Original Article

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Genetic Polymorphisms in *RNF138*, *ABCA1* and *ESRRG-GPATCH2* Genes and their Role in Insulin Resistance Risk among Normal BMI Individuals in Indian Population: A Case-control Study

Biotechnology Section

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ABSTRACT

Introduction: India, characterised as the "diabetes capital" of the world, faces a rapidly increasing diabetes crisis, with over 65 million cases diagnosed. Despite the growing prevalence, the genetic underpinnings of Insulin Resistance (IR) among Indians with a normal Body Mass Index (BMI) remains understudied.

Aim: To fill the knowledge gap by investigating the association of specific gene variants (*RNF138, ABCA1*, and Oestrogen-Related Receptor γ (*ESRRG*)-*GPATCH2*) with IR risk in this demographic.

Materials and Methods: This study was a case-control study conducted in Bengaluru, Karnataka, India on a total of 191 participants (90 men and 101 women). The study, including data collection and analysis, was completed over a period of six months. Participants were categorised into cases (Homeostasis Model Assessment Insulin Resistance (HOMA2-IR) >2) and controls (HOMA2-IR <2) based on HOMA2-IR values. Genotyping for rs4799327 (*RNF138*), rs2275543 (*ABCA1*), and rs1497828 (*ESRRG-GPATCH2*) was performed using the Illumina Infinium Global Screening Array (GSA). Statistical analyses, including Odds Ratios (ORs), 95% Confidence Intervals (CIs), and inheritance model analysis, were conducted to assess the association between genotypes and IR.

Results: Significant associations were found between IR and genetic variants rs4799327 (OR=2.74, 95% CI: 1.28-5.88, p-value=0.006) in *RNF138*, rs1497828 (OR=2.90, 95% CI: 1.51-5.57, p-value=0.0011) in *ESRRG-GPATCH2* (dominant inheritance model), and rs2275543 (OR=3.50, 95% CI: 1.17-10.42, p-value=0.011) in *ABCA1* (additive model). The study highlights a notable susceptibility to IR linked to these genetic markers among individuals with a normal BMI in the Indian population.

Conclusion: This study underscores the importance of genetic factors in the risk of developing IR among Indians with a normal BMI, suggesting a complex interplay of genetics beyond traditional risk factors. These findings necessitate further research into the functional significance of these associations and their potential implications for targeted interventions and preventive strategies in high-risk populations.

Keywords: Diabetes mellitus, Insulin resistance, Metabolic syndrome, Oestrogen-related receptor gamma, RNF138 protein, Single nucleotide polymorphism

INTRODUCTION

The global prevalence of diabetes presents a daunting challenge to public health, marking it as a significant global health crisis. With India accounting for over 77 million diagnosed cases, the nation has emerged as the epicenter of the diabetes epidemic worldwide [1]. The alarming rise in diabetes cases underscores the urgent need for comprehensive research and intervention strategies. India, in particular, is grappling with a staggering increase in diabetes cases. Over the past few decades, the country has witnessed a steady rise in diabetes prevalence, with current estimates indicating that more than 77 million individuals are living with diabetes [2]. Alarmingly, approximately 57% of adults with diabetes in India remain undiagnosed, highlighting significant gaps in healthcare access and screening efforts.

Diabetes, as part of Metabolic Syndrome (MetS), presents a multifaceted challenge in management and treatment. BMI fails to fully capture metabolic nuances; individuals with a normal BMI may still have metabolic abnormalities, while those with higher BMIs may exhibit better metabolic health than expected [3]. This limitation underscores the need for comprehensive measures that consider factors such as waist circumference, body composition and metabolic biomarkers to ensure accurate assessment and appropriate management strategies for metabolic disorders like diabetes [4].

IR stands as a critical precursor to Type 2 Diabetes (T2D), a condition with increasing prevalence worldwide, particularly pronounced in populations like India, where genetic predispositions and lifestyle factors converge to amplify the risk. This concept underscores the intricate interplay between genetic and environmental factors in metabolic dysregulation, highlighting the need for comprehensive research to elucidate the underlying mechanisms. IR, a key factor in metabolic disorders such as Type 2 Diabetes Mellitus (T2DM), is closely associated with altered body composition parameters, including increased visceral adiposity and reduced muscle mass, even among individuals with a normal BMI [5].

These alterations instigate systemic inflammation and disrupt the balance of adipokines, cytokines and metabolic signalling molecules, exacerbating IR. Changes in body composition, accompanied by alterations in metabolic markers such as Glycated Haemoglobin (HbA1c), Fasting Plasma Glucose (FPG), Triglycerides (TG), Low-Density Lipoprotein Cholesterol (LDL-C), High-Density Lipoprotein Cholesterol (HDL-C) and Total Cholesterol (TC), indicate IR and its associated metabolic disorders, raising the risk of cardiovascular diseases. Through the exploration of genes such as Ring Finger

Protein 138 (*RNF138*), ATP Binding Cassette Subfamily A Member 1 (*ABCA1*) and Oestrogen Related Receptor Gamma-G-Patch Domain Containing (*ESRRG-GPATCH2*), which are crucial in metabolic regulation, this study aims to investigate the genetic pathways underlying IR and its metabolic implications in the Indian population, providing insights into disease susceptibility and potential therapeutic targets [6-8].

