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# Frequencies of *Insulin- Promoter Factor-1* (*IPF-1*) gene mutations in a Cohort of Sudanese Patients with Type 2 Diabetes Mellitus

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**ABSTRACT**

Diabetes mellitus (DM) refers to a group of metabolic disorders with defective insulin secretion, insulin action or both. *Insulin promoter factor-1* (*IPF-1*) gene plays a central role in the development of the pancreas and regulation of insulin gene expression in β cells. This study aimed to determine the frequencies of *C18R,D76N,* and *R197H* mutations in the coding region of the *IPF*-1gene in a cohort of Sudanese patients with type 2 DM. Following informed consent, 96 individuals in eleven families with one or more diabetic members.DNA was extracted from EDTA-venous blood and buccal washes using the phenol-chloroform-iso-amyl alcohol (PCI) technique. Three variants (*C18R, D76N,* and *R197H*) were screened for by PCR-RFLP and the following primers:
[F: CATGAACGGCGAGGAGCAG][R: GCCATGTACAGGCACGCAG]
[F: TCCCGTACGAGGTGCCCCCCCTCGCCGTC] [R: CGGTTGGGCTCCTCCAGGAC]
[F: GGTGGAGCTGGCTGTCATGTTG] [R: AGGGCTGTGGCGACGCGTAAG] primers and:
[*NlaIII*, *SalI* and *Fnu4HI*] restriction enzymes for C18R, D76N and R197H mutations respectively.
A third (31/96, 32.3%) of the study individuals were diabetics with a mean age of 53±14.2years compared to 28±17.1years in non-diabetics. More than 95% of the diabetics were in the first and second generations. *C18R* gene mutation was detected in 3.2% of the diabetics and in 4.9% of non-diabetics. The *D76N* was seen in 3.1% of non-diabetic subjects only. The *R197H* gene variant was not detected in the study population. There was a strong correlation between maternal history of DM and the incidence of diabetes in the study families. *C18R* and *D76N*genes mutations play little part in the development of DM type 2 in Sudanese patients. Maternal family history correlates strongly to the development of type 2 DM.

## 1. INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder of multiple etiologies that is characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolisms. Defects in insulin secretion or insulin action or both are the most important mechanisms behind the metabolic derangement. Worldwide prevalence of DM has risen dramatically over the last decades. It is projected that the number of individuals with DM will continue to increase in the near future. There is considerable geographic variation in the incidence of type 1 and type 2 DM with Scandinavia having the highest rate of type 1 DM, while the lowest rates were seen in Pacific Rim. Type 2 DM is more prevalent in certain Pacific Islands compared to India, the United States, Russiaand China. This variabilityis mostlikely due to genetic and environmental factors. There is an also considerable variation in DM prevalence among different ethnic populations within a given country. It has been estimated that a third of the children born in the year 2000 will suffer from diabetes at some point in their lifetime [1, 2, 3, 4].In Sudan, DM is a growing health problem with an increasing incidence in all socio-economic classeswith major impacts on the economy. DM is associated with a high prevalence of complications and a low quality of life [5, 6, 7].Diagnosis and classification of DM is now unified based on WHO and the National Diabetes Data Group (NDDG) guidelines [8, 9].

Genetic defect of beta-cell function, is frequently characterized by onset of mild hyperglycaemia at an early age (<25 years). Patients with these forms of diabetes, formerly referred to as maturity onset diabetes of the young (MODY 1-4)with impaired insulin secretion with minimal or no defect in insulin structure.Genetic defects in insulin action that have metabolicabnormalities associated with mutations of the insulin receptor may range from hyper-insulinaemia, modest hyperglycaemia to symptomatic DM.Insulin counter-acts

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the concerted action of a number of hyperglycaemia-generating hormones maintaining low blood glucose levels. It stimulates lipogenesis, diminishes lipolysis and increases amino acid transport into cells. Insulin also modulates transcription, altering the cell content of numerous mRNAs. It stimulates growth, DNA synthesis and cell replication effects that it holds in common with the insulin-like growth factors (IGFs) and relaxin[10].

