ORIGINAL ARTICLE

CONTENTS

Impact of INSR (rs2229429) G>A genetic polymorphism on response to exogenous insulin in type 1 diabetic Iraqi patients

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ABSTRACT

Aim: To examine prevalence of genotypic distribution, particularly assessing how genetic polymorphisms in Insulin Receptor gene influence effectiveness of insulin therapy in a sample of Iraqi population.

Materials and Methods: Effect of Single Nucleotide Polymorphisms rs2229429 G>A have been investigated in 99 T1DM individuals, with a mean age of 12.3 years. These patients were managed with exogenous insulin through a basal-bolus monotherapy regimen. Genotyping was performed using an allele-specific polymerase chain reaction technique, and the data were statistically analyzed.

Results: The prevalence of the minor allele frequency is 12% in a sample of Iraqi population. Homozygous mutant carriers of rs2229429 G>A were 10.479 times at higher risk for developing poor glycemic control (HbA1c >86 mmol/mol) compared to wild genotype in type 1 diabetes mellitus, p=0.008. Ultimately poor responders to exogenous insulin, demonstrating significantly higher plasma insulin receptors levels p<0.001.

Conclusions: The investigated Single Nucleotide Polymorphisms is significantly associated with hyperglycemia in type 1 diabetes mellitus and contributes to the development of double diabetes.

KEY WORDS: pharmacogenetics, SNPs, insulin receptor, hyperglycemia

Wiad Lek. 2025;78(1):71-81. doi: 10.36740/WLek/199949 DOI

INTRODUCTION

Pharmacogenomic studies in drug discovery offers significant advantages for individuals with genetic predispositions, introducing novel approaches for personalized medicine and genetic diagnostics [1]. Thereby the efficacy of pharmacotherapy is enhanced, and the risk of drug-induced toxicity is minimized through this approach [2]. Notably, approximately 25-60% of patients exhibit interindividual variability, leading to divergent pharmacological responses to the same medication. The present pharmacogenetic research has the potential to elucidate the underlying factors contributing to individual differences in drug responses among patients. It underscores the importance of employing advanced methodologies for rapid, high-throughput DNA testing, which enables the prompt identification of numerous genetic variations [1, 2]. This study particularly focuses on variations in a specific gene sequence located within the insulin receptor (INSR) gene on the short arm of chromosome 19 (19p13.2). It provides a detailed illustration of the impact of these variations on exogenous insulin monotherapy in type 1 diabetes mellitus (T1DM). According to a previous study, lifelong administration of exogenous insulin is crucial for T1DM individuals. It has been proven to be highly effective in achieving glycemic control and increasing life expectancy [3]. Nonetheless, despite strict adherence to exogenous insulin protocols, a significant number of T1DM individuals exhibit insufficient responsiveness to insulin therapy. As a result, they experience persistent difficulty in attaining glycemic control, leading to chronic hyperglycemia [4]. As a result, uncontrolled T1DM can lead to serious complications, such as nephropathy, retinopathy, neuropathy, diabetic foot ulcers, diabetic ketoacidosis, infections, and serve as risk factors for cardiovascular diseases. Furthermore, environments conducive to the proliferation of cancer cells may be generated. Genetic variations in pharmacognosy can affect the interaction between insulin and its receptor in T1DM, significantly influencing glycemic control [5, 6]. Approximately 99.9% of nucleotide bases are identical across all individuals; however, the remaining 0.1% accounts for roughly 1.4 million unique variations among humans. These dispersed SNPs arise approximately every 300 to 2000 base pairs throughout the genome. Occurring in at least 1% of the population, they contribute significantly to human diversity [7]. Previous studies have

