

Evaluation of relationship between HNF-1 α and GLP-1R polymorphisms and type 2 diabetes in a population living in northeast of Iran

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Abstract

The prevalence of type 2 diabetes mellitus (T2DM) is rising dramatically in the Middle East, especially in the Islamic Republic of Iran, but the genetic basis of type 2 diabetes in Iran is poorly understood. Polymorphisms of hepatocyte nuclear factor-1 α (HNF-1 α) and glucagon-like peptide-1 receptor (GLP-1R) genes showed association with type 2 diabetes in several ethnic groups. In this study, we evaluated whether these markers confer susceptibility to T2DM in a diabetic population living in Mashhad (northeast of Iran). Genotyping of Ala98Val (HNF-1 α) and Thr149Met (GLP-1R) was done by the restriction fragment length polymorphism-PCR (RFLP-PCR) method in the following groups: 1) early-onset diabetes (age at onset \leq 35 years); 2) late-onset diabetes (age at onset $>$ 35 years); and 3) control. Our results showed that CT (Ala/Val) genotype of HNF-1 α was higher in the early-onset type 2 diabetic group compared to the controls but difference was not significant. We did not find the GLP-1R Thr149Met mutation in all participants. The prevalence of the HNF-1 α (Ala98Val) and (GLP-1R) Thr149Met mutations has not been previously reported in Iranian participants. We conclude that these mutations are not a common cause of T2DM in our studied population.

Keywords: Type 2 diabetes; Hepatocyte nuclear factor-1 α ; Glucagon-like peptide-1 receptor; Polymorphism

Introduction

Type 2 diabetes is a disease with a rising prevalence worldwide (Gadsby, 2002). The prevalence of diabetes in Iran ranges from 1.3% in rural areas to 14.5% in large cities and most patients have type 2 diabetes (Azizi et al., 2003a; Azizi et al., 2003b). It is predicted that the developing countries will contribute 77.6% of the total number of diabetic patients in the world by the year 2030 (Azizi et al., 2003b; Hussain et al., 2007). In addition to a sedentary lifestyle related to recent affluence leading to obesity and insulin resistance, the rising prevalence has been attributed to genetic predisposition (Bener et al., 2005; Yaturu et al., 2005). Impairment in insulin secretion plays a major role in the pathogenesis of type 2 diabetes in addition to insulin resistance (Association, 2009). Therefore, genetic variants in insulin secretion pathways are plausible candidate genes for type 2 diabetes. In the present study, single nucleotide polymorphisms (SNPs) in the genes regulating

insulin secretion (TCF1 [encoding HNF-1 α], and GLP-1R) were evaluated as risk factors for type 2 diabetes mellitus in a diabetic population living in Mashhad (northeast of Iran).

The most frequent monogenic forms of T2DM with profound defect in insulin secretion is autosomal dominant diabetes and its early onset form is called MODY (maturity onset diabetes of the young). Mutations in the hepatocyte nuclear factor-1 α (HNF-1 α) cause the type 3 of MODY (MODY3). HNF-1 α is an important transactivating factor that is involved in pancreatic B-cell glucose sensing because it increases the transcription of many genes participating in the insulin secretion process (Bowden et al., 1997; Mahtani et al., 1996; Shaw et al., 1998). Common variations in this gene were associated with impaired insulin secretion and the risk of type 2 diabetes (Holmkvist et al., 2006; Urhammer et al., 1997). HNF-1 α mutations are the most common mutations in Western and Asian countries (Ellard, 2000; Herman et al., 1994; Iwasaki et al., 1997; Lee et al., 2001).

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Previous reports in Danish Caucasians have shown that insulin responses to an oral glucose load have decreased in Val carriers of the HNF-1 α Ala98Val polymorphism compared with the Ala homozygous individuals, suggesting that this amino acid replacement might influence β -cell function (Urhammer et al., 1997). Earlier studies in a Finnish population showed an association between Ala98Val polymorphism and T2DM with a prevalence of 13.2% (Lehto et al., 1999).

