ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN-POLONIA
VOL. LXI, N1, 6 SECTIO D 2006

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Genetic polymorphism of alcohol dehydrogenase 3 and CYP2E1 in digestive tract alcohol damage

The abuse of ethyl alcohol leads to the damage of many organs: the liver, pancreas, gastric mucous membrane, cerebral tissue and results in the loss of behaviour control. In Poland, people with alcohol dependence syndrome account for about 3% of the population. Cirrhosis develops in 10–20% of alcohol-addicts, chronic pancreatitis in 5% and both in 1% (6).

The main alcohol metabolism occurs in the liver. There are three groups of oxidizing enzymes of ethanol. These are: alcohol dehydrogenase (ADH), microsomal ethanol oxidizing system (MEOS) and catalase. ADH oxidizes 80–90% of ethyl alcohol. In alcoholic liver disease 1/3–1/4 of ethanol consumed is metabolized by MEOS. The most important enzyme of this metabolic pathway is cytochrome P-450 CYP2E1. Catalase plays the least important role in alcohol metabolism.

ADH is encoded by 7 genes located on the 4th chromosome, and CYP2E1 by the gene located on the long arm of chromosome 10. ADH and CYP2E1 exhibit polymorphism, therefore they occur in the population in more than one form encoding various subunits of different properties. ADH occurs in over 20 isoenzymes which are due to structural and functional differences, kinetic properties. The gene ADH3 exhibit polymorphism thereby are present in more than one form, encoding various forms of subunit γ of different properties. ADH3 forms two alleles ADH3*1, ADH3*2 encoding subunits γ1 and γ2 , respectively, The subunit γ1 shows higher ethanol activity than the subunit γ2 (1). Genetic polymorphism of enzymes involved in alcohol metabolism plays a relevant role in etiopathogenesis of alcohol disease. The alcoholic disease of the liver does not develop in all drinking individuals. Even in those consuming large amounts of alcohol systematically the liver damage occurs in 77% of cases. It is assumed that this fact is associated with genetically determined differences in alcohol metabolism (5). CYP2E1 forms two alleles encoding respective subunits c1 and c2. C2 shows higher activity for ethanol than c1. The genotype c1/c1 was defined as type A, genotype c1/c2 as type B and homozygotes c2/c2 as type C CYP2E1.

The aim of the present study was to find in the Polish population the ADH3 and CYP2E1 genotypes, which are likely to be responsible for higher susceptibility to alcohol disease of the liver and chronic alcohol pancreatitis; are there any differences in the ADH3 and CYP2E1 genotypes and alleles in the Polish population comparing patients addicted to alcohol with cirrhosis, chronic alcohol pancreatitis, those addicted but without liver and pancreas damage and non-drinkers?

MATERIAL

The study encompassed 198 patients: 48 women and 150 men. Average age was 45 ± 9.44 years. Group I included the patients with chronic alcohol abuse: 57 patients with alcohol liver cirrhosis (group IA), 44 patients with alcohol chronic pancreatitis (group IB) and 43 patients abus-

ing alcohol but without damage to gastrointestinal organs – healthy drinkers (group IC). Group II consisted of 54 non-drinking patients with functional alimentary disorders. All the patients gave informed consent for examinations. Group I included patients consuming on average over 80 g of pure ethanol a day for at least two years, average 160.86±43.93 g daily.

All the patients examined had positive CAGE test. The diagnosis of cirrhosis and chronic pancreatitis was based on the generally accepted criteria (3, 8). Other non-alcoholic causes of liver cirrhosis and chronic pancreatitis were excluded. The patients with alcoholic disease without alimentary organ damage (group IC) underwent the examinations to exclude alimentary pathology, particularly of the liver and pancreas. Group II consisted of the patients in whom the history allowed to confirm that they did not drink alcohol, the organic alimentary pathology was excluded and functional disorders were diagnosed.

METHODS

Genomic DNA was isolated from 15 ml of peripheral blood with 0.3 ml of 0.5 M EDTA.

Genotyping of ADH3 gene polymorphism. The detection of polymorphisms in ADH3 gene was performed using polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP). The amplified product was digested with SspI and subjected to electrophoresis in 3% agarose gel (or 12% polyacrylamide gel), stained with ethidium bromide (11).

Genotyping of CYP2E1 gene polymorphisms. The detection of polymorphisms in 5'-flanking region of P4502E1 gene was performed using polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP). The amplified product was digested with PstI or RsaI, and subjected to electrophoresis in 2% agarose gel, stained with ethidium bromide (13).

