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Research paper

Polymorphism −116C/G of the human X box binding protein 1 gene is associated with risk of type 2 diabetes in a Chinese Han population☆

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ABSTRACT

Evidence has been obtained showing that endoplasmic reticulum (ER) stress is closely associated with the development of type 2 diabetes (T2D) and that the human X box binding protein 1 (XBP1) is an important transcription factor involved in the development of ER stress. The study aimed to analyze the potential association between polymorphism −116C/G of *XBP1* and the risk of T2D. The association between *XBP1* polymorphism −116C/G and T2D risk was assessed among 1058 consecutive unrelated subjects, including 523 T2D patients and 535 healthy controls, in a case control study. The −116GG genotype and −116G allele were more frequent in T2D subjects compared with control subjects by statistical analysis, showing that the −116GG homozygote polymorphism of *XBP1* might lead to increased susceptibility to T2D in a Chinese Han population. T2D subjects with the −116GG genotype had higher fasting plasma glucose levels, fasting insulin levels, and HbA1c and worse HOMA-IR than the T2D subjects with −116CG and −116CC genotypes ($P < 0.0001$). The study supports a role for −116C/G polymorphism of the *XBP1* promoter in the pathogenesis of T2D in a Chinese Han population, and more studies are needed to further evaluate our results.

1. Introduction

Recently, evidence has been obtained showing that development of type 2 diabetes (T2D) is importantly associated with endoplasmic reticulum (ER) stress. Studies from human tissues indicated that ER stress occurred in T2D, and proteins related to ER stress such as BiP (Grp78), CHOP and P582PK were prominently elevated in pancreas sections of humans with T2D compared to controls without T2D (Laybutt et al., 2007). Moreover, in addition to the increase in markers related to ER, the ER density volume was significantly increased in β cells of patients with T2D, suggesting dysfunctional morphological changes due to ER stress (Marchetti et al., 2007). Studies of cell models also demonstrated that ER overload caused ER stress and led to cell apoptosis via Chop induction in mouse MIN6 β cells (Oyadomari et al., 2002b). Moreover, ER stress inhibited insulin receptor signaling and insulin action by a JNK-dependent serine phosphorylation of IRS-1 in rat Fao liver cells with tunicamycin (Ozcan et al., 2004). Therefore, it is possible that ER stress leads to β cell dysfunction and IR linked to T2D pathogenesis.

Under stress, human X box binding protein 1 (XBP1), an important transcription factor, has been shown to protect β cells against apoptosis and maintain glucose homeostasis. Marchetti et al. reported that XBP1 was not higher in T2D β cells or isolated islets cultured at low glucose (5.5 mmol/l) than in ND samples, whereas XBP1 was significantly increased in T2D β cells or isolated islets cultured at high glucose (11.1 mmol/l) but not in ND (Marchetti et al., 2007). Lee et al. showed that XBP1 deficiency could compromise the ER stress response in β cells, and the combined effects of XBP1 deficiency on the canonical ER stress response and its negative feedback activation of IRE1α caused β cell dysfunction in XBP1 mutant mice (Lee et al., 2011). Ozcan et al. showed that after induction of ER stress, reduction in the compensatory capacity through down-regulation of XBP1 led to suppression of insulin receptor signaling in intact cells (Ozcan et al., 2004). In addition, function loss of

Abbreviations: ER, endoplasmic reticulum; T2D, type 2 diabetes; XBP1, X box binding protein 1; β, pancreatic beta; IR, insulin resistance; HDLC, high-density lipoprotein cholesterol; LDLC, low-density lipoprotein cholesterol; WHO, World Health Organization; BMI, body mass index; HPLC, high pressure liquid chromatography; HOMA, homeostatic model assessment; HWE, Hardy–Weinberg equilibrium; CIs, confidence intervals; ORs, odds ratios.

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an *XBP1* allele in mice led to enhanced ER stress, reduced insulin receptor signaling, systemic insulin resistance and T2D, whereas wild type *XBP1* improved these factors (Ozcan et al., 2004). Based on these backgrounds, XBP1 protein may play an important role in the pathogenesis of T2D.

XBP1, the gene encoding for X-box binding protein 1, is located on chromosome 22q12 and is a pivotal gene involved in a complex cascade of events in ER stress (Liou et al., 1990). Recent studies have confirmed that a genetic variant (−116C/G, rs2269577, changing the consensus motif ACGT into AGGT) at nucleotide −116 of the *XBP1* gene, identified by Kakiuchi et al., was associated with significantly decreased *XBP1* transcriptional activity (Kakiuchi et al., 2003). The −116C → G substitution in the promoter region of the *XBP1* gene was demonstrated to eliminate the binding motif of XBP1 itself and impair the XBP1 loop in the ER stress response, leading to decreased transcription activity by the XBP1 protein (Kakiuchi et al., 2003). The role of this variant has been extensively studied in many diseases including vitiligo (Ren et al., 2009), schizophrenia (Chen et al., 2004), lithium prophylaxis in bipolar disorder (Masui et al., 2006).

However, whether the −116C/G polymorphism in the *XBP1* gene is associated with T2D in the population has not yet been reported. Thus, a hospital-based case control study was performed to explore the association of the −116C/G polymorphism of *XBP1* gene with T2D and related traits in a Chinese Han population.

