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Identification of I27L polymorphism in the HNF-1α gene in Western Indian population with late-onset of diabetes

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Background: Hepatocyte nuclear factor 1α (HNF-1α) gene mutations cause both early- and late-onset of diabetes mellitus. **Aim:** The purpose of this study was to determine the role of HNF-1α gene in the etiology of diabetes in Western Indian population. **Materials and Methods:** Blood samples from 100 diabetes patients and 50 normal individuals were collected and genomic DNA was isolated from the leucocytes. Exon 1 and exon 4 of the HNF-1α gene were PCR amplified and single stranded conformational polymorphism (SSCP) was performed with the PCR products. Direct sequencing of the PCR products was carried out to identify the polymorphism in the HNF-1α gene. **Results:** Polymorphisms were detected in 12 patients with respect to exon 1 (-30delC relative to the initiation codon, L17L, I27L) and in 5 patients with respect to exon 4 (G288G, IVS4nt+64G>T, IVS4nt+42A>G) of the HNF-1α gene in the population set under investigation. Normal individuals did not show any polymorphism. **Conclusions:** In about 12% of the patients with late onset of diabetes (age of onset between 51 to 60 years), I27L polymorphism was detected.

KEYWORDS: Type 2 diabetes, late-onset of diabetes, hepatocyte nuclear factor 1α, single nucleotide polymorphism, I27L polymorphism

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Introduction

Hepatocyte nuclear factor 1-alpha (HNF-1α) gene located on the chromosome 12 (12q24.2) codes for a transcription factor, which is expressed in pancreatic β-cells and the hepatocytes. Mutations in HNF-1α gene have been shown to cause maturity-onset diabetes of the young (MODY 3) which is proposed to be a clinically heterogeneous group of disorders characterized by an autosomal-dominant mode of inheritance, where the onset is before the age of 25 years, with a primary defect of pancreatic β-cell function.[1] Variants in this gene have been described in Japanese,[2] Danish Caucasians,[3] Finnish, and Chinese[4] subjects with late-onset of the type 2 diabetes mellitus (T2 DM). Variations in the HNF-1α gene were also reported to cause type 1 diabetes mellitus (T1 DM) in Japanese subjects.[5-7] Thus, HNF-1α gene mutations cause both early- and late-onset of diabetes mellitus.

Evidence for the mutational hotspot in exon 4 of HNF-1α has been demonstrated by the studies in Germany[8] and in Finnish and North American populations.[9] A prevalent amino acid polymorphism at codon 98–Ala98Val (exon 1) of the HNF-1α was shown to be associated with diabetes in the South Indian population.[10] Therefore, this study was designed to investigate the role of HNF-1α gene with reference to exon 1 and exon 4 in the etiology of diabetes in a sample of Western Indian population in Maharashtra.

Materials and Methods

Blood samples from 100 patients (11 T1 DM, 85 T2 DM, and 4 impaired glucose tolerance [IGT]) were collected, diagnosed according to the WHO criteria. Blood samples were also collected from 50 normal individuals. Informed consent was obtained from all the patients participating in the study [Table 1].

DNA was isolated from leucocytes by phenol chloroform method. Exon 1 and exon 4 were PCR amplified using primers described earlier[1]—Exon 1: forward primer (5′-3′)—GGCAGGCAAACGCAACCCACG and reverse primer (5′-3′)—GAAGGGGGGCTCGTTAGGAGC; Exon 4: forward primer (5′-3′)—CAGAACCCTCCCCTTCATGCC and

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reverse primer (5′-3′)—GGTGACTGCTGTCAATGGGAC. PCR reaction was set in a volume of 25 µL: 1× buffer, 1.5 mM $MgCl_2$, 150 µM dNTP (Promega), 12 pM of each primer (Invitrogen), 0.1 U/µl *Taq*DNA polymerase (Invitrogen/Promega), and 100 ng genomic DNA. The PCR conditions were: initial denaturation at 95 °C for 5 min followed by 35 cycles of denaturation (94 °C for 45 s), annealing (62 °C for 30 s), and extension (72 °C for 1 m 45 s). This was followed by final extension at 72 °C for 10 min.

