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Identification of (Ala98Val) and (Ile27Leu) mutation in exon 1 of *HNF1-α* (MODY 3) And their Association with early-onset Diabetes in Sudanese families

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ABSTRACT
Maturity onset diabetes of the young (Mody3) is a monogenic form of diabetes. Gene defects in the Hepatocyte Nuclear Factor -1 alpha (*HNF1-α*) causes MODY3. *HNF1-α* gene located in the chromosome (12q24.2).A prevalent amino acid polymorphism at Ala98Val,I27L inexon-1 of *HNF1-α* was shown to be associated with diabetes in different study in many countries Asian, there for the aim of the study to see the classification and percentage of the patients have MODY have mutations of *HNF1-α* (MODY 3) gene with references to exon 1 by amplifying and sequenceing the coding regions of the gene in the etiology of diabetes in a sample of Sudanese families in Khartoum State. This was a case control study, conducted at the period from October 2013 to August 2017, in Khartoum state. Blood samples were collected from 80 diabetic patients, and 80 non-diabetic controls. Age, sex, weight, and height were recorded. Biochemical profiles of blood glucose level, HbA1c and C-peptide were estimated. From extracted DNA, exon 1 in *HNF1-α* gene was amplified using Polymerase Chain Reaction (PCR). Restriction Fragment Length Polymorphism (RFLP) was carried out to identify the occurrence of A98V mutation. Then DNA sequencing was done to detect other mutation in coding region of exon 1. *HNF1-α* A98V mutation was investigated in all patients and controls by PCR- RFLP, this mutation was not detected in all participants, and the result was confirmed by using DNA sequencing, while other two SNPs missense(I27L) and synonymous (L17L) were also detected. The study showed the occurrence of two mutations I27L &L17L, in one Sudanese diabetic family while L17L mutation was detected alone in 4 different Sudanese families

KEYWORDS: hepatocyte nuclear factor1α, MODY3, PCR- RFLP, mutation, TCF1.
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المستخلص
سكري الشبان الا ضجين يرمز إلى حالة مختلفة عن السكري الأول والثاني اللذان يتطلبان عوامل جينية وبيئية أكثر تعقيداً .
الجِ ن *HNF1-α* احدى الجينات المسؤولة عن تطور سكري الشبان الناضجين. وقد تم اخذ 80 عينة دم من 9 عوائل لذين

لهم تاريخ مرضى للسكر الوراثى و80 شخص من الاصحاء من ذين ليس لديهم تاريخ مرضى للسكر فى العائلة لمسح جين *HNF1-α* للطفرا ات الوراثية (le27Leu ,Ala98Val,) فى الاكسون 1 (xon-1) باستخدام نظام التحليل (PCR-RFLP) لم يتم تحديد الطفرة الوراثية المسببة لسكرى الشباب , وبارسال بعض العينات لتحليل ال DNA sequences تم تحدد طفرة وارثية مسببة للسكرى (Ile27Leu) فى اربعة اشخاص من اسرة واحدة، ويعتبر هذا البحث الاول فى السودان بالنسبة للطفرات الوراثيةالمسببة لمرض سكري الشبان الناضجين وبان الفحص الجيني هوالطريقة المثلي في التشخيص المبكر، تحديد نوعية العلاج ومتابعة تطور المرض

INTRODUCTION

Maturity-onset diabetes of the young (MODY) is a clinically heterogeneous group of disorders inherited by autosomal dominant allele, the occurrence of diabetes usually before the age of 25 year, that result in reduction of insulin secretion. MODY can be a result of mutations in any of at least the following six genes; *HNF-1β, HNF-4α,* hepatocyte nuclear factor *HNF1-α* insulin promoter factor-1, neurogenic differentiation 1/β-cell E-box transactivator 2 and glucokinase enzyme) (Bjørkhaug L, et al.2003).