2. Materials and methods

2.1. Study population and characteristics

Consecutive new patients with T2D in the absence of anti-diabetic therapy were enrolled as a case series in several hospitals from Beijing and the Harbin area of northern China. T2D was diagnosed based on the diagnostic criteria defined by WHO in 1999 (Alberti and Zimmet, 1998) and the American Diabetes Association in 2003 (Genuth et al., 2003) (fasting plasma glucose ≥7.0 mmol/l and/or 2-h plasma glucose ≥11.1 mmol/l). Meanwhile, unrelated controls without T2D were recruited from healthy people who underwent physical examination in the above hospitals. Subjects with evidence of a history of cancers, cardiovascular and cerebrovascular diseases, mood disorders, type diabetes 1, and alcoholism were excluded. Written informed consent was obtained from each participant after the study was explained in detail. Ethical approval was obtained for the study from the relevant ethics committees.

The demographic, physical and biochemical characteristics of both the T2D subjects and the control subjects were extensively assessed. The characteristics considered in our study included age, gender, smoking, drinking, height, weight, body mass index (BMI), waist/hip ratio, fasting glucose, fasting insulin, HbA1c, HOMA-IR, total cholesterol, triglyceride, high-density lipoprotein cholesterol (HDLC), and low-density lipoprotein cholesterol (LDLC).

2.2. Measurement methods

Height and weight were measured in light indoor clothes and without shoes, and BMI was calculated as weight (kg)/height^2 (m^2). Waist circumference was measured in an upright position to the nearest 0.5 cm at the umbilical level, according to WHO recommendation. The hip is recorded at the widest part of the hips, corresponding to the groin level for women and approximately 2–3 in. below the navel in men. The plasma concentration of glucose was analyzed by an automated glucose oxidase method. Plasma insulin was measured with the ALPCO Human Insulin ELISA Kit (ALPCO, USA). The levels of HbA1c were evaluated using ion-exchange high-pressure liquid chromatography (HPLC). Insulin sensitivity was assessed using homeostatic model assessment (HOMA), in which the homeostasis model of insulin resistance (HOMA-IR) = [fasting insulin (μU/ml)] × [fasting glucose (mmol/l)] / 22.5 (μU/ml = pmol/l ÷ 6.965).

2.3. SNP genotyping

Genomic DNA was extracted from peripheral blood leukocytes using a QIAGEN QIAamp DNA Mini Blood Kit (Germany). The SNP was detected using GeneScan analysis software (ABI, Foster City, CA). The details of primers, probes and reaction conditions are available upon request (https://products.appliedbiosystems.com/ab/en/US/adirect/ab). PCR was performed on the ABI PRISM 7900 HT Sequence Detection System using the TaqMan Universal Master Mix without UNG (Applied Biosystems, Foster City, CA) and heated to 95 °C for 10 min followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min.

2.4. Statistical analysis

Before statistical analysis, the normality of the distribution was evaluated using the Shapiro–Wilk test, and the variables underwent a logarithmic transformation when necessary. The U-test or ANOVA test for quantitative data and the $\chi 2$-test for qualitative data were used to determine whether there were significant differences in characteristic factors between cases and controls. The $\chi 2$-test was performed to assess the deviations from the Hardy–Weinberg equilibrium (HWE) and the frequencies of genotype and allele in *XBP1* among the cases and controls. Odds ratios (ORs) and corresponding 95% confidence intervals (CIs) were estimated to compare cases to controls in association with genotypes, alleles and models. All statistical tests were 2-sided and performed using the Statistical Package for Social Science (SPSS 11.0 for windows, SPSS Inc., Chicago, IL, USA), and statistical significance was taken as a *P* value less than 0.05.

3. Results

3.1. Baseline characteristics

The baseline characteristics of all participants in the study are summarized in Table 1. Of 1058 participants, 523 were T2D patients and 535 were healthy controls. The T2D and control subjects were matched by age and gender. The mean age was 58.56 years (±5.87 years; range, 30–78 years) for the T2D subjects and 58.94 years (±6.83 years; range, 31–80 years) for the controls (P = 0.3248). The gender (male/female) ratio was 1.29:1 in the T2D patients and

Table 1
Baseline characteristics of patients with type 2 diabetes and controls.

Characteristics	Diabetic cases	Controls	P value
Total, N	523	535	
Mean age (years)	58.56 ± 5.87	58.94 ± 6.83	0.3248
Gender			0.7240
Male	295 (56.41%)	296 (55.33%)	
Female	228 (43.59%)	239 (44.67%)	
Smoking	76 (14.53%)	98 (18.32%)	0.0967
Drinking	64 (12.24%)	85 (15.89%)	0.0879
Family history of diabetes	204 (39.01%)	226 (42.24%)	0.2838
BMI (kg/m^2)	25.28 ± 4.21	24.64 ± 4.14	0.0128
Waist/hip ratio	0.89 ± 0.02	0.86 ± 0.01	<0.0001
Fasting glucose (mmol/l)	8.93 ± 1.38	5.19 ± 0.81	<0.0001
Fasting insulin (pmol/l)*	86.1 ± 4.37	53.6 ± 2.82	<0.0001
HbA1c (%)	73 ± 13 (8.8 ± 1.16)	34 ± 10 (5.3 ± 0.92)	<0.0001
HOMA-IR*	5.31 ± 0.46	2.08 ± 0.19	<0.0001
Total cholesterol (mmol/l)	4.35 ± 0.79	4.49 ± 0.85	0.0006
Triglyceride (mmol/l)	1.72 ± 0.98	1.64 ± 0.87	0.1603
HDL-C (mmol/l)	1.08 ± 0.21	1.15 ± 0.30	0.0005
LDL-C (mmol/l)	2.71 ± 0.78	2.78 ± 0.79	0.1474

Continuous data are expressed as the means ± SEM.
* The logarithms of these variables were used for the analysis.