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Original article

Evidence from genetic studies among rs2107538 variant in the *CCL5* gene and Saudi patients diagnosed with type 2 diabetes mellitus

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ABSTRACT

Type 2 diabetes mellitus (T2DM) is a chronic and metabolic disorder that affects the adult population. Chemokines are proinflammatory cytokines that play a role in the development of chronic diseases such as obesity, gestational diabetes, and T2DM. The C-C Motif Chemokine Ligand 5 (*CCL5*) gene plays a role in antiviral immunity, tumor development, obesity, impaired glucose tolerance, and T2DM. This study aimed to investigate the genetic role of the rs2107538 variant in the *CCL5* gene in Saudi patients with T2DM. Sixty subjects with T2DM patients and 60 healthy controls participated in this prospective case-control study. Prior to Sanger sequencing, genomic DNA was extracted and amplified with Polymerase chain reaction (PCR), after which the PCR products were purified. The collected data were used to conduct various statistical analyses to determine the relationship between T2DM and control subjects. The findings of the current study revealed a positive association for most parameters between T2DM and control subjects ($p < 0.05$). The frequency of genotypes ($p = 0.002$, AA vs.GG: $p = 0.008$, GA + AA vs. GG: $p = 0.0002$) and alleles (A vs. G: $p = 0.0007$) revealed a strong risk association. Multiple logistic regression with individual effects revealed a link between SBP and HDLc levels ($p = 0.03$). In patients with T2DM, waist ($p = 0.001$), TG ($p = 0.0007$), and LDLc ($p = 0.0004$) levels were all associated with the ANOVA. Finally, the rs2107538 variant was linked to an increased risk of T2DM in the Saudi Population. The GA and AA genotypes were strongly connected to the T2DM subjects. In order to rule out disease-causing variants in the global population, future research should use a large sample size.

1. Introduction

Diabetes is defined by the World Health Organization (WHO) as a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbances in carbohydrate, lipid, and protein metabolism resulting from defects in insulin secretion, insulin action, or a combination of both (Timasheva et al., 2023). According to reports from the International Diabetes Federation, the prevalence of diabetes may continue to rise from 537 million in 2021 to 783 million in 2045 (Mohammed et al., 2023). Furthermore, most cases of diabetes were attributed to type 2 diabetes mellitus (T2DM). This chronic, multifactorial, and metabolic disease is recognized worldwide; it is characterized by elevated serum glucose levels (Wu et al., 2022). Several molecular studies have been conducted to elucidate the molecular mechanisms of T2DM, which include β-cell dysfunction, insulin resistance, and impairment of incretin signaling, as well as multiple diseases associated with single nucleotide polymorphisms (SNPs) (Azarova et al., 2023). Additionally, genetic risk mechanism loci were found to reduce insulin secretion rather than its action (Saxena et al., 2023).

The inflammatory response is mediated by cytokines and chemokines. Altered cytokine and chemokine expression have been reported in inflammatory and auto-immunological illnesses, which may influence susceptibility to disease-disease development. The

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pathogenesis of diabetes is heavily influenced by cytokines and chemokines (Teler et al., 2017). Previous research has identified a link between T2DM and chemokine inflammatory markers, which are related to immunology via anti-inflammatory drugs and have also been shown to improve insulin sensitivity and β-cell function. Chemokines are associated with key signaling molecules that regulate T2DM pathophysiology. They significantly impact obesity and glucose intolerance, which can be caused by different types of diabetes (Pan et al., 2021). The C–C chemokine motif ligand 5 (CCL5) and its ligand have been associated with the pathogenesis of type 1 and T2DM (Zhang et al., 2020). Typically, human *CCL5* is found on chromosome 17 (17q11.2-q12), which comprises three exons and two introns. *CCL5* is polymorphic, and its rs2107538 variant is suggested to affect CCL5 expression (Sheng and Qi, 2020a; Zhang et al., 2020).

CCL5 is associated with obesity, diabetes, cardiovascular disease, coronary artery disease, myocardial infarction, tuberculosis, and specific cancers. However, limited studies with the rs2107538 variant/SNP have been limited due to the lack of restriction enzymes. As a result, the variant rs2107538 can be studied using tetra-arms PCR, Sanger sequencing, or qPCR analysis. This study used Sanger sequencing analysis as the preferred technique to detect SNPs and no studies with the *CCL5* were conducted in any form of human disease(s) in Saudi Arabia. We used T2DM patients along with control subjects confirmed to have obesity and those without obesity in this study because the variant rs2107538 has been linked to obesity and diabetes. This study sought to investigate the role of the rs2107538 variant in patients confirmed with T2DM.

2. Materials and methods

2.1. Institutional Review Board approval

This study was approved by the Institutional Review Board of the College of Medicine at King Saud University (KSU). All patients involved in this study signed informed consent forms before they participated in this study. This study was also carried out according to the standards of the Declaration of Helsinki.

