Thi-Qar Medical Journal (TQMJ):Vol.(22),No.(2),2021
Web Site: https://jmed.utq.edu.iq Email: utjmed@utq.edu.iq
ISSN (Print):1992-9218, ISSN (Online):1992-9218

Association of Common Variants of FGF21 Gene Polymorphism Rs499765 and Rs838133 with Type 2 Diabetes Mellitus in Iraqi Population

Abdulhusein A. F. Algenabi
Majid Kadhum Hussain
Thakra Al-Kashwan
Rasha Mohamed Abood

SUMMERY

Background:
Type 2 diabetes mellitus is a major public health issue. FGF21 is a key endocrine regulating protein factor involved in ontogenesis and metabolism.

Aim: To assess the association of FGF21 gene polymorphism (rs499765 and rs838133) in Iraqi type 2 diabetic patients.

Methods: To assess the relationship between SNPs (rs499765 and rs838133) of the FGF21 gene and T2DM, a case-control study of 200 people (100 T2DM and 100 controls) was undertaken. Tetra ARMS-PCR was used to genotype SNPs (rs499765 and rs838133) using specified primers after extracting DNA from blood. Various statistical analyses were used to analyze the results.

Results: The differences in total cholesterol, VLDL, TG and HDL between healthy and diabetic groups are significant. Genotyping of FGF21 gene for rs838133 SNP revealed a significant elevation of the number of the AC carriers in the patient's group versus the controls group under the co-dominant model, the dominant model and the over dominant were significantly increased the risk of T2DM by 1.91, 1.8 and 1.94 folds (OR=1.91, P=0.0320), (OR=1.87, P=0.037) and (OR=1.94, P=0.0270) respectively. The analysis of the rs499765 SNP failed to exhibit a significant variation under all examined inheritance models.

Conclusion: Carriers of the AC genotype of rs838133 SNP in FGF21 gene have a 1.9 folds risk to develop T2DM in comparison to those of the wild genotype, rs499765 (C>G), is not linked to the T2DM in the Iraqi population.

1. Introduction

T2DM is a worldwide public health concern, and the Middle East has the world's second highest diabetes rate. Around 10% of T2DM patients lived in the Middle East, Nearly half of them had not been diagnosed (International Diabetes Federation. Diabetes Atlas, 2019). T2DM affects more than 90% of diabetes patients and causes microvascular and macrovascular complications, as well as considerable psychological and physical distress in both patients and healthcare professionals, in addition to a significant financial strain on health services. Despite a better understanding of T2DM risk factors and evidence of effective preventive programs, the disease's prevalence and incidence continue to rise worldwide (Chatterjee, Khunti, and Davies 2017). Patients with T2DM are more likely to have visceral obesity, and it is connected to insulin resistance. Dyslipidemia (high TG and lower HDL cholesterol levels; postprandial hyperlipidemia) and hypertension are also common in these patients (Baynest 2015). The inadequacy of understanding of the biology of diseases like type 2 diabetes and obesity stymies efforts to develop better therapeutic and preventative therapies. It is hoped that discovering DNA polymorphisms that impact disease predisposition would give insight into disease development mechanisms. This would not only promote translational research but would also pave the way for personalized medicine by

allowing for more precise risk assessment and disease subtype classification (McCarthy 2010). FGF21 is an important endocrine regulatory protein factor that has a role in ontogenesis and metabolism (Engineering and Biotechnology 2021). FGF21 is a hormone generated mostly by the liver that plays a number of activities, including signalling to the hypothalamus paraventricular nucleus to limit alcohol and sugar consumption, increasing glucose absorption by adipocytes, and working as an insulin sensitivity enhancer (von Holstein-Rathlou et al., 2016; Berglund et al., 2010). Gene FGF21 codes for a member of the fibroblast growth factor group. FGFs are involved in a wide variety of biological activities and have a broad spectrum of cell-survival characteristics and mitogenic. This protein is a secretory endocrine factor that is crucial for metabolism. In adipose tissue, the encoded protein increases glucose absorption. It's on chromosome 19q13.33, with four exons and a size of 2,810 bases (Nishimura T, et al., 2000). In the current study, two of the flanking SNPs (rs499765 and rs838133) of FGF21 gene will be analyzed for the association with type 2 diabetic patients.

The rs838133 variant aligns to exon 1 of the FGF21 gene, as well as a gene-dense area on chromosome 19 that comprises the genes IZUMO1, RASIP1, FUT1and FUT2 all of them are within 100 kilobytes. The synonymous SNP rs838133 is found in the first exon of the FGF21 gene, which codes for FGF21 21 (Chu et al. 2013). The rs499765 variant is found in the flanking region of the FGF21 gene, and it has the potential to affect gene expression, resulting in translational dynamics or abnormal splicing of FGF21 (Yu et al. 2019).

2. Materials and methods

2.1. Study subjects

1. From August to October 2020, researchers from the Clinical Biochemistry Department of the University of Kufa's College of Medicine conducted a case-control study. We had 200 volunteers divided into two groups: healthy patients (group A) and T2DM patients (group B). Participants were diagnosed by specialist physicians. They were recruited from AL-Medical Sadr's Endocrinology Center in Najaf city. The patients' group was chosen using the patient's group inclusion criteria; were: (1) Physicians have diagnosed all patients with type 2 diabetes, according to WHO guidelines; (2) Diabetic is verified if the fasting blood glucose level was higher than 126 mg/dl (7.0 mmol) with diabetes symptoms. Exclusion criteria included:
(1) Type 1 diabetes, (2) CVD patients, (3) Acute kidney disease, (4) Liver disease, and (5) Pregnant or breastfeeding women. All healthy participants who are free of chronic diseases such as heart disease, renal disease, liver disease, and diabetes mellitus are chosen. Biochemical methodologies were used in the Kufa University Medical Faculty Biochemical Department's laboratory. The Kufa University Medical Faculty's Ethics Committee authorized the research, and all participants completed a signed informed consent form.