RNF138, *ABCA1* and *ESRRG-GPATCH2* have been chosen for investigation in present research due to their recognised involvement in metabolic pathways associated with insulin sensitivity and glucose homeostasis [9]. *RNF138*, a type of E3 ubiquitin ligase, is believed to play a role in several cellular functions, including insulin signalling and glucose metabolism, suggesting its potential involvement in the development of IR [10]. Similarly, *ABCA1*, a key regulator of cellular cholesterol efflux, plays a crucial role in lipid metabolism and has been implicated in insulin sensitivity and glucose uptake, making it a promising gene for exploring the risk of IR [11]. Furthermore, *ESRRG*, a member of the nuclear receptor superfamily, serves as a transcriptional regulator of genes involved in energy metabolism and mitochondrial function, indicating its potential role in modulating insulin sensitivity and glucose utilisation [12].

By focusing on these genes, the study seeks to elucidate the intricate genetic pathways contributing to IR risk among individuals with normal BMI, thereby enhancing our understanding of the genetic architecture of metabolic dysregulation in the Indian population. Leveraging advanced genetic analyses and statistical methodologies, the study aims to identify specific Single-Nucleotide Polymorphisms (SNPs) within *RNF138*, *ABCA1* and *ESRRG-GPATCH2* that are significantly associated with IR, shedding light on their functional significance and potential implications for diabetes risk.

The current study aims to bridge the gap in our understanding of the genetic factors contributing to IR risk among individuals with a normal BMI in the Indian population. By elucidating the genetic underpinnings of IR and its implications for diabetes risk, the study endeavours to inform public health strategies aimed at preventing and managing diabetes in high-risk populations, thereby addressing a critical aspect of the global diabetes epidemic.

The current case-control study aims to achieve the following objectives:

1. **Investigate genetic factors:** To identify and analyse specific SNPs within the *RNF138*, *ABCA1* and *ESRRG-GPATCH2* genes that are significantly associated with IR among individuals with a normal BMI in the Indian population.
2. **Assess metabolic markers:** To examine the relationship between identified genetic variants and key metabolic markers such as HbA1c, FPG, TG, LDL-C, HDL-C and TC.
3. **Public health implications:** To provide insights that can inform public health strategies aimed at preventing and managing diabetes and related metabolic disorders, particularly in high-risk populations with a genetic predisposition to IR.

By achieving these objectives, this study seeks to enhance our understanding of the genetic architecture of IR and its metabolic implications, contributing valuable knowledge for developing targeted interventions and improving health outcomes in the Indian population.

MATERIALS AND METHODS

The case-control study involved 191 Indian participants (90 men and 101 women), aged between 18 to 65 years, recruited from January 2022 to December 2023. The study was conducted in Bengaluru, Karnataka, India. This study was approved under AERC (Answergenomics Ethics Review Committee) Number: 10001/AERC/24, in accordance with the National Ethical Guidelines for Biomedical and Health Research Involving Human Participants, ICMR (2017). The participants were divided into cases and controls based on their HOMA2-IR values [13]. Those with HOMA2-IR >2 were considered cases (57 participants), while those with HOMA2-IR <2 were regarded as controls (134 participants). HOMA2-IR values were determined using the online tool provided by the Medical Science Division of The University of Oxford (www.OCDEM.ox.ac.uk). Demographic information, including self-reported height (in centimeters) and weight (in kilograms), was collected through an electronic registration questionnaire. BMI was computed as weight (in kilograms) divided by height (in meters) squared. Additionally, measurements such as Body Fat Percentage (BFP) and fat mass were obtained.

Inclusion criteria: Study participants were adults aged 18 years with a normal BMI (18.5-24.9 kg/m^2). Participants were classified as cases (HOMA-IR >2) or controls (HOMA-IR <2) based on insulin resistance. All participants provided informed consent for genetic testing and data collection.

Exclusion criteria: Individuals with a history of cancer, cardiovascular or renal failure, mental illness, pregnancy, or lactation were excluded from the study to ensure participant safety and adhere to ethical guidelines.

Study Procedure

Following a 12-hour fast, venous blood samples were drawn from participants to assess various metabolic markers. A total of three vials of venous whole blood were withdrawn from each participant in a single blood draw session. For participants where blood drawing was not feasible, a possible saliva sample was collected for genotyping purposes.