HNF1-α (OMIM 142410) is expressed in the pancreatic α-cell and mutations in this gene lead to α-cell dysfunction and diabetes mellitus. The most common cause of MODY diabetes in great majority of population' heterozygous mutation in *HNF1-α* gene (Shields, et al.2010; Winckler W, et al 2007). Mutations in *HNF1-α* gene are reported in up to 60%and 1–2% of all patients with diabetes (Murphy R, et al. 2008). It has been shown that common variants in the *HNF-1α* gene (I27L, A98V) are associated with an increased risk of type 2 diabetes (Holmkvist J, et al. 2008; Weedon MN, et al. 2005).

Hepatocyte nuclear factor-1 alpha (*HNF1-α*), now known as transcription factor-1 [*TCF1*]), which is responsible for MODY3, is a transcription factor regulating a number of liver- and beta-cell-specific genes. The gene is located on chromosome 12q24, (Lindgren CM, et al. 2002; Wiltshire S, et al. 2004; Reynisdottir I, et al. 2003). Common variations in the *HNF1-α* gene have been associated with impaired insulin secretion (Urhammer S, et al. 1997; Chiu KC, et al. 2003)

The *HNF1-α* gene composed of 3 functional domains, which contains 10 exons codes for 628 amino acids. The domains are as follows; a short N terminal dimerization domain encoded by exon 1, a homeobox DNA binding domain encoded by exons 2,3,4 and a C-terminal transactivation domain encoded by exons 5-10(Kaisaki, et al. 1997).

A prevalent amino acid polymorphisms I27L (rs1169288)and A98V(rs1800574) of *HNF-1α* was shown to be associated with diabetes in, Western Indian population (Shekher Anuradha M, et al. 2005). U K Caucasians (U .K. 2005). Nepal (Shakya P, et al.2014). Southern Brazil (Bonatto N, et al. 2012). Czech republic (S. Pruhova1, et al. 2003). USA (Holmkvist J, et al. 2006). Therefore this study was designed to determine the incidence of different types of mutation *HNF-1α*, among type 2 Sudanese diabetic families in Khartoum state

MATERIALS & METHODS

Subjects

A case control study of Type 2 diabetes conducted to evaluate the polymorphisms of *HNF1-α* gene mutation in nine Sudanese families in Khartoum State. Type2 diabetes was diagnosed according to WHO criteria (WHO. 2006). A total number of 80family

members33 males and 47 females, whose clinical presentation was suggestive of MODY3. Inclusion criteria were: early onset (by 25 years) of diabetes, mild hyperglycemia, no autoimmune markers, without obesity and positive family history of diabetes for at least two consecutive generations for most of the patients. Informed written consent was obtained from all families.

Clinical studies

Blood samples were obtained in Fluoride Oxalate and EDTA tubes from all participants after an overnight (8-12-h) fasting. Plasma was used for the determination of blood glucose, glyco-Sylated hemoglobin (HbA1c), and C-peptide determination

DNA extraction

Genomic DNA from all patients and controls were extracted from whole blood using Genomic DNA Purification kit according to manufacturer recommend-dations (Blood DNA Preparation Kit, Jena Bioscience, and Germany). Extracted DNA was quantified and then used for PCR amplification .

Genotyping of SNPs in exon 1

Primer design

The following primers were designed by Primer3 software-
Forward primer, 5
AGTTTGGTTTGTGTCTGCCG 3;
And reverse
primer,5GGTCATGGGGACTCAACTCA 3
(Macrogen, Korea).

Polymerase chain reaction (PCR)

The PCR was carried out in a total volume of 25 µL containing; 5 µLPCR premix (iNtRON Biotechnology, Korea), 1 µL of each primer, 2 µL DNA and 16 µL D.W. The PCR conditions set were: Initial denaturation (95°C for 5 minutes), followed by 35 cycles of second denaturation (95°C for 30 s), annealing (61°C for 30 s), extension (72°C for 30 s), and a final extension (72°C for10 min).DNA bands size was estimated in relation to standard 100bp DNA ladder .

Restriction fragment length polymorphism (RFLP)

PCR-RFLP was used to screen for A98V polymorphism of the HNF1-αgene. The amplified fragment was cut with restriction enzyme *Hae*III and the result was confirmed by DNA sequencing. Three units of the enzyme *Hae*III was incubated with 15µL of PCR products at 37˚C for 24hrs. The cleaved products were detected by electrophoresis on 3% agarose gel after staining with ethidium bromide (0.5 g/ml). The variations in the digested fragments were visualized under UV gel-documentation system (Table 1).

