

Gastrointestinal Symptoms and Ethanol Metabolism in Alcoholics

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Excessive alcohol intake frequently results in gastrointestinal discomfort. It is an empirical fact that the severity of gastrointestinal discomfort induced by alcohol abuse is subject to interindividual variation. The aim of this study was to determine whether genetic polymorphism in alcohol dehydrogenase 3 (*ADH3*) and cytochrome P450 2E1 (*CYP2E1*), important first-pass enzymes in the metabolism of ethanol, predispose to the development of gastrointestinal symptoms in alcoholics. Blood samples were obtained from 92 adult alcoholics admitted for detoxification. The samples were analyzed for genetic polymorphism in *ADH3* and *CYP2E1* by polymerase chain reaction followed by restriction fragment length polymorphism analyses. During an interview on the first day of hospital admission, patient characteristics and gastrointestinal symptoms in the week before admission were assessed. A total of 75 of 92 alcoholics (83%) reported symptoms: 66 patients had upper gastrointestinal symptoms (72%), 70 patients had lower gastrointestinal symptoms (76%), and 59 patients reported alarming symptoms (64%). Patients with gastrointestinal symptoms less often abused beer in comparison to those without gastrointestinal symptoms ($P = 0.05$). The numbers of patients with the homozygous $\gamma 1\gamma 1$ genotype, the heterozygous $\gamma 1\gamma 2$ genotype, and the homozygous $\gamma 2\gamma 2$ genotype in *ADH3* who reported gastrointestinal symptoms were 20 (83%), 34 (76%), and 15 (88%), respectively. The number of patients with the heterozygous *c1c2* *CYP2E1* genotype (5%) and the heterozygous *DC* *CYP2E1* genotype (14%) was low and also unrelated to gastrointestinal symptoms. Our data suggest that the ethanol concentrations of the consumed beverages, and not interindividual variations in the activities of first-pass alcohol-metabolizing enzymes, are associated with gastrointestinal symptoms in alcoholics.

KEY WORDS: ethanol; gastrointestinal symptoms; metabolism; alcohol dehydrogenase; cytochrome P450 2E1.

Ethanol consumption is considered to be an important risk factor for the development of gastrointestinal discomfort and complications (1). Side effects of ethanol consumption on upper and lower gastrointestinal tract are common. However, not every alcohol consumer develops discomfort or complications. Consequently, it might be possible that

susceptibility could be influenced by interindividual variations in the activities of ethanol-metabolizing enzymes (2). Ethanol is metabolized predominantly by the liver, whereas 20% of ethanol metabolism may already occur in the gastric mucosa. Ethanol is detoxified in two steps during the first passage through the liver. The speed of every step is enzyme dependent. The enzymes involved with the first step are alcohol dehydrogenase 3 (*ADH3*) and cytochrome P450 2E1 (*CYP2E1*) (3–6). A reduction in first-pass metabolism accumulates the amount of ethanol for a longer period in the body.

The local alcohol toxicity is mediated by acetaldehyde on the gastric mucosa. Acetaldehyde inhibits mucosal regeneration and forms stable adducts with mucosal proteins

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causing gastric injury and is therefore being considered as a local carcinogen in the gastrointestinal tract. Excessive alcohol abusers are known to have more chronic gastritis than moderate or non-alcohol users (10). Persons consuming excessive amounts of alcohol with the rapid metabolizing alcohol dehydrogenase genotype have a greater risk for upper gastrointestinal malignancies in comparison to persons with other genotypes (11).

The rate of ethanol metabolism variability is genetic in origin and varies between racial populations. The *ADH3* gene carries two alleles, *ADH3*1* and *ADH3*2*, which differ by one amino acid, and encodes the subunits γ_1 and γ_2 , respectively. The isoenzyme $\gamma_1\gamma_1$ metabolizes ethanol 2.5-fold as fast as the $\gamma_2\gamma_2$ isoenzyme (4). The *CYP2E1* enzyme particularly contributes to ethanol metabolism in chronic alcohol consumers. Genetic polymorphisms in *CYP2E1* have been demonstrated in the transcription-regulation region (alleles *c1* and *c2*) and in intron 6 (alleles *D* and *C*) (5). The presence of the *c2* or *C* allele was associated with enhanced enzyme activity (5, 6). The aim of this study was to determine whether polymorphism in the genes for *ADH3* and *CYP2E1* is associated with the development of gastrointestinal symptoms in alcoholics.