The gut hormone glucagon-like polypeptide-1 (GLP-1) is an incretin hormone that is released from epithelial cells of the gastrointestinal mucosa after food intake. Secreted GLP-1 binds to the GLP-1 receptor (GLP-1R) on the pancreatic β -cell and markedly increases glucose-dependent insulin secretion (Drucker, 2006). The GLP-1R is a candidate gene for diabetes mellitus, as mutations may induce the impaired insulin response that is a characteristic feature of non-insulin-dependent diabetes mellitus (NIDDM) (Zhang et al., 1994). Studies of the GLP-1R have been directed towards identifying polymorphisms in the GLP-1R gene that may be a contributing factor in the pathogenesis of diabetes. A GLP-1R polymorphism in which threonine 149 is substituted with a methionine residue has been identified in a patient with type 2 diabetes but was not found in non-diabetic control participants (Tokuyama et al., 2004). Expression of a nonsynonymous single nucleotide polymorphism, which results in substitution of methionine for threonine at position 149 of GLP-1R in cell systems, decreases binding affinity for glucagon-like peptide (GLP)-1 and intracellular signaling after hormone receptor binding (Beinborn et al., 2005). The Thr149Met substitution may alter the conformation of the binding pocket by indirect mechanisms, and thereby impair high-affinity interactions between the receptor and the amino-termini of cognate ligands. These functional effects suggest that genetic variation in GLP-1R may alter responsiveness to GLP-1 in vivo (Sathanathan et al., 2010).

No information is available on the prevalence of the HNF-1 α Ala98Val and GLP-1R Thr149Met mutations among the Iranian population and their association with type 2 diabetes. The present study was undertaken to investigate the prevalence of these polymorphisms in a diabetic population admitted to the Shahid Ghodsi hygiene center, Mashhad, Iran.

Materials and Methods

Participants

This study was performed on 150 unrelated participants from the city of Mashhad (North east of Iran, Khorasan Razavi province) who were referred to Shahid Ghodsi hygiene center. Mutational analysis was performed in 50 healthy control participants (37 men and 13 women) with a mean body mass index (BMI) of 24.81 ± 3.37 kg/m² and 100 patients with type 2 diabetes mellitus whom were divided into two groups on according to the age at which diabetes were diagnosed. One group with early-onset diabetes ≤ 35 years included 41 patients (male/female: 12/29) with a mean BMI of 26.57 ± 2.94 kg/m² and the other group with late-onset diabetes > 35 years included 59 patients (male/female: 22/37) with a mean BMI of 26.47 ± 2.97 kg/m². Since MODY3 often takes place in youth, patients had been assigned to such division in order to compare patients with early-onset diabetes with those of late-onset diabetes. Before participation, the purpose and risks of the study were carefully explained and informed consents were obtained. The protocol was approved by the committee of ethics in Ferdowsi University of Mashhad.

DNA Analysis

Genomic DNA was extracted from peripheral blood leukocytes of T2DM patients and control participants using a genomic DNA purification kit (Ferments, Canada) and quantitated by ultra violet (UV) absorption at 260 nm.

Genotyping of polymorphism

HNF-1 α Ala98Val and GLP-1R Thr149Met were genotyped by restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR) using the listed primers (Table 1).

Table 1. Primers for amplification of HNF-1 α and GLP-1R exons.

Gene	Primers	Product size (bp)
HNF-1 α	Fwd:	424
	CGTGGCCCTGTGGCAGCCGA	
	Rvs:	
GLP-1R	GGGCTCGTTAGGAGCTGAGGG	380
	Fwd:	
	CTGCTTCATTCCTCTATCTGGG	
	Rvs:	
	TGTATTCACCTCTCTGGCCTTG	

Table 2. Association of HNF-1 α Ala98Val polymorphism with type 2 diabetes.

