RESEARCH

Association of leptin–melanocortin gene polymorphisms with the risk of obesity in northwest Indian population

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Abstract

Background Obesity, a multifaceted endocrine issue, is adversely affecting all age groups and is posing a significant public health challenge. The genetic polymorphisms of the melanocortin 4 receptor (*MC4R*) and leptin (*LEP*) genes likely contribute to the development of obesity. The present study aimed to explore the effects of *MC4R* and *LEP* gene polymorphisms on obesity among the northwest Indian population.

Methods The present study was conducted among 333 obese cases and 338 non-obese controls (aged 18–50 years). All subjects underwent measurements for anthropometric, physiometric, as well as biochemical parameters. Genotyping for *MC4R* and *LEP* gene variants was performed using the polymerase chain reaction (PCR)-based restriction fragment length polymorphism (RFLP) method. However, 10% of the samples for each variant were confirmed using the Sanger sequencing method.

Results The polymorphisms of leptin–melanocortin pathway genes (*MC4R-LEP*) were found to be significantly associated with various obesity-related parameters like waist circumference: $p = 0.017$, waist-to-height ratio: $p = 0.009$, total cholesterol: $p = 0.0001$ and triglycerides: $p = 0.0001$. Both the *LEP* gene variants rs2167270 and rs7799039 conferred 2.4- and 2.2-fold risk toward obesity under the recessive genetic model [OR (95% CI) 2.42 (1.44–4.07), $p = 0.001$; OR (95% CI) 2.26 (1.41–3.60), $p = 0.0001$, respectively]. All four polymorphisms of the *MC4R* and *LEP* genes demonstrated a strong interaction of 82.1% with the lifestyle factor ($p = 0.001$). The haplotype combinations A–A for rs571312 and rs12970134 conferred twofold risk [OR (95% CI) 2.61 (1.10–6.20), $p = 0.028$]. However, the combination A–G for rs2167270 and rs7799039 predicted sixfold risk [OR (95% CI) 6.02 (3.39–10.68), $p = 0.0001$] toward the obesity development in this population.

Conclusion Our study revealed a connection between *MC4R* (rs571312, rs12970134) and *LEP* (rs2167270, rs7799039) gene variants with obesity, highlighting their prominent role in assessing the risk of obesity among the northwest Indian population.

Keywords Melanocortin-4 receptor, Leptin, Gene polymorphism, Obesity, Environment gene interaction

Introduction

Obesity is a complex endocrine disorder characterized by excessive increase in body weight due to abnormal fat deposition in the adipose tissue, afflicting individuals of all ages and thereby decreasing their life expectancy. It exerts a detrimental impact on practically all physiological systems of the body and is a substantial public health problem [1]. Although exposed to the widespread

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obesogenic environmental factors, not everyone becomes obese, implying a significant role of the genetic component in the etiology of obesity. Studies have found that BMI heritability is high, spanning from 40 to 70% [2, 3]. The rising incidence of obesity presents a global health problem, as excess weight increases the risk of various diseases, especially chronic diseases like type 2 diabetes, cardiovascular diseases and cancer [4]. Numerous factors, including lifestyle choices, dietary habits, and socioeconomic status, significantly influence the development of obesity. Recent studies conducted by the World Health Organization (WHO) propose that adults up to the age of 64 can sustain a healthy lifestyle by participating in moderate aerobic physical activity for 150–300 min or vigorous activity for 75–150 min daily [5]. Research also indicates that non-vegetarians are more prone to obesity than vegetarians [6]. It has been forecasted that by 2040, a significantly larger segment of the rural as well as urban population will be overweight or obese [7].

The genome-wide association studies (GWAS) have recently revealed that *MC4R* and *LEP* are the two important genes involved in the pathophysiology of common obesity. The melanocortin 4 receptor (*MC4R*) belongs to the G-protein-coupled receptor (GPCR) family found throughout the brain. Its major role is to regulate food intake, maintain energy homeostasis and control body weight [8, 9]. Deficiency in melanocortin receptors can lead to hyperphagia, elevated body fat and hyperinsulinemia (increase in insulin secretion). *MC4R* is an intronless gene that encodes a 332-amino acid transmembrane protein. It is present on chromosome 18q21.3 and is predominantly expressed in the hypothalamus, whereas leptin is one of the major adipokines, and its primary physiological job is to reduce body fat by lowering food intake and boosting energy expenditure. Leptin functions as a satiety hormone that restricts orexigenic peptides, such as neuropeptide Y and agouti-related protein, which increase hunger, while enhancing anorexigenic peptides, such as alpha-melanocyte stimulating hormone and corticotrophin-releasing hormone, which suppress feeding. Leptin is a 16-kDa hormone with 167 amino acids. It exhibits a globular protein tertiary structure and is located on chromosome 7q32.1 [10].

Several investigators have discovered that polymorphisms near the *MC4R* and *LEP* genes are linked to quantitative factors that determine metabolic diseases, as well as alterations in anthropometric parameters and feeding behavior [11]. Several previous investigations involving various ethnic populations (Portuguese, Asian, Czech, Malaysian, Tatar, Chinese and Iranian) found polymorphisms in *MC4R* (rs571312C/A and rs12970134G/A) to be associated with obesity, proving the connection of *MC4R* variants with obesity [12–19]. Similarly, many investigators have found both positive as well as contradictory results regarding *LEP* (rs2167270A/G and rs7799039G/A) gene polymorphisms among different populations [20–25]. Thus, it could be predicted that variants near the *MC4R* and *LEP* gene may influence the etiology and progression of obesity through direct impacts on anthropometric parameters, lipid profile, cardiovascular activities, metabolic profile of adipose tissue as well as body fat management.

In India, foreign population migration through Punjab has significantly shaped the genetic composition, fostering a distinct gene pool within the region. As a wealthy northwestern state, Punjab grapples with rising obesity rates attributed to high-calorie diets and sedentary lifestyles. Genetics also plays a significant role in the development of complex conditions such as obesity. Single-nucleotide polymorphisms in the leptin–melanocortin pathway genes, *MC4R* and *LEP*, are associated with obesity. Inspired by this, we conducted a case–control study in northwest India, genotyping *MC4R* (rs571312C/A and rs12970134G/A) and *LEP* (rs2167270A/G and rs7799039G/A) variants to explore their link to obesity risk. This study marks the first investigation of these gene variants concerning obesity in this population. Identifying risk alleles could guide targeted therapies, alleviating the societal and economic burdens of obesity while improving quality of life.

Materials and methods
Study subjects
The present study enrolled a total of 671 participants among which 333 were obese and 338 were healthy controls. All study participants were adults recruited from various districts within the Punjab state. Obesity classification was established according to BMI criteria specific to the Asian and South Asian populations [26]. The inclusion criteria for obese participants were adults aged 18–50 years with a body mass index (BMI) exceeding 25 kg/m^2 (mean age: 39.65 ± 10.25 years, mean BMI: 30.37 ± 2.94 kg/m^2). Controls consisted of healthy individuals with a BMI ranging from 18.5 to 22.9 kg/m^2 (mean age: 37.46 ± 11.56 years, mean BMI: 21.42 ± 1.22 kg/m^2). Exclusion criteria encompassed pregnant and lactating women, individuals with significant muscle mass, diabetes, kidney disease, and other cardiovascular conditions. To mitigate selection bias in this present case–control study, controls were gathered randomly from the identical population, matching ages in a 1:1 ratio, thereby enhancing the study's statistical power. The procedures for our case–control study were reviewed and approved by the Guru Nanak Dev University's Ethics Committee (ethical code-1609/HG) in Amritsar, Punjab.

