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ASSOCIATION OF *RARRES2* CHEMERIN GENE POLYMORPHISM WITH TYPE 2 DIABETES MELLITUS AND METABOLIC SYNDROME: A CASE-CONTROL STUDY

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ABSTRACT

Background: Chemerin is type of adipokine that involved in metabolism, inflammation and glucose homeostasis of adipose tissues. Genetic variations in the *RARRES2* gene may come up with metabolic abnormalities of type 2 diabetes mellitus (T2DM) in association with metabolic syndrome (MetS). The present study investigated the association of gene polymorphism of *RARRES2 rs17171368* gene with T2DM with and without MetS and evaluated its relationship with selected metabolic parameters.

Methods: A case-control study was conducted among 294 participants aged 25–75 years, comprising 98 patients with T2DM with MetS, 98 patients with T2DM without MetS and 98 healthy controls. Anthropometric, clinical and biochemical parameters were assessed. Serum chemerin levels were estimated using ELISA. Genotyping of the RARRES2 polymorphism was performed using the t-ARMS (allele specific PCR). Allelic and genotypic frequencies were compared among the study groups, and associations with age, BMI and biochemical parameters were evaluated.

Results: The distribution of RARRES2 genotypes was significantly varied among the study groups. The GG genotype was more frequently observed in patients with T2DM with MetS [33 (33.7%)] than in patients with T2DM without MetS [12 (12.2%)], with a statistically significant difference ($p<0.001$). Variations in genotype distribution were also observed across selected age and BMI categories. Level of serum chemerin were elevated in patients with T2DM with MetS in comparison to other study groups. The findings of this study advocate the association of *RARRES2 rs1717368* genetic variation with metabolic phenotype of T2DM, particularly in the presence of MetS.

Conclusion: This study exhibits a prominent association between *RARRES2 rs1717368* gene polymorphism and T2DM with MetS. The higher frequency of the GG genotype in the T2DM with MetS categories suggests that this genetic variant may contribute to susceptibility to metabolic complications. Further studies involving larger and ethnically diverse populations are warranted to confirm these findings.

KEYWORDS: RARRES2; Chemerin; Type 2 diabetes mellitus; Metabolic syndrome; Gene polymorphism; Adipokine; Genotype

1. INTRODUCTION

Type-2 diabetes mellitus (T2DM) is an example of endocrine disorder with a strong hereditary component. Genetic polymorphisms in gene responsible for T2DM disorder may affect genetic susceptibility to a disease which is characterized by islets disfunction and insulin resistance(1). Metabolic syndrome (MetS) specifies a group of metabolic disfunctions like high blood pressure, central obesity, insulin resistance, and unusual cholesterol levels. These issues greatly enhance the risk of developing atherosclerotic cardiovascular diseases (CVD) and T2DM(2,3). Metabolic syndrome is diagnosed when an individual presents with at least three of the associated health issues. With rising obesity rates, more than one-fifth of individuals in America and Europe are now affected by this condition. Early identification and intervention are crucial for improving health outcomes(4). Chemerin, a type of adipokine, firstly discovered in psoriasis patients, is mainly secreted by adipose tissue(5). It promotes the macrophagic adhesion to proteins of extracellular matrix and facilitates the chemotaxis, which helps the recruitment of macrophages to inflammation sites(6). Yang et.al.,2019, has reported a significant association between chemerin and several inflammatory markers like C-reactive protein (CRP), tumour necrosis factor-alpha (TNF-α), and interleukins-6 (IL-6)(7). Chemerin role has been positively discovered as inflammatory mediator, as well as in promotion of obesity pathogenesis and metabolic syndrome (MetS) too (8). Level of circulating chemerin remains higher among T2DM suffering individuals than healthy individuals. Because in diabetic condition, chemerin may exacerbate glucose intolerance, reduce serum insulin levels, and cause resistance to insulin secretion(9). Genetic polymorphisms in chemerin gene can play an essential role in vulnerability of disease and phenotypic variations in T2DM. Several previous studies have reported the association of single-nucleotide polymorphisms (SNPs) in the *RARRES2* gene with an increased incidence of T2D and CAD(10). The *RARRES2 rs17173608* SNP, located in the potential site of mutation, intron 2 of *RARRES2,* significantly associated with metabolic syndromes and obesity(11). In this study, we targeted to investigate the relationship of gene polymorphism of *RARRES2 rs17171368* and T2DM susceptibility, with and without metabolic syndrome.

