

Polymorphisms in genes encoding acetylsalicylic acid metabolizing enzymes are unrelated to upper gastrointestinal health in cardiovascular patients on acetylsalicylic acid

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Background

As acetylsalicylic acid is metabolized by UDP-glucuronosyltransferase 1A6 (UGT1A6) and cytochrome P450 2C9 (CYP2C9), interindividual differences in activity of these enzymes may modulate the effects and side-effects of acetylsalicylic acid. The objective of this study was to assess whether polymorphisms in UGT1A6 and CYP2C9 genes are related to the prevalence of upper gastrointestinal symptoms in cardiovascular patients using acetylsalicylic acid for secondary prevention of ischaemic heart disease.

Methods

Blood samples were taken from acetylsalicylic acid using patients admitted to the Coronary Care Unit. Dyspepsia-related health was evaluated at week 2, using a validated upper gastrointestinal complaint questionnaire. A subset of 160 patients responded to a survey and were eligible to participate in this study. DNA was isolated and UGT1A6 and CYP2C9 genotypes were determined using polymerase chain reaction restricted fragment length polymorphism techniques.

Results

Seventy per cent of the patients returned the questionnaire. UGT1A6 and CYP2C9 variant polymorphisms were found in 103 (63%) and 56 (35%) patients, respectively. There was no association between gastrointestinal symptoms and UGT1A6 (OR = 0.80, 95% CI = 0.41–1.56) or CYP2C9 polymorphisms (OR = 0.85, 95% CI = 0.44–1.67).

Conclusions

There was no association between polymorphisms in genes encoding for acetylsalicylic acid metabolizing enzymes on the prevalence of gastric complaints in cardiovascular patients on acetylsalicylic acid.

Introduction

The efficacy of acetylsalicylic acid (aspirin) for secondary prevention of ischaemic heart diseases has been well established [1, 2]. However, gastrointestinal side-effects

are frequently seen in patients on the drug [3–5]. Acetylsalicylic acid, just like other NSAIDs, blocks gastrointestinal cyclo-oxygenase activity, leading to reduced synthesis of mucosal-protective prostaglandin.

As a result even low doses of acetylsalicylic acid increase the risk for gastric mucosal damage and related complaints.

The major enzymes involved in the metabolism of acetylsalicylic acid are enzymes responsible for hydroxylation and glucuronidation of the drug, and include cytochrome P450 2C9 (CYP2C9) and UDP-glucuronosyltransferase 1A6 (UGT1A6). Both enzymes are polymorphic. There are two known variant alleles for UGT1A6, resulting in a change at amino acid 181 (T→A) and amino acid 184 (R→S). Both variants are mostly (>98%) in complete linkage disequilibrium. The amino acid changes 181 (T→A) and 184 (R→S) result in a 30–50% reduced enzyme activity compared with the wild-type allele [6]. The variant alleles for CYP2C9 (R144C and I359L) also produce enzymes bearing some 5–30% of the activity of the wild-type enzyme [7].

Because of major differences in metabolic efficacy between the wild type and the variant phenotypes, it is tempting to reason that carriers of these variant alleles differ in their acetylsalicylic acid metabolism. This may account for the presence of adverse events in acetylsalicylic acid users.

The enzyme UGT1A6 can be found through the entire human gastrointestinal tract, except in the small intestine [8–10]. Similar expression is described for CYP2C9, and in addition, this enzyme is highly expressed in liver tissue [11, 12].

The aim of this study was to assess whether interindividual variability in the UGT1A6- or CYP2C9-gene is related to the prevalence of gastric complaints in hospitalized cardiovascular patients using acetylsalicylic acid for secondary prevention of ischaemic heart disease.

Methods

Subjects

This study was conducted at the Departments of Cardiology and Gastroenterology and Hepatology of the Radboud University Nijmegen Medical Centre, the Netherlands and was approved by the local ethics committee. A total of 402 consecutive cardiovascular patients (mean age 67 (SD = 12) years and 250 (61%) males) were asked to participate in the study that took place between April 2002 and August 2003 at the Coronary-Care Unit of the Heart Centre. Individual patients participating in the study gave their informed consent. In 70% ($n = 281$, mean age 67 (SD = 11) years and 191 (68%) males) of patients acetylsalicylic acid was started on admission because of a cardiovascular event.

Data collection

Blood samples were taken immediately after admission. Two weeks after discharge a questionnaire was sent, that allowed assessment of dyspeptic complaints. The severity of dyspeptic complaints was measured on a 7-point Likert scale that contained the following items: abdominal pain, epigastric pain, heartburn, regurgitation, bloating, belching, flatulence and nausea [13]. While analysing the questionnaires we reduced the 7-point scales into dichotomous variables by using a cut-off value into event-positive or -negative, and a value >2 was considered as event-positive.

