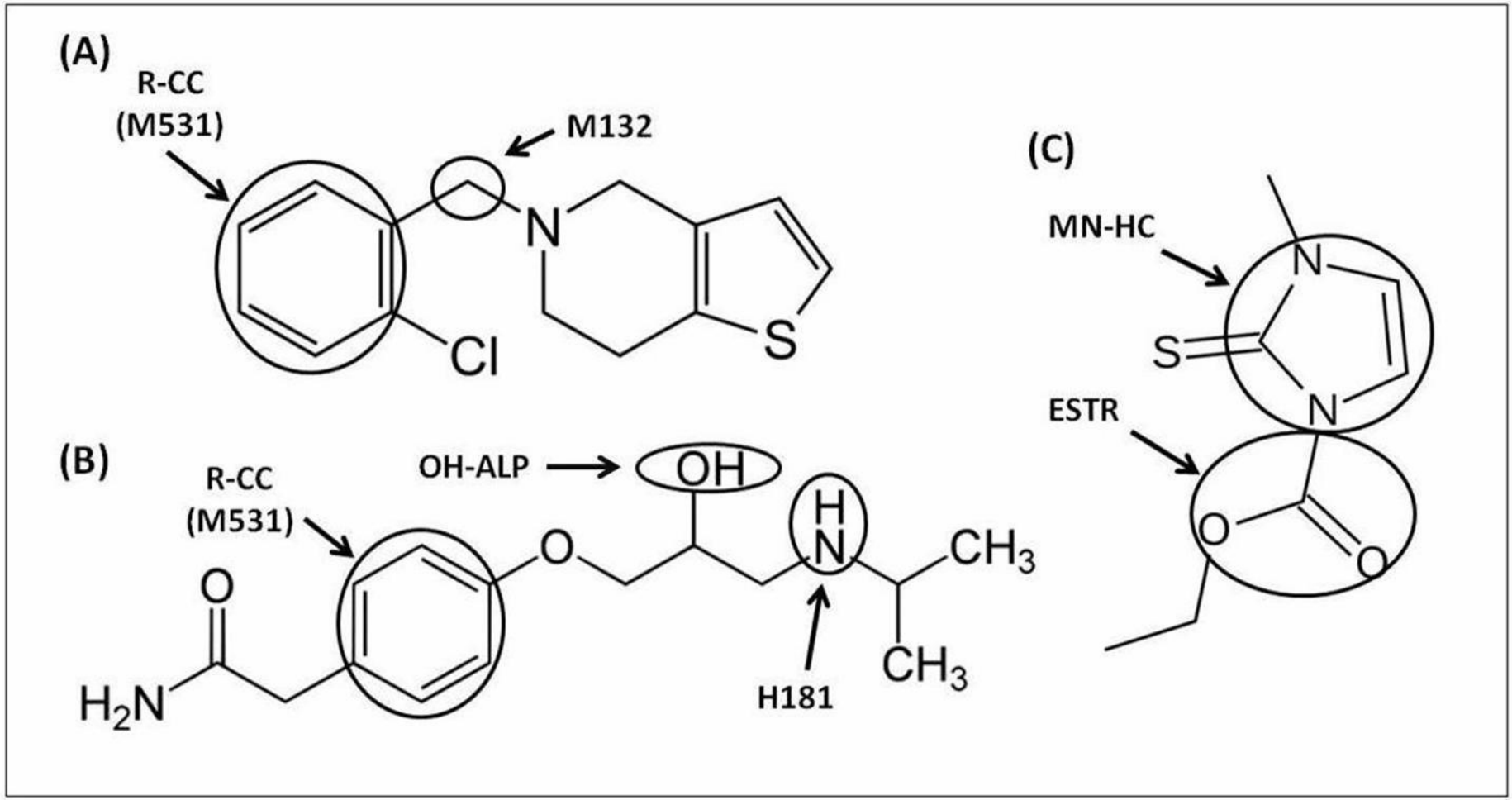
**Table 5**. Hepatotoxicity risk associated with chemical moieties in homozygous and heterozygous *ABCB11* c.1331C carriers

