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• BRIEF REPORTS •

Relationship between genetic polymorphism of cytochrome P450IIE1 and fatty liver

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Abstract

AIM: To study the correlation between genetic polymorphism of cytochrome P450IIE1 (CYPIIE1) and fatty liver.

METHODS: Peripheral blood mononuclear cells were collected in 56 patients with fatty liver, 26 patients without fatty liver and 20 normal controls. Then PCR-RFLP was used to analyze genetic polymorphism of CYPIIE1 in monocytes on the region of Pst I and Rsa I.

RESULTS: The frequency of homozygotic C1 gene in patients with alcoholic fatty liver (28.6 %), obese fatty liver (38.5 %), or diabetic fatty liver (33.3 %) was significantly lower than that of the corresponding patients without fatty liver (100 %, 100 % and 80 % respectively), while the frequency of C2 genes, including C1/C2 and C2/C2, was significantly higher (71.4 %/0 %, 61.5 %/0 %, and 66.7 %/20 %) (P<0.01). The frequency distribution of the above genes of non-fatty liver patients (100 %/0, 100 %/0, and 80 %/20 %) was not significantly different from that of the normal controls (85 %/15 %) (P>0.05).

CONCLUSION: The genetic polymorphism of CYPIIE1 on the position of Pst I and Rsa I is related to the susceptibility of fatty liver. Besides, C2 gene may play a key role in the pathogenesis of fatty liver.

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INTRODUCTION

Cytochrome P450 (CYP) is a group of isoenzymes encoded by genes with similar structure and function, whose molecular weight is 40-60KD. CYP is a kind of monooxygenase, located in the smooth endoplasmic reticulum of cells. According to the similarity of amino acid sequence, CYP is divided into CYPI, CYPII, CYPIII, and CYPIV gene families. The subfamily is named alphabetically, and every enzyme is named in number order. Cytochrome P450IIE1 (CYPIIE1) is a N-nitrosodimethylamine demethylase, which is mainly expressed in the liver with an evident racial and individual difference in activity. It not only takes part in the metabolism of drugs, but also activates a lot of precarcinogens and prepoison[1-4]. Human CYPIIE1 is located in 10q2403-qter. It is 1104Kb consisting of 9 extrons and 8 introns, encoding a 493-amino acid protein[5]. The polymorphism of CYPIIE1 gene is significantly different among races and individuals. It may be related to some genetic factors. CYPIIE1 has 6 restriction fragment length polymorphisms (RFLP), among which 5' -flanking region of Pst I and Rsa I affects CYPIIE1 expression at the transcription level. C2 allelic gene can enhance the transcription, which causes the different activities of CYPIIE1[6-11].

Fatty liver is common and is resulted frequently from alcohol excess, obesity, diabetes or drugs[12-17]. Its pathogenesis remains unclear. Studies on the relationship between genetic polymorphisms of CYPIIE1 and the development of alcoholic fatty liver has been reported, but the results are disputable[17-21]. In this study, we used PCR-RFLP to study the relationship between genetic polymorphisms of CYPIIE1 and alcoholic or non-alcoholic fatty liver.

MATERIALS AND METHODS

Reagents

Heparin and lymphocyte separation medium were purchased from Tianjin Hematologic Institution of Chinese Academy of Medical Sciences. The primers of CYPIIE1, PCR buffer, dNTP, and Taq enzyme were obtained from Roche (America). Restriction endonucleases (PstIand RsaI), their buffer, and pUC19-DNA marker were obtained from MBI Ferments.

Patients and controls

From October 1998 to January 2001, 82 patients from several hospitals in Jilin Province were studied, among them 28 cases had alcoholic fatty liver, 8 cases had alcoholism but no liver disease, 13 cases had obese fatty liver, 8 cases had obesity but no fatty liver, 15 cases had diabetic fatty liver, and 10 cases had diabetes but no fatty liver. Twenty health blood donors were used as normal controls. The standard of alcoholism for female was drinking alcohol >40 g/d, for male drinking alcohol >80 g/d, for at least 5 years. The age and sex distribution of the two groups were similar. Five ml venous blood was taken from every person and anticoagulated with heparin.