MATERIALS AND METHODS

Study Population: A total of 294 subjects in the 25–75-year age group, including 196 cases and 98 healthy controls, were enrolled in this study. Each participant was compulsorily asked to sign a written, after explaining about study in their native language to participate in this study. The study protocol was approved by the Institutional Ethics Committee of SAI Tirupati University, Udaipur, Rajasthan, India (STU/IEC/2024/284), and all study methodologies adhered to the Helsinki guidelines. Metabolic syndrome (MetS) diagnosis was performed according to global agreement of MetS as per regulations of the International Diabetes Federation, 2005(12). Inclusion criteria of participants to involve in this study was as following. The patients must have possess the central obesity i.e., their body mass index (BMI) must be more than 30kg/m², along with these, participants must fulfil any two out of the remaining four factors of MetS diagnosis. These four factors were more than 150 mg/dL concentration of elevated triglycerides values (or any other specific treatment for this lipid abnormality), low level of HDL- cholesterol value i.e., less than 40 mg/dL and less than 50 mg/dL concentration among male and female respectively, patient must have been diagnosed with hypertension (systolic blood pressure ≥ 130 mm Hg or diastolic blood pressure ≥ 85 mmHg) and either diagnosed with T2DM or elevated fasting plasma glucose (FPG) i.e., 126 mg/dl. Cases were identified according to the World Health Organization criteria(13). Patients with type one diabetic mellitus, smokers, pregnant women and chronic alcoholics, were excluded from this study.

Anthropometric Measurement: Anthropometric parameters like weight (kg), circumference of waist (cm) and participants height (cm) were measured. BMI of participants were calculated by dividing the weight (kg) of individual to square of their height (m^2). Waist to height ratio (WtHR) was calculated by dividing waist circumference (cm) to height (cm), and percentage of body fat (BF%) were calculated by suing the formula (1.20 x BMI) + (0.23 x age) - (10.8 x sex) - 5.4 (Sex: male=1, female=2)(14).

Blood Pressure: Systolic blood pressure (SBP) and diastolic blood pressure (DBP) (mmHg) of participants were measured using Sphygmomanometer in sitting position. MAP = DBP + (SBP-DBP)/3formula was used to calculate the mean arterial pressure (MAP) (14).

Laboratory Investigations: Participants were asked for overnight fasting before sample collection. Ten mL of peripheral venous blood was collected from each participant and distributed into plain, EDTA, and fluoride vials according to the requirements of the respective investigations. Blood collected in plain vials was used for estimation of serum chemerin and lipid profile. Blood collected in EDTA vials was used for HbA1c estimation, genomic DNA extraction, and genotyping. Fluoride-preserved blood was used for blood glucose estimation. Serum and plasma were separated by centrifugation after allowing the samples to stand for approximately 1 hour, and the separated samples were stored appropriately until further analysis. Fasting and postprandial blood glucose levels were measured using the glucose oxidase-peroxidase protocol. Total serum cholesterol, triglycerides, and high-density lipoprotein cholesterol (HDL-C) were estimated using the Erba Chem 7 semi-automatic biochemistry analyzer (Erba Diagnostics Mannheim GmbH, Mannheim, Germany). Very-low-density lipoprotein cholesterol (VLDL-C) and Low-density lipoprotein cholesterol (LDL-C) were estimated using the Friedewald formula(15). HbA1c was estimated using the Erba Chem 7 semi-automatic biochemistry analyzer (Erba Diagnostics Mannheim GmbH, Mannheim, Germany). Serum chemerin concentration was measured using a commercially available sandwich ELISA kit (Real Gene, Cat. No. 3011953).