documented that various homozygous and heterozygous mutations in the INSR gene indicate a potential link to altered insulin sensitivity. These alterations result from receptor dysfunction in either the extracellular ligand-binding domain (alpha-domain). Substantial evidence supports the association between insulin resistance and single nucleotide polymorphisms (SNP) induced dysfunction in the INSR alpha-domain [8, 9]. Different forms of insulin resistance resulting from mutations in the extracellular binding domain have been described in numerous studies. These mutations reduce or completely inhibit insulin binding affinity, leading to impaired intracellular signal transduction, contributing to the development of insulin resistance [10, 11]. Insulin resistance type A, Rabson-Mendenhall syndrome, Leprechaunism syndrome, Donohue syndrome, and Polycystic Ovary Syndrome are well-documented examples of genetic forms of extreme insulin resistance. Additionally, patients with double diabetes and reduced responsiveness to platinum-based chemotherapy in epithelial ovarian cancer demonstrate insulin resistance, which are all linked to dysfunction in the alpha-domain of the INSR gene [12, 13]. Additionally, many polymorphisms in the coding region of in the INSR gene had shown correlation with insulin resistance and type 2 diabetes mellitus (T2DM) [14]. Insulin resistance is a condition, mainly prevalent in T2DM, and develops when tissues demonstrate a decreased response to insulin, resulting in hyperglycemia. Studies of insulin resistance and T2DM have suggested that insulin-signaling abnormalities may occur due to decreased cellular INSR content and/or reduced tyrosine kinase activity [15]. Causes of insulin resistance can be divided into pre-receptor, insulin receptor, and post-receptor defects. Given the crucial role of the INSR in initiating insulin action, a mutation in the INSR gene is a likely candidate for explaining genetic forms of insulin resistance [16]. The molecular mechanism of insulin resistance includes defects in receptor maturation and folding, improper transport from the nucleus to the cell surface, or impaired receptor accelerated degradation. As a result, a decreased number of INSR are produced of target cells surfaces or reduced INSR function [10]. A defective INSR function leads to decreased insulin binding affinity and reduced tyrosine kinase activity [17]. Consistent with this hypothesis, T1DM individuals enrolled in the current study have been proposed to exhibit insulin resistance due to receptor defects. Individuals with T1DM who are overweight and have a family history of type 2 diabetes or exhibit clinical signs of insulin resistance are considered to have "double" diabetes [18]. A specific SNP rs2229429 G>A exist in the coding region of exon 8 in the alpha-subunit in the INSR gene and have been connected with insulin resistance. Therefore, we look to explore the prevalence of genotypes of INSR gene rs2229429 G>A among T1DM individuals. Additionally, to investigate predictive role of this polymorphism in a sample of T1DM Iraqi patients treated with exogenous insulin monotherapy. Furthermore, to analyze if there is any association between different genotypes of INSR gene rs2229429 and hyperglycemia in insulin-treated patients with T1DM. This could assist in the diagnosis of double diabetes, thereby allowing for the concurrent consideration of alternative therapeutic options. The SNP rs2229429 G>A has been characterized in the alpha-subunit (FnIII-1) domain of the INSR gene. It is thought to be a benign synonymous mutation. Although the protein sequence is unchanged, the synonymous variant (rs2229429) G>A may influence gene expression, mRNA stability, translation efficiency, and protein folding [19]. The synonymous variant (rs2229429) G>A impact the abundance of insulin receptors on cell surfaces through multiple mechanisms. The secondary structure of mRNA might be altered, and may result in the production of fewer receptors. Furthermore, it affects the speed and efficiency with which ribosomes translate mRNA into mature INSR. Thereby, potentially affecting post-translational modifications and eventually produce misfolded proteins. Moreover, the transport of receptors to the cell surface may be disrupted, or the rate of degradation of existing receptors may be increased [20]. Previous evidence suggests that misfolded proteins may impede insulin binding by altering the three-dimensional structure of the insulin-binding pockets within the receptor's ectodomain, a consequence of defective cleavage into subunits. Since the investigated SNP (rs2229429) G>A is located within the (FnIII-1) alpha-subunit domain, it is expected to distort the docking sites. The (FnIII-1) structural domains are essential for receptor dimerization and high-affinity Insulin binding. Therefore, insulin binds with low affinity to the deformed (FnIII-1) alpha-subunit [21, 22]. Almost any deformation to an amino acid inside the binding interface, alters the receptor's affinity for exogenous insulin. Consequently, preventing the receptor from going through the required conformational changes to activate its intracellular signaling machinery. This in turn reduces receptor activation, which disrupts the signal transduction pathway [21-23]. The underlying cause of this phenomenon is a geometrical constraint, the introduction of a bulky side chain into the narrow binding pocket obstructs access to the active site. This affects the stability and functionality of the INSR, which in turn causes insulin molecules to have difficulty in binding, and even when they do, the connection is weaker and more prone

Table 1. Primers sequences of rs2229429 G>A genetic polymorphism

Primers	Primer sequence (5 '->3 ')	Primer size (bp)	Product size (bp)	Reference
Forward Primer	AACCTCACTGCATCAGCCT	19	319	Current study
Reverse Primer Allele G	CAGAATGTGACGGAGTTCGAC	21		
Reverse Primer Allele A	CAGAATGTGACGGAGTTCGAT	21		

Table 2. Distribution of Genetic Variants in INSR rs2229429 G>A among T1DM individuals

Gene	Genotype	patients	Frequency %	Allele		p-value
rs2229429	GG wild	83	83.8	G	A	<0.0001
	GA heterozygous mutant	8	8.1	(174) 0.88	(24) 0.12	
	AA homozygous mutant	8	8.1			
	Total	99	100			

Table 3. Hardy-Weinberg equilibrium for the INSR rs2229429 G>A genotypes among T1DM individuals

Genotype Observed % N=99		HWE Expected %			Fisher exact test	P-value
G	A	G	A	GA/AG	GG/observed vs GG/expected	<0.0001
88	12	77.23	1.47	21.3	GA/observed vs GA/expected	<0.0001
					AA/observed vs AA/expected	<0.0001

HWE: Hardy-Weinberg Equilibrium, numbers are presented as numbers and percentages, p-value<0.05 is indicated as significant.