| | | | | *ABCB11* 1331T>C | | |
|---|---|---|---|---|---|---|
| | **CC (%)** | **OR (95% CI)** | ***Pc*** | **C (%)** | **OR (95% CI)** | ***Pc*** |
| ***R-CC (M531+M532+M533) with or without other groups*** | | | | | | |
| DILI, n=115 | 50 (43) | 1.7 (1.1-2.7) | **0.031** | 154 (67) | 1.7 (1.2-2.3) | **0.004** |
| ***R-CC (M531+M532+M53) with other groups*** | | | | | | |
| DILI, n=81 | 33 (41) | 1.6 (0.9-2.6) | 0.097 | 104 (64) | 1.5 (1.0-2.1) | 0.079 |
| ***R-CC (M531+M532) only*** | | | | | | |
| DILI, n=34 | 17 (50) | 2.3 (1.1-4.6) | 0.057 | 50 (74) | 2.3 (1.3-4.0) | **0.008** |
| NSAID, n=19 | 12 (63) | 3.9 (1.5-10.2) | **0.010** | 30 (79) | 3.1 (1.4-6.8) | **0.010** |
| ***M531 only*** | | | | | | |
| DILI, n=23 | 11 (48) | 2.1 (0.9-4.9) | 0.223 | 34 (74) | 2.3 (1.2-4.6) | **0.031** |
| ***M532 only*** | | | | | | |
| DILI, n=11 | 6 (55) | 2.7 (0.8-9.1) | 0.261 | 16 (73) | 2.2 (0.8-5.6) | 0.249 |
| ***Other groups only*** | | | | | | |
| DILI, n=35 | 14 (40) | 1.5 (0.7-3.1) | 0.604 | 41 (59) | 1.2 (0.7-1.9) | 1 |

Other groups: any other chemical moieties described by Hirano and co-workers such as M132, ESTR, H181, MN-HC and OH-ALP, M531: one carbocyclic system with at least one aromatic ring, M532: two carbocyclic systems with at least one aromatic ring, M533: three carbocyclic systems with at least one aromatic ring, HC: hepatocellular type of damage, CI: confidence intervals, OR: odds ratio, *Pc* after Bonferroni's correction

Figure legend

Figure 1. Chemical moieties associated with BSEP-mediated taurocholate transport inhibition (13) exemplified in chemical drug structures. (A) Ticlopidine presenting a carbocyclic system with one aromatic ring (R-CC, M531) and a ring-linking group containing one carbon atom (M132). (B) Atenolol presenting an OH group bonded to an aliphatic carbon atom (OH-ALP) and an amine bonded to an aliphatic carbon atom (H181) in addition to an R-CC structure. (C) Carbimazole presenging a mononuclear heterocycle (MN-HC) and one ester group bonded to a heterocyclic carbon atom (ESTR).


(A)
R-CC
(M531)
M132
N
Cl
S
(B)
R-CC
(M531)
OH-ALP
OH
O
H
N
CH3
O
H2N
CH3
H181
(C)
MN-HC
N
S
N
ESTR
O
O

**Supplementary table 1.** A. Genotype distribution of the *ABCB11* c.1331T>C polymorphism in 188 drug-induced liver injury (DILI) patients and 91 sex, age and drug-matched controls. B. Genotype distribution of the *ABCB11* c.1331T>C polymorphism in 91 sex, age and drug-matched controls and 219 healthy controls.

**A**

| ***ABCB11* 1331T>C** | **Drug-matched controls n=91** | **DILI n=188** | **HC n=89** |
|---|---|---|---|
| **CC, n (%)** | 26 (28) | 79 (42) | 44 (49) |
| **OR (95% CI)** | | 1.8 (1.1-3.1) | 2.4 (1.3-4.5) |
| ***P**** | | **0.030** | **0.004** |
| **TC, n (%)** | 48 (53) | 82 (44) | 33 (37) |
| **TT, n (%)** | 17(19) | 27 (14) | 12 (14) |
| **Frequency C allele, %** | 55 | 64 | 68 |
| **OR (95% CI)** | | 1.4 (1.0-2.1) | 1.7 (1.1-2.7) |
| ***P*** | | **0.044** | **0.011** |
| **Frequency T allele, %** | 45 | 36 | 32 |

HC: hepatocellular type of damage, CI: confidence intervals, OR: odds ratio
**P* after recessive genetic model for the major allele.
Comparison of proportions (CC genotype): DILI *Pc*=0.07; HC *Pc*=0.01
Armitage's test for trend (C allele) DILI *P*= 0.046; HC *P*= 0.013

**B**

| ***ABCB11* 1331T>C** | **Drug-matched controls n=91** | **Healthy controls n=219** |
|---|---|---|
| **CC, n (%)** | 26 (28) | 69 (32) |
| ***P**** | | 0.610 |
| **TC, n (%)** | 48 (53) | 103 (47) |
| **TT, n (%)** | 17 (19) | 47(21) |
| **Frequency C allele, %** | 55 | 55 |
| ***P*** | | 0.986 |
| **Frequency T allele, %** | 45 | 45 |

Comparison of proportions (CC genotype): *Pc*=1
Armitage's test for trend (C allele) *P*= 0.986

