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Original Article

Association of polymorphisms of *adiponectin* gene promoter-11377C/G, *glutathione peroxidase-1* gene C594T, and cigarette smoking in nonalcoholic fatty liver disease

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Abstract

Background: The number of studies on *adiponectin*, *GPx-1* gene polymorphisms, and nonalcoholic fatty liver disease (NAFLD) susceptibility is increasing, but none have investigated the effect of cigarette smoking in combination with the gene polymorphisms on the susceptibility to NAFLD. In order to understand the distribution of adiponectin and GPx-1 in the local population, to explore the possible association of cigarette smoking with *adiponectin* and *GPx-1* gene polymorphisms in the pathogenesis of NAFLD, we conducted this research, examining the distribution of polymorphisms of *adiponectin* and *GPx-1* in NAFLD patients and healthy controls, analyzing the association between these polymorphisms and cigarette smoking.

Methods: Two hundred nonalcoholic simple fatty liver (NAFL), 200 nonalcoholic steatohepatitis (NASH), and 200 nonalcoholic fatty hepatic cirrhosis (NAFHC) cases from the First Affiliated Hospital of Xinxiang Medical College in China from February 2011 to November 2014 were selected for this study, and 200 healthy individuals as a control group. No significant difference among the four groups in age, sex, ethnicity, and birthplace was observed. The genetic polymorphisms of *adiponectin* gene promoter-11377C/G and *GPx-1* gene C594T were analyzed using polymerase chain reaction-restriction fragment length polymorphisms in peripheral blood leukocytes of the above-mentioned cases. The interaction between the two mutants and the gene-environment association of the genotypes with cigarette smoking were analyzed.

Results: The frequencies of *adiponectin* gene promoter-11377C/G(CG), -11377C/G (GG), *GPx-1* gene C594T (CT) and C594T (TT) were 24.50%, 26.00%, 24.00%, and 25.50% in the NAFL group, 34.50%, 37.00%, 35.00%, and 36.00% in the NASH group, 42.00%, 46.00%, 43.50%, and 45.50% in the NAFHC group, and 14.00%, 14.50%, 13.00%, and 14.00% in the control group, respectively. Statistical tests showed a significant difference in the frequencies among each group ($p < 0.01$). The risk of NAFLD significantly increased in patients with *adiponectin* gene promoter-11377C/G (CG) genotype [odds ratio $(OR)_{NAFL} = 2.5278$; $OR_{NASH} = 6.1823$; $OR_{NAFHC} = 17.8570$), in those with -11377C/G (GG) genotype ($OR_{NAFL} = 2.5900$; $OR_{NASH} = 6.4017$; $OR_{NAFHC} = 18.9023$), in those with *GPx-1* gene *C594T* (CT) genotype ($OR_{NAFL} = 2.6687$; $OR_{NASH} = 6.7772$; $OR_{NAFHC} = 22.2063$), and in those with C594T (TT) genotype ($OR_{NAFL} = 2.6330$; $OR_{NASH} = 6.4729$; $OR_{NAFHC} = 21.5682$). Combined analysis of the polymorphisms showed that percentages of *adiponectin* gene promoter -11377C/G (GG)/*GPx-1* gene C594T (TT) in the NAFL, the NASH, NAFHC, and control groups was 7.00%, 13.50%, 21.00%, and 2.00%, respectively ($p < 0.01$). The people who carried the *adiponectin* gene promoter -11377C/G (GG)/*GPx-1* gene *C594T* (TT) had a high risk of NAFLD ($OR_{NAFL} = 7.2800$; $OR_{NASH} = 41.2941$; $OR_{NAFHC} = 363.9724$), and statistical analysis suggested a positive association between -11377C/G (GG) and C594T (TT) in increasing the risk of NAFLD ($\gamma_{2NAFL} = 2.2071$, $\gamma_{4\ NAFL} = 2.0773$; $\gamma_{2\ NASH} = 2.1084$; $\gamma_{4NASH} = 2.0543$; $\gamma_{2\ NAFHC} = 2.1387$; $\gamma_{4NAFHC} = 2.0004$). Likewise, there were also positive association in the pathogenesis of NAFLD between -11377C/G (CG) and C594T (TT), -11377C/G (CG) and C594T (CT), -11377C/G (GG), and C594T (TT) (CT).The frequencies of smoking index (SI) $\leq$ 400 and SI > 400 were 22.50% and 26.50% in the NAFL group, 29.00% and 40.50% in the NASH group, 34.00% and 51.50% in the NAFHC group, and 15.50% and