2.2. Measurements

Height, weight, BMI have all been measured as anthropometric measurements. The lipid profile, which included HDL, triacylglycerol (TG), LDL-C, VLDL, total cholesterol, as well as insulin and insulin resistance levels, were determined.

2.3. Genotyping

DNA was extracted from blood samples using a DNA extraction kit (G-spin). The amplification refractory mutation system (ARMS) method was used to detect polymorphisms in the FGF21 gene (rs499765 and rs838133). The tetra-primers were designed and are illustrated in Tables 1 and 2. The primer sequences for PCR amplification of the FGF21 gene (rs499765 and rs838133) were created by Company (Macrogen / Korea) using the online software Primer 3 (https://primer3.ut.ee). The PCR was carried out in a 25µl volume with an initial denaturation at (94°C for 5 min), denaturation on (94°C for 30 s), annealing at (56°C for 30 s), extension on (72°C for 60 s) for 35 cycles, and a final extension at (72°C for 5min). In a 2 percent agarose gel using Redsafe dye, electrophoresis was applied to resolve the result for genotyping.

2.4. Statistical analysis

SPSS was used to examine all of the data (V20, IBM SPSS Statistics for Mac). Hardy-Weinberg equilibrium and the Chi-square test were used to assess polymorphisms in the FGF21 distribution. The data were normal checked. The standard deviation (SD) and mean of continuous variables

were calculated. For numerous comparisons of means across groups, the one-way ANOVA test was used. We computed the odds ratio (OR) and confidence intervals (CI). A p-value < 0.05 was considered statistically significant. The OSSE website is used to calculate genetic power (osse.bii.a-star.edu.sg).

Results

A successful T-PCR-ARMS product was obtained from 200 amplified DNA samples (100 patients and 100 controls). T-ARMS-PCR genotype of FGF21 gene rs499765 C>G polymorphism showed Individuals with normal homozygous (CC) revealed that two bands (283, 187 bp), and variant homozygous (GG) also two bands of (283, 139bp), while the heterozygous (CG) gives three bands (283,187, and 139 bp), as shown in Figures 1. T-ARMS-PCR genotype of FGF21 gene rs838133 A>C polymorphism showed Individuals with normal homozygous (AA) revealed that two bands (496, 235bp), and variant homozygous (CC) also two bands of (496, 298bp), while the heterozygous (AC) gives three bands (496,235, 298bp), as shown in Figures 2. TG, Total cholesterol, LDL, VLDL, and HDL are statistically significant between healthy control and patient groups.

The frequencies of genotype and allele of FGF21 SNPs (rs49765) and (rs838133) are demonstrated in Tables 4 and Table 5 respectively. When compared to the healthy population, diabetes patients have higher frequencies of certain alleles and genotypes. In Table 5, In the codominant model, the number of AC genotypes of rs838133 in the patients' group is considerably greater than in the control group, increasing the odds ratio (OR = 1.91, CI 95% = 1.03-3.36, p = 0.032). T2DM risk is also increased by dominant and over dominant models, according to odds ratios (OR = 1.87, CI 95% = 1.03- 3.40 , p = 0.037; OR = 1.94, CI 95% = 1.07–3.49, p = 0.027) respectively. No-statistically significant differences in FGF21 (rs499765) SNP were observed, as shown in Table 4. Regarding the effect of rs499765 and rs838133 on biochemical characteristics of the studied patient under co-dominant model, In Table 6 and Table 7 were no statistically significant associations between the different allele frequency and other biochemical characteristics of patients groupT2DM.

4. Discussion

Multiple genes connected to Diabetes mellitus type 2 have genetic functions that are currently being explored. The main objectives of these studies are to understand more about the disease's genesis, improve treatment choices, and identify persons who are at high risk of acquiring diabetes. Two flanking SNPs (rs499765 and rs838133) of the FGF21 gene were investigated for their connection with type 2 diabetic patients in the current study, to improve disease treatment programs in Iraq. The relationship between FGF21 gene polymorphism and the progression of T2DM in humans is poorly understood. Because the FGF21 genotype is more likely to affect human characteristics over the course of a lifetime, it may have various effects than an acute, experimental, or pharmacological intervention.

The genetic powers of two FGF21 gene polymorphisms (rs838133) and (rs499765) were realized to be 90.3% and 99 %, respectively, creditable evidence of the results at a level of 80% is realized to be adequate in documenting an appropriate decision. It is crucial to mention that such values have no bearing on the decision-making process. However, because the current case-control study was the first to deal with FGF21 gene polymorphism in the Iraqi population, the current findings might be used as a pilot base for future research. The small sample size was most likely a hint to the study's power (Ellis, 2010). The increase in the number of people submitted to the analysis is a suitable answer to such a problem. Unfortunately, the circumstances surrounding the COVID-19 pandemic confused us and prevented us from including additional patients and controls.

One of the examined SNPs (rs499765) in the FGF21 gene of the control group was found to be compatible with HWE, indicating that the genotype distribution in the Iraqi population remains constant from generation to generation. However, SNP of FGF21 gene (rs838133) was revealed to be inconsistent with Hardy- Weinberg equilibrium. A variety of factors, including mutations, inbreeding, population substructure, and sample size, might explain the disparity (Evans and Purcell, 2012). The smallest sample

size is most likely to blame (Schnermann,2015). The low sample size is most likely the most notable reason since we were unable to increase the number of examined people owing to the COVID-19 epidemic.