Statistical analysis. The ADH3 genotypes and alleles were compared using the χ^2 homogeneity test. The calculations were performed by STATISTICA PL software.

RESULTS

Table 1 and Table 2 show the presence of ADH3 and CYP2E1 alleles and ADH3 and CYP2E1 genotype in the groups examined.

Table 1. The presence of ADH3 and CYP2E1 alleles in the groups examined

Group	ADH3*1	ADH3*2	c1	c2	Total
Alcohol liver cirrhosis – IA	71 (62,3%)	43 (37,7%)	110 (96,5%)	4 (3,5%)	114
Alcohol chronic pancreatitis – IB	60 (68,2%)	28 (31,8%)	86 (97,7%)	2 (2,3%)	88
Healthy drinkers – IC	51 (59,3%)	35 (40,7%)	86 (100%)	0 (0%)	86
Non-drinkers – II	44 (40,7%)	64 (59,3%)	108 (100%)	0 (0%)	108

Table 2. The presence of ADH3 and CYP2E1 genotypes in the groups examined

Group	ADH3*1/*1	ADH3*1/*2	ADH3*2/*2	c1/c1	c1/c2	c2/c2	Total
IA	26 (45,6%)	19 (33,3%)	12 (21,1%)	53 (93%)	4 (7%)	0	57
IB	17 (38,6%)	26 (59,1%)	1 (2,3%)	42 (95,5%)	2 (4,5%)	0	44
IC	17 (39,5%)	17 (39,5%)	9 (21,0%)	43 (100%)	0 (0%)	0	43
II	10 (18,5%)	24 (44,5%)	20 (37,0%)	54 (100%)	0 (0%)	0	54

Table 3. The comparison of ADH3 genotype frequency in the selected groups

Genotype ADH3	Group		Group		Group		Group	
	I (%)	II (%)	IA (%)	IC (%)	IA (%)	IB (%)	IB (%)	IC (%)
ADH3*1/ADH3*1	41,67	18,52	45,62	39,53	45,62	38,64	38,64	39,53
ADH3*1/ADH3*2	43,05	44,44	33,33	39,53	33,33	59,09	59,09	39,53
ADH3*2/ADH3*2	15,28	37,04	21,05	20,94	21,05	2,27	2,27	20,94
	χ^2= 14,74 p<0,001		χ^2= 0,47 p>0,05		χ^2= 21,93 p<0,001		χ^2= 22,47 p<0,001	

Table 4. The comparison of CYP2E1 genotype frequency in the selected groups

Genotype CYP2E1	Group		Group		Group	
	I (%)	II (%)	IA (%)	II (%)	IB (%)	II (%)
c1/c1	95,83	100	92,98	100	95,5	100
c1/c2	4,17	0	7,02	0	4,5	0
	χ^2= 2,32 p>0,05		χ^2= 3,93 p<0,05		χ= 2,51 p>0,05	

In the examined population encompassing 198 subjects the allele ADH3*1 was present in 57% of patients, and the allele c2 was present only in 1.5% of patients. The c2 allele was found only in patients abusing alcohol. In the group of patients with alcoholic cirrhosis it was present in 3.5% of cases while in patients with chronic alcoholic pancreatitis in 2.3%.

The genotype ADH3*1/ADH3*1 was found to be significantly more frequent in alcohol abusers compared to non-drinkers (p<0.001). The genotype ADH3*2/ADH3*2 was more common in non-drinkers (p<0.001). The allele ADH3*1 was found to be more statistically significantly frequent in alcohol abusers than in non-drinkers (p<0.001). The examinations of allele ADH3*1 presence among alcohol abusers showed that alleles ADH3*1 were more frequent in patients with cirrhosis hepatis and chronic pancreatitis than in patients without alimentary organ damage. But differences were not statistically significant (p>0.05). The examinations of the group of alcohol abusers showed that the genotype ADH3*2/ADH3*2 occurred statistically significantly less frequently in patients with chronic pancreatitis than in those without alimentary lesions (p<0.001) and in patients with cirrhosis (p<0.001).

Among 198 subjects the genotype c1/c2 (type B) was present in 3.0% of subjects. The genotype c2/c2 (type C) was not found in any patient. The genotype c1/c1 (type C) occurred in 97.0% of cases. Heterozygotes c1/c2 were present only in patients consuming excessive amounts of ethanol; in 7% of patients with alcoholic cirrhosis and in 4.5% of those with chronic alcoholic pancreatitis. The genotype c1/c2 was not demonstrated in any of the patients abusing alcohol but without alimentary damage. The genotype c1/c2 occurred statistically significantly more frequently in patients with alcohol cirrhosis than in non-drinkers (p<0.05).