2.2. Involvement of Saudi patients with diabetes

In this case-control study, 60 T2DM patients and 60 healthy controls were recruited. All patients were recruited from KSU premises. Initially, 320 case-control patients were recruited based on inclusion and exclusion criteria, as well as patient-signed consent forms and completed questionnaires for clinical data. Lastly, a minimum of 60 T2DM cases and 60 controls were recruited based on a previous publication. All T2DM cases recruited in this study had a family history of T2DM on the paternal or maternal side. The cases were recruited based on the WHO criteria, which describe that glucose levels should not exceed 126 mg/dL or > 7.0 mmol/L. Exclusion criteria for patients with T2DM are based on low glucose levels, i.e., below 7.0 mmol/L. Healthy controls were recruited without any family history of diabetes and normal glucose levels. Elevated glucose levels were excluded if documented in the control subjects. T2DM cases and controls were selected based on the sample size calculated as described in a previous publication.

2.3. Anthropometric, biochemical, and clinical details

We recorded anthropometric measurements such as age, weight (kg), height (cm), body mass index (BMI), waist, hip, systolic blood pressure (SBP), and diastolic blood pressure (DBP) for hypertension (HTN) values between T2DM cases and controls. Additionally, we measured several biochemical parameters, such as fasting blood glucose (FBG), glycated haemoglobin (Hb1Ac), and lipid profile parameters, such as total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDLc), and low-density lipoprotein cholesterol (LDLc). Subsequently, 5 ml of peripheral blood was collected from each patient; 3 ml was stored in an anticoagulant tube, while the remaining 2 ml was in an EDTA tube for molecular analysis.

2.4. Molecular analysis

DNA analysis was conducted using peripheral blood collected in an EDTA tube using a Norgen DNA isolation kit with the recommended protocol. Concentrated DNA measurement was performed based on a NanoDrop spectrophotometer, and finally, all genomic DNA samples were converted to 50 ng/µl. All genomic DNA samples were stored at − 80℃ to perform the molecular analysis in the G141 Laboratory, affiliated with this work apart from Sanger sequencing. A 96-well gradient thermocycler (Applied Biosystems) was used to perform PCR. Next, to perform 50 µl of a PCR reaction, a master mix kit was used (10X Buffer, 50 ml of $MgCl_2$, and 10 U of Taq DNA polymerase), dNTPs, 10p moles of rs2107538 SNP primers of both reactions were used along with 5 µl of DNA. Amplification was performed using initial denaturation (95 °C for 30 sec), annealing (64 °C for 30 sec), extension (72 °C for 45 sec), and the final extension was 72 °C for 5 mins. The total reaction was completed after 35 cycles and after completing the PCR analysis, the temperature was set at 4°C until the machine was turned off. In this study, (F-TCATCAGTTTCCTCTTTGACC and R-CTGCCTCAATTTACAGTGTG) primers were used to screen rs2107538 SNP at the promoter region − 403G > A. The PCR products were run on 2% agarose gel and prepared with ethidium bromide along with a 100 bp DNA ladder (Fig. 1). The gel image was captured with a UVI gel doc and the bands were documented based on the base pairs of band size.

2.5. Sanger sequencing analysis

Undigested PCR products were purified prior to the Sanger sequencing analysis being conducted. DNA sequencing analysis was conducted outside the G-141 laboratory on the KSU premises. The presence of alleles G and A was confirmed using bidirectional Sanger sequencing using Applied Biosystem. Fig. 2 defines the GG, GA, and AA genotypes represented in the DNA chromatogram sequence for the rs2107538 SNP

2.6. Statistical analysis

In this study, SPSS software (version 27.0) was used to analyze the subjects present in T2DM and control subjects. Additionally, both qualitative and quantitative variables were used in this study; the unpaired student *t*-test was used in Table 1, while the genotype and allele frequencies are shown in Table 2. Hardy–Weinberg equilibrium analysis (HWE) analysis was carried out in the rs2107538 variant; p-value < 0.05 is considered non-significant and $p > 0.05$ is considered significant. Multiple linear regression (Table 3) and individual effects (Table 4) between independent and dependent factors were studied in subjects along with genotypes. The analysis of variance (ANOVA) analysis is studied in Table 5. Fig. 2 was generated using Origin software (version 22.0) for T2DM and control genotypes and R software was used to generate the Pearson correlation coefficient graph (Fig. 4). A p-value<0.05 is considered statistically significant.

