Meta Gene 12 (2017) 109–112

Contents lists available at ScienceDirect

Meta Gene

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

Association of *ACACB* gene polymorphism (rs2268388, G>A) with type 2 diabetes and end stage renal disease in Pakistani Punjabi population

Maryam Zain [a,b,c,d,*], Fazli Rabbi Awan [a,c], Syeda Sadia Najam [a], Mehboob Islam [a], Abdul Rehman Khan [a], Ahmed Bilal [e], Naima Bellili [b], Michel Marre [b], Ronan Roussel [b], Frederic Fumeron [b]

[a] Diabetes and Cardio-Metabolic Disorders Laboratory, Health Biotechnology Division, National Institute for Biotechnology and Genetic Engineering, Jhang Road, P.O. Box: 577, Faisalabad, Pakistan
[b] INSERM, UMR_S 1138, Centre de Recherche des Cordeliers, F-75006, Paris, France
[c] Pakistan Institute of Engineering and Applied Sciences (PIEAS), Islamabad, Pakistan
[d] Department of Biochemistry and Biotechnology, The Women University, Multan, Pakistan
[e] Allied Hospital/Punjab Medical college (PMC), Faisalabad, Pakistan

ARTICLE INFO

Article history:
Received 24 November 2016
Revised 26 January 2017
Accepted 15 February 2017
Available online 16 February 2017

Keywords:
Acetyl-CoA Carboxylase beta (ACACB)
End Stage Renal Disease (ESRD)
Genotyping
Pakistani diabetics
rs2268388
Single Nucleotide Polymorphism (SNP)
Type 2 Diabetes Mellitus (T2DM)

ABSTRACT

The Acetyl-CoA Carboxylase Beta (ACACB) is a regulator of fatty acid metabolism. A SNP (rs2268388, G>A) in this gene has been recently associated with diabetes, diabetic nephropathy, obesity as well as metabolic syndrome in some populations. Therefore, the aim of present study was to test this SNP for its association with diabetes and end stage renal disease (ESRD) in Pakistani Punjabi patients. For this case-control study, a total of 206 subjects comprising of four groups (controls, type 2 diabetics, type 2 diabetics with ESRD, and non-diabetics with ESRD) were genotyped for rs2268388 (G>A) using real time PCR based TaqMan Assay. Using statistical analysis, it was found to be associated with type 2 diabetes as OR (95% CI): 2.54 (1.18–5.46), $p = 0.01$. The BMI of the persons having AA genotype was found to be raised ($p = 0.009$) as compared to persons having GA and AA genotypes. We found that this SNP rs2268388 (G>A) is significantly associated with type 2 diabetes and obesity in Pakistani Punjabis, which may have implications for the management of diabetes in the target population.

1. Introduction

Diabetes mellitus is a common metabolic disease across the world which results in high blood glucose levels due to defects in insulin secretion, action or both (ADA, 2008). In Pakistan, the prevalence of diabetes along with impaired glucose tolerance is estimated at 22% (Shera et al., 2007). Important biochemical pathways of central metabolism involving carbohydrates, lipids and protein metabolism; whence dysregulated could lead to the development of diabetes and its associated complications (Khan and Awan, 2012; Zain and Awan, 2014). The problems in fatty acid metabolism can lead to insulin resistance which is a precursor for type 2 diabetes. Hence, impaired fatty acid metabolism owing to a functional abnormality in one of its regulator, the Acetyl-CoA Carboxylase Beta (ACACB) (Abu-Elheiga et al., 2003) is investigated in this study.

