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Thr54 allele of fatty-acid binding protein 2 gene is associated with obesity but not type 2 diabetes mellitus in a Caucasian population

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ABSTRACT

Aim: The fatty acid-binding protein 2 (*FABP2*) *A54T* polymorphism has been associated with type 2 diabetes mellitus (T2DM) and obesity in many but not all studies. Our aim was to investigate possible associations of *FABP2 A54T* polymorphism with T2DM and/or obesity in a Greek Caucasian population.

Methods: 242 subjects with T2DM and 188 control subjects were genotyped for the *FABP2 A54T* polymorphism using PCR-RFLP method. Of the total subjects included in both groups, 172 were classified as obese (BMI $\geq$30 kg/m^2) and 258 were classified as nonobese (BMI $<$30 kg/m^2).

Results: In the whole population, 218 subjects (50.7%) were genotyped as AA, 175 subjects (40.7%) as AT, and 37 subjects (8.6%) as TT for the *FABP2 A54T* polymorphism. According to the dominant model, the frequency of AA genotype was significantly lower in obese than in nonobese subjects (43.0% vs 55.8%, $p = 0.009$). No significant difference was observed in genotypes between diabetic and nondiabetic subjects. According to the additive model, the presence of TT genotype was significantly associated with obesity after adjusting for age, sex, and the presence of T2DM (OR 2.32, $p = 0.028$).

Conclusion: *FABP2 A54T* polymorphism may help identify Caucasian subjects at risk for obesity.

1. Introduction

Obesity and type 2 diabetes mellitus (T2DM) are spreading rapidly worldwide affecting life quality and expectancy. The close relationship between obesity and diabetes led to the term ''diabesity'' in the 1970s [1]. Epidemiological studies show a wide variety in the prevalence of both diseases in different populations, ages, and genders [2]. The fact that these diseases are multifactorial can explain the complexity of understanding their genetic and environmental traits, common or not [3].

The *FABP2* (fatty acid binding protein 2) gene is located in the 4q28–4q31 chromosomal region, consists of approximately 3.4 kbs [4] and codes for the intestinal fatty acid binding protein (I-FABP). *FABP2* is a member of the FABPs superfamily that produces intracellular proteins which bind hydrophobic ligands reversibly [5,6]. I-FABP acts in enterocytes and

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participates in the uptake, transportation and metabolism of long chain fatty acids and their acyl-CoA esters [4]. I-FABP has a high affinity for both saturated and unsaturated long chain fatty acids [7] and is involved in the synthesis of triglyceride-rich lipoproteins. There is evidence that the physiologic role of I-FABP may be to inhibit the transfer of dietary fatty acids to the circulation. A high level of I-FABP expressed in a differentiated enterocyte model inhibited fatty acid incorporation [8]. Moreover, experiments with I-FABP null mice support the idea that I-FABP is not essential for dietary fat absorption but may function as a lipid-sensor for energy homeostasis that affects body weight gain in a gender-specific fashion [9].

Many polymorphisms of *FABP2* have already come to light [10]. The most extensively studied polymorphism is the *Ala* to *Thr* substitution at codon 54 (*A54T*) that results from a G to A nucleotide substitution and affects primary protein structure [11]. Hitherto, evidence suggests that metabolic interactions that involve lipids and carbohydrates are affected by the *FABP2 A54T* polymorphism, leading to changes in essential metabolites that are associated with obesity and/or T2DM [11]. Due to the excessive absorption of fatty acids attributed to the *T54* variant, skeletal muscles preferentially use fatty acids for energy rather than glucose, resulting in increased glucose levels. It has been shown that the *FABP2 A54T* polymorphism is associated with insulin resistance and decreased lipid oxidation in Pima Indians, a population with a high prevalence of obesity and T2DM [12]. However, results are contradictory since in some studies *FABP2 A54T* polymorphism has been associated with insulin resistance and T2DM while in others not (for review see [11]). Moreover, previous studies found contradictory associations between *FABP2* genotypes and obesity in different ethnic groups [13–17]. The aim of this study was to investigate possible associations of *FABP2 A54T* polymorphism with T2DM and/or obesity in a Greek Caucasian population.