DISCUSSION

Genetic factors determine the individual susceptibility to alcoholism and alcoholic damage to the alimentary tract. It would be important to find the genes responsible for the susceptibility to alcohol addiction and to conduct the screening examinations defining whether a particular individual is genetically loaded, which might reduce this phenomenon.

In the Caucasians, the ADH3*1 alleles are found in 50–60% of the population and their influence on the development of alcoholic hepatic damage varies in the European inhabitants. The alleles ADH3*1 were observed in 55.6% of healthy non-drinking volunteers of the European population and ADH3*2 in 44.4%. In alcohol abusers with cirrhosis and without hepatic parenchyma damage – 57.7% and 42.3%, respectively (2).

The study examining ADH polymorphism in the Spanish population demonstrated higher but not statistically significant frequency of ADH3*1 alleles in alcoholics than in non-drinkers (9). Our results demonstrate that the ADH3*1 allele is found in 62.3% of patients with alcoholic hepatic cirrhosis, in 68.2% of those with alcoholic chronic pancreatitis and in 59.3% of alcohol abusers without alimentary damage. It should be noticed that in non-drinkers the presence of this allele is statistically significantly lower while that of ADH3*2 higher. Therefore it may be thought that ADH3*1 promotes alcoholism while ADH3*2 is likely to be a protective factor in the Polish population. Likewise, the genotype ADH3*1/ADH3*1 is more common in alcohol abusers than in non-drinkers. It promotes alcoholic cirrhosis and chronic alcoholic pancreatitis.

The findings in the Asian population are different. The ADH3*1 allele in this population is more commonly spread, its frequency is estimated at 90%. Mulligan in her study suggests that the presence of ADH3*1 decreases the risk of alcohol addiction in an American Indian population (15). Another study examined ADH3 polymorphism in the population of 371 Kenyans and found no statistically significant differences in alleles and genotypes between alcohol addicts and non-drinkers (16). It may be concluded that the role of ADH3 polymorphism is different in different races and varies markedly from one population to another.

Drenth et al. demonstrated that ADH3 polymorphism may be the risk factor of chronic pancreatitis. Chronic pancreatitis was more common in ADH3*1 and ADH3*2 homozygotes than in heterozygotes. The authors concluded that the presence of heterozygotous alleles have a protective role in the etiology of chronic pancreatitis in the group examined (7).

The results of our studies are different. In the group of patients with alcoholic pancreatitis higher frequency of the heterogenous ADH3*1/ADH3*2 genotype was observed while the presence of homozygotous ADH3*2/ADH3*2 was estimated at 2.3%. It may be thought that the possession of this genotype generally does not favour alcoholism and protects against chronic pancreatitis. On the other hand, ADH3*1 alleles evidently showed coexistence with alcoholic chronic pancreatitis.

The influence of genetic ADH3 polymorphism on the development of alcohol addiction, alcoholic cirrhosis or alcoholic pancreatic damage should be considered in terms of individual races. The presence of ADH3 alleles and genotypes is different in the Caucasian population and in Asians. The factors which seem to protect against alcoholism and alcoholic pathologies of the gastrointestinal tract in the Polish population play a different role in other races and populations.

In all populations alleles c2 are significantly less widespread than c1. However, they are more common in the Asian race than in Caucasian one. The studies evaluating 695 patients from different geographical regions revealed that alleles c2 were present in 28% of the Taiwan population, in 8% of Afro-Americans and only on 4% of Americans of European origin (10). The individuals possessing the genotype c2/c2 consume more alcohol than those with the genotype c1/c1 and alcohol liver diseases might be associated with the presence of the allele c2. Therefore, the presence of alleles c2 may be thought to predispose to alcohol abuse (13).

This is confirmed by the study conducted in the population of Americans of Mexican origin: alleles c2 were found to be more frequent in alcoholics (34.7%) than in non-drinkers (22.1%) (14).

The study involving 82 patients of the Chinese population confirmed statistically significant higher prevalence of the homozygous genotype c2 amongst patients with alcoholic fatty degeneration of the liver than that in the control group (17). In the Caucasian population, similarly to other ethnic groups, the frequency of alleles c1 is higher than the frequency of c2. The occurrence of heterozygous genotype c1/c2 in the Italian population of alcoholics was estimated at 5%, the remaining 95% had the genotype c1/c1 (4). Likewise, the allele c2 is rare in the Polish population. In our study it was present only in 1.5% of subjects and exclusively in those abusing alcohol with liver and pancreas pathology. The findings presented confirm the results of studies by Grove et al. The authors suggest that the allele c2, although rare in the Caucasian population, increases the risk of alcoholic liver disease and favours alcohol abuse (12).