Type 2 DM is a heterogeneous multifactorial disease that is caused by both genetic and environmental factors with a minority of cases caused by single-gene defect such as MODY. The candidate gene approach has identified several genes that regulate insulin signaling and secretion, but their contribution to DM are small. Recent genome scan studies have been conducted to identify major susceptibility loci that are linked with type 2 DM. Increasing evidence suggests that defects in genes encoding transcription factors that are expressed in the pancreatic β cells may be important contributors to the genetic basis of type 2 diabetes mellitus [11, 12, 13].An important transcription factor is the insulin promoter factor-1 (*IPF-1*)/pancreatic duodenal homeobox-containing factor-1 (PDX-1).*IPF-1* plays a central role in the development of the pancreas and the regulation of the insulin gene expression in the mature pancreatic beta cells [14, 15, 16].Human *IPF-1*contains two exons and spans a regionof about 6 kb on human chromosome6 bands 13q12.*IPF-1* is a homeodomain-containing proteinthat is thought to be a key regulator of pancreatic islet development and insulin gene transcription in β-cells. The human *IPF-1* gene coding region shows 83% nucleotide identity with the mouse *IPF-1* gene and was encoded for by two exons that extended over a 5 kb region of human genome.The *IPF-1*gene deletion results in a frame shift at the COOH-terminal border of the trans-activation domain of *IPF-1*, resulting in a protein that lacks a domain that is crucial for DNA binding. Probandshomozygous for this mutation completely lack pancreatic tissue [17, 18, 19, 20, 21].

Three novel *IPF-I* missense mutations (*CI8R, D76N and R197H*) in patients with type 2 diabetes demonstrated decreased binding activity to the human insulin gene promoter and reduced activation of the insulin gene in response to hyperglycaemia in the human β-cell line Nes2y. These mutations are present in 1 % of the population and predispose the subjects to type 2 diabetes with a relative risk of 3.0. These mutations are a rare cause of MODY and pancreatic agenesis [12, 16, 22].Furthermore, these variants can be as high as 6% in subjects with a strong family of type 2 DM [23]. Other reports from the Far East suggested that *IPF-1*mutations are not commonly associated with type 2 DM [24].

## 2. MATERIALS & METHODS

The study protocol was reviewed and ethically approved by the Scientific and the Ethics Committeesof the Institute of Endemic Diseases, University of Khartoum. Written Informed consents were obtained from all individuals.This was a cross-sectional,prospective, analytical and community-based study. Consenting families with adocumented history of type 2 DM with one or more members were enrolled. In addition,an extendedfamily of17 members with an unconfirmedfamily history of DM type 2 from the centre was also enrolled.

Demographic and clinical data was collected with specialemphasis on duration of DM, type of treatment, history of maternal DM, glucose level control, history of hypertension, renal problems, retinopathy, periodonitis/dental caries, children affected with DM, family history of obesity and dietary history.

Five mls of blood were collected in EDTA vacotainers from adults and 15 mls of buccal wash in phosphate buffered saline(PBS) were collected from children.

DNA was extracted from venous blood using either phenol cholorformisoamyl alcohol or salting out techniques as described by Sambrook et al. 1989 and Miller, 1988[25, 26] respectively.

DNA from the deposit of the buccal washes was extracted using the guanidine chloroform method. Breifly: centrifugemouth wash fluid $10X10^3$ rpm,drain the supernatant. Add 2 ml of lysis buffer (STE+20%; SDS = 9: 1), 10 µl of proteinase K(10mg/ml), 1 ml of Guanidine chloride (6mol/l) and 300µl of Ammonium acetate to the pellet. Incubate at 55ºC for 2 hours (or at 37ºC for overnight). Leave tubes to cool at room temperature, transfer the mixture to 15 ml polypropylene Falcon tube and add 2 mls of pre-chilled cold chloroform (-20ºC), vortex and centrifuge for 3 minutes at 2500 rpm and leave for 1 minute to clear. Collect the upper layer in a fresh 15 ml polypropylene Falcon tube and add 10 mls of cold absolute ethanol. Stir and keep at $-20^0$C for at least 2 hours. Spin the tube at 3000 rpm for 15 minutes and drain the supernatant carefully and invert the tube on tissue paper for 5 minutes. Wash the pellet in 4 mls of 70% ethanol, centrifuge at 3000 rpm for 15 minutes.Drain the supernatantand leave the pellet to dry for 2-3 hours. For re-suspension, add 100 µl of sterile double distilled water, vortex and keep at 4ºC for 1-2 days. Aliquot DNA and keep at -20ºC for later analysis. The PCR was carried for C18R, R197H and D76N as described previously[16, 22, 16]. For each mutation a set of forward and reversed primers were used to amplify the target fragment. The following primers were used:

For *C18R*(fragment size 117 bp)
***C18R-F:***CATGAACGGCGAGGAGCAG
***C18R-R:***GCCATGTACAGGCACGCAG
*For R197H (fragment size 287 bp)*
***R197H-F*:**GGTGGAGCTGGCTGTCATGTTG
***R197H-R:*** AGGGCTGTGGCGACGCGTAAG
*For D76N (fragment size 146 bp)*
***D76N-F:*** TCCCGTACGAGGTGCCCCCCCTCGCCGTC
***D76N-R:*** CGGTTGGGCTCCTCCAGGAC

Two µ liters of template DNA were used in the 25 µl reaction mixture containing magnesium chloride 25mM; dNTPs (dATPs, dGTPs, dCTPs, dTTPs) 10 mM (Promega, Madison WI, USA), 0.2 µM from each of forward and reverse

primers(Biomers.net GmbH, Germany) and 0.3µm of *Taq* polymerase (Promega, Madison WI, USA). The reaction mixture was heated in a Flexigenethermal cycler ((Techne, UK)as follows: hot start at 94ºC for 7 minutes, this was followed by 35 cycles as follows: 94ºC for 1 minute (denaturing) , 61ºC for 1 minute (annealing), 72ºC for 1 minute(extension) and a final extension at 72ºC for 10 minutes. The PCR products were electrophoresed using 3% Agrose gel stained with ethdium bromide (SIGMA E.1510). A DNA marker of 50 bp (Promega, Madison WI, USA) was used in each run. The gel was visualized using Syngene documentation system, (Syngene LTD, USA).Ten µL of the PCR product were digested in a 20 µL reaction volume containing 2 µL of Nla III enzyme (Fermentas, Germany)2 µL of the 10X buffer (supplied by the manufacturer), and 16 µL of the nuclease-free water. Similarly, 10µL of the PCR product were digested in a 20µl reaction volume containing 2 µL of *Fnu4HI* enzyme (Fermentas, Germany), 16 µLof nuclease-free distilled water and 2 µL of 10X buffer. Finally, 10µL of PCR product was digested in 20µL reaction volume containing 2 µL of *SalI* enzyme (Fermentas, Germany), 2 µL of 10X buffer, and 16 µL of nuclease-free distilled water. The mixture was mixed gently and spun for few seconds, then incubated at 37ºC for 1-16 hours. Following incubation, the enzymes were inactivated at 65°C for 20 minutes. Then 4µL of the gel loading buffer (0.25% bromophenol blue, 40% sucrose in water) was added, and the samples were loaded onto 3% NuseiveAgarose gel (1:3). The digested fragments were visualized under UV light.Body mass index (BMI)was calculated using the formula: BMI= Weight (kg) / height$^2$ (m). Cutoff points recommended by the CDC were used to classify 20 year old and older adults as underweight (<18.5), normal weight (18.5 – 24.9), overweight (25.0 – 29.9), obese (>30.0). For children, BMI was corrected for age, also according to the CDC recommendations, with percentile cutoffvalues used to classify children as either underweight (< 5$^{th}$ percentile), healthy weight (5$^{th}$ – 84$^{th}$ percentiles), at risk foroverweight (85$^{th}$–94$^{th}$ percentiles) or overweight (≥95thpercentile). Data was collected and a master sheet was constructed. The data was analyzed using Statistical Package of Social Sciences (SPSS) version 11. Continuous data was expressed as mean and standard deviation. The significance of difference between two mean values among cases and non-cases group were determined by the Student independent t test, or ANOVA when comparing more than two means with $p<0.05$ considered significant.Significance testing of difference between proportions was conducted using the Chi-square test where applicable, adjusted by Pearson's or Fisher's exact test, depending on the number of observations, with a value corresponding to $p <0.05$ for significance unless otherwise stated.

## 3. RESULTS

### 3.1 Demographic and clinical data analysis of the study groups

Ten families with a history of late-onset type 2 DM (n=79), and one extended family with an unconfirmed history of DM (n=17) were enrolled. TheM:Fwas 1:2, with32% (31/96) diabetics. The study individuals were categorized into 1$^{st}$ generation (grandfathers and grandmothers), 2$^{nd}$ generation(sons and daughters)and 3$^{rd}$ generation (grandsons and granddaughters) with mean ages of 61.9±8.9, 44.1±12.1and 16.1±8.7 respectively (Table 1). The tribes of the enrolled families included:Gaaleen, Robatab, Nobioon (Mahas and Danagla), Shaygeya, Kawahla, Four, Noba and Dewaiheya (Table 1).Type 2 DM was present in more than half (53.3%) of the study population. The frequency of type 2 DM between the study tribes was not significantlydifferent ($p = 0.4$).