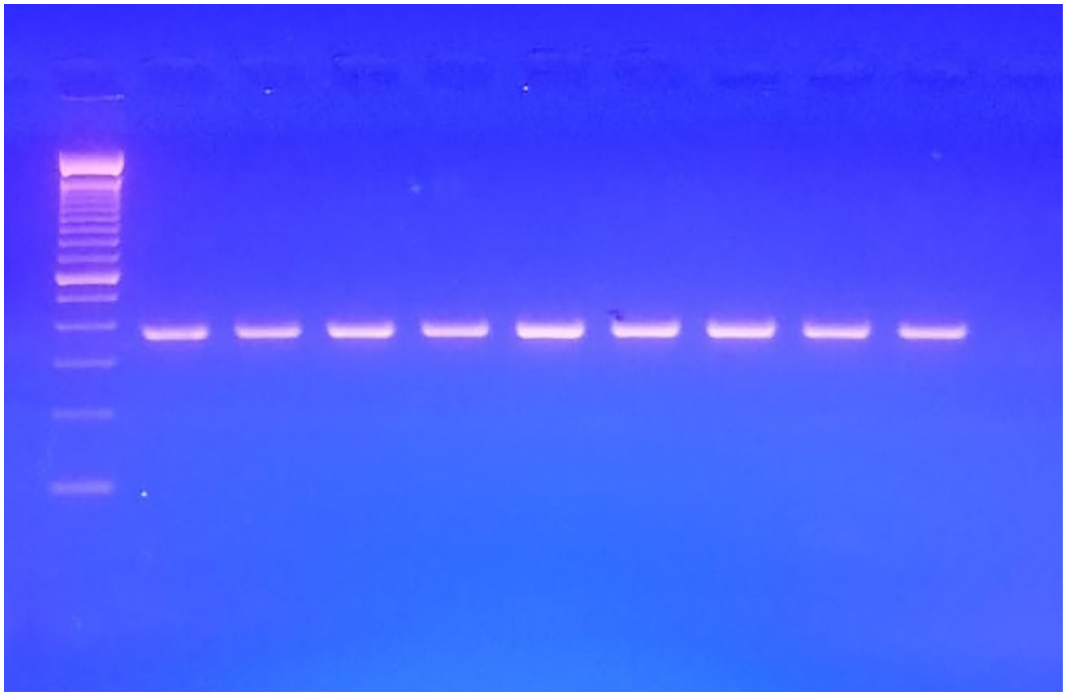

Fig. 1. Representation of 2% agarose gel image for 338 bp PCR products in rs2107538 variant.

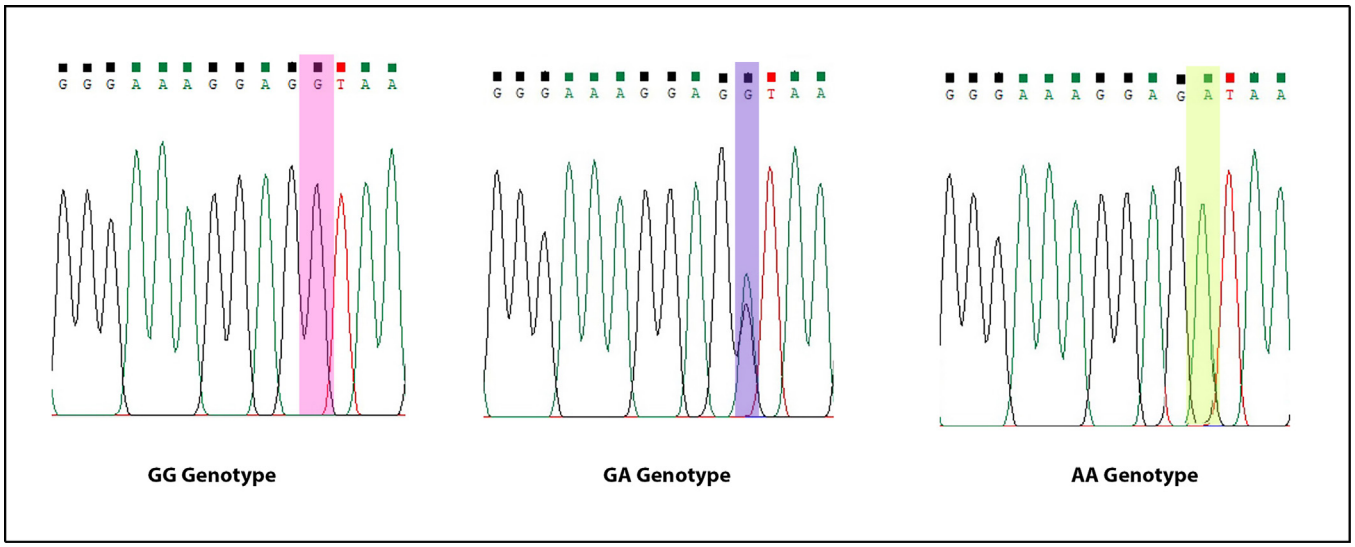

Fig. 2. Sanger sequencing analysis carried out in GG, GA and AA genotypes in rs2107538 variant.

