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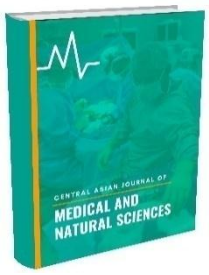

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Polymorphism of acyl-CoA *synthetase 1 ACSL1* Gene rs2292899 in Iraqi Patients with type2 Diabetes Mellitus

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Abstract: The aim of this study was to investigate the association of polymorphism of acyl-CoA synthetase 1 (ACSL1) rs2292899 to the susceptibility of type2 diabetes mellitus in diabetic patients from Wasit province using TaqMan SNP genotyping assay. A total of 80 participants (45 confirmed patients with type2DM and 35 healthy individuals as controls) were selected by using a convenient sampling method. The results of the current study displayed that in both in patients and control groups, the distribution frequencies of the genotypes and alleles of acyl-CoA synthetase 1 rs2292899 were according to Hardy Weinberg equilibrium P>0.05, indicating that the frequency of the gene has reached the genetic equilibrium and the selected samples were representative of the population. G allele is a major one in the studied groups. This allele is more frequent in T2DM patients (92.22%) compared to controls (84.29%). Two genotypes were detected in acyl-CoA synthetase 1 rs2292899.The homozygous GG genotype was the main genotype in studied group with higher percentage in T2DM patients 84.44% compared with 68.57% in controls, p=0.09166.The heterozygous AG genotype was lower in patients (15.56%) vs. (31.43%) in healthy controls. These results suggest that the G allele might be a risk factor in T2DM while the A allele may be considered as a protective allele against the disease. The association analysis showed that the GG genotype increases the association with T2DM than that in controls OR=2.4881 (CI95% [0.8478 to 7.3022]), p = 0.0970. The heterozygous genotype of AG decreases the association with T2DM OR=0.4019(CI95% [0.1369 to 1.1796]), p = 0.097.The subgroup analysis among females demonstrated that the genotypes GG or AG was not associated with T2DM with OR=1.00 for each genotype. OR of 1.00 indicates that the risk is comparable in the two groups.

The association analysis showed that the GG genotype increases significantly T2DM risk among the males patients OR= 6.7083 (CI95% [1.2018 to 37.4450]), p = 0.0300. The heterozygous AG genotype decreases significantly the association with the disease OR= 0.1491 (CI95% [0.0267 to 0.8321]), p = 0.0300.In conclusion, the *acyl-CoA synthetase 1* rs2292899 have possible roles in type2 diabetes mellitus susceptibility.

Introduction

Type 2 diabetes mellitus (T2DM) is an expanding global health problem closely linked to the epidemic of obesity) De Fronzo *etal*.,2015). This problem is considered a severe public health problem in terms of human life and average life expectancy (Khan *etal*.,2020). In recent years, there has been an incidence increase, affecting individuals of all ages. The global burden of T2DM on people 20–79 years old is projected to increase to 629 million in 2045 compared to 425 million in 2017 (Al-Rifai *etal*.,2019). The breakthrough discovery of a new family of naturally endogenous, small (~22 nucleotides), microRNAs that are small, conserved non-coding RNA molecules which are involved in most cellular processes through interaction with 3'-UTR region of the genes.(Ameres and Zamore,2013). MiRNAs regulate RNAs and gene expression in various common diseases such as T2DM (Kong *etal*.,2011) .MiRNAs seem to play a role in the development of pancreatic islets and differentiation of insulinproducing cells (Poy *etal*.,2007) . Single nucleotide polymorphisms (SNP) are variations in single nucleotides of genomic DNA sequence, which have modified potentials on gene functions) Shen *etal*.,2013(. SNPs affect the miRNA binding efficiency, giving rise to increased or decreased miRNA regulation (Ghaedi *etal*.,2015). Single-nucleotide polymorphisms that reside in the microRNA target site can affect the binding of miRNA to mRNA, which can either create illegitimate-binding sites or abolish existing-binding sites(Chen *etal*.,2008). Recent studies have indicated that polymorphisms in miRNA target sites can affect gene and protein expression and lead to influence the risk of certain human diseases, including Tourette's syndrome and cancers(Abelson *etal*.,2005:Chin *etal*.,2008). Limited data are available that has investigated the association between gene polymorphisms in miRNA-binding sites and susceptibility to type2 diabetes mellitus especially acyl-CoA synthetase 1 ACSL1 rs2292899.