Table 1. The restriction site and RFLP of *HNF1a* gene

Analyzed HNF1A region	Restriction enzyme	PCR product Size (bp)	Restriction site	RFLP Fragment size (bp)	
				Normal	Mutant
Exon1	*HaeIII*	525	5’ …GG▼ CC... 3’ 3□... CC ▲ GG... 5□	167 80 61	253 57

Sequencing of HNF-1α Gene

DNA purification and standard sequencing was performed for both strands of HNF1-α Gene by Macrogen Company (Seoul, Korea). The sequences chromatogram was viewed by FinchTV program (http://www.geospia.com/products/finchtv.s html).

Then the nucleotides sequences of the HNF1-α- gene were matched to similar sequences using nucleotide BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi.) (Atschul SF, et al. 1997). Highly similar sequences were retrieved from NCBI and subjected to multiple sequence alignment using BioEdit software (Hall TA 1999). Chimera and I-mutant softwares were used for prediction of effect of I27L on HNF1 alpha protein (NP_001293108.1) structure and function. (Huang CC, et al. 2014; E.Capriotti, P. et al. 2005).

RESULTS

Demographic data

The number of the study population was 80 individuals from 9 different families who had strong family history of diabetes (3 or more successive generations). 33 males (41.25%) and 47 females (58.75 %), the age of the nine family members ranged between 7 to 75 years old.

Biochemical investigations

Biochemical measurements were performed with a COBAS INTEGRA 400 plus system for Glucose and HbA1c and C-peptide with Elecsys and cobas immunoassay analyzers. Table2 shows the biochemical and anthropometric measurement of diabetic family member and control individuals.44 (55%) males and 36(45%), females the age range between 6 to 80 years old. The family members and the controls were in cross matching state regarding the age and sex ($p > 0.005$). Fasting blood glucose and HbA1c levels were highly significant among the family member than the controls ($p. = 0.000$). The mean Body Mass Index (BMI) was 26.1 ±4 among the diabetic family members and 24 ±4.0 among the control ones, ($p. = 0.004$). C-peptide level showed highly significant difference between diabetic families (2.0 ± 0.7) and control (2.4 ±0.6), ($p. = 0.004$).

Table 2. Biochemical anthropometric measurement of diabetic families and control subjects

Parameter	families members (N= 80) [1] (mean ± SD)	Controls (N = 80) (mean ± SD)	p. value
Age (mean ± SD)	35.6 ± 16.9	31.4±13.1	0.078
Sex (F/M)	(47/33)	(42/38)	0.157
BMI (kg/ m^2) [2]	26.1 ±4.0	24 ±4	0.004
Underweight < 20 (kg/ m^2)	7 (9%)	11 (13.7)	
Normal weight 20 – 25 (kg/ m^2)	25 (31%)	37 (46.3%)	
Overweight 26 – 30 (kg/ m^2)	31(39%)	28(35%)	
Obese > 30 (kg/ m^2)	17 (21%)	4 (5%)	
FBS (mg/dL) [3]	196 ± 84	99 ± 14	0.000
HbA1c (%)[4]	6.6 ±2	5.2±3	0.000
C-peptide (nmol/L)[5]	2.0 ± 0.70	2.4 ±0.6	0.000

[1]Data is represented as mean ± SD.
[2]BMI: Body Mass Index.
[3] FBS: Fasting Blood Sugar; (80 – 126 mg/dL)
[4]The reference range (HbA1c =3.5 – 6.5%)
[5]The reference range of C-peptide is 0.8-3.1 ng/mL with type I < 0.8

HNF-1α gene A98V polymorphism

The A98V MODY3 mutation was absent in all subjects, by using PCR –RFLP (Fig. 1, 2).