**Supplement table 2.** Chemical moieties in the hepatotoxicity causative agents present in the study cohort

| THERAPEUIC GROUPS | ACTIVE INGREDIENTS | CHEMICAL GROUPS | | | | | |
|---|---|---|---|---|---|---|---|
| | | ***M132*** | ***ESTR*** | ***R-CC*** | ***H181*** | ***MN-HC*** | ***OH-ALP*** |
| ***Alimentary tract and metabolism*** | | | | | | | |
| **A02BA02** | **RANITIDINE** | 0 | 0 | 0 | 1 | 1 | 0 |
| **A02BC01** | **OMEPRAZOLE** | 0 | 0 | 0 | 0 | 1 | 0 |
| **A03FA** | **CINITAPRIDE** | 1 | 0 | 1 | 1 | 1 | 0 |
| **A07EC01** | **SULFASALAZINE** | 0 | 0 | 2 | 0 | 1 | 0 |
| **A10BX02** | **REPAGLINIDE** | 0 | 0 | 2 | 0 | 1 | 0 |
| ***Blood and blood forming organs*** | | | | | | | |
| **B01AC04** | **CLOPIDOGREL** | 1 | 0 | 1 | 1 | 0 | 0 |
| **B01AC05** | **TICLOPIDINE** | 1 | 0 | 1 | 1 | 0 | 0 |
| ***Cardiovascular system*** | | | | | | | |
| **C07AB03** | **ATENOLOL** | 0 | 0 | 1 | 1 | 0 | 1 |
| **C08DB01** | **DILTIAZEM** | 0 | 0 | 1 | 1 | 0 | 0 |
| **C09AA01** | **CAPTOPRIL** | 0 | 0 | 0 | 0 | 1 | 0 |
| **C09AA02** | **ENALAPRIL** | 0 | 0 | 1 | 1 | 1 | 0 |
| **C09CA01** | **LOSARTAN** | 1 | 0 | 2 | 0 | 2 | 1 |
| **C09CA04** | **IRBESARTAN** | 1 | 0 | 2 | 0 | 2 | 0 |
| **C09CA07** | **TELMISARTAN** | 1 | 0 | 2 | 0 | 0 | 0 |
| **C10AA01** | **SIMVASTATIN** | 0 | 0 | 0 | 0 | 1 | 0 |
| **C10AA02** | **LOVASTATIN** | 0 | 0 | 0 | 0 | 1 | 0 |
| **C10AA04** | **FLUVASTATIN** | 0 | 0 | 1 | 0 | 0 | 2 |
| **C10AA05** | **ATORVASTATIN** | 0 | 0 | 3 | 0 | 1 | 2 |
| **C10AB04** | **GEMFIBROZIL** | 0 | 0 | 1 | 0 | 0 | 0 |
| **C10AB05** | **FENOFIBRATE** | 0 | 0 | 2 | 0 | 0 | 0 |
| ***Genito urinary system and sex hormones*** | | | | | | | |
| **G03GB02** | **CLOMIFENE** | 1 | 0 | 3 | 1 | 0 | 0 |
| ***Systemic hormonal preparations*** | | | | | | | |
| **H03BB01** | **CARBIMAZOLE** | 0 | 1 | 0 | 0 | 1 | 0 |
| **H03BB02** | **THIAMAZOLE** | 1 | 0 | 1 | 1 | 1 | 0 |
| ***Antiinfectives for systemic use*** | | | | | | | |
| **J01AA08** | **MINOCYCLINE** | 0 | 0 | 0 | 0 | 0 | 0 |
| **J01CA04** | **AMOXICILLIN** | 0 | 0 | 1 | 1 | 0 | 0 |
| **J01CR02** | **AMOXICILLIN-CLAVULANATE** | 0 | 0 | 1 | 1 | 0 | 1 |
| **J01DC02** | **CEFUROXIME** | 0 | 0 | 0 | 0 | 1 | 0 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **J01DC04** | **CEFACLOR** | 0 | 0 | 1 | 1 | 0 | 0 |
| **J01DD04** | **CEFTRIAXONE** | 0 | 0 | 0 | 0 | 2 | 0 |
| **J01EE01** | **SULFAMETHOXAZOLE + TRIMETHOPRIM** | 1 | 0 | 2 | 0 | 2 | 0 |
| **J01FA01** | **ERYTHROMICIN** | 0 | 0 | 0 | 0 | 3 | 0 |
| **J01FA03** | **MIDECAMYCIN** | 0 | 0 | 0 | 0 | 3 | 0 |
| **J01FA06** | **ROXITHROMYCIN** | 0 | 0 | 0 | 0 | 3 | 0 |
| **J01FA10** | **AZITHROMYCIN** | 0 | 0 | 0 | 1 | 3 | 0 |
