Gene 665 (2018) 133–140

Contents lists available at ScienceDirect

Gene

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

Research paper

CREB1 functional polymorphisms modulating promoter transcriptional activity are associated with type 2 diabetes mellitus risk in Chinese population

Yao Xu[a,*], Ruhui Song[a], Wenlin Long[a], Hui Guo[a], Weilin Shi[a], Shiliang Yuan[b], Geng Xu[c], Tongcun Zhang[a,*]

[a] Institute of Biology and Medicine, College of Life Science and Health, Wuhan University of Science and Technology, Wuhan, Hubei 430081, China
[b] Tianyou Hospital Affiliated to Wuhan University of Science and Technology, Wuhan, Hubei 430064, China
[c] Department of pathology, Xinzhou People's Hospital, Wuhan, Hubei 430400, China

ARTICLE INFO

Keywords:
CREB1 gene
Promoter variations
Association
Type 2 diabetes

ABSTRACT

The cAMP responsive element binding protein 1 (CREB1) is a ubiquitous transcription factor that contributes to the regulation of gluconeogenesis. The mechanisms of the CREB1 function remain largely unknown. In this study, we aimed to explore genetic variations in *CREB1* promoter region and determine whether these loci affect transcriptional activity and risk on type 2 diabetes (T2D). Three polymorphisms were identified and designated as MU_1, MU_2 and MU_3, respectively. Genotypic distribution analysis revealed that MU_1 genotypes presented similar distribution between T2D and healthy controls ($P > 0.05$), while the MU_2 and MU_3 showed significant differences ($P < 0.05$). Haplotypic blocks of the three loci were constructed, and H1-TGA, H2-TTT and H3-ATT had higher frequencies in T2D patients than those in controls. Association studies revealed that the three loci significantly affected plasma glucose, glycated hemoglobin and insulin secretion. Disequilibrium analysis identified that the MU_2 and MU_3 variants were strongly linked in T2D ($r^2 = 0.348$, D' = 1.0). Further analysis indicated that MU_2 (TT vs GG, OR = 2.38, 95%CI = 1.19–4.77, $P = 0.01$) and MU_3 (AA vs TT, OR = 1.16, 95%CI = 1.19–4.77, $P = 0.04$) were significantly associated with T2D in dominant genotypes. Luciferase assay showed that T-A haplotype from the highly linked MU_2 and MU_3 exhibited maximal promoter activity, which was consistent with the correlation results. We concluded that the TT genotype of MU_2 and the AA genotype of MU_3 could be used as molecular markers for evaluating the risk on T2D.

1. Introduction

Type 2 diabetes mellitus (T2D) is a complex metabolic disorder with a remarkably increasing prevalence worldwide. The typical characteristics of T2D are elevated blood glucose and attenuated insulin action, which are attributed to multifactorial effects (Fukushima et al., 2004). According to the investigation of World Health Organization, genetic factors do predispose to the risk of T2D susceptibility (Antosik and Borowiec, 2016). Encouraging results showed that monogenic or polygenic polymorphisms could be molecular markers for evaluating and diagnosing the disease development (Babey et al., 2011). Identification of genetic variations is the most promising T2D research field because elucidation of the causative genotypes (alleles) will highlight their further applications in the therapeutic intervention, treatment and prevention of T2D.

Glycometabolism dysregulation, specifically gluconeogenesis, is a key contributor to fasting hyperglycaemia in T2D patients (Cambuli et al., 2012). Previous study reported that continuous hepatic glucose increase resulting in insulin resistance could be causative pathogenesis of long-standing diabetes (Gastaldelli et al., 2000). Cyclic adenosine monophosphate (cAMP) response element binding protein 1 (CREB1), a member of the cAMP activating transcription factor family, plays a central role in gluconeogenic regulation, lipid metabolism and insulin signaling pathways (Herzig et al., 2001). Cyclic AMP signaling leads to