DNA Extraction: Genomic DNA(gDNA) was isolated from whole blood samples, collected in EDTA vials, using standard organic extraction method(16). The purity and concentration of the extracted gDNA was determined spectrophotometrically using a NanoDrop Lite Plus spectrophotometer (Thermo Fischer Scientific, Waltham, MA, USA) by measuring absorbance ratio at A260/A280. The DNA was kept at -20° C for genotyping.

Genotyping: The *RARRES2 rs17173608* polymorphism was genotyped using the T-ARMS-PCR (Tetra-Amplification Refractory Mutation System- Polymerase Chain Reaction) PCR (11). 25 µL PCR reaction mixture was prepared containing, 12.5 µL LEO PRIME™ Genotyping Master mix (2X) (Cat. No. #R8220; DXBIDT / Rubizon Pvt. Ltd., Bengaluru, India), 1 µL of each primer (100 pmol/µl), 1 µL of genomic DNA (100 ng/ µL) and 7.5 µL nuclease free water. The primers of detection of RARRES2 rs17173608, SNP were as follows(17): Forward Inner (G Allele): 5′-ATTGCTATAGTCCAGTGCCCTTCG-3′, Reverse Inner (T Allele): 5′-CCAGTTCCCTCTGTCGGCTTAA-3′, Forward Outer (F-Com): 5′-GTCAGACCCATGCAGTTTTCAAAC-3′, Reverse Outer (R-com): 5′-GAGTTCCTCTCTCAAGCATCAGGG-3′. PCR amplification was done with 5 min. initial denaturation step at 95°C, followed by 30 cycles of denaturation at 95 for 30 s, annealing at 56°C for 30 s and extension at 72°C for 30 s, followed by a final extension step at 72°C for 10 min. Amplified products were resolved over 2% agarose gel electrophoresis using 0.5 µg/mL ethidium bromide (EtBr) , which was visualized using iGlow Gel Documentation System (Invitrogen, Thermo Fischer Scientific, Waltham, MA, USA).

Statistical Analysis: Observed data were analyzed using SPSS v.29 (SPSS Inc, Chicago, IL, USA). Two means were compared using t-test and for the comparison of more than two means one-way ANOVA (analysis of variance) followed by post hoc analysis were used. Pearson's correlation test was used to correlate biochemical parameter and MetS criteria. Serum chemerin level, independent factors and MetS criteria were correlated to each other with multiple linear regression. Proportions among qualitative parameters, genotype, and allelic frequencies between the study groups were analysed with chi-square test. Value of odd ratio (OR) at 95% confidence interval (95% CI) of MetS criteria were analysed using logistic

regression analysis.

RESULTS

Demographic characteristics and anthropometric parameters (Table 1) exhibit mean age was higher in Group 1 (53.32 ± 11.80) than in Group 3 (42.02 ± 12.20). The proportion of males was highest in Group 2 (71.4%), whereas females accounted for 36.7% of Group 1. Gender distribution did not differ significantly among the groups. All anthropometric parameters, including BMI, waist circumference, hip circumference, body fat percentage, and waist-to-hip ratio, differed significantly among the groups (all $p<0.001$). Group 1 showed the highest values for these parameters.

Table 1. Demographic Characteristics of participants

Parameter	G1:T2DM with MetS (n=98)	G2: T2DM without MetS (n=98)	G3: Healthy Control (n=98)	Test statistics	p-value
Age (years) Mean ± SD	53.32 ± 11.80	51.71 ± 14.04	42.02 ± 12.20	F = 22.63	0.002
Age range (years)	25–75	27–75	25–75		
Male n (%)	62 (63.3%)	70 (71.4%)	63 (64.3%)	$\chi^2 = 1.43$	0.489 NS
Female n (%)	36 (36.7%)	28 (28.6%)	35 (35.7%)		
Waist Circumference (cm)	158.85 ± 22.66	90.56 ± 8.97	86.29 ± 7.51	F=749.355	<0.001
BMI (kg/m²)	31.97 ± 7.25	26.13 ± 5.25	23.98 ± 2.93	F=56.599	<0.001
Body Fat % (BF%)	35.02 ± 7.70	27.80 ± 6.66	18.39 ± 7.38	F=129.335	<0.001
Waist-to-Hip Ratio (WtHR)	1.21 ± 0.34	0.90 ± 0.11	0.79 ± 0.09	F=104.170	<0.001
Hip Circumference (cm)	136.52 ± 26.01	101.32 ± 8.61	111.22±19.30	F=86.238	<0.001