METHODS

Consecutive patients were recruited between January and November 2002 at four detoxification clinics. Patients were included if they have reached the age of 18 and were ethanol dependent following DSM-IV criteria. Patients were asked to participate on the first day of admission. After written informed consent, blood samples were taken and stored in EDTA tubes at -20°C . Patients were interviewed to assess the frequency of gastrointestinal symptoms in the week before admission. The interview included inquiries on the presence of the following gastrointestinal symptoms: abdominal pain, abdominal distension, epigastric pain, heartburn, regurgitation, bloating, flatulence, nausea, dysphagia, weight loss, hematemesis, melena, colorectal bleeding, and diarrhea. Symptoms were scored on a 4-point Likert scale: none, mild (can be ignored), moderate (cannot be ignored but does not affect lifestyle), and severe (affects lifestyle). Serological screening for *Helicobacter pylori* infection was performed with the Pyloriset, which was locally validated (Pyloriset test kit; Imphos, the Netherlands). The Pyloriset is a commercially available enzyme-linked immunosorbent assay that measures IgG antibodies to *H. pylori* infection. Furthermore, patient characteristics and ethanol consumption-related information were collected. The ethics committee of the hospital approved the study protocol.

Genotyping. DNA was isolated from whole blood using the Pure Gene DNA isolation kit, and isolation was performed according to the instructions of the manufacturer (Gentra systems). The genotyping for the polymorphism in *ADH3* was carried out by polymerase chain reaction (PCR) amplification according to the method of Xu *et al.* (7). Subsequently the PCR product was digested using the restriction enzyme *SspI* (PCR/RFLP). The *ADH3*1* allele contains an additional restriction site for *SspI*,

yielding fragments of 63 and 67 bp, which is not present in the *ADH3*2* allele. The *CYP2E1* genotypes were determined by PCR/RFLP methods as described elsewhere (8). The restriction enzymes *RsaI* and *PstI* were used to discern the *c1* and *c2* alleles of the 5'-flanking region, while the enzyme *DraI* was used to detect the *D* and *C* polymorphisms in intron 6.

Analyses. We estimated that 50% of the patients with the $\gamma_2\gamma_2$ slower metabolizing genotype and 80% of the patients with the $\gamma_1\gamma_1$ or $\gamma_1\gamma_2$ rapid metabolizing genotype would report gastrointestinal symptoms. To detect the difference of 30% with a power of 0.80, an α of 0.05 (one-sided), and taking into account the unequal distribution of *ADH3* polymorphism (a ratio of 0.3), a total of 90 patients were required.

Chi-square and *t*-test statistics were used to estimate differences in baseline characteristics among patients with and without gastrointestinal symptoms. Abdominal pain, abdominal distension, and diarrhea were classified as lower gastrointestinal symptoms; epigastric pain, heartburn, regurgitation, bloating, flatulence, and nausea, as upper gastrointestinal symptoms. Dysphagia, weight loss, hematemesis, melena, and colorectal bleeding were classified as alarming symptoms. Logistic regression analysis was performed to study the association between polymorphism and upper gastrointestinal symptoms, lower gastrointestinal symptoms, and alarming symptoms in order to estimate an odds ratio with 95% confidence interval (CI) for these variables. Because other factors besides interindividual variations in the activities of ethanol-metabolizing enzymes might be related to the presence of gastrointestinal symptoms, we performed a multivariate regression analysis to adjust for acid secretion inhibitory therapy and *H. pylori* infection. Data were analyzed by SAS version 8.0.

RESULTS

We included 92 Caucasian patients in the study; 73 were male and the average age was 42 years. The patients reported on average a daily ethanol consumption of 200 g for more than 10 years. A total of 75 of 92 patients (83%) suffered from gastrointestinal symptoms: 66 reported upper gastrointestinal symptoms (72%), 70 reported lower gastrointestinal symptoms (76%), and 59 patients had symptoms suspicious for a malignancy (64%) (Table 1). Thirty-seven patients reported their alarming symptoms as mild, 26 as moderate, and 3 as severe. Patients with gastrointestinal symptoms less often abused beer in comparison to those without gastrointestinal symptoms ($P = 0.05$). All other patient characteristics were similar between patients with and those without gastrointestinal symptoms (Table 2). Many patients (39%) were using acid-suppressive drugs to control gastrointestinal symptoms. From patients using medication to control the gastrointestinal symptoms, 31% reported complete symptom relief, while in the other 69% symptoms did not disappear.