The genotype and allele assessment of rs838133 A>C SNP under the codominant model exhibited a significant (OR= 1.91) rise of AC genotype carriers in the patient's group relevant to the control group. This observation suggests a relative risk factor equals 1.9 to develop T2DM in Iraqi individuals carrying the AC genotype. However, data of rs499765 C>G SNP failed to point out a significant association with the disease. To fully comprehend the molecular role of the two studied SNPs in the pathogenesis of T2DM, it is necessary to consider the impact of FGF21 on T2DM as well as the impact of the two SNPs on its gene.

FGF21 is mostly generated in the liver, although it may also be found in the muscle, stomach, brain, adipose tissue and pancreas (Laeger T, et al.2014). FGF21 was the first known endocrine signal to be triggered by protein restriction rather than calorie restriction, and it was thought to be induced by fasting (Laeger T, et al. 2016). Nishimura et al. were the first to clone FGF21 (Nishimura et al., 2000). FGF21 was identified to be a new metabolic regulator with biological functions in 2005 (Kharitonenkov A, 2005). According to Y. Panahi et al., serum FGF21 concentrations in persons with poorly managed diabetes are substantially higher than in those with healthy controls and well-controlled diabetes. Another conclusion was that the group with healthy controls and well-controlled diabetes participants had similar FGF21 serum levels (Y. Panahi et al., 2016). High blood levels of FGF21 were linked to insulin resistance and impaired glucose metabolism in adults in another investigation by Semba et al. (Samba et al., 2012). In research by Kralisch et al, mean FGF21 serum concentrations in type 2 diabetes patients were 2.1 times greater than in the control group. Non-diabetic individuals with undetectable FGF21 levels by ELISA had a better metabolic profile than those with detectable FGF21 (Kralish et al., 2011).

Frayling et al found no relationship between the FGF21 expression in the liver and FGF21 rs838133 allele, implying that the impact of this variation, or a linking disequilibrium, on FGF21 gene regulation is too small to be detected as an eQTL, even in the relevant organ (Frayling et al.,2018). Despite the fact that the variant occurs in an exon of FGF21, we can't rule out the potential that it is mediated by a nearby gene, because the rs838133 allele is linked to the expression of the adjacent FUT2 gene. It is involved in the metabolism of vitamin B12 (Hazra et al., 2008).

Food preferences are influenced by gene polymorphisms, which is a fascinating example of gene-environment interaction. Despite the fact that there is no substantial difference in overall calorie consumption, the FGF21 “A” allele at rs838133 is linked to higher carbohydrate intake and a predilection for sweet foods (Chu AY, 2013; Tanaka T, 2013; Soberg S, 2017). The rs838133 minor A allele has been related to a high waist-hip ratio and hypertension, but not to a low total body fat percentage, according to Frayling et al., (2018). Those with the AA genotype of rs838133 had considerably greater vitamin A consumption. Females that were homozygous for the rs838133 minor A allele ingested much more vitamin C, vitamin D, carbohydrates, vitamin B1, B2, and B6 than other genotypes (Saber-Ayad et al. 2020).

The SNP rs499765 is positioned in the flanking region of the FGF21 gene, and it might impact gene expression, resulting in aberrant splicing, or translational dynamics of FGF21 (Yu et al., 2019). Researchers found a novel risk-associated SNP for NAFLD patients, rs499765. The rs499765 variant showed a substantial association with blood FGF21 levels, according to a recent study. Despite the fact that the SNP rs499765 on chromosome 19 is situated downstream of the FGF21 gene, it is impossible to rule out the possibility that this variation impacts the FGF21 gene (Jiang et al., 2014).

Regarding the effect of rs499765 and rs838133 on biochemical characteristics of the studied patient under co-dominant model, the current study demonstrated in Table 6, Analyzing of biochemical data stratified to the genotypes (CG, CC, and GG) of rs499765 (C>G) polymorphism non statistically significant associations between the different allele frequency and other biochemical characteristics of patients group (T2DM). In Table 7, the analysis of biochemical data stratified to the genotypes (AA, AC, and CC) of rs838133 (A>C) polymorphism no statistically significant associations between the different

allele frequency and other biochemical characteristics of patients group (T2DM). The previous studies found non-significant differences were noted between the two SNPs (rs499765G>C and rs838133C>T) and other biochemical data, including serum levels of total cholesterol, TG, high-density lipoprotein-cholesterol and LDL-C (Jiang et al. 2014). Other study found FGF21 variant rs838133A>G related to higher triglycerides, higher LDL-cholesterol but minimal if any effect on BMI (Frayling TM,2018). The minor G-allele was shown to be substantially related to reduced LDL-C in another research by Epperlein et al, (2021).

In the current investigation, we discovered a link between the C allele of the rs838133 A>C SNP and T2DM in an Iraqi population sample. Previous studies, Chu et al., (2013); Soberg et al., (2017); Tanaka et al., (2013) showed that a common allele at rs838133 (A>G, minor allele frequency = 44.7%), which causes a synonymous alteration to FGF21's first exon, is linked to increased carbohydrate consumption and lower protein and fat intake, but does not affect total calorie intake. According to Soberg et al. (2017), carbohydrate preferences are specific to sweet food and may enhance alcohol consumption. According to an animal study, FGF21 send signals to reward centres in the brain (Talukdar et al., 2016; von Holstein-Rathlou et al., 2016). The human genetic investigation found no discernible effect on the risk for T2DM and only nominal evidence for an impact on BMI. Soberg et al. (2017) showed that rs838133 A-allele carriers also had superior glycemic control than non-carriers, with lower plasma glucose and serum insulin during fasting and 2 hours following an oral glucose tolerance test. The SNP rs838133 has recently been linked to increased intake of alcohol and cigarettes, as well as other types of reward seeking behavior (Schumann G et al., 2016).