All participants were asked about their background, lifestyle and risk factors. Anthropometric as well as physiometric parameters such as weight, height, hip circumference (HC), waist circumference (WC), skinfold measurements, systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured for all the study subjects. Each participant was asked to sign a written informed consent form. Individuals' height and weight were measured to the nearest 0.5 cm and 0.1 kg, respectively, while wearing lightweight clothes with no shoes. Standard anthropometric techniques were used to measure HC and WC to the closest 0.1 cm with the help of Steel tape (Markson measuring tape). The skinfold measurements were taken with the help of a Lange calliper. The waist-to-hip ratio (WHR) was computed by dividing the waist circumference (cm) by the hip circumference (cm), whereas Steel tape (Markson the waist-to-height ratio (WHtR) was computed by dividing the waist circumference (cm) by the height (m) of the subject. The body mass index (BMI) was obtained by dividing the weight in kilograms by the square of the height in meters. The mercury sphygmomanometer was used to measure systolic and diastolic blood pressure (SBP and DBP) following a 5-min reset period. A sedentary lifestyle was defined as spending n minutes per day on leisure activities ($n = 30$ min per day for males and 25 min per day for women) [27]. Dietary patterns were examined, distinguishing between those who consumed vegetarian and non-vegetarian diets.

Biochemical analysis

To measure the lipid profile, 5 ml of venous blood was obtained after an overnight fast and serum was separated. Various lipid parameters such as triglycerides (TG), total cholesterol (TC) and high-density lipoproteins (HDL-C) were measured using Erba-Mannheim kits (Trans Sasia Bio-medicals Ltd., Solan, India). The Friedewald formula was used to compute low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) [28].

Genotyping

The organic approach (Phenol chloroform isoamyl; PCA method) was used to extract the genomic DNA from blood leukocyte pellets. The DNA obtained was quantified and diluted to 50 ng of working concentration before being stored at -20 °C until genotyping. The genotyping of *MC4R* polymorphisms (rs571312C/A and rs12970134G/A) and *LEP* polymorphisms (rs2167270A/G and rs7799039G/A) was done by polymerase chain reaction-based restriction fragment length polymorphism method (PCR–RFLP). The annealing temperature and the sequence of primers are given in Table 1.

The PCR results were validated by running them over a 1.5% agarose gel stained with ethidium bromide (EtBR). The PCR products were then subsequently digested with restriction enzymes. For variant rs571312C/A, enzyme *Hpy166II* was used to digest the PCR products of 210 bp, whereas, for rs12970134G/A, enzyme *DdeI* was used to digest PCR products of 124 bp. For variants rs2167270A/G and rs7799039, enzymes *HPYCH4III* and *HhaI* were used

Table 1 List of primers and restriction enzymes used for genotyping of *MC4R* (rs571312, rs12970134); *LEP* (rs2167270, rs7799039)

| SNP	Major allele	Minor allele	Primer sequence	Annealing temperature (°C)	PCR product (bp)	Restriction enzyme	Fragment length (bp)
rs571312	C	A	F:5'-TTTACACAAACATCGGGTCA-3'	57	210	*Hpy166II*	C:210
			R: 5'-TGAATGTATTGTGTGCCACTGA-3'				A:185,25
rs12970134	G	A	F: 5'-GACTCTTACCAAACAAAGCCTG-3'	61	124	*DdeI*	G:104/20
			R: 5'-TGCTAGGTTGGTCCTGGTTG-3'				A:124
rs2167270	A	G	F: 5'-CAGATTAAGGTGATGGGTTG-3'	58–62	386	*HPYCH4III*	A:296/90
			R: 5'-CTCTGGAGGGACATCAAGA-3'				G:386
rs7799039	G	A	F: 5'-GCTTTCTAAGCCAAGGCAA-3'	59–62	500	*HhaI*	G:320/180
			R: 5'-GCTCTTTTTCAAGGTGCACG-3'				A:500

to digest PCR products of 386 bp and 500 bp, respectively. The digestion was done at 37 °C for 12–14 h. The digested fragments mixture was loaded onto a 2.5% agarose gel stained with 10% ethidium bromide (EtBR). In addition, Sanger Sequencing was performed to corroborate the genetic pattern in 10% of all samples for *MC4R* (rs571312 and rs12970134) and *LEP* (rs2167270 and rs7799039) polymorphisms (Figs. 1, 2, 3, and 4).