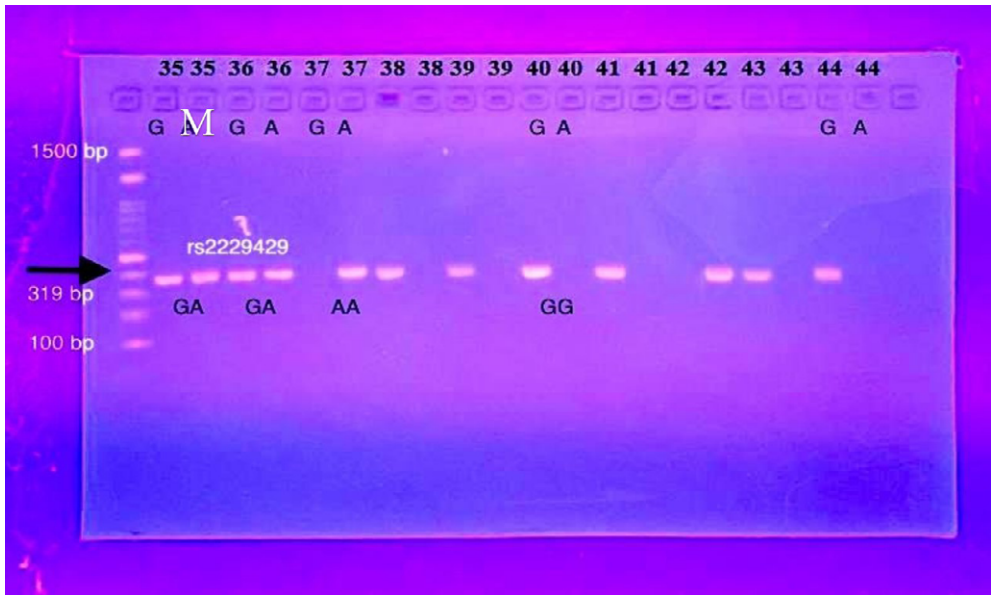

Fig. 1. Genotyping of rs2229429 genetic polymorphism G>A by Allele-Specific PCR Technique.

to breaking. The intended metabolic effects of exogenous insulin are lessened because of this weak or unstable binding. Thus, chronic hyperglycemia in Type 1 diabetic patients in the current study with the above-mentioned SNPs is further explained by the weak INSR activation due impairment in the binding site, as well as reduced protein amount and increased rate of degradation [24]. A decreased insulin binding affinity hinders tyrosine kinase activity to a certain extent. Therefore, impairs the ability of the receptor to transmit signals across the membranes. This suggests that T1DM individuals possessing a SNP in the (FnIII-1) domain may develop insulin resistance, due homozygosity for INSR gene mutations [25]. We hypothesized that the SNP rs2229429 G>A affects glycemic control.

To investigate this, we genotyped patients with T1DM for the SNP rs2229429 G>A. Subsequently, an evaluation of this SNP has been performed to determine whether they are linked to the circulating levels of HbA1c or soluble insulin receptor (sIR) levels in patients who are on insulin basal-bolus monotherapy.

AIM

The primary objective of this study is to examine the prevalence of genotypic distribution, particularly as-

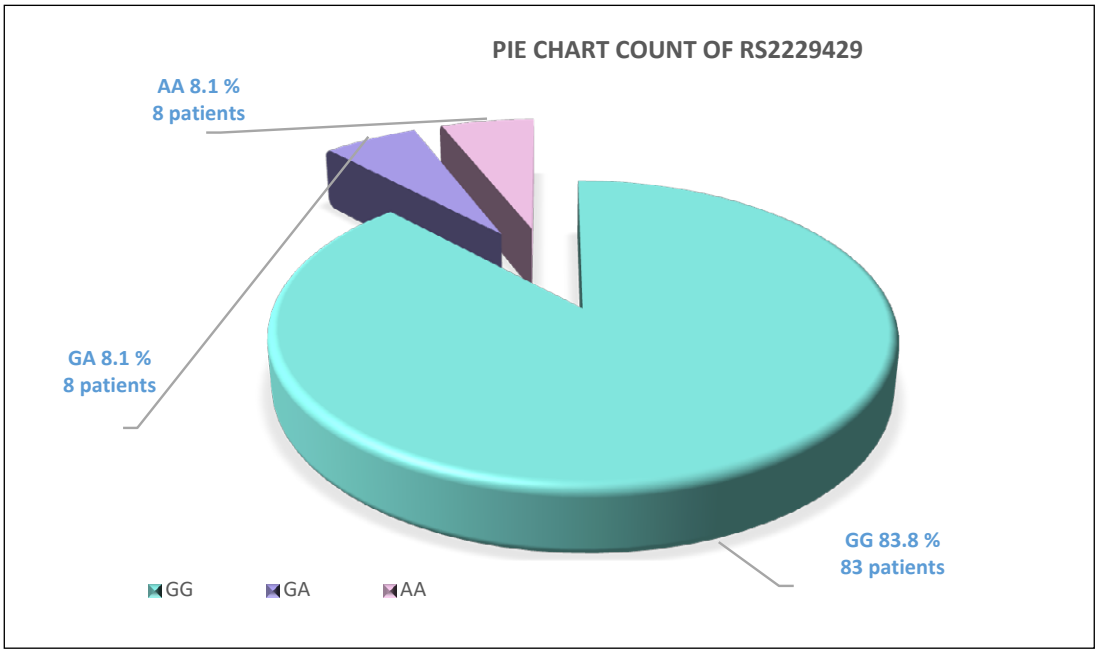

Fig. 2. Distribution of genetic variants of rs2229429 across three genotypes among T1DM individuals.