Preliminary analysis of the PCR samples was performed with the help of single stranded conformational polymorphism (SSCP) which was performed with 7.5% acrylamide gel. The SSCP protocol which was followed, was modified from the method described earlier.[4,9] 15 µL of the loading dye (95% formamide + 0.05% BPB + 0.05% xylene cyanol) was added to 2 µL of the PCR product. This was kept at 95 °C for 5 min; snap cooled and immediately loaded on the 7.5% acrylamide gel. Gel was electrophoresed at 150 V for approximately 2 h. The gel was then silver stained according to the following procedure: gel fixed with 10% acetic acid for 20 min, two washes of 10 min each; washed with MilliQ water for 9 min, three washes of 3 min each; stained with freshly prepared solution of – 0.150 g $AgNO_3$ (silver nitrate, Merck) + 150 µL formaldehyde (Sigma) + 100 mL MQ water for 30 min; washed with 100 mL MilliQ water for 90 s, three washes of 30 s each; developed with freshly prepared developer – 3 g Na_2CO_3 (sodium carbonate, Merck) + 100 µL formaldehyde (Sigma) + 20 µL of sodium thiosuphate (Merck) solution from 10 mg/ml stock + 100 mlLMilliQ water – 3 min; fixed with 10% acetic acid and dried.

Direct sequencing of the samples was carried out with the kit from Applied Biosystems (Big Dye Terminator 3.1) using the ABI prism 3730 sequencing machine. Examples of the SSCP of the PCR amplified exon 1 and exon 4 of the patients are depicted in Figure 1.

Results

Polymorphisms were detected in 12 patients with respect to exon 1 (-30delC relative to the initiation codon, L17L, I27L) and in five patients with reference to exon 4 (G288G, IVS4nt+64G>T, IVS4nt+42A>G). Table 2 summarizes type of polymorphism detected and the number of patients who showed the respective polymorphism. Normal individuals did not show any polymorphism.

Studies have reported that the I27L polymorphism is associated with the increased risk of T2 DM, particularly in the elderly and overweight (BMI > 25 kg/m^2).[11] In the current investigation also, it is seen that the I27L polymorphism was found in patients with late-onset age of T2 DM and BMI of four patients out of the five who showed the I27L polymorphism was more than 25 kg/m^2 [Table 3]. Investigations have showed that the subjects with LL genotype were observed to be more insulin-resistant than the subjects with IL and II genotypes.[12] In this study, in spite of the patients taking oral medication

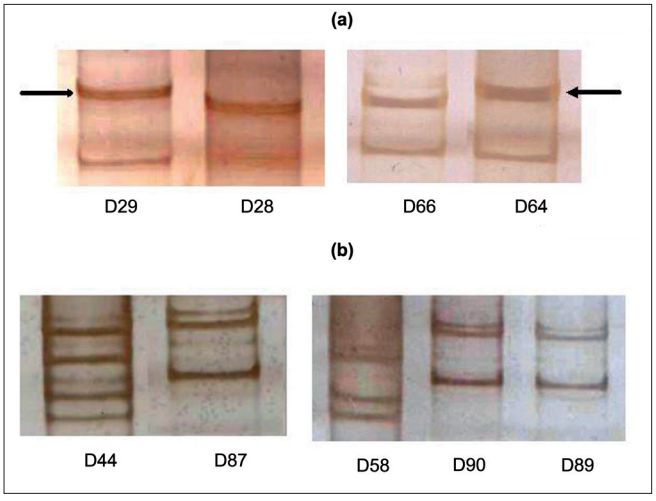

Figure 1: (a) SSCP of PCR amplified exon 1 of the patients. Samples D29 and D64 show a shift in the mobility of the band (shown by arrow) due to the polymorphism L17L and I27L, which was confirmed by sequencing. Samples D28 and D66 had no polymorphism in exon 1. (b) SSCP of PCR amplified exon 4 of the patients. Samples D44 (G288G) and D58 (G288G and IVS4nt+42A>G) show a shift in the mobility of the band due to polymorphism which was confirmed by sequencing. Samples D87, D89, and D90 had no polymorphism in exon 4.