PCR-RFLP

Peripheral blood mononuclear cells (PBMC) were separated by density gradient centrifugation. Then DNA was extracted. The two primers of CYPIIE1 were 5' -ccagtcgagtctacattgtca-3' (1 370-1 349) and 5' -ttcattctgtcttctaactgg-3' (999-978) respectively. PCR was conducted for 40 cycles with denaturing at 94 ℃ for 1 min, annealing at 50 ℃ for 1 min, extending at 72 ℃ for 1 min, and then designed to extend at 72 ℃ for 10 min. The PCR products were digested with Pst I or Rsa I at 37 ℃ for 2 hours, then loaded onto a 20 g/L agarose gel for electrophoresis. At last, the DNA fragments were observed under ultraviolet lamp.

Statistical analysis

Analysis of data was performed using χ^2 test. P<0.05 was considered to be statistically significant.

RESULTS

Genetic polymorphism of CYPIIE1

The PCR products were fragments of 410 bp (Figure 1). After digestion with restriction enzyme Pst I or Rsa I, CYPIIE1 was divided wild type homozygote group (C1/C1, type A), heterozygote group (C1/C2, type B) and mutant homozygote group (C2/C2, type C) (Figure 2). C1 referred to wild type gene (PstI+, RsaI-), and C2 referred to mutant gene (PstI-,RsaI+).

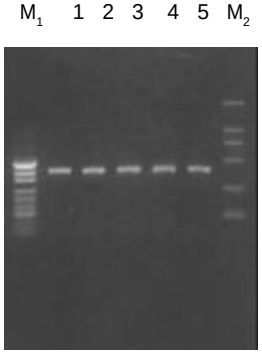

Figure 1 Electrophoregram of PCR products (Lanes 1-5). The signals of pUC19-DNA marker (M_1) present 489 bp, 404 bp, 331 bp, 242 bp, 190 bp, 147 bp and 110 bp from up to bottom. The signals of DL2000-DNA marker (M_2) present 2 000 bp, 1 000 bp, 750 bp, 500 bp, 250 bp and 100 bp from up to bottom.

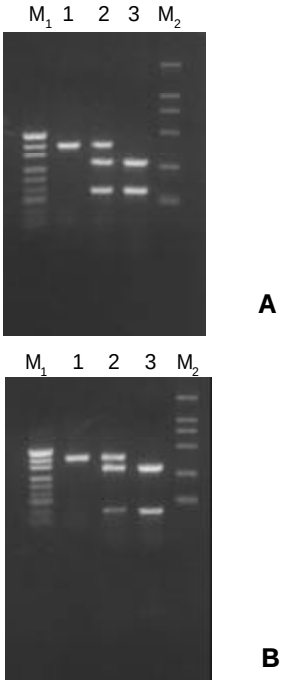

Figure 2 After digested with restriction enzyme PstI(A) or RsaI (B), CYPIIE1 was divided into three types, namely wild type homozygote (C1/C1) (Lane 1), heterozygote (C1/C2)(Lane 2) and mutant homozygote (C2/C2)(Lane 3). C1 means wild type gene (PstI+, RsaI-), and C2 mutant gene (PstI-, RsaI+). pUC19-DNA marker (M_1) and DL2000-DNA marker (M_2).

Genotype distribution

The genotype distribution of each group of patients and controls are presented in Table 1.

Comparison of gene frequency

The frequency of homozygotic C1 gene in patients with alcoholic, obese, or diabetic fatty liver was significantly lower than that of patients with corresponding diseases but without fatty liver, while the frequency of C2 genes, including C1/C2 and C2/C2, was significantly higher (P<0.01) (Table 2). Compared with healthy controls, the frequency of homozygotic C1 gene of the patients with alcoholic, obese, or diabetic fatty liver was apparently lower and the frequency of C2 gene was apparently higher (P<0.01). At the same time, there was no obvious difference in homozygotic C1 gene or C2 gene between healthy controls and patients with alcoholism, obesity or diabetes but without fatty liver (P>0.05).

