CYP2C9 variants as a risk modifier of NSAID-related gastrointestinal bleeding: a case–control study
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Objectives The aim of this study was to assess whether the CYP2C9*2 and/or *3 variants might modify the risk for NSAID-related upper gastrointestinal bleeding (UGIB) in NSAID users.

Patients and Methods We conducted a multicenter, case–control study in which cases were patients aged more than 18 years with a diagnosis of UGIB, and controls were matched (1:3) by sex, age, date of admission, and hospital. Exposure was defined as the mean number of defined daily doses (DDDs) of NSAIDs metabolized by CYP2C9 in the week preceding the index date. Three DDD categories were defined (0, ≤ 0.5, and > 0.5). Exposure was constructed taking both NSAID use and CYP2C9 polymorphisms into account. Patients of non-European origin were excluded from the analysis.

Results A total of 577 cases and 1343 controls were finally included in the analysis: 103 cases and 89 controls consumed NSAIDs metabolized by CYP2C9, and 88 cases and 177 controls were CYP2C9*3 carriers. The adjusted odds ratios (aORs) of UGIB associated with the CYP2C9*2 and wild-type alleles proved to be similar [OR = 8.79 (4.50–17.17) and 10.15 (2.92–35.35), respectively] and lower than those of the CYP2C9*3 allele [aOR = 18.07 (6.34–51.53)] for consumers taking more than 0.5 DDDs of NSAIDs metabolized by CYP2C9. Grouping genotypes into carriers and noncarriers of the CYP2C9*3 variant resulted in aORs of 16.92 (4.96–57.59) for carriers and 9.72 (4.55–20.76) for noncarriers, where DDDs were greater than 0.5.

Conclusion The presence of the CYP2C9*3 variant increases the risk for UGIB associated with NSAID for DDDs greater than 0.5. The presence of the CYP2C9*2 allele shows no such effect.

Keywords: CYP2C9*2, CYP2C9*3, gastrointestinal hemorrhage, NSAID

Introduction
Upper gastrointestinal bleeding (UGIB) related to NSAIDs is one of the most frequent and severe adverse drug reactions (ADRs) [1–4]. This ADR has been observed to have a very important idiosyncratic component, which might be associated with some variants of the gene that codes for enzyme CYP2C9 (the main metabolizer of NSAIDs) [5–7]. This gene has two variants that have functional consequences on enzyme activity and are quite specific in populations of European origin, with estimated prevalences of around 14% for CYP2C9*2 and 8% for CYP2C9*3 [8–10]. These variants – and CYP2C9*3 in particular – can decrease the metabolism of many NSAIDs [11,12]. Accordingly, carriers of these variants could suffer a relative overdose, which would increase their risk for UGIB, given the latter’s dose-dependent nature [4,8]. Furthermore, the cost of determining these single-nucleotide polymorphisms (SNPs) has been greatly reduced, which would ease their clinical use for the purposes of preventing this ADR.

To date, six different studies have assessed the role of the CYP2C9*2 and CYP2C9*3 variants in the risk for NSAID-related UGIB [13–18] but their results have been inconsistent [19]. Accordingly, this paper reports the first full case–control study conducted with the aim of ascertaining whether the presence of the CYP2C9*2 and/or *3 variants might modify the risk for NSAID-related UGIB, depending on the NSAID dosage consumed.
Patients and methods

Study settings and design

An incident case–control study was implemented at hospitals in four cities in northern Spain (Santiago de Compostela, Valladolid, Galdakao, and Barcelona) and one in northern Italy (Verona). Patients were recruited from January 2004 through November 2007. Informed consent was obtained from all eligible patients, and the study was approved by the ethics committee of each participant hospital.