The blood collection and testing were conducted using the following tubes and quantities:

- HbA1c: A 5 mL blood sample was collected in an EDTA vial for the analysis of HbA1c.
- Glucose Fasting: A 5 mL blood sample was collected in a fluoride vial to ensure accurate fasting glucose measurements.
- Total Cholesterol (TC), Serum LDL Cholesterol, TG, Serum HDL Cholesterol and Insulin Fasting: A 5 mL blood sample was collected in a Serum Separator Tube (SST) vial for these serum-based tests.

The assessments were conducted using the Beckman DxC 700 AU for all markers except HbA1c, which was analysed with the Tosoh G-8 and fasting insulin levels were measured by the Beckman UniCel DxI 800, adhering to the protocols provided by the manufacturers. The reference ranges were as follows: FPG (70-100 mg/dL), TC (0-100 mg/dL), TG (0-150 mg/dL), HDL-C (40-60 mg/dL) and LDL-C (0-100 mg/dL) [14-16]. The HOMA2-IR formula, calculated as fasting insulin (mU/L) multiplied by FPG (mmol/L), was used to test for IR [17]. Using a HOMA2-IR cut-off of 2, participants were then classified as either insulin sensitive or resistant.

Genotyping and Single-Nucleotide Polymorphism (SNP) selection: This study aimed to investigate the genetic factors influencing IR by analysing participants' DNA. DNA extraction was conducted using the Qiagen blood extraction kit, known for its efficiency in isolating high-quality genomic DNA from blood specimens. For genotyping, the Illumina Infinium GSA V3 platform was employed, offering extensive coverage of genetic variants across the genome. The genotyping process utilised the Illumina iScan system, ensuring high-throughput and precise scanning of SNP arrays. Data interpretation, quality control and export were facilitated by Genome Studio V2 software [18] ensuring the accurate calling of genotypes and preparation for further statistical analysis [19].

In this study, three genes were focused-*RNF138*, *ABCA1* and *ESRRG*-selected based on a thorough review of existing scientific literature that identified their potential involvement in metabolic pathways and their impact on IR. The SNPs within these genes were chosen for their strong associations with metabolic traits such as insulin sensitivity and glucose homeostasis. This careful selection process aimed to identify genetic variants with significant associations with IR in the Indian population, providing valuable insights into the

genetic architecture of metabolic disorders [Table/Fig-1] shows the description of the three single-nucleotide polymorphisms.

Gene	Chromosome	RSID	Major/minor alleles
RNF138	18:29674992	rs4799327	G/A
ABCA1	9:107651174	rs2275543	T/C
ESRRG- GPATCH2	1:217527024	rs1497828	C/G

[Table/Fig-1]: Description of the three single-nucleotide polymorphisms. *RNF138*- ring finger protein 138, *ABCA1*- ATP Binding Cassette Subfamily A Member 1-, ESRRG- GPATCH2- oestrogen related receptor gamma-G-Patch Domain Containing 2

Genotype data analysis: The study employed PLINK software for quality control of genotype data, which involved filtering out samples with low genotyping rates, excluding SNPs with considerable missing data and removing rare variants due to their limited statistical power [20]. Heterozygosity checks were performed to verify the accuracy of genotype distribution. Following quality control, SNPstats software was utilised to investigate the association between specific SNPs and IR [21]. The analysis produced Odds Ratios (ORs) along with their corresponding 95% Confidence Intervals (CIs). In the control group, adherence to Hardy-Weinberg equilibrium was maintained at a significance level of 0.05 [21]. Calculating OR and the associated 95% CIs was the method used to estimate risk. The Linkage Disequilibrium (LD) between the SNPs was also investigated in the study, using the LD coefficient D, which was determined using the SNPstats software Additionally, power analyses were performed based on power and sample size calculations [22]. Each SNP underwent Bonferroni adjustment to mitigate the risk of type I errors resulting from multiple tests.

STATISTICAL ANALYSIS

The statistical evaluation began by calculating the differences in age, glucose levels and lipid metrics, including TC, LDL-C, HDL-C, TGs and HbA1c, between the two groups. Due to the non parametric nature of the data, the analysis employed the Mann-Whitney U test, utilising the SciPy library. Additionally, the distribution of gender across the groups was assessed using the Chi-square test, with comprehensive results outlined in the table below.

RESULTS

In this study, a total of 191 participants were included and all underwent genotyping. The clinical characteristics, as well as the glucose and lipid metabolic parameters of the subjects, are detailed in [Table/Fig-2]. The analysis conducted on the data did not reveal any significant statistical differences in terms of age or gender between the case and control groups, nor among males and females within those groups (p-value >0.05). However, notable variations were observed in BMI (p-value=0.01), fat mass (p-value=0.007), HbA1c (p-value=0.01), fasting glucose (p-value=0.001) and serum TGs (p-value=0.02) when comparing cases to controls. It is important to note that the p-values for BFP, TC and serum HDL-C are all above the significant levels, as indicated in [Table/Fig-2].