Biochemical parameters (Table 2) show SBP, DBP, and MAP were significantly higher in Group 1 than in the other groups (all $p<0.001$). Similarly, FBS, PPBS, and HbA1c were significantly higher in Group 1 (all $p<0.001$). The lipid profile showed higher TC, TG, LDL-C, and VLDL levels and lower HDL-C levels in Group 1. The difference in HDL-C between Groups 1 and 2 was not statistically significant ($p=0.691$). Serum chemerin levels were highest in Group 1 (318.12 ng/mL), followed by Group 2 (270.14 ng/mL) and Group 3 (143.17 ng/mL).

Table 2. Comparison of Biochemical parameters Across Study Groups

Parameter	G1:T2DM with MetS Mean±SD	G2: T2DM without MetS Mean±SD	G3: Healthy Control Mean±SD	F-value	p-value
SBP (mmHg)	170.24 ± 20.33	131.08 ± 11.70	109.11 ± 10.00	F=433.712	0.016
DBP (mmHg)	102.60 ± 11.94	89.32 ± 8.48	73.53 ± 6.51	F=242.437	0.023
MAP (mmHg)	126.95 ± 19.29	103.24 ± 6.73	85.69 ± 5.88	F=278.999	0.041
FBS (mg/dL)	197.79 ± 50.48	155.21 ± 24.96	97.39 ± 9.02	F=229.493	0.001
PPBS (mg/dL)	279.46 ± 72.62	220.49 ± 32.00	133.37 ± 17.48	F=240.508	0.031
HbA1c (%)	9.51 ± 2.60	8.21 ± 0.96	5.16 ± 0.80	F=175.929	0.027
TC (mg/dL)	241.39 ± 65.28	200.45 ± 29.14	179.14 ± 45.20	F=41.133	0.001
TG (mg/dL)	259.33 ± 109.54	189.74 ± 42.50	164.12 ± 81.79	F=34.818	<0.001
HDL-C (mg/dL)	41.80 ± 10.44	42.31 ± 7.02	46.95 ± 12.25	F=7.688	<0.001
LDL-C (mg/dL)	146.62 ± 67.14	120.19 ± 31.69	101.88 ± 39.73	F=20.974	<0.001
VLDL (mg/dL)	51.85 ± 21.91	37.95 ± 8.50	32.31 ± 16.13	F=36.615	<0.001
Chemerin (ng/mL)	318.12 ± 42.52	270.14 ± 37.95	143.17±10.34	F=716.165	0.001

Genotypic and allelic analyses of *RARRES2 rs17173608* polymorphism (Table 3) demonstrated statistically significant heterogeneity across the three-study cohort ($\chi^2 = 20.02$, df= 4, $p<0.001$). within T2DM with MetS group (G1), The heterozygous TG genotype was the most prevalent in both the G1 and G2 population. Pairwise genotype comparison marked a significantly elevated frequency of the homozygous GG genotype in G1 in contrast to G2 ($\chi^2 = 18.12$, $p<0.001$), alongside a significant reduction in the TT genotype when comparing G1 with G3 ($\chi^2 = 9.92$, $p<0.007$). no significant

genotypic difference was observed between G2 and G3 ($\chi^2 = 1.40$, p<0.496). Overall allelic frequencies varied significantly among the cohorts ($\chi^2 = 19.47$, df= 2, p<0.023). the mutant G allele predominated in G1 (58.7%) in comparison to its lower representation in G2 (37.2%) and G3 (42.9%), where the wild type T allele remained dominant. Allelic pairwise tests confirmed significant differences between G1 and G2 (($\chi^2 = 17.18$, p<0.001) as well as between G1 and G3 ($\chi^2 = 9.19$, p=0.002), while G2 and G3 were statistically non-significant ($\chi^2 = 1.06$, p<0.303).

