CYP2C9 variants as a risk modifier of NSAID-related gastrointestinal bleeding: a case–control study

Adolfo Figueiras[a,b], Ana Estany-Gestal[a], Carmelo Aguirre[c], Borja Ruiz[d], Xavier Vidal[e], Alfonso Carvajal[f], Inés Salado[f], Angel Salgado-Barreira[b], Luca Rodella[g], Ugo Moretti[h] and Luisa Ibáñez[e]; on behalf of the EMPHOGEN group

Objective **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. *Pharmacogenetics and Genomics* 26:66–73**

Pharmacogenetics and Genomics 2016, **26**:66–73

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

[a]Department of Preventive Medicine and Public Health, Santiago de Compostela University, Santiago de Compostela, [b]Biomedical Research Network Consortium of Epidemiology and Public Health (*CIBER en Epidemiología y Salud Pública – CIBERESP*), [c]Basque Country Pharmacovigilance Unit, Galdakao-Usansolo Hospital, Galdakao, [d]Department of Pharmacology, Basque Country University School of Medicine, Leioa, Basque Country, [e]Department of Pharmacology, Therapeutics and Toxicology, Autonomous University, Catalonian Institute of Pharmacology, Clinical Pharmacology Service, Vall d'Hebron University Hospital, Barcelona, [f]Pharmacoepidemiology Institute, University of Valladolid, Valladolid, Spain, [g]Service of Digestive Endoscopy and [h]Clinical Pharmacology Unit, Verona University Hospital, Verona, Italy

Correspondence to Adolfo Figueiras, PhD, Department of Preventive Medicine and Public Health, Faculty of Medicine, University of Santiago de Compostelac/ San Francisco s/n., 15 786 Santiago de Compostela, A Coruña, Spain
Tel: +34 981 95 11 92; fax: +34 881 81 24 33; e-mail: adolfo.figueiras@usc.es

Received 11 July 2015 **Accepted** 12 October 2015

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.

DOI: 10.1097/FPC.0000000000000186

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, septoplasty, 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.

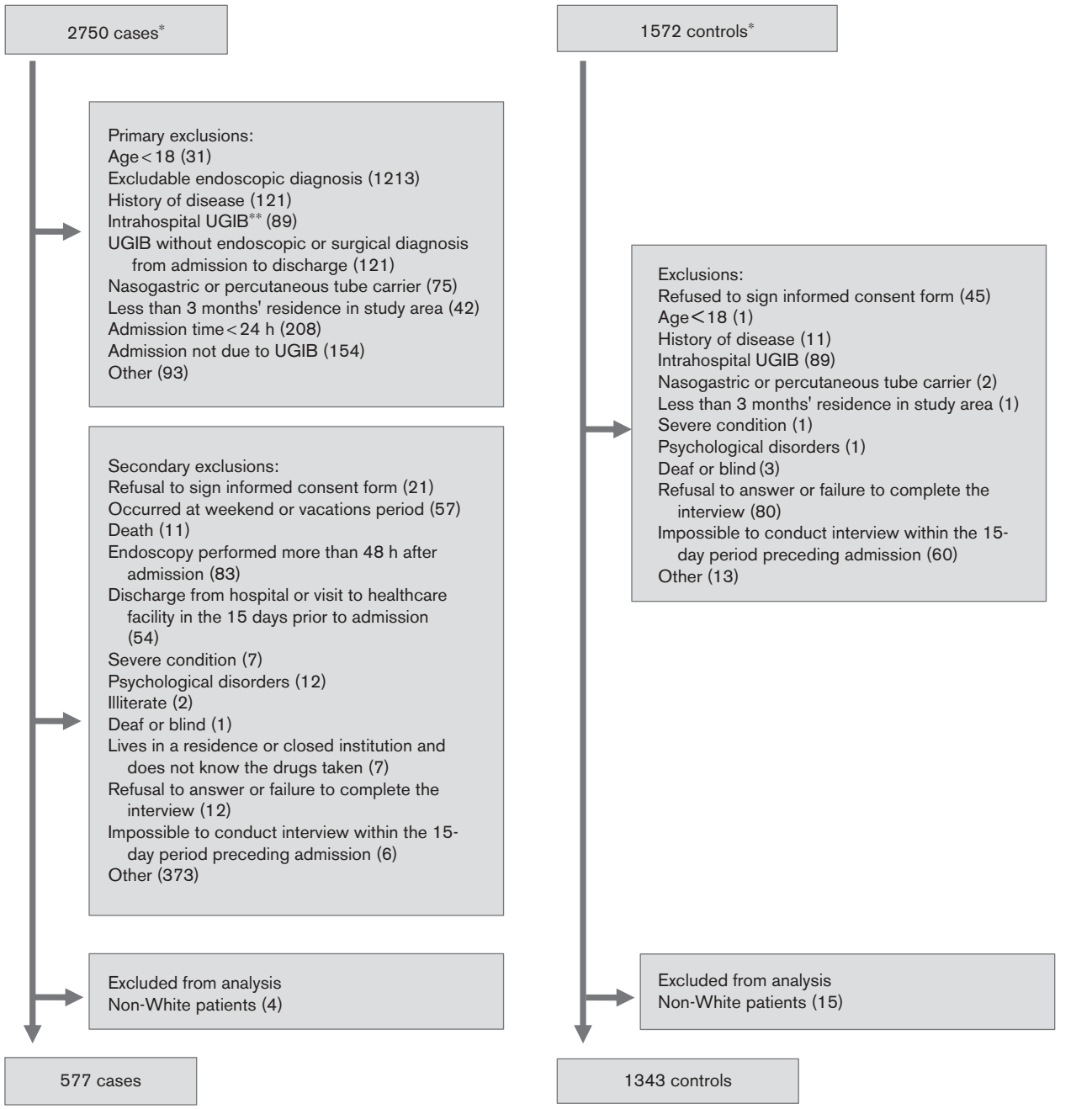
Single-nucleotide polymorphism genotyping