Parameters	Cases median (Q1-Q3)	Controls median (Q1-Q3)	U statistic	p-value
BMI[a]	23.05 (21.89-24.09)	22.235 (20.84-23.61)	4656.5	0.01
BFP[a]	26.11 (19.47-29.01)	23.02 (17.9-27.52)	4409.5	0.09
Fat Mass[a]	15.85 (13.59-17.79)	13.62 (11.78-16.04)	4761.5	0.007
HbA1c[a]	5.4 (5.3-5.5)	5.3 (5.1-5.5)	4695.5	0.01
Insulin fasting[a]	11.02 (9.67-13.88)	5.31 (3.9-6.92)	7143	1.96E-21
Glucose fasting[a]	92.6 (86.9-96.2)	87.05 (82.42-92.27)	4937.5	0.001
Total cholesterol[a]	186.0 (166.0-227.0)	185.0 (161.25-211.0)	4146	0.35
Serum LDL cholestrol[a]	46.5 (42.9-52.5)	49.5 (44.03-54.68)	3383.0	2.128e-01
Serum triglycerides[a]	102.0 (85.0-127.0)	88.0 (70.0-126.0)	4631	0.02
Serum HDL cholesterol[a]	46.5 (42.9-52.5)	49.5 (44.025-54.67)	3383	0.21

[Table/Fig-2]: Clinical characteristics and glucose and lipid metabolic parameters of the subjects enrolled in the present study.
[a]Mann-Whitney test
[b]χ^2 test. Data are presented as the median (Q1-Q3) for age, BMI, Fat Mass, Hba1c, total cholesterol, triglycerides, HDL, LDL, Glucose fasting, insulin fasting. Data are presented as n for gender. p<0.05 was considered to indicate a statistically significant difference. BFP: Body fat percentage; BMI: Body mass index; LDL: Low-density lipoprotein; HDL: High-density lipoprotein BMI (kg/m^2): 18.5-24.9 kg/m^2; Body fat percentage (BFP): Men: 6-24%, Women: 14-31%; HbA1c (%): <5.7% (Normal); Insulin fasting (µU/mL): 2.6-24.9 µU/mL; Glucose fasting (mg/dL): 70-100 mg/dL; Total cholesterol (mg/dL): <200 mg/dL; Serum LDL cholesterol (mg/dL): <100 mg/dL; Serum triglycerides (mg/dL): <150 mg/dL; Serum HDL cholesterol (mg/dL): Men: >40 mg/dL, Women: >50 mg/dL

The allelic and genotypic distribution of the three SNPs is provided in [Table/Fig-3]. All the controls and cases for the SNPs rs4799327, rs2275543 and rs1497828 were in compliance with the Hardy-Weinberg equilibrium (p-value >0.05). This was evaluated through a Fisher's exact test.

Gene	RSID	Risk allele	Best fit model	Statistical significance
RNF138	rs4799327	A	Dominant	(OR)=2.74; 95% (CI): 1.28-5.88; p-value=0.006
ABCA1	rs2275543	C	Log additive	(OR)=3.50; 95% (CI): 1.17-10.42; p-value=0.011
ESRRG-GPATCH2	rs1497828	C	Dominant	(OR)=2.90; 95% (CI): 1.51-5.57; p-value=0.0011

[Table/Fig-3]: The risk alleles for the polymorphisms along with the best fit model and statistical significance.

Chi-square tests were performed to evaluate the association between genotypes and allele frequencies of each SNP with the phenotype (IR). All the SNPs showed significant differences between the cases and controls after the Bonferroni correction, with respective p-values of 0.0063, 0.011 and 0.0013 for the genotypic frequencies (p-value <0.016). Thus, the association between these three polymorphisms and IR is quite significant. The allelic frequency difference between the cases and controls for all the SNPs considered also stands significant (p-value <0.05) [Table/Fig-4].

Chi-squared analyses were performed to investigate the relationship between the genotypic and allelic variations of the three SNPs (rs4799327, rs2275543 and rs1497828) within two groups distinguished by their HOMA2-IR levels (>2 and <2). The obtained p-values indicate significant associations between SNP genotypes and IR status. The rs4799327 variant in the *RNF138* gene showed a significant association with IR under a dominant inheritance model, with a risk allele of A. The Odds Ratio (OR) for this variant was 2.74 (95% CI: 1.28-5.88, p-value=0.006), indicating that individuals with the A allele had a 2.74-fold increased risk of developing IR compared to those without the allele. For the rs2275543 variant in the *ABCA1* gene, the analysis revealed a significant association with IR using a log-additive model. The risk allele C was linked to an OR of 3.50 (95% CI: 1.17-10.42, p-value=0.011), suggesting that individuals carrying the C allele were at a significantly higher risk of developing IR. The rs1497828 variant in the *ESRRG-GPATCH2* gene demonstrated a significant association with IR under the dominant inheritance model, with a risk allele of C. The OR for this variant was 2.90 (95% CI: 1.51-5.57, p-value=0.0011), indicating a strong relationship between the C allele and increased susceptibility to IR [Table/Fig-3].