***ADH3* Genotyping.** In 86 of the 92 patients we were able to evaluate the *ADH3* genotype. The number of patients with the homozygous $\gamma_1\gamma_1$ genotype, the heterozygous $\gamma_1\gamma_2$ genotype, and the homozygous $\gamma_2\gamma_2$

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TABLE 1. FREQUENCY OF GASTROINTESTINAL SYMPTOMS IN THE WEEK BEFORE ADMISSION

	Number (%)			
	None	Mild	Moderate	Severe
Overall	17 (18)	33 (36)	36 (39)	6 (7)
Upper gastrointestinal symptoms	26 (28)	39 (42)	23 (25)	4 (4)
Epigastric pain	63 (68)	20 (22)	8 (9)	1 (1)
Heartburn	53 (58)	22 (24)	17 (19)	0
Regurgitation	52 (57)	28 (30)	10 (11)	2 (2)
Bloating	46 (50)	28 (30)	15 (16)	3 (3)
Nausea	54 (59)	27 (29)	11 (12)	0
Alarm symptoms	33 (36)	37 (40)	19 (21)	3 (3)
Dysphagia	71 (77)	16 (17)	3 (3)	2 (2)
Weight loss	58 (63)	20 (22)	13 (14)	1 (1)
Hematemesis	85 (92)	5 (5)	2 (2)	0
Meleana	75 (82)	13 (14)	4 (4)	0
Colorectal bleeding	79 (86)	11 (12)	1 (1)	1 (1)
Lower gastrointestinal symptoms	22 (24)	41 (45)	26 (28)	3 (3)
Abdominal pain	57 (62)	33 (36)	2 (2)	0
Abdominal distension	35 (38)	33 (36)	22 (24)	2 (2)
Diarrhea	49 (53)	32 (35)	10 (11)	1 (1)

genotype was 24 (30%), 45 (52%), and 17 (20%), respectively (Table 3). There were no statistical differences in the distribution of the homozygous $\gamma 1\gamma 1$ genotype, the heterozygous $\gamma 1\gamma 2$ genotype and the homozygous $\gamma 2\gamma 2$ genotype among patients with upper gastrointestinal symptoms, lower gastrointestinal symptoms and alarming symptoms in comparison to patients without these symptoms. Even after adjustment for *H. pylori* infection and acid-suppressive therapy no association was found.

TABLE 2. PATIENT CHARACTERISTICS BY GASTROINTESTINAL SYMPTOMS

	Symptoms	
	Absent (n = 17)	Present (n = 75)
Male/female (No.)	14/3	57/18
Mean age (years)	42	42
<i>Helicobacter pylori</i> seropositive (%)	47	28
Smoking (%)	93	75
Coaddiction		
Opiates (%)	14	10
Cocaine (%)	21	19
Cannabis (%)	21	21
Medication		
H2-RA (%)	6	8
PPI (%)	6	19
Alcohol intake (g ethanol/day)	189.1	195.3
Mean addiction duration (years)	13	17
Previous detoxification (%)	79	78
Beverage		
Beer (%)*	100	74
Wine (%)	14	22
Liquor (%)	7	10

*P = 0.05.

TABLE 3. GASTROINTESTINAL SYMPTOMS AND ADH3 POLYMORPHISM

Gastrointestinal symptoms	ADH3, number (%)		
	$\gamma 1\gamma 1$ (n = 24)	$\gamma 1\gamma 2$ (n = 45)	$\gamma 2\gamma 2$ (n = 17)
Any			
Absent	4 (24)	11 (65)	2 (12)
Present	20 (29)	34 (49)	15 (22)
Upper			
Absent	7 (28)	13 (52)	5 (20)
Present	17 (29)	32 (51)	12 (20)
Lower			
Absent	5 (23)	12 (54)	5 (23)
Present	19 (30)	33 (51)	12 (19)
Alarm			
Absent	8 (25)	21 (66)	3 (9)
Present	16 (30)	24 (44)	14 (26)

CYP2E1 Genotyping. In 78 of the 92 patients included we were able to evaluate *CYP2E1* genotypes. The number of patients with the homozygous *c1c1* genotype, the heterozygous *c1c2* genotype, and the homozygous *c2c2* genotype was 74 (95%), 4 (5%), and 0 (0%), respectively. The number of patients with the homozygous *DD* genotype, the heterozygous *DC* genotype, and the homozygous *CC* genotype was 67 (86%), 11 (14%), and 0 (0%), respectively. Despite the fact that 10 of the 11 patients (91%) with the heterozygous *DC CYP2E1* genotype reported upper gastrointestinal symptoms, 9 lower gastrointestinal symptoms (82%), and 8 alarming symptoms (73%), no statistical association was found between the *CYP2E1* genotype and symptom clustering.

