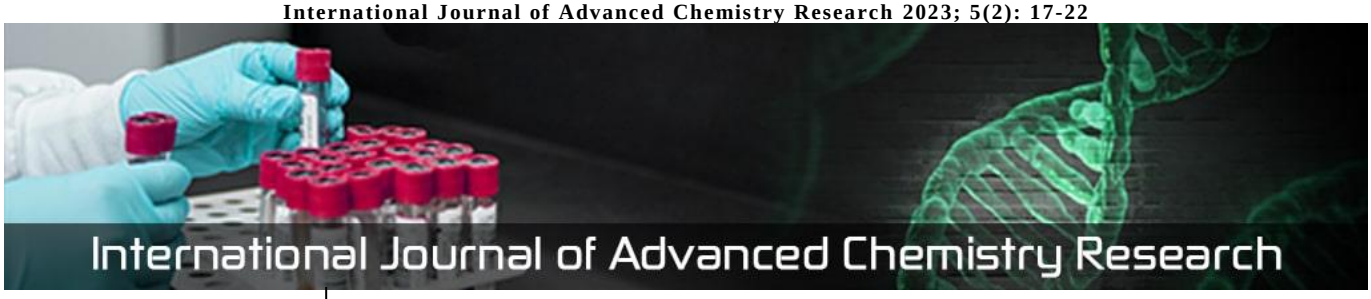

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Association of some biochemical parameters in FHCL females with TCF7L2 polymorphism

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Abstract
Genetic variants of transcription factor7, such as the TCF7L2 gene, have been found to be highly linked diabetes to type 2. Familial combined hyperlipidaemia (FCHL) is defined by hypercholesterolemia, hypertriglyceridemia, or together. Furthermore, abnormalities of glucose metabolism are frequent in FCHL. As a result, the present study suggested hypothesized that TCF7L2 could be linked to the genetic predisposition for this frequent dyslipidemia in a very female populace of Iraq. As a result, we proposed that TCF7L2 could be linked to the hereditary predisposition to this frequent dyslipidemia in a predominantly female Iraqi population. The current investigation included 50 DM2 with FCHL female patients and 50 samples that were healthy. A single primer for amplification was used for analyzing the gene, and the PCR approach was combined with sequencing. This study looked at the effect of TCF7L2 polymorphism on FCHL and the component characteristics lipid profile and kidney function test in serum by using chemical testing equipment. The findings: For total subjects, (G/A vs. AG + GT) were substantially higher in FHCL cases than in control groups, and TCh,TG, ApoB, or hyperglycemia were linked with G>A and G>T genotypes of the TCF7L2 in Iraqi females which increased the risk of FHCL. In the TCF7L2 gene the allele G was more common in FHCL cases than in controls subjects. Conclusions: Our findings suggested that TCF7L2 genetic variants were substantially correlated to increased G>A and G>T in FCHL together with DM2 in Iraqi females.

Keywords: TCF7L2, FHCL, polymorphism, hypercholesterolaemia

Introduction
Intronic mutations in the transcription factor 7-like 2 gene were linked to type 2 diabetes (DM2) in cases from the Iceland and United States [1]. These results have been strongly confirmed in several studies of diabetes type 2 [2, 7]. Europids [1, 4] contain indications of a relationship with DM2. TCF7L2 is a versatile box including transcription component that participant in the pathway of WNT signaling. The proteins of WNT are superior cell differentiation and proliferation, and the proteins in their signaling pathway also have a direct affect cell adhesion and transcription of gene [8]. Mutations in the WNT signaling system have been identified in a variety of people disorders, such as cardiovascular, skeletal, cancer, and neurological issues [9, 10]. A complicated hereditary disorder FCHL (familial mixed hyperlipidemia) is linked to coronary artery disease. Persons with FCHL may develop hypertriglyceridemia, hypercholesterolemia, and mixed hyperlipidemia [11]. Furthermore, Medical characteristics of DM2, such as impaired glucose tolerance, hypertriglyceridemia, and hyperinsulinemia, have been associated with FCHL profiles [12, 13].
We reasoned that, given this type of phenotype coverage, the TCF7L2 mutations previously related to DM2 might also affect the FCHL phenotype. The role of TCF7L2 mutations, which have recently been related to FHCL, will be examined in the present study [1].