Model of inheritance analysis: In this study, genetic inheritance models (codominant, dominant, recessive, overdominant and log-additive models) for SNPs rs4799327, rs2275543 and rs1497828 were investigated. [Table/Fig-5-7] provide detailed data on these models, including the evaluation of the lowest Akaike Information

SNP	Group	A1	A2	χ^2	χ^2 p-value	A1A1	A1A2	A2A2	p-value	HWE p-value
rs4799327	Cases	G 102 (0.89)	A 12 (0.11)	5.182	0.02282	A/A 1 (0.02)	G/A 10 (0.18)	G/G 46 (0.81)	0.0063	0.48
	Controls	G 214 (0.8)	A 54 (0.2)			A/A 4 (0.03)	G/A 46 (0.34)	G/G 84 (0.63)		0.59
rs2275543	Cases	T 110 (0.96)	C 4 (0.04)	5.017	0.02509	C/C 0 (0)	T/C 4 (0.07)	T/T 53 (0.93)	0.011	1
	Controls	T 240 (0.9)	C 28 (0.1)			C/C 1 (0.01)	T/C 26 (0.19)	T/T 107 (0.8)		1
rs1497828	Cases	C 27 (0.24)	G 87 (0.76)	9.264	0.002338	C/C 4 (0.07)	G/C 19 (0.33)	G/G 34 (0.6)	0.0013	0.053
	Controls	C 107 (0.4)	G 161 (0.6)			C/C 18 (0.13)	G/C 71 (0.53)	G/G 45 (0.34)		0.73

[Table/Fig-4]: Comparison of genotypic and allelic distribution of three SNPs (rs4799327, rs2275543 and rs1497828) between the two groups HOMA2 IR >2 and HOMA2 IR <2. In the Chi-square association analysis of cases and controls, the four SNPs (rs920590, rs7274134, rs6762208 and rs2078267) exhibited statistically significant associations with the trait after Bonferroni correction, with their respective p-values falling below the adjusted threshold of 0.016

Model	Genotype	Status=Ca	Status=Co	OR (95% CI)	p-value	AIC	BIC
Codominant	G/G	46 (80.7%)	84 (62.7%)	1.00	0.024	238.8	268.1
	G/A	10 (17.5%)	46 (34.3%)	2.80 (1.27-6.17)			
	A/A	1 (1.8%)	4 (3%)	2.21 (0.24-20.66)			
Dominant	G/G	46 (80.7%)	84 (62.7%)	1.00	0.0063	236.8	262.9
	G/A-A/A	11 (19.3%)	50 (37.3%)	2.74 (1.28-5.88)			
Recessive	G/G-G/A	56 (98.2%)	130 (97%)	1.00	0.64	244.1	270.1
	A/A	1 (1.8%)	4 (3%)	1.66 (0.18-15.30)			
Overdominant	G/G-A/A	47 (82.5%)	88 (65.7%)	1.00	0.0084	237.3	263.4
	G/A	10 (17.5%)	46 (34.3%)	2.72 (1.24-5.98)			
Log-additive	---	---	---	2.39 (1.18-4.84)	0.0095	237.6	263.6

[Table/Fig-5]: Inheritance models analysis of the SNP rs4799327 (RNF198) between the cases and controls.
rs4799327 association with response status (n=191, adjusted by gender+age.cat
Significant threshold after Bonferroni correction for multiple comparisons is less than 0.0125; AIC: Akaike information criterion; BIC: Bayesian information criterion; CI: Confidence interval; Ca: Cases; Co: Controls

Model	Genotype	Status=Ca	Status=Co	OR (95% CI)	p-value	AIC	BIC
Codominant	T/T	53 (93%)	107 (79.8%)	1.00	0.036	239.7	268.9
	T/C	4 (7%)	26 (19.4%)	3.39 (1.12-10.30)			
	C/C	0 (0%)	1 (0.8%)	NA (0.00-NA)			
Dominant	T/T	53 (93%)	107 (79.8%)	1.00	0.012	238	264
	T/C-C/C	4 (7%)	27 (20.1%)	3.54 (1.17-10.75)			
Recessive	T/T-T/C	57 (100%)	133 (99.2%)	1.00	0.36	243.4	269.5
	C/C	0 (0%)	1 (0.8%)	NA (0.00-NA)			
Overdominant	T/T-C/C	53 (93%)	108 (80.6%)	1.00	0.018	238.6	264.7
	T/C	4 (7%)	26 (19.4%)	3.35 (1.10-10.16)			
Log-additive	---	---	---	3.50 (1.17-10.42)	0.011	237.8	263.8

[Table/Fig-6]: Inheritance models analysis of the SNP rs2275543 (*ABCA1*) between the cases and controls.
rs2275543 association with response status (n=191, adjusted by gender+age.cat)
Significant threshold after Bonferroni correction for multiple comparisons is less than 0.0125; AIC: Akaike information criterion; BIC: Bayesian information criterion; CI: Confidence interval; Ca: Cases; Co: Controls