DISCUSSION

Gastrointestinal symptoms and complications associated with ethanol abuse are common. The results from this study confirm that persons abusing ethanol often have gastrointestinal symptoms; even alarming symptoms were frequently reported. Gastrointestinal symptoms were significantly related to non-beer abusers. Our study was designed to evaluate whether interindividual variations in the activities of ethanol-metabolizing enzymes were associated with gastrointestinal symptoms. However, interindividual variations in the activities of *ADH3* and *CYP2E1* enzymes were not associated with gastrointestinal symptoms. This finding is, however, in line with the fact that symptoms are poorly correlated with the severity of mucosal injury. Furthermore, because most patients reported persistent symptoms during acid-suppressive therapy, the impact of medical management of gastrointestinal symptoms in alcoholics on symptom improvement seems minimal.

The only positive finding of this study was a significant negative correlation between gastrointestinal symptoms and beer drinking. This is most likely because gastrointestinal disturbances depend on the ethanol concentration of the consumed beverages and are due either to a direct effect of highly concentrated ethanol or to the activity of enzymes with a high K_m for ethanol known to prevail in the upper digestive tract, such as σ -ADH (a class IV ADH), which plays no role at systemic concentrations of ethanol but could at the very high concentrations of ethanol achieved in the upper digestive tract after drinking. The role of the two enzymes we have investigated is minimal in some of the organs where the upper gastrointestinal symptoms may be generated. The principal catalyst of ethanol in the gastroesophageal area is ADH7, a class IV alcohol dehydrogenase (σ -ADH). However, so far no major polymorphisms of the *ADH7* gene have been found, except for a point mutation (16). The frequency of this point mutation is very low in the Caucasian population and therefore it is probably not responsible for the development of gastrointestinal symptoms.

Alcohol dehydrogenase is an important first-step enzyme in the oxidation of alcohol to acetaldehyde (4, 9). Especially, *ADH3* is the most important enzyme in the oxidation of ethanol to acetaldehyde in Caucasians. However, oxidation of alcohol by the *CYP2E1* enzymes is also important, especially in chronic alcohol abusers, as it is inducible up to 10-fold by consumption of alcohol. Only few patients were heterozygous for the *CYP2E1* genotype. To detect a difference in frequency of genotype between patients with and those without symptoms, many more patients need to be included. However, *CYP2E1* is probably not an important risk factor for gastrointestinal symptoms because few patients are heterozygous for its genotypes. Furthermore, taking into consideration that most patients in our study had been abusing alcohol for more than 10 years, there might be an enhancement of induction of increased enzyme metabolism activity after long-term alcohol intake.

Several other factors may be related to the occurrence of gastrointestinal symptoms. For example, *Helicobacter pylori* infection leads to morphologic gastric mucosa changes which might be associated with a decrease in alcohol metabolism (12–14). It is, however, unclear whether this reflects the bioavailability of alcohol or the amount of its first-pass metabolism. In our study, however, we did not find an association between symptoms and *Helicobacter pylori* infection. This finding might be a consequence of the limited number of alcoholics in our study.

Despite the high prevalence of gastrointestinal symptoms, it is not known whether drugs for management of dyspeptic symptoms in the general population are equally

effective in alcoholics. The impact of medical management of gastrointestinal symptoms in alcoholics seems to be minimal. Most patients using drugs for upper gastrointestinal symptoms have persistent symptoms. Traditionally, patients who present with gastrointestinal discomfort are being managed with acid-suppressive medication. Acid-suppressive medication might even increase symptoms. Treatment with proton pump inhibitors increases acetyldehyde production from ethanol (15). Furthermore, previous studies have shown that H2 receptor antagonists, especially cimetidine, are associated with increased blood alcohol levels by an effect on gastric alcohol dehydrogenase activity (3). The results from this study showed that despite many of the patients using gastric acid inhibitors for their upper gastrointestinal symptoms, many failed to respond adequately. Because of the poor results with acid-suppressive therapy, other drugs or management strategies to prevent gastrointestinal discomfort should be evaluated.

In conclusion, more than three-quarters of these alcoholics who had on average abused ethanol for more than 10 years reported gastrointestinal symptoms. The combination of excessive drinking and the *ADH3* and *CYP2E1* genotypes was not associated with gastrointestinal symptoms.

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