Definition of cases and controls

Cases were defined as any person over 18 years of age who met one of two conditions: (i) admitted to hospital with a primary diagnosis of UGIB (hematemesis, vomitus of red blood or ‘coffee-grounds’ material; melena; and/or hematochezia) and shown by endoscopy to present with acute lesions of the gastric mucosa or erosive duodenitis; or (ii) having no clinical symptomatology of UGIB but having undergone an endoscopy within 48 h of admission in which signs of recent bleeding were evident.

Controls were recruited from the same hospitals as cases. Both in Spain and Italy, hospitals have a defined geographical area of influence, and thus individuals living in a particular area are allocated to a specific hospital. As geographical origin may be associated with the likelihood not only of exposure but even of the prevalence of the polymorphisms studied, recruiting cases and controls from the same source population prevents possible selection biases [20]. Three controls were selected for each case and matched by hospital, sex, age (± 5 years), and date of admission (±3 months). To prevent the selection of controls from being associated with overestimation of exposure to NSAIDs, controls were recruited from among patients in the preoperative unit who were about to undergo minor surgery for nonpainful clinical processes unassociated with NSAID use (e.g. cataracts, prostate adenoma, sepsplasty, or lipoma removal).

The main exclusion criterion for cases and controls was history of cancer, coagulopathy, Mallory–Weiss syndrome, or esophageal varices (Fig. 1). Individuals who were not resident in the study area or had no reliable interview data were also excluded. All exclusion criteria considered are set out more fully in Fig. 1.

Clinical data collection

A comprehensive interview was conducted with both cases and controls by trained health personnel, using a questionnaire purpose-designed to collect sociodemographic information on patients, their personal clinical history, toxic habits, grounds for admission, underlying symptomatology (cases only), reason for the surgery for which they had been scheduled (controls only), previous episodes of gastric diseases, and exposure to drugs.

Four complementary strategies were pursued to obtain the most complete pharmacologic anamnesis possible: (i) participants were asked direct questions, including about daily dose and indication, about any drugs (as well as over-the-counter ones) that they had taken during the preceding 2 months; (ii) they were also asked about frequent symptoms for which NSAIDs are indicated, and the treatments used to mitigate such symptoms; (iii) for ease of recall, participants were shown prompt cards of the most popular NSAIDs in each of the study areas; and (iv) when a participant failed to remember any of the data requested, either the interview was repeated at a later date or, if the patient had been discharged, he/she was contacted by telephone.

We defined the index date on the basis of patients’ clinical history but blind to their drug use: for cases, this was the date of appearance of hematemesis, coffee-ground vomitus, melena, or bloody stools (with hemorrhoids being ruled out); and for controls the index date was deemed to be the date of interview. In line with other studies that have analyzed the relationship between exposure to NSAIDs and risk for UGIB [21,22], we considered a 7-day etiologic window dating from the index date.

Helicobacter pylori determination

Presence of anti-H. pylori IgG antibodies was determined in the plasma of participants using a commercial ELISA kit [23]. The serologic techniques used for H. pylori determination are not affected by the presence of UGIB or use of proton pump inhibitors (PPIs) [24]. To avoid false positives due to old infections, we asked patients whether they had received treatment for H. pylori infection.

Single-nucleotide polymorphism genotyping

Two different methods were used for genotyping participants’ CYP2C9*2 and CYP2C9*3 variants: SNaPshot technology and TaqMan Drug Metabolism Genotyping Assays (Foster City, California, USA). Concordance between the two methods was 100% [8]. All determinations were masked as regards patients’ case or control status and exposure to NSAIDs.