Model	Genotype	Status=Ca	Status=Co	OR (95% CI)	p-value	AIC	BIC
Codominant	G/G	34 (59.6%)	45 (33.6%)	1.00	0.0049	235.6	264.9
	G/C	19 (33.3%)	71 (53%)	2.85 (1.43-5.66)			
	C/C	4 (7%)	18 (13.4%)	3.15 (0.96-10.41)			
Dominant	G/G	34 (59.6%)	45 (33.6%)	1.00	0.0011	233.7	259.7
	G/C-C/C	23 (40.4%)	89 (66.4%)	2.90 (1.51-5.57)			
Recessive	G/G-G/C	53 (93%)	116 (86.6%)	1.00	0.25	243	269
	C/C	4 (7%)	18 (13.4%)	1.91 (0.60-6.04)			
Overdominant	G/G-C/C	38 (66.7%)	63 (47%)	1.00	0.01	237.7	263.7
	G/C	19 (33.3%)	71 (53%)	2.33 (1.20-4.50)			
Log-additive	---	---	---	2.21 (1.30-3.76)	0.0023	235	261

[Table/Fig-7]: Inheritance models analysis of the SNP rs1497828 (ESRRG- GPATCH2) between the cases and controls.
rs1497828 association with response status (n=191, adjusted by gender+age.cat)
Significant threshold after Bonferroni correction for multiple comparisons is less than 0.0125; AIC: Akaike information criterion; BIC: Bayesian information criterion; CI: Confidence interval; Ca: Cases; Co: Controls

Criterion (AIC) and Bayesian Information Criterion (BIC) values to determine the best-fit model for each SNP [23].

For SNP rs4799327, located in the *RNF138* gene and rs1497828, located in the intergenic region of *ESRRG-GPATCH2*, the top model identified was dominant, with respective p-values of 0.0063 and 0.0011, accompanied by the lowest AIC and BIC values.

Specifically, for SNP rs4799327, the risk genotype was identified as G/A-A/A compared to G/G, exhibiting a significant association (p-value=0.0063; OR=2.74; 95% CI: 1.28-5.88), as outlined in [Table/Fig-5].

Regarding SNP rs2275543, the log-additive model emerged as the best fit, characterised by the lowest AIC and BIC values. The risk allele for this model was determined to be C (OR=3.50; 95% CI: 1.17-10.42; p-value=0.011), as depicted in [Table/Fig-6].

Finally, for SNP rs1497828, the risk genotype was identified as G/C-C/C in contrast to G/G, displaying a significant association (p-value=0.0011; OR=2.90; 95% CI: 1.51-5.57), as shown in [Table/Fig-7]. These findings provide valuable insights into the genetic basis of IR, shedding light on the specific polymorphisms associated with increased susceptibility to this metabolic condition.

DISCUSSION

Understanding the genetic underpinnings of IR and its association with metabolic disorders is crucial for advancing our knowledge of disease mechanisms and developing targeted interventions. In this discussion, we delve into the genetic factors identified in present study-specifically, the roles of *RNF138*, *ABCA1* and *ESRRG-GPATCH2* polymorphisms in influencing insulin sensitivity and glucose homeostasis. By elucidating the functional significance of these genetic variants and their implications for metabolic health, present study aim to contribute to a broader understanding of IR pathogenesis and its potential therapeutic interventions.

Genetic inheritance analysis involves assessing how genetic variants contribute to the inheritance patterns of certain traits or diseases within populations. Different genetic inheritance models are evaluated to understand the relationship between genotypes and phenotypes. These models include codominant, dominant, recessive, overdominant and log-additive models. The choice of the best-fit model is determined based on statistical criteria such as the Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) values, with lower values indicating a better fit.

RNF138 belongs to the RING Finger (RNF) family and it is specifically classified within the UIM subfamily [24]. RING finger proteins contain the RING domain, which serves as an E3 ubiquitin ligase that is important for carrying out a highly specific post-translational modification known as ubiquitination [25]. E3 ubiquitin ligase is known to contribute to IR due to its proteolytic and degradation effects on the insulin receptor and insulin receptor substrates, as reported by Bai T et al., [26]. Yang XD et al., propose two methods by which E3 ubiquitin ligase affects IR and diabetes: one method involves the direct degradation of insulin receptor substrates, insulin receptors and other important insulin signalling molecules by E3 ubiquitin ligases via the ubiquitin-proteasome system; the second method involves E3 indirectly regulating insulin signalling through the modulation of pro-inflammatory mediators such as TNF-α [6].

The E3 ubiquitin ligase activity mediated by the RING domain is essential for the covalent attachment of ubiquitin to target proteins, directing them for proteasomal degradation. Dysregulation of RNF proteins, including *RNF138*, has been implicated in various diseases, highlighting their importance in maintaining cellular homeostasis. In the context of metabolic disorders, RNF proteins may influence key pathways related to insulin signalling, thus potentially contributing to the development of IR [27].