Polymorphisms selection

The single-nucleotide polymorphisms near the *MC4R* (rs571312C/A, rs12970134G/A) and *LEP* (rs2167270A/G, rs7799039G/A) gene are associated with obesity in many previous studies among different ethnic populations, thereby suggesting a strong impact of *MC4R* and *LEP* gene on obesity [14, 29–36]. However, on the contrary, some studies showed no link between these polymorphisms and obesity [22, 37–41].
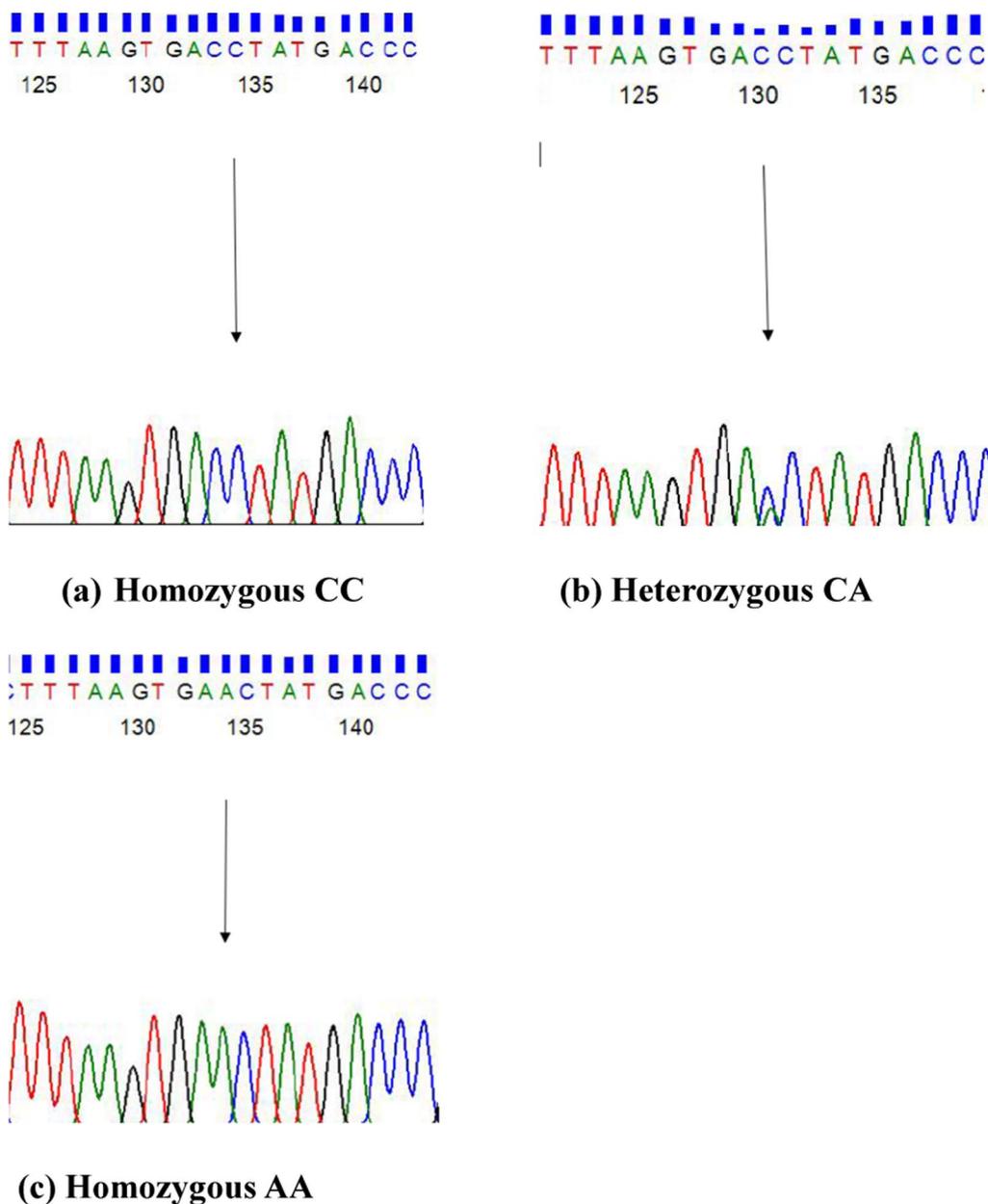
Fig. 1 Sequencing chromatograms showing the genotypes for variant rs571312 (**a**) homozygous wild genotype (**b**) heterozygous genotype (**c**) homozygous mutant genotype
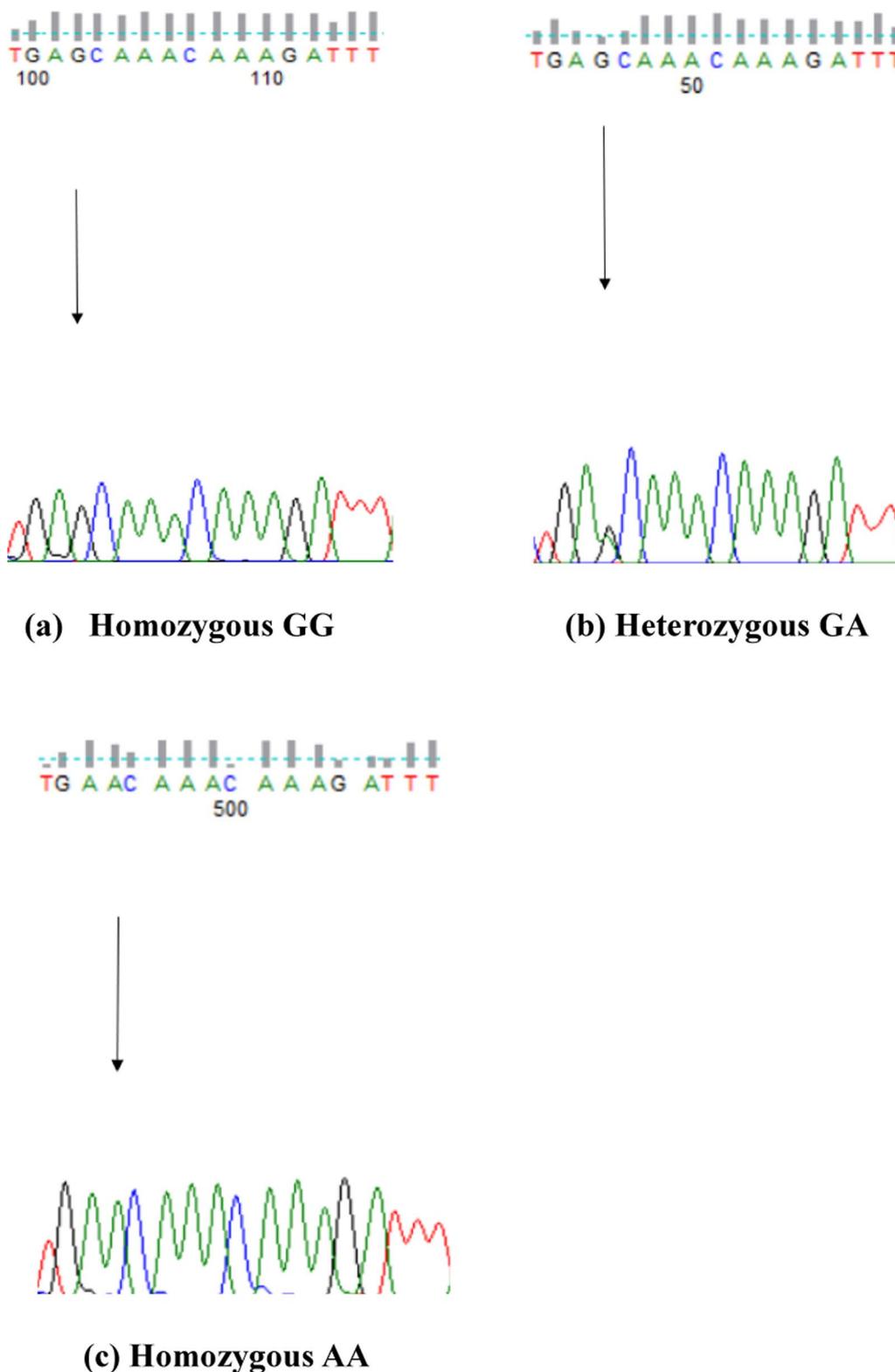
Fig. 2 Sequencing chromatograms showing the genotypes for variant rs12970134 (**a**) homozygous wild genotype (**b**) heterozygous genotype (**c**) homozygous mutant genotype
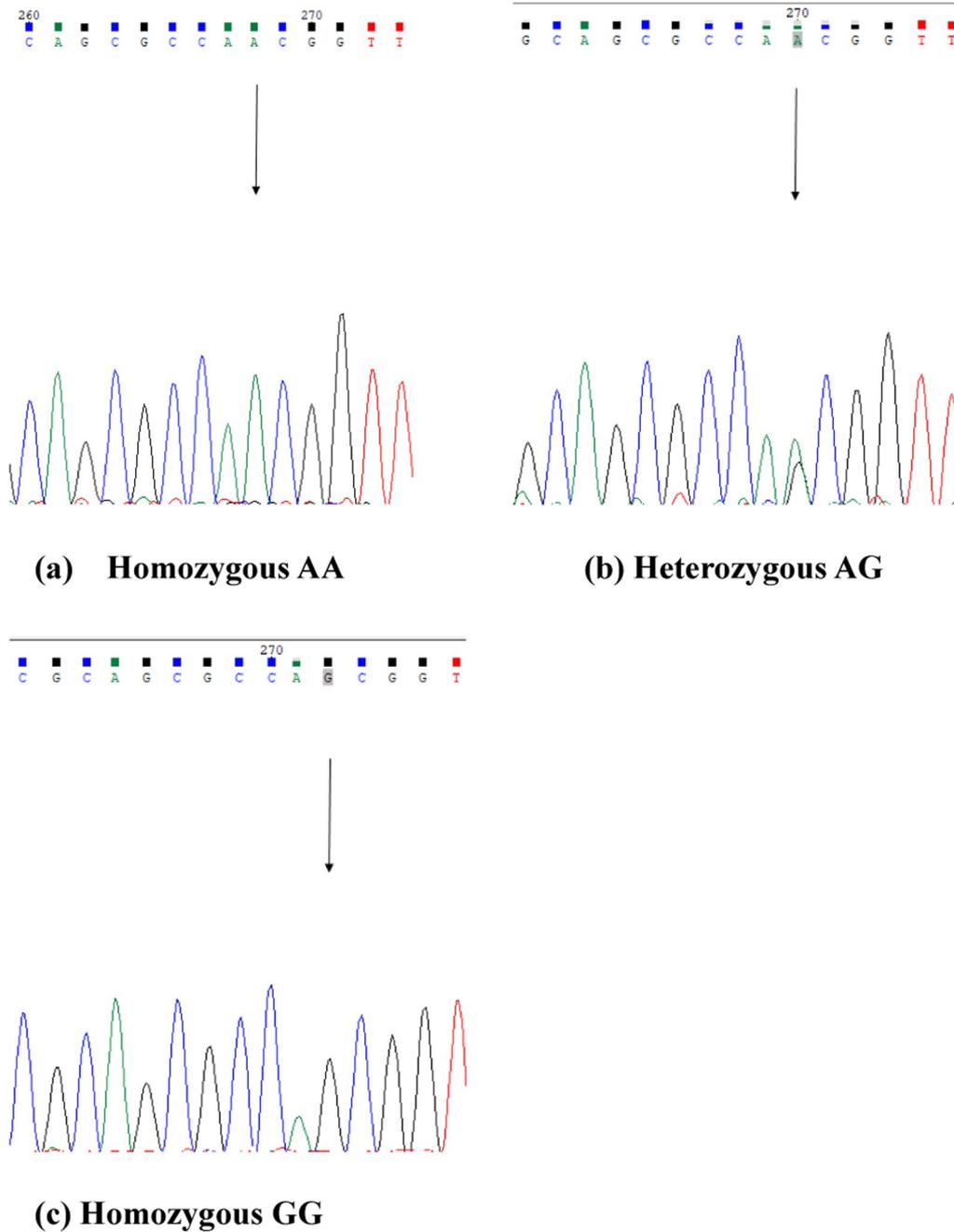
Fig. 3 Sequencing chromatograms showing the genotypes for variant rs2167270 (**a**) homozygous wild genotype (**b**) heterozygous genotype (**c**) homozygous mutant genotype