Definition of variables

An NSAID consumer was defined as any person shown by pharmacologic anamnesis to have consumed some medication belonging to this therapeutic group in the week preceding the index date. Because of the low prevalence of exposure to every single NSAID, it was not possible to detect the interaction between each NSAID and the CYP2C9 polymorphisms studied. Hence, to obtain sufficient statistical power, the NSAIDs had to be grouped. To this end, all NSAIDs that relied on the above enzyme for at least 50% of their biotransformation were considered: according to the literature, these were celecoxib, diclofenac, ibuprofen, naproxen, aceclofenac, indomethacin, lornoxicam, and piroxicam [9]. Finally, to
perform an analysis of the NSAID dose effect, we calculated the defined daily dose (DDD) and, as the amount of no specific NSAID is comparable to that of another, the DDD was then taken as reference. The DDD is the average adult maintenance dose used for the main indication [25], and, in view of the fact that the main indication for the use of NSAIDs is the same, we used the proportion of any given participant’s DDDs that corresponded to NSAIDs metabolized by CYP2C9 as an approximation of his/her level of exposure. Thus, the dose–response effect was evaluated using three categories: (i) NSAID nonusers; (ii) NSAID users of 0.5 DDD or less; and (iii) NSAID users of over 0.5 DDD. Acetylsalicylic acid was deemed to be an antiplatelet agent when pharmacologic anamnesis showed that a patient had taken it for a heart complaint.
The main drugs that might be related to gastrointestinal events were also taken into account – that is, PPIs, antiplatelet agents, and oral anticoagulants. For alcohol, caffeine, and tobacco use, mean daily consumption over the 2 months preceding the interview date was calculated.

### Statistical analysis

Possible bias in the selection of controls was evaluated by reference assessment of the Hardy–Weinberg equilibrium in both variants [26–29], using the SNPassoc Library of the R package (v2.12.2) [30]. Deviation from the Hardy–Weinberg equilibrium in the control group indicates the probability of genotyping errors, and selection or other bias [31]. To eliminate the risk of stratification bias, all non-White patients (i.e. of non-European origin) were excluded from the analysis.

To assess the risk for bleeding, adjusted odds ratios (aORs) and their 95% confidence intervals (CIs) were estimated by generalized linear mixed models for dependent binomial-type variables (case or control) [32,33]. The analysis was performed with this method instead of the typical logistic regression because of the nature of the design (a multicenter, matched case–control study). To establish the effect on individuals, it was thus necessary to consider three types of strata: level 1, patients; level 2, cases and matched controls; and level 3, hospitals.

To construct the models, we first performed a bivariate analysis, and then selected all independent variables having a P-value lower than 0.2 for multivariate analysis. Second, the variables so selected were then studied in a multivariate analysis. The variables with the highest level of statistical significance were successively eliminated, provided that the coefficients of the principal variables of exposure changed by no more than 10% [34] and provided that the coefficients of the principal variables of exposure changed by no more than 10% [34] and improved the Schwartz’s Bayesian Information Criterion [35], until the most appropriate model had been obtained. The reference category considered in interactions between DDDs of NSAIDs and genotype or allele was absence of exposure to NSAIDs and wild-type genotype or wild-type allele [36]. The lmer function was used for estimation of the models: it is implemented in the lme4 library of the R package (v2.12.2) [33].

We also calculated the additive interaction [S (95% CI)] as the rate between the combined effects of genetic variants and NSAIDs, and the sum of the effects considered separately. S has been shown to be the most reliable measure of additive interaction when adjusting for confounding [37].

### Results

Of the 4325 patients who were interviewed, 577 cases and 1343 controls were finally included in the analysis (Fig. 1). Table 1 shows the demographic and clinical characteristics of cases and controls.
The CYP2C9 genotype was obtained in 91.0% of individuals. Table 2 shows the distribution of CYP2C9 genotype frequencies for cases and controls. Calculation of the Hardy–Weinberg equilibrium showed that controls were in equilibrium in terms of both the *2 (P = 0.745) and *3 variants (P = 0.538).