3. Results

3.1. Normal characteristics of Saudi participants

The clinical characteristics and biochemical parameters studied in the T2DM cases and controls are shown in Table 1. Both groups were not matched in this study, apart from the sample size. The main criteria of this study were to enroll all patients who should have a family history of diabetes. The T2DM cases (67.06 ± 7.28) were older than controls (49.53 ± 7.55); they had a high incidence of male participants (66.7%) in T2DM cases and females (40%) in the control subjects. The age range of T2DM was found to be 59–86 years and in controls, it was found to be 41–78 years. A high incidence of age ($p < 0.0001$), gender ($p < 0.0001$), weight ($p = 0.0003$), BMI ($p < 0.0001$), waist ($p = 0.03$), hip ($p < 0.0001$), SBP ($p < 0.0001$), DBP ($p = 0.03$), FBG ($p < 0.0001$), HbA1c ($p < 0.0001$), TG ($p = 0.01$), TC ($p = 0.01$), and family history ($p < 0.0001$) were present in T2DM cases. However, height ($p = 0.85$), HDLc ($p = 0.14$), and LDLc ($p = 0.68$) showed a negative correlation.

3.2. HWE analysis and genotype distribution

In this study, the HWE analysis was not associated with controls ($x^2 = 5.9$, $p = 0.01$) but instead was associated with patients ($x^2 = 2.95$, $p = 0.08$). The SNP rs2107538 in the *CCL5* gene consists of GG, GA, and AA genotypes in the promoter region at position 403. The combination of thermal PCR and Sanger sequencing anal-

Table 1
Clinical and demographic characteristics between T2DM cases and controls.

Covariates	T2DM cases (n = 60)	Controls (n = 60)	P value
Age (Years)	67.06 ± 7.28	49.53 ± 7.55	<0.0001
Gender (Male. Female)	40 (66.7%). 20 (33.3%)	24 (40%). 36 (60%)	<0.001
Weight (kgs)	82.84 ± 13.60	74.05 ± 12.23	0.0003
Height (cms)	158.19 ± 8.18	158.45 ± 7.72	0.85
BMI (kg/m^2)	32.90 ± 4.84	28.02 ± 3.91	<0.0001
Waist (cms)	97.71 ± 18.73	91.01 ± 15.57	0.03
Hip (cms)	110.35 ± 9.12	97.76 ± 6.75	<0.0001
SBP (mmHg)	125.52 ± 11.58	115.25 ± 7.27	<0.0001
DBP (mmHg)	78.27 ± 6.27	75.91 ± 6.01	0.03
FBG (mmol/L)	12.79 ± 4.29	5.44 ± 0.44	<0.0001
Hb1Ac	7.01 ± 0.81	5.30 ± 0.46	<0.0001
TG (mmol/L)	2.51 ± 2.44	1.70 ± 0.87	0.01
TC (mmol/L)	5.60 ± 1.17	5.12 ± 0.88	0.01
HDL-c (mmol/L)	0.73 ± 0.34	0.65 ± 0.25	0.14
LDL-c (mmol/L)	3.77 ± 1.08	3.70 ± 0.81	0.68
Family History of T2DM	60 (100%)	31 (51.7%)	<0.0001

ysis confirmed the genotype analysis results, which are presented in Table 2. The GG genotype was high at 80% in controls and 48.30% in cases of T2DM. The GA and AA genotypes were found to be high in T2DM at 35% and 16.70%, as well as 15% and 5% in controls (Fig. 3). Furthermore, A allele was found to be 34.20% in cases and 12.50% in controls, while 65.80% was present in the G allele in T2DM and 87.20% in control subjects. The results of the current study confirmed genotype (GA vs. GG: odds ratio (OR) – 3.86 [95% CI: 1.56–9.56]; p = 0.002 and AA vs. GG: OR – 5.51 [95% CI: 1.40–21.71]; p = 0.008), the dominant model (OR – 4.27 [95% CI: 1.90–9.61]; p = 0.0002) and allele frequency (OR – 3.63 [95% CI: 1.87–7.02]; p = 0.0007) showed a significant relationship among these factors.

3.3. Multiple regression analysis studied in single effect and combined effect in patients with T2DM

Table 3 displays the combined effect of multiple regression analysis, with the variant rs2107538, FBG, and HbA1c combined as independent variables, and the remaining dependent variables including age, weight, height, SBP, DBP, waist and hip circumference and lipid profile measured. The study results confirmed that there was no association between the dependent variables and the independent variables (p > 0.05). Individual effects of genotype, FBS, and HbA1c were studied separately with dependent variables in Table 4, and the current study revealed a correlation between FBG and SBP (p = 0.03) and HDLc (p = 0.03). This suggests a strong association between FBG, SBP and HDLc.