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

Fig. 1



Flow of participants through the study. Each patient may have been excluded for more than one criterion. UGIB, upper gastrointestinal bleeding. *Subjects may have been excluded on the basis of more than one criterion. **It was considered upper gastrointestinal bleeding from 48 hours before hospitalization.

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 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 these effects considered separately. *S* has been shown to be the most reliable measure of additive interaction when adjusting for confounding [37].

Table 1 Description of cases and controls[a]

	N (%)		
	Cases ($n=577$)	Controls ($n=1343$)	*P*-value[b]
Demographic variables			
Sex (men)	426 (73.8)	940 (70.0)	0.077
Age [mean (SD)]	62.77 (16.856)	63.45 (15.585)	0.376
Interview variables			
Number of interviews conducted			<0.001[c]
1	500 (86.7)	1278 (95.2)	
≥2	77 (13.3)	65 (4.8)	
Reliability of interview			0.020[c]
<5	29 (5.0)	36 (2.7)	
5–7	67 (11.6)	158 (11.8)	
7–9	252 (43.7)	564 (42.0)	
≥9	229 (39.7)	585 (43.6)	
Comorbidity			
BMI [mean (SD)]	26.58 (4.351)	26.60 (4.01)	0.959
Diabetes	95 (16.5)	185 (13.8)	0.144
Cardiovascular disease	153 (27.0)	280 (21.0)	0.005[c]
Arterial hypertension	227 (39.4)	506 (38.0)	0.654
Elevated cholesterol	174 (30.5)	379 (28.5)	0.356
Positive *Helicobacter pylori*	338 (91.4)	911 (80.6)	<0.001[c]
Personal history of GI disorders			<0.001[c]
None or dyspepsia	371 (64.6)	1147 (85.8)	
Ulcer	81 (14.1)	105 (7.9)	
Bleeding	122 (21.3)	85 (6.4)	
Arthrosis	169 (30.8)	444 (35.3)	0.048[c]
Arthritis	63 (5.1)	34 (6.2)	0.270
Consumption of caffeine	486 (84.2)	1133 (84.4)	0.766
Smoking habit			0.056
Nonsmoker/ex-smoker	426 (73.8)	1043 (77.7)	
Moderate	68 (11.8)	150 (11.2)	
Heavy	83 (14.4)	150 (11.2)	
Alcohol			0.056
Abstainer	468 (34.8)	196 (34.0)	
Little	676 (50.3)	274 (47.5)	
Moderate	175 (13.0)	86 (14.9)	
Heavy	24 (1.8)	21 (3.6)	
Comedication			
NSAID			–
DDD of NSAID = 0	455 (78.9)	1229 (91.5)	
0 > DDDs of NSAID ≤ 0.50	66 (11.4)	65 (4.8)	
DDDs of NSAID > 0.50	56 (9.7)	49 (3.6)	
Exposure to NSAID not metabolized by CYP2C9	186 (32.2)	209 (15.6)	<0.001[c]
Exposure to PPIs	53 (9.2)	170 (12.7)	0.029[c]
Exposure to antiplatelets	116 (20.1)	186 (13.8)	<0.001[c]
Exposure to anticoagulants	43 (7.5)	63 (4.7)	0.011[c]
Exposure			
NSAID metabolized by CYP2C9			<0.001[c]
DDD of NSAID = 0	474 (82.1)	1254 (93.4)	
0 > DDDs of NSAID ≤ 0.50	54 (9.4)	55 (4.1)	
DDDs of NSAID > 0.50	49 (8.5)	34 (2.5)	
CYP2C9 genotype			0.095
CYP2C9*1/*1	299 (59.4)	742 (59.6)	
CYP2C9*1/*2	105 (20.9)	295 (23.7)	
CYP2C9*2/*2	11 (2.2)	31 (2.5)	
CYP2C9*1/*3	66 (13.1)	142 (11.4)	
CYP2C9*2/*3	13 (2.6)	27 (2.2)	
CYP2C9*3/*3	9 (1.8)	8 (0.6)	