Due to discrepancies in earlier research findings across various populations, the current study selected four polymorphisms of the *MC4R* and *LEP* genes to investigate their association with obesity.

Statistical analysis

The statistical program for social sciences (SPSS) for Windows v. 21.0 was used to analyze the data. The Student's *t*-test was used to compare continuous variables,
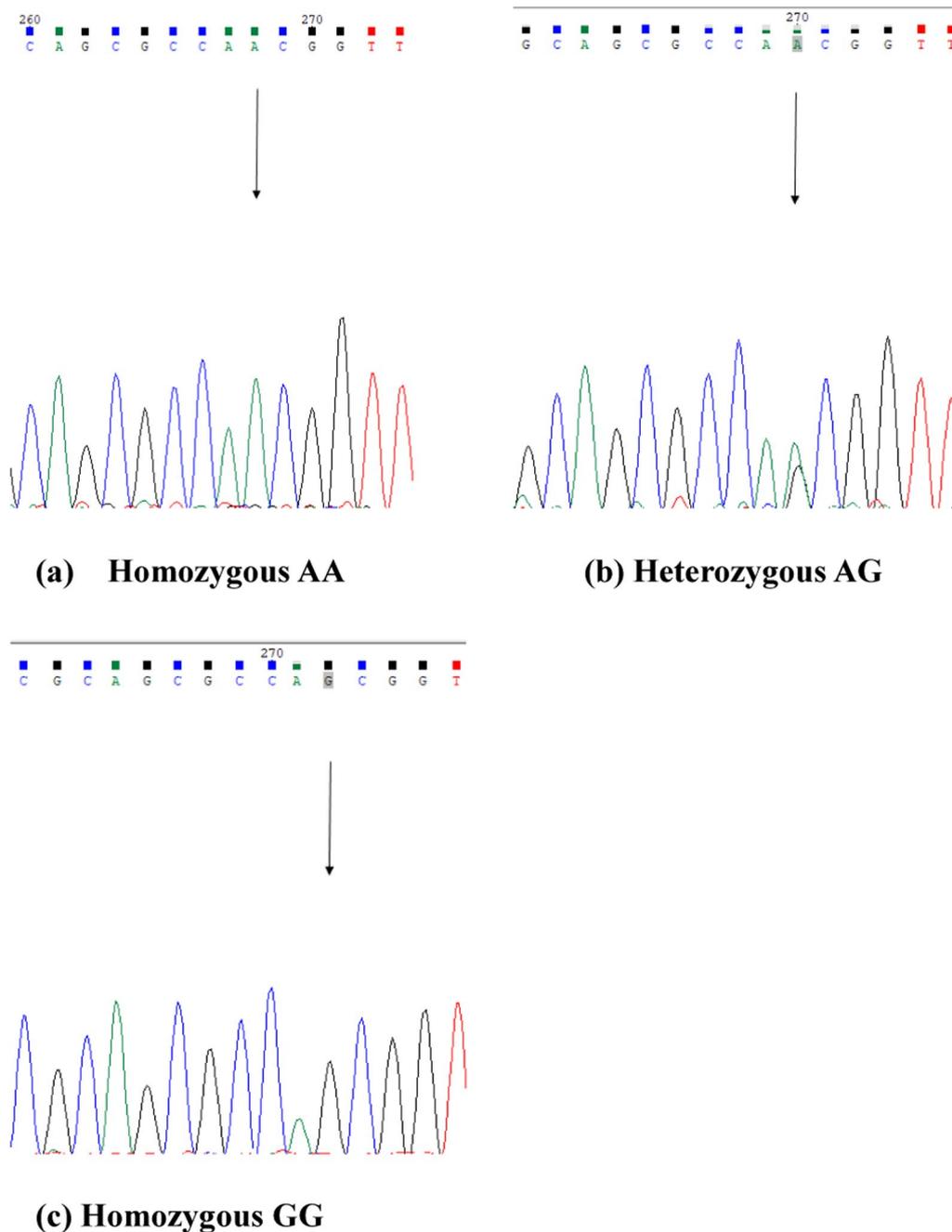
Fig. 4 Sequencing chromatograms showing the genotypes for variant rs7799039 (**a**) homozygous wild genotype (**b**) heterozygous genotype (**c**) homozygous mutant genotype

and one-way analysis of variance (ANOVA) was used to analyze quantitative data from two groups. The chi-square test (χ^2) assessed the allelic and genotypic frequencies between obese cases and nonobese controls. After controlling for numerous confounding factors, logistic regression was used to examine the gene-environment interaction and the association of analyzed variations under assumed genetic models. The interaction between genetic and environmental factors by using software Multifactor dimensionality reduction MDR v 3.0.2.

A linear regression analysis was used to investigate the effect of *MC4R* and *LEP* gene variations on obesity-defining features. The genotypes were used to create a

linkage disequilibrium (LD) map and haplotype within polymorphisms of the *MC4R* and *LEP* gene using Haploview software. As a measure of the connection between the SNPs and the occurrence of obesity, haplotype frequencies, odds ratios (ORs), two-tailed *p* values, and 95% confidence intervals (CIs) were determined. A two-tailed *p* value of 0.05 was deemed statistically significant.

Results

Anthropometric, demographic and clinical characteristics in obese versus non-obese subjects

Table 2 summarizes the data for several anthropometric, clinical and biochemical variables within the obese cases and non-obese healthy controls. A statistically significant difference was observed between the obese and non-obese groups. As compared to non-obese subjects, the obese subjects had higher means of weight, BMI, waist circumference (WC), hip circumference (HC), arm circumference (AC), calf circumference (CC), waist-to-hip ratio (WHR), waist-to-height ratio (WHtR), triceps skinfold (T-skf), biceps skinfold (B-skf), sub-scapular skinfold (sub-skf), supra-iliac skinfold (sup-skf), sum of skinfolds (sum-skf) ($p < 0.0001$). The physiometric parameters were also found to be higher in obese individuals than non-obese [systolic blood pressure (SBP): $p = 0.0001$, diastolic blood pressure (DBP): $p = 0.045$, mean arterial blood pressure (MBP): $p = 0.028$, pulse pressure (PP): $p = 0.002$, pulse rate (PR): $p = 0.042$]. Among lipid variables except for HDL-C levels, the rest of the variables such as TC, TG and LDL were higher in obese subjects. However, there was no significant variation in the frequencies of height and VLDL-C between obese cases and non-obese controls.