3.4. ANOVA analysis of the rs2107538 T2DM variant

The ANOVA analysis between GG, GA, and GG genotypes and dependent parameters such as age, weight, height, BMI, waist, hip, SBP, DBP, FBG, HbA1c, TC, TG, HDLc, and LDLc was studied as depicted in Table 5. The elevated levels found in the GG genotype were weight (84.08 ± 14.91), height (159.15 ± 8.95), DBP (79.40 ± 5.54), FBG (13.58 ± 4.59), HbA1c (7.10 ± 0.75) and (0.76 ± 0.38). The heterozygous genotype had age (67.52 ± 7.53), SBP (127.00 ± 12.87), TC (5.82 ± 0.97), and LDLc (3.99 ± 0.79) as elevated levels, and in the homozygous AA variant group, BMI (33.53 ± 4.28), waist (104.38 ± 34.87), hip (111.50 ± 6.05) and TG (3.53 ± 5.12) has high levels among the GG, GA, and AA genotypes. ANOVA analysis confirmed that a significant association was found between the waist circumference (p = 0.001), TG (p = 0.0007), and LDLc (p = 0.0004).

4. Discussion

In this hospital-based study, we sought to determine whether the rs2107538 variant previously reported (Böger et al., 2005, Herder et al., 2008, Kochetova et al., 2019, Lee et al., 2021, Prasad et al., 2007, Jang et al., 2007, Joo et al., 2007) is associated with T2DM subjects in our population. A high rate of consanguinity in the Saudi population may play a significant role in its prevalence and studies indicate that Saudis absorb glucose more quickly than Africans. It appears Saudi Arabia has a genetic predisposition to develop T2DM (Elhadd et al., 2007). Based on available data, the prevalence of T2DM in Saudi Arabia has grown from 17.1 to 24.3% (Alshammari and Alnasser, 2021). One of the main factors contributing to the increase in T2DM is a lack of physical activity, poor nutrition, and elevated BMI levels (Alwin Robert et al., 2017). The role of the variant rs2107538 or any part of *CCL5* in any human disease in the Saudi population has not been studied. As a result, this study aimed to determine whether the variant rs2107538 is associated with a high prevalence of T2DM in the Saudi population. Additionally, Sanger sequencing analysis confirmed that 35% of heterozygous and 16.70% of variant genotype AA have a significant impact on the Saudi population. Furthermore, genetic analysis confirmed that genotypes play a significant role in the pathogenesis of T2DM. The findings of this study are supported by the results of Sanger sequencing analysis performed on 60 T2DM cases and 60 healthy controls (without diabetes).

The results of the current study confirmed the t-test association in age, weight, BMI, waist, hip, SBP, DBP, FBG, HbA1c, TG, TC, and family history of T2DM (p < 0.05). The association showed a strong association in the genotypes of GA (p = 0.002) and AA (p = 0.008), the dominant model (p = 0.0002), and the allele frequencies (p = 0.0007). In addition, an individual effect showed an association between FBG as an independent variable with SBP and HDLc as dependent variables (p = 0.03). The ANOVA also correlated with waist circumference, TG, and LDLc (0 < 0.05). There have been limited studies on T2DM among the global population that has been associated with lower and higher risk (Böger et al., 2005, Herder et al., 2008, Kochetova et al., 2019, Lee et al., 2021, Prasad et al., 2007) and our study was highly associated with an average risk × 4.3. Consequently, the variant rs2107538 has been studied in various human diseases such as GDM (Teler et al., 2017), PTDM

Table 2
Genotype and allele frequencies for rs2107538 variant in T2DM cases and controls.

Genotypes and Alleles	T2DM cases (n = 60)	Controls (n = 60)	OR (95%CI)	P value
GG	29 (48.30%)	48 (80.0%)	**Reference**	**Reference**
GA	21 (35.0%)	09 (15.0%)	3.86 (95%CI. 1.56–9.56)	0.002
AA	10 (16.70%)	03 (05.0%)	5.51 (95%CI. 1.40–21.71)	0.008
GA + AA vs GG	31 (51.70%)	12 (20.0%)	4.27 (95%CI. 1.90–9.61)	0.0002
GG + AA vs GA	39 (65.0%)	51 (85.0%)	0.32 (95%CI. 0.13–0.79)	0.01
GA + GG vs AA	50 (83.30%)	57 (95.0%)	0.26 (95%CI. 0.06–1.01)	0.03
G allele	79 (65.80%)	105 (87.20%)	**Reference**	**Reference**
A allele	41 (34.20%)	15 (12.50%)	3.63 (95%CI. 1.87–7.02)	0.0007

Table 3
Multiple Linear regression analysis carried out to find the effect of FBG, Hb1Ac and genotypes on different variables.

