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SPECIAL REPORT

# Genetic predisposition to acute gastrointestinal bleeding after NSAIDs use

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Impaired drug metabolism is a major cause of adverse drug reactions, and it is often caused by mutations at genes coding for drug-metabolising enzymes. Two amino-acid polymorphisms of cytochrome *P*4502C9 (CYP2C9), an enzyme involved in the metabolism of several nonsteroidal anti-inflammatory drugs (NSAIDs), were studied in 94 individuals with acute bleeding after NSAIDs use and 124 individuals receiving NSAIDs with no adverse effects. The frequency of *CYP2C9* variant alleles was increased in overall bleeding patients, with a significant trend to higher risk with increasing number of variant alleles ($P=0.02$). The odds ratio for bleeding patients receiving CYP2C9 substrates ($n=33$) was 2.5 for heterozygous and 3.7 for homozygous carriers of mutations ($P<0.015$), suggesting that the inherited impairment of CYP2C9 activity increases the risk for severe adverse drug reactions after NSAIDs use.
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**Abbreviations:** CYP2C9, Cytochrome *P*4502C9; NSAIDs, nonsteroidal anti-inflammatory drugs

**Introduction** Severe adverse effects secondary to the use of nonsteroidal anti-inflammatory drugs (NSAIDs) occur in a small percentage of subjects. Such effects are of high importance because of their clinical consequences and because millions of people are daily treated with NSAIDs. Acute gastrointestinal bleeding is an unwanted side effect common to all chemical types of NSAIDs, and occurs either with oral or parenteral administration of the drugs, thus indicating that a local irritation mechanism is not enough to explain gastrointestinal bleeding after NSAIDs use.

Interindividual variability in drug metabolism is a major cause of adverse drug effects. In many cases, such variability is linked to polymorphisms in genes coding for drug-metabolising enzymes. Individuals carrying enzyme-inactivating mutations display impaired drug metabolism. Higher plasma drug concentrations and lower clearance rates occur in carriers of inactivating mutations when treated at standard doses.

The enzyme CYP2C9 is responsible for the metabolism of several NSAIDs (Goldstein & de Morais, 1994). The gene coding for the CYP2C9 enzyme is polymorphic, and several allelic variants of the gene have been described (Xie *et al*., 2002). Two of these variant alleles occur with a high population frequency and are related with impaired drug metabolism in white subjects. These variant alleles designated as *CYP2C9*2* and *CYP2C9*3* consist of single-nucleotide substitutions that cause the amino-acid changes R144C and I359L, respectively. Both variant alleles lead to decreased enzyme activity on CYP2C9 substrates, as compared with the wild-type allele, designated as *CYP2C9*1* (Haining *et al*., 1996; Crespi & Miller, 1997). Major clinical implications of *CYP2C9* genotype have been shown with warfarin; individuals carrying variant alleles require low doses and are at increased risk for major bleeding complications during therapy (Aithal *et al*., 1999). Since most NSAIDs are metabolised by the CYP2C9 enzyme, it can be hypothesised that individuals carrying variant alleles, and therefore with low enzyme activity *in vivo*, should be at a higher risk of developing adverse drug reactions with NSAIDs use. This study was carried out to investigate such a hypothesis.

**Methods** The study group consisted of 94 patients suffering from acute gastrointestinal bleeding after NSAIDs use and 124 individuals who consumed NSAIDs, at similar doses as patients and that reported no adverse effects (Table 1). All consecutive patients who matched the inclusion criteria (i.e. evidence for upper gastrointestinal bleeding with immediate antecedents of NSAID therapy and the absence of other factors that may have caused gastrointestinal bleeding) were required to participate in the study and all of them agreed. To avoid confounding factors, patients taking NSAIDs because of gastric pain, as well as patients under concomitant therapy with drugs that are substrates or inhibitors of CYP2C9 were not included. Endoscopy was carried out in 93 bleeding patients, and the infection by *Helicobacter pylori* was investigated in 62. Control individuals were consecutively

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selected from patients with diverse pathologies who required NSAIDs therapy, and who attended the participating hospitals. Over 95% of control individuals requested agreed to participate. Informed consent was a prerequisite for inclusion. Indication for NSAID therapy was both, for bleeding patients and controls, as follows: individuals under long-term therapy received NSAIDs due to arthrosis, rheumatoid arthritis or as anti-aggregation therapy. Individuals with short-term therapy received NSAIDs because of acute osteoarticular pain. Data concerning concomitant drug use, including proton-pump inhibitors and other drugs used to prevent gastric ulcerations, were collected for bleeding patients and control individuals. The study was approved by the ethics committees of the participating hospitals.