Association of *MC4R* and *LEP* polymorphisms with obesity under different genetic inheritance model

The genotypic and allele frequency distribution of *MC4R* rs571312, rs12970134 and *LEP* rs2167270, rs7799039 polymorphisms in obese cases and non-obese controls is presented in Table 3. To investigate the association between *MC4R* and *LEP* gene variants with obesity risk, the data were subjected to logistic regression analysis using several genetic models. For both polymorphisms of *MC4R* as well as the *LEP* gene, the differences observed in overall genotype frequencies were statistically significant ($p = 0.024$ for rs571312 and $p = 0.011$ for rs12970134 variants; $p = 0.002$ for both rs2167270 and rs7799039 variants). In both the variants of the *MC4R* gene, the homozygous recessive AA genotype was more prevalent among obese subjects (18.92%; $p = 0.007$ in rs571312 and 18.31%; $p = 0.011$ in rs12970134). The prevalence of wildtype C-allele (rs571312) and G-allele (rs12970134) was higher in the control group than in the cases (66.57% and 65.24%, respectively). There was a significant association of polymorphism rs571312 with the risk of obesity in both dominant and recessive genetic models [OR (95% CI) 1.38 (1.01–1.87); $p = 0.042$]; [OR (95% CI) 1.69 (1.10–2.59), $p = 0.015$], respectively. However, the rs12970134 variant showed a significant association with obesity under the recessive genetic model [OR (95% CI) 1.94 (1.24–3.03); $p = 0.003$].

Among the two *LEP* variants, the homozygous recessive GG (rs2167270) and AA (rs7799039) genotypes were higher in obese cases (15% and 18%, respectively) as shown in Table 3. Both the polymorphisms showed significant association with obesity risk under the recessive model. Polymorphism rs7799039 conferred twofold higher risk toward the development of obesity

Table 2 Anthropometric, clinical and biochemical characteristics of obese patients and non-obese healthy controls

Variables	Cases (*n* = 333)	Controls (*n* = 338)	*p* value
Height (cm)	161.35 ± 9.64	161.63 ± 8.34	0.787
Weight (kg)	79.16 ± 10.87	56.09 ± 6.45	**0.0001**
BMI (kg/m²)	30.37 ± 2.94	21.42 ± 1.22	**0.0001**
WC (cm)	101.94 ± 16.19	81.37 ± 9.41	**0.0001**
HC (cm)	112.97 ± 17.15	92.38 ± 9.49	**0.0001**
AC (cm)	30.09 ± 4.23	25.52 ± 3.50	**0.0001**
CC (cm)	35.15 ± 4.49	30.38 ± 3.34	**0.0001**
WHR	0.89 ± 0.09	0.86 ± 0.07	**0.001**
WHtR	0.63 ± 0.09	0.49 ± 0.05	**0.0001**
T-skf (mm)	20.11 ± 5.18	16.74 ± 5.41	**0.0001**
B-skf (mm)	16.35 ± 5.97	10.82 ± 4.49	**0.0001**
Sub-skf (mm)	25.08 ± 7.27	18.80 ± 7.30	**0.0001**
Sup-skf (mm)	23.17 ± 6.00	17.06 ± 6.42	**0.0001**
Sum-skf (mm)	84.73 ± 17.99	63.43 ± 15.60	**0.0001**
SBP (mmHg)	124.86 ± 16.45	17.76 ± 15.56	**0.0001**
DBP (mmHg)	81.37 ± 12.65	78.68 ± 9.73	**0.045**
MBP (mmHg)	96.49 ± 13.87	93.08 ± 14.33	**0.028**
PP (mmHg)	44.46 ± 14.27	39.71 ± 13.18	**0.002**
PR (counts/min)	77.91 ± 9.44	80.12 ± 11.00	**0.042**
TC (mg/dl)	186.73 ± 42.30	167.51 ± 39.79	**0.028**
TG (mg/dl)	120.90 ± 71.21	88.19 ± 50.26	**0.015**
HDL-C (mg/dl)	46.21 ± 14.69	51.47 ± 14.52	**0.049**
VLDL-C (mg/dl)	24.40 ± 14.34	19.47 ± 11.79	0.074
LDL-C (mg/dl)	117.79 ± 15.67	100.05 ± 31.14	**0.0004**

Values are presented in Mean ± standard deviation

Statistically significant values are represented in bold ($p < 0.05$)

BMI body mass index; *W-crc* waist circumference; *H-crc* hip circumference; *A-crc* arm circumference; *C-crc* calf circumference; *WHR* waist-to-hip ratio; *WHtR* waist-to-height ratio; *T-skf* triceps skinfold; *B-skf* bicep skinfold; *Sub-skf* sub-scapular skinfold; *Sup-skf* supra-iliac skinfold; *SBP* systolic blood pressure; *DBP* diastolic blood pressure; *MBP* mean blood pressure; *PP* pulse pressure; *PR* pulse rate; *TC* total cholesterol; *TG* triglyceride; *HDL-C* high-density lipoprotein-cholesterol; *VLDL-C* very low density lipoprotein cholesterol; *LDL-C* low-density lipoprotein cholesterol

Table 3 Genotype and allele distribution of *MC4R* (rs571312, rs12970134) and *LEP* (rs2167270, rs7799039) polymorphisms in obese patients and non-obese healthy controls

Genotypes	Cases (*n* = 333)%	Controls (*n* = 338)%	OR (95% CI)	*p* value
rs571312				**0.024**
CC	125 (37.54)	153 (45.27)	1.00	–
CA	145 (43.54)	144 (42.60)	1.23 (0.88–1.71)	0.214
AA	63 (18.92)	41 (12.13)	1.88 (1.18–2.97)	**0.007**
Dominant (CC vs. CA + AA)	–	–	1.38 (1.01–1.87)	**0.042**
Recessive (AA vs. CC + CA)	–	–	1.69 (1.10–2.59)	**0.015**
C allele	395 (59.3)	450 (66.57)	1.36 (1.09–1.70)	**0.006**
A allele	271 (40.7)	226 (33.43)		
HWE p value	0.07	0.43	–	–
rs12970134				**0.011**
GG	130 (39.04)	138 (40.83)	1.00	–
GA	142 (42.65)	165 (48.81)	0.91 (0.65–1.26)	0.589
AA	61 (18.31)	35 (10.36)	1.85 (1.14–2.98)	**0.011**
Dominant (GG vs. GA + AA)	–	–	1.08 (0.79–1.47)	0.636
Recessive (AA vs. GG + GA)	–	–	1.94 (1.24–3.03)	**0.003**
G allele	402 (60.36)	441 (65.24)	1.23 (0.98–1.53)	0.06
A allele	264 (39.64)	235 (34.76)		
HWE *p* value	0.047	0.061	–	–
rs2167270				**0.002**
AA	145 (43.5)	150 (44.4)	1.00	–
AG	138 (41.5)	165 (48.8)	0.86 (0.62–1.19)	0.377
GG	50 (15)	23 (6.8)	2.24 (1.30–3.87)	**0.003**
Dominant (AA vs. AG + GG)	–	–	1.03 (0.76–1.40)	0.827
Recessive (GG vs. AA + AG)	–	–	2.42 (1.44–4.07)	**0.001**
A allele	428 (64.3)	465 (69)	1.22 (0.97–1.53)	0.079
G allele	238 (35.7)	211 (31)		
HWE *p* value	0.074	0.012	–	–
rs7799039				**0.002**
GG	147 (44.1)	165 (48.8)	1.00	–
GA	126 (37.9)	143 (42.3)	0.98 (0.71–1.37)	0.942
AA	60 (18)	30 (8.9)	2.24 (1.37–3.66)	**0.001**
Dominant (GG vs. GA + AA)	–	–	1.21 (0.89–1.64)	0.225
Recessive (AA vs. GG + GA)	–	–	2.26 (1.41–3.60)	**0.0001**
G allele	420 (63.06)	473 (70)	1.36 (1.08–1.71)	**0.007**
A allele	246 (36.94)	203 (30)		
HWE *p* value	0.001	0.901	–	–

Statistically significant values are represented in bold (*p* < 0.05)

OR odds ratio; *CI* confidence interval; *HWE* Hardy–Weinberg equilibrium

[OR (95% CI) 2.26 (1.41–3.60), *p* = 0.0001]. In obese individuals, variants rs571312 and rs2167270 were in agreement with HWE (*p* > 0.05), whereas variants rs12970134 and rs7799039 were not in HWE (*p* < 0.05). Among the control group except rs2167270, all the rest of the polymorphisms were in agreement with HWE.