Allele/genotype	Diabetic subjects		Control subjects	OR (95% CI)	p
	Early-onset Diabetes	Late-onset Diabetes			
Number (n)	41	59	50		
C/C frequency% (n)	90.2 (37)	100 (59)	96 (48)	Reference	
C/T frequency% (n)	9.8 (4)	0 (0)	4 (2)	1 (0.13–11.42)	0.99
Allele C	95.1 (78)	100 (118)	98 (98)	Reference	
Allele T	4.9 (4)	0 (0)	2 (2)	1.96 (0.27–22.06)	0.68

Note: No subjects had the T/T genotype

In order to amplify HNF-1 α gene, the 50 μ l reaction mixture contained 0.2 μ g of DNA, 100 μ M of each dNTP, 2 U Taq DNA polymerase, 1.5 mM MgCl₂, and 10 pmol of each primer. The PCR conditions were 5 min at 94 °C; 35 cycles for 30 s at 95 °C, 15 s at 64 °C, 3 min at 72 °C with a final extension of 5 min (Lim et al., 2008). For GLP-1R, the 50 μ l reaction mixture contained 0.2 μ g of DNA, 200 μ M of each dNTP, 2 U Taq DNA polymerase, 3 mM MgCl₂, and 10 pmol of each primer. PCR was performed as follows: DNA denaturation for 5 min at 94 °C; 35 cycles of: 30 sec at 96 °C, 15 sec at 60 °C, 3 min at 72 °C, and a final extension for 5 min at 72 °C (Tokuyama et al., 2004). The fragments were amplified in a thermal cycler (Primus 96 advanced ® / Primus 96 advanced ® Gradient).

A 10 μ l of PCR product was digested in a total volume of 25 μ l with 2.5 U of restriction enzyme HaeIII (HNF-1 α exon 1 polymorphism Ala98Val) or NlaIII (GLP-1R exon 5 polymorphism Thr149Met) in the buffer provided by the manufacturer (Fermentas GmbH, Germany) at 37 °C for 4 and 6 hours, respectively. The expected sizes of HaeIII digestion products are as follows: the CC (Ala/Ala) genotype 61, 85, and 167 bp; the CT (Ala/Val) genotype 61, 85, 167, and 252 bp; and the TT (Val/Val) genotype 61 and 252 bp. For the GLP-1R PCR product, the sizes of the restriction fragments digested with NlaIII are as follows: the CC (Thr/Thr) genotype 207, 133, 29, and 11 bp; the CT (Thr/Met) genotype 207, 135, 133, 72, 29, and 11 bp; and the TT (Met/Met) genotype 135, 133, 72, 29, and 11 bp.

The PCR and restriction fragments were electrophoresed on 2% agarose gel and visualized by ethidium bromide staining in a gel documentation system (Kalagene Pajooh, Iran).

Biochemical Assays

Fasting blood samples were taken from the patients and control participants for the estimation of glucose and lipids. Serum glucose, triglyceride,

cholesterol, LDL-cholesterol (LDL-C), and HDL-cholesterol (HDL-C) were determined by enzymatic colorimetric assays using standard kits (Pars Azmoon, Mashhad, Iran).

Statistical Analysis

Data was analyzed with the SPSS program (version 18.0). Results are given as mean \pm S.D. or percentages. To compare quantitative data in groups of carriers of different genotypes, the unpaired Student's t-test was used. Odd ratios (OR) and 95% confidence intervals (CI) were used for estimating the strength of association between different groups and alleles or genotypes of HNF-1 α and GLP-1R genes polymorphism. To provide separate ORs for each genotype, dummy variables were used, with a wild-type genotype used as a reference group. p- Values of less than 0.05 were considered significant.

The significance of interaction between clinical characteristics and polymorphic variants was assessed using a multivariate analysis of variance (MANOVA).

Table 3. Clinical and biochemical features of the studied population.

