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Research Article

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Hypoxia Inducible Factor-1α Gene rs11549465 Might be Protective Factor for the Development of Type 1 Diabetes Mellitus

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Abstract

Introduction: Pancreas is a highly vascularized tissue which makes oxygen a fundamental element for its metabolism. As a consequence of the variations in its delivery, the expression of genes that maintain homeostatic levels of oxygen supply a regulated by hypoxia-inducible factor 1 α (HIF-1α). Here we tested the role of single nucleotide polymorphism (SNP) of HIF-1α as markers for susceptibility or protection to develop pancreatic injury in Mexican patients with type 1 diabetes mellitus (T1DM).

Methods: We studied 55 patients with T1DM that conformed to the criteria of the American Diabetes Association (ADA); in them, we extracted DNA from peripheral blood cells and studied the gene that encodes HIF-1α. The SNP selection was done in accordance to the SNP database (https://www.ncbi.nlm.nih.gov/SNP/). Three polymorphisms were included: rs11549465, rs11549467 and rs2057482. Statistical analysis was performed by the EpiInfo statistical program (version 6), using $p<0.05$ as the level as statistical significance; gene frequencies were compared to those present in 66 healthy ethnically matched individuals.

Results: We observed in patients a significant decreased frequency of the polymorphism rs11549465 as compared to the group of healthy controls (p=0.0018; OR=0.07 [0.0-0.48]). We observed a change in the domain oxygen dependent degradation domain "ODDD" which converts Pro-582 to Ser.

Conclusion: The T allele of the polymorphism rs11549465 seems to be a protective factor for the development of T1DM.

Keywords

Type 1 diabetes mellitus; Hypoxia inducible factor-1 α

Introduction

T1DM is an autoimmune disease due to destruction of the β pancreatic cells and a decrease in insulin synthesis resulting in an imbalance of glucose homeostasis [1]. In addition, there is a decrease in oxygen supply to β pancreatic cell, turning the environment of this tissue hypoxic [2]. With the purpose of maintaining homeostatic oxygen levels, the pancreatic cells activate the hypoxia-inducible factor 1 alpha (HIF-1α), whose activity is relevant for survival of pancreatic cells. HIF-1α, encoded in the 14q21-24 region, has 826 aminoacids and a molecular weight of 120-130 kDa. The active site of this protein is the ODDD, which works as an oxygen sensor [3]. Inside the nucleus, the HIF complex has the capacity to bind specifically to hypoxia-response elements (HRE) through TACGT sequences that are present in the promoter regions of several genes [4]. The metabolic function of the proteins regulated by HIF-1α may suggest a role for the development of T1DM, T2DM and other clinical entities as well [5]. HLA-DR3/DQA1*0501/DQB1*0201 and HLA-DR4/DQA1*0301/DQB1*0302 are risk factors for the genetic susceptibility to T1DM [6]. Here we investigated the genetic background associated to the disease by analyzing the frequencies of the SNP within the HIF-1α gene in Mexican patients with T1DM.

Materials and Methods

Patients

We studied 55 patients with T1DM (mean age 9.1 years old), samples were collected from a referral hospital in Mexico City. Symptoms of diabetes and a casual plasma glucose ≥200 mg/dL (11.1 mmol/L): to make the diagnosis of T1DM, the criteria of ADA were used and conformed to guidelines described by the WHO. The control group involved 66 first grade relatives of both genders and clinically healthy at the time of sampling, according to the criteria of the ADA. Participants signed a consent letter.

We used the following single nucleotide polymorphism of HIF-1α: rs11549465 (Pro582Ser, C1772D), rs11549467 (Ala588Thr, G1790A) and rs2057482 (C191T), located in the 14 chromosome. In our study the patient group was composed by 22 males (40%) and 33 females (60%) with a mean age of 9.1 ± 3.9; the mean body mass index was 17.3 ± 4.1 kg/m^2; and a mean serum glucose of 501.2 ± 212.9. The control group was conformed by 7 males (10.6%) and 59 females (89.39%) giving total group of 66 healthy individuals according to American Diabetes Association, they have a mean serum glucose of 102.4 ± 77.9 and a BMI of 26.9 ± 4.6.

Genotyping SNPs and selection of HIF-1α

DNA was obtained by fresh blood from peripheral mononuclear cells, following the manufacturer's instructions QIAGEN (Hilden, Germany). Genotyping of the HIF-1α polymorphisms were Carried out using 5 'exonuclease TaqMan Allelic Discrimination Assays (Applied Biosystems, Foster City, USA) on a Rotor-Gene Q Real-time PCR system according to manufacturer's instructions (QIAGEN, Hilden, Germany). Selection of SNPs in the HIF-1α gene was done based on the information from the dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/). Three polymorphisms were included into the genotyping tests: rs11549465, rs11549467 and rs2057482. PCR was performed in a full volume of 10 µl, which contained 40 ng DNA (1 µl), 1X TaqMan Universal PCR Master Mix (5 µl), TaqMan Probes 20X (0.5 µl), water (3.5 µl). Thermal cycling conditions as follows:

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95°C for 10 min, followed by 40 cycles of 92°C for 15 sec and 60°C for 1 min.

Statistical analysis

Gene frequencies of the HIF-1α alleles from both study groups were calculated by direct count. For comparison between the groups, we used Chi-square analysis including 2x2 contingency tables, and Fisher's exact test when appropriate, statistical significance were defined as p values less than 0.05. Analysis of Hardy-Weinberg equilibrium test, revealed normal distribution, then was performed by the Chi-square test in http://www.oege.org, in the cases and controls separately for each variant association before analysis. For estimating risks, we employed odds ratio (OR) with a 95% confidence interval (95% CI). These calculations were performed by using the EpiInfo statistical program (version 6, Centers for Disease Control and Prevention, Atlanta, GA, USA).