Haplotype analysis

Tables 4 and 5 present the haplotype distribution and pairwise linkage disequilibrium (LD) among all the studied SNPs of *MC4R* and *LEP* genes in obese cases and controls. Based on the genotype distributions of *MC4R* and *LEP* gene polymorphisms in obese cases and healthy

Table 4 Evaluation patterns of LD of *MC4R* rs571312C/A and rs12970137G/A polymorphisms in obese cases and non-obese controls

Haplotypes		Obese % (n) (333)	Non-obese % (n) (338)	OR (95 CI%)[a]	p^a-value	p^b-value	p^c-value
rs571312C/A	rs12970134G/A						
C	G	42.5 (141)	48.4 (164)	Reference	–	0.126	0.119
A	G	26.5 (88)	25.7 (87)	1.17 (0.81–1.70)	0.392	0.920	
C	A	25.7 (86)	23.5 (79)	1.26 (0.86–1.85)	0.222	0.516	
A	A	5.2 (18)	2.4 (8)	2.61 (1.10–6.20)	**0.028**	0.066	
LD measure		**Obese cases**				**Non-obese controls**	
		D′	r^2			D′	r^2
rs571312C/A and rs12970134G/A		0.469	0.059			0.668	0.07

Statistically significant values are presented in bold

[a] *p* values and OR values derived from comparing each haplotype with the baseline haplotype (C-G)

[b] *p* values derived from comparing a specific haplotype with the other three

[c] *p* value derived from Comparison between obese and non-obese subjects for overall haplotypes

Table 5 Evaluation patterns of LD of *LEP* rs7799039G/A and rs2167270A/G polymorphisms in obese cases and non-obese controls

Haplotypes		Obese % (n) (333)	Non-obese % (n) (338)	OR (95 CI%)[a]	p^a-value	p^b-value	p^c-value
rs7799039G/A	rs2167270A/G						
G	A	37.1 (124)	45.1 (153)	Reference	-	**0.041**	**0.0001**
A	A	19.3 (64)	23.4 (79)	0.99 (0.66–1.50)	0.998	0.222	
G	G	18.7 (62)	26.4 (89)	0.85 (0.57–1.28)	0.460	**0.021**	
A	G	24.9 (83)	5 (17)	6.02 (3.39–10.68)	**0.0001**	**0.0001**	
LD measure		**Obese cases**				**Non-obese controls**	
		D′	r^2			D′	r^2
rs7799039G/A and rs2167270A/G		0.231	0.052			0.441	0.034

Statistically significant values are presented in bold

[a] *p* values and OR values derived from comparing each haplotype with the baseline haplotype (G-A)

[b] *p* values derived from comparing a specific haplotype with the other three

[c] *p* value derived from Comparison between obese and non-obese subjects for overall haplotypes

controls, the LD values between all pairs of polymorphisms were determined using Haploview software (v 4.1) (Fig. 5). In *MC4R* polymorphisms, the C-G haplotype, carrying both wildtype alleles (rs571312C and rs12970134G), was the most common haplotype among the studied groups and considered as baseline haplotype. After comparing with the baseline haplotype, the AA haplotype comprised of high-risk rs571312A and high-risk rs12970134A dramatically elevated 2 folds risk of obesity [OR (95% CI) 2.61 (1.10–6.20), $p = 0.028$]. The LD measurements described that the two variants of *MC4R* were in slight LD among the cases than control groups ($D' = 0.469$, $r^2 = 0.059$ for obese cases); ($D' = 0.668$, $r^2 = 0.07$ for non-obese controls) (Fig. 5a and b), whereas, in the two polymorphisms of the *LEP* gene, the total distribution of haplotype frequencies differed markedly between obese cases and non-obese controls ($p = 0.0001$). The lower-risk haplotype G-A of rs7799039G and

rs2167270A was the baseline and most prevalent haplotype among the studied group. When compared to the low-risk baseline G-A haplotype, the high-risk A-G haplotype revealed a sixfold increase in the development of obesity [OR (95% CI) 6.02 (3.39–10.68), $p = 0.0001$]. Based on the LD metrics, it could be hypothesized that both the SNPs of the *LEP* gene were in slight LD among obese cases compared to nonobese ones ($D' = 0.231$, $r^2 = 0.052$ for obese cases); ($D' = 0.441$, $r^2 = 0.034$ for non-obese controls) (Fig. 5c and d) (Tables 4 and 5).

Interaction analysis

Tables 6 and 7 demonstrate the interaction analysis of various diplotype combinations of the studied SNPs. Table 6 reveals that the combination of the risk genotype AA for rs571312 and AA for rs12970134 exhibited 3.91 ($p = 0.024$) fold risk toward the development of obesity in the studied population. Apart from this, the

(a)   **(b)**

(c)   **(d)**
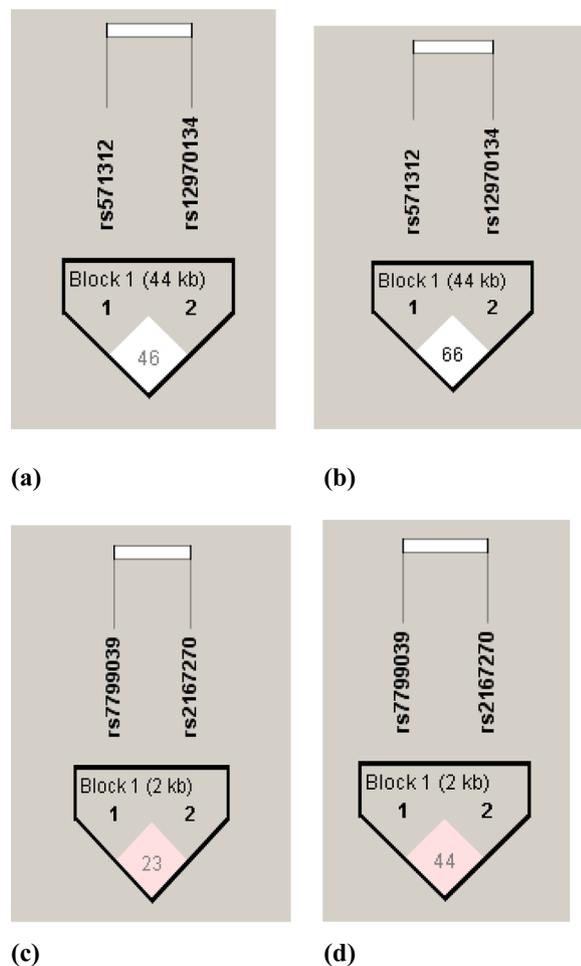
Fig. 5 LD plot demonstrating the position of two *MC4R* and two *LEP* variants and pairwise *D*′ values observed in the study population with respect to (**a**) obese cases of *MC4R* variants, (**b**) non-obese controls of *MC4R* variants, (**c**) obese cases of *LEP* variants and (**d**) non-obese controls of *LEP* variants

combination of CA-AA also conferred twofold risk of obesity ($p = 0.029$). However, in Table 7, the genotype combinations AA–AA, GG–GG and GG–GA showed a risk toward obesity but among them, the risk genotype combination (GG–AA) conferred a huge risk of about 5 folds toward the development of obesity ($p = 0.041$).