DDD, defined daily dose; GI, gastrointestinal; PPI, proton pump inhibitors.
[a]Non-European patients were excluded.
[b]The *P*-value for this variable is polychotomous – that is, it is the joint *P*-value for the entire variable.
[c]Variables included in the multivariate model.

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 NSAIDs 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 ($P < 0.05$).

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

	Cases		Controls	
Genotypes	Expected prevalence (%)	Real prevalence [*n* (%)]	Expected prevalence (%)	Real prevalence [*n* (%)]
CYP2C9*1/*1	59.5	299 (59.4)	59.6	742 (59.6)
CYP2C9*1/*2	22.9	105 (20.1)	22.9	295 (23.7)
CYP2C9*2/*2	2.4	11 (2.2)	2.4	31 (2.5)
CYP2C9*1/*3	11.9	66 (13.1)	11.9	142 (11.4)
CYP2C9*3/*3	1.0	9 (1.8)	0.9	8 (0.6)
CYP2C9*2/*3	2.3	13 (2.6)	2.3	27 (2.2)
Variants	HWE (*P*-value)[†]		HWE (*P*-value)[†]	
*CYP2C9*2* (C430T)	0.578		0.745	
*CYP2C9*3* (A1075C)	0.036		0.538	

HWE, Hardy–Weinberg equilibrium.
[†]$P \leq 0.05$ was deemed statistically significant.

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

	Adjusted OR[a] (95% CI)	*P*-value
Allele CYP2C9*1		
DDDs of NSAIDs = 0	1.00 (ref)	–
0 < DDDs of NSAIDs ≤ 0.50	3.19 (1.76–5.78)	< 0.001
DDDs of NSAIDs > 0.50	8.79 (4.50–17.17)	< 0.001
Allele CYP2C9*2		
DDDs of NSAIDs = 0	1.05 (0.77–1.42)	0.776
0 < DDDs of NSAIDs ≤ 0.50	1.31 (0.36–4.71)	0.679
DDDs of NSAIDs > 0.50	10.15 (2.92–35.35)	< 0.001
Allele CYP2C9*3		
DDDs of NSAIDs = 0	1.54 (1.04–2.26)	0.030
0 < DDDs of NSAIDs ≤ 0.50	2.61 (0.62–11.10)	0.193
DDDs of NSAIDs > 0.50	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.
[a]ORs adjusted for the following confounding variables: personal history of gastrointestinal disorders; presence of *H. pylori*; osteoarthritis; number of interviews conducted with the patient; reliability of the interview; patients exposed to proton pump inhibitors, antiplatelet agents, oral anticoagulants, and NSAIDs not metabolized by CYP2C9.