The present study was designed to analyze the polymorphism of *ACSL1* rs2292899, and to determine whether there is an association between this polymorphism and the type2 diabetes mellitus phenotype in an Iraqi population from Wasit province.

Materials and Methods

This is a case –control study, The participants included 45 confirmed type2 diabetes mellitus patient (25males and 20 females) their age 40–70 years (mean ± standard deviation: 51.92± 51.50 years, median= 51 years). The control group comprised of 35 healthy individuals (19males and 16 females), their age 40-70 years (mean ± standard deviation: 51.52 ± 47.18 years, median=48years). Data collection encompassed a range of factors, including demographic details, medical history, and sample collection date, gathered from participants who met global diagnostic criteria. Samples were collected from Alzahraa Teaching Hospital in Kut, Iraq. For blood sample collection, 3 ml of blood was obtained via vein puncture from each participant, with the collected blood then transferred to sterile ethylenediaminetetraacetic acid –k3 (EDTA) tubes, labelled, and stored at -20°C for subsequent DNA extraction and genotyping.

Genomic DNA Extraction:

Genomic DNA was extracted from whole blood utilizing the Quick-DNA™ Blood MiniPrep kit (Zymo, USA) Catalogue Nos. D3024 & D3025. The quality of the extracted genomic DNA was

assessed via Nanodrop, measuring the A260/A280 absorbance ratio within the range of 1.8 to 2.0, indicative of high quality.

SNPs Genotyping:

The TaqMan custom SNP genotyping assay from Thermo Fisher Scientific was utilized for genotyping the SNP rs2292899 in the acyl-CoA synthetase 1(ACSL1) rs2292899 gene. Real-time PCR was employed for the allele-specific discriminating approach. The reference and alternative alleles for rs2292899 were referred to from NCBI.

Statistical Analysis:

The data analysis was carried out using SPSS 21.0 software. The significance level (P-value) was categorized as follows: Sig. denoting Significant (P<0.05), and NS representing non-Significant. Analysis of variance (ANOVA) was employed to assess group differences.

Results

Forty-five Patients with type2DM (25males and 20 females) and 35 healthy individuals (19 males and 16 females)) were genotyped for of diabetes mellitus rs2292899 gene polymorphism.In both patients and control groups, the distribution frequencies of the genotypes and alleles *of acyl-CoA synthetase 1* rs2292899 were according to Hardy Weinberg equilibrium P>0.05, indicating that the frequency of the gene has reached the genetic equilibrium and the selected samples were representative of the population. The allele and genotype frequencies of rs2292899 gene polymorphisms were used to estimate the odds ratio (OR), confidence intervals (95% CIs), χ 2, and p-value.The distribution of genotypes and allele frequencies of rs2292899 in T2DM patients is shown in Table (1). G allele is a major one in the studied groups. This allele is more frequent in T2DM patients (92.22%) compared to controls (84.29%). Two genotypes were detected in *acyl-CoA synthetase 1* rs2292899.The homozygoue GG genotype was the main genotype in studied group with higher percentage in T2DM patients 84.44% compared with 68.57% in cintrols,P=0.09166.The heterozygoue AG genotype was lower in patients (15.56%) vs. (31.43%) in healthy controls.These results suggest that the G allele might be a risk factor in T2DM while the A allele may be considered as a protective allele against the disease.

Table (1) Distribution of genotypes and allele frequency of *acyl-CoA synthetase 1* rs2292899 A/G in T2DM patients and controls

Groups	Genotype (%)			Allele frequency (%)	
	GG	AG	AA	G	A
Control	24(68.57)	11(31.43)	0.00	(84.29%)59	(15.71%)11
Patients	38(84.44)	7(15.56)	0.00	(92.22%)83	7 (7.78%)
Chi square	2.845			2.484	
P-value	0.09166			0.11501	
Significance	Ns.			Ns.	