Genotyping

Each patient that gave informed consent, and returned the questionnaire while on acetylsalicylic acid was eligible for genotyping. Genomic DNA was extracted from EDTA blood of the remaining 160 patients, using the Puregene isolation Kit[®] (Gentra Systems, Minneapolis, MN). Genotyping for the different alleles of UGT1A6 and CYP2C9 was performed using PCR-RFLP analyses. A DNA fragment of 992 bp containing both the T181A and R184S mutations of UGT1A6 was amplified using the primers 5'-GGAAAATACCTAGGAGCCCTGTGA-3' (forward) and 5'-AGGAGCCAAATGAGTGA GGGAG-3' (reverse). Cycling conditions were 95 °C for 4 min, followed by 37 cycles of 95 °C for 30 s, 64 °C for 60 s and 72 °C for 60 s, followed by a final 5 min extension period at 72 °C. The PCR product was subjected to digestion with the restriction enzyme *NsiI* for *T181A* and *Fnu4HI* for *R184S*, respectively. Fragmentation patterns are shown in Figure 1. After *NsiI* digestion 992 bp were found for the wild type, 992 + 616 + 376 bp for the heterozygous variant allele and 616 + 376 bp for the homozygous variant allele. Digestion with *Fnu4HI* generated 833 + 159 bp products for the wild type, 833 + 464 + 369 + 159 bp products for heterozygous samples and 464 + 369 + 159 bp fragments for homozygous variant samples [14].

The CYP2C9 genotype was analysed using the forward and reverse primers 5'-TACAAATACAAT GAAAATATCATG-3' and 5'-TAACAACCAGACTCA TAATG-3' for CYP2C9*2 and 5'-TGCACGAGGTCCA GAGGTAC-3' and 5'-GATACTATGAATTTGGGACT TC-3' for CYP2C9*3 generating PCR products of 691 and 131 bp, respectively (26). PCR conditions were the same as for UGT1A6 with only slight modifications. For CYP2C9*2 the annealing temperature was 50.7 °C and for CYP2C9*3 an annealing temperature of 53.7 °C was used. Aliquots of each PCR product were digested with 10 U of the restriction endonuclease *AvaII* (Promega, Madison, WI USA) for CYP2C9*2 and 10 U of *KpnI*

