Association between polymorphism in the AKT1 gene and type 2 diabetes mellitus in a mexican population

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ABSTRACT

Background: AKT1 is a serine/threonine protein kinase that plays an important role in the insulin pathway. In order to determine whether the AKT1 gene plays a role in the pathogenesis of type 2 diabetes mellitus characterized by insulin resistance, in this paper we analyze the AKT1 gene c.-350G > T rs1130214 polymorphism as a risk factor for type 2 diabetes mellitus in an adult population of western Mexico. Materials and methods: A total of 199 subjects including 70 patients with type 2 diabetes mellitus and 129 healthy controls of Mexican origin were enrolled. Three milliliter of peripheral blood samples were collected in tubes containing ethylenediamine tetraacetic acid. Genomic DNA was isolated from peripheral blood leukocytes by standard methods and stored at −20 °C. Genotyping of the c.-350G > T rs1130214 polymorphism in the AKT1 gene were determined by employing the polymerase chain reaction-restriction fragment length polymorphism method. Genotypes were tested for association with type 2 diabetes mellitus.

RESUMEN

La insulina ejerce su efecto al activar una serie de cascadas reguladas por cinasas. AKT1 es una proteína cinasa de serina/threonina que multiplica la señal iniciada por la insulina y regula muchos procesos celulares esenciales de crecimiento, supervivencia, desarrollo y metabólicos. Entre los procesos metabólicos se encuentra la captación celular de glucosa. Varias publicaciones presentan las bases en desórdenes de la vía de acción de la insulina, los cuales incluyen obesidad, síndrome metabólico y diabetes mellitus tipo 2 (DM tipo 2). Con base en lo anterior analizamos el polimorfismo rs1130214 del gen AKT1 como factor de riesgo para DM tipo 2 en población adulta del occidente de México. En este estudio de casos y controles participaron 199 individuos mexicanos no emparentados, cuyo genotipo se identificó por PCR-RFLP. De las personas estudiadas, 67 fueron diagnosticadas con DM tipo 2 según los criterios de la OMS. La distribución de genotipos estuvo en equilibrio de Hardy-Weinberg. El genotipo GT fue
with type 2 diabetes mellitus. **Results:** The genotype frequencies were in Hardy-Weinberg equilibrium. The GT genotype frequency was higher in the diabetic group as compared with the control group. **Conclusions:** Our findings suggest that the c.-350G > T polymorphism of the AKT1 gene is associated with susceptibility to type 2 diabetes mellitus in the population studied. (REV MEX ENDOCRINOL METAB NUTR. 2015;2:167-70)

**Key words:** Type 2 DM. Type 2 diabetes mellitus. AKT1. v-AKT murine thymoma viral oncogene homolog 1. Polymorphism.

**BACKGROUND**

Type 2 diabetes mellitus (T2DM) is a chronic, degenerative, complex, polygenic disorder characterized by impaired insulin resistance in peripheral tissues, insulin secretion, and dysregulation of carbohydrate, lipids, and protein metabolism with environmental and genetic factors. Insulin mediates multiple metabolic responses in skeletal muscle, including glucose and fatty acid uptake and metabolism as well as gene-regulatory responses. The insulin actions are mediated by intracellular signaling, including activation of the phosphatidylinositol 3-kinase (PI3K/AKT) pathway. The AKT1 gene is particularly important in mediating several metabolic actions of insulin.

AKT1 is a serine/threonine kinase that phosphorylates and regulates the function of many cellular proteins involved in processes that include metabolism. The AKT1 activation is carried out by two phosphorylations: one at serine-473 by the rictor-mTOR complex and the other one at threonine 308 by phosphoinositide-3-dependent protein kinase-1. AKT1 phosphorylates and inhibits ASC160, which facilitates GLUT4 translocation from the vesicles to cytoplasmic membrane. Activation of Akt by insulin in adipocytes is reduced in T2DM, and experimental reduction of AKT leads to decreased insulin sensitivity and reduced glucose disposal. However, several association studies in the insulin pathway suggested different candidate genes for T2DM in many populations. The genetic polymorphism of AKT1 gene provides a basis for studying the association between genetic variants and the development of T2DM. Based on a literature review, the AKT1 gene rs1130214 polymorphism has been the most studied and associated with different metabolic parameters. In this report we study the rs1130214 polymorphism and its relationship with T2DM in an adult cohort. We show that the GT genotype occurs most often in people with T2DM whose statistical parameters suggest that it is a risk factor.