Ns.: non-significant P>0.05

Susceptibility analysis of *acyl-CoA synthetase 1* rs2292899 A/G gene polymorphism withT2DM

Table (2) shows the association of each genotype of rs2292899 gene with susceptibility toT2DM. The GG genotype increases the association with T2DM than that in controls OR=2.4881 (CI95% [0.8478 to 7.3022]) ,P = 0.0970. The heterozygous genotype of AG decreases the association with T2DM OR=0.4019(CI95% [0.1369 to 1.1796]), P = 0.0970.

Table (2): Odds ratio of *acyl-CoA synthetase 1* rs2292899 A/G in T2DM patients and controls .

Genotypes	Control no.(%)	Patients no.(%)	OR	OR95%CI	P value	Significance
GG	24(68.57)	38(84.44)	2.4881	0.8478 to 7.3022	0.0970	Ns.
AG	11(31.43)	7(15.56)	0.4019	0.1369 to 1.1796	0.0970	Ns.
AA	0.00	0.00				

Ns.: non-significant P>0.05

The odds ratio among females of *acyl-CoA synthetase 1*rs2292899 A/G gene in studied groups

The association analysis of acyl-CoA synthetase 1 rs2292899 among females T2DM patients and controls revealed that the genotypes GG or AG are not associated with T2DM with OR=1.00 for each genotype table 3.OR of 1.00 indicates that the risk is comparable in the two groups.

Table (3): Odds ratio of *acyl-CoA synthetase 1* rs2292899 A/G among female T2DM patients and control.

Genotypes	Control	Patients	OR	OR95%CI	P value	Significance
GG	12	15	1.0000	0.2191 to 4.5640	1.0000	Ns.
AG	4	5	1.0000	0.2191 to 4.5640	1.0000	Ns.
AA	0.00	0.00				

Ns.: non-significant P>0.05

The Odds ratio among males of *acyl-CoA synthetase 1* rs2292899 A/G gene in studied groups

The association analysis showed that the polymorphism rs2292899 of the GG genotype increases significantly T2DM risk among the males patients OR= 6.7083 (CI95% [1.2018 to 37.4450]), P = 0.0300. The heterozygous AG genotype decreases significantly the association with the disease OR= 0.1491 (CI95% [0.0267 to 0.8321]) P = 0.030.

Table (4): Odds ratio among males of *acyl-CoA synthetase 1* rs2292899 A/G in studied groups.

Genotypes	Control	Patients	OR	OR95%CI	P value	Significance
GG	12	23	6.7083	1.2018 to 37.4450	0.0300	Sig. *
AG	7	2	0.1491	0.0267 to 0.8321	0.0300	Sig. *
AA	0.00	0.00				

*P< 0.05

Discussion

The present study revealed that allele and genotype frequencies of *acyl-CoA synthetase 1* rs2292899were according to Hardy Weinberg equilibrium. The G allele is more frequent in T2DM patients compared to controls .Two genotypes were detected in *acyl-CoA synthetase 1* rs2292899.The homozygoue GG genotype was the main genotype in studied group with higher percentage in T2DM patients compared with in cintrols,.The heterozygous AG genotype was lower in T2DM patients compred to healthy controls. In previous studies that have investigated the association of polymorphisms of several factors and other biomarkers among patients with type2 DM from Wasit province, (Yousif and Ghali,2021), revealed that IL-10 is a major contributor to the onset of type 2 diabetes mellitus and there may be a correlation between low levels of interleukin-10 and type two diabetes(Al-Sarray and Ahmed ,2021) found that may be a correlation between high levels of TNF-α and type 2 diabetes mellitus.(Shamkhi and Ahmed ,2021), displayed that levels of SIRT1 may be not

associated with type2 diabetes mellitus. Furthermore, the cell free mitochondrial DNA increases significantly in patients with type2 diabetes mellitus (Hussein and Ghali,2022). COX-1 is a major contributor to the onset of type 2 diabetes and there may be an association between low levels of cyclooxygenase-1and type 2 diabetes (Jebil and Ghali,2021). The association analysis of IL-17AG197A gene polymorphism with T2DM displayed that heterozygous AG genotype of IL-17AG197A showed a risk association among T2DM with OR=1.24 CI95% (0.31 - 5.01) p-value =1.00 and the G allele was associated with an increased risk ofT2DM (Khidhum and Ahmed,2022). (Mahmood and Ghali,2022), revealed that there was an association between the polymorphism of Osteoprotegerin (OPG) polymorphism and susceptibility to type2 diabetes mellitus. (Mahmood and Ghali,2022b), found also that there may be a correlation between high levels of OPG and T2DM.