Linear regression analysis

Table 8 reveals the impact of the investigated polymorphisms on the numerous anthropometric and biochemical parameters among obese cases. The evaluation was done by linear regression analysis under the additive genetic model of inheritance that was adjusted for gender, diet and lifestyle. The *MC4R* gene variant rs12970134 showed a statistically significant association with WC ($\beta = 0.557$, $p = 0.017$) and WHtR ($\beta = 0.612$, $p = 0.009$) which depicted an increased trend in the model. On the other hand, the presence of *LEP* rs7799039 polymorphism significantly contributed to a 2.24% and 4.15% rise in total cholesterol (TC) and triglycerides (TG) levels among obese subjects ($\beta = 2.246$, $p = 0.0001$; $\beta = 4.154$, $p = 0.0001$, respectively). These findings indicated an increasing trend of obesity-related parameters with each addition of a minor allele of the corresponding variant. However, the *MC4R* polymorphism rs571312 and the *LEP* polymorphism rs2167270 had no significant association with any of the investigated anthropometric and biochemical parameters.

Gene-environment interaction analysis

The present work analyzed the interaction of 7 factors consisting of 4 single-nucleotide polymorphisms (rs571312, r12970134, rs2167270, rs7799039) and 3 obesity-related parameters (WHR, lifestyle and diet) in this population (Table 9). It was observed that except

Table 6 Frequency distribution of *MC4R* rs571312 and rs12970134 polymorphisms and interaction analysis of various diplotypes combination in obese cases and non-obese healthy controls

Genotype combination rs571312 rs12970134	Obese cases n(%)	Non-obese controls n(%)	χ^2	OR (95% CI)	*p* value
CC-GG	49 (14.6)	64 (18.98)	Reference	–	–
CC-GA	53 (15.9)	76 (22.4)	0.05	0.91 (0.54–1.51)	0.720
CC-AA	23 (6.8)	16 (4.8)	2.24	1.87 (0.89–3.92)	0.094
CA-GG	56 (16.9)	58 (17.13)	0.54	1.26 (0.74–2.12)	0.384
CA-GA	62 (18.5)	68 (20.22)	0.3	1.19 (0.71–1.97)	0.499
CA-AA	26 (7.9)	15 (4.33)	4.07	2.26 (1.08–4.72)	**0.029**
AA–GG	25 (7.3)	17 (4.97)	2.59	1.92 (0.93–3.94)	0.075
AA–GA	27 (8)	20 (5.86)	2.11	1.76 (0.88–3.50)	0.105
AA–AA	12 (3.45)	4 (1.258)	4.43	3.91 (1.19–12.89)	**0.024**

Statistically significant values are presented in bold

The interactive association was assessed between the genotype status of the studied polymorphisms by 2*2 chi-square contingency table

OR odds ratio, *CI* confidence interval

Table 7 Frequency distribution of *LEP* rs2167270 and rs7799039 polymorphisms and interaction analysis of various diplotypes combination in obese cases and non-obese healthy controls

Genotype combination rs2167270 rs7799039	Obese cases n(%)	Non-obese controls n(%)	χ^2	OR (95% CI)	*p* value
AA–GG	64 (19.2)	73 (21.6)	Reference	–	–
AA–GA	55 (16.4)	63 (18.7)	0.01	0.99 (0.60–1.63)	0.986
AA–AA	26 (7.8)	13 (3.9)	4.07	2.28 (1.08–4.80)	**0.030**
AG–GG	61 (18.2)	81 (23.8)	0.26	0.85 (0.53–1.37)	0.528
AG–GA	52 (15.6)	70 (20.6)	0.29	0.84 (0.51–1.38)	0.508
AG–AA	25 (7.4)	15 (4.3)	2.49	1.90 (0.92–3.91)	0.081
GG–GG	22 (6.6)	11 (3.3)	3.47	2.28 (1.02–5.06)	**0.042**
GG–GA	19 (5.6)	10 (2.8)	2.67	2.16 (0.93–5.00)	0.069
GG–AA	9 (2.7)	2 (0.6)	3.71	5.12 (1.06–24.63)	**0.041**

Statistically significant values are presented in bold

The interactive association was assessed between the genotype status of the studied polymorphisms by 2*2 chi-square contingency table

OR odds ratio, *CI* confidence interval

for the 6-locus model (G1 G2 G3 G4 E1 E2), the cross-validation consistencies (CVC) for the rest of the models were maximal (10/10). However, the accuracy of the test sample was greatest in the 5-locus interaction model (G1, G2, G3, G4, E2) (82.1%) and the accuracy of the training sample (TrBA) was highest in the 6-locus interaction model (G1, G2, G3, G4, E1, E2) (88%). At the 5% level, all the interaction models were found to be significant. The testing balance accuracy (TBA) evaluates the frequency of accurately classifying individuals concerning their case–control status. A TBA score exceeding 55% is deemed satisfactory. In this analysis, the 5-locus interaction model (G1, G2, G3, G4, E2) exhibited the highest TBA of 82.1%. This indicates that the combined interaction of these four genetic variations and the lifestyle factor collectively produced the most effective model for predicting obesity progression. The findings underscore the significant influence of lifestyle factor on the interplay between these genetic variants in obesity development.

The dendrogram given in Fig. 6 represents that the clusters, i.e., G2–G4, had a stronger synergistic effect in predicting the susceptibility toward the obesity risk than G1–G3. Both these clusters also showed a lesser degree of interaction with one other. Furthermore, the 3 environmental factors (E1, E2, E3) demonstrated midway interactions between synergy and redundancy with all the polymorphisms in the development of obesity. In Fig. 7, it was observed that E2 (Lifestyle factor) played a prominent role by contributing the highest entropy, accounting for 19.7%, to the progression of obesity independently. The entropy model showed a strong synergistic interaction between G2 and G4 (15.97%) regarding obesity risk. G1 demonstrated moderate synergistic interaction with G4 (5.30%), G3 (7.51%) and G2 (3.50%) concerning the

risk of developing obesity, whereas E1, E2 and E3 showed a midway interaction between synergy and redundancy with each other and with the rest of the genetic markers.

Discussion

Obesity stands out as a paramount public health concern due to its strong associations with cancer, type 2 diabetes, and cardiovascular diseases [42–44]. Unravelling the intricate mechanisms underlying obesity and its comorbidities reveals a substantial contribution from genetic factors, as evidenced by emerging research. In our investigation, we investigated the association between genetic variants in the *MC4R* and *LEP* genes and obesity risk through a case–control study encompassing 671 individuals (333 cases and 338 controls) in Punjab, a northwest state of India. Our study revealed a significant correlation between the genotypes *MC4R* rs571312AA, rs12970134AA, and *LEP* rs2167270GG, rs7799039AA with an elevated risk of obesity and aberrant lipid profiles within this northwest Indian population. Individuals carrying the rs2167270GG and rs7799039AA genotypes exhibited a twofold higher susceptibility to obesity compared to non-carriers, underscoring the potential significance of these *LEP* gene variants in obesity etiology within this population. Similarly, carriers of both polymorphisms in the *MC4R* gene (rs571312AA and rs12970134AA) faced a 1.8-fold increased risk of obesity.