Results

As displayed on (Table 1), patients with T1DM have a lower frequency of the T allele of rs11549465 [p=0.0018; OR=0.07 (0.01-0.48)] and of the CT genotype for this SNP [p=0.0002; OR=0.01 (0.0-0.3)]. Moreover, patients with T1DM had an increase frequency of the CC genotype of rs11549465 [p=0.0009; OR=17.6 (2.3-369)] and the C allele [p=0.018; OR=15.3 (2.0-314)]. There were no significant differences between the patients with T1DM and controls regarding to the frequency of rs11549467 and rs2057482 allele or HIF-1α variants. Patients with T1DM have a lower frequency of the T allele of rs11549465 [p=0.0018; OR=0.07 (0.01-0.48)] and of the CT genotype for this SNP [p=0.0002; OR=0.01 (0.0-0.3)]. Moreover, patients with T1DM had an increase frequency of the CC genotype of rs11549465 [p=0.0009; OR=17.6 (2.3-369)] and the C allele [p=0.018; OR=15.3 (2.0-314)].

Discussion

We found that patients with T1DM have a lower frequency of the T allele of the polymorphism rs11549465 as compared to controls. We propose the possibility that the beta cells of the pancreas are conditioned to a better response towards hypoxia due to the presence of the variant rs11549465. Previously, it was found an increased frequency of the T allele of the rs11549465 polymorphism in the control group as compared with patients with T1DM in a Caucasian population [7]. Likewise, it was observed that the frequency of the minor allele (T) of the same polymorphism was increased in the control group as compared with patients with T2DM in a Japanese population [8]. Moreover, the implication of the T allele in the natural history of the disease has shown that it is important for the expression of the late stage complications. For instance, diabetic nephropathy and foot ulcers are more common in patients that carry the studied SNP [9]. These results suggest that the polymorphism rs11549465 of the gene HIF-1α plays an important role for the physiopathology of two metabolic diseases with different physiopathology. Diverse mechanisms can mediate the protective effect of the rs11549465 variant in the development of T1DM. For instance, of the 35 polymorphisms of HIF-1αthat has been identified, only three are localized in coding segments of the gene. A more detailed examination of this genetic region allowed researchers to establish the relation between a SNPs and the malfunction of the proteins. Likewise, a change from a C to a T inHIF-1αgives rise to rs11549465 that translates a serine instead of a proline in the position 582 of the N-terminal domain of transactivation (N-TAD) [10]. This domain is a component of the ODDD, a critical binding region for the Von Hippel-Lindau factor protein. Although this change doesn't intervene with the hydroxylation of the proline residues in the position 564 of HIF-1α, it generates a molecule that resists degradation, making it more prone to translocate to the nucleus. Therefore, the transcriptional activity of the HIF complex is increased in those individuals with the rs11549465 in relation to the common isoform. In turn, the products of the transcription of HIF-1α, including vascular endothelial growth factor "VEGF", have several effects that can be protective against T1DM in more than one steps of its physiopathology. Although this study advances the understanding of the genetic factors involved in the development of T1DM, it also

Table 1: Distribution of polymorphisms in T1DM patients and controls.

Gene	T1DM	Controls	OR (95% IC)	*p**
Genotype/Allele				
	N (%)	***N (%)***		
HIF-1α (rs11549465)				
CC	54 (98.2)	49 (75.4)	17.6 (2.3 – 369)	0.0009
CT	0 (0.0)	16 (24.6)	0.0 (0.0 – 0.3)	0.0002
TT	1 (1.8)	0 (0.0)	NS	
C	109 (99.0)	114 (87.7)	15.3 (2.0 – 314)	0.0018
T	1 (1.0)	16 (12.3)	0.07 (0.0 – 0.48)	0.0018
HIF-1α (rs11549467)				
GG	53 (96.4)	64 (98.5)	NS	
GA	2 (3.6)	1 (1.5)	NS	
AA	0 (0.0)	0 (0.0)	NS	
G	108 (98.2)	129 (99.0)	NS	
A	2 (1.8)	1 (1.00)	NS	
HIF-1α (rs2057482)				
CC	45 (88.2)	53 (82.8)	NS	
CT	6 (11.8)	11 (17.2)	NS	
TT	0 (0.0)	0 (0.0)	NS	
C	96 (94.1)	117 (91.4)	NS	
T	6 (5.9)	11 (8.6)	NS	

T1DM, type 1 diabetes mellitus patients; OR, odds ratio; CI, confidence interval; **p* value corrected <0.05; NS, not significant

faces several limitations. We didn't observe any complications of the disease and therefore, we can't make conclusions regarding the natural history of the disease. Future studies should explore the development of diabetic foot, stroke, retinopathy, neuropathy and cardiac ischemia to establish the role of the T allele in the late stages of the disease. On the other hand, other genetic factors were not taken into account for this study. Functional investigations using cells derived from carriers of this SNP will be important to find out its relation with physiology.

Declarations

Competing interests,Authors have declared that no competing interests exist,Authors' contributions;authors' contributions jce, gj: conception of the research idea, study design, reviewing the manuscript, ftj, lra: molecular experiments, cvmp, leg , mava, mbp: data collection, analysis and interpretation and the drafting of the manuscript, reviewing the manuscript. All authors read and approved the final version of the manuscript.

Ethical Approval

All authors hereby declare that all experiments have been examined and approved by the ethics committee from Children's Hospital of Mexico Federico Gómez and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Consent for Publication

All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this case report. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal.

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