Characteristic	Early-onset diabetes (N=41)	Late-onset diabetes (N=59)	Control (N=50)
Male/Female	22/37	12/29	13/37
BMI (kg/m ²)	26.59 \pm 2.88	26.37 \pm 3.01	24.82 \pm 3.36
FBS (mg/dL)	203.73 \pm 60.12	175.59 \pm 62.31	87.90 \pm 9.60
Triglycerides (mg/dL)	156.04 \pm 46.54	155.13 \pm 54.71	106.51 \pm 36.73
Cholesterol (mg/dL)	202.60 \pm 42.64	198.13 \pm 34.88	174.20 \pm 39.01
LDL-C (mg/dL)	132.47 \pm 34.77	126.93 \pm 38.43	109.62 \pm 35.26
HDL-C (mg/dL)	36.60 \pm 5.80	40.45 \pm 7.22	41.84 \pm 10.05

BMI, Body mass index; FBS, Fasting plasma glucose; LDL-C, Low density lipoprotein-cholesterol; HDL, High density lipoprotein-cholesterol; Data are presented as means \pm SEM.

Results

The results of investigating HNF-1 α Ala98Val and GLP-1R Thr149Met polymorphisms are shown in Table 2. We have not detected restriction fragment length polymorphism in the exon five of human GLP-1R gene by NlaIII digestion. The Thr149Met mutation (ACG \rightarrow ATG) was absent in all patients and controls. By RFLP-PCR, we were able to clearly distinguish the 2 control participants and 4 patients of the early-onset T2DM group with HNF-1 α Ala98Val (GCC \rightarrow GTC) mutation (Figure 1).

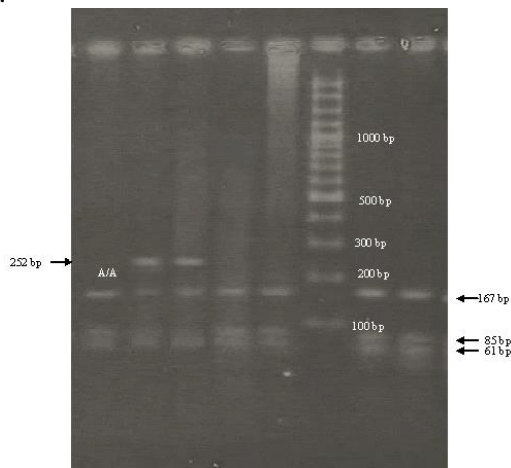


Figure 1. Genotyping of Ala98Val HNF-1 α by *HealIII* RFLP-PCR. Lines 1–5: The early-onset group \leq 35; Line 6: Gene rulers™ 100 bp DNA ladder; Lines 7–8: The late-onset $>$ 35 group. A/V: Alanine/ Valine genotype; A/A: Alanine/Alanine genotype.

No mutation was detected in all patients of late-onset $>$ 35 group. There were no rare homozygotes (TT) for all participants. Of case diagnosed \leq 35 years and control participants, 9.8% and 4% respectively were heterozygous (CT), giving an OR of 1 (95% CI: 0.13 to 11.42, $p = 0.99$). No significant change in T-allele frequency was detected in the early-onset diabetic patients (OR: 1.96, 95% CI: 0.27 to 22.06, $p = 0.68$). The mean ages of onset of diabetes and their mean BMI in participants with HNF-1 α mutation were 32.5 ± 3.1 years and 27.4 ± 1.9 kg/m², respectively. Table 3 shows the clinical and biochemical characteristics of the studied participants. Mean fasting glucose, triglyceride, cholesterol, and LDL-C levels showed an increase in the early-onset and the late-onset groups compared with the control group ($p < 0.02$). The early-onset diabetic patients had lower mean serum HDL-C levels than the healthy control participants ($p < 0.004$). No significant differences were found between the early-onset \leq 35 group and the late-onset $>$ 35 group cases in all clinical features (except fasting glucose and HDL-C). To

study whether the HNF-1 α Ala98Val mutation had consequences on the phenotype, we compared clinical variables between the diabetic participants with and without the HNF-1 α mutation. There were no significant differences in age, BMI, fasting glucose concentration, blood triglyceride, cholesterol, LDL-C, and HDL-C between these patients (Table 4).