Abbreviations: CREB1, cAMP responsive element binding protein 1; T2D, type 2 diabetes mellitus; ZDF, Zucker diabetic fatty; STZ, streptozotocin; SNP, single nucleotide polymorphism; GWAS, genome-wide association study; OGTT, oral glucose tolerance test; aCRS-RFLP, amplification created restriction site - restriction fragment length polymorphism; EM, expectation maximization; H, heterozygosity; PIC, polymorphism information content; TC, total cholesterol; TG, triglycerides; LDL, low density lipoprotein; HDL, high density lipoprotein

* Corresponding authors at: No.2 Huangjiahu west Road, Institute of Biology and Medicine, College of Life Science and Health, Wuhan University of Science and Technology, Wuhan, Hubei 430081, China.
E-mail addresses: xuyao0307@wust.edu.cn (Y. Xu), zhangtongcun@wust.edu.cn (T. Zhang).

https://doi.org/10.1016/j.gene.2018.05.002
Received 1 February 2018; Received in revised form 14 April 2018; Accepted 2 May 2018
Available online 03 May 2018
0378-1119/ © 2018 Elsevier B.V. All rights reserved.

CREB1 phosphorylation at Ser133, which binds to the cAMP response element (CRE) to stimulate cellular gene expression (Pesiri et al., 2015). The CREB-dependent gluconeogenic gene program is required for maintenance of glucose homeostasis, while its dysregulation induces morbidity of many metabolic diseases including T2D. Erion et al. reported that *CREB* knock down in liver dramatically reduced fasting plasma glucose, cholesterol and triglyceride concentrations in Zucker diabetic fatty (ZDF) rats, *ob/ob* mice, and streptozotocin (STZ)-treated, high-fat-fed rat model of T2D (Erion et al., 2009). In addition, the decrease of *CREB1* gene expression by epigenetic modification was associated with kidney injury of diabetic mice (Shan et al., 2016). These results suggested that *CREB1* could be a promising causative gene for pathogenesis and development of T2D.

Numerous reports have investigated on the genomic basis of T2D, and showcased the effect of single nucleotide polymorphism (SNP) in functional gene corresponding to risk prediction of diabetic phenotype (Hubacek et al., 2018). As a key regulator of glucose and insulin action, however, to the best of our knowledge, the genetic associations of *CREB1* variants with diabetic risk have not been established in various ethnic groups of T2D. Only several SNPs of *CREB1* gene have been reported in non-diabetic disorders. Pal et al. found SNPs from several exonic regions of *CREB1* gene altering its protein expression, and further influenced the susceptibility to opioid, alcohol and narcotic addictions (Pal et al., 2014). Recently, a genome-wide association study (GWAS) revealed the SNP, rs7591784, near *CREB1* gene was significantly associated with *CREB1* expression as well as opioid response (Nishizawa et al., 2014). Therefore, in this report, we designed a case-control study and screened *CREB1* SNPs to examine the association between selected SNPs and diabetic phenotypes in Chinese population. The integrated heritability identification and clinical data may provide convincing evidence for the extensive application of significant SNP locus.

2. Material and methods

2.1. Ethics statement

This study was approved by the Tianyou Hospital ethical committee of Wuhan University of Science and Technology. Written informed consents were obtained from all patients prior to the study. All experimental protocols were in accordance with the ethical principles for medical research involving human subjects of the Helsinki Declaration (http://www.wma.net/e/policy/b3.htm).

2.2. Study subjects

The T2D subjects were randomly recruited from patients attending the inpatient clinic of the Tianyou affiliated Hospital, Wuhan University of Science and Technology from May 2015 to June 2016. The non-diabetic subjects were collected from an unselected population undergoing routine health checkups at the Hospital of Wuhan University from May 2015 to March 2016. All subjects were recruited on a rigorous set of criteria. For cases clinically diagnosed T2D patients as defined by world health organization (WHO) criteria when the venous plasma glucose concentration was ≥200 mg/dl 2 h after a 75 g oral glucose load, the fasting plasma glucose was ≥126 mg/dl. Patients with other morbidities such as malnutrition, pancreatitis, anemia and malignant cancer were excluded from the study. For healthy controls, the inclusion criteria were as follows: (i) fasting glucose < 126 mg/dl and glycated hemoglobin < 6.0% (ii) no history of diabetes or other autoimmune disease in the degree relatives, (iii) no hypertension. A total of 209 diabetic subjects and 223 healthy controls were enrolled in the present study. All individuals were belonging to Chinese ethnicity. There was no bias in geographic distribution of cases and controls. Whole blood samples of all subjects were collected and genomic DNA was extracted by phenol-chloroform extraction.