The significance of our findings is due to two factors. The genetic association data, for starters, give theories regarding the possible negative and positive impacts of FGF21-based treatments. To develop these ideas, we utilized rs838133, a common, naturally occurring variation in the human FGF21 gene. Second, the findings add to our understanding of the wide variety of consequences that variations in the FGF21 gene have in people.

It's worth to mention up the research's limitations. First, because of the COVID-19 pandemic and the resulting quarantine, the number of T2DM patients and controls is limited. Second, due to the absence of financing, the concentration of FGF21 was not determined.

Acknowledgements

The authors wish to thank the workers at the Al-Najaf endocrinology center in Najaf, Iraq, for their support in collecting blood samples from patients. Unlimited thanks should also be expressed to all patients of the current study.

Table1: Primers of Nested T-ARMS PCR SNP rs499765 C/G

Primer	Sequence (5'-3')
Outer forward	5'-GAAGCAATTGAATGTTGAATTG-3'
Outer reverse	5'-CTGGCCCGAAATTTTAATTTTT-3'
Inner forward	5'-CTCTTTGGTTGAGTGAAATCAGC-3'
Inner reverse	5'-GTTTGCAAAGGATTTGGGTTC-3'

Thi-Qar Medical Journal (TQMJ):Vol.(22),No.(2),2021
Web Site: https://jmed.utq.edu.iq Email: utjmed@utq.edu.iq
ISSN (Print):1992-9218, ISSN (Online):1992-9218

Table 2: Primers of Nested T-ARMS PCR SNP rs838133 A/C

Primer	Sequence (5'-3')
Outer forward	5'-ATATCATGGTTCAGGCGCAG-3'
Outer reverse	5'-CTCTGGCCCACACTCACTTT-3'
Inner forward	5'-CGGGTTCGAGCACTCAAGA-3'
Inner reverse	5'-CAGCACAGAAACCCACGGG-3'

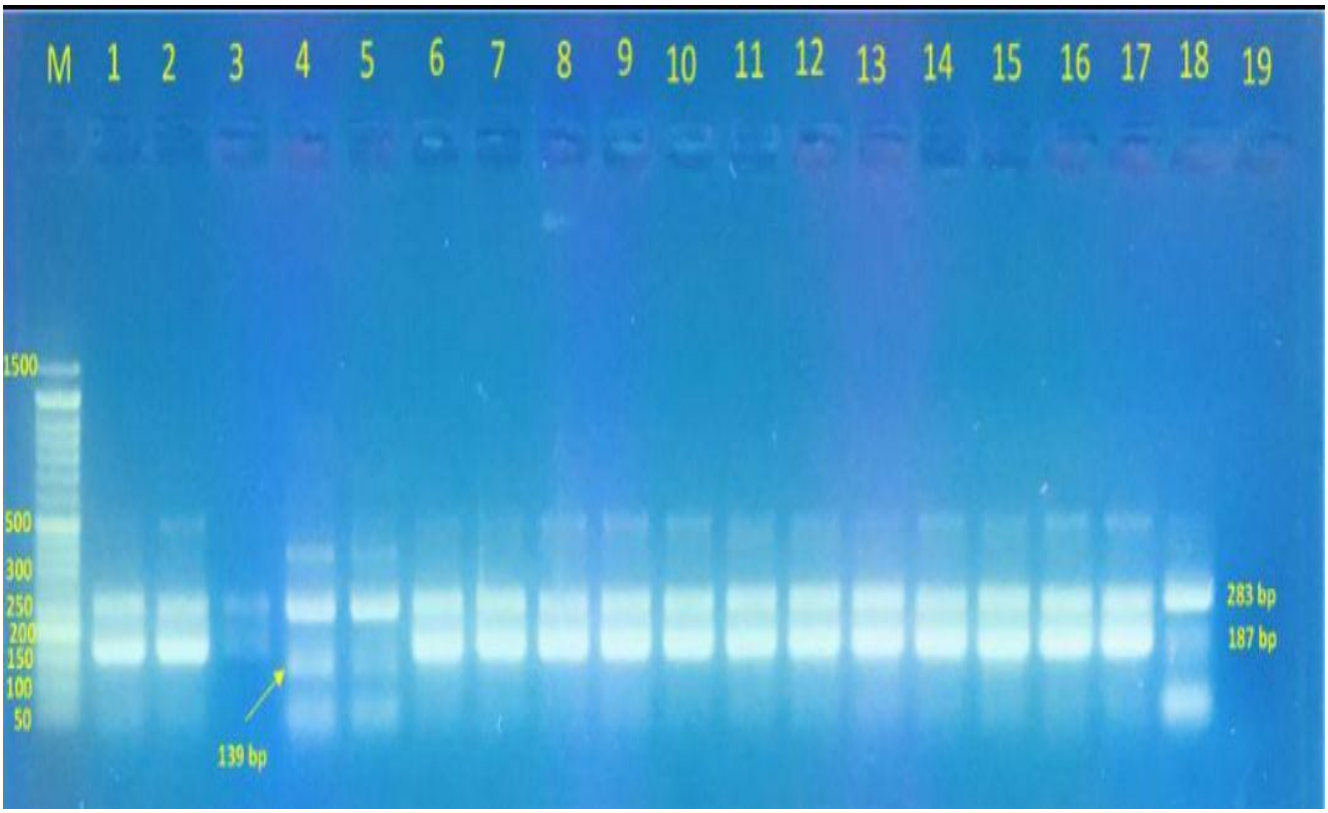

Figure 1: T-ARMS-PCR genotype of FGF21 gene rs499765 C>G polymorhpism on 2% agarose gel electrophoresis . M : Molecular weight of DNA 50bp step ladder marker, has 17 bands, ranging from 50bp – 1.5kb. Lane 1,2,3,6,7,8,9,10,11,12,13,14,15,16,17,18,19 (CC),lane 4,5 (GG)