| **J01MA02** | **CIPROFLOXACIN** | 0 | 0 | 0 | 1 | 1 | 0 |
| **J01MA12** | **LEVOFLOXACIN** | 0 | 0 | 0 | 1 | 1 | 0 |
| **J01MA14** | **MOXIFLOXACIN** | 0 | 0 | 0 | 0 | 0 | 0 |
| **J02AB02** | **KETOCONAZOLE** | 1 | 0 | 2 | 0 | 3 | 0 |
| **J04AC01** | **ISONIAZID** | 0 | 0 | 0 | 0 | 1 | 0 |
| **J04AK01** | **PYRAZINAMIDE** | 0 | 0 | 0 | 0 | 1 | 0 |
| **J05AB11** | **VALACICLOVIR** | 0 | 0 | 0 | 1 | 0 | 0 |
| ***Antineoplastic and immunomodulating agents*** | | | | | | | |
| **L02BB01** | **FLUTAMIDE** | 0 | 0 | 1 | 0 | 0 | 0 |
| **L02BG03** | **ANASTROZOLE** | 1 | 0 | 1 | 0 | 1 | 0 |
| **L04AA13** | **LEFLUNOMIDE** | 0 | 0 | 1 | 0 | 1 | 0 |
| **L04AX01** | **AZATHIOPRINE** | 0 | 0 | 0 | 0 | 1 | 0 |
| ***Musculo-skeletal system*** | | | | | | | |
| **M01AB01** | **INDOMETACIN** | 0 | 0 | 1 | 0 | 0 | 0 |
| **M01AB05** | **DICLOFENAC** | 0 | 0 | 2 | 0 | 0 | 0 |
| **M01AB15** | **KETOROLAC** | 0 | 0 | 1 | 0 | 0 | 0 |
| **M01AE01** | **IBUPROFEN** | 0 | 0 | 1 | 0 | 0 | 0 |
| **M01AE02** | **NAPROXEN** | 0 | 0 | 1 | 0 | 0 | 0 |
| **M01AE17** | **DEXKETOPROFEN** | 0 | 0 | 2 | 0 | 0 | 0 |
| **M01AH02** | **ROFECOXIB** | 0 | 0 | 2 | 0 | 1 | 0 |
| **M01AX17** | **NIMESULIDE** | 0 | 0 | 2 | 0 | 0 | 0 |
| **M03BX08** | **CYCLOBENZAPRINE** | 0 | 0 | 0 | 1 | 0 | 0 |
| **M05BA04** | **ALENDRONIC ACID** | 0 | 0 | 0 | 1 | 0 | 1 |
| ***Nervous system*** | | | | | | | |
| **N02BA01** | **ACETYLSALICYLIC ACID** | 0 | 0 | 1 | 0 | 0 | 0 |
| **N02BB02** | **METAMIZOLE SODIUM** | 0 | 0 | 1 | 0 | 1 | 0 |
| **N02BE01** | **PARACETAMOL** | 0 | 0 | 1 | 0 | 0 | 0 |
| **N02CC03** | **ZOLMITRIPTAN** | 1 | 0 | 0 | 1 | 1 | 0 |
| **N03AB02** | **PHENYTOIN** | 0 | 0 | 2 | 0 | 1 | 0 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **N03AF01** | **CARBAMAZEPINE** | 0 | 0 | 0 | 0 | 0 | 0 |
| **N03AG01** | **VALPROIC ACID** | 0 | 0 | 0 | 0 | 0 | 0 |
| **N05AA01** | **CHLORPROMAZINE** | 0 | 0 | 0 | 1 | 0 | 0 |
| **N05AX08** | **RISPERIDONE** | 0 | 0 | 0 | 1 | 1 | 0 |
| **N05BA21** | **CLOTIAZEPAM** | 0 | 0 | 1 | 0 | 0 | 0 |
| **N05BA** | **BENTAZEPAM** | 0 | 0 | 1 | 0 | 0 | 0 |
| **N05CM02** | **CLOMETHIAZOLE** | 0 | 0 | 0 | 0 | 1 | 0 |
| **N06AA09** | **AMITRIPTYLINE** | 0 | 0 | 0 | 1 | 0 | 0 |
| **N06AB03** | **FLUOXETINE** | 0 | 0 | 2 | 1 | 0 | 0 |
| **N06AB04** | **CITALOPRAM** | 0 | 0 | 1 | 1 | 0 | 0 |
| **N06AB05** | **PAROXETINE** | 0 | 0 | 1 | 1 | 1 | 0 |
| **N06AB06** | **SERTRALINE** | 0 | 0 | 1 | 0 | 0 | 0 |
| **N06AX11** | **MIRTAZAPINE** | 0 | 0 | 0 | 0 | 0 | 0 |
| ***Respiratory system*** | | | | | | | |
| **R03DC01** | **ZAFIRLUKAST** | 1 | 0 | 2 | 0 | 0 | 0 |
| **R03DC03** | **MONTELUKAST** | 0 | 0 | 2 | 0 | 0 | 1 |