Dependent variables	R-value	Adjusted R square	F	p value
Age	0.217	−0.004	0.922	0.436
Weight	0.138	−0.034	0.360	0.782
Height	0.057	−0.05	0.06	0.98
Waist	0.207	−0.009	0.833	0.481
Hip	0.216	−0.005	0.911	0.442
SBP	0.291	0.36	1.732	0.171
DBP	0.188	−0.016	0.682	0.567
TG	0.177	−0.021	0.601	0.617
TC	0.263	0.020	1.391	0.255
HDL-C	0.278	0.028	1.569	0.207
LDL-C	0.252	0.013	1.268	0.294

Table 3. Multiple Linear regression analysis and its role in FBG, Hb1Ac and genotypes as independent variables to study in different variables.

Table 4
Individual effects with genotypes, FBS and HbA1c on different dependent variables (outcome variables).

Outcome variables	Genotype			FBS			HbA1c		
	Standardized Co-efficient β	t value	P value	Standardized Co-efficient β	t value	P value	Standardized Co-efficient β	t value	P value
Age	0.006	0.044	0.965	0.165	0.971	0.336	−0.280	−1.660	0.103
Weight	−0.066	−0.491	0.626	0.136	0.789	0.434	−0.048	−0.282	0.779
Height	−0.147	−1.117	0.269	0.178	1.048	0.299	−0.055	−0.327	0.745
Waist	0.068	0.514	0.609	0.094	0.553	0.582	0.129	0.766	0.447
Hip	−0.026	−0.198	0.844	0.175	1.030	0.307	0.050	0.299	0.766
SBP	0.012	0.092	0.927	0.354	2.127	0.038	−0.121	−0.735	0.465
DBP	−0.173	−1.302	0.198	−0.106	−0.619	0.539	0.006	0.036	0.972
TC	−0.026	−0.200	0.842	0.324	1.931	0.059	−0.270	−1.620	0.111
TG	0.161	1.210	0.231	0.118	0.689	0.494	−0.035	−0.204	0.839
HDL-c	−0.023	−0.180	0.858	0.354	2.120	0.038	−0.236	−1.425	0.160
LDL-c	−0.037	−0.284	0.777	0.198	1.174	0.245	−0.321	−1.920	0.060

Table 5
Anova analysis performed between T2DM genotypes and covariates.

Dependent variables	GG (n = 29)	GA (n = 21)	AA (n = 10)	P value
Age (Years)	66.89 ± 7.77	67.52 ± 7.53	66.60 ± 5.75	0.57
Weight (kgs)	84.08 ± 14.91	81.73 ± 10.41	81.55 ± 16.47	0.17
Height (cms)	159.15 ± 8.95	158.42 ± 7.17	154.90 ± 7.75	0.56
BMI (kg/m^2)	33.15 ± 4.28	32.25 ± 5.87	33.53 ± 4.28	0.26
Waist (cms)	92.50 ± 10.37	98.87 ± 7.80	104.83 ± 34.87	0.001
Hip (cms)	107.20 ± 11.08	113.43 ± 7.93	111.50 ± 6.05	0.07
SBP (mmHg)	124.82 ± 11.09	127.00 ± 12.87	124.44 ± 11.02	0.74
DBP (mmHg)	79.40 ± 5.54	78.55 ± 5.32	73.75 ± 9.16	0.08
FBG (mmol/L)	13.58 ± 4.59	11.87 ± 3.98	12.41 ± 3.98	0.75
Hb1Ac	7.10 ± 0.75	7.00 ± 0.91	6.90 ± 0.96	0.53
TG (mmol/L)	2.31 ± 1.69	2.28 ± 1.08	3.53 ± 5.12	0.0007
TC (mmol/L)	5.57 ± 1.37	5.82 ± 0.97	5.23 ± 0.93	0.17
HDL-c (mmol/L)	0.76 ± 0.38	0.72 ± 0.30	0.71 ± 0.34	0.53
LDL-c (mmol/L)	3.72 ± 1.38	3.99 ± 0.79	3.43 ± 0.44	0.0004

(Joo et al., 2007, Jeong et al., 2010), cardiovascular (Mohtavinejad et al., 2021) and coronary artery diseases (Lee et al., 2021, Ting et al., 2015, Liu et al., 2012, Duell et al., 2006), myocardial infarction (Tereshchenko et al., 2011), asthma (Nahas et al., 2012, Lu et al., 2012), prostatic hyperplasia (Pang et al., 2019), and cancers such as prostate cancer (Kidd et al., 2012), oral and pancreatic cancers (Weng et al., 2010, Duell et al., 2006). Furthermore, these variants were studied in *meta*-analyses alongside tuberculosis (Sheng and Qi, 2020b) and cancer (Ying et al., 2014).

