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Relationship between PNPLA3 Gene rs738409 Polymorphism and Non-alcoholic Fatty Liver in Mazandaran Province

Mohammad Ali Saremi [1*], Mahsa Alikarami [1]

[1] Personalized Medicine Research Center of AmitisGen, Tehran, Iran

** Corresponding author: Mohammad Ali Saremi, Personalized Medicine Research Center of AmitisGen, Tehran, Iran. Email: masaremi@yahoo.com*

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Abstract

Nonalcoholic fatty liver disease (NAFLD) is a common cause of chronic liver disease. Non-alcoholic fatty liver disease is a spectrum of simple steatosis that is benign to non-alcoholic steatohepatitis (NASH) associated with fibrosis and inflammation. Genetic factors play an important role in this disease. The PNPLA3 gene (C > G) rs738409 polymorphism has been defined to be associated with increased liver fat content and liver damage in metabolic disorders. In this study, the association of this polymorphism with non-alcoholic fatty liver disease was investigated in the Mazandaran province population. Our study population consisted of 85 patients and 85 controls. Blood samples were collected from patients and DNA were then extracted by boiling method. Genotypes of this polymorphism were determined by PCR-RFLP method. There was a significant relationship between CG genotype and risk of NAFLD (P = 0.012).

INTRODUCTION

The liver is one of the most important organs of the body, which is involved in regulation of glucose and fat metabolism [1]. Normal liver contains about five grams of fat per 100 grams of body weight, when fat makes up > 5% of liver weight, it is called fatty liver [2]. Non-alcoholic fatty liver disease is a common cause of chronic liver disease. It has been first identified in 1980 by Ludwig et al. (1980) in patients without use of alcohol [3]. Non-alcoholic fatty liver disease (NAFLD) has been described as a spectrum of simple steatosis to non-alcoholic steatohepatitis [4], which may be accompanied by fibrosis, lobular inflammation and balloon degeneration, leading to progression of hepatic cirrhosis and liver failure [5, 6], as well as cancer. The liver may lead to a liver transplant [7]. The cause of this disease is excessive accumulation of fatty acids and triglycerides in the liver. Patients with NAFLD are usually asymptomatic. It is diagnosed only among non-alcoholic individuals after finding abnormal laboratory tests or abdominal ultrasound during routine health examinations or other illnesses [3]. The prevalence of NAFLD is estimated to be 20-30% in the general population and 67-75% in obese individuals [8]. In Iran, studies have been conducted on the prevalence of NAFLD and NASH, where its prevalence vary in different studies, ranging from 2.9% (8) to 7.1% [9, 10], however, a study in 2013 by Bagheri Lankrani et al. reported a prevalence of 21.5% for adults in southern Iran, which is higher than the previous studies in Iran [11].

The PNPLA3 gene (Patatin-Like Phospholipase Domain-Containing Protein-3) was identified by Baulande in adipose tissue [12]. This gene is located on the long arm of chromosome 22 (Chr22q13.31). In 2008, Romeo et al. reported that rs738409 is the single nucleotide polymorphism of the PNPLA3 gene leading to a non-synonymous I148M sequence variation and a strong association of this polymorphism with increased fat content has been revealed [13]. The single nucleotide polymorphism (C > G) rs738409 on PNPLA3 encodes a 481-amino-acid protein, which is conserved from potato to human and shows expression level up to 10-fold higher in liver than in adipose tissue [14]. This single nucleotide polymorphism (rs738409) has been identified in the third exon of the PNPLA3 gene by substituting G for C and converting isoleucine to methionine at amino acid 148 (I148M) in the PNPLA3 gene [7]. Simple modeling indicates that this substitution could lead to the spatial inhibition of the protein's catalytic domain which results in loss of function of PNPLA3 gene [15]. In humans, PNPLA3 is predominantly expressed in the liver and belongs to a patatin-like phospholipase family, which contains the triglyceride lipase, a key protein involved in the hydrolysis of triglycerides to diglycerides. Its activity is regulated by the hormonal pathways involved in

regulation of lipid deposition in the liver. Observations indicates that PNPLA3 is capable of encoding a key molecule involving in mediation of the pathological processes of liver injury in metabolic disorders [16]. PNPLA3 gene variation contributes to ethnic and interpersonal differences in liver fat content and susceptibility to non-fatty liver disease [13]. In this study, we investigated the effect of rs738409 polymorphism PNPLA3 gene on non-alcoholic fatty liver in northern population of Iran.