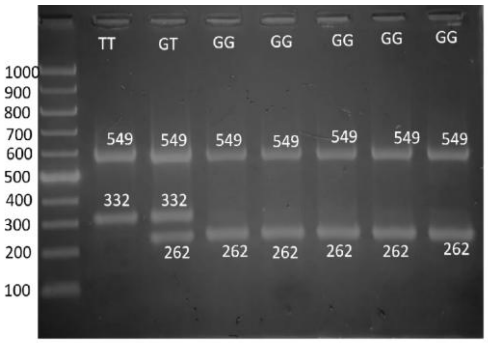

Figure 1: Agarose gel electrophoresis for the detection of RARRES2 rs17173608 gene polymorphism. Product sizes were 262 bp for G allele, 332 bp for T allele, and 549 bp for two common primers.

Table 3. Distribution of genotypic and allelic frequency of *RARRES2 rs17173608* among the three study groups

Parameter	G1: T2DM with MetS (n=98)	G2: T2DM without MetS (n=98)	G3: Healthy Control (n=98)	Total (N=294)	Overall χ^2 (df)	Overall p-value	Pairwise comparison	Pairwise χ^2	Pairwise p-value
Genotype, n(%)									
TT	16 (16.3%)	37 (37.8%)	31 (31.6%)	84 (28.6%)	20.2(4)	<0.001	G1 vs G2	18.12	<0.001
TG	49 (50.0%)	49 (50.0%)	50 (51.0%)	148 (50.3%)			G1 vs G3	9.92	0.007
GG	33 (33.7%)	12 (12.2%)	17 (17.3%)	62 (21.1%)			G2 vs G3	1.40	0.496(ns)
Alleles, n(%)									
T	81(41.3)	123(62.8)	112(57.1)	316(53.7)	19.47(2)	0.023	G1 vs G2	17.18	<0.001
G	115(58.7)	73(37.2)	84(42.9)	272(46.3)			G1 vs G3	9.19	0.002
							G2 VS G3	1.06	0.303 (ns)
ns = non-significant									

Subsequent age-stratified analysis across five chronological brackets (25-35, 36-45, 46-55, 56-65- and 66-75-years age group) revealed distinct distribution patterns within each group (table 4). In the G1 cohort, where participants were concentrated in older age group (31.6%), the GG genotype displayed a prominent upward trend with age, rising from 0.0% in 25–35-year age group to 57.1% in 66-75 age category, although overall intra-group variation did not reach statistical significance ($\chi^2 = 12.484$, p=0.131). Conversely, the G2 study group demonstrated a statistically significant genotypic variation across age group ($\chi^2 = 18.173$, p=0.020), driven by a predominance of the TT genotype among early year age group (25-35 year) individuals (55.6%), a surge in TG heterozygosity in 46–55-year age group (73.8%) and a subsequent concentration of GG genotype carriers in 66-75 age category (30.4%). The healthy cohort (G3) was skewed toward younger age category (34.7% at 25–35-year age group), it was entirely absent among individuals aged 56 year and older, though overall age stratified differences within G3 remained statistically non-significant ($\chi^2 = 13.63$, p=0.092).

Table 4: Distribution of Chemerin *RARRES2 rs1717368* genotype according to age group

Age Group (years)	TT n(%)	TG n(%)	GG n(%)	Total n(%)	χ^2	p-value
G1: T2DM with MetS (N=98)						
25-35	2 (33.3%)	4 (66.7%)	0 (0.0%)	6 (6.1)	12.484	0.131
36-45	4 (21.1%)	10 (52.6%)	5 (26.3%)	19 (19.4)		
46-55	5 (23.8%)	11 (52.4%)	5 (23.8%)	21 (21.4)		
56-65	5 (16.1%)	15 (48.4%)	11 (35.5%)	31 (31.6)		