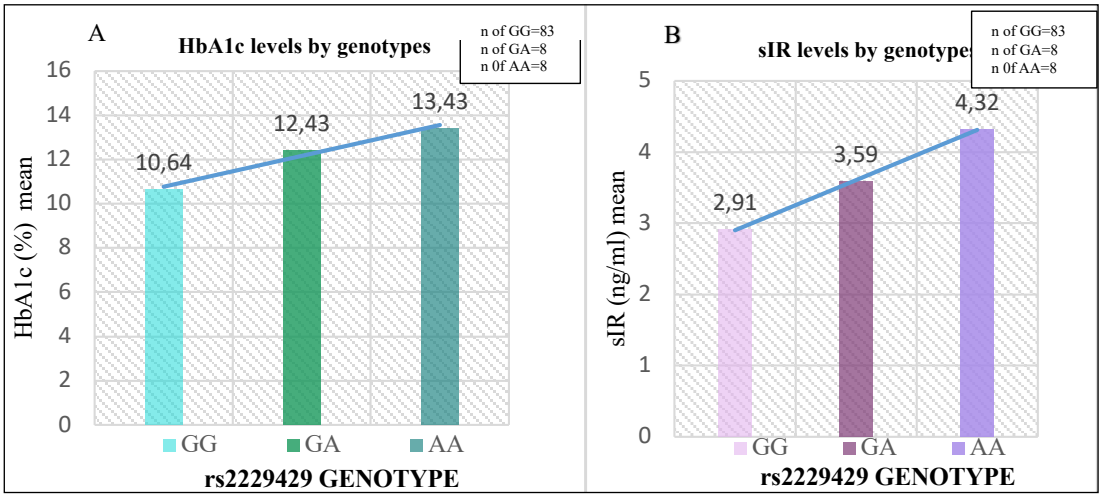

Fig. 3. Comparison of HbA1c levels (A) and sIR Levels (B) by rs2229429 genotypic distribution among T1DM individuals.

sessing how genetic polymorphisms in the INSR gene influence the effectiveness of insulin therapy in a sample of the Iraqi population.

MATERIALS AND METHODS

PATIENTS SELECTION

This study was designed as a cross-sectional study. Ninety-nine T1DM individuals, ranging in age from 5 to 17 years old, were retained in this study during their visit to the Al-Hassan Metabolism and Diabetes Center, Kerbala, Imam Alhassan Almojtaba Teaching hospital/Kerbala, Almarjan Hospital/Babylon and Alzahraa Hospital, Al Najaf City between September 2023 and March 2024. Patients were previously diagnosed according to diagnostic criteria, they received a daily dose of the exogenous insulin basal-bolus monotherapy regimen according to their body weight for at least 1 year, and they followed both inclusive criteria exclusive criteria.

SOLUBLE INSULIN RECEPTOR MEASUREMENT

Shedding of soluble insulin receptors in human plasma may reflect biological response to hyperglycemia. Elevated sIR levels have been associated with diabetes [26]. The determination of human sIR by commercially available INSR-specific sandwich ELISA system, Sunlong Biotech Co (China). Following manufacturer's instructions, specifically designed to detect sIR concentrations in human serum samples, sandwiched by two antibodies.

Table 4. Distribution of genotypic variations of INSR rs2229429 G>A gene across socio-demographic variable in T1DM

Variable		INSR rs2229429 genotypes mean (±SD)						p-value		
		n	GG (n=83)	n	GA (n=8)	n	AA (n=8)	GG vs GA	GG vs AA	GA vs AA
Age (years)	5-12	38	9.58±2.29	2	10.50±2.12	5	10.20±1.79	0.48	0.56	0.86
	13-17	45	15.02±1.44	6	15.00±1.41	3	15.00±2.00	0.97	0.98	>0.99
BMI (kg/m$^{2)}$	Underweight	39	14.89±1.23	1	14.92±0.0	2	17.05±4.03	0.98	0.03	0.74
	Normal	34	21.60±1.96	2	22.40±1.69	2	21.90±0.99	0.57	0.83	0.75
	Overweight	7	28.80±0.89	2	26.60±0.71	3	27.90±0.98	0.01	0.19	0.21
	Obese	3	30.27±0.46	3	30.27±0.37	1	30.01±0.0	0.98	0.67	0.59
DM duration (years)	1 -5	83	4.40±2.96	8	4.00±2.62	8	3.94±1.94	0.71	0.67	0.96
Duration of Rx (years)	1-3	83	2.60±1.39	8	2.25±1.04	8	2.56±1.04	0.49	0.94	0.51

BMI: body mass index, DM duration: diabetes mellitus duration, Duration of Rx Duration of insulin therapy, n=number of patients.

Table 5. Impact of exogenous insulin on glycemic control in respect to INSR rs2229429 G>A gene variations in T1DM

Variable	INSR rs2229429 genotypes			p- value	Reference
	GG (n=83) Mean (± SD)	GA (n=8) mean (± SD)	AA (n=8) mean (± SD)		
FBS (mg/dL)	188.76±52.99	222.38±90.32	233.1±25.78	0.03	Current study
RBS (mg/dL)	317.27±73.79	375.63±72.19	344.00±112.20	0.09	
HbA1c %	10.64±1.44	12.43±1.03	13.43±1.39	< 0.001	
sIR (ng/ml)	2.91±0.87	3.59±0.92	4.32±0.89	< 0.001	

FBS: fasting blood sugar, RBS: random blood sugar, HbA1c: glycated hemoglobin presented in percentage, sIR: soluble insulin receptor, n=numbers of patients

GENETIC ANALYSIS

The genomic DNA was extracted from frozen blood of T1DM patients employing the Favorprep TM Blood gDNA extraction mini kit favorgen. Molecular studies of the INSR gene were performed using Allele specific Polymerase chain reactions (AS-PCR). PCR was performed using specific primer pairs designed for (rs2229429) G>A (Macrogen, Korea) [27]. The primers were designed using Primer Blast Software [28], primers sequences utilized for amplification analysis of *INSR* gene for SNP identification is shown in Table 1.

STATISTICAL ANALYSIS

Statistical analysis of data was performed using Statistical Package for Social Sciences (SPSS), version 26 software (IBM, USA). Independent t test was performed to assess significant differences between means. Chi-square was used to assess significant differences among percentages. To test for statistically significant differences in basal characteristics among the genotypes, a one-way Analysis of Variance (ANOVA) was utilized. For each test, a p-value of <0.05 was considered statistically significant. Haldane-Anscombe correction and Hardy-Weinberg Equilibrium (HWE) (MedCalc Software for HWE) were performed as well. Odds ratio was also estimated.