Thi-Qar Medical Journal (TQMJ):Vol.(22),No.(2),2021
Web Site: https://jmed.utq.edu.iq Email: utjmed@utq.edu.iq
ISSN (Print):1992-9218, ISSN (Online):1992-9218

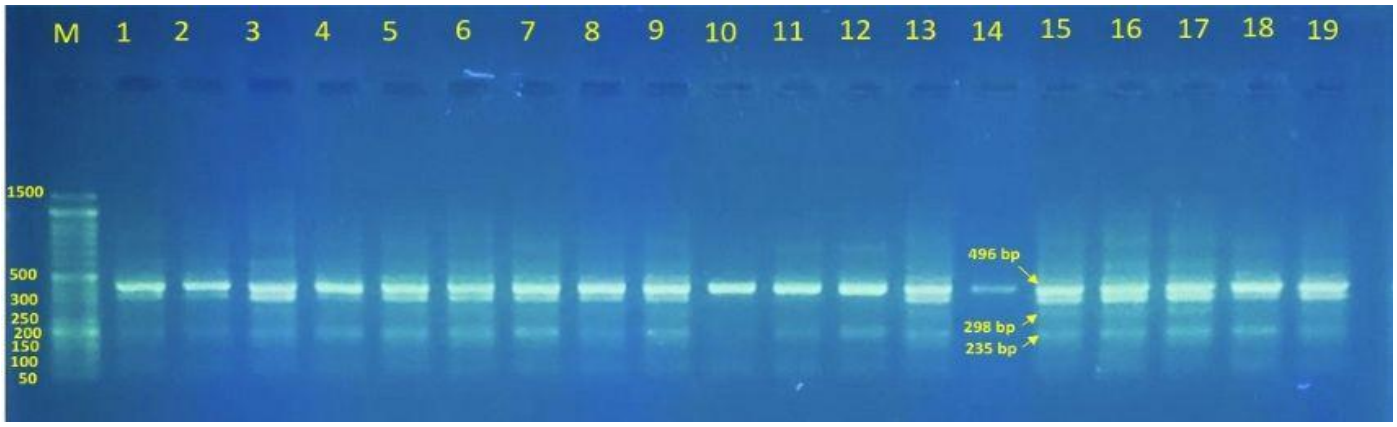

Figure 2: T-ARMS-PCR genotype of FGF21 gene rs838133 A>C polymorhpism on 2% agarose gel electrophoresis . M : Molecular weight of DNA ladder marker has 17 bands, ranging from 50bp – 1.5kb. Lane 1,2,4,10,11,12 (AA) , lane 3,5,6,7,8,9,13,15,16,19,18,17(AC).

Table 3: Demographic and Biochemical Features of Study Participants

Parameters	Control persons (Mean± SD)	T2DM patients (Mean± SD)	P value
No (M/F)	100(47/53)	100(53/47)	
M/F ratio	0.88	1.12	
Age (y)	52.9± 7.54	54.1± 7.90	0.307
FBS (mg/dl)	87.96± 2.104	266±5.06	0.0001
BMI (kg/m2)	28.51 ± 4.12	29.15 ± 5.93	0.118
Insulin (µU/ml)	15.6 ± 4.1	28.9± 8.74	0.0001
HOMA-IR	3.273 ± 0.946	18.62± 5.482	0.0001
Cholesterol (mg/dl)	182.3±18.7	200.7±39.	0.0001
Triglycerides (mg/dl)	148.9± 23.8	188.7 ± 58.5	0.0001
VLDL-C (mg/dl)	26.3± 5.23	37.2 ± 10.1	0.0001
LDL-C (mg/dl)	112.61 ± 19.7	123.1 ± 39.5	0.017
HDL-C (mg/dl)	44.4± 7.47	40± 4.14	0.0001

p* < 0.05 indicates statistical significant

Thi-Qar Medical Journal (TQMJ):Vol.(22),No.(2),2021
Web Site: https://jmed.utq.edu.iq Email: utjmed@utq.edu.iq
ISSN (Print):1992-9218, ISSN (Online):1992-9218

Table 4: Genotype and allele frequency of gene SNP at rs499765 (C>G) SNP between patients and Control

Genotype allele	All (n=200)		Controls (n=100)		Patients (n=100)		χ^2	P-Value	OR	95% CI
	No.	%	No.	%	No.	%				
Co dominant :										
CC	**192**	0.96	**98**	0.98	**94**	0.94	Ref.	Ref.	Ref.	Ref.
CG	**5**	0.025	**1**	0.01	**4**	0.04	1.87	0.171	4.17	0.45 – 37.99
GG	**3**	0.015	**1**	0.01	**2**	0.02	0.371	9.543	2.08	0.18 – 23.38
Dominant :										
CC	**192**	0.96	**98**	0.98	**94**	0.94	Ref.	Ref.	Ref.	Ref.
CG+GG	**8**	0.04	**2**	0.02	**6**	0.06	0.28	0.363	0.49	0.10 – 2.35
Recessive :										
CC+CG	**197**	0.98	**99**	0.99	**98**	0.98	Ref.	Ref.	Ref.	Ref.
GG	**3**	0.02	**1**	0.01	**2**	0.02	0.33	0.561	2.02	0.18 – 22.64
Over dominant :										
CC+GG	**195**	0.97	**99**	1	**96**	0.96	Ref.	Ref.	Ref.	Ref.
CG	**5**	0.03	**1**	0	**4**	0.04	1.84	0.174	4.12	0.45 – 37.57
C	**289**	0.99	**197**	0.98	**192**	0.96	Ref.	Ref.	Ref.	Ref.
G	**11**	0.01	**3**	0.02	**8**	0.04	2.18	0.139	2.65	0.69 – 10.14