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 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[a] (95% CI)	*P*-value
Genotypes without CYP2C9*3[b]		
DDDs of NSAIDs = 0	1.00 (ref)	–
0 < DDDs of NSAIDs ≤ 0.5	3.22 (1.68–6.20)	< 0.001
DDDs of NSAIDs > 0.5	9.72 (4.55–20.76)	< 0.001
Genotypes with CYP2C9*3[c]		
DDDs of NSAIDs = 0	1.37 (0.90–2.11)	0.140
0 < DDDs of NSAIDs ≤ 0.5	3.88 (0.85–17.81)	0.080
DDDs of NSAIDs > 0.5	16.92 (4.96–57.59)	< 0.001

CI, confidence interval; DDD, assumed average maintenance dose per day for a drug used for its main indication in adults.
[a]ORs adjusted for the following confounding variables: personal history of gastrointestinal disorders; presence of *Helicobacter pylori*; osteoarthritis; number of interviews conducted with the patient; reliability of the interview; patients exposed to proton pump inhibitors, antiplatelet agents, oral anticoagulants, and NSAIDs not metabolized by CYP2C9.
[b]*1/*1,*1/*2, or *2/*2.
[c]*1/*3,*3/*3, or *2/*3.

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 (*n* = 103)	CYP2C9*3 (*n* = 88)	NSAID plus CYP2C9*3 (*n* = 16)	None (*n* = 402)
Erosions				
Gastric	41 (39.8)	25 (28.4)	4 (25.0)	102 (25.4)
Duodenal	32 (31.1)	22 (25.0)	8 (50.0)	55 (13.7)
Ulcers				
Gastric	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	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 contrast, 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 noncarriers 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 therapeutic 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 presence 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 gastrointestinal 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 contribute to variability in the NSAIDs dose–risk relationship 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* testing 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.

Acknowledgements

The authors thank Michael Benedict for his help with the English version of this paper.

This research/work has been funded in part by Instituto de Salud Carlos III (PI12/02414)/Plan Estatal de I + D + I 2012-2016 and Fondo Europeo de Desarrollo Regional (FEDER). This research was also funded by grants PI021512, PI021364, PI020661, PI021572 (Health Research Fund/Fondo de Investigación Sanitaria), SAF2002-04057 (Ministry of Health & Consumer Affairs, Spain), PGIDIT03PXIC20806PN (Galician Regional Authority, Spain). The study was also supported in part by the Novartis, Pfizer and Dr Esteve pharmaceutical companies.

The EMPHOGEN project (multicentric study of genetic polymorphisms) consists of A. Figueiras, P. López, P. Sánchez-Diz, A. Salgado-Barreira, A. Ruano, E. Domínguez, A. Estany-Gestal (Santiago de Compostela, Spain, co-ordinating group); S. Ortega, A. Carvajal (Valladolid, Spain); B. Ruiz, C. Aguirre, M.M. de Pancorbo, P. Ruiz (Bilbao, Spain); L. Ibáñez, X. Vidal (Barcelona, Spain) and A. Conforti, R. Leone (Verona, Italy).

Conflicts of interest

There are no conflicts of interest.