The mean of Body mass index (BMI) of the study population was 26.3 ±3.4 and was not significantly different between adult diabetics and non-diabetics ($p = 0.2$).A quarter (26.7%) of the individuals were in the habit of physical exercisewith no significant difference between diabetics and non-diabetics ($p = 0.1$) (Table 2).

Maternal history of type DM in of the 1$^{st}$ generation was significantly higher compared to the other generations ($p = 0.000$) (Table 2).Medical complications among 1$^{st}$generation diabetics were as follows: hypertension (46.7%; $p$= 0.001), retinopathy (20%; p= 0.04), renal problems (13.3%; p= 0.09), periodonitis(13.3%; p= 0.1) and dental caries (40%; p= 0.02). Only hypertension and dental caries were significantly different between diabetics and non-diabetics. Means BMI was not significantly different between diabetics and non-diabetics (26.4 ±3.8 and 26.2± 3.1 respectively ($p = 0.9$).

Fifty per cent of individuals from the 2$^{nd}$generation were diabetics with about a third (31.8%, $p = 0.1$)on regular physical exercise. Eighty eight per cent of the 2$^{nd}$ generation individuals had a family history of type 2 DM ($p = 0.00$), the majority(86.4%)was from the maternal side($p$= 0.00) (Table 2). Medical complications were reported as follows: hypertension (27.3%; $p = 0.001$), retinopathy (9.1%; p = 0.04), renal problems (11.4%; p = 0.09), periodonitis (6.8%; p = 0.1) and dental caries (25%; p = 0.02). Hypertension, retinpathy and dental caries were significantly different between diabetics and non-diabetics. Means BMI were not significantly different between diabetics and non-diabetics (26.2 ±4.7 and 25.0± 5.4 respectively; $p = 0.9$)

Three per cent of the 3$^{rd}$ generation were diabetics,half of these were regular physical exercisers ($p = 0.1$). Eighty six per cent of the 3$^{rd}$ generation diabetics had a family history of type 2 DM ($p = 0.00$) with 73.0% on the maternal side ($p = 0.00$)(Table 3). Medical complications in 3$^{rd}$ generationdiabetics were as follows: hypertension (2.7%; $p = 0.001$) and dental caries (25%; p = 0.02). Hypertension and dental caries were significantly different between diabetics and non-diabetics.Means BMI were not significantly different between diabetics and non-diabetics (26.2 ±4.7 and 25.0± 5.4 respectively; $p = 0.9$).

Amplification for *C18R* mutation yielded a fragment size of 117 bp in four samples,one diabetic and 3 non-diabetics (Fig.1). Two samples from non-diabetic subjects showed the *D76N*mutation with a fragment size of 146 bp (Fig. 2). The *R197H* mutation was not detected in any sample.

**Table. 1:** Demographic characteristics of the study population (n=96).

| | | 1st Generation | 2nd Generation | 3rd Generation | *p* |
|---|---|---|---|---|---|
| **NUMBER** | | 15 | 44 | 37 | |
| **Age** | Mean ± SD | 61.9 ± 8.9 | 44.1 ± 12.1 | 16.1 ± 8.7 | 0.000 |
| **Gender** | Male | 4 (26.7%) | 13 (29.5%) | 14 (37.8%) | 0.6 |
| | Female | 11 (73.3%) | 31 (70.5%) | 23 (62.2%) | |
| **Tribe** | Gaaleen | 1 (6.7%) | 12 (27.3%) | 14 (37.8%) | 0.1 |
| | Robatab | 7 (46.7%) | 9 (20.5%) | 5 (13.5%) | |
| | Nobioon | 2 (13.3%) | 12 (27.3%) | 11 (29.7%) | |
| | Others | 5 (33.3%) | 11 (25.0%) | 7 (18.9%) | |
| **Occupation** | Laborer | 2 (13.3%) | 7 (15.9%) | 0% | 0.000 |
| | Clerk | 3 (20.0%) | 10 (22.7%) | 4 (12.2%) | |
| | Student | 0% | 2 (4.5%) | 28 (84.8%) | |
| | Unemployed | 10 (66.7%) | 25 (56.8%) | 1 (3.0%) | |

