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ORIGINAL ARTICLE

Association study of *PNPLA2* gene with histological parameters of NAFLD in an obese population

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Summary
Introduction: The prevalence of non-alcoholic fatty liver disease (NAFLD) and the closely associated metabolic syndrome is high and is related to risk factors such as obesity and type 2 diabetes. A genetic basis for NAFLD has been suggested, but only few causal genes have been identified. The most significant association reported to date is the robust association of the *PNPLA3* I148M variant with susceptibility to NAFLD. We therefore hypothesized that the *PNPLA2* gene might also be involved in NAFLD pathogenesis, because of its close sequence similarity with *PNPLA3* and its possible involvement in ectopic fat accumulation.
Methods: In this study, we investigated the association of *PNPLA2* polymorphisms with the development of non-alcoholic fatty liver disease in a prospectively recruited Belgian obese population comprising 633 individuals with varying degrees of fatty liver disease. We selected 3 *PNPLA2* SNPs for genotyping, including 2 tagSNPs that cover most information on common genetic variation in the selected region.

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Results: After performing linear regression analysis, we found that 2 of the analyzed *PNPLA2* SNPs were associated with anthropometric and metabolic parameters. In our subcohort of patients that underwent liver biopsy ($n = 372/633$ or 58.7%), we assessed the influence of the *PNPLA2* variants on the severity of histologically determined liver damage, but we did not find convincing evidence for association.
Conclusion: Although we found evidence for moderate association between *PNPLA2* tagSNPs and anthropometric and metabolic parameters in our cohort, no evidence for association between polymorphisms in the *PNPLA2* gene and the presence and severity of NAFLD was identified.

Introduction

The global increase in the prevalence of obesity has led to a rise in the associated incidences of the metabolic syndrome and non-alcoholic fatty liver disease (NAFLD). The metabolic syndrome is an important risk factor for type II diabetes and cardiovascular disease [1,2] and recent findings suggest an important role for hepatic steatosis and abdominal adipose tissue in the development of the syndrome. Furthermore, the metabolic syndrome appears to be strongly associated with the development of NAFLD, which is considered to be the hepatic manifestation or hepatic component of the metabolic syndrome [3,4]. NAFLD has become the most common chronic liver disease in Western countries, with an estimated prevalence of 20—30% in adults in developed countries. The prevalence of hepatic steatosis increases with BMI, reaching a prevalence of 65—85% in obese and morbidly obese individuals [5,6]. NAFLD comprises a disease spectrum of hepatic disorders ranging from simple steatosis (triglyceride accumulation) to steatohepatitis, liver cirrhosis, and hepatocellular carcinoma [7]. Several studies show that NAFLD relates to obesity and its metabolic consequences such as insulin resistance and dyslipidemia [8], but the underlying mechanisms explaining this relationship are still poorly understood.

The heritability of NAFLD is estimated between 20 and 39 percent [9,10], indicating that genetic factors play an important role in the etiology of the disease. In order to identify DNA sequence variations that contribute to inter-individual differences in NAFLD, Romeo et al. carried out a genome-wide association study. In this study, a nonsynonymous variant (rs738409—I148M) in the *PNPLA3* (patatin-like phospholipase domain-containing 3 or adiponutrin) gene was found to be associated with increased hepatic fat levels and hepatic inflammation and thus susceptibility to NAFLD [11]. This original association was found using a liver phenotype based on magnetic resonance spectroscopy, but has been robustly replicated in several independent studies, including our own study and was also confirmed in subjects with histologically characterized NAFLD [12—15].

PNPLA3 is a member of the patatin-like phospholipase domain-containing protein (PNPLA) family, most closely resembling PNPLA2 (also known as adipose triglyceride lipase (ATGL) or desnutrin) [16,17], which is a major triglyceride lipase in adipose tissue [18]. Because of its close sequence similarity with PNPLA3 and its possible involvement in ectopic fat accumulation [19], the lipase PNPLA2 has caught our attention as it is likely to be involved in NAFLD pathogenesis. Results from animal experiments have shown that liver-specific deletion of *PNPLA2* renders mice to be more prone to hepatic steatosis [20]. Furthermore, polymorphisms in the *PNPLA2* gene have been found to be associated with plasma free fatty acids, triglycerides and type 2 diabetes [21], indicating involvement of PNPLA2 in pathways related to the development of metabolic syndrome.

To the best of our knowledge, no prior association study exploring the role of *PNPLA2* in the pathogenesis of NAFLD has already been performed. In this study, we therefore aim to evaluate the contribution of *PNPLA2* gene polymorphisms to the development of NAFLD using an extensive and well-phenotyped cohort of individuals with varying degrees of fatty liver disease.

Materials and methods

Study population

A total of 633 obese individuals (182 men and 451 women), presenting with a problem of overweight or obesity were consecutively recruited from the outpatient obesity clinic at the Antwerp University Hospital. All patients underwent a standard metabolic work-up combined with a liver-specific program. Patients known to have diabetes were excluded from the study. Patients were also excluded from further analysis in case of significant alcohol consumption (> 20 g/day) [22] or if another liver disease was diagnosed. All study subjects are of Belgian Caucasian origin. Population characteristics are summarized in Table 1. The study was approved by the local ethics committee and all participants gave their written informed consent once the aim and design of the study had been explained.

Metabolic work-up

A detailed questionnaire was completed by all patients and a clinical examination including anthropometry measures was performed. All measurements were performed under fasting conditions. Height was measured to the nearest 0.5 cm and body weight was measured with a digital scale to the nearest 0.2 kg. BMI was calculated as weight in kilograms over height in meters squared. Waist circumference was