Risk for UGIB associated with each allele was also analyzed, taking the NSAID dose consumed into account (Table 3). For DDDs of NSAID in excess of 0.5, risk for UGIB was observed to be similar for the *2 and wild-type variants [OR = 8.79 (4.50–17.17) and 10.15 (2.92–35.35), respectively], and very much lower than that for the *3 variant [aOR = 18.07 (6.34–51.53)]. In view of these results, patients were grouped into two categories: carriers and noncarriers of the *3 allele (Table 4). From DDDs of 0.5 upward, risk for UGIB among carriers of genotypes with the *3 variant was 16.92 (4.96–57.59; P < 0.001) versus 9.72 (4.55–20.76; P < 0.001) among carriers of genotypes with the *2 and/or wild-type variants. Despite the absence of statistical significance, this interaction might well be additive [S = 1.75 (0.40–7.69)]. All these models were adjusted for personal history of gastrointestinal disorders, presence of H. pylori, osteoarthritis, number of interviews conducted with each patient, reliability of the interview, exposure to PPIs, antiplatelet agents, oral anticoagulants, and NSAIDs not metabolized by CYP2C9.

Lastly, when the number, site, and type of lesion (erosion or ulcer) were analyzed (Table 5), it was observed that NSAID consumers and carriers of CYP2C9*3 had more lesions/patient (2.0) than did NSAID consumers (1.7) or carriers of the *3 variant (1.7). It is noteworthy that 93.7% (15/16) of NSAID consumer carriers of CYP2C9*3 presented with a duodenal ulcer versus 62.0% (314/506) of the remaining cases (P < 0.05).

Discussion
In this full case–control study, we found that the presence of the CYP2C9*3 variant increases the risk for UGIB associated with NSAID consumption; this occurs among persons taking doses higher than that regarded as half the average dose (>0.5 DDD). In view of the fact

### Table 2 Prevalence of CYP2C9 genotypes and Hardy–Weinberg equilibrium test

| Genotypes          | Cases         | Controls       |
|--------------------|---------------|----------------|
|                    | Expected       | Real           | Expected       | Real           |
|                    | prevalence (%) | [n (%)]       | prevalence (%) | [n (%)]       |
| CYP2C9*1/*1        | 59.5 (59.4)    | 59.6 (59.6)    |
| CYP2C9*1/*2        | 22.9 (20.1)    | 22.9 (23.7)    |
| CYP2C9*2/*2        | 2.4 (2.2)      | 2.4 (2.5)      |
| CYP2C9*1/*3        | 11.9 (13.1)    | 11.9 (11.4)    |
| CYP2C9*2/*3        | 1.0 (1.8)      | 0.9 (0.6)      |
| CYP2C9*3/*3        | 22.9 (20.1)    | 22.9 (23.7)    |
| CYP2C9*1/*1/*3     | 59.5 (59.4)    | 59.6 (59.6)    |
| CYP2C9*2/*1/*3     | 22.9 (20.1)    | 22.9 (23.7)    |
| CYP2C9*2/*2/*3     | 2.4 (2.2)      | 2.4 (2.5)      |
| CYP2C9*3/*3/*3     | 11.9 (13.1)    | 11.9 (11.4)    |
| CYP2C9*1/*2/*3     | 1.0 (1.8)      | 0.9 (0.6)      |

HWE, Hardy–Weinberg equilibrium. *P = 0.05 was deemed statistically significant.

### Table 3 Risk for upper gastrointestinal bleeding associated with each allele of CYP2C9

| Allele CYP2C9*1 | Adjusted OR (95% CI) | P-value |
|----------------|----------------------|---------|
| DDDs of NSAIDs = 0 | 1.00 (ref)          | –       |
| 0 < DDDs of NSAIDs ≤ 0.5 | 3.19 (1.78–5.78) | < 0.001 |
| 0.5 < DDDs of NSAIDs ≤ 0.5 | 8.79 (4.50–17.17) | < 0.001 |

| Allele CYP2C9*2 | Adjusted OR (95% CI) | P-value |
|----------------|----------------------|---------|
| DDDs of NSAIDs = 0 | 1.05 (0.77–1.42) | 0.776    |
| 0 < DDDs of NSAIDs ≤ 0.5 | 1.31 (0.36–4.71) | 0.679    |
| 0.5 < DDDs of NSAIDs ≤ 0.5 | 10.15 (2.92–35.35) | < 0.001 |
| 0.5 < DDDs of NSAIDs ≤ 0.5 | 15.4 (1.04–22.6) | 0.030    |
| 0 < DDDs of NSAIDs ≤ 0.5 | 2.61 (0.62–11.10) | 0.193    |
| 0.5 < DDDs of NSAIDs ≤ 0.5 | 18.07 (6.34–51.53) | < 0.001 |