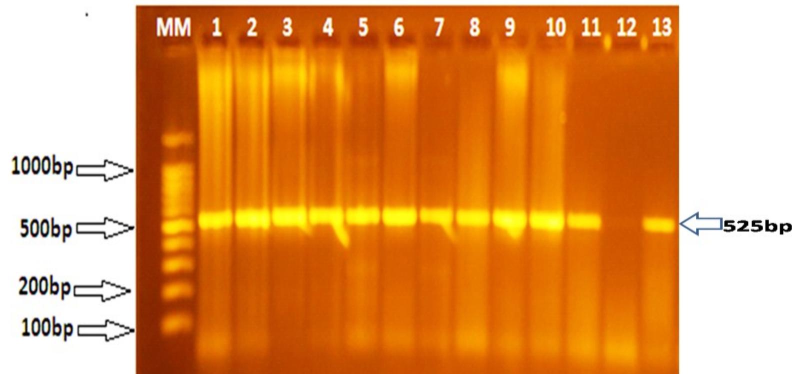

Fig. 1: PCR amplification of HNF1-α gene on 1.5%agarose gel electrophoresis.

Lane MMDNA ladder: (100 bp).

Lane (1- 13) showing typical band size (525) corresponding to the molecular size of MODY3gene,

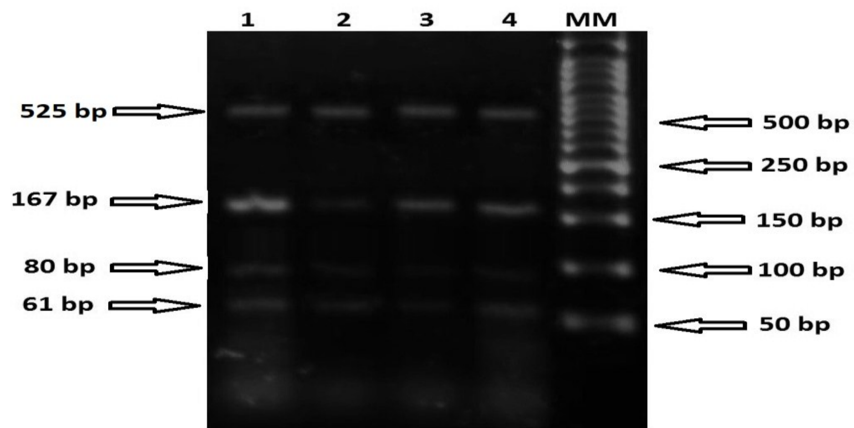

Fig. 2: 3% agarose gel showing restriction products of exon 1 of HNF1-α gene by HaeIII

Lane MM: 100 bp DNA ladder. Other Lane: Restriction PCR product

DNA sequencing

The DNA sequencing confirmed the absence of A98V mutation ;(Fig. 3).Two mutations were detected in theHNF1-αgene.One of them is missense mutation (I27L: ATC>CTC) found in4 patients from the same family (their body mass index showed overweight) and the other is nonsense mutation (L17L: CTC>CTG) in 4 different families (Fig. 4 and 6), (Table 3)

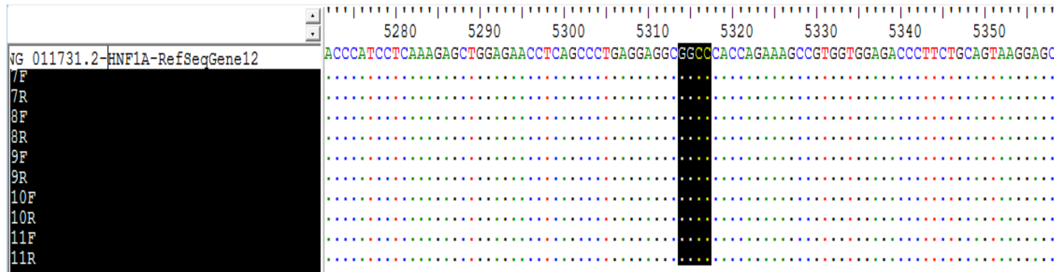

Fig. 3. Alignment determining by using BioEdit software. Highlight sequences Indicated the position of *Hae*III cutting site in A98V MODY3 (exon 1)