66-75	0 (0.0%)	9 (42.9%)	12 (57.1%)	21 (21.4)		
25-35	2 (33.3%)	4 (66.7%)	0 (0.0%)	6 (6.1)		
G2: T2DM without MetS (N=98)						
25-35	10 (55.6%)	7 (38.9%)	1 (5.6%)	18 (18.4)		
36-45	6 (42.9%)	7 (50.0%)	1 (7.1%)	14 (14.3)		
46-55	4 (17.4%)	18 (78.3%)	1 (4.3%)	23 (23.5)	18.173	0.020
56-65	9 (45.0%)	9 (45.0%)	2 (10.0%)	20 (20.4)		
66-75	8 (34.8%)	8 (34.8%)	7 (30.4%)	23 (23.5)		
G3: Healthy Control (N=98)						
25-35	16 (47.1%)	15 (44.1%)	3 (8.8%)	34 (34.7)		
36-45	9 (31.0%)	15 (51.7%)	5 (17.2%)	29 (29.6)		
46-55	6 (31.6%)	9 (47.4%)	4 (21.1%)	19 (19.4)	13.63	0.092
56-65	0 (0.0%)	7 (77.8%)	2 (22.2%)	9 (9.2)		
66-75	0 (0.0%)	4 (57.1%)	3 (42.9%)	7 (7.1)		

Chi-square test statistics revealed a significant association between chemerin *RARRES2 rs1717368* genotype frequencies and body mass index (BMI) categories across all three studied cohort (table 5). Across all groups, the homozygous TT genotype was consistently enriched among leaner individuals and predominant among underweight (UW) individuals (G1-=75.0%, G2= 66.7% and G3=100%) and normal weight (NW) individuals (G1=34.6%, G2= 51.6% and G3=41.7%), while being marked reduced or absent in the higher obesity strata. Conversely, carriage of the polymorphic G allele was positively associated with increasing adiposity, the heterozygous TG genotype was the most prevalent form among overweight (OW) participants (G1=85.7%, G2= 54.3% and G3= 61.8%) and the homozygous mutant GG genotype reached its highest concentration in the severe obesity classes.

Table 5: Distribution of Chemerin *RARRES2 rs1717368* genotype according to BMI

Age Group (years)	**TT n(%)**	**TG n(%)**	**GG n(%)**	**Total n(%)**	**χ^2**	**p-value**
G1: T2DM with MetS (N=98)						
UW	3 (75.0%)	0 (0.0%)	1 (25.0%)	4 (4.08)		
NW	9 (34.6%)	11 (42.3%)	6 (23.1%)	26 (26.5)		
OW	1 (14.3%)	6 (85.7%)	0 (0.0%)	7 (7.14)	30.460	<0.001
Obese I	0 (0.0%)	11 (68.8%)	5 (31.2%)	16 (16.33)		
Obese II	3 (8.3%)	17 (47.2%)	16 (44.4%)	36 (36.7)		
Obese III	0 (0.0%)	4 (44.4%)	5 (55.6%)	9 (9.2)		
G2: T2DM without MetS (N=98)						
UW	6 (66.7%)	3 (33.3%)	0 (0.0%)	9 (9.2)	17.651	0.024
NW	16 (51.6%)	15 (48.4%)	0 (0.0%)	31 (31.6)		
OW	10 (28.6%)	19 (54.3%)	6 (17.1%)	35 (35.7)		
Obese I	5 (27.8%)	9 (50.0%)	4 (22.2%)	18 (18.4)		
Obese II	0 (0.0%)	3 (60.0%)	2 (40.0%)	5 (5.1)		
Obese III	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0)		
G3: Healthy Control						
UW	3 (100.0%)	0 (0.0%)	0 (0.0%)	3 (3.06)		
NW	25 (41.7%)	29 (48.3%)	6 (10.0%)	60 (61.2)		
OW	3 (8.8%)	21 (61.8%)	10 (29.4%)	34 (34.7)	24.324	<0.001
Obese I	0 (0.0%)	0 (0.0%)	1 (100.0%)	1 (1.02)		
Obese II	0 (0.0%)	0 (0.0%)	0 (0.0%)	0		
Obese III	0 (0.0%)	0 (0.0%)	0 (0.0%)	0		

The distribution of chemerin *RARRES2 rs1717368* genotype across genders (table 6) exhibited a statistically significant differences only among group 2 population ($\chi^2 = 9.811$, p= 0.007), driven by a higher frequency of the TT genotype in female participants (57.1%). And TG genotype in male participants (60.0%). Conversely, no sex based variations were noted in G1 ($\chi^2 = 0.297$, p= 0.862) and G3 ($\chi^2 = 1.178$, p= 0.555), where genotype frequencies remained comparable between males and females.