CI, confidence interval; DDD, assumed average maintenance dose per day for a drug used for its main indication in adults.

### Table 4 Dose of NSAIDs metabolized by CYP2C9, genotypes carrying the CYP2C9*3 variant, and related risk for upper gastrointestinal bleeding

| Genotype/NSAID dose (in DDDs) | Adjusted OR (95% CI) | P-value |
|-------------------------------|----------------------|---------|
| CYP2C9*3                      |                      |         |
| DDDs of NSAIDs = 0             | 1.00 (ref)           | –       |
| 0 < DDDs of NSAIDs ≤ 0.5      | 3.22 (1.68–6.20)     | < 0.001 |
| 0.5 < DDDs of NSAIDs ≤ 0.5    | 9.72 (4.55–20.76)    | < 0.001 |

### Table 5 Number and type of localization of lesions, by exposure to NSAID metabolized by CYP2C9 and presence of CYP2C9*3

| Types and locations of lesions | NSAID metabolized by CYP2C9 | NSAID metabolized by CYP2C9*3 | NSAID plus CYP2C9*3 | NSAID plus CYP2C9*3* |
|--------------------------------|-----------------------------|-------------------------------|---------------------|----------------------|
| Erosions                       | 41 (39.8)                   | 25 (28.4)                     | 4 (25.0)            | 102 (25.4)           |
| Gastric                        | 32 (31.1)                   | 22 (25.0)                     | 8 (50.0)            | 55 (13.7)            |
| Ulcers                         | 55 (53.4)                   | 39 (44.4)                     | 5 (31.3)            | 125 (31.1)           |
| Duodenal                       | 56 (54.4)                   | 59 (67.0)                     | 15 (93.7)           | 199 (49.5)           |
| Pyloric                        | 8 (7.8)                     | 0 (0)                         | 0 (0)               | 13 (3.2)             |

Figures in brackets show the corresponding percentages. Each patient may have more than one lesion.
that NSAIDs are one of the most widely used treatment
groups worldwide [38], that ~14% of the population of
European origin carries genotypes with the *3 variant
[8–10], and that genotyping costs are very low, our
finding could enable the risk for UGIB in this population to
be substantially reduced.

We observed that patients who consumed mean DDDs
of NSAIDs in excess of 0.5 and who carried the *3 allele
had a nearly two-fold increased risk of suffering from
UGIBs as compared with patients who consumed the
same dose but were noncarriers of this variant. In con-
trast, we found that the pattern of behavior of the *2
allele was very similar to that of the wild type, thereby
enabling patients to be grouped into carriers or non-
carriers of the *3 allele, and important differences to be
detected in CYP2C9-related risk of suffering from UGIB.
This finding is analogous to the results of studies
undertaken with other drugs that have a narrow thera-
petic index and are also metabolized by CYP2C9, such as
warfarin [39], phenprocoumon [28], and phenytoin
[29], where the increased risk is higher for carriers of the
*3 allele. Our results are also in agreement with in-vitro
and in-vivo studies that report that, compared with
CYP2C9*2, CYP2C9*3 has a high impact on the clearance
of most NSAIDs [12].

