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Short Communication

Association of *peroxisome proliferator activated receptor-γ* gene with non-alcoholic fatty liver disease in Asian Indians residing in north India ☆

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ABSTRACT

Background: Genetics of non-alcoholic fatty liver (NAFLD) in Asian Indians has been inadequately studied. We investigated the association of polymorphisms C161T and Pro12Ala of *peroxisome proliferator-activated receptor gamma* (*PPARγ*) with clinical and biochemical parameters in Asian Indians with NAFLD.
Methods: In this case–control study, 162 NAFLD cases and 173 controls were recruited. Abdominal ultrasound, clinical and biochemical profiles, fasting insulin levels and value of homeostasis model assessment of insulin resistance were determined. Polymerase chain reaction–restriction fragment length polymorphisms of two polymorphisms were performed. The association of these polymorphisms with clinical and biochemical parameters was analysed.
Results: Higher frequency of Ala and T alleles of *PPARγ* was obtained in cases. Ala/Ala genotype of *PPARγ* (Pro12Ala) was associated with significantly higher serum triglycerides (TG), alkaline phosphatase (ALK) and waist–hip ratio in cases as compared to controls. In C161T polymorphism, TT genotype was significantly increased TG ($p=0.04$), total cholesterol ($p=0.01$), ALK ($p=0.04$) and gamma-glutamyl transpeptidase ($p=0.007$) in cases. The linkage disequilibrium for these two single-nucleotide polymorphisms of *PPARγ* was differed in cases (D1 = 0.1; $p=0.006$) and controls (D1 = 0.07; $p=0.1$). Using a multivariate analysis after adjusting for age, sex and body mass index, the presence of NAFLD was linked to these two polymorphisms (odds ratio 1.64 (95% CI: 1.09–2.45, $p=0.05$)].
Conclusion: Asian Indians in north India carrying the alleles Ala and T of *PPARγ* (Pro12Ala and C161T) polymorphisms are predisposed to develop NAFLD.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is usually in the form of steatosis but may progress to non-alcoholic steatohepatitis (NASH), fibrosis and cirrhosis, and occasionally to hepatocellular carcinoma. NAFLD is now considered to be hepatic manifestation of the metabolic syndrome and is closely associated with abdominal obesity and insulin resistance (Tsochatzis et al., 2009). It is the most common cause of chronic liver disease in the United States and other developed countries (Mohanty et al., 2009; Shneider et al., 2006). According to limited data available in Asian Indians living in India, the prevalence of NAFLD ranges from 15 to 32% (Bajaj et al., 2009).

Peroxisome proliferator-activated receptor-γ (PPAR-γ) is a member of the nuclear hormone receptor superfamily that is expressed at high levels in adipose tissues, monocytes/macrophages, and colon and at lower levels in other tissues (Desvergne and Wahli, 1999). Two common polymorphisms, a Pro12Ala substitution located at codon 12 (Deeb et al., 1998) and a synonymous C161T substitution in exon 6 (Meirhaeghe et al., 1998), have been detected in the *PPARγ*.

PPARγ increases expression of lipoprotein lipase, an enzyme that serves to partition fat to adipocytes, limiting fatty acid flux to the liver. Hui et al. (2008) showed that CT/TT genotypes of *PPARγ*, serum triglycerides (TG), waist-to-hip ratio (WHR) and homeostasis

Abbreviations: BMI, body mass index; NAFLD, nonalcoholic fatty liver disease; PPARγ, peroxisome proliferator-activated receptor gamma; NASH, non-alcoholic steatohepatitis; AFLD, alcoholic fatty liver disease; CVD, cardiovascular disease; T2DM, type 2 diabetes mellitus; RIA, radioimmunoassay.

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model assessment of insulin resistance (HOMA-IR) are risk factors for NAFLD. The linkage of the Ala allele of *PPARγ* to develop severe steatohepatitis and fibrosis in patients with alcoholic fatty liver disease (AFLD) indicates a more prominent anti-inflammatory impact of *PPARγ* in progression of AFLD than of NAFLD (Rey et al., 2010).

We hypothesized that the Pro12Ala and C161T of *PPARγ* gene may have an association with NAFLD in non-diabetic Asian Indians. In this study, we assessed the association of polymorphisms Pro12Ala and C161T of *PPARγ* with the NAFLD in the Asian Indians in north India.

2. Methods

2.1. Study subjects

This study was conducted in the Departments of Medicine and Biochemistry, All India Institute of Medical Sciences, and Fortis Hospital, New Delhi between May 2006 and January 2012. The study was approved by the institutional ethics committee and informed consent was obtained from each subject. In a sex- and age-matched case–control study, a total of 335 overweight/obese subjects [body mass index (BMI > 23 kg/m^2)], 162 with NAFLD (cases) and 173 without NAFLD (controls), were enrolled in this study. Recruitment of obese subjects with NAFLD was based on liver ultrasonography (see below). Subjects with known type 2 diabetes mellitus (T2DM), cardiovascular disease (CVD), presence of other liver diseases, severe end organ damage, human immunodeficiency virus (HIV) infection, pregnancy and lactation, and drug induced liver damage were excluded from the study. Weight, height, waist circumference (WC), hip circumference (HC) and blood pressure were evaluated as described previously (Misra et al., 2009).

