Contents lists available at ScienceDirect

Diabetes Research and Clinical Practice

journal homepage: www.elsevier.com/locate/diabres

Polymorphisms in fatty acid binding protein 5 show association with type 2 diabetes☆

Liming Bu, Lorena M. Salto, Kevin J. De Leon, Marino De Leon *

Center for Health Disparities and Molecular Medicine and Department of Basic Sciences, Loma Linda University, School of Medicine, Loma Linda, CA 92350, United States

ARTICLE INFO

Article history:
Received 16 September 2010
Received in revised form
29 December 2010
Accepted 10 January 2011
Published on line 1 February 2011

Keywords:
Fatty acid binding protein (FABP)
Single nucleotide polymorphism (SNP)
Type 2 diabetes mellitus (T2DM)

ABSTRACT

Genes for the fatty acid binding proteins (FABP) family encode small 14–15 kDa cytosolic proteins and can be regulated during type 2 diabetes mellitus (T2DM) and obesity. This study compared association of single nucleotide polymorphisms (SNPs) in FABP1-5 with T2DM in different ethnic groups. Associations with T2DM of SNPs in these proteins were assessed in African American (AA), non-Hispanic White (NHW), and Hispanic American (HA) individuals. A total of 650 DNA samples were genotyped; control samples were obtained from Coriell's North American Human Variation Panel Repository (NAHVP) of apparently healthy individuals and T2DM cases were taken from the American Diabetes Association GENNID Study. The rs454550 SNP of FABP5 showed a significant association with T2DM in NHW (OR: 9.03, 95% CI: 1.13–71.73, $p = 0.014$). Our analysis also identified a new FABP5 SNP (nSNP) that showed a significant association with T2DM in NHW (OR: 0.44, 95% CI: 0.19–0.99, $p = 0.045$) and AA (OR: 0.17, 95% CI: 0.03–0.80, $p = 0.016$). The Ala54Thr FABP2 polymorphism was significantly associated with T2DM in HA individuals only (OR: 1.85, 95% CI: 1.05–3.27, $p = 0.032$). All other FABP SNPs did not show association with T2DM. These findings suggest a potential distinct role(s) of SNPs in FABP5 and FABP2 genes in T2DM in different populations.

1. Introduction

Type 2 diabetes mellitus (T2DM) is a complex, multi-factorial metabolic disorder that exhibits a wide range of disparities among different ethnic groups [1–4]. Genetic linkage analyses and T2DM association studies have shown a dynamic interplay between genetic and environmental components [5]. In the United States alone, about 11% of the population 20 years of age and older has T2DM and 40% of these cases may be undiagnosed [1,4]. Hispanic American (HA) and African American (AA) individuals exhibit twice the incidence of T2DM when compared to non-Hispanic Whites (NHW). Due to the strong genetic component of T2DM, there is a need to identify promising target genes to develop adequate therapies and preventive educational interventions.

The fatty acid binding protein 5 (FABP5), also known as E-FABP, DA11, mal1 and PAFABP, belongs to the family of fatty acid-binding proteins (FABPs), a group of small intracellular 14–15 kDa cytoplasmic proteins that bind and transport long-chain free fatty acids. FABP5 is expressed in different tissues and organs including the skin, liver and the nervous system [6]. The unique properties of FABPs as free fatty acids carriers in the cytoplasm have been the focus of intense studies because fatty acids can affect glucose metabolism by altering insulin receptor sensitivity [10]. For instance, high levels of free fatty acids may result in insulin resistance and in glucose intake capacity reduction [7]; members of the FABP family can

☆ This work was supported by NIH grants 5P20MD001632-05 and 2R25GM060507-09.

* *Corresponding author at*: Center for Health Disparities and Molecular Medicine, Loma Linda University, School of Medicine, 142 Mortensen Hall, 11085 Campus Street, Loma Linda, CA 92350, United States. Tel.: +1 909 558 7174; fax: +1 909 558 0196.
E-mail addresses: bliming@llu.edu (L. Bu), lsalto@llu.edu (L.M. Salto), kdeleon@llu.edu (K.J. De Leon), madeleon@llu.edu (M. De Leon).
0168-8227/$ – see front matter
doi:10.1016/j.diabres.2011.01.005

be involved in regulating this process in cells. FABP5/E-FABP/mal1 may play a role in T2DM and obesity by protecting myocytes from the cellular toxicity of cholesterol, free fatty acids and lipid oxidants [8,9]. Studies using the mal1/FABP5−/− mice have shown that these animals exhibit an increased systemic insulin sensitivity [10]. This and other findings reported independently by several groups, prompted studies to determine if single nucleotide polymorphisms (SNPs) in members of FABP family of proteins are important regulators of diseases such as T2DM and obesity. Genetic association studies analyzing FABP1, FABP2, FABP3 and FABP4 genes have identified SNPs associated with an increased risk of developing T2DM and/or obesity in several populations [11–15]. However, while FABP5 expression has been increasingly associated with T2DM and obesity, the literature does not report studies examining significant polymorphism associations.