**Table. 2:** Health indicators and family history of the study families (n=96).

| | | 1st Generation | 2nd Generation | 3rd Generation | *p* |
|---|---|---|---|---|---|
| | | (n=15) | (n=44) | (n=37) | |
| **BMI** | Mean ± SD | 26.3 ± 3.4 | 26.1 ± 5.0 | 24.0 ± 6.4 | 0.2 |
| **Exercise** | Yes | 4 (26.7%) | 14 (31.8%) | 19 (51.4%) | 0.1 |
| | No | 11 (73.3%) | 30 (68.2%) | 13 (48.6%) | |
| **Family history** | Yes | 6 (40.0%) | 39 (88.6%) | 32 (86.5%) | 0.000 |
| | No | 9 (60.0%) | 5 (11.4%) | 5 (13.5%) | |
| **Maternal family history** | Yes | 5 (33.3%) | 38 (86.4%) | 27 (73.0%) | 0.000 |
| | No | 10 (66.7%) | 6 (13.6%) | 10 (27.0%) | |

**Table. 3:** Age, BMI, Hypertension &retinopathyin relation to IPF-1 mutations in diabetics & non-diabetics (n=96).

| | | Diabetics | Non-diabetics | *p* value |
|---|---|---|---|---|
| | | n=31 (32.3%) | n=65 (67.7%) | - |
| **Age** | Mean ± SD | 53.1 ± 14.2 | 28.0 ± 17.1 | 0.000* |
| **BMI** | Mean ± SD | 26.2 ± 4.3 | 24.9 ± 5.9 | 0.250 |
| **Hypertension** | Yes | 15 (48.4%) | 5 (7.7%) | 0.000* (OR=3.6) |
| | No | 16 (51.6%) | 60 (92.3%) | |
| **Retinopathy** | Yes | 5 (16.1%) | 2 (3.1%) | 0.03* (OR=2.5) |
| | No | 26 (83.9%) | 63 (96.9%) | |
| **Gene Mutation** | C18R | 1 (3.2%) | 3 (4.6%) | 0.6 |
| | D76N | 0 0% | 2 (3.1%) | |

**Table. 4:** *IPF-1* gene mutations among the three study generations (n=96).

| | | 1st Generation | | 2nd Generation | | 3rd Generation | | |
|---|---|---|---|---|---|---|---|---|
| | | Freq. | % | Freq. | % | Freq. | % | *P* |
| | | 15 | 15.6 | 44 | 45.8 | 37 | 38.6 | - |
| **C18R Mutation** | Yes | 0 | 0 | 2 | 4.5 | 2 | 5.4 | 0.7 |
| | No | 15 | 100 | 42 | 95.5 | 35 | 94.6 | |
| **D76N Mutation** | Yes | 0 | 0 | 1 | 2.3 | 1 | 2.7 | 0.8 |
| | No | 15 | 100 | 43 | 97.7 | 37 | 97.3 | |

*Statistically significant if $p \leq 0.05$

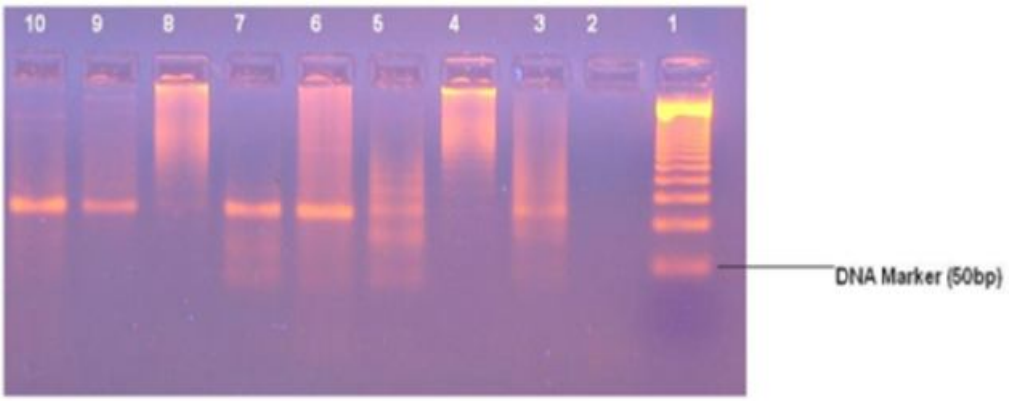


**Fig. 1:** PCR amplifications of 9 samples +DNA marker (50 bp) for C18R mutation. Lanes [2-4, 5 & 8] no products. Lanes 6,7, 9,10 showed bands of 146 bp