2. Subjects and methods

2.1. Subjects

A total of 430 unrelated subjects (241 males and 189 females) were genotyped. All subjects were Caucasians of Greek ethnic origin. The diabetic study population consisted of 242 subjects diagnosed as having T2DM (119 males and 123 females). The control (nondiabetic) study population consisted of 188 subjects (122 males and 66 females). The inclusion of subjects having T2DM was based on the criteria recommended by WHO for T2DM (http://www.aafp.org/afp/981015ap/mayfield.html). According to these criteria, diagnosis of T2DM was based on two measurements of fasting plasma glucose levels of 126 mg/dl (7.0 mmol/l) or higher or two casual glucose readings of 200 mg/dl (11.1 mmol/l) or higher. All diabetic patients were regular patients at the hospital diabetes clinic and received antidiabetic medication. Subjects included in the control group had been at the hospital for reasons unrelated to T2DM and had received a thorough medical examination, including specific evidence (medical and drug history taken by a specialist clinician, fasting plasma glucose measured at least twice) to exclude T2DM.

Of the total subjects included in both groups, 172 (80 males and 89 females) were classified as obese (BMI $\geq$30 kg/m^2) and 258 (157 males and 94 females) were classified as nonobese (BMI $<$30 kg/m^2). Other variables measured were blood glucose levels, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, systolic and diastolic blood pressure, urea, and creatinine. All subjects participated after being informed about the study by their attending clinician and giving written consent. The protocol of the study was approved by the Scientific Council and the Ethics Committee of the Academic General Hospital of Alexandroupolis (Greece) and was conducted according to the Declaration of Helsinki.

2.2. Genotyping of FABP2 polymorphism

Genomic DNA was extracted from peripheral whole blood with Puregene DNA Purification System (Gentra, Minnesota, MI, USA). PCR was used to amplify the sequence of interest as described previously with some modifications [18]. The following pair of primers were used for amplification: 5′-CTACCGAGTTTTCTTCCCACC-3′ (forward) and 5′-AATTAAAC-CATCCAATGAAATAGAGC-3′ (reverse). The reaction mix was composed by: 5 μl 10× buffer, 2 μl $MgCl_2$ 1 mM, 0.4 μl dNTPs 25 mM, 0.5 μl forward primer 144 pmol/μl, 0.6 μl reverse primer 116.5 pmol/μl, 0.5 μl Taq polymerase (5U-HyTest), 3 μl DNA (final volume: 50 μl). PCR started with initial denaturation at 94 °C/5 min and followed by 35 cycles under the following conditions: denaturation at 94 °C/1 min, annealing at 62 °C/54 s and extension at 72 °C/45 s. The final extension was carried out at 72 °C/10 min. All PCR amplifications were carried in the PCR-engine apparatus PTC-200 of MJ Research (Watertown, MA, USA). 10 μl of PCR product (376 bps) was digested in a 20 μl reaction volume with 5 U of HhaI restriction enzyme (Takara Bio Inc.) at 37 °C/2 h. The digested products were resolved on 2%, w/v, agarose gel electrophoresis, visualized by staining with ethidium bromide and identified with a 50 bps molecular weight ladder (Invitrogen). HhaI digested the wild-type allele (intact restriction site), which yielded two products of 207 bps and 169 bps, while the Thr substitution lacked the HhaI restriction site.

2.3. Statistical analysis

Quantitative data are reported as mean ± SD (standard deviation). Comparisons for continuous variables between two or more groups were performed with independent *t*-test or one-way ANOVA respectively. Categorical data were compared by chi-square test. Allele frequencies were estimated by the gene counting method. Genotypes were assessed according to dominant, recessive, and an additive genetic model. Each genetic model comprised two groups: the combined group of variant homozygotes and heterozygotes vs wild-type homozygotes for the dominant model; variant homozygotes vs the combined group of wild-type homozygotes and heterozygotes for the recessive model; and variant homozygotes vs wild-type homozygotes for the additive model. Multiple logistic regression analysis with a stepwise forward selection procedure was performed with obesity or