The initial objective of this study was to identify and characterize SNPs in the FABP5 gene and to determine if significant associations with T2DM exist. To have a more comprehensive view of our findings, SNPs reported for other FABPs were also genotyped and used for comparison. The potential polymorphisms were characterized using established DNA collections from different ethnic groups and validated using more than one method of analysis. Our results support several key findings: (1) two SNPs in the FABP5 gene show significant associations with T2DM. The FABP5 rs454550 SNP association with T2DM was significant in NHW, but not in the HA and AA cohorts. A new SNP, nSNP, was identified in the regulatory region of the FABP5 gene. The results show an association between the minor allele of the nSNP and absence of T2DM in AA and NHW but not in HA. (2) The Ala54Thr SNP in the FABP2 gene is associated with T2DM in HA but not in AA or NHW.

2. Materials and methods

The study analyzed a total of 650 DNA samples obtained from the Coriell Institute for Medical Research Repository, Camden, NJ, USA. Three hundred thirty (330) DNA samples, 110 for each ethnic group respectively, were obtained from the North American Human Variation Panel (NAHVP) collection of apparently healthy individuals as the control group. DNA samples from participants of the Genetics of Non-Insulin Dependent Diabetes Mellitus (GENNID) study repository [16] were selected at random from non-related individuals of AA ($n = 100$), NHW ($n = 100$) and HA ($n = 120$) ethnicities for the type 2 diabetes mellitus cases. The Coriell Institute for Medical Research Repository obtains DNA samples from clinicians and researchers who use a standardized National Institute of General Medical Sciences informed consent for all donors/subjects. The DNA samples are anonymized and all donors have provided informed consent for the use of their DNA in research. The GENNID study collected informed consents for all sib-pairs (index cases) and family members enrolled in the study once the complete family pedigree was admitted into the study [17]. According to the GENNID study, the criteria used to define type 2 diabetes mellitus cases is based on the criteria set forth by the National Diabetes Data Group at the collection of the samples. At this time, type 2 diabetes was defined as having a fasting plasma glucose concentration of 140 mg/dl or greater on more than one occasion. Index cases could also be included in the study if they had a plasma glucose concentration of 200 mg/dl or greater, in the 2-h sample and in at least one other sample during an oral glucose tolerance test. The criterion for defining type 2 diabetes mellitus using fasting plasma glucose concentration has changed. The National Diabetes Data Group now defines type 2 diabetes as having a fasting plasma glucose concentration of 126 mg/dl or greater on more than one occasion. The cut point for the oral glucose tolerance test concentration has remained the same. All of the cases defined as having type 2 diabetes mellitus in the original study would currently qualify as type 2 diabetes mellitus cases. According to the Coriell Institute, the DNA control samples used for the study are classified as ''apparently healthy'' by the investigators who submit the samples to the repository. Along with each sample collected, a submission form is used to request available clinical, molecular, cytogenetic and biochemical data (http://ccr.coriell.org/Sections/Support/NIGMS). These samples were first used to characterize seven putative SNPs in the FABP5 gene reported in the NCBI and SNPper (http://www.ncbi.nlm.nih.gov/SNP and http://snpper.chip.org) databases: rs454550 (C/G), rs391842 (A/G), rs162115 (C/T), rs408070 (A/G), rs11542504 (C/T), rs3180883 (C/T), and rs10098288 (G/T). Furthermore, genotyping and sequencing resulted in the identification of a new SNP in FABP5 (nSNP) that was also used to perform T2DM association analyses. SNPs in FABP1, FABP2, FABP3 and FABP4 genes were selected from four published papers: rs52241883 in FABP1 [18]; Al54Thr in FABP2 [19]; rs2271072 and rs2279885 in FABP3 [20]; and SNP −87T > C in FABP4 [21]. Procedures used included PCR combined with confronting two-pair primers (PCR-CTPP) [22] and TaqMan Allelic Discrimination Assay (Applied Biosystems Inc., Foster City, CA, USA). The SDS 2.3 Allelic Discrimination software was employed for genotyping characterization. We did a final confirmation using direct DNA sequencing of the SNP region of interest in FABP5. Each SNP was confirmed with at least two of these methods getting the same results. For the PCR-CTPP approach, two overlapping PCR products were amplified covering the selected SNP region. The primers used for the rs454550 SNP were: 5′-GGCAGGGACTTGAAATGAAA-3′ and 5′-GGAAGTGATTGATCGGCTGT-3′; 5′-GAAACCACACTTCCCGAGAC-3′ and 5′-GGGACCTAGGGACAGACGAC-3′. The primers used for the nSNP were: 5′-TGG GAG ATA GCA AAC CAA CC-3′ and 5′-TGC CTG TTG GAA GTC AGA TG-3′; 5′-CTA TAA AGT GCC CAG GAA GCA CA-3′ and 5′-TGA GTA GCT ACT AAG TGC TAG AC-3′. Power analysis for the study was calculated using the CaTS power calculator [23]. The linkage disequilibrium (LD) value (D') was calculated using Haploview [24,25]. Statistical analyses were performed using PASW Statistics v 18.0 (IBM: SPSS Inc.). Distributions of genotypes, allele frequencies and their association with T2DM were evaluated by Pearson's chi-square and by Fisher's exact test. The ethnic groups were separated for the analysis. The minor allele genotypes were collapsed to compute odds ratios (ORs) and 95% confidence intervals (95% CIs). Due to poor age-matching between the cases and the controls, an odds ratio was computed, age-crude, for the full sample and for an ethnic-specific age-range