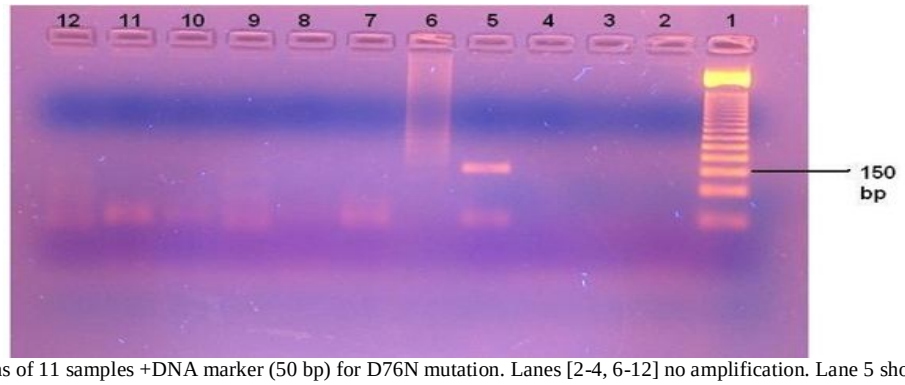


**Fig. 2:** PCR amplifications of 11 samples +DNA marker (50 bp) for D76N mutation. Lanes [2-4, 6-12] no amplification. Lane 5 showed a band with size 146 bp.

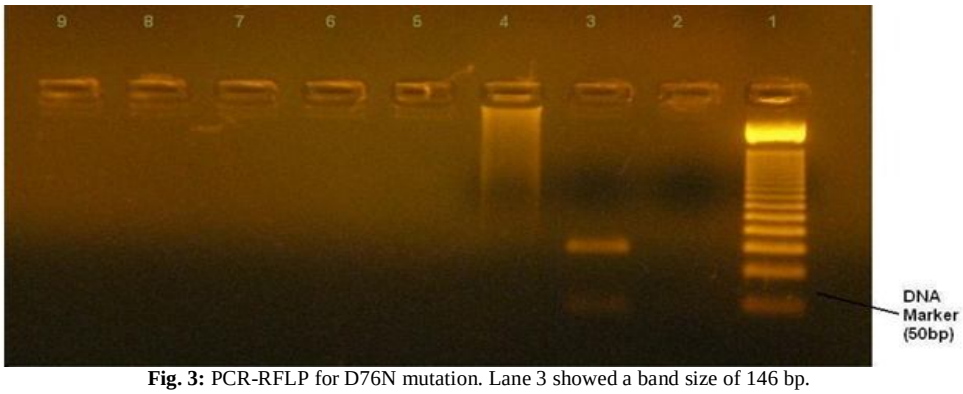


**Fig. 3:** PCR-RFLP for D76N mutation. Lane 3 showed a band size of 146 bp.

For *C18R* mutation, four positive samples after restriction by *Nla III* enzyme and gel electrophoresis showed fragments size of 111 bp, as a confirmatory positive result for the *C18R* mutation. For *D76N* mutation, SalI restriction enzyme was used to confirm the mutation. A fragment size of 146 bp was detected and was taken as a confirmatory positive result for the *D76N* mutation (Figure 3) [16].The *C18R* variant was seen only in one diabeticpatient out of the 31 tested (3.2%), and in 3 non-diabeticsubjects out of the 61tested (4.9%). The diabetic carrier was a 45 years old man, diagnosed at age 43, with a known history of diabetes. He is obese and hypertensive. The mutation was not found in his mother or his daughters and son, and his father was not included in the study.*D76N* variant was also seen in the two non-diabetic subjects (3%) who also carried the C18R variant. One of them was 43 years-old-man, hypertensive and overweight. The other one was a 19 years oldwith a normal weight. The mutation was not foundin their mothers or their fathers. All the non-diabetic carriers had a known family history of hypertension.