The ACACB enzyme catalyzes the carboxylation of acetyl-CoA to malonyl-CoA. Malonyl CoA is the major precursor in fatty acid synthesis. However, when in excess, it controls the fatty acid oxidation by inhibiting Carnitine Palmitoyl Transferase I enzyme which is involved in this metabolic process (Munday and Hemingway, 1999). Mice which lack *acacb* gene have a normal life span due to their higher rate of fatty acid oxidation, which results in lower amounts of fat, as ACACB is involved in the accumulation of fat (Abu-Elheiga et al., 2005; Fullerton et al., 2013; Shah et al., 2013). Of various factors which predominantly contribute to the development of diabetes and kidney diseases, the insulin resistance, and accumulation of free fatty acids are very important. The enzyme Acetyl-CoA carboxylase beta, is involved in the fatty acid metabolism is believed to play a key role in the insulin resistance (Assimacopoulos-Jeannet et al., 1997). ACACB enzyme is involved in the conversion of the acetyl to malonyl CoA. Malonyl CoA is the major precursor for the fatty acids synthesis and storage and it catalysis the first step in the synthesis of fatty acids. ACACB is thus said to be the regulatory enzyme which is involved in the regulation of fatty acids production by having a control on the regulation of short and long chain fatty acids and its storage and deposition (Munday and Hemingway, 1999). This enzyme is also involved in the oxidation of fatty acid and in the insulin sensitivity and its production. This fact is also supported by some experimental evidence of the gene knockout studies on mice which showed that the mice lacking *acacb* gene had a higher rate of fatty acids oxidation and fatty acid storage or deposition was reduced and their fat storage capacity is 50% less as compared to normal mice (Abu-Elheiga et al., 2001; Abu-Elheiga et al., 2003). ACACB might affect insulin sensitivity via alteration of fatty acid metabolism.

* Corresponding author at: Department of Microbiology and Molecular Genetics, The Women University, Multan, Pakistan.
E-mail address: maryam.9002@wum.edu.pk (M. Zain).

http://dx.doi.org/10.1016/j.mgene.2017.02.003
2214-5400/

As some experimental evidences showed that *ACACB* gene was expressed in human kidneys thus contributing some important metabolic functions which are implicated in the development of diabetic nephropathy (Maeda et al., 2010; An et al., 2015; Wei et al., 2015). The ACACB is therefore a good candidate for susceptibility to type 2 diabetes and kidney complications and one of its SNP (rs2268388, G/A) has already been explored for susceptibility in few populations. In Japanese and Spanish cohorts, the A allele frequency was higher in proteinuric, diabetic and obese subjects (Maeda et al., 2010; Riancho et al., 2011). Similar results were reported in European and Americans subjects for its association with type 2 diabetes and proteinuria (Tang et al., 2010). *ACACB* gene polymorphism results in obesity, increased BMI (Body Mass Index), lipotoxicity as well as the increased C– Reactive protein levels in several populations (Kotani et al., 2012).

Several meta-analysis and other studies involving the exome sequencing and expression study showed its involvement in hyperinsulinemia, accumulation of fats, increased incidence of BMI, diabetes and nephropathy complications (Ma et al., 2013; Proverbio et al., 2013; An et al., 2015; Li et al., 2015).

Therefore, considering the importance of *ACACB* gene in diabetes and nephropathy, the present study was focused to analyze *ACACB* gene SNP (rs2268388, G/A) and its association with DN as well as the other clinical outcomes of diabetes and kidney complications in Pakistani Punjabi population.

2. Material and methods

2.1. Study subjects, collection of samples and biochemical analysis

A total of 206 human subjects aged ≥35 years (males and females) were included in this study. After explaining the nature of study, a written informed consent was obtained from each subject, and the study was approved by the institutional ethics review committee. Various demographic, anthropometric measurements were taken along with blood samples for DNA and biochemical analysis. The genomic DNA was extracted using phenol chloroform method as described earlier (Islam et al., 2014). Biochemical analytes were measured on semi-automated clinical chemistry analyzer (Microlab 300, Merck). The subject groups included in this study were:**G1-C** (*Control subjects with no diabetes and no nephropathy*, $n = 52$),**G2-D** (*Type 2 diabetes patients but without nephropathy*, $n = 51$),**G3-DN** (*Type 2 diabetes patients with nephropathy/ESRD*: *End Stage Renal Disease*, $n = 60$),**G4-CN** (*Non- diabetes patients with nephropathy/ESRD as control nephropathy*, $n = 43$).

The diagnosis of diabetes mellitus was made according to the American Diabetes Association criteria (ADA, 2008). Samples from the **G1-C** and **G2-D** were collected from Faisalabad, Pakistan by arranging diabetes camps or through personal contacts. Samples from **G3-DN** and **G4-CN** were collected from the Dialysis Units of Allied Hospital and District Headquarters Hospital, Faisalabad, Pakistan.