**MATERIALS AND METHODS**

A total of 70 T2DM subjects were collected from the Hospital Civil Fray Antonio Alcalde. Subjects were selected as adults > 30 years old in whom a T2DM diagnosis was established between 30 and 55 years ago according to WHO criteria. A questionnaire was obtained to assess the clinical antecedents and genetic risk factors for chronic diseases. Informed consent was obtained from all the subjects who participated in this study. A total of 129 control individuals were recruited from the Blood Bank healthy respondents who did not have T2DM arterial hypertension.
at the time of sample collection. The subjects who had been diagnosed with any other disease were excluded from this study.

The blood samples were collected by venous puncture from the respective subjects. The genomic DNA was extracted from white blood cells using the salting out method and used for amplification of the candidate gene. Polymerase chain reaction (PCR) standardization was done. The concentration of the extracted DNA was estimated using the NanoDrop in two optical density wavelengths: 260 nm and 280 nm.

Genotyping was done based on endpoint PCR GeneAmp 2400 (Applied Biosystems) followed by XcmI restriction enzyme digestion for allele G identification. The 102 bp PCR product was sequenced and confirmed the product. The PCR primers were forward primer 5'-CAG AGG CGC TGT GGT TTA GGA -3' and reverse primer 5'-GAT GCA GGC CAC TGG CGC AGA -3'. The PCR was performed in a volume of 20 μl. The temperature was kept at 95 °C for five minutes for denaturation, followed by 40 cycles of 20 seconds at 95°C, 10 seconds at 55°C, and 20 seconds at 72 °C.

 Allelic frequencies were calculated by the gene counting method and the genotype distribution was calculated with Hardy-Weinberg expectations by a chi-squared test. Statistical analyses were performed using the Statistical Package for Social Science (SPSS) v.10 to analyze the data in this study. Descriptive statistics were utilized to analyze the genotypes of all the study subjects; moreover, all of these factors were compared by using Student's t-test; one-way analysis of variance (ANOVA) test was utilized to compare the group means and a level of p < 0.05 was considered as statistically significant.

RESULTS

A total of 199 subjects were recruited in this study and were divided into two groups: 122 controls and 70 T2DM subjects. The mean age of the control group was 45.5 ± 6.25 years, and the case group was 50.4 ± 9.01 (Table 1). The body mass index, systolic and diastolic blood pressure, high-density lipoprotein, triglyceride, low-density lipoprotein, and cholesterol among T2DM patients and control group was not different (p > 0.05).

Analysis of c.-350G > T polymorphism with 102 bp PCR product and the genotypic and allelic frequencies, as shown in table 2, demonstrated the significant difference of genotypes of this polymorphism.

DISCUSSION

In the present study we demonstrate that the GT genotype of rs1130214 polymorphism of AKT1 gene is significantly associated with T2DM in individuals from western Mexico, which represents a susceptibility
gene for this disease. A major question concerns the mechanism by which rs1130214 influences the risk for T2DM due to rs1130214 in the 5' untranslated region of the AKT1 mRNA. It is noteworthy that previous work has identified, in murine cell lines and mouse cell lines, that the G allele region functions as a repressor in undifferentiated muscle cell and undifferentiated fat cells, and the allele T functions as weak activator in differentiated muscle cell and functions as repressor in differentiated fat cells. The enhancer activity is weak when compared with two other polymorphisms analyzed in the study and that are in linkage disequilibrium with rs1130214.

If we analyze the amount of protein necessary for optimal function of each cell, besides the level of transcription of the AKT1 gene, we must consider the regulation of AKT1 kinase activity, the phosphorylation pattern in response to insulin, which has been analyzed in the muscle of diabetic patients, defects to insulin signaling and was associated with altered AKT1-Thr(308) phosphorylation.

It is known that in patients with T2DM, fasting hyperinsulinemia and resistance to insulin action are key components of this disease. The genetic risk factors in insulin signaling in Mexicans have been poorly studied; reports show association of the INS gene with fasting hyperinsulinemia and association of the IRS1 gene with T2DM. We have been unable to identify other reports with genes involved in the insulin signaling associated with T2DM in Mexicans. The present study provides evidence that the GT genotype of rs1130214 polymorphism of AKT1 gene is a susceptibility gene for T2DM in the Mexican population.

CONCLUSION

The present study has showed a genetic association for rs1130214 polymorphism of AKT1 gene among Mexican T2DM patients compared to control subjects.