Material and Methods
Samples
In this study, a total of 100 females (aged 25-45 years) were enrolled, including 70 patients with FHCL and DM2 who consulted the national diabetes center, Baghdad, Iraq from November 2022 to March 2023, and 50 healthy controls group. The Center Ethical Committee approved the trial since physicians made diagnoses for every patient. Using the American Diabetes Association criteria [14], fifteen individuals were classified as DM2 with FHCL.

Each participant provided written educated consent. The FCHL consideration criteria were: Total cholesterol (TCh) and triacylglycerol (TG) (the levels age/sex were 110th) population of Iraqi families. Furthermore, belated CHD was used as a deciding criteria for the samples to guarantee that families weren't impacted by severe specific FCHL norms CHD was confirmed via angiography or after a myocardial infarction [11, 15, 16].

In this group, 50 girls were assigned DM2 with FCHL. The morals committees of the taking an interest centers approved the examination configuration. This study excluded samples with compromised chronic inflammatory, malignancy, renal function, and connective tissue diseases.

Biochemical tests

All samples were obtained when fasting (for at least 8 hours). The blood specimen were placed into plain tubes, with the serum sample examined for biochemical characteristics and EDTA tubes examined for molecular analysis. Al-Nahrain University's Molecular and Biotechnology Laboratory's Biotechnology Research achieved all of the estimates in the female Iraqi FCHL families.We evaluated the concentration of FBS, (TCh), (LDL), (TG), (HDL), (Cr), (UA)and (BUN) in serum by using apparatus for chemical tests [15, 16, 17]. Because 30 days have been noticed through clinical examination as a time of break for statins according to LDL kinetic and statins elimination rate data, sample individuals who took lipid dropping medicines were assessed after their lipid dropping treatments was ceased for roughly 30 days.

Genetic analysis

To begin, complete blood samples were drawn from the veins of 50 patients with FHCL and 50 controls as healthy. We using a DNA extraction kit (Geneaid extraction kit, Korea) to obtained DNA from the above specimens.

To scan TCF7L2 gene, one primer for amplification was designed. Then amplified a segment 196 bp by using reverse primer R: 5′- AGATGCAGCAAAGCCAAAGT -3′ and forward primer F: 5′-GGCCTCTTTCATCACAGACC-3′ and. The PCR procedure was performed out in a 201 whole volume, which included 5 l of master mix (From bioneer, USA), 1 l for each primer, and 2 l of DNA template, before being completed in a total volume of 20l with 11 l of distal water. The procedure of PCR were programmed in the following order: begin denaturation at 95 °C for 5min, then proceed to 35 cycles of 95 °C for 30 second then 58 °C for 30second, and 72 °C for 30s, preceded by final extension at 72 °C for 5 minutes. Shortly after red safe staining, electrophoresis was used to separate the products of PCR on 1.5% agarose gels and examined under ultraviolet light (302nm).

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS 22.0) was employed for all statistical analyses. The mean and standard deviation (SD) were used for the expression of all continuous variables. To contrast different means, the t test was employed. Comparing categorical data, such as genotype frequencies, was done using the x^2 test. A 95% confidence interval (CI) and the odds ratio (OR) were utilized to analyze the effects of significant risk factors. Statistical significance is thought to exist when the p value is less than 0.05.

Results

The biochemical parameters of FHCL cases as well as control samples in female Iraqi patients are shown in Table 1. The serum concentrations (g/l) of Apo B and the serum concentration (mmol/L) of FBS, TC, TG,LDL, BUN in FHCL patients were significantly greater when compare to control groups (p<0.05), while FHCL patients' serum HDL and UA concentrations (measured in mmol/L) were considerably significantly lower than those of the control groups (p<0.05). Among FHCL cases and control subjects, there was no discernible significant change in the blood concentration (mmol/L) of (Cr) (p>0.05).

Table 1: Clinical parameters of FHCL patients and control samples

Parameters	FHCL with DM2	Healthy Control	*p* value
Number (n)	50	50	
FBS	8.12 ± 2.14	4.11 ± 0.88	0.003*****
TCh	6.71 ± 1.02	4.54 ± 1.02	0.02 *
TG	3.01 ± 0.91	2.15 ± 0.58	0.04*
LDL	3.17 ± 0.75	2.45 ± 0.10	0.01*
HDL	1.12 ± 0.31	1.89 ± 0.75	0.02*
Apo B	1.95± 0.13	1.12± 0.11	0.002*
BUN	4.98 ± 0.16	4.01 ± 0.45	0.009 *
UA	249.21 ± 63.24	254.01 ± 74.51	0.001 *****
Cr	55.98 ± 14.49	63.55 ± 15.49	0.1

The *p* value ***** $p \leq 0.05$=Significant.