The role of genetic polymorphism of CYP2E1 in chronic alcoholic pancreatitis is poorly defined and remains unclear. The studies carried out in the Caucasian population did not demonstrate statistically significant differences in the presence of alleles c2 amongst patients with chronic

alcoholic pancreatitis, idiopathic pancreatitis, healthy alcoholics and controls (18). These results were the same as ours.

Our studies suggest that in the Polish population examined the ADH3*1 allele and ADH3*1/ADH3*1 genotype occur more frequently in alcohol abusers and promote alcoholism, alcoholic cirrhosis and chronic pancreatitis while the ADH3*2 allele and ADH3*2/ADH3*2 genotype may protect against alcohol abuse. Among the patients consuming excessive amounts of alcohol, the ADH3*2/ADH3*2 genotype is extremely rare in chronic pancreatitis individuals, thus it is likely to be the protective factor of this disease.

The frequency of alleles c2 in the Polish population is low. They were present only in alcoholic cirrhosis and alcoholic pancreatitis and can increase the risk of alcoholic liver disease and pancreatitis disease. In non-drinkers and excessive drinkers without alimentary tract pathology was not demonstrated. The genotype c1/c2 occurred statistically significantly more frequently in patients with alcohol cirrhosis than in non-drinkers.

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SUMMARY

Genetic polymorphism of enzymes involved in alcohol metabolism plays a relevant role in etiopathogenesis of alcohol disease. The aim of the present study was to find in the Polish population the ADH3 and the CYP2E1 genotypes, which are likely to be responsible for higher susceptibility to alcohol disease of the liver and chronic alcohol pancreatitis. The ADH3 and the CYP2E1 genotype and ADH3*1, ADH3*2, c1 and c2 alleles frequency were examined in 198 patients. Genotyping of the ADH3 and the CYP2E1 was performed using polymerase chain reaction-restriction fragment length polymorphism methods on white cell DNA. The genotype ADH3*1/ADH3*1 was found to be significantly more frequent in alcohol abusers compared to non-drinkers. The examinations of the group of alcohol abusers showed that the genotype ADH3*2/ADH3*2 occurred statistically significantly less frequently in patients with chronic pancreatitis than in those without alimentary lesions and patients with cirrhosis. Thus it is likely to be the protective factor of chronic pancreatitis. The allele c2 was present only in 1.5% of patients. It was found only in patients abusing alcohol. The genotype c1/c2 was present in 3% of subjects. The genotype c2/c2 was not found in any patient. Heterozygotes c1/c2 were present only in patients consuming excessive amounts of ethanol. The presence of c2 promotes alcoholic damage to alimentary organs amongst Poles.

Polimorfizm genetyczny ADH3 i CYP2E1 w alkoholowych uszkodzeniach przewodu pokarmowego

Genetyczny polimorfizm enzymów metabolizujących alkohol odgrywa istotną rolę w etiopatogenezie choroby alkoholowej. Celem pracy jest poszukiwanie genotypów ADH3 i CYP2E1 w populacji polskiej, które mogłyby być odpowiedzialne za większą podatność na rozwój alkoholowej choroby wątroby i alkoholowego przewlekłego zapalenia trzustki. U 198 badanych oznaczano genotyp ADH3 i CYP2E1 oraz częstość występowania alleli ADH3*1, ADH3*2, c1 i c2. Genotyp ADH3 oceniano opierając się na metodzie RELP. Stwierdzono znamiennie częstsze występowanie alleli ADH3*1 i genotypu ADH3*1/ADH3*1 wśród pacjentów nadużywających alkoholu niż u niepijących. Wśród nadużywających alkoholu genotyp ADH3*2/ADH3*2 występował istotnie statystycznie rzadziej u chorych z przewlekłym zapaleniem trzustki niż u chorych bez zmian w przewodzie pokarmowym i u pacjentów z marskością wątroby, zatem może on stanowić czynnik ochronny przed tym schorzeniem. Allel c2 obecny był tylko u 1,5% badanych i tylko wśród nadużywających alkoholu. Genotyp c1/c2 występował u 3%, nie stwierdzano obecności genotypu c2/c2. Heterozygoty c1/c2 były istotnie statystycznie częściej obecne wśród pacjentów z alkoholową marskością wątroby niż u niepijących. Obecność alleli c2 sprzyjała alkoholowym uszkodzeniom narządów przewodu pokarmowego.