RESULTS

RESULTS OF AMPLIFICATION REACTION OF THE EXTRACTED HUMAN DNA

The gene polymorphism rs2229429 produced a clear band with a molecular size of 319 bps amplicon using a UV-transilluminator, performed at the Research Center for Genetic Testing (Fig.1). The size of the amplicon was estimated by comparing it to a 100-1500 bp DNA ladder, indicated by lane M. Lane 40 represented individuals with the GG genotype (wild type), lane 37 represented individuals with the AA genotype (mutant type), and lanes 35 and 36 represented individuals with the GA genotype (heterozygous). The gel electrophoresis was conducted at 45 volts. This analysis successfully distinguished between different genotypes of the rs2229429 G>A polymorphism, providing valuable genetic information that may have clinical implications, such as drug response.

Figure 1 illustrates the results by using UV-transilluminator after having performed the Agarose gel electrophoresis by using 1.5% agarose per TBE buffer.

Table 6. Association between INSR rs2229429 G>A genotypes and poor glycemic control in T1DM

SNP allele		HbA1c ≤ 86 mmol/mol				HbA1c > 86 mmol/mol				OR (95% CI)	p-value
		rs2229429 G>A Genotypes				rs2229429 G>A Genotypes					
		n	GG	GA	AA	n	GG	GA	AA		
rs2229429 G>A	G	33	33 (100%)	0	0	50	50 (75.8%)	0	0	10.479 (1.964-55.916)	0.008
	A	0	0	0	0	16	0	8 (12.1%)	8 (12.1%)		
Total patients (99)		33 (100 %)				66 (100%)					

SNP: single nucleotide polymorphism, OR: Odds ratio, 95 % CI: 95 % confidence interval, Haldane-Anscombe correction used for OR, HbA1c: glycated hemoglobin, n=numbers of patients are shown as numbers and frequencies.

Table 7. Relationship between HbA1c levels according to INSR rs2229429 G>A genotypes in T1DM

Variable	Genotype	HbA1c ≤ 10% Mean (±SD)	HbA1c > 10 % Mean (±SD)	p-value	Reference
rs2229429 G>A	GG	9.34±0.57	11.51±1.16	<0.001	Current study
	GA	0±0	12.43±1.03	<0.001	
	AA	0±0	13.43±1.39	<0.001	

HbA1c: glycated hemoglobin presented in percentage, independent sample t-test, p-value (zero-inflated model)

Table 8. Comparison between two age groups according to according to INSR rs2229429 G>A genotypes in T1DM

Variable	Genotype	Age (5-12 years) HbA1c %, mean (±SD)	Age (13-17 years) HbA1c %, mean (±SD)	p-value	Reference
rs2229429 G>A	GG	10.74±1.59	10.57±1.3	0.60	Current study
	GA	11.95±0.07	12.59±1.17	0.49	
	AA	13.1±1.63	13.99±0.87	0.43	
	Total	11.05±1.73	10.98±1.58		

HbA1c: glycated hemoglobin presented in percentage; independent sample t-test used

GENOTYPE AND ALLELE FREQUENCIES ANALYSIS FOR INSR rs2229429 G>A GENOTYPES IN T1DM INDIVIDUALS

The data presented in Table 2 and Figure 2 show the different genotypes distribution among the 99 enrolled patients. Results of allele frequency revealed that patients with the allele A are significantly at a high risk for developing insulin resistance in T1DM patients as compared with patients with the allele G as indicated by a P-value of <0.0001.

HARDY-WEINBERG EQUILIBRIUM ANALYSES FOR INSR rs2229429 G>A GENOTYPES

The following findings underscore the presence of genetic deviations from the Hardy-Weinberg equilibrium (HWE) within this sample across all three genotypes among the enrolled 99 patients. Table 3 revealed the observed and expected genotype counts and Hardy-Weinberg frequencies. The observed frequencies of genotypes (GG: 83.8, GA: 8.1, AA: 8.1) significantly differ from the expected frequencies (GG: 77.23, GA: 21.3, AA: 1.47). The P-value of <0.0001 indicates that the observed genotype frequencies significantly differ from the expected frequencies under HWE, implying that the data supports a conclusion of departure from HWE. We reject or the null hypothesis that the population is at H-W equilibrium

GENOTYPIC VARIATIONS OF INSR rs2229429 G>A GENE ACROSS SOCIO-DEMOGRAPHIC VARIABLE IN T1DM

Table 4 compares basal characteristics, including age, BMI, duration of diabetes and therapy across three genotypes of the rs2229429 gene in 99 T1DM patients. Mean basal bolus daily Insulin dose for the GG genotype was 1.06±0.25 IU, for GA genotype 1.27±0.17 IU and for AA genotype 1.16±0.14 IU. Age, the BMI across genotype groups shows statistical significance only for GA genotype of overweight (p=0.01) patients and AA genotype of underweight (p=0.03) patients compared to the wild type.