Genetic testing

The results presented that two 196-bp segments of the (TCF7L2) gene were amplified in FHCL and control samples as characterized in Figure 1.

Table 2 indicates the alterations in the TCF7L2 area for the fragment 196 bp of female FHCL with DM2 belonging to healthy.

Table 2: Alterations in the TCF7L2 area for the fragment 196 bp of

Sample	Number	Substitution type	Location	Nucleotide	Range of nucleotide
FHCL with DM2	33	Transition	15810	G>A	15660 to 15812
	4	Transition	15657	G>A	15650 to 15812
	6	Transition	15658	A>G	15650 to 15812
	7	Transversion	15661	G>T	15650 to 15812
Controls	5	Transition	15810	G>A	15662 to 15812
	45	----------			15660 to 15809

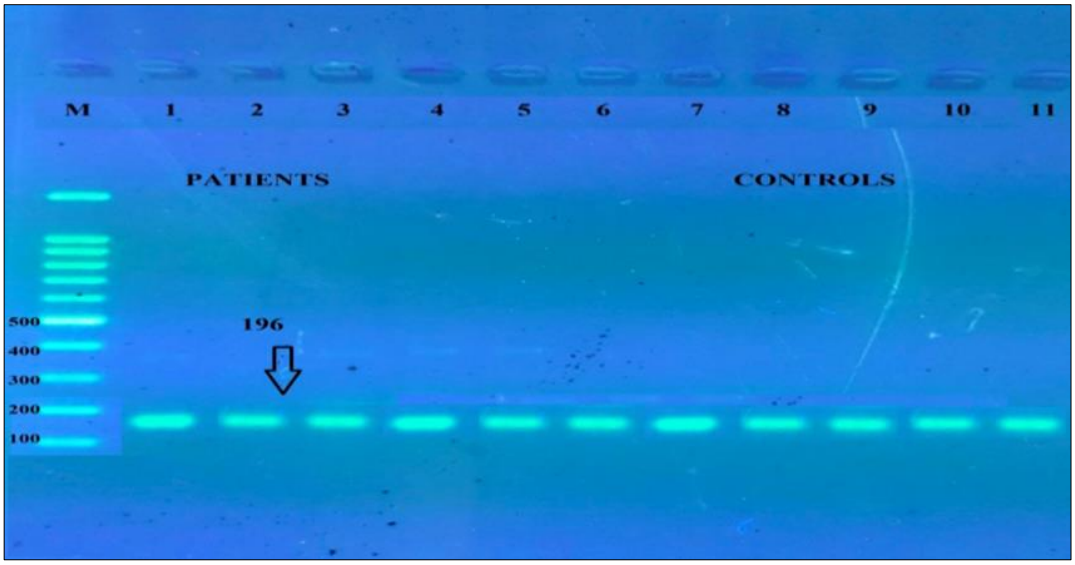

Fig 1: The amplified TCF7L2 with segment 196 bp from healthy and FHCL with DM2 samples was separated into bands by 1.5% agarose gel electrophoresis (5 v/cm, 2 h, 1x tris-acetoc buffer dyed with red safe dye), which were thereafter visible under ultraviolet light. Lanes (1, 2, 3, 4, 5) characterized patients' samples, while lanes (7, 8, 9, 10, 11) characterized healthy samples.

The genotype frequency and distribution (AG + GT vs. T/G) revealed a significant difference between FHCL and control participant as shown in Table 3. G allele was statistically significantly higher in FHCL cases than in y controls for female members.

Table 3: Genotype variations and frequencies in groups of individuals with FHCL cases and controls.

Genotype	FHCL	Control	*p* value
	N (%)	N (%)	
G/A	39(78%)	16(32%)	0.002 *
A/G	5(10%)	0(0%)	
G/T	6(12%)	0(0%)	
Allele			
G	25(50%)	8(50%)	0.003*
A	22(44%)	8(50%)	
T	3(6%)		

For all subjects, there were a significantly differente in the frequency and distribution of genotypes (G/A *vs.* AG + GT) between FHCL and control groups ($p \leq 0.05$), and for female samples, G allele was significantly larger in FHCL with DM2 than in control groups (25% *vs.* 8%).