Table 6: Genotype distribution according to gender

		TT	**TG**	**GG**	**Total**	**χ^2**	**p-value**
Male	G1	11 (17.7%)	30 (48.4%)	21 (33.9%)	62	0.297	0.862
Female		5 (13.9%)	19 (52.8%)	12 (33.3%)	36		
Male	G2	21 (30.0%)	42 (60.0%)	7 (10.0%)	70	9.811	0.007
Female		16 (57.1%)	7 (25.0%)	5 (17.9%)	28		

Male	G3	21 (33.3%)	33 (52.4%)	9 (14.3%)	63	1.178	0.555
Female		10 (28.6%)	17 (48.6%)	8 (22.9%)	35		

Genetic model analysis (table 7) demonstrated that the chemerin (*RARRES2*) variant was significantly associated with risk in the T2DM with MetS group (G1), but showed no significant association in the T2DM-MetS group (G2) when compared to healthy controls. In G1, risk was significantly elevated under the dominant model (OR-2.37, 95% CI: 1.20-4.70, p=0.019), the recessive model (OR-2.42, 95% CI: 1.24-4.73, p=0.014), and the allelic model (OR-1.89, 95% CI: 1.27-2.83, p=0.002), while the over-dominant model showed no significant effect (OR=0.96, p=1.00). in contrast, for the T2DM without MetS participant group none of the evaluated models i.e., dominant (OR=0.76, p=0.453), recessive (OR=0.66, p=0.421), over-dominant (OR=0.96, p=1.000), or allelic (OR=0.79. p=0.303) showed significant association, which indicates that the risk-conferring effect of the G allele was specifically prominent among individuals presenting with comorbid metabolic syndrome.

Table 7: Inheritance (Genetic) Model Analysis - Chemerin (RARRES2) Genotype, Cases vs Healthy Controls

Case Group	Genetic Model	Case (exp/unexp)	Control (exp/unexp)	OR	95% CI	p-value
G1: T2DM+MetS	Dominant (TG+GG vs TT)	82/16	67/31	2.37	1.20–4.70	0.019
G1: T2DM+MetS	Recessive (GG vs TG+TT)	33/65	17/81	2.42	1.24–4.73	0.014
G1: T2DM+MetS	Over-dominant (TG vs TT+GG)	49/49	50/48	0.96	0.55–1.68	1.000
G1: T2DM+MetS	Allelic (G vs T allele)	115/81	84/112	1.89	1.27–2.83	0.002
G2: T2DM-MetS	Dominant (TG+GG vs TT)	61/37	67/31	0.76	0.42–1.38	0.453
G2: T2DM-MetS	Recessive (GG vs TG+TT)	12/86	17/81	0.66	0.30–1.48	0.421
G2: T2DM-MetS	Over-dominant (TG vs TT+GG)	49/49	50/48	0.96	0.55–1.68	1.000
G2: T2DM-MetS	Allelic (G vs T allele)	73/123	84/112	0.79	0.53–1.19	0.303

DISCUSSION:

The present study demonstrated significant differences in anthropometric and metabolic characteristics among T2DM with Mets, T2DM without Mets and healthy controls. Age differed significantly among the groups (p=0.002), whereas sex distribution was comparable (p=0.489). Waist circumference, BMI, body fat percentage, waist to hip ratio and hip circumference were significantly higher in T2DM with Mets group (all p=0.001), indicating greater adiposity and central obesity in individual with Mets(18).

The biochemical profile showed significantly higher blood pressure, FBS, PPBS, HbA1C, total cholesterol, triglycerides, LDL-C and VLDL-C with lower HDL-C in the diabetic groups compared with healthy controls. These findings reflects the characteristics clustering of hyperglycemia, hypertension and dyslipidemia in T2DM with Mets. Serum chemerin was also markedly elevated in T2DM with Mets (318.12±42.52 ng/ml) compared with T2DM without Mets (270.14±37.95 ng/ml) and healthy controls (143.17±10.34 ng/ml; p=0.001). This observation is consistent with previous studies reporting increased circulating chemerin in obesity, insulin resistance, T2DM and Mets(19,20).