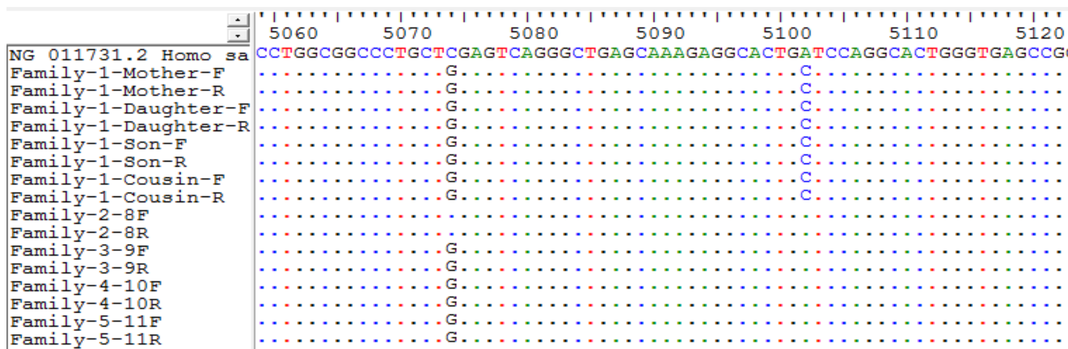

Fig. 4) HNF1-αgene multiple sequence alignment for five families show mutant CTC>CTG (L17L) and mutant ATC> CTC (I27L).

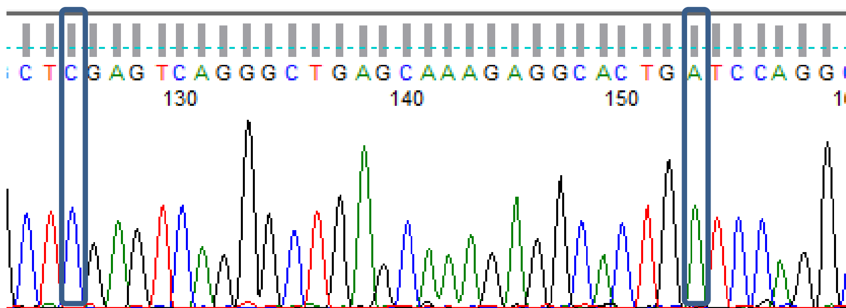

Fig. 5): *HNF1-α* gene normal sequences chromatogram

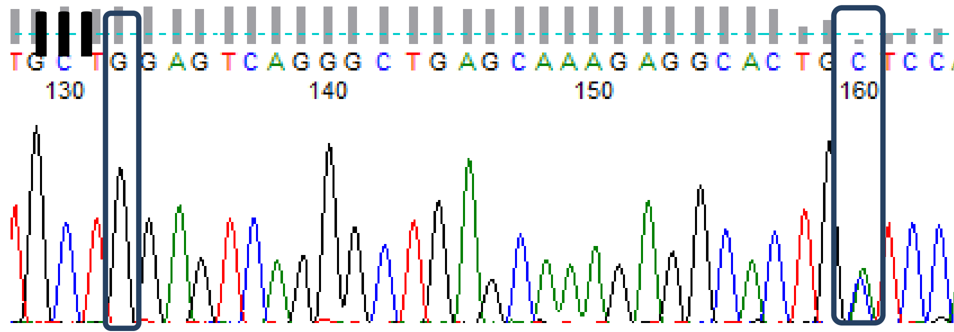

Fig. 6): *HNF1-α* gene mutant sequences chromatogram

Chromatogram of *HNF1-α* gene, the square sequences begin with mutant G and end with mutant C (hnf1 mutant). The Chromatogram was viewed by Finch TV program

Table 3.biochemical anthropometric measurement for the Family 1.