METHODS

A total of 85 blood samples were obtained from patients diagnosed with fatty liver based on ultrasound findings and 85 samples from healthy control. DNA was extracted by boiling method, and then quantified by nanodrop. Polymerase chain reaction was perform by specific primer for extended region rs738409 (Table 1). PCR product was visualized by 1.5% agarose gel electrophoresis (Fig 1). Furthermore, RFLP (Restriction Fragment Length Polymorphism) method was used for genotyping, therefore, PCR product digested by BtsCI restriction enzyme and product of this process visualized by 2% agarose gel electrophoresis. Statistical analysis was performed by SPSS software.

Table 1: Primer Sequence of rs738409

Primer	sequence
Rs738409	F: CCCTGCTCACTTGGAGAAAG R: CTGCAGGCAGGAGATGTGT

RESULTS

In terms of gender, study populations consisted of 27 male (38.9%) and 48 female (61.1%) and control group consisted of 34 male (44.3%) and 51 female (55.7%). No significant difference was found between the two groups (P value = 0.51). Fragments of the enzyme digestion were observed in the present study (227bp was considered as CC genotype, followed by CG genotype (227-119-108), and GG genotype (119-108 bp). Figure 2 shows the products of BtsCI enzymatic digestion on gel electrophoresis.

The frequency of genotypes and alleles were calculated between groups in which the genotype CG was found to be significantly different between the control and patient groups (P = 0.012), suggesting CG as susceptible genotype; those with CG genotype were 2.63 times more likely to develop the disease. Moreover, a significant difference was found in terms of GG genotype between control and patient groups (P = 0.002), indicating that GG genotype was the disease-protecting genotype in our study.

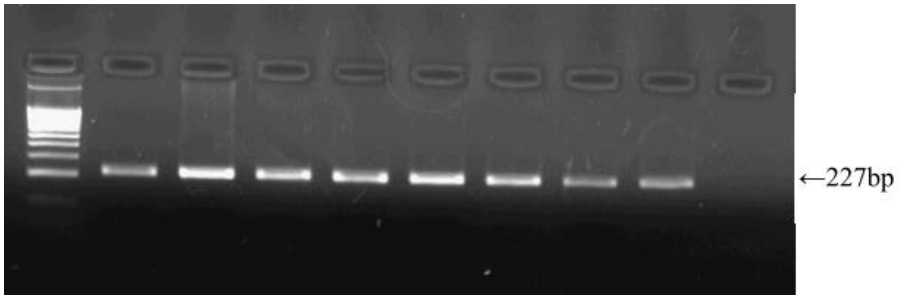

Figure 1: rs738409 PCR Product Based on 1.5% Agarose Gel Electrophoresis

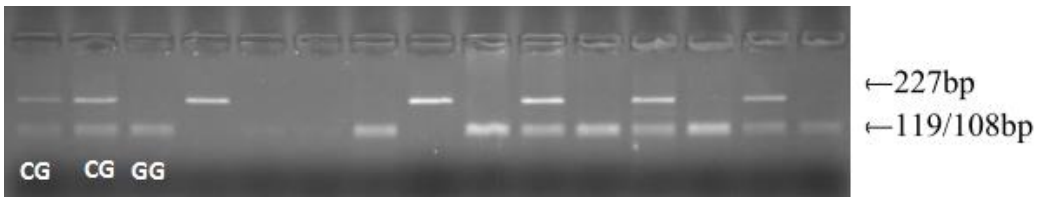

Figure 2: Fragment of Digested Products, BtsCI

DISCUSSION

Non-alcoholic fatty liver disease is a common cause of chronic liver disease and has emerged as a major global health problem. Advances in genome analysis have greatly helped to understand the genetic factors of NAFLD. The development of NAFLD involves numerous genetic factors that interact with lifestyle and environment [6]. Studies have shown that genetic factors play an important role in the pathogenesis of NAFLD. The PNPLA3 gene was identified in 2001 by Baulande et al. The rs738409 C > G polymorphism is a common variant of this gene that has been reported in the Dallas Heart Study of different ethnicities with liver fat content [12]. It has been shown that the rs738409 G allele is associated not only with fat accumulation in the liver, but also with liver damage. Rotman et al. also confirmed the association of the rs738409G allele with steatosis in the American white population [4]. In this study, we investigated the effect of rs748409 polymorphism as a risk factor on non-alcoholic fatty liver disease. The results indicate a significant

relationship of CG genotype of this polymorphism with non-alcoholic fatty liver disease. Based on the findings presented herein, it is suggested that this polymorphism can be investigated in other ethnicities of Iran with gender-and age-matched controls and patients.