A significant difference was observed in the distribution of RARRES2 rs17173608 genotypes among the three groups (χ^2=20.02, df=4, p<0.001). The GG genotype was more frequent in T2DM+MetS (33.7%) than in T2DM without MetS (12.2%) and healthy controls (17.3%). Similarly, the G allele frequency was highest in T2DM+MetS (58.7%), with a significant difference in allele distribution (χ^2=19.47, df=2, p=0.023). These findings suggest a possible association of the G allele, particularly the GG genotype, with the metabolic phenotype of T2DM with MetS. Similar associations between the RARRES2 rs17173608 G allele and MetS have been reported previously(11). However, contradictory findings have also been reported, particularly regarding its association with T2DM, indicating possible population and phenotype-specific effects(11,21).

Age-stratified analysis showed no significant genotype association in T2DM+MetS (p=0.131) or healthy controls (p=0.092), whereas a significant difference was observed in T2DM without MetS (p=0.020). BMI-stratified analysis demonstrated significant genotype variation in T2DM+MetS (p<0.001), T2DM without MetS (p=0.024), and healthy controls (p<0.001). These findings suggest that genotype distribution may vary across adiposity categories; however, the small numbers in some age and BMI subgroups warrant cautious interpretation(22).

Gender-stratified analysis showed no significant genotype difference in T2DM+MetS (p=0.862) or healthy controls (p=0.555), while a significant difference was observed in T2DM without MetS (p=0.007). Overall, the findings indicate that RARRES2 rs17173608 may contribute to metabolic susceptibility, particularly in T2DM accompanied by MetS, although larger studies with adjusted genetic models and adequate subgroup sizes are required to confirm this association(23). Inheritance model analysis revealed a significant association of the Chemerin (RARRES2) polymorphism

with T2DM accompanied by metabolic syndrome (G1). Significant associations were observed under the dominant model (OR=2.37, 95% CI: 1.20–4.70, p=0.019), recessive model (OR=2.42, 95% CI: 1.24–4.73, p=0.014), and allelic model (OR=1.89, 95% CI: 1.27–2.83, p=0.002), indicating a higher susceptibility associated with the G allele. In contrast, no significant association was observed in T2DM without metabolic syndrome (G2) in any genetic model (p>0.05). These findings suggest that the RARRES2 polymorphism may contribute more specifically to the metabolic-syndrome-associated phenotype of T2DM(24). This observation is supported by previous studies reporting an association of RARRES2 variants with metabolic syndrome and metabolic traits(25). A study reported that the G allele of RARRES2 rs17173608 increased the risk of metabolic syndrome (OR=1.78, 95% CI: 1.14–2.78, p=0.012)(26). Genetic variation within RARRES2 has also been shown to influence circulating chemerin levels, supporting its potential role in metabolic regulation(27).

CONCLUSION:

The present study demonstrates that T2DM accompanied by metabolic syndrome is characterized by greater central adiposity, adverse metabolic alterations, and markedly elevated circulating chemerin levels compared with T2DM without metabolic syndrome and healthy controls. The significant association of RARRES2 rs17173608, particularly the G allele and GG genotype, with T2DM with metabolic syndrome suggests a potential genetic contribution to the metabolic-syndrome-associated phenotype. The significant dominant, recessive, and allelic associations further support the possible role of this polymorphism in metabolic susceptibility. However, the findings should be interpreted cautiously because of the limited subgroup sizes, and larger studies with appropriate adjustment for potential confounders are warranted to validate the genetic and metabolic associations observed.

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Author contribution

The conception or design of the study: AP, PS
The acquisition, analysis or interpretation of the data: AP, PS, AKT, SD
Sample Collection: AP
Writing of the manuscript: AP, AKT
AP and AKT share equal contribution for this work.
All authors read and approved the manuscript.

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