Table 4. Clinical and biochemical features of the diabetic subjects with and without the HNF-1 α Ala98Val Mutation

	Diabetic mutation Carriers	Diabetic subjects without mutation
Number (n)	4	96
Age (years)	49.3 \pm 7.5	52.77 \pm 10.59
BMI (kg/m ²)	27.4 \pm 1.9	26.43 \pm 2.99
FBS (mg/dL)	196.5 \pm 57.8	186.74 \pm 63.12
Triglycerides (mg/dL)	147.5 \pm 27.8	155.84 \pm 52.08
Cholesterol (mg/dL)	230.8 \pm 72.4	198.69 \pm 36.15
LDL-C (mg/dL)	150.2 \pm 45.6	128.33 \pm 36.52
HDL-C (mg/dL)	41.3 \pm 9.0	38.78 \pm 6.85

BMI, Body mass index; FBS, Fasting plasma glucose; LDL-C, Low density lipoprotein-cholesterol; HDL, High density lipoprotein-cholesterol; Data are presented as means \pm SEM.

Discussion

We have identified Ala98Val mutation in the HNF-1 α gene in four of 41 patients whose diabetes onset was before 35 years of age. No mutation was detected in patients whose diabetes onset was after 35 years of age. Although the prevalence of the HNF-1 α Ala98Val mutation has been reported for many countries, the prevalence of the HNF-1 α Ala98Val mutation in type 2 diabetes has not been previously reported in Iranian patients. In the present study, the HNF-1 α Ala98Val mutation was found in 9.8% of the early-onset type 2 diabetes. Sahu et al. reported that the overall frequency of HNF-1 α Ala98Val mutation was 14% in Indian patients with early-onset type 2 diabetes (Sahu et al., 2007). Anuradha et al. showed that in Asian Indians, the Ala98Val polymorphism of HNF-1 α is associated with earlier age at onset of type 2 diabetes (Anuradha et al., 2005). Common variations in the HNF-1 α gene have been associated with impaired insulin secretion (Chiu et al., 2003; Urhammer et al., 1997). The Ala98Val variant may influence transcriptional activity and insulin secretion in vivo, data from Caucasians suggests that the association is likely to only modestly increase the risk of adult T2DM (Holmkvist et al., 2006).

A previous study in Scandinavian participants has suggested an association of valine 98 allele with early-onset familial T2DM (Lehto et al., 1999). The SNPs rs1920792, rs1169288 (Ile27Leu), and rs1800574 (Ala98Val) showed nominal association with type 2 diabetes in the Scandinavian samples. However, these SNPs were not associated with type 2 diabetes in another sample of 4,400 individuals from North America and Poland (Weedon et al., 2005). A concurrent large association study in individuals from the UK indicated that common variation in HNF-1 α is not associated with type 2 diabetes, with the exception of the Ala98Val polymorphism (3%) (Weedon et al., 2005; Winckler et al., 2005). In the present study, this allele was not associated with an early-onset of T2DM. This is the first study on the association of the HNF-1 α Ala98Val polymorphism with early-onset of type 2 diabetes from Iran.

We did not find any association of GLP-1R Thr149Met polymorphism with type 2 diabetes. The GLP-1R is one of the key targets in the management of type 2 diabetes mellitus with actions including regulation of insulin biosynthesis and secretion (Drucker and Nauck, 2006). Several groups have examined normal and diabetic populations for natural mutations in the GLP-1R. The Thr149Met polymorphism was discovered in a diabetic patient with defective glucose-induced insulin secretion but was not found in non-diabetic control participants (Tokuyama et al., 2004).

Our data revealed that the HNF-1 α Ala98Val and GLP-1R Thr149Met polymorphisms are not associated with an increased risk of type 2 diabetes in the studied population. Screening for the HNF-1 α Ala98Val and GLP-1R Thr149Met mutations was performed only in the 100 type 2 diabetes individuals, and hence we cannot provide an exact frequency for these mutations in T2DM. The association with HNF-1 α Ala98Val and GLP-1R Thr149Met polymorphisms needs to be confirmed in a larger cohort of Iranian patients with T2DM.

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