REFERENCES

1. Nasiri Toosi M, Shams Ardekani MR, Minaie MB, Nazim I, Esfahani MM, Khadem A. Fatty Liver Disease From the Perspective of Traditional Iranian Medicine. Quran Med. 2012;1(4):117-8. doi: 10.5812/quranmed.11092
2. Savadkooh F, Hosseini Tabatabai M, Shahabinejad S. [The prevalence of fatty liver on ultrasound patients without a history of liver disease and its association with cholesterol and triglyceride]. TabibShargh. 2003;5(3):177-83.
3. Orangi E, Ostadrahimi A, Mahdavi R, Svmy M, Tarzmani M. [Indicators related to oxidative stress and antioxidant status in patients with nonalcoholic fatty liver]. J EndocrinolMetab. 2010;12(5):499-3.
4. Rotman Y, Koh C, Zmuda JM, Kleiner DE, Liang TJ, Nash CRN. The association of genetic variability in patatin-like phospholipase domain-containing protein 3 (PNPLA3) with histological severity of nonalcoholic fatty liver disease. Hepatology. 2010;52(3):894-903. doi: 10.1002/hep.23759 pmid: 20684021
5. Valenti L, Alisi A, Galmozzi E, Bartuli A, Del Menico B, Alterio A, et al. I148M patatin-like phospholipase domain-containing 3 gene variant and severity of pediatric nonalcoholic fatty liver disease. Hepatology. 2010;52(4):1274-80. doi: 10.1002/hep.23823 pmid: 20648474
6. Zain SM, Mohamed R, Mahadeva S, Cheah PL, Rampal S, Basu RC, et al. A multi-ethnic study of a PNPLA3 gene variant and its association with disease severity in non-alcoholic fatty liver disease. Hum Genet. 2012;131(7):1145-52. doi: 10.1007/s00439-012-1141-y pmid: 22258181
7. Romeo S, Huang-Doran I, Baroni MG, Kotronen A. Unravelling the pathogenesis of fatty liver disease: patatin-like phospholipase domain-containing 3 protein. Curr Opin Lipidol. 2010;21(3):247-52. doi: 10.1097/mol.0b013e328338ca61 pmid: 20480550
8. Kotronen A, Peltonen M, Hakkarainen A, Sevastianova K, Bergholm R, Johansson LM, et al. Prediction of non-alcoholic fatty liver disease and liver fat using metabolic and genetic factors. Gastroenterology. 2009;137(3):865-72. doi: 10.1053/j.gastro.2009.06.005 pmid: 19524579
9. Sohrabpour AA, Rezvan H, Amini-Kafiabad S, Dayhim M, Merat S, Pourshams A. Prevalence of nonalcoholic steatohepatitis in Iran: a population based study. Middle East J Digest Dis. 2010;2(1):14.
10. Alavian SM, Mohammad-Alizadeh AH, Esna-Ashari F, Ardalan G, Hajarizadeh B. Non-alcoholic fatty liver disease prevalence among school-aged children and adolescents in Iran and its association with biochemical and anthropometric measures. Liver Int. 2009;29(2):159-63. doi: 10.1111/j.1478-3231.2008.01790.x pmid: 18492015
11. Lankarani KB, Ghaffarpasand F, Mahmoodi M, Lotfi M, Zamiri N, Heydari ST, et al. Non alcoholic fatty liver disease in southern Iran: a population based study. Hepat Mon. 2013;13(5):e9248. doi: 10.5812/hepatmon.9248 pmid: 23922564
12. Baulande S, Lasnier F, Lucas M, Pairault J. Adiponutrin, a transmembrane protein corresponding to a novel dietary- and obesity-linked mRNA specifically expressed in the adipose lineage. J Biol Chem. 2001;276(36):33336-44. doi: 10.1074/jbc.M105193200 pmid: 11431482
13. Romeo S, Kozlitina J, Xing C, Pertsemlidis A, Cox D, Pennacchio LA, et al. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. Nat Genet. 2008;40(12):1461-5. doi: 10.1038/ng.257 pmid: 18820647
14. Sanyal AJ, American Gastroenterological A. AGA technical review on nonalcoholic fatty liver disease. Gastroenterology. 2002;123(5):1705-25. doi: 10.1053/gast.2002.36572 pmid: 12404245
15. Chang CY. Understanding the relationship between PNPLA3, NAFLD and insulin resistance: do ethnic differences bring more questions or more answers? Liver Int. 2011;31(9):1246-9. doi: 10.1111/j.1478-3231.2011.02612.x pmid: 22093451
16. Li Q, Qu HQ, Rentfro AR, Grove ML, Mirza S, Lu Y, et al. PNPLA3 polymorphisms and liver aminotransferase levels in a Mexican American population. Clin Invest Med. 2012;35(4):E237-45. doi: 10.25011/cim.v35i4.17153 pmid: 22863562