References

1 Pirmohamed M, James S, Meakin S, Green C, Scott AK, Walley TJ*, et al*. Adverse drug reactions as cause of admission to hospital: prospective analysis of 18 820 patients. *BMJ* 2004; **329**:15–19.
2 Wolfe MM, Lichtenstein DR, Singh G. Gastrointestinal toxicity of nonsteroidal antiinflammatory drugs. *N Engl J Med* 1999; **340**:1888–1899.
3 Green GA. Understanding NSAIDs: from aspirin to COX-2. *Clin Cornerstone* 2001; **3**:50–60.
4 Laporte JR, Ibáñez L, Vidal X, Vendrell L, Leone R. Upper gastrointestinal bleeding associated with the use of NSAIDs: newer versus older agents. *Drug Saf* 2004; **27**:411–420.
5 Miners JO, Birkett DJ. Cytochrome P4502C9: an enzyme of major importance in human drug metabolism. *Br J Clin Pharmacol* 1998; **45**:525–538.
6 Ingelman-Sundberg M, Sim SC, Gomez A, Rodriguez-Antona C. Influence of cytochrome P450 polymorphisms on drug therapies: pharmacogenetic, pharmacoepigenetic and clinical aspects. *Pharmacol Ther* 2007; **116**:496–526.
7 Kirchheiner J, Brockmöller J. Clinical consequences of cytochrome P450 2C9 polymorphisms. *Clin Pharmacol Ther* 2005; **77**:1–16.
8 Sánchez-Diz P, Estany-Gestal A, Aguirre C, Blanco A, Carracedo A, Ibáñez L, *et al*. Prevalence of CYP2C9 polymorphisms in the south of Europe. *Pharmacogenomics J* 2009; **9**:306–310.
9 Lee CR, Goldstein JA, Pieper JA. Cytochrome P450 2C9 polymorphisms: a comprehensive review of the in-vitro and human data. *Pharmacogenetics* 2002; **12**:251–263.
10 Xie HG, Prasad HC, Kim RB, Stein CM. CYP2C9 allelic variants: ethnic distribution and functional significance. *Adv Drug Deliv Rev* 2002; **54**:1257–1270.
11 Wang L, McLeod HL, Weinshilboum RM. Genomics and drug response. *N Engl J Med* 2011; **364**:1144–1153.
12 Agúndez JA, García-Martín E, Martínez C. Genetically based impairment in CYP2C8- and CYP2C9-dependent NSAID metabolism as a risk factor for gastrointestinal bleeding: is a combination of pharmacogenomics and metabolomics required to improve personalized medicine? *Expert Opin Drug Metab Toxicol* 2009; **5**:607–620.
13 Martin JH, Begg EJ, Kennedy MA, Roberts R, Barclay ML. Is cytochrome P450 2C9 genotype associated with NSAID gastric ulceration? *Br J Clin Pharmacol* 2001; **51**:627–630.
14 Vonkeman HE, van de Laar MA, van der Palen J, Brouwers JR, Vermes I. Allele variants of the cytochrome P450 2C9 genotype in white subjects from the Netherlands with serious gastroduodenal ulcers attributable to the use of NSAIDs. *Clin Ther* 2006; **28**:1670–1676.
15 Pilotto A, Seripa D, Franceschi M, Scarcelli C, Colaizzo D, Grandone E*, et al*. Genetic susceptibility to nonsteroidal anti-inflammatory drug-related gastroduodenal bleeding: role of cytochrome P450 2C9 polymorphisms. *Gastroenterology* 2007; **133**:465–471.
16 Ma J, Yang XY, Qiao L, Liang LQ, Chen MH. CYP2C9 polymorphism in non-steroidal anti-inflammatory drugs-induced gastropathy. *J Dig Dis* 2008; **9**:79–83.
17 Blanco G, Martínez C, Ladero JM, Garcia-Martin E, Taxonera C, Gamito FG*, et al*. Interaction of CYP2C8 and CYP2C9 genotypes modifies the risk for nonsteroidal anti-inflammatory drugs-related acute gastrointestinal bleeding. *Pharmacogenet Genomics* 2008; **18**:37–43.
18 Carbonell N, Verstuyft C, Massard J, Letierce A, Cellier C, Deforges L*, et al*. CYP2C9*3 loss-of-function allele is associated with acute upper gastrointestinal bleeding related to the use of NSAIDs other than aspirin. *Clin Pharmacol Ther* 2010; **87**:693–698.
19 Estany-Gestal A, Salgado-Barreira A, Sánchez-Diz P, Figueiras A. Influence of CYP2C9 genetic variants on gastrointestinal bleeding associated with nonsteroidal anti-inflammatory drugs: a systematic critical review. *Pharmacogenet Genomics* 2011; **21**:357–364.
20 Rothman KJ, Greenland S. *Modern epidemiology*, 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 1998. p. 119.
21 Lanas A, Bajador E, Serrano P, Fuentes J, Carreño S, Guardia J*, et al*. Nitrovasodilators, low-dose aspirin, other nonsteroidal antiinflammatory drugs, and the risk of upper gastrointestinal bleeding. *N Engl J Med* 2000; **343**:834–839.
22 Laporte JR, Carné X, Vidal X, Moreno V, Juan J. Upper gastrointestinal bleeding in relation to previous use of analgesics and non-steroidal anti-inflammatory drugs. Catalan Countries Study on Upper Gastrointestinal Bleeding. *Lancet* 1991; **337**:85–89.
23 TrinityBiotech. *Helicobacter pylori* test. Available at: *http://www.trinitybiotech.com/products/uni-gold-h-pylori-antigen/*. [Accessed 11 February 2009].
24 Gisbert JP, Abraira V. Accuracy of *Helicobacter pylori* diagnostic tests in patients with bleeding peptic ulcer: a systematic review and meta-analysis. *Am J Gastroenterol* 2006; **101**:848–863.
25 WHO Collaborating Centre for Drug Statistics Methodology. Norwegian Institute of Public Health. Definition and general considerations. Available at: *http://www.whocc.no/ddd/definition_and_general_considera/*. [Accessed 1 May 2012].
26 Salanti G, Amountza G, Ntzani EE, Ioannidis JP. Hardy–Weinberg equilibrium in genetic association studies: an empirical evaluation of reporting, deviations, and power. *Eur J Hum Genet* 2005; **13**:840–848.
27 Chen J, Chatterjee N. Exploiting Hardy–Weinberg equilibrium for efficient screening of single SNP associations from case–control studies. *Hum Hered* 2007; **63**:196–204.
28 Puehringer H, Loreth RM, Klose G, Schreyer B, Krugluger W, Schneider B, Oberkanins C. VKORC1 −1639G > A and CYP2C9*3 are the major genetic predictors of phenprocoumon dose requirement. *Eur J Clin Pharmacol* 2010; **66**:591–598.
29 Aynacioglu AS, Brockmöller J, Bauer S, Sachse C, Güzelbey P, Ongen Z*, et al*. Frequency of cytochrome P450 CYP2C9 variants in a Turkish population and functional relevance for phenytoin. *Br J Clin Pharmacol* 1999; **48**:409–415.
30 González JR, Armengol L, Guinó E, Solé X, Moreno V. SNPassoc: SNPs-based whole genome association studies. R package version 1.6-0; 2009. Available at: *http://CRAN.R-project.org/*. [Accessed 3 September 2012].
31 Liu L, Shao JH, Wang K, Hua FZ. Methodological concerns about a recent meta-analysis of the influence of the I148M variant of patatin-like phospholipase domain containing 3 on the susceptibility and histological severity of nonalcoholic fatty liver disease. *Hepatology* 2011; **53**:2145.
32 Brown H, Prescott R. *Applied mixed models in medicine*, 2nd ed. New Jersey: John Wiley and Sons; 2006. pp. 320–321.
33 Bates D, Sarkar D. lme4: linear mixed-effects models using S4 classes. R package version 0.999375-26; 2007. Available at: *http://CRAN.R-project.org/*. [Accessed 30 May 2012].
34 Mickey RM, Greenland S. The impact of confounder selection criteria on effect estimation. *Am J Epidemiol* 1989; **129**:125–137.
35 Schwarz G. Estimating the dimension of a model. *Ann Stat* 1978; **6**:461–464.