This is the first study to our knowledge to analyze the
role of enzyme CYP2C9 in the risk for UGIB considering
NSAID dosage. We observed that at low doses the pre-

cence of the *3 allele did not increase the risk for UGIB
with respect to noncarriers but that from a DDD of 0.5
there was an important increase in risk among carriers of
the *3 allele. This finding is in agreement with (i) the
known dose-dependent nature of NSAID-induced gas-

trointestinal damage [4]; and (ii) the increase in plasma
NSAID levels among carriers of the *3 allele [5,9].
Accordingly, at low doses there would be no increase in
the risk for UGIB, regardless of the presence of the
variants studied. For medium and high doses, however,
the presence of the *3 variant plays a relevant role in the
increase in risk, whereas the *2 allele shows a similar risk
to the wild-type allele, probably because CYP2C9*3 has
a higher impact than CYP2C9*2 on the clearance of most
NSAIDs [12]. These results would indicate that the
presence of the *3 variant can be used as a UGIB risk
marker among consumers of NSAIDs metabolized
through this pathway.

Our study has a number of strengths and limitations. (i)
Its principal strength lies in the fact that it was the first
full case–control study to assess the present hypothesis.
Most of the previous studies [13–17] had an exposed-
only (or partial case–control) design [40], thus making it
impossible to analyze this interaction or effect modifier
because data on unexposed individuals were lacking [19].
Another earlier study [18] had a case-only design
but this design allows for multiplicative interactions;
nevertheless, additive interactions are far more common
in biology [41]. (ii) In contrast to the other full
case–control study [42], which did not take NSAID
exposure into account, we assessed whether CYP2C9
variants might modify the risk of the relationship
between NSAIDs and UGIB. (iii) A further strength is
the fact that the control group in our study fulfilled the
Hardy–Weinberg equilibrium, which was not the case in
other studies [13–16]. Lastly, another advantage of our
study is that stratification bias was controlled for [43,44]
by excluding non-Whites from the analysis.

The main limitation of our study pertains to the sample
size: despite it being the largest with respect to that of
other papers published to date on this topic [13–18],
the sample size used was not high enough to conduct an
analysis by genotype. However, our sample size was large
enough to analyze the effect of each allele. Another
possible limitation was our failure to determine the
CYP2C8 and CY2C19 polymorphisms. We nevertheless
feel that not having determined CYP2C8 does not affect
the study’s validity, as CYP2C8 plays a very marginal role
in the clearance of NSAIDs [12]: its partial metabolizing
role has only been described for the (R)-enantiomer of
ibuprofen [12], and in-vitro studies have shown this role
to be very marginal [45]. Furthermore, if its role were
indeed important, then an increased residual risk would
be observed in CYP2C9*2 carriers, because of its partial
binding disequilibrium with CYP2C9*3 [12]. In our
study, the risk associated with the *2 allele was found to
be very similar to that of the *1 allele, something that
would also support the negligible role played by CYP2C8
in NSAID clearance. With respect to CYP2C19, we
found no evidence to indicate that it might be associated
with NSAIDs-related UGIB. Moreover, there is no
description of it playing a relevant metabolizing role in
any NSAID [12]. Some direct association with risk for
UGIB has been found but this is in no way related to
NSAID use [42].

All in all, understanding that pharmacogenetics con-
tribute to variability in the NSAIDs dose–risk relation-
ship may help when it comes to choosing the NSAID and
the dosage that is safest and most effective. We found
that patients carrying the *3 variant experienced a higher
risk for NSAID-related UGIB. The use of CYP2C9 test-
ing could be a method for identifying such higher-risk
patients who are candidates for taking lower NSAID
doses or for using NSAIDs not metabolized by the
CYP2C9 enzyme. If our results are confirmed by other
studies and, bearing in mind (i) that NSAIDs are one of
the most widely used therapeutic groups, (ii) the elevated
incidence of NSAID-related UGIBs, and (iii) the current
low cost of SNP determination, these findings could have
significant implications for clinical practice and public
health.
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Conflicts of interest
There are no conflicts of interest.

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