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The ACE gene I/D polymorphism does not affect the susceptibility to or prognosis of PBC

ACE gen I/D polimorfizmi primer biliyer siroz için bir risk değildir

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Background/aims: *Primary biliary cirrhosis is an autoimmune liver disease that is strongly influenced by poorly defined, complex genetic factors. Alterations of the renin-angiotensin system have been implicated in the pathogenesis of various diseases. A deletion polymorphism of a 287-bp fragment of intron 16 of the angiotensin converting enzyme gene allele results in higher levels of circulating enzyme. Angiotensin converting enzyme deletion genotype has been linked to hypertension and sarcoidosis and has been reported to regulate liver fibrosis in HCV-mediated liver disease. We investigated the frequency of the Angiotensin converting enzyme gene insertion/deletion polymorphism in primary biliary cirrhosis patients.* ***Methods:*** *52 biopsy-proven primary biliary cirrhosis patients and 98 healthy controls were evaluated. Angiotensin converting enzyme insertion/deletion polymorphism was detected by polymerase chain reaction amplification of a genomic DNA fragment on intron 16 of the angiotensin converting enzyme gene. Clinical phenotype of primary biliary cirrhosis was verified with positive anti-mitochondrial antibody or M2 antibody, demonstration of cholestatic liver enzymes, and staging of liver biopsy. The differences between these variables among different genotypes were noted.* ***Results:*** *There was no significant difference in genotypes and allele frequency between the primary biliary cirrhosis group and controls. The D allele frequency was 54% in primary biliary cirrhosis cases and 55% in controls (p=ns). Furthermore, there was no significant difference in clinical features between patients with angiotensin converting enzyme-insertion or insertion/deletion genotypes vs. patients with angiotensin converting enzyme-deletion genotype.* ***Conclusions:*** *In our limited sample, the angiotensin converting enzyme deletion genotype did not make a significant contribution to the pathogenesis or progression of primary biliary cirrhosis.*

Key words: Primary biliary cirrhosis, polymorphism, renin angiotensin system

Amaç: *Primer biliyer siroz nedeni tam bilinmeyen, kompleks genetik faktörler tarafından etkilenen, otoimmün bir karaciğer hastalığıdır. Renin anjiotensin sistemindeki değişiklikler pekçok hastalığın patogenezinden sorumlu tutulmuştur. Anjiotensin konverting enzim geninin 16. intonundaki 287 baz delesyon polimorfizmi, dolaşımdaki anjiotensin konverting enzim miktarını artırmaktadır. Anjiotensin konverting enzim gen delesyon polimorfizmi hipertansiyon, sarkoidoz, HCV'e bağlı karaciğer sirozu gibi pekçok hastalıkla ilişkilendirilmiştir. Bu çalışmada anjiotensin konverting enzim gen insersiyon/ delesyon polymorphizmi primer biliyer siroz hastalarında incelenmiştir.* ***Yöntem:*** *52 biopsi ile doğrulanmış primer biliyer siroz hastası ve 98 sağlıklı kontrol çalışmaya dahil edilmiştir. Anjiotensin konverting enzim insersiyon/delesyon polymorfizmi anjiotensin konverting enzim geninin 16 intronunun PCR ile amplifikasyonu yöntemine dayanarak incelenmiştir. Klinik veriler pozitif anti-mitokondrial antikor (M2), kolestatik karaciğer enzimleri ve biopsy evresine göre sınıflanmıştır.* ***Bulgular:*** *Primer biliyer siroz grubu ile kontrol grubu arasında anjiotensin konverting enzim genotipleri ve allel sıklığı arasında fark izlenmemiştir. D allel sıklığı primer biliyer siroz olgularında %54, kontrollerde %55 olarak saptanmıştır. Anjiotensin konverting enzim genotipiyle klinik bulgular arasında korelasyon mevcut değildir.* ***Sonuç:*** *Anjiotensin konverting enzim gen insersiyondelesyon polimorfizmi varlığının primer biliyer siroz gelişimine ve ilerlemesine katkısı yoktur.*

Anahtar kelimeler: Primer biliyer siroz , polimorfizm, renin anjiotensin sistemi

INTRODUCTION

Primary biliary cirrhosis (PBC) is a chronic autoimmune inflammatory liver disease characterized by the presence of an intrahepatic mononuclear cell infiltrate, as well as circulating auto-antibodies (1, 2). The occurrence of hepatic angiogenesis in the liver of PBC patients is a novel finding (3). The excessive accumulation of inflammatory infiltrates, together with the accumulation of extracellu-

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lar matrix and development of fibrosis in the livers of PBC patients may result in an increased resistance of the tissue to blood flow and to the decreased delivery of oxygen. Under these circumstances, an angiogenic switch occurs, leading to the formation of neovessels, as described for other chronic inflammatory liver diseases (4). Angiotensins are important mediators in angiogenesis (5).