Chemokines are a type of cytokine that binds to specific G protein-coupled receptors on target cells. These low-molecular-weight proteins attach to several transmembrane proteins found on the surface of leukocytes and regulate their movement throughout the body. Proinflammation mediates the recruitment and switching of macrophages of M1 and M2 phenotype macrophages; the chemokine receptors CCL2, CCL3, CCL5, CCL7, CCL8, and CCL11 are activated in adipose tissue. A subtype of CCL5 cell is present in the CCR1 receptor, which is expressed in T-cells, epithelial cells, and activated platelets. Furthermore, the process also drags on in natural killer cells and leukocytes. Subsets of leukocytes, such as T-cells, monocytes, basophils, and eosinophils, are activated by CCL5. Additionally, CCL5, which has a crucial role in the immune response, is regulated on activation and is normally produced and released by T-cells. Moreover, obesity, impaired glucose tolerance, and T2DM all have a significant function in chemokines, and the gene *CCL5,* in particular, plays an important role in their etiology (Qidwai, 2016, Herder et al., 2008; Kochetova et al., 2019, Zhang et al., 2020).

As genes are transmitted from both parents to their offspring and between generations, the family history of any human disease plays a significant role in human life. Familial diabetes is thought to be a strong predictor of chronic diseases, including metabolic

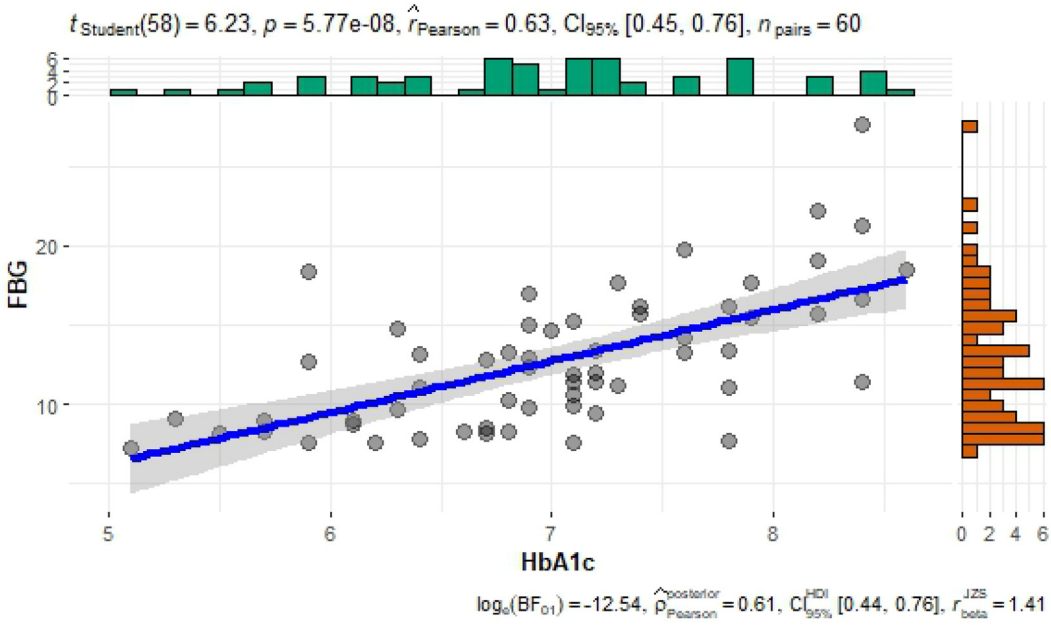

Fig. 4. Pearson correlation coefficient was studied between FBG and HbA1c in rs2107538 variant.