Thi-Qar Medical Journal (TQMJ):Vol.(22),No.(2),2021
Web Site: https://jmed.utq.edu.iq Email: utjmed@utq.edu.iq
ISSN (Print):1992-9218, ISSN (Online):1992-9218

Table 5: Genotype and allele frequency of gene SNP at rs838188 (A>C) SNP between patients and Control

Genotype allele	All (n=200)		Controls (n=100)		Patients (n=100)		χ^2	P-Value	OR	95% CI
	No.	%	No.	%	No.	%				
Co dominant :										
AA	**68**	0.34	**41**	0.41	**27**	0.27	Ref.	Ref.	Ref.	Ref.
AC	**129**	0.63	**57**	0.57	**72**	0.72	4.62	**0.032***	1.91	1.03 - 3.36
CC	**3**	0.03	**2**	0.02	**1**	0.01	0.04	0.825	0.759	0.06 – 8.79
Dominant :										
AA	**68**	0.34	**41**	0.41	**27**	0.27	Ref.	Ref.	Ref.	Ref.
AC+CC	**132**	0.66	**59**	0.59	**73**	0.73	4.36	**0.037***	1.87	1.03 – 3.40
Recessive :										
AA+AC	**197**	0.98	**98**	0.98	**99**	0.99	Ref.	Ref.	Ref.	Ref.
CC	**3**	0.02	**2**	0.02	**1**	0.01	0.33	0.561	0.495	0.04 – 5.54
Over dominant :										
AA+CC	**71**	0.35	**43**	0.43	**28**	0.28	Ref.	Ref.	Ref.	Ref.
AC	**129**	0.65	**57**	0.57	**72**	0.72	4.19	**0.027***	1.94	1.07 – 3.49
Alleles										
A	**365**	0.66	**139**	0.69	**126**	0.63	Ref.	Ref.	Ref.	Ref.
C	**135**	0.34	**61**	0.31	**74**	0.37	1.89	0.169	1.33	0.88 – 2.02

Thi-Qar Medical Journal (TQMJ):Vol.(22),No.(2),2021
Web Site: https://jmed.utq.edu.iq Email: utjmed@utq.edu.iq
ISSN (Print):1992-9218, ISSN (Online):1992-9218

Table 6 : Biochemical features of T2DM patients in relevance to the genotypes of rs499765 (C/G) of FGF21 gene polymorphism examined under codominant model:

Clinical characteristic	Genotype Mean ± SD			P-Value
	CC(n=94)	CG (n= 4)	GG(n=2)	
Cholesterol (mg/dl)	199.30 ± 37.82	148.50 ± 34.64	220 ± 72.01	0.114
TG (mg/dl)	186.63 ± 57.80	241.50 ± 70.0	174.50 ± 50.0	0.377
VLDL (mg/dl)	36.41 ± 9.41	42.0 ± 2.82	40.75 ± 13.45	0.493
HDL (mg/dl)	40.11 ± 4.09	35.50 ± 3.53	39.50 ± 6.13	0.298
LDL (mg/dl)	122.52 ± 37.98	71.0 ± 41.01	139.7 ± 69.37	0.129
Insulin (µIU / ml)	29.02 ± 8.89	32.25 ± 2.05	25.82 ± 8.85	0.679
HOMA-IR	18.70 ± 5.55	18.65 ± 4.03	16.8 ± 6.58	0.799
FBG (g/dl)	266.84 ± 5.16	266.0 ± 1.41	266.2 ± 5.07	0.951

p* < 0.05 indicates statistical significant

Table 7: Biochemical features of Patient T2DM in relevance to the genotypes of rs838133 (A>C) FGF21 gene polymorphism examined under codominant model

Clinical characteristic	Genotype Mean ± SD			P-Value
	AA(n=27)	AC (n= 72)	CC (n=1)	
Cholesterol (mg/dl)	207.5± 43.54	199.1 ± 38.54	162.5 ± 9.19	1.39
TG (mg/dl)	189.9 ± 63.97	188.2 ± 57.44	192.0 ± 38.18	0.989
VLDL (mg/dl)	38.6 ± 10.50	36.1 ± 10.01	42.0 ± 2.82	0.663
HDL (mg/dl)	39.5 ± 4.31	40.3 ± 4.11	37.5 ± 2.12	0.715
LDL (mg/dl)	129.3 ± 45.88	121.8 ± 36.81	83.0 ± 14.14	0.245
Insulin (µIU / ml)	30.5 ± 9.57	28.0 ± 8.35	35.8 ± 7.42	0.224
HOMA-IR	19.2 ± 5.32	18.2 ± 5.54	23.15 ± 5.16	0.368
FBG (g/dl)	264.5 ± 0.70	266.2 ± 4.52	267.1 ± 5.26	0.586

p* < 0.05 indicates statistical significant

References

1- Anon. 2020. "A Polymorphism in the FGF21 Gene Is a Novel Risk Variant for Metabolic-Associated Steatohepatitis Asmaa Elsayed A Thesis Submitted in Fulfilment of the Requirements for the Degree of Master of Philosophy School of Medicine January 2020." (January).

2- Baynest, Habtamu Wondifraw. 2015. "Classification, Pathophysiology, Diagnosis and Management of Diabetes Mellitus." *Journal of Diabetes & Metabolism* 06(05). doi: 10.4172/2155-6156.1000541.