2.2. Biochemical analysis

Fasting blood glucose (FBG), total cholesterol (TC), TG, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein (VLDL), alkaline phosphatase (ALK), aspartate transaminase (AST), Alanine aminotransferase (ALT) and gamma-glutamyl transpeptidase (GGT) were analyzed as previously described (Bajaj et al., 2009). Fasting insulin levels were measured using commercially available radioimmunoassay (RIA) kits (Immunotech, France) (Vikram et al., 2006). The intra- and interassay percentage coefficient variables were 2.3% and 1.9% for insulin.

2.3. Ultrasound imaging

Liver ultrasound was carried out using 3.5 MHz curvilinear probe (Siemens-G 60S 2004, Germany) by a trained operator who was blinded to all clinical and laboratory data. A complete examination requires both sub-costal and inter-costal scanning. The definition of fatty liver was based on a comparative assessment of image brightness relative to the kidneys, in line with previously reported diagnostic criteria (Brunt et al., 1999).

2.4. Genetic analyses

Genomic DNA was isolated from peripheral blood leukocytes by rapid non-enzymatic method (Lahiri and Nurmberger, 1991). DNA amplification and restriction fragment length polymorphism of the *PPARγ* Pro12Ala and C161T polymorphisms were performed by standard protocols (Bhatt et al., 2012; Hui et al., 2008b).

2.5. Definitions

Overweight was defined as BMI ≥23 kg/m^2 (Misra et al., 2009). WC cut-offs of ≥90 cm for males and ≥80 cm for females were considered an indicator of abdominal obesity (Misra et al., 2009). Other cutoffs were as follows: FBG ≥100 mg/dl, serum TG ≥150 mg/dl, blood pressure ≥130/85 mmHg and HDL-C; males <40 mg/dl, and females <50 mg/dl (Misra et al., 2009). IR was measured by two surrogate measures: fasting hyperinsulinemia and HOMA-IR. Hyperinsulinemia was defined as values in the highest quartile as described previously (Lambert et al., 2004). The value of HOMA denoting insulin resistance was termed as HOMA-IR and was calculated as = {fasting insulin (μU/ml) × fasting glucose (mmol/l)/22.5 (Matthews et al., 1985)}. AST value of up to 50 IU, ALT up to 50 IU, and ALK >80 IU and <240 IU were defined as normal.

2.6. Statistical analysis

Data were recorded on a pre-designed excel sheet (Microsoft Corp., USA). The allelic and genotypic frequencies were determined by manual counting. Statistical analysis was performed using STATA Version 9 (Stata Corp, Texas, USA). After confirming the normality aspect of quantitative variables, descriptive statistics were computed using mean ± SD and student *t* test. Difference between proportions was tested using chi-square test. The allelic and genotypic frequencies were determined by manual counting. In order to determine if observed allele frequency was in conformity with the expected frequency (Hardy–Weinberg equilibrium), chi-square analysis was done. Between-group differences in proportions of alleles or genotypes were compared using chi-square test and a two-tailed Fisher's exact test. The influence of the genotype on the clinical parameters was estimated by ANOVA. Haplotype frequencies and linkage disequilibrium between the two polymorphisms (Pro12Ala and C161T) were estimated using SNP Alyze 7.0 (Dynacom, Kanagawa, Japan). Multivariate analysis was carried out to identify the independent predictors of NAFLD considering age, sex and BMI. The odds ratio (OR) and 95% confidence interval were used as a measure of strength for the association between different Pro12Ala and C161T genotypic combinations with the disease. A p value <0.05 was considered as significant.

3. Results

3.1. Clinical and biochemical profiles

The clinical and biochemical profiles are presented in Table 1. Significantly higher systolic blood pressure ($p = 0.001$), diastolic blood pressure ($p = 0.003$), weight ($p = 0.02$), BMI ($p = 0.006$), WC ($p = 0.001$) and HC ($p = 0.005$) were observed in cases than in controls. Further, significantly higher values of FBG ($p = 0.04$), TG ($p = 0.002$), TC ($p = 0.002$), LDL-C ($p = 0.03$), VLDL ($p = 0.01$), ALT ($p = 0.05$), GGT ($p = 0.0001$), fasting insulin ($p = 0.0008$), and HOMA ($p = 0.009$) were recorded in cases as compared to controls (Table 1).

3.2. Genotype distribution

The genotype and allele frequency distribution of C161T and Pro12Ala of *PPARγ* in cases and controls is shown in Table 2. Significantly higher frequency of Ala ($p = 0.02$) and T ($p = 0.008$) alleles of Pro12Ala and C161T polymorphisms respectively, of *PPARγ* was seen in cases than controls.

3.3. Haplotype distribution

Haplotype frequencies for the Pro12Ala and C161T polymorphisms were estimated via an expectation maximization algorithm (Table 3). The Ala/C ($p = 0.04$ and Pro/T ($p = 0.03$ haplotype were prevalent in cases as compared to controls, whereas the Pro/C haplotype was more prevalent in controls ($p = 0.004$). The LD for these two SNPs (Pro12Ala and C161T) differed in cases (D1 = 0.1; $p = 0.006$) and controls (D1 = 0.07; $p = 0.1$).