36 Figueiras A, Domenech-Massons JM, Cadarso C. Regression models: calculating the confidence interval of effects in the presence of interactions. *Stat Med* 1998; **17**:2099–2105.
37 Skrondal A. Interaction as departure from additivity in case-control studies: a cautionary note. *Am J Epidemiol* 2003; **158**:251–258.
38 McGettigan P, Henry D. Use of non-steroidal anti-inflammatory drugs that elevate cardiovascular risk: an examination of sales and essential medicines lists in low-, middle-, and high-income countries. *PLoS Med* 2013; **10**:e1001388.
39 Higashi MK, Veenstra DL, Kondo LM, Wittkowsky AK, Srinouanprachanh SL, Farin FM, Rettie AE. Association between CYP2C9 genetic variants and anticoagulation-related outcomes during warfarin therapy. *JAMA* 2002; **287**:1690–1698.
40 Liu X, Fallin MD, Kao WH. Genetic dissection methods: designs used for tests of gene-environment interaction. *Curr Opin Genet Dev* 2004; **14**:241–245.
41 Albert PS, Ratnasinghe D, Tangrea J, Wacholder S. Limitations of the case-only design for identifying gene-environment interactions. *Am J Epidemiol* 2001; **154**:687–693.
42 Musumba CO, Jorgensen A, Sutton L, Van Eker D, Zhang E, O'Hara N, *et al.* CYP2C19*17 gain-of-function polymorphism is associated with peptic ulcer disease. *Clin Pharmacol Ther* 2013; **93**:195–203.
43 Cardon LR, Palmer LJ. Population stratification and spurious allelic association. *Lancet* 2003; **361**:598–604.
44 Lee WC, Wang LY. Simple formulas for gauging the potential impacts of population stratification bias. *Am J Epidemiol* 2008; **167**:86–89.
45 Chang SY, Li W, Traeger SC, Wang B, Cui D, Zhang H, *et al.* Confirmation that cytochrome P450 2C8 (CYP2C8) plays a minor role in (S)-(+)- and (R)-(−)-ibuprofen hydroxylation in vitro. *Drug Metab Dispos* 2008; **36**:2513–2522.