3- Berglund ED, Kang L, Lee-Young RS, Hasenour CM, Lustig DG, Lynes SE. *et al*. Glucagon and lipid interactions in the regulation of hepatic AMPK signaling and expression of PPARalpha and FGF21 transcripts in vivo. American journal of physiology Endocrinology and metabolism. 2010;**299**:E607-14.

4- Chatterjee, Sudesna, Kamlesh Khunti, and Melanie J. Davies. 2017. "Type 2 Diabetes." The Lancet 389(10085):2239–51. doi: 10.1016/S0140-6736(17)30058-2.

5- Chu, Audrey Y., Tsegaselassie Workalemahu, Nina P. Paynter, Lynda M. Rose, Franco Giulianini, Toshiko Tanaka, Julius S. Ngwa, Qibin Qi, Gary C. Curhan, Eric B. Rimm, David J. Hunter, Louis R. Pasquale, Paul M. Ridker, Frank B. Hu, Daniel I. Chasman, and Lu Qi. 2013. "Novel Locus Including FGF21 Is Associated with Dietary Macronutrient Intake." Human Molecular Genetics 22(9):1895–1902. doi: 10.1093/hmg/ddt032.

6- Ellis PD. The Essential Guide to Effect Sizes: An Introduction to Statistical Power, Meta-Analysis, and the Interpretation of Research Results. Cambridge University Press, Cambridge. 2010.

7- Engineering, Forestry, and Herbivorous Animal Biotechnology. 2021. "FGF21 Polymorphisms of FGF21 Gene and Their Association Analysis with Growth and Carcass Traits in Ovine (Ovis Arise) with Different Genders." 29(02):298–306. doi: 10.3969/j.issn.1674-7968.2021.02.010.

8- Epperlein, S.; Gebhardt, C.; Rohde, K.; Chakaroun, R.; Patt, M.; Schamarek, I.; Kralisch, S.; Heiker, J.T.; Scholz, M.; Stumvoll, M.; et al. The Effect of FGF21 and Its Genetic Variants on Food and Drug Cravings, Adipokines and Metabolic Traits. Biomedicines **2021**, 9, 345. https:// doi.org/10.3390/biomedicines9040345.

9- EVANS, D. M. & PURCELL, S. 2012. Power calculations in genetic studies. Cold Spring Harb Protoc, 2012, 664-74.

10- Frayling, Timothy M., Robin N. Beaumont, Samuel E. Jones, Hanieh Yaghootkar, Marcus A. Tuke, Katherine S. Ruth, Francesco Casanova, Ben West, Jonathan Locke, Seth Sharp, Yingjie Ji, William Thompson, Jamie Harrison, Cecilia M. Lindgren, Niels Grarup, Anna Murray, Rachel M. Freathy, Michael N. Weedon, Jessica Tyrrell, and Andrew R. Wood. 2017. "A Common Allele in FGF21 Associated with Preference for Sugar Consumption Lowers Body Fat in the Lower Body and Increases Blood Pressure." *BioRxiv*. doi: 10.1101/214700.

11- Hazra, A., Kraft, P., Selhub, J., Giovannucci, E.L., Thomas, G., Hoover, R.N., Chanock, S.J., and Hunter, D.J. (2008). Common variants of FUT2 are associ- ated with plasma vitamin B12 levels. Nat. Genet. 40, 1160–1162.

12- International Diabetes Federation. Diabetes Atlas; International Diabetes Federation: Brussels, Belgium, 2019, 9th ed. p. 167. Available online: https://www.diabetesatlas.org/en/resources accessed on 27 July 2020).

13- Jiang, Shan, Rong Zhang, Huating Li, Qichen Fang, Feng Jiang, Xuhong Hou, Cheng Hu, and Weiping Jia. 2014. "The Single Nucleotide Polymorphism Rs499765 Is Associated with Fibroblast Growth Factor 21 and Nonalcoholic Fatty Liver Disease in a Chinese Population with Normal Glucose Tolerance." Journal of Nutrigenetics and Nutrigenomics 7(3):121–29. doi: 10.1159/000367943.

14- Kharitonenkov A, Shiyanova TL, Koester A, Ford AM, Micanovic R, Galbreath EJ, Sandusky GE, Hammond LJ, Moyers JS, Owens RA, et al. FGF-21 as a novel metabolic regulator. *J Clin Invest.* 2005;115:1627–1635. doi: 10.1172/JCI23606.
15- Kralisch S, Fasshauer M. Fibroblast growth factor 21: effects on carbohydrate and lipid metabolism in health and disease. Curr Opin
Clin Nutr Metab Care 2011; 14(4):354-359.

16- Laeger T, Albarado DC, Burke SJ et al (2016) Metabolic responses to dietary protein restriction require an increase in FGF21 that is delayed by the absence ofGCN2. Cell Rep 16:707–716.

17- Laeger T, Henagan TM, Albarado DC et al (2014) FGF21 is an endocrine signal ofprotein restriction. J Clin Invest 124:3913–39224.
18- McCarthy, Mark I. 2010. "Genomics, Type 2 Diabetes, and Obesity." *New England Journal of Medicine* 363(24):2339–50. doi: 10.1056/nejmra0906948.

19- Nishimura T, Nakatake Y, Konishi M, Itoh N. Identification of a novel FGF, FGF-21, preferentially expressed in the liver. Biochim Biophys Acta 2000;1492:203–6.