Blood samples were immediately frozen after collection and kept at −80 °C until analysis. Genomic DNA was prepared from peripheral leucocytes, and dissolved in sterile 10 mM Tris–HCl, pH 8.0, 1 mM ethylenediaminetetraacetic acid at a final concentration of 400–600 $\mu g/ml^{-1}$. The samples were stored at 4 °C in sterile plastic vials. The presence of *CYP2C9* variant alleles was investigated by the use of polymerase chain reaction (PCR) and restriction mapping as described elsewhere (Sullivan-Klose *et al*., 1996). The intergroup comparison values were calculated by using the $\chi^2$-test, unless the conditions for the application of this test were not valid. In such cases, Fisher's exact test was used to calculate the *P* value. Intergroup comparisons were considered as statistically significant when *P* values were below 0.05. The 95% confidence intervals (CI) were calculated by using the SPSS (10.0) statistical package.

**Table 1** Descriptive data of bleeding patients and control subjects involved in the study

| *Variable* | *Bleeding patients (n = 94)* | *Control patients (n = 124)* |
|---|---|---|
| *Age (years)* | | |
| Mean (s.d.) | 62.2 (19.9) | 68.3 (12.7) |
| *Gender* | | |
| Men | 53 | 58 |
| Women | 41 | 66 |
| *Duration of NSAID therapy (days)* | | |
| 1 | 13 | 0 |
| 2–3 | 15 | 0 |
| 4–7 | 31 | 0 |
| Over 7 | 35 | 124 |
| Mean (days; s.d.) | 249 (713) | 1084 (1536) |
| *Types of NSAID* | *Number, daily dose (min–max, mg)* | *Number, daily dose (min–max, mg)* |
| Aceclofenac | 2 (100–200) | 4 (100–200) |
| Celecoxib | 3 (200–600) | 4 (200–400) |
| Diclofenac | 8 (50–300) | 13 (50–250) |
| Ibuprofen | 9 (600–1800) | 10 (600–1800) |
| Indomethazine | 1 (150) | 3 (75–150) |
| Lornoxicam | 1 (16) | 3 (8–16) |
| Piroxicam | 7 (20–200) | 8 (20–120) |
| Naproxen | 2 (500–1000) | 5 (500–1000) |
| Salicylates | 51 (100–2000) | 59 (100–2000) |
| Paracetamol | 2 (500) | 4 (500–1000) |
| Metamizole | 1 (575) | 1 (575) |
| Ketorolac | 4 (10–70) | 6 (10–60) |
| Dexketoprofen | 3 (25–75) | 4 (25–75) |

**Results** The *CYP2C9* genotypes and allele frequency of bleeding patients and controls are shown in Tables 2 and 3. The frequency for *CYP2C9* variant alleles was increased in patients with acute bleeding, with an odds ratio (OR) value of 1.64 (95% CI 1.05–2.58; $P = 0.023$). Assuming that the risk is linked to the possession of variant alleles, the OR for individuals carrying variant alleles is 1.76 (0.99–3.13; $P = 0.042$) and the $\chi^2$-value for linear test for trend is 5.35 ($P = 0.020$), with an OR of 1.61 for heterozygous and 3.10 for homozygous carriers of mutations.

Nine bleeding patients and 34 control individuals concomitantly received proton-pump inhibitors and/or other drugs to prevent gastric ulcerations. When these individuals are not

**Table 2** *CYP2C9* genotyping results in patients with acute gastrointestinal bleeding secondary to NSAID and in control individuals who reported no adverse effects during NSAID therapy

| *Genotype* | *Bleeding patients num, (%)* | *Control patients num, (%)* | *Crude OR* | *95% CI* | P |
|---|---|---|---|---|---|
| *CYP2C9*1/*1* | 43 (45.7) | 74 (59.7) | 0.57 | 0.32–1.02 | 0.041 |
| *CYP2C9*1/*2* | 30 (31.9) | 29 (23.4) | 1.54 | 0.81–2.93 | 0.161 |
| *CYP2C9*1/*3* | 12 (12.7) | 16 (12.9) | 0.99 | 0.41–2.35 | 0.976 |
| *CYP2C9*2/*2* | 6 (6.4) | 2 (1.6) | 4.16 | 0.74–30.6 | 0.078[a] |
| *CYP2C9*2/*3* | 2 (2.1) | 1 (0.8) | 2.67 | 0.19–75.7 | 0.579[a] |
| *CYP2C9*3/*3* | 1 (1.1) | 2 (1.6) | 0.66 | 0.02–9.39 | 0.731[a] |
| Total | 94 (100) | 124 (100) | – | – | – |

[a]Fisher's test was used for these comparisons.