Table 4: Mean TCF7L2 genotypes with analyzed parameters in the FHCL patients and controls groups

Samples	Analyzed parameters	Genotypes		
		G/A	A/G	G/T
FHCL	TCh	5.1±0.91	6.0±0.60	5.3±1.14
	TG	2.3±0.60	3.1±0.77	1.9±0.71
	FBS	5.1±0.21	4.7±0.52	4.8±1.01
	ApoB	1.0±0.20	1.3±0.29	1.0±0.29
Controls	TCh	-	4.9±1.22	-
	TG	-	1.9±0.75	-
	FBS	-	4.5±0.50	-
	ApoB	-	1.1±0.37	-

The mean TCF7L2 genotypes with analyzed parameters in the female Iraqi population are shown Table 4. The G/A and G/T genotypes were not detected in controls, however the A/G genotype was found in both participants. The (TCh), ApoB, and (TG) levels were significant greater in FHCL cases females than in controls. (p value ≤0.05).

Table 5: A logistic regression evaluation that included genotypes in study groups

Risk Factors	OR	95% CI	P
G/A *vs.* AG + GT	1.210	1.020–1.450	0.022 *
TG	0.757	0.911–0.899	0.005 *
TCh	1.499	1.350–1.698	0.003 *
HDL	0.031	0.020–0.050	0.004 *
LDL	0.801	0.599–0.899	0.002 *
Apo B	0.031	0.017-0.051	0.006*

Table 5 depicts a multiple logistic regression test that included genotypes with the a number of parameters: TCh, TG, HDL, LDL, and ApoB serum concentrations were the most bothersome factors for FHCL. After multivariate alteration, all of our members in the current study remain completely associated to FHCL.

Discussion

Variations in the TCF7L2 gene were linked to FHCL and DM2 in an Iraqi female population. In this study, we looked into how TCF7L2 genotypes, which were previously linked to DM2, affect FCHL segment characteristics and fasting blood glucose levels in an FCHL Iraqi female. Our findings suggest that TCF7L2 variants provide strong evidence for a link with great TG for a same risk genotype in both assays.

Previous research has highlighted the importance of TCF7L2 in cancer and oncogenesis cancer development [34-36]. To investigate the significance of TCF7L2 in diabetes type 2 and in what way variations of this gene affect sensitivity to diabetes type 2, functional investigations are required.

Diabetes type 2 is distinguished by decreased insulin production in response to elevated demand on metabolism. This is due to decreased beta-cell bulk and/or poor beta-cell activity [37]. This flaw in beta-cell compensation appears to be the result of a combination of genetic influences and environmental. TCF7L2 regulation, according to Shu *et al.* [38], may play a significant role in the control of both beta-

cell function and survival, and targeting its expression may be a new technique to preserve beta-cell survival in DM2.

Furthermore, Common TCF7L2 variations were shown to be associated with an elevated risk of diabetes in individuals with reduced glucose tolerance by Florez *et al*. [7]. They also provided evidence that TCF7L2 risk polymorphisms were associated with impaired beta-cell activity yet not with insulin resistance. TCF7L2 is found in the majority of human tissues, especially mature pancreatic beta-cells, with the possible exception of muscle cells in the skeleton [20]. Obese people with DM2 had lower levels of TCF7L2 expression in their adipose tissue [20]. The normal Wnt signaling pathway, one of the main mechanisms for controlling the formation and proliferation of cells, is transcribed by the transcription factor TCF7L2. Wnt signaling controls glucagon-like peptide (GLP-1) production in intestinal cells [39, 40, 41] as well as beta-cell insulin secretion and proliferation [38, 39, 40]. Additionally, Lyssenko *et al*. [42] showed how the enteroinsular axis, islet gene expression, and insulin production are all impacted by the elevated risk of DM2 brought on by TCF7L2 mutations. It is uncertain how TCF7L2 specifically affects glucose metabolism and the pathogenesis of DM2, but it is plausible that TCF7L2 controls the release of glucose-sensitive insulin from beta cells.