Conflicts of interest: The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

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12.00% in the control group, respectively. Statistical tests showed a significant difference in the frequencies among each group (all $p < 0.01$). The risk of NAFLD significantly increased in patients with SI $\leq$ 400 ($OR_{NAFL} = 2.0636$; $OR_{NASH} = 4.4474$; $OR_{NAFH\ C} = 10.9677$) and in those with SI > 400 ($OR_{NAFL} = 3.1393$; $OR_{NASH} = 8.0225$; $OR_{NAFHC} = 21.4583$), and statistical analysis suggested a positive association between cigarette smoking and -11377C/G (CG), -11377C/G (GG), *C594T* (CT), and *C594T* (TT) in increasing the risk of NAFLD (all $\gamma > 1$).
Conclusion: *Adiponectin* gene promoter -11377C/G (CG), -11377C/G (GG), *GPx-1* gene C594T (CT), C594T (TT), and cigarette smoking are risk factors in NAFLD, and the significant association between genetic polymorphisms of -11377C/G, C594T, and cigarette smoking amplify the risk of NAFLD.

Keywords: adiponectin gene promoter -11377C/G; cigarette smoking; glutathione peroxidase-1 gene C594T; nonalcoholic fatty liver disease; polymorphism

1. Introduction

The exact mechanism of nonalcoholic fatty liver disease (NAFLD) is unclear, but the two-hit theory is more popular than other hypotheses. The theory states that insulin resistance can cause liver fat accumulation in hepatocytes, the first hit in the pathogenesis of NAFLD, which makes the liver more vulnerable to oxidative stress and subsequent lipid peroxidation, among the factors constituting the second hit, thus progressing to inflammation and fibrosis, and eventually NAFLD.[1,2]

Cigarettes release nicotine and carbon monoxide in the combustion process. These ingredients interfere with lipid metabolism, and multiple ingredients of cigarettes also stimulate and promote free radical lipid peroxidation, which participates in the development of NAFLD.[3] Adiponectin, one of the major adipocyte-secreted proteins, has attracted scientific interest in recent years and has been extensively studied in both human and animal models. Adiponectin exerts insulin-sensitizing effects through binding to adiponectin receptors, leading to activation of adenosine monophosphate-activated protein kinase, peroxisome proliferators activated receptor-α, and potentially other unknown molecular pathways. The role of adiponectin in improving insulin sensitivity is such that it has become an important factor in inhibiting the progress of NAFLD.[4] Glutathione peroxidase-1 (GPx-1) has the function of scavenging free radicals and derivatives, and phospholipid hydroperoxide glutathione peroxidase constitutes with catalase and glutathione-S-transferase (an organic hydroperoxide reduction system) at different levels of substrate specificity, reducing the formation of lipid peroxides and enhancing resistance to oxidation damage, an important factor that controls the progression of NAFLD.[5] *Adiponectin* and *GPx-1* genes have polymorphisms which have multiple alleles with different alleles encoding different adiponectin or GPx-1 activities. Polymorphisms in *adiponectin* or *GPx-1* genes can affect the reaction of the body to the external environment (such as smoking), which is an important factor that determines its susceptibility to NAFLD.

The number of studies on *adiponectin*, *GPx-1* gene polymorphisms, and NAFLD susceptibility is increasing, but none have investigated the effect of cigarette smoking in combination with the gene polymorphisms on the susceptibility to NAFLD. In order to understand the distribution of adiponectin and GPx-1 in the local population, to explore the possible association of cigarette smoking with *adiponectin* and *GPx-1* gene polymorphisms in the pathogenesis of NAFLD, we conducted this research examining the distribution of polymorphisms of *adiponectin* and *GPx-1* in NAFLD patients and healthy controls, and analyzed the relationship between these polymorphisms and smoking status.

2. Methods

2.1. Diagnostic criteria

The diagnosis of NAFLD was based on guidelines for diagnosis and treatment of NAFLD revised by the Fatty Liver and Alcoholic Liver Disease Study Group of the Chinese Liver Disease Association in 2010.[6]

2.2. Inclusion criteria

(1) The patients conformed to the above diagnostic criteria of NAFLD; (2) the patients were aged from 17 years to 69 years, men or women; (3) alanine transaminase or aspartate transaminase level was less than 80 U/L; (4) the patients voluntarily signed an informed consent form and passed ethic evaluation.

2.3. Exclusion criteria

(1) History of alcohol intake > 20g/d; (2) co-existence of other liver diseases, such as viral, drug-induced, auto-immune hepatitis, and so on; (3) suspicion of liver cirrhosis or liver cancer; (4) co-existence of other severe systematic disease or infectious disease, such as malignant neoplasm, severe cardiopulmonary disease, neurological disorders, human immunodeficiency virus infection, and so on; (5) currently pregnant, breastfeeding, pregnancy anticipated during study, or planning to conceive; (6) co-existence of mental disorders or severe neurosis, or unable to express symptoms subjectively, and hindering of connection and cooperation with researchers because of dysgnosia or aphasis.