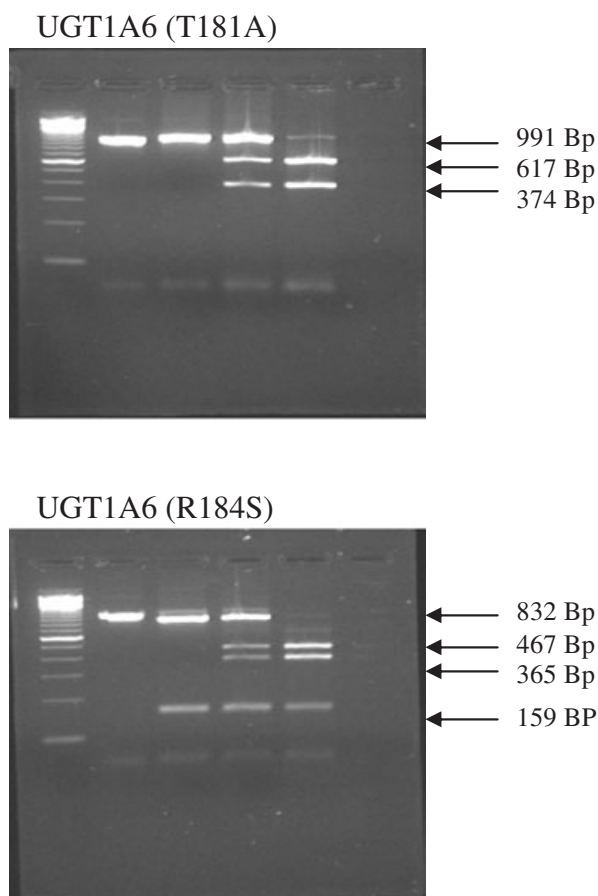


Figure 1
Fragmentation patterns of UGT1A6 genetic polymorphisms

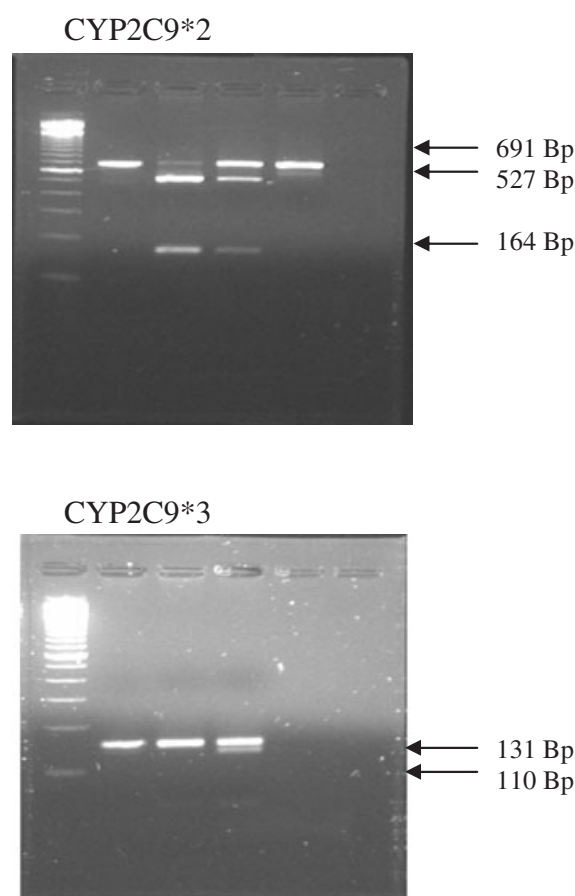


Figure 2
Fragmentation patterns of CYP2C9 genetic polymorphisms

(Gibco BRL[®]) for the detection of CYP2C9*3 at 37 °C for 1 h. The fragment sizes after *AvaII* digestion were 527 + 164 bp for the wild type, 691 + 527 + 164 bp for the heterozygous variant and 691 bp for the homozygous variant. Digestion with *KpnI* generated products of 131 bp for the wild type, 131 + 110 + 21 bp for heterozygous variant alleles and 110 + 21 bp for homozygous variant alleles (Figure 2). Digested samples were run on a 3% agarose gel and stained with ethidium bromide.

Data analysis

Based on the reported frequency of the UGT1A6 (57%) and CYP2C9 (31%) polymorphism of in the healthy population we calculated that to detect a difference in prevalence of gastrointestinal complaints of 20% it was necessary to study 141 patients on acetylsalicylic acid in order to obtain 0.80 power and alpha values of 0.05 for UGT1A6 and 161 patients for CYP2C9 [7].

We prepared a frequency table (Table 1) containing

prevalence of gene variants. Next, CYP2C9 was dichotomized into wildtype and variant, whereas UGT1A6 was subdivided into three groups, wildtype, heterozygote (WT/R184S + T181A and WT/R184S) and homozygote variant (R184S + T181A/R184S and R184S + T181A/R184S + T181A), respectively. The differences between combinations of gene variants and gastric complaints were evaluated by the Pearson's chi-squared test or Fisher's exact test where appropriate.

Results

Our study population consisted of 160 patients with a mean age of 67 years (SD = 11) and 68% ($n = 109$) males. UGT1A6 and CYP2C9 gene variants were detected in 63% and 35% of patients, respectively (Table 1). In Table 2 we categorized the gene variants for both genes into six possible combinations, and we found that the combinations of CYP2C9*wildtype with both UGT1A6*wildtype (25%) or UGT1A6*heterozygote (29%) were the most common.

Genetic polymorphisms			n (%)
UGT1A6	UGT1A6*1*1	WT/WT	61 (37)
	UGT1A6*1*2	WT/T181A + R184S	74 (45)
	UGT1A6*1*3	WT/R184S	6 (4)
	UGT1A6*2*2	T181A + R184S/T181A + R184S	18 (11)
	UGT1A6*2*3	T181A + R184S/R184S	5 (3)
CYP2C9	CYP2C9*1*1	WT/WT	104 (65)
	CYP2C9*1*2	WT/R144C	34 (21)
	CYP2C9*2*2	R144C/R144C	2 (1)
	CYP2C9*1*3	WT/I359L	18 (11)
	CYP2C9*1*4	WT/R144C + I359L	2 (1)

Table 1

Prevalence of UGT1A6 and CYP2C9 polymorphisms in cardiovascular patients using acetylsalicylic acid for secondary prevention of ischaemic heart disease ($n = 164$)

Category	CYP2C9	UGT1A6	Patients with complaints (%)	Total
1	Wildtype	Wildtype	24 (60%)	40
2	Variant	Wildtype	17 (81%)	21
3	Wildtype	Heterozygote	35 (74%)	47
4	Variant	Heterozygote	14 (48%)	29
5	Wildtype	Homozygote variant	8 (47%)	17
6	Variant	Homozygote variant	3 (50%)	6

Table 2

Association between the studied combinations of gene variants subdivided into categories with the prevalence of any gastrointestinal complaints ($n = 160$)