2.3. Biological measurements

Clinical characteristics were collected for further association study. Blood samples were drawn in the fasting state, and fasting plasma glucose, HbA_{1c}, total cholesterol, triglycerides, low density lipoprotein cholesterol and high density lipoprotein cholesterol were measured using Hitachi 912 auto-analyzer (Roche, Basel, Switzerland) according to manufacturer's instructions. In addition, the aforementioned T2D patients were adjusted diet for maintaining weight about 3 days before an oral glucose tolerance test (OGTT). For the OGTT, the patients underwent an overnight fast and then ingested 75 g glucose; blood was drawn before ingesting the glucose and at 60, 120, and 180 min, respectively. Plasma glucose concentrations were measured by the glucose oxidase-peroxidase method using commercial kits (Shanghai Biological Products Institution, Shanghai, China). Insulin levels were measured by radioimmunoassay (Linco Research, St Charles, MO, USA).

2.4. Identification and genotyping of the CREB1 SNPs

Based on the sequence information of the *CREB1* gene (accession number: NC_000002.12) retrieved from GenBank, two primer pairs were designed to amplify the *CREB1* promoter region using Primer v5.0 software. A total of 60 DNA samples (30 from T2D cases, 30 from non-diabetic controls) were randomly selected to construct the screening DNA pool. In detail, all selected DNA samples were dissolved to 50 ng/μl, and then each contributed 1 μl to the pool without compromising pipette error. PCR was carried out using the DNA pool as template, and the amplified products were sequencing on both strands using an ABI PRISM BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA) and an ABI 3730XL automated sequencer (Applied Biosystems). All PCR conditions are available upon request.

Combining the sequencing maps and BLASTN assay, genetic variations of the *CREB1* gene were in silico identified in the representative pooling samples (Fig. 1A). In this study, amplification created restriction site - restriction fragment length polymorphism (aCRS-RFLP) was established to genotype MU_1 and MU_2 loci, in which a point mutation (not the SNP locus) was deliberately introduced to create a restriction enzyme recognized site (Fig. 1B). The remodeling primers were shown in Table 1. For MU_3 locus, genotyping assay was carried out by direct-RFLP, because this SNP occurring can alter a natural restriction enzyme recognized site (Fig. 1B). Aliquots of 10 μl PCR products of three loci were digested with 5 U of restriction enzyme *Afa* I, *Hha* I, and *Xsp* I (TaKaRa, Dalian, China) for 2 h at 37 °C, and subsequently the RFLP fragments were separated on 3.0% agarose gels with ethidium bromide staining.

2.5. Haplotype and linkage disequilibrium analysis

Haplotypes of the three SNP loci (MU_1, MU_2 and MU_3) within the *CREB1* gene were constructed by the SHEsis software (http://analysis.bio-x.cn/myAnalysis.php), and haplotype frequency distribution was directly calculated in the T2D and non-diabetic controls. Linkage disequilibrium was analyzed by the expectation maximization (EM) algorithm, as obtained through the Haploview software (Barrett et al., 2005).

2.6. Plasmid construction, transient transfection and luciferase assay

The genomic region harboring two closely linked SNP loci (MU_2 and MU_3) were amplified by PCR from case or control individuals, thus, four pairwise sets were formed due to the different combinations of the genotypes. The amplified fragments were cloned into the luciferase reporter gene vector pGL3-Basic (Promega, Madison, WI) by the *Nhe*I and *Hind*III digestion. All constructed plasmids were sequenced to verify correct insertion sequence and proper orientation. 293T cells

Download English Version:

https://daneshyari.com/en/article/8644918

Download Persian Version:

https://daneshyari.com/article/8644918

Daneshyari.com