The renin-angiotensin system (RAS) is a circulatory cascade system primarily involved in the regulation of blood pressure and serum electrolytes (6). The key enzyme in this system is the angiotensin converting enzyme (ACE), which converts angiotensin I to the potent vasoconstrictor angiotensin II (7). The RAS has been shown to play a role in the pathogenesis of several diseases including fibrosis in the heart, kidney, lung and liver during chronic inflammation through the regulation of cell growth, inflammation, oxidative stress, angiogenesis and fibrosis (8, 9).

The ACE gene insertion/deletion (I/D) polymorphism was first identified in 1990. The ACE-D, a deletion polymorphism of a 287-bp fragment of intron 16 of the ACE gene allele, has been shown to result in higher levels of circulating enzyme in a dose-dependent manner (10). The role of the ACE gene I/D polymorphism as a risk factor has been investigated in several diseases (11, 12, 13). However, the prevalence of the ACE I/D polymorphism in PBC and its contribution to the course of the disease has not yet been defined. We therefore investigated the occurrence of the ACE I/D polymorphism in PBC patients.

MATERIALS AND METHODS

Fifty-two consecutive patients with PBC admitted to the University of Pittsburgh Medical Center were recruited. Clinical phenotype of PBC was verified with positive anti-mitochondrial antibody or M2 antibody, demonstration of cholestatic liver enzymes and staging of liver biopsy (14). Ninety-eight healthy volunteers recruited through the North America Pancreatitis 2 study were used as the control group.

Laboratory Procedure

Genomic DNA was purified from peripheral blood cells by using the Puregene DNA isolation kit, Gentra systems (Minneapolis, MN) (15). To determine the ACE genotype, a genomic DNA fragment on intron 16 of the ACE gene was amplified by polymerase chain reaction (PCR) according to Rigat's method (10). The amplified ACE gene fragments without insertion (D allele) and with insertion (I allele) of approximate 190 and approximate 490 bp, respectively, were detected on a 1% agarose gel containing ethidium bromide. To increase the specificity of DD genotyping, PCR amplifications were performed with an insertion-specific primer pair (5 prime TGGGACCACAGCGCCCGCCACTAC 3 prime and 5 prime TCGCCAGCCCTCCCATGCCCATAA 3 prime); only the I allele produces a 335-bp amplicon. The 335-bp fragment was identified on a 1.5% agarose gel containing ethidium bromide. The reaction yields no products in the samples of DD genotype (16).

Ethics: The study was carried out with the approval of the institutional review board of the University of Pittsburgh Medical Center. Informed written consent was obtained from each patient and the study protocol conforms to the ethical guidelines of the Declaration of Helsinki.

Results are given as mean ± SD or allele frequencies. The differences between clinical and laboratory variables among patients with different ACE genotypes were noted. Genotype frequencies were statistically analyzed by Armitage trend test. Standard odds-ratio analyses and the one and two way analysis of variance (ANOVA) were used to compare cases and control subjects.

RESULTS

Fifty-two PBC patients (47 F, 5 M; mean age: 58.2±7.6 years) and 98 controls (41 M, 57 F; mean age: 67.06±9.96 years) were evaluated. The mean age of healthy controls was significantly higher than of the PBC group, meaning that they are healthy and have very low chance of developing any disease after that age ($p<0.001$). The female gender was more frequent in the PBC group since the disease is more common in females ($p<0.01$). Peak alkaline phosphatase (ALP) level was 390±275 U/L in PBC patients. Liver biopsies proved stage 4

Table 1. The demographic and clinical characteristics of primary biliary cirrhosis (PBC) patients

	PBC	Controls	P value
F/M	47/5	57/41	P<0.01
Mean age (years)	58.2±7.6	67.06±9.96	P<0.001
Histologic Stage			
I	16	-	-
II	11	-	-
III	15	-	-
IV	10	-	-

Table 2. Distribution of ACE I/D genotype in the study groups

ACE Genotype	PBC (n: 52)	Control (n: 98)	PBC/Female (n: 47)	Controls/Female (n: 57)
II	9	19	7	12
ID	30	50	28	30
DD	13	29	12	15
P value	ns		ns	

ACE: Angiotensin converting enzyme. PBC: Primary biliary cirrhosis.

disease in 10 patients and stage 3 disease in 15 patients (Table 1). Five patients had liver transplantation for end–stage liver failure.