2.2. Genotyping

Genotyping was performed by Real Time Polymerase Chain Reaction (RT-PCR) based TaqMan allelic discrimination assay on ABI PRISM 7000 sequence detector (Applied Biosystems, USA). For a subset of 100 randomly selected subjects, genotyping was repeated with 100% concordance for this SNP.

2.3. PCR setup and primer design

2.3.1. Probe sequence and PCR conditions for ACACB (G/A) polymorphism

The PCR conditions for TaqMan assay were 95 °C for 5 min, 60 °C for 1 min, 25 cycles of 92 °C for 15 s and 60 °C for 2 min and by using the absolute blue QPCR mix (Thermoscientific Company).

2.3.1.1. Probe sequence. 5′-GAGGGGTAGAGGGTGGGCAGGAAAC [G/A] GAGTGTTCTCTGCTGGGAGAACAGC-3′

2.4. Statistical analysis

For the comparisons of genotype and allele frequencies as well as phenotypic traits, one way ANOVA, chi-square (χ^2) and logistic regression analysis were performed using SYSTAT (version 11, Systat Software Inc., Chicago, Illinois, USA) and SPSS12 software (version 12, Chicago, Illinois, USA).

3. Results

Baseline characteristics of all four groups are given in Table 1.

The Table 1 indicates the differences in the biochemical aspects (glucose, hemoglobin, urea, creatinine, triglycerides, cholesterol) which were significantly different ($p < 0.05$) in all the four groups. This indicates that the nephropathy and diabetes greatly affect the biochemistry and physiology of diabetic and nephropathy subjects versus control. The glucose ranges are higher in the diabetic subjects (G2-D and G3-DN) as compared to the non- nephropathy and control subjects (G1-C and G4-N). While Hemoglobin (Hb) levels are higher in Control (G1-C) and Diabetic subjects (G2-D). The urea and creatinine levels were higher in diabetic nephropathy and nephropathy patients (G3-DN and G4-N) while the control and diabetic subjects have normal ranges of both the parameters. The lipid profile (cholesterol and triglyceride) as well as systolic and diastolic ranges of the nephropathy groups (G3-DN and G4-N) were higher as compared to the control and diabetic people (G1-C and G2-D).

The genotype and allele frequencies (Table 2) for the *ACACB* gene (rs2268388, G>A) are in accordance with Hardy-Weinberg equilibrium ($\chi^2 = 0.55$, $p = 0.46$). The common genotype for *ACACB* in our study subjects was GG with a prevalence of 157 (76.2%), followed by GA genotype which had a frequency of 47 (22.8%) and AA genotype frequency of 2 (1%). Whereas, the G allele frequency was 0.88 and A allele frequency was 0.12 in participants. The association of ACACB gene polymorphism was also tested with various biochemical parameters. The BMI of the participants group wise was also calculated (Table 1). The **G2-D** group BMI was found to be higher with Mean ± SD of 27 ± 5 as compared to the other three groups (**G1-C**, **G3-DN** and **G4-CN**) groups. Although there are only two diabetic people who have AA genotype but they are obese with BMI range of 45.5 and 31.3 respectively. Thus BMI of the persons having AA (38.4 ± 10.0) ($p = 0.009$) genotype was found to be raised as compared to GA and AA genotypes (28.3 ± 6.1 and 26.4 ± 4.1) respectively. Whereas the overall diabetic group has BMI almost in normal range which is 27 ± 5, although most of the diabetics have BMI range > 25. This result thus needs to be further investigated by specifically genotyping the diabetic and obese subjects having BMI range > 25 in order to reach to a meaningful conclusion.

The genotype distribution for rs2268388 (G>A) polymorphism between patients and controls is shown in Table 2. There was an increase in frequency of A carriers in patients with diabetes (**G2-D group** + **G3-DN group**) when compared to subjects without diabetes (**G1-C** + **G4-CN** groups) (Table 2). The association persists after further adjustment for nephropathy status (OR of 2.54, 95% CI: 1.18–5.46, $p = 0.01$). No such association was found conferring risk of this SNP for nephropathy status (Table 2).

4. Discussion

The prevalence of diabetes and its complications is rapidly rising in all populations of the world every year. In developing countries, burden of diabetes is more prominent, as healthcare and economic conditions are very poor to cope with this problem. According to one report for Asian Indians, it was observed that the Indians were more prone to insulin resistance as compared to Caucasians (Jahan et al., 2009). Diabetes