**Table 3** Frequencies for *CYP2C9* variant alleles in patients with acute gastrointestinal bleeding secondary to NSAID and in control individuals who reported no adverse effects during NSAID therapy

| *Variant allele* | *Bleeding patients num, (%)* | *Control patients num, (%)* | *Crude OR* | *95% CI* | P |
|---|---|---|---|---|---|
| *CYP2C9*1* | 128 (68.1) | 193 (77.8) | 0.61 | 0.39–0.95 | 0.022 |
| *CYP2C9*2* | 44 (23.4) | 34 (13.7) | 1.92 | 1.14–3.25 | 0.009 |
| *CYP2C9*3* | 16 (8.5) | 21 (8.5) | 1.01 | 0.48–2.08 | 0.987 |
| Total | 188 (100) | 248 (100) | – | – | – |

included in the comparison, the OR for variant *CYP2C9* alleles is 1.69 (95% CI 0.94–3.06), almost identical to that obtained when concomitant drug use was not taken into consideration (data not shown).

Confounders such as infection by *H. pylori*, gender and type of gastrointestinal lesion (i.e. peptic ulceration or acute bleeding gastropathy) did not significantly influence the genotype results. In all, 13 bleeding patients had a history of acute gastrointestinal bleeding, and the frequency of variant alleles in this subgroup of patients did not differ from the overall bleeding patients group. The increase in the frequency of variant alleles among bleeding patients was independent of the time elapsed from the treatment initiation until the onset of acute bleeding. The lack of impact of these possible confounder factors in the association of *CYP2C9* polymorphism and bleeding risk can be evaluated by comparing the data of subgroups of bleeding patients, shown in Table 4, with the data from control patients shown in Tables 2 and 3.

Regarding the drug type, the relative risk for carriers of variant alleles was analysed independently among bleeding patients treated with NSAIDs associated with a high and low risk of major gastrointestinal complications, according to the estimated relative risks reported elsewhere (Henry *et al*., 1996). Such factors did not significantly influence the bleeding risk associated to *CYP2C9* genotype. The OR for carriers of variant alleles among bleeding patients receiving drugs with a high relative risk for gastrointestinal complications, such as piroxicam, indomethazine or naproxen was 1.6. This value is similar to that of bleeding patients receiving drugs with a low relative risk for gastrointestinal complications such as ibuprofen or diclofenac, with an OR of 1.7.

In contrast, the association of *CYP2C9* genotype and bleeding risk depended on whether the drug is a substrate of the CYP2C9 enzyme. In all, 33 bleeding patients were under therapy with NSAIDs, who undergo extensive CYP2C9 metabolism, including aceclofenac, celecoxib, diclofenac, ibuprofen, indomethazine, lornoxicam, piroxicam and naproxen (Goldstein & de Morais, 1994; Lee *et al*., 2002). Among these patients, the OR for carriers of variant alleles, as compared with 50 control subjects receiving such drugs was 2.60 (95% CI 1.10–6.19; $\chi^2$ 5.68, $P=0.017$). The $\chi^2$-value for the linear test for trend is 5.92 ($P=0.015$), with an OR of 2.47 for heterozygous and 3.70 for homozygous carriers of mutations. In the rest of the bleeding patients, a lower influence of CYP2C9 genotype in bleeding risk was observed. In all, 53 patients were treated with NSAIDs that are partially metabolised by CYP2C9, such as salicylates and paracetamol (Patten *et al*., 1993; Miners & Birkett, 1998). Among these patients, the OR for carriers of variant alleles is 1.49 (1.33 for heterozygous and 1.53 for homozygous individuals) when compared with 63 controls treated with these drugs. Eight bleeding patients received drugs in whose metabolism the role of CYP2C9 has not been fully elucidated, including metamizole, ketorolac and dexketoprofen. The OR for carriers of variant alleles, as compared with 11 control subjects receiving the same drugs, is 0.89.