Family name	Age	[1]BMI (kg/m2)	[2] FBS (mg/dL)	[3] C-peptide	[4]HbA1c (%)
mother	55	27.7	2 17	1.7	6.9
daughter	19	29.1	380	1.5	11.2
son	23	28.9	199	2.2	6.8
cousin	22	29.4	124	2.9	5.2

[1] Overweight 26 – 30 (kg/ m^2)
[2]normal FBS (80 – 126 mg/dL)
[3]The reference range of C-peptide is 0.8-3.1 ng/mL with type I < 0.8
[4]The reference range (HbA1c 3.5 – 6.5%)

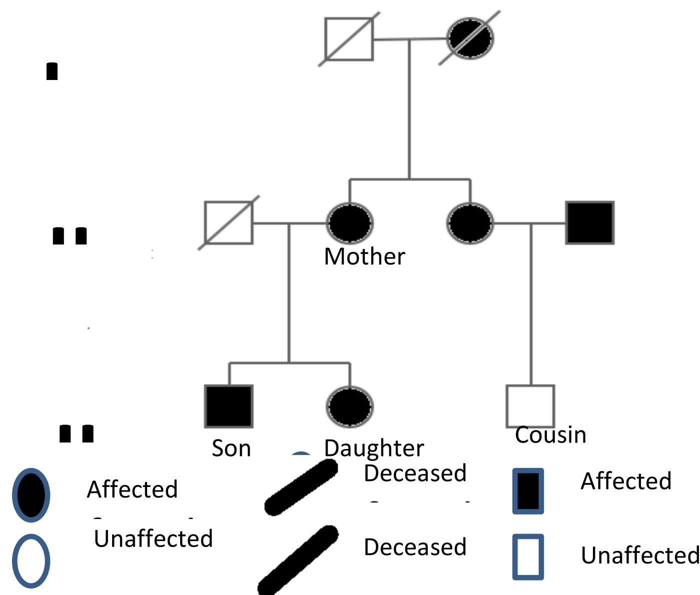

Figure 7.Family 1 pedigrees tree.

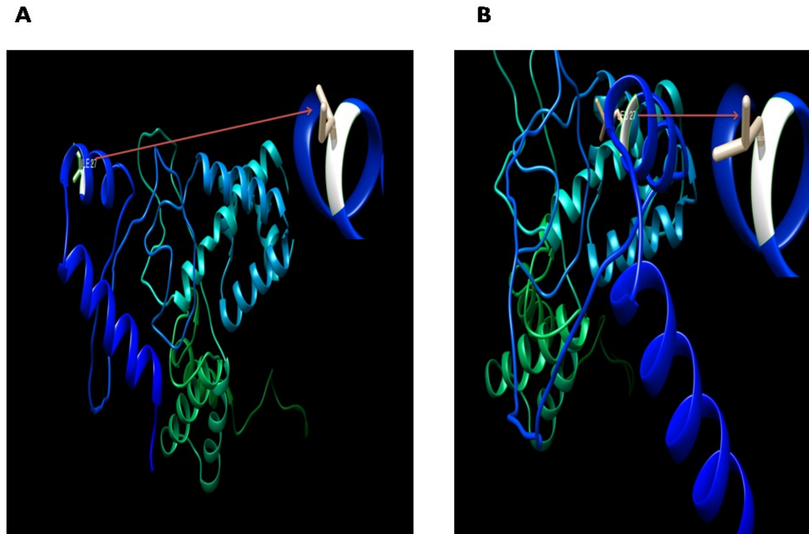

Fig. 8: Homology modeling of the *HNF1-α* protein with and without p.I27L

Homology modeling of the *HNF1-α* protein,
A=showed normal Isoleucine at position 27;
B=showed the mutant leucine at position 27.By using (Chimera software version 1.9.)
The mutation p.I27L in exon 1 of HNF1-αis located between a α-helix on the surface and a loop in the protein Structure,