The finding of genetic variations of the TCF7L2 gene that contribute to hereditary susceptibility to DM2 is a significant step forward in the genetic study of the disease. Grant *et al*. were the first to report on the link between genetic variants in the TCF7L2 gene and DM2 [4], they found a substantial relationship of TCF7L2 polymorphisms with greater risk of DM2 in Icelandic persons, which was replicated in US and Danish individuals. Numerous reported research have explored this gene since its original publication, with significant replication across various ethnic populations. Indeed, the TCF7L2 gene is thought to be one of the most important in determining hereditary vulnerability to DM2 in humans. Van and colleagues [43] investigated whether TCF7L2 gene variants affect DM2 susceptibility in a Dutch population and found that DM2 patients had higher percentages of the heterozygous (GT) and homozygous (TT) genotypes as well as the minor allele of the rs12255372, which is significantly more common than it is in controls. The allele frequencies discovered in this study for Finns are in good agreement with the frequencies of alleles already identified in Finns for type 2 diabetes [30]. The findings in the present study show a substantial link between TCF7L2 gene variations and elevated TG levels in FCHL from two distinct groups. Despite the fact that Huertas-Vazquez *et al*. [23] discovered differences in genotypes frequencies for these polymorphisms in Mexican and Finnish populations, they found considerable evidence for relation with high AG for the same SNPs. Importantly, gene expression study of 47 fat samples from Mexican FCHL patients and controls validated the findings, this result agreement with our study. TCF7L2 gene expression was shown to be considerably lower in unrelated TG and FCHL patients than in unrelated normolipidaemic controls. It is worth mentioning, however, that TCF7L2 expression was lower in DM2 patients in prior investigation. TCF7L2's function in adipose tissue is unknown. TCF7L2 and its as-yet unidentified variations may be implicated in adipose function or adipogenesis via affecting transcriptional control of genes that lead to TG accumulation [31-33].

According to Chandak *et al*. [26], genetic variation in the TCF7L2 gene may be significantly related with DM2 in Indian populations, as previously documented in European populations. In our investigation, we discovered that TCF7L2 polymorphisms were related with the risk of FHCL and DM2 in the Iraqi female population. The genetic distribution of TCF7L2 differed significantly between FHCL patients as well as controls. The frequency of alleles G of the TCF7L2 gene was greater in FHCL patients compared to control people. The G allele of the TCF7L2 gene was found to be a risk factor for FHCL in female patients. After multivariate correction for confounding factors such as serum concentrations of TCh, TG, LDL, and HDL for total subjects, the (G/A vs. AG + GT) was substantially greater in persons with FHCL than in controls. This finding suggested that the existence of the GA and GT genotypes of the TCF7L2 gene elevated the risk of FHCL in Iraqi females. The G allele frequency of the TCF7L2 gene was greater in FHCL patients compared to control people. This outcome could be due to two factors. For starters, the sample size of Iraqi females was modest, limiting statistical power to identify a relationship between TCF7L2 and FHCL in females. Second, it could be due to estrogen. Estrogen is involved in numerous processes that are both physiological and pathological, including lipid metabolism, glucose metabolism, and insulin-related pathways for signaling [44]. Several earlier investigations have suggested that TCF7L2 plays an important function in glucose homeostasis. As a result, estrogen influences the function of the TCF7L2 gene, either directly or indirectly.

The frequency of the G allele and A allele of the TCF7L2 gene was significantly greater in the Iraqi female subjects in our study. The current study included some limitations. For starters, the current study was constrained by a small subject size of Iraqi females. When estimating ORs, this could have resulted in low statistical significance and broad confidence intervals. A bigger sample size case-control study is necessary to evaluate the connection between TCF7L2 and FHCL polymorphisms in Iraqi females. Second, more research is needed to determine the fundamental molecular mechanism that links TCF7L2 polymorphisms to FHCL. Although the precise molecular mechanism behind the link of the TCF7L2 gene and FHCL with DM 2 risk is unknown, these consistent findings suggest that the TCF7L2 gene is an important locus for predicting hereditary susceptibility to FHCL.

Abbreviations

FHCL: Familial combined hyperlipidaemia, TCF7L2: Transcription Factor 7 Like 2 gene, DM2: Diabetes type 2, FBS: Fasting Blood suger, ApoB: apolipoprotein B, Cr: creatinine BUN: blood urea nitrogen, UA: uric acid, HDL: high density lipoprotein, TCh: Total cholesterol, LDL: low density lipoprotein, TG: Triglyceride.

Conclusions

Taking everything into account, to the best of our knowledge, these findings show that the G alleles in TCF7L2 are all linked to high GA levels in Iraqi female FCHL families, and that ApoB, TCh, and TG levels were greater in FHCL cases than in control groups. Useful proof that TCF7L2 is in fact implicated in the genesis of FCHL is

provided by the observed substantial difference in TCF7L2 between Iraqi FCHL- and GA-influenced participants and neither molipidaemic nor healthy control people. This finding may improve our knowledge of genetic variations and disease-association research.

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