20- Saber-Ayad, Maha, Sarah Hammoudeh, Hadia Radwan, Shaista Manzoor, Hussein Jabbar, Rahaf Wardeh, Ahmed Ashraf, Peter Habib, Alsamman M. Alsamman, and Rifat Hamoudi. 2020. "The FGF-21 Genetic Variants Rs838133 and Rs838145 Are Associated with High Salt Intake in the Emirati Population." *Journal of Advanced Research* 24(July):485–94. doi: 10.1016/j.jare.2020.05.020.
21- Semba RD, Sun K, Egan JM, Crasto C, Carlson OD, Ferrucci L. Relationship of serum fibroblast growth factor 21 with abnormal glucose metabolism and insulin resistance: the Baltimore Longitudinal Study of Aging. J Clin Endocrinol Metab 2012; 97(4):1375-1382.

22- Schnermann, J. 2015. One physiology does not fit all: a path from data variability to "physiogenetics"? Am J Physiol Renal Physiol, 309, F29-32.
23- Schumann G,.KLB is associated with alcohol drinking, and its gene product b-Klotho is necessary for FGF21 regulation of alcohol preference. Proc Natl Acad Sci USA. 2016;113(50):14372–14377.
24- Søberg, S.; Sandholt, C.H.; Jespersen, N.Z.; Toft, U.; Madsen, A.L.; von Holstein-Rathlou, S.; Grevengoed, T.J.; Christensen, K.B.; Bredie, W.L.P.P.; Potthoff, M.J.; et al. FGF21 Is a Sugar-Induced Hormone Associated with Sweet Intake and Preference in Humans. Cell Metab. 2017, 25, 1045–1053.e6.

25- Talukdar, S., et al., A Long-Acting FGF21 Molecule, PF-05231023, Decreases Body Weight and Improves Lipid Profile in Non-human Primates and Type 2 Diabetic Subjects. Cell Metab, 2016. 23(3): p. 427-40.
26- Tanaka, T., et al., Genome-wide meta-analysis of observational studies shows common genetic variants associated with macronutrient intake. Am J Clin Nutr, 2013. 97(6): p. 1395- 402.
27- von Holstein-Rathlou, S.; BonDurant, L.D.; Peltekian, L.; Naber, M.C.; Yin, T.C.; Claflin, K.E.; Urizar, A.I.; Madsen, A.N.; Ratner, C.; Holst, B.; et al. FGF21 Mediates Endocrine Control of Simple Sugar Intake and Sweet Taste Preference by the Liver. Cell Metab. 2016, 23, 335–343.
28- Y. Panahi1, S. Bonakdaran2, M.A. Yaghoubi.,et al .(2016). Serum levels of fibroblast growth factor 21 in type 2 diabetic patients. General Endocrinology. 10.4183/aeb.2016.257.

29- Yu, Weihui, Hong Zhu, Xiong Chen, Xuejiang Gu, Xingxing Zhang, Feixia Shen, Weiping Jia, and Cheng Hu. 2019. "Genetic Variants Flanking the FGF21 Gene Were Associated with Renal Function in Chinese Patients with Type 2 Diabetes." *Journal of Diabetes Research* 2019. doi: 10.1155/2019/9387358.

Thi-Qar Medical Journal (TQMJ):Vol.(22),No.(2),2021
Web Site: https://jmed.utq.edu.iq Email: utjmed@utq.edu.iq
ISSN (Print):1992-9218, ISSN (Online):1992-9218

ترافق المتغيرات الشائعة للانماط الوراثية (rs499765 و rs838133) لجين FGF21 لدى العراقيين المصابين بداء السكري من النوع الثاني

منى عبد الرضا البرقعاوي علي مجيد عماره ذكرى عبد جبر الكيشوان
عبد الحسين الجنابي ماجد كاظم حسين رشا محمد عبود

الخلاصة
الخلفية: مرض السكري النوع الثاني يعتبر مشكلة صحية عالمية. FGF21 يعتبر البروتين المنظم لعمل الغدد الصم يعمل على تنظيم عمليات الايض وتوليد الخلايا .
هدف الدراسة : تقييم علاقة تعدد الاشكال للمتغيرات الشائعة لجين FGF21 (rs499765 و rs838133) لدى مرضى داء السكري النوع الثاني .
طريقة العمل: لغرض تقييم العلاقة بين تعدد الاشكال للمتغيرات الشائعة لجين FGF21 (rs499765 و rs838133) وداء السكري من النوع الثاني، تم تصميم دراسة من نوع الحالات والشواهد، شملت 200 مشاركا (100 مريض بداء السكري النوع الثاني و 100 من الأصحاء) تم اختيارهم عشوائيا بعد ان تم تشخيصهم من قبل الأطباء المختصين في مركز الغدد الصماء في مدينة الصدر الطبية / محافظة النجف الاشرف. تضمنت المقاييس التي تم تقييمها: مستوى الأنسولين ، مستوى الدهون وحساسية الانسولين ، كما تم دراسة التنميط الوراثي لجين FGF21 باستخدام تقنية TARMS-PCR .
النتائج : وجد فرق معنوي بين الاصحاء ومرضى السكري النوع الثاني في المستويات الكولسترول ، VLDL، TG و HDL.
دل تنميط جين FGF21، rs838133 SNP ، عن إرتفاع معنوي في عدد حاملي النمط الجيني المتخالف AC في مجموعة المرضى مقارنة بمجموعة الأصحاء عند فحصهما بوساطة النموذج المشترك المهيمن (OR=1.94) والمشترك (OR=1.8) وفوق المشترك (OR=1.94) . وقد فشل تحليل معطيات نتائج ال rs499765 SNP في إظهار فروقات معنوية عند فحصها بوساطة جميع النماذج الوراثية.
الاستنتاج : ان النمط الوراثي AC لجين FGF21، rs838133 ، يشكل عامل خطورة لنشوء مرض السكري النوع الثاني في السكان العراقيين قدره 1.9 مرات.