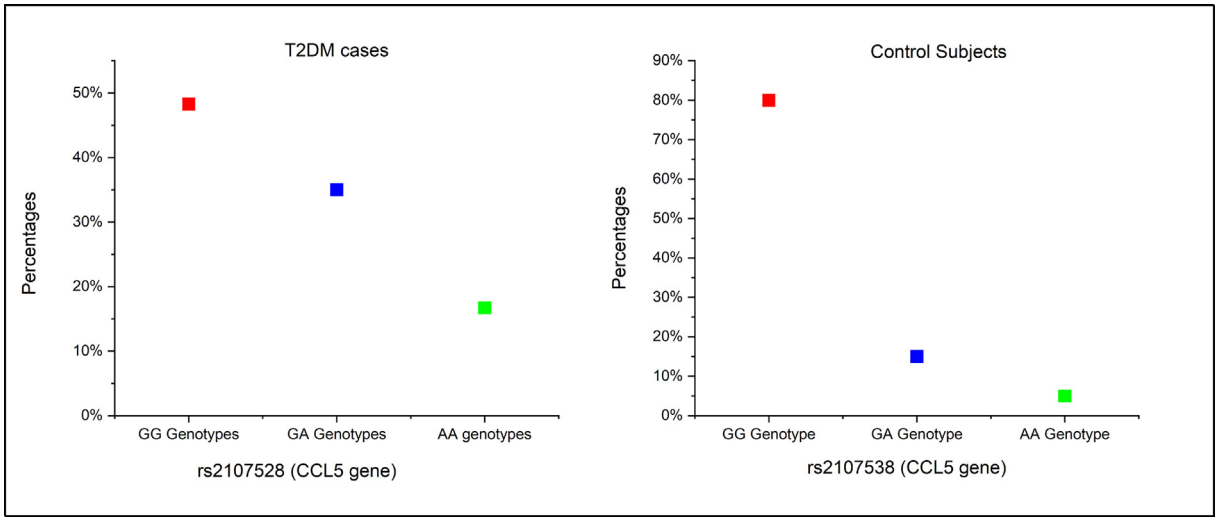

Fig. 3. Genotype frequencies of GG, GA and AA genotypes in T2DM cases and control subjects in rs2107538 variant in *CCL5* gene.

disorders and T2DM. Previous reports indicate that individuals with a family history of T2DM are six times more likely to develop the disease in their lifetime (Annis et al., 2005). Family history may play a significant role in Saudi Arabia due to the high rates of consanguinity. The high rate of consanguinity marriage in the Saudi population to polygenic diseases such as T2DM (Elhadd et al., 2007). In a retrospective study, by Anokute et al. (1992) confirmed that the 6:2 OR indicates a causal association between diabetes in familial aggregation. Thus, our current study focuses on family history since all of the T2DM cases evaluated had a 100% family history of T2DM, while 51.7% of controls had a family history of T2DM. In T2DM cases , 35% of patients had GA genotypes, and 10% had AA genotypes. Furthermore, in control subjects with a family history of T2DM, 11.7% have AG genotypes, and 5% have AA genotypes.

FBG and HbA1c are the most effective method for monitoring the diagnosis. HbA1c can be used to determine the average plasma glucose levels for at least 90 days. In this study, we confirm T2DM and control subjects using FBG, as recommended by the American Diabetes Association criteria. The normal level of FBG was 5.5 mmol/L, and in our study, the minimum documented value was 7.2 mmol/L, and the maximum documented value was 27.67 mmol/L. The mean FBG level was 12.79 mmol/L. The normal range for HbA1c was <5.6%; however, in our study, 93.4% of participants had levels above this threshold. Furthermore, the median HbA1c level measured was 7.0%. A Pearson correlation was performed in patients with the combination of FBG and HbA1c. Consistent with previous studies, we observed that the HbA1c levels were not correlated (p = 0.61) with FBG present in the rs2107538 variant (Fig. 4)(Sherwani et al., 2016).

Obesity is a condition linked to chronic and noncommunicable diseases, such as T2DM, which remains the focus of many ongoing studies. The relationship between T2DM and obesity was confirmed to be one of the risk factors for both diseases, i.e. T2DM

can be a risk factor for obesity; obesity can be a risk factor for patients with T2DM. Likewise, HTN is a common factor for T2DM patients with obesity (Colosia et al., 2013). Obesity-related insulin resistance contributes to the development of cardiovascular risk factors such as dyslipidemia, T2DM, and HTN. Furthermore, a combination of HTN and obesity can result in T2DM as a vascular complication (Colosia et al., 2013). Dyslipidemia is defined as an increase or decrease in plasma lipid fraction, with elevated levels of TC, TG, and HDLc and low levels of HDLc (Silitonga et al., 2019). Elevated levels of VLDL, LDLc, and TG and decreased levels of LDLc predict the development of CAD (Biadgo et al., 2017). In this study, we found no subjects with elevated glucose levels, lipid profile levels, HTN values, and abnormal BMI, including AC or CC genotypes. Thus, we believe that the combination of these factors may result in the development of predicted human diseases such as metabolic syndrome, CAD, heart disease and various cancers in the future.

In this study, we enrolled Saudi participants and patients with a family history of T2DM. Furthermore, in the controls used, the family history of T2DM was >50% of the participants, which was identified via Sanger sequencing. However, this study contained notable limitations; the recruitment of a small sample size in both T2DM and control subjects and the limited screening of a single variant.

5. Conclusion

This study summarized the function of the rs2107538 variant of the *CCL5* gene in Saudi Arabian T2DM patients. Both GA/AA genotypes and ANOVA analyses were associated with T2DM patients in the Saudi population. To our knowledge, this study is the first to analyze the rs2107538 variant and its role in the Saudi population. Thus, future research should include via larger sample sizes and screening with multiple variants in the *CCL5* gene. Furthermore, functional studies may be conducted, which may more substantially contribute to our understanding of the role of gene variants in the development of human disease.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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