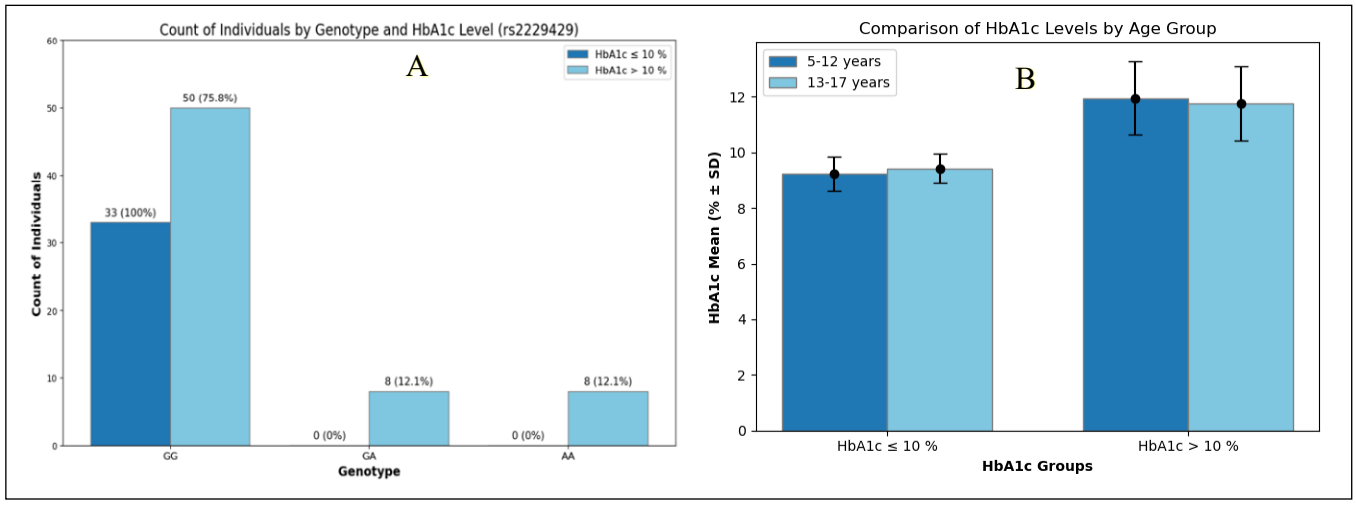

Fig. 4. The distribution of rs2229429 G>A genotypes and HbA1c level groups (A) and age groups (B) among T1DM individuals.

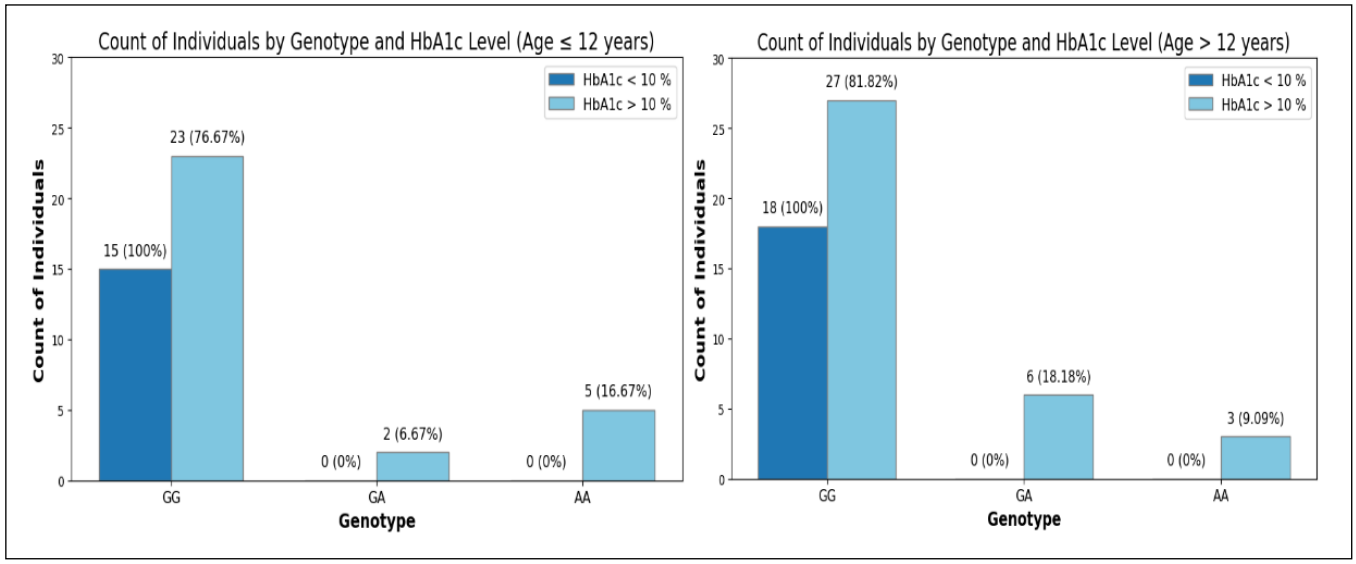

Fig. 5. Counts of T1DM individuals by two age groups and HbA1c levels according to INSR rs2229429 G>A genotypes in T1DM.

Table 9. Relationship between serum sIR levels and HbA1c groups according INSR rs2229429 G>A genotypes in T1DM patients

Variable sIR mean (±SD)		HbA1c ≤ 10 % group	HbA1c > 10 % group	p-value	Reference
		sIR mean (±SD)			
rs2229429	GG	2.74±0.56	3.02±1.01	0.16	Current study
	GA	0±0	3.59±0.92	<0.001	
	AA	0±0	4.32±0.89	<0.001	

sIR: soluble insulin receptor, independent sample t-test used (zero-inflated model)

THE IMPACT OF EXOGENOUS INSULIN ON LABORATORY PROFILES IN RESPECT TO INSR rs2229429 G>A GENETIC VARIATIONS IN T1DM

Patients were on a mean daily dose of 1.16±0.08 IU according to their body weight. None of the patients enrolled in the study received a drug dosage of 2 or more IU kg/day. In Table 5 mean FBS and HbA1c levels show a statistically significant difference of p=0.03 and p < 0.001, with the AA genotype group having the highest mean HbA1c. The GA group exhibits the highest mean RBS; however, it shows no significant association between the different genotypes. Plasma sIR levels (p<0.001) vary noticeably, with the GA and AA genotypes having higher mean values than the GG genotype.