The frequency of ACE I/D genotypes is summarized in Table 2. There was no significant difference in genotypes and allele frequencies between the PBC group and controls. The D allele frequency was 54% in PBC cases and 55% in controls (p=ns). ACE DD genotype was present in 25% of PBC patients and 30% of controls. Since PBC was more common in females, we selected only females in both the PBC and control groups to eliminate the effect of gender for further analysis. Results showed that there was no significant difference in allele frequency of ACE I/D polymorphism between female PBC patients and healthy females.

Laboratory and biopsy findings did not differ between patients with different ACE genotypes. Peak ALP levels were similar in patients with II, ID or DD genotypes (365.6±73.9, 435.8±56.1 and 304.6±61.1, respectively). Furthermore, there was no significant difference in clinical features between patients with ACE-insertion or I/D genotypes vs. patients with ACE-deletion genotype. When all patients were categorized according to their fibrosis stage (stage 1 or 2 was considered as mild disease, stage 3 or 4 as severe disease), the distribution of ACE genotype was similar between groups (Table 3). ACE genotype also did not affect the progression of PBC to end-stage liver disease or the need for liver transplantation.

DISCUSSION

Primary biliary cirrhosis is an autoimmune disease characterized by chronic inflammation. Genetic predisposition to PBC is indicated by the higher familial incidence of the disease, particularly among siblings, and the high concordance rate among monozygotic twins (17). Accordingly, several genetic polymorphism studies have been conducted to explain the genetic background of PBC (18, 19).

Components of the RAS contribute to the pathogenesis of various inflammation-associated diseases (20). The key enzyme in the RAS system is an ACE that converts angiotensin-I to the potent vasoconstrictor angiotensin-II (7). In the liver, angiotensin II is recognized as one of the most potent fibrogenic molecules that activates hepatic stellate cells (21). Furthermore, angiotensin II encourages myofibroblast contraction and proliferation and promotes release of inflammatory cytokines as well as the deposition of extracellular matrix in chronic liver disorders (22). Increased ACE activity might lead to higher angiotensin II levels, which in turn causes liver fibrosis in different disorders.

Based on the above observations, we hypothesized that functional polymorphisms in the ACE gene would increase susceptibility or alter the clinical course of PBC. However, we showed that the ACE gene I/D polymorphism was not associated with susceptibility to PBC or severity of PBC in an American population. The ACE gene I/D allele frequencies were similar to previously reported frequencies in American studies, suggesting that the study sample was representative of the larger population (22). Although the age and gender distribution of the control group was different than in PBC patients, the ACE I/D polymorphism was not affected by gender or age. Sub-group analysis of fe-

Table 3. Genotype distribution of ACE I/D polymorphism according to PBC stage

PBC Stage/ACE genotype	Scheuer's stage I+II	Scheuer's stage II+III	P value
I (Allele frequency)	42%	50%	ns
D (Allele frequency)	58%	50%	ns
ACE-II	3	6	ns
ACE-ID	16	14	ns
ACE-DD	7	6	ns

ACE: Angiotensin converting enzyme. PBC: Primary biliary cirrhosis.

male subjects revealed that gender has no impact on the results. On the other hand, it has been noted that the pathological risk of ACE D/D genotype varies between populations with different genetic and environmental backgrounds (13). Possibility of an important effect of this polymorphism in PBC cannot be excluded in different populations.

The natural history of PBC has improved significantly during the last decades. Most patients are diagnosed early with asymptomatic PBC. Some will remain asymptomatic, whereas others progress to symptomatic PBC (23) with fatigue and pruritus. Powell et al. (24) indicated that host genetic factors may account for some of the variability in the rate of disease progression seen in patients with chronic HCV infection by showing the striking relationship between angiogenetic genotypes and the development of progressive hepatic fibrosis. However, we did not find any relation between ACE genotype and PBC stage or progression. Our study suggests that although the ACE I/D polymorphism increases the systemic ACE levels, its effect on hepatic tissue is unknown. Different regulatory pathways and molecules might be working in the liver for the regulation of local RAS.

The present study showed that there is no relation between pathogenesis and progression of PBC and the ACE I/D polymorphism. These results might also suggest that locally regulated RAS may be a more important factor in chronic liver disorders, independent from the systemic RAS.

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