In a murine knockout model study by Bhagwandin C et al., it was concluded that MARCH1, which is a member of the RING finger family, exhibits a negative regulatory effect on insulin receptors through ubiquitination, which ultimately affects insulin sensitivity [25]. Hu X et al., found that *RNF186* increased hepatic Triglyceride (TG) buildup, dysregulated insulin sensitivity and could increase hepatic inflammation when subjected to a high-fat diet, while Lee JH et al., discovered that *RNF20* regulates liver TG synthesis [28,29]. According to research by Sarkar P and Thirumurugan K, *RNF213* is linked to adipogenesis and insulin control, connecting TNF-mediated pathways to obesity [30]. Present research shows a strong correlation between IR and rs4799327 in the *RNF138* gene according to the dominant model of inheritance (OR=2.74; 95% CI: 1.28-5.88; p-value=0.006).

The ATP-binding cassette transporter (*ABCA1*) is essential for the transfer of cellular cholesterol and phospholipids to lipid-poor apolipoproteins, forming precursor HDL particles and aiding in lipid metabolism. The expression of *ABCA1* in adipose tissue and its relationship to IR in obesity have been studied [31]. Their findings showed that obese individuals had significantly reduced levels of *ABCA1* expression in their visceral adipose tissue. Reduced *ABCA1* expression was observed in insulin-resistant individuals and this was independently linked to increased insulin sensitivity.

The study by Zaidi A et al., focuses on the expression analysis of the *ABCA1* gene in type 2 diabetic patients in Pakistan, both with and without dyslipidaemia and its correlation with the glycaemic index and lipid profile. The researchers found that *ABCA1* expression was significantly downregulated in patients with diabetic dyslipidaemia compared to those with diabetes alone and healthy controls. This downregulation was negatively correlated with fasting blood sugar and positively correlated with HbA1c and lipid profile in diabetic patients, suggesting that *ABCA1* plays a critical role in managing cholesterol levels and glycaemic control in Type 2 Diabetes (T2D). The study emphasises the potential of *ABCA1* as a therapeutic target for managing diabetic dyslipidaemia [32].

The review by Babashamsi MM et al., highlights the multifaceted role of *ABCA1* in MetS, emphasising its involvement in HDL and VLDL production, insulin-glucose homeostasis, inflammation suppression and obesity regulation [33]. It elucidates how *ABCA1* mediates key steps in HDL biogenesis, impacts VLDL production in the liver and influences insulin secretion and sensitivity in pancreatic β-cells, adipocytes and skeletal muscle cells. Furthermore, it underscores the association between abnormal *ABCA1*-regulated phenotypes and the development of MetS, offering insights into potential therapeutic strategies targeting *ABCA1* to mitigate MetS-related complications.

Present study complements these findings by revealing a significant association between the SNP rs2275543 in the *ABCA1* gene and IR. The identification of the risk allele C (OR=3.50; 95% CI: 1.17-10.42; p-value=0.011) further accentuates *ABCA1*'s role in metabolic disorders, aligning with the review's emphasis on *ABCA1*'s involvement in insulin-glucose homeostasis and underscoring its potential as a therapeutic target for addressing MetS-related complications.

de Haan W et al., examined how the absence of *ABCA1* specifically in adipocytes affects lipid metabolism and glucose regulation using *ABCA1*-deficient mice [34]. Their research showed that without *ABCA1*, there was a buildup of cholesterol and TGs in adipose tissue, leading to larger fat deposits and increased body weight when fed a high-fat and high-cholesterol diet. Additionally, *ABCA1*-deficient mice displayed impaired glucose tolerance, reduced sensitivity to insulin and lower insulin production. These results emphasise the importance of *ABCA1* in controlling lipid levels and glucose metabolism, suggesting that it could be a promising target for treating metabolic disorders. Present study reveals a significant association between IR and the *ABCA1* gene's SNP rs2275543.

The risk allele C is linked to increased IR (OR=3.50; 95% CI: 1.17-10.42; p-value=0.011), shedding light on its potential role in metabolic disorders such as T2DM.

The ESRRG is part of the orphan nuclear hormone receptor family, which includes steroid hormone receptors. It functions as a continuous transcription activator [35]. This group of receptors is involved in various regulatory functions, managing both homeostatic and metabolic activities [36]. ESRRG is implicated in the pathophysiology related to both the pancreas and the liver. In the pancreas, ESRRG regulates pancreatic β-cell function and insulin secretion, affecting glucose homeostasis. In the liver, ESRRG influences hepatic glucose production and lipid metabolism, contributing to insulin sensitivity and glucose utilisation. Dysregulation of ESRRG signalling pathways can disrupt insulin action and exacerbate IR, thereby playing a role in the development and progression of metabolic disorders like T2DM.