It is shown are serum HbA1C levels of patients with T1DM (n=99) in relation to (Fig.3A) INSR rs2229429 (p <0.001), significant difference is shown in GA heterozygous mutant allele (jade green bar) compared

to homozygous GG (turquoise bars). Elevated HbA1C levels were observed in patients with the mutant AA (teal bar) genotypes. Differences in sIR levels among groups based on rs2229429 genotypes ($p<0.001$) elevated soluble insulin receptor levels were observed in patients with the mutant GA (lilac bar) and AA (amethyst purple bar) genotypes, compared to GG genotypes (lavender bar) (Fig.3B).

DISTRIBUTION OF GENOTYPES INSR rs2229429 G>A IN RELATION TO HBA1C IN T1DM INDIVIDUALS

Table 6 presents the association between rs2229429 genotypes and HbA1c levels (≤ 86 mmol/mol and >86 mmol/mol). Genotype distribution of GA and AA genotypes is highly prevalent in HbA1c > 86 mmol/mol group, with identical distributions for GA (8 patients) and AA (8 patients) genotypes. The data suggest that carriers of the homozygous mutant type are more likely to develop insulin resistance than the homozygous wild type and are 10.479 at higher risk developing insulin resistance.

Figure 4A illustrates the distribution of the different genotypes among HbA1c level groups. Genotype GA and AA is highly prevalent in the HbA1c >10 % level group. Figure 4B shows near equal HbA1c levels among both age groups. A significant association between rs2229429 genotypes and HbA1c mean levels is illustrated in Table 7. HbA1c mean levels are presented in percentage. GA (12.43±1.03) and AA (13.43±1.39) genotypes is higher than GG (11.51±1.16) genotypes in HbA1c > 10% group. Additionally, GG genotypes of the HbA1c >10% groups are higher than the GG genotype of the HbA1c ≤ 10% group.

The HbA1c levels among different genotypes showed no statistical significance among the two age groups (Table 8).

Patients with the heterozygote and homozygote genotype demonstrating HBA1c levels > 10% are distributed over two age groups (Fig. 5).

RELATIONSHIP BETWEEN SIR AND HBA1C GROUPS ACCORDING TO INSR rs2229429 G>A GENETIC DISTRIBUTION IN T1DM INDIVIDUALS

Serum soluble insulin receptor levels were analyzed according to genotypic distribution and HbA1c groups are illustrated in Table 9. sIR values according to manufacturer's protocol for normal individuals was identified by a range from 1 to 3 ng/ml. HbA1c >10 % group shows higher mean values for the minor allele of rs2229429 compared to the major allele and is of statistical significance.

DISCUSSION

SNP selection of rs2229429 G>A was based on a literature search of previously reported associations with glycemic control and reduced insulin sensitivity in T1DM and was proposed to be associated with insulin resistance. The synonymous SNP exists in the coding region at exon 8, and is located in the FnIII-1 domain on the in the alpha-subunit of the INSR gene, on chromosome 19 (locus chr19:7166377). This SNP is quite common in European ethnicity with a heterozygote frequency of at least 8 % [19]. A minor allele frequency for the A allele in Qatari population was of 29 % and 0.05 % in Korean and Japanese ethnicity, however the minor allele frequency in the Caucasian ethnicity was reported to be 19 % for the A allele. Our results are consistent with earlier studies in populations according to 1000 Genomes Project Consortium. The results of the DNA amplification reaction of the investigated polymorphism in this study revealed three genotypes. GG, GA, and AA, with a minor allele frequency of 12 % for the A allele in a sample of Iraqi population. Our data reveals that the most common genotype prevalence was for the homozygous mutant GG (83.8%), followed by an equal distribution for the heterozygous GA (8.1%) and homozygous AA (8.1%). Recent excellent researches identified that SNPs within the INSR gene ectodomain (alpha-chain), are associated with hyperglycemia [14]. This hypothesis meets our results. We examined if the SNP in INSR gene rs2229429 G>A is associated with high levels of HbA1c in T1DM. The results showed that the enrolled T1DM patients had poor glycemic control with a HbA1c total mean of 11.01±1.64. We observed that patients who were heterozygous for the minor allele A in rs2229429 tended to have higher HbA1c levels and consequently, indicating poor glycemic control than patients who were homozygous for the wild type G allele. From hypotheses from many studies, we assume according to evidence that the synonymous SNP rs2229429 might have led to reduced insulin sensitivity. Consequently, leading to an unstable signaling cascade. As a results, we propose that insulin resistance could be associated to the enrolled patients carrying mutant alleles in the *INSR* gene possessing over a high BMI [29, 30]. The results suggest that the Odds Ratio 10.479 for the rs2229429 SNP A genotype is of statistical significance (p-value = 0.008), indicating difference of having > 86 mmol/mol with the G allele as reference. These results demonstrated that patients carrying the A allele are significantly at high risk of 10.479 times for