## 4. DISCUSSION

Diabetes mellitus Type 2 is a multi-factorial disease that comprises subtypes that are strongly associated with environmental factors at one end of the spectrum and geneticfactors at the other end. Genetic factors have been largely elucidated in the last few years by identification of 5 genes responsible for the autosomal dominant MODY subtype and key role in the development of the endocrine pancreas or in the expression of glucose metabolism genes. Apart from the monogenic forms of type 2 diabetes mellitus little is known about the nature of the genetic factors involved. *CI8R, D76N, Q59L* and *R197H* are well described novel *IPF-l* missense mutations in Caucasians patients with type 2 diabetes mellitus. As was shown previously, *D76N* and *Q59L* mutations have reduced penetrance and are more prevalent with a relative risk of 12.6 and with decreased glucose stimulated insulin-secretion in non diabetic subjects [22].*D76N* mutation was seen only in non-diabetic in our cohort, which is in agreement with other reports [16, 22, 23, 24].The low frequency of *C18R* variant in our cohort of diabetic patients is concordant with previous studies that showed that it is not prevalent amongpatients with a strong family history of type 2 DM [16, 23]. The *R197H* variant was not detected in our study subjects, which is in agreement with findings in other ethnic groups in the Far East. The mutation was reported in Britons only, which may probably point to the fact that it is restricted to certain ethnicities [16, 24].As far as we are aware, our study is the first study to be carried in a heterogeneous population like Sudanese that descend from different roots such as Africans, Arabs and Caucasians. The presence of these mutations in non-diabetics in our cohort clearly showed that the presence of these mutations does not necessary mean that the individuals will develop DM. Other environmental factors seem to play important roles in the development of the disease. The current data could help

incounselingindividuals at risk and help themmodify their diets and life style as a measure to delay the development of DM. The study data suggests that maternal family history may be a strong predisposing factor to type 2 DM, supportingprevious reports in caucasians, South Africans and Iranians[27, 28, 29].

**In conclusion,** *C18R* and the *D76N* mutations play a small part in development of DM type 2 in Sudanese individuals. Frequencies of *IPF-1* gene variant mutations arehigher than that reported in other populations, differences that might be related to sample size or the multi-ethnic nature of Sudan.Maternal family history correlates strongly with the development of type 2 DM.

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## 6. CONFLICT OF INTEREST: NON DECLARED.