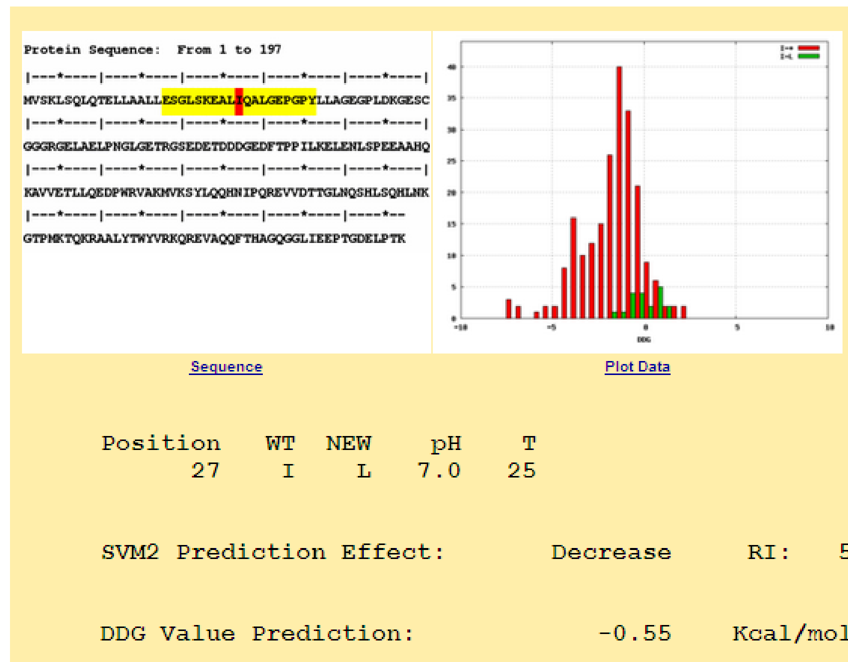

Fig. 9: Predictor of protein stability change

Predicting stability changes at position p.I27L mutation result in decrease in protein function

DISCUSSION

HNF1-α is a common cause of MODY in many populations, with more than 193 mutations being described (S. Ellard and K. Colclough, 2006). Defective insulin secretion is the hallmark of patients with *HNF1-α* (i.e. MODY3) mutations (Lehto M, et al. 1997). In the present study the MODY3 genes *HNF1-α* in Sudanese diabetic families were screened. This is the first report to investigate a possible association between non insulin-dependent Diabetes Mellitus and SNPs from *HNF1-α* gene in Sudanese population.

In spite of Ala98Val MODY3 mutation was detected in South Indian (Anuradha S et al. 2005). Nepal (Shakya P, et al.2014). U.K. Caucasian (Chiu KC, et al. 2003). And Iran population (Sepahi S, et al. 2013). This mutation was absent in all investigated Sudanese subjects by using PCR–RFLP and DNA sequencing. This may be due to difference in populations or sample size. A study with larger samples size from different parts of Sudan would help to establish exact relation between Ala98Val polymorphism and diabetes in Sudanese population

DNA sequencing of exon 1 of *HNF1-α* revealed two mutations (I27L, L17L) other than Ala98Val, in one Sudanese diabetic family (family 1).This result is in line with other studies conducted in Western Indian(Ranade SS, et al. 2010). And Southern Brazilian (Bonatto N, et al. 2012). population, where two mutations were detected in the same population. While synonymous L17L mutation was detected alone in 4 different Sudanese families. This finding is correlates with results obtained in Czech (S. Pruhova1, et al. 2003). And Chinese families (Yang Y, et al. 2016).

I-mutant and Chimera software revealed that the presences of I27L SNP in *HNF-1α,* affected protein function and structure. This may be due that this SNP located at highly conserved region and also occurs within the dimerization domain of *HNF-1α* (Giuffrida FMA, et al. 2009). The second finding of this study is that body mass index of all 4 patients of the missense mutation (I27L) are overweight, this observation in close agreement with study of Ranade et al. (2010) in Western Indian population (Ranade SS, et al. 2010). And Luna-arias and Boldo-le, (2016) in Norway population, they found there is association between overweight (Luna-arias JP, et al 2016).

and I27Lpolymorphism. On the other handI27Lpolymorphism found as significant risk factor of non insulin-dependent Diabetes Mellitus in normal-weight subjects in Japan (Morita K, et al. 2014). There was one diabetic patient without any mutation in exon 1 of HNF1-α. The cause of diabetic in this patient may be due to presence of other mutation in other exons of this gene or in other MODY genes, that not covered by our study. A study on larger number of sample can help to establish adjust relationship between I27L polymorphism and diabetes in Sudanese population.

CONCLUTION

This study revealed for the first time in Sudan the absence of Ala98Val in patients with diabetes. While this study detect the presence of other two mutations (Ile27Leu and Leu17 Leu) that have been previously reported in patient with diabetes.

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