Table 1 Genotype distribution of each group of patients and controls

Group	*n*	A (C1/C1)	B (C1/C2)	C (C2/C2)
Patients with alcoholic fatty liver	28	8	14	6
Patients with alcoholism but without liver diseases	8	8	0	0
Patients with obese fatty liver	13	5	6	2
Patients with obese but without fatty liver	8	8	0	0
Patients with diabetic fatty liver	15	5	8	2
Patients with diabetes but without fatty liver	10	8	2	0
Healthy controls	20	17	3	0

Table 2 Comparison of gene frequency of each group (%)

Group	A	B	C	C2 frequency
Patients with alcoholic fatty liver	28.6	50.0	21.4	71.4
Patients with alcoholism but without liver diseases	100	0	0	0
Patients with obese fatty liver	38.5	46.1	15.4	61.5
Patients with obese but without fatty liver	100	0	0	0
Patients with diabetic fatty liver	33.3	53.4	13.3	66.7
Patients with diabetes but without fatty liver	80	20	0	20
Healthy controls	85	15	0	15

DISCUSSION

There are three metabolic pathways of ethanol in the liver, alcohol dehydrogenase (ADH) in cytoplasm, microsomal ethanol oxidazing system (MEOS) in smooth endoplasmic reticulum, and catalase in peroxidase. The major component of MEOS is CYPIIE1[22,23]. When concentration of ethanol in blood and liver tissue is low, most of ethanol is oxidized by ADH. While for the alcoholism or the people in whose liver tissue the concentration of ethanol is higher than 10 mmol/L, the activation of MESO plays a key role in metabolism of ethanol. In the pathogenesis of alcoholic fatty liver, the function of CYPIIE1 was mainly to take part in lipid peroxidation (LOP) reaction and to increase the contents of microsomal oxygen and carbonyl free radical[16,24,25]. It has been proved in rat models that generation of microsomal oxygen and carbonyl free radical formed from oxidated ethanol was related to CYPIIE1[26,27]. It was presumed that these oxygen-derived free radicals might impair the liver by directly damaging liver cells, affecting the sensititity of the liver to LPO, and inducing antibody-dependent cytotoxic effect through combination with CYPIIE1 on the cell membrane[28-30]. Not every alcoholic abuser could inevitably induce liver injury. Iwahashi K and colleagues[31] reported that in the people who had C2 allele, CYPIIE1 activity was much

higher, and metabolic ability on ethanol was much stronger. Our results showed that homozygotic C1 gene frequency of the patients with alcoholic fatty liver was significantly lower than that of the controls or the patients with alcoholism but without fatty liver, while C2 gene frequency was much higher. It suggested that C2 gene might induce the expression of CYPIIE1 and facilitate genesis of alcoholic fatty liver.

LPO also took part in the pathogenesis of non-alcoholic fatty liver induced by obese or diabetes[32,33]. Now the precise stimulator of LPO reaction is unclear. The expression of CYPIIE1 in the animal models and patients with nonalcoholic fatty liver might be related to the induction of acetone and fatty acid[34,35]. It has been proved that the level of CYPIIE1 in the rats with obesity was four times as high as that of the rats without obesity[36]. The rising concentration of fatty acid and pyruvic acid in the liver might be a risk factor in pathogenesis of nonalcoholic fatty liver. When the increased fatty acid concentration in blood of patients with obesity could not be oxidated by mitochondria completely, CYPIIE1 would have the ability to oxidize fatty acid and in turn is activated by it so as to strengthen the oxidation ability. During oxidation of fatty acid, high reactivity carbonyl free radicals would be produced, which then stimulated the production of LPO, at last injured the liver[37]. In patients with diabetes, the ketone bodies produced by the liver could not be totally utilized by extrahepatic tissues, and the level of acetone would rise in the liver. The acetone could not only induce CYPIIE1 activation, but also be resolved by it. A great many of free radicals were produced, thus injuring the liver[38]. Not all patients with obesity or diabetes suffer from fatty liver. Our results showed that C2 gene frequency in patients with obese fatty liver or diabetic fatty liver was obviously higher than that of the patients without fatty liver. In conclusion, C2 gene frequency in patients with alcoholic or nonalcoholic fatty liver is much higher than that of controls. So C2 gene may be important for the pathogenesis of fatty liver.

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