These results suggest that the G allele might be a risk factor in T2DM while the A allele may be considered as a protective allele against the disease. Susceptibility analysis showed that the GG genotype increases the association with T2DM than that in controls. The heterozygous genotype of AG decreases the association with T2DM.The subgroup analysis displayed remarkable results between males and females. Among females ,the association analysis revealed that the genotypes GG or AG are not associated with T2DM ,whereas among males the GG genotype increases significantly T2DM risk among the males patients. The heterozygous AG genotype decreases significantly the association with the disease. These results are not consistent with Zhao *etal*., 2013 who found that (ACSL1) rs2292899GA heterozygote genotype was associated with a significantly increased risk of T2DM.

However, the results of this study agreed with Zhao *etal*.,2013 that the GG genotype is the dominant genotype and that the homozygous AA genotype was not associated with the disease. The SNP rs2292899 was located on 3′-UTR of the ACSL1 gene. ACSL1, the major acyl-CoA synthetase of adipocytes, is involved in peroxisome proliferator-activated receptor signaling pathway. Peroxisome proliferator-activated receptors have been identified as the key regulators of fatty acid and lipoprotein metabolism, glucose homeostasis, cellular proliferation/differentiation and the immune response.

Although ACSL1 clearly has an important role relevant to lipid metabolism and fatty-acid-induced insulin resistant, rare studies to date have investigated the association between genetic variants of ACSL1 and T2DM(Lobo etal.,2009) .The SNP rs2292899 of ACSL1 gene was predicted to be located on binding site of miR-34a seed. Expression of miR-34a increased in the β-cell line MIN6B1 or pancreatic islets after exposed to palmitate in islets of diabetic db/db mice. Elevated miR-34a resulted in sensitization to apoptosis and impaired nutrient-induced insulin secretion (Lovis *etal*.,2008; Kong *etal*.,2010) showed that hypertriglyceridemia as one of the characteristic blood lipid abnormalities in diabetes could favor the onset of diabetes via higher serum level of miR-34a.(Manichaikul *etal*.,2016)provided an evidence that humans of ACSL1 SNPs associated with fasting glucose, diabetes, and subclinical atherosclerosis. In metabolically active tissues the acyl-CoAs go toward mitochondrial ß-oxidation. ACSL1 can also specify phospholipid synthesis, and these can serve as a source of arachidonic acid-CoA metabolites that support prostaglandin synthesis to fuel inflammation in diabetes that exacerbates atherosclerosis(Kanter etal.,2012). Whereas Acsl1 mRNA expression is controlled by inflammation and hyperglycemia, the mechanisms underlying the induction of Acsl1 mRNA by these stimuli have remained enigmatic. (Klöting *et al*.2009) performed a global miRNA gene expression assay in different fat depots of overweight and obese individuals and found that the expression of miR-132 had a role in the link between adipose tissue dysfunction and the development of obesity-associated disorders including T2DM.

Results from the study of (Ning *et al*., 2011)demonstrated that insulin and insulin signaling induced insulin resistance through interactions with fat, which was in turn likely caused by the excessive accumulation of activated long-chain acyl-CoAs in mitochondria. It indicated that fat may not be able to induce insulin resistance without insulin,while ACSL1 and the fatty-acid-binding protein 2

(FABP2) genes have a major role in fatty acid and lipoprotein metabolism. Variations in assays, sample sizes, population characteristics and design make it difficult to harmonize available data

Conclusion

A SNPs located within miRNA-binding sites:*acyl-CoA synthetase 1* rs2292899 have possible roles in type2 diabetes mellitus susceptibility. The homozygous GG genotype of *acyl-CoA synthetase 1* rs2292899 is associated with type2 DM .The G allele might be a risk factor in T2DM while the A allele may be considered as a protective allele against the disease. Female genotypes GG and AG of *acyl-CoA synthetase 1* rs2292899 are not associated with T2DM.

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