developing chronic hyperglycemia associated with insulin resistance as compared to the G allele carriers in the same HbA1c group. Furthermore, the current study demonstrated a significant association between rs2229429 G>A genotypes and HbA1c mean levels in T1DM patients, along with the p-value (<0.001). HbA1c mean±SD levels of GA (12.43±1.03) and AA (13.43±1.39) genotypes is higher than GG (11.51±1.16) genotypes in HbA1c > 10% group. This suggests a possible association between genotype and insulin sensitivity in T1DM patients. Similarly, the results of the mean HbA1c were compared in two age groups, despite that the adolescents experiencing pubertal and hormonal changes. Their mean HbA1c levels were lower than the school-aged group, however results showed no statistical association between both age groups and the mean HbA1c levels of those with the wild type genotype. This analysis suggests that patients carrying the "wild type" who experiences hyperglycemia, might be due to poor compliance, dehydration, stress, physical inactivity, hormonal changes and sedentary lifestyle. T1DM individuals carrying the minor allele of rs2229429 showed an increased prevalence of chronic hyperglycemia, regardless of the age. There is a direct correlation between HbA1c and insulin resistance, where HbA1c has been shown to be more strongly associated with the insulin sensitivity in healthy subjects with normal glucose tolerance. As a result, HbA1c is a reliable biomarker and an excellent indicator of insulin resistance for testing individuals for diabetes [31]. A family history of diabetes has been reported in 39 % of patients in the current study. The two patients who were overweight and were heterozygote for the minor allele (p=0.01) have reported to have parental history of diabetes. This may suggest the possibility that these patients exhibit characteristics of double diabetes. Further investigation for lipid profile, metabolic biomarkers, blood pressure, waist circumference is fundamental to the 9 patients with a high BMI possessing over the investigated SNP are essential to exclude double diabetes. Based on the hypothesis that soluble sIR may exist in human plasma, we attempted to detect sIR in human plasma in T1DM individuals [31, 32]. Previous research has proposed that the increased soluble IR ectodomain level appears to be a more rapid glycemic marker than A1C or glycoalbumin. This hypothesis is consistent with the current study, sIR levels have been analyzed and have shown significant differences (p<0.001) across genotypes. This indicates a possible association between genotype and sIR levels. The half-life of the insulin receptor was discovered to be 7–12 hours, however a previous study estimated a half-life of 6 hours in diabetic patients [32]. This is explained by the fact that shedding of the insulin receptor appeared to be parallel to the blood glucose levels, which is consistent with our results. Healthy normoglycemic individuals' results for sIR have shown normal values (data not shown).

CONCLUSIONS

The prevalence of minor allele frequency of the INSR rs2229429 G>A gene polymorphism is 12 % in a sample of T1DM Iraqi individuals. The investigated SNP is identified as a biomarker for predicting poor glycemic control. Results indicate that patients carrying the minor allele are 10.479 times (p=0.008) at a higher risk as compared with patients with the G allele for developing poor glycemic control (HbA1c >10 %). Ultimately, we suggest that elevated levels of the soluble insulin receptor may serve as a rapid glycemic biomarker for hyperglycemia.

RECOMMENDATIONS FOR FUTURE PERSPECTIVES

This research prompts further inquiries that require additional investigation, including the clinical, metabolic, and immunological profiles of affected individuals, which would be highly valuable. Extensive, long-term clinical trials are also essential to evaluate the safety and effectiveness of non-insulin antidiabetic medications for patients with insulin-resistant T1DM or double diabetes. Future research should focus on therapies targeting insulin resistance, such as metformin and pramlintide, as well as investigate various drug classes, including thiazolidinediones, GLP-1 receptor agonists, SGLT2 inhibitors, and DPP-4 inhibitors. Further, in-depth exploration of the novel once-weekly basal insulin formulation, insulin Efsitora alpha, is crucial. Hypoglycemia concerns were among the reasons why the U.S. Food and Drug Administration recently voted against its approval for type 1 diabetes, though it has been approved in several other countries. Consequently, conducting comprehensive studies on patients with double diabetes would be highly beneficial. Researchers have been exploring the potential of CRISPR-Cas9, an innovative and powerful gene-editing technology, for correcting genetic mutations in stem cells, with the goal of transforming them into healthy cells. Currently, CRISPR-Cas9 research is in early stages, primarily focused on preclinical models. Although this technology shows great promise, it may take several years before it is ready for cww linical application. Therefore, further research is essential to establish comprehensive ethical guidelines, regulatory frameworks, and public policies to create globally accepted standards for CRISPR use in human genetic modification. While preclinical trials have shown the potential of CRISPR-Cas9, the long-term

effects of gene editing in humans remain largely unknown, emphasizing the need for studies that prioritize long-term safety assessments to evaluate the stability of edited genes and possible immune responses. Additionally, developing computational models to simulate soluble insulin receptor interactions and predict the impact of modulating its levels or activity could provide insights into insulin sensitivity and glucose regulation.

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This study was carried out in accordance with ethical guidelines to ensure the protection of participants. Informed consent was obtained from all participants, who were made fully aware of the study's objectives and procedures. Participation was voluntary, and individuals were free to withdraw from the study at any time without any restrictions or sanctions. All participant data were anonymized and securely stored in password-protected files, accessible solely to the research team. The study received approval from the Research Committee of Kerbala Health Directorate (2023167/Kerbala) in 8/09/2023.

CONFLICT OF INTEREST

The Authors declare no conflict of interest

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RECEIVED: 25.11.2024
ACCEPTED: 05.01.2025