Table 1: Patients included in the molecular analysis with reference to HNF-1α gene

Type 1 DM		
Total patients: 11		
Age of onset in years	Female	Male
5–15	4	2
16–25	3	2
Type 2 DM		
Total patients: 85		
Age of onset in years	Female	Male
20–30	1	1
31–40	5	6
41–50	6	25
51–60	16	18
61–70	2	5
IGT		
Total patients: 4		
Age of onset in years	Female	Male
41–50	1	–
51–60	2	–
61–70	1	–

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Table 2: Polymorphisms detected in exon 1 and exon 4 of HNF-1α

Polymorphism	Number of patients, DM type, sex and age of onset in years	Total number of patients
Exon 1		
-30delC relative to the initiation codon	1 T2 DM (male, 60)	1
-30delC relative to the initiation codon and L17L: CTC>CTG	1 IGT (female, 50) and 1 T2 DM (male, 45)	2
L17L: CTC>CTG and I27L: ATC>CTC	4 T2 DM (male, 53; male, 51; male, 48; female, 59)	4
L17L: CTC>CTG	3 T2 DM (female, 43; male, 54; female, 20) and 1 T1 DM (female, 12)	4
I27L: ATC>CTC	1 T2 DM (male, 59)	1
Exon 4		
G288G: GGG>GGC and IVS4nt+64G>T	1 T2 DM (male, 50)	1
G288G: GGG>GGC	2 T2 DM (male, 50; male, 70)	2
G288G: GGG>GGC and IVS4nt+42A>G	2 T1 DM (male, 22; female, 22)	2

Table 3: Clinical characteristics of the patients with I27L polymorphism

Patient ID	D29	D61	D64	D65	D89
Genotype	IL	IL	IL	IL	LL
Sex	Male	Male	Male	Male	Female
Age of onset (years)	53	51	48	59	59
BMI (Kg/m^2) Normal: 25 kg/m^2	27.12	25.16	28.76	24.28	25.61
Waist–hip ratio	0.84	1	0.93	0.91	0.77
Fasting sugar (mg%) Normal: 70–110	104	100	105	216	134
Fasting serum insulin (ul U per mL) Normal: 2–25	19.82	18.29	23.45	19.02	33.88

Note: Fasting glucose: Blood glucose estimation was done by GOD/POD method; Fasting Insulin: Techniques and kits used: CLIA—Fully automated. Chemiluminescence system, Alpha Lite, France.

for the treatment of T2 DM, the LL genotype has shown insulin resistance compared to the IL genotype [Table 3].

Discussion

The investigation with reference to the South Indian population concluded that Ala98Val polymorphism of HNF-1α gene is associated with MODY 3 and with earlier age of onset of T2 DM among Asian Indians.[10] This study in Western Indian population reports polymorphism in the HNF-1α gene, thereby giving further evidence for the presence of variation in this gene is associated with diabetes in Indians from the Western part of the country as well. South Indian population study showed the association of HNF-1α variation with early-onset of diabetes[10] in contrast to the present analysis which reports the association of late-onset of diabetes with HNF-1α polymorphism. The Val allele (exon 1, codon 98 of HNF-1α) was reported to be associated with earlier diagnosis of diabetes in South Indian population,[10] whereas the present investigation demonstrates the association of Leu allele (exon 1, codon 27 of HNF-1α) with late-onset of diabetes.

This investigation is the first report of genetic polymorphism in an Indian population in Maharashtra with diabetes, with reference to the HNF-1α gene, which has resulted in identification of some polymorphisms in its coding as well as in the non-coding regions. This study has identified I27L polymorphism in about 12% of the patients with late-onset of diabetes (age of onset between 51 and 60 years), from Western Indian population who showed mild complications (hypertension). The location of isoleucine at 27th position is conserved in many species in the HNF-1α.[13] As the I27L polymorphism occurs within the dimerization domain of HNF-1α and is also a highly conserved amino acid;[13] this polymorphism could have a probable biological significance, which remains to be demonstrated. Therefore, the study of this polymorphism on larger sample size of diabetes patients and the associated phenotype would help in analyzing its association with diabetes and defining its possible role, which is not clearly understood. In summary, from the results of the earlier study in South Indian population[10] and this study in Western Indian population, exon 1 of HNF-1α appears to be polymorphic with reference to the Indians. Further studies with the population from the rest of India would substantiate whether this is indeed true only for the Southern and Western population or also for general Indian population. Since establishing associations becomes useful for genetic susceptibility and pharmacogenomic analysis, this will provide a background assessment of the population and diversity.

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