## 7. REFERENCES

1. King H, Aubert RE, Herman WH. Global burden of diabetes, prevalence, numerical estimates, and projections. Diabetes Care. 1998;21(9):1414-1431.
2. Hogan P, Dall T, Nikolov P. Economic costs of diabetes in the US in 2002. DiabetesCare. 2003; 26(3):917-932.
3. Narayan KM, Boyle JP, Thompson TJ, Sorensen SW, Williamson DF. Lifetime risk for diabetes mellitus in the United States. JAMA. 2003; 290(14):1884-1890.
4. Dean L, McEntyre J, and Beck B. (Editors). Genetic Landscape of Diabetes. [Online]. 2004 Jul 7. Available from: URL: http://www.ncbi.nlm.nih.gov/books/bv. Access date: 2008 Sept 20.
5. Elamin, A., Altahir H, Ismail B, Tuvemo T. Clinical pattern of childhood type 1 (insulin dependent) diabetes mellitus in Sudan. Diabetologia. 1992;35(7):645-8.
6. Elbagir MN,Eltom MA, Elmahadi MA, Kadam IM, and Berne C. A population-based study of the prevalence of diabetes and impaired glucose tolerance in adults in northern Sudan. Diabetes Care. 1996; 10:1126-1128.
7. Elbagir MN, Eltom MA, Elmahadi EM, Kadam IM, Berne C. A high prevalence of diabetes mellitus and impaired glucose tolerance in the Danagla community in northern Sudan.DiabetMed. 1998 ;15:164-9.
8. National Diabetes Data Group. Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. Diabetes. 1979;28:1039–1057.
9. World Health Organization. Definition, Diagnosis and Classification ofDiabetes Mellitus and its Complications: Report of a WHO Consultation.Part 1: Diagnosis and Classification of Diabetes Mellitus. Geneva, World Health Orgnization. 1999
10. King, MW. Insulin Action. [Online][4]. Available from: URL:http://themedicalbiochemistrypage.org/home.html. Access date: 2008 Jun 28.
11. Ahlgren, U, Jonsson, J, Simu, K&Edlund, H. β–cell specific inactivation of the mouse IPF1/Pdx1 gene results in loss of the β-cell phenotype and maturity onset diabetes. Genes Dev. 1998;12:1763-1768.
12. Hansen L, Urioste S, Petersen HV, Jensen J, Eiberg H, Barbetti F, Serup P, Hansen T, and Pedersen O. Missense Mutations in the Human Insulin Promoter Factor-1 Gene and Their Relation to Maturity-Onset Diabetes of the Young and Late-Onset Type 2 Diabetes Mellitus in Caucasians. J ClinEndocrinolMetab. 2000;85(3):1323-6.
13. Baeza N, Hart A, Ahlgren U, and Edlund H. Insulin promoter factor-1 controls several aspects of (beta)-cell identity. Diabetes. 2001;50 (1):S36.
14. Ohlsson,H.,Karlsson, K, Edlund, T. IPF-I ,ahomeodomain-containing trans-activator of insulin gene. EMBOJ. 1993; 12:4251-4259.
15. Johnsson JL, Carlsson T, Edlund H. Insulin promoter factor 1 is required for the pancreas development in mice.Nature. 1994;371:606-609.
16. Macfarlane WM., Frayling TM,Ellard S, Evans JC , Allen LI, Bulman MP, Ayres S, Shephered M, Clark P,MillwardA,DemaineA,Wilkin T,Docherty K, and Hattersley AT.(1999). Missense mutations in the insulin promoter factor 1 gene predispose 2 diabetes.J Clin Invest104:r33-r39.
17. Inoue H,Riggs AC, Tanizawa Y, Ueda K, Kuwano A, Liu L, Donis-Keller H, Permutt MA. Isolation, characterization, and chromosomal mapping of the human insulin promoter factor-1 (IPF-1) gene. Diabetes. 1996;45:789-794.
18. Stoffers DA,Zinkin NT, Stanijovic V, Clarke WL, Habener JF. Pancreatic agenesis attributable to a single nucleotide deletion in the human IPF-1 gene coding sequence.Nat Genet. 1997;15:106-110.
19. Chevre, JC, Hani, EH, Stoffers, A, Habener, JF, Froguel, P. Insulin promoter factor 1 gene (IPF-1) is not a major cause of maturity onset diabetes of the young in French Caucasians. Diabetes. 1998;47:843-844.
20. Hara M, Linder TH, Paz VP, Wang X, Iwaski N, Ogata M, Iwamoto Y, Bell GI. mutations in the coding region of the insulin promoter factor 1 gene are not a common cause of maturity-onset diabetes of the young in Japanese subjects.Diabetes. 1998;46:720-725.
21. Stride, A, Hattersley, AT. Different genes, different diabetes: lesson from maturity-onset diabetes of the young. Ann Med. 2002;34:207-16.
22. Hani EH.,Stoffers DA, Chevre JC, Durand E, Stanojevic V, Dina C. Defective mutations in the insulin promoter factor-1 (IPF-1)gene in late-onset type 2 diabetes mellitus. JClinInvest. 1999;104:41:48.
23. Reis A.F., Ye, WZ., Dubois-Laforgue, Bellane-Chantelot, C., Timsit, J., and Velho, G. Mutations in the insulin promoter factor-1 gene in late-onset type 2 diabetes mellitus. EurJ Endocrinol. 2000; 143:511-513.
24. Shiau, MY, Huang,CN, Liao, JH, Chang, YH. Missense mutations in the human insulin promoter factor-1 gene are not a common cause of type 2 diabetes mellitus in Taiwan. Endocrinol Invest. 2004; 2711:1076-80.
25. Sambrook J, Fritsch E F, and Manitis T. (Editors). MolecularCloning A Laboratory Manual.2nded. New York. Cold Spring Harbor Laboratory Press, Cold Spring Harbor. p. 21. 1998
26. MillerSA,Dykes DD, &Polesky HF.A simple salting out procedure forextracting DNA from human nucleated cells. Nucleic AcidsRes. 1988; 16: 1215.
27. Bjørnholt JV, Erikssen G, Liestøl K, Jervell J, Thaulow E, and Erikssen J. Type 2 diabetes and maternal family history: An impact beyond slow glucose removal rate and fasting hyperglycemiain low-risk individuals? Results from 22.5 years of follow-up of healthynondiabetic men. Diabetes Care. 2000; 23(9):1255–1259.
28. Erasmus R, Blanco E, Okesina A, Arana J, Gqweta Z, and Matsha T.Importance of family history in type 2 black South African diabetic patients. Postgrad Med J. 2001;77(907): 323–325.
29. Ostovan MA. Familial Inheritance in Diabetes Mellitus in South Iranian Population. SEMJ, 2007; 8(4).

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