**Discussion** This study provides evidences for inherited susceptibility of developing severe adverse drug reactions during NSAIDs use. Such susceptibility is linked to amino acid polymorphisms of the CYP2C9 enzyme. The association of

**Table 4** Summary of genotyping data in subgroups of bleeding patients

| *Variable* | *Overall bleeding patients* | *NSAID type* | | *Age* | | *Gender* | | *Type of lesion* | | *Helicobacter pylori* + | *Other factors* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *Extensive CYP2C9 metabolism* | *Partial CYP2C9 metabolism* | *Age <median* | *Age ⩾median* | *Men* | *Women* | *Peptic ulcer* | *Acute gastric lesion* | | *Antecedents of acute bleeding* | *Days of therapy ⩽median* |
| *Allele variant* | | | | | | | | | | | | |
| *CYP2C9*1* | 128 (68.1) | 42 (63.6) | 73 (68.9) | 68 (69.4) | 60 (66.7) | 74 (69.8) | 54 (65.9) | 91 (66.9) | 21 (70.0) | 56 (65.1) | 17 (65.4) | 65 (70.7) |
| *CYP2C9*2* | 44 (23.4) | 17 (25.8) | 24 (22.6) | 23 (23.5) | 21 (23.3) | 24 (22.6) | 20 (24.4) | 33 (24.3) | 6 (20.0) | 21 (24.4) | 8 (30.8) | 21 (22.8) |
| *CYP2C9*3* | 16 (8.5) | 7 (10.6) | 9 (8.5) | 7 (7.1) | 9 (10.0) | 8 (7.6) | 8 (9.8) | 12 (8.8) | 3 (10.0) | 9 (10.5) | 1 (3.9) | 6 (6.5) |
| Total alleles | 188 (100) | 66 (100) | 106 (100) | 98 (100) | 90 (100) | 106 (100) | 82 (100) | 136 (100) | 30 (100) | 86 (100) | 26 (100) | 92 (100) |
| *Genotype* | | | | | | | | | | | | |
| Nonmutated | 43 (45.7) | 12 (36.4) | 25 (47.1) | 23 (46.9) | 20 (44.4) | 25 (47.2) | 18 (43.9) | 29 (42.6) | 8 (53.3) | 17 (39.5) | 6 (46.1) | 22 (47.8) |
| Heterozygous | 42 (44.7) | 18 (54.5) | 23 (43.4) | 22 (44.9) | 20 (44.4) | 24 (45.3) | 18 (43.9) | 33 (48.5) | 5 (33.3) | 22 (51.1) | 5 (38.5) | 21 (45.7) |
| Homozygous | 9 (9.6) | 3 (9.1) | 5 (9.4) | 4 (8.1) | 5 (11.1) | 4 (7.5) | 5 (12.2) | 6 (8.8) | 2 (13.3) | 4 (9.3) | 2 (15.3) | 3 (6.5) |
| Total subjects | 94 (100) | 33 (100) | 53 (100) | 49 (100) | 45 (100) | 53 (100) | 41 (100) | 68 (100) | 15 (100) | 43 (100) | 13 (100) | 46 (100) |

Genotyping data for control patients are shown in Tables 2 and 3. For details on NSAIDs that undergo extensive or partial CYP2C9 metabolism, see the text. The median values are the following: age, 67 years; days of therapy until the onset of bleeding, 7 days.

variant *CYP2C9* alleles and the risk of acute gastrointestinal bleeding shows a gene–dose effect (i.e. the risk increases with the number of mutated allelic variants), and it is higher in patients receiving drugs that are mainly metabolised by the CYP2C9 enzyme. This suggests that *CYP2C9* genotyping may identify a subgroup of individuals who are at a potentially increased risk of acute gastrointestinal bleeding when treated with NSAIDs. It should be pointed out that in this study the observed risk is mainly related to the *CYP2C9*2* allele, either in heterozygosity or homozygosity (Tables 2 and 3). This is surprising since the *CYP2C9*2* variant allele does not contain mutations that affect substrate binding capacity (Lee *et al*., 2002). A possible explanation for the association of *CYP2C9*2* with NSAID-related bleeding risk may be related to a combined effect of mutations on *CYP2C8* and *CYP2C9* genes. In fact, a linkage disequilibrium between *CYP2C9*2* and *CYP2C8*3* variant alleles has been shown (Yasar *et al*., 2002). Further studies on the individualised role of both *CYP2C8* and *CYP2C9* variant alleles on the clearance *in vivo* of NSAIDs, as well as analyses on the impact of *CYP2C8*/*CYP2C9* linkage disequilibrium in diverse human populations will help to clarify this point.

According to our findings, the use of NSAIDs that undergo extensive CYP2C9 metabolism should be cautious in individuals carrying mutations at the *CYP2C9* gene. Since interethnic differences in the frequency of *CYP2C9* variant alleles exist (Lee *et al*., 2002; Xie *et al*., 2002), the findings obtained in the present study should not be extrapolated to individuals other than white subjects. Our findings should stimulate further research involving individuals from other ethnic origins, and the impact of the CYP2C9 polymorphism in the risk of developing adverse effects during therapy with different types of NSAIDs should be investigated.

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