GPATCH2, which stands for G-Patch Domain Containing 2, is a gene that encodes a protein known to be involved in RNA processing, an essential step in the production of proteins from genes. While GPATCH2 is not directly implicated in the typical pathways associated with insulin signalling or glucose metabolism, its role in cellular processes may indirectly influence factors related to insulin resistance or the development of diabetes [37].

The association between the ESRRG rs1890552 A>G polymorphism and urine 8-epi-PGF2α levels was investigated in a study by Kim M et al., involving 1,933 Korean individuals to determine whether it was related to Impaired Fasting Glucose (IFG) or the onset of T2D [38]. The results showed that, relative to the control group, those with IFG or T2D had higher plasma Malondialdehyde (MDA), urinary 8-epi-PGF2α and brachial-ankle pulse wave velocity (baPWV). Furthermore, there was a significant increase in the frequency of the ESRRG rs1890552 GG genotype in individuals with T2D or IFG, suggesting that this genotype may contribute to an increased vulnerability to these metabolic disorders. Our study identified a significant association between SNP rs1497828 and T2D risk, with the G/C-C/C genotype showing a higher risk compared to G/G genotype carriers (p-value=0.0011; OR=2.90; 95% CI: 1.51-5.57). This emphasises the importance of genetic variations in predisposing individuals to T2D and the utility of genetic markers in identifying those at risk for metabolic disorders.

Present study identified genetic polymorphisms, specifically rs4799327, rs2275543 and rs1497828, associated with IR in individuals with a normal BMI in the Indian population, which has significant public health implications. This research highlights the potential of incorporating genetic screening into routine healthcare assessments to identify individuals at higher risk of IR and T2D, especially where traditional factors like BMI may not be reliable indicators. While genetic screening offers a promising solution for early detection, its widespread implementation could face challenges, such as costs and accessibility, particularly in low-resource settings.

Early identification of these genetic predispositions enables targeted interventions, such as personalised lifestyle modifications or clinical treatments, which could help delay or prevent the onset of IR and its progression to T2D. This approach holds promise for improving outcomes and reducing the burden of diabetes in high-risk populations. However, integrating genetic screening into standard healthcare would require careful consideration of ethical, logistical and societal factors to ensure equitable access and appropriate use of the information.

The rationale behind selecting *RNF138*, *ABCA1* and *ESRRG-GPATCH2* lies in their well-established roles in metabolic pathways implicated in insulin sensitivity and glucose homeostasis, making them compelling genes for genetic studies focused on IR risk in the Indian population. Furthermore, understanding the genetic determinants of IR holds significant implications for public health interventions and personalised medicine approaches, paving the way for targeted therapeutic interventions aimed at mitigating the burden of diabetes in high-risk populations.

Limitation(s)

This study provides important preliminary insights into genetic markers associated with IR in the Indian population; however, it has some limitations. The small sample size and focus on a specific population may limit the generalisability of the findings. The case-control design and reliance on self-reported data introduce potential biases and limit the ability to infer causality. Additionally, the study analysed only a limited number of SNPs and lacked detailed phenotypic data, such as lifestyle habits, physical activity levels and inflammatory markers, which would have provided a more comprehensive understanding of how these factors interact with genetic predispositions to influence IR. Despite these constraints, the findings offer a valuable foundation for future research, which should involve larger, more diverse populations and a broader range of genetic and phenotypic data to confirm and expand on these results. Moreover, while present study identifies significant genetic associations with IR, it does not include functional analyses to explore the underlying biological mechanisms. Future research should focus on in-depth functional studies to better understand how these genetic variations contribute to IR and metabolic disorders.

CONCLUSION(S)

In conclusion, present study indicates that *RNF138, ABCA1* and *ESRRG-GPATCH2* play significant roles in the genetic predisposition to IR within the Indian population. Through a meticulous examination of relevant SNPs and a comprehensive analysis of metabolic markers, present stidy identified notable associations between genetic variations and IR status. Specifically, the findings reveal compelling links between certain genotypes and an increased risk of IR, shedding light on the intricate genetic architecture underlying metabolic dysfunction. Moreover, present study underscores the importance of further research to validate and extend these findings in larger, prospective longitudinal cohorts. By deepening the understanding of the genetic determinants of IR, present study lays a crucial foundation for future investigations aimed at developing targeted interventions and personalised therapeutic approaches to mitigate the burden of diabetes and related metabolic disorders in high-risk populations.

Authors' contribution: ST: Spearheaded the research efforts, providing invaluable expertise in genetic analysis and study design. SF: Contributed to data analysis, interpretation and drafting of manuscript. NK: Statistically analysed the genetic data and majorly drafted and reviewed the manuscript. JAKG: Analysed large scale genetic dataset, providing genetic associations and complex genetic patterns. KS: Contributed to the editing and proofreading of the script. VB: Contributed his clinical insights by offering valuable perspectives. BAR and RR: Conceptualised the study idea and provided the platform to carry out the research.

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