Complaints	Gene category						P
	1	2	3	4	5	6	
Any complaint	24 (60)	17 (81)	35 (74)	14 (48)	8 (47)	3 (50)	0.06
Abdominal pain	9 (24)	6 (30)	15 (35)	4 (15)	2 (13)	0 (0)	0.25
Epigastric pain							
Day	8 (23)	6 (30)	7 (17)	4 (15)	2 (14)	2 (40)	0.63
Night	5 (14)	3 (16)	5 (13)	4 (15)	2 (13)	2 (40)	0.72
Heartburn	6 (16)	6 (32)	10 (26)	5 (19)	1 (6)	1 (20)	0.48
Regurgitation	11 (29)	11 (52)	11 (28)	5 (19)	2 (13)	0 (0)	0.06
Bloating	10 (27)	8 (40)	12 (32)	5 (19)	3 (19)	2 (40)	0.59
Belching	11 (29)	10 (48)	17 (40)	6 (22)	4 (27)	1 (20)	0.39
Flatulence	15 (41)	11 (55)	24 (56)	9 (33)	6 (38)	1 (20)	0.28
Nausea	11 (29)	4 (21)	12 (28)	6 (23)	3 (20)	3 (50)	0.76

Table 3

Gastrointestinal complaints per gene category

A total of 101 patients (63%) had experienced any gastrointestinal symptoms in the 2 weeks following discharge. Patients using acetylsalicylic acid reported various symptoms on the questionnaire: abdominal pain (25%), epigastric pain (21%), heartburn (22%), regurgitation (29%), bloating (28%), belching (34%), flatulence (45%) and nausea (26%).

We tried to find associations between the prevalence

of complaints and gene categories. Gene categories were not associated with the prevalence of any upper gastrointestinal complaint (Table 2, $P = 0.06$). Table 3 shows the associations per complaint. For example, as depicted in Table 3, regurgitation was more common in patients with gene category 2 (variant CYP2C9 alleles and wildtype UGT1A6 alleles) (52%), but this was statistically nonsignificant ($P = 0.06$).

Discussion

We detected UGT1A6 and CYP2C9 gene variants in 63% and 35% of patients, respectively, on acetylsalicylic acid for secondary prevention of cardiovascular disease. The allele frequencies in our study group are similar to those found in the normal population [6, 7]. Sixty-one per cent of our patients had experienced upper gastrointestinal symptoms in the 2-week period following discharge, but there was no statistically significant correlation between symptoms and functional gene variants. In 1999, Lampe *et al.* described UGT1A6 allele frequency in a multiethnic population [6]. They reported genotype frequencies for UGT1A6 of 0.478, 0.392, 0.029, 0.090, 0.012 for wild-type (wt)/wt, wt/T181A + R184S, wt/R184S, T181A + R184S/T181A + R184S and T181A + R184S/R184S, respectively. We found that 63% of patients possessed variant alleles of UGT1A6 in comparison with 53% variants in the population of Lampe *et al.* It is possible that ethnic differences account for the different allele frequencies.

Bigler *et al.* first described the effects of the interindividual differences of UGT1A6 and CYP2C9 genotypes on acetylsalicylic acid metabolism [7]. They found that the protective effect of acetylsalicylic acid on colon adenoma risk was modulated by UGT1A6 and to a lesser extent by CYP2C9.

Our study has several limitations. First, our results do not indicate noncausality in the relation between the onset of gastrointestinal symptoms and the use of acetylsalicylic acid. In the study of Laheij *et al.* more than half of the general cardiovascular patient population (53%) reported upper gastrointestinal symptoms [15]. They did find that patients with symptoms more often used acetylsalicylic acid or calcium channel antagonists, but the study emphasizes that their results do not indicate causality. Further, we did not perform upper gastrointestinal endoscopy to investigate the underlying pathology of the upper gastrointestinal complaints nor did we study the role of concomitant medication. Moreover, the use of adverse event self-reporting, rather than observation by a clinician, might limit the conclusions drawn from the results. Finally, we sampled our data on gastrointestinal complaints at a single time point. Adverse events can develop any time, and it is entirely possible that the more severe complications of the drug take longer to develop. However, in our experience, most gastrointestinal symptoms occur in the first 2 weeks after start of acetylsalicylic acid use [15, 16].

There was a high prevalence of upper gastrointestinal symptoms in our patient population. Sixty per cent of the patients had experienced any upper gastrointestinal

symptom in the 2 weeks after discharge, and these symptoms impair the health-related quality of life (measured by the Short-Form 36 questionnaire) [15]. All patients in this study used relatively low doses of acetylsalicylic acid. However, cardiovascular patients use the drug for prolonged time and their plasma levels are higher than incidental users of acetylsalicylic acid. In conclusion, we found no effect of functional variants in genes that encode for acetylsalicylic acid metabolizing enzymes on the prevalence of upper gastrointestinal symptoms.

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