These findings align harmoniously with a plethora of prior studies, corroborating the association of risk with the minor alleles of the aforementioned polymorphisms [25, 36, 45–47]. For instance, Hong et al. [48] demonstrated a 1.43-fold increase in obesity risk associated with the rs571312 variant, while Huang et al. [45] reported a nearly threefold risk linked to rs12970134. Likewise,

Table 8 Linear regression analysis: impact of studied polymorphisms on anthropometric and biochemical parameters associated with obese cases under additive model

| Gene	SNP	BMI		WC		WHR		WHtR		TC		TG	
		β (SE)	p	β (SE)	p	β (SE)	p	β (SE)	p	β (SE)	p	β (SE)	p
MC4R	rs571312	0.473 (0.240)	0.054	0.070 (0.239)	0.769	− 0.149 (0.097)	0.129	0.014 (0.239)	0.953	2.789 (1.574)	0.137	− 3.093 (2.776)	0.316
	rs12970134	− 0.002 (0.013)	0.901	0.557 (0.226)	**0.017**	− 0.019 (0.162)	0.909	0.612 (0.226)	**0.009**	− 0.512 (0.227)	0.109	0.549 (0.515)	0.365
LEP	rs2167270	0.344 (0.306)	0.267	0.217 (0.227)	0.343	0.195 (0.175)	0.271	0.374 (0.234)	0.117	− 1.327 (0.922)	0.209	− 1.620 (1.262)	0.256
	rs7799039	− 0.141 (0.248)	0.573	0.105 (0.214)	0.624	− 0.025 (0.122)	0.838	0.127 (0.263)	0.631	2.246 (0.290)	**0.0001**	4.154 (0.331)	**0.0001**

$p < 0.05$ was considered statistically significant and adjusted for gender, diet and lifestyle. $β$ represents change in the respective units of traits with increase in minor allele

Table 9 Gene-environment interaction model identified by Multifactor Dimensionality Reduction method for obese cases and controls

No. of Loci	Interacting SNPs	TrBA	TBA	CVC	Sign test (*p* value)
1	E2	0.7426	0.7426	10/10	10 (0.001)
2	E1 E2	0.782	0.782	10/10	10 (0.001)
3	G2 G4 E2	0.8078	0.8032	10/10	10 (0.001)
4	G1 G2 G4 E2	0.8275	0.8168	10/10	10 (0.001)
5	G1 G2 G3 G4 E2	0.8598	0.821	10/10	10 (0.001)
6	G1 G2 G3 G4 E1 E2	0.8802	0.8078	6/10	10 (0.001)
7	G1 G2 G3 G4 E1 E2 E3	0.2991	0.8168	10/10	10 (0.001)

p value < 0.05 was considered statistically significant

G1 = rs571312; G2 = rs12970134; G3 = rs2167270; G4 = rs7799039; E1 = Waist–hip ratio; E2 = Lifestyle; E3 = Diet; *TrBA* training balance accuracy, *TBA* testing balance accuracy, *CVC* cross validation consistency
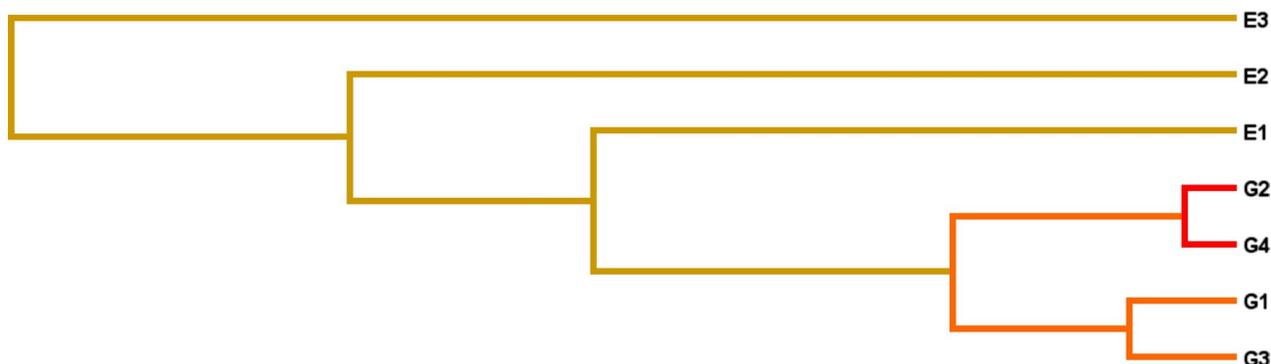
Fig. 6 The dendrogram representing the nature of interaction between the 4 single-nucleotide polymorphisms (rs571312, rs12970134, rs2167270 and rs7799039) and environment factors for obese cases and controls. Color scheme used—red = high synergistic interaction (synergy), orange = lesser degree of interaction, green = weak interaction, blue = no interaction (redundancy), gold = midway interaction between synergy and redundancy
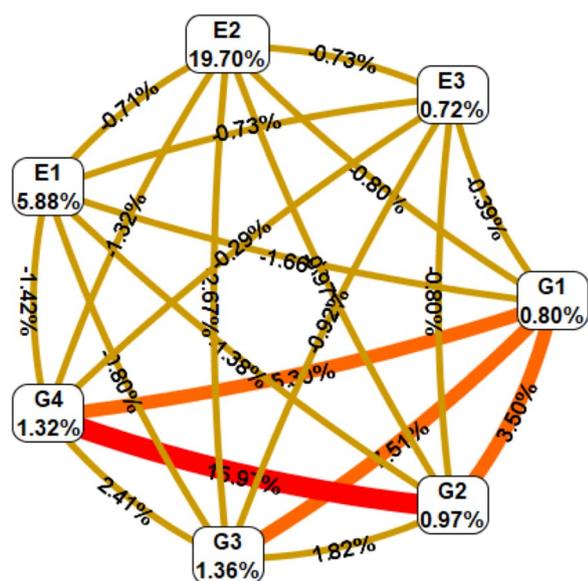
Fig. 7 Interaction entropy model analyzed by multifactor dimensionality reduction (MDR) for obese cases and controls

Dasgupta et al. [25] observed significant elevations in obesity risk among south Indian cohorts, with a 3.2-fold increase tied to rs2167270 and a 1.8-fold increase associated with rs7799039.

Employing a gene candidate strategy in the obese cohort revealed potential contributions of *MC4R* rs12970134 and *LEP* rs7799039 polymorphisms to heightened susceptibility to obesity, as evidenced by increases in obesity-related traits including waist circumference (WC), waist-to-height ratio (WHtR), total cholesterol (TC), and triglycerides (TG). These findings were consistent with prior investigations conducted across diverse populations, encompassing Portuguese, Indian, Iranian, Tunisian, and Moroccan cohorts [12, 13, 19, 34, 49, 50]. Linear regression analysis further validated these associations, with *MC4R* rs12970134 demonstrating elevated WC and WHtR, while *LEP* rs7799039 was associated with significant elevations in TC and TG levels among obese individuals, suggesting that these variants are pertinent markers for obesity-related indices in

this population. However, conflicting results exist, as evidenced by studies such as those conducted by Kochetova et al. [17], Constantin et al. [51], Vogel et al. [52] and Mohamed et al. [53] which reported no significant associations between these genetic polymorphisms and obesity-related traits ($p > 0.05$).

In the hypothalamus, the interplay between leptin and melanocortin receptors constitutes the fundamental components of the leptin–melanocortin circuit, crucial for regulating energy intake and expenditure. Notably, genes within this pathway, particularly *MC4R* and *LEP*, serve as key players in the modulation of energy homeostasis and have been identified as primary determinants of monogenic obesity [54]. Previous investigations have elucidated that polymorphisms in the *MC4R* gene can influence the receptor's functionality by impeding the establishment of binding sites on the cell surface. Such genetic variations may disrupt the standard inhibitory activity of *MC4R* in the hypothalamus, consequently leading to perturbations in energy balance and contributing to the development of obesity. Likewise, within the hypothalamus, leptin functions to curb food intake while simultaneously boosting energy expenditure. Polymorphisms such as rs2167270 and rs7799039, located in the 5' untranslated region and promoter region of the leptin gene, respectively, have been implicated in modulating serum lipid levels and altering leptin gene expression [29, 30]. Aboelros et al. [55] suggested that the rs7799039 variant may underlie the aberrant metabolism of insulin and leptin hormones, potentially resulting in resistance to their physiological effects.

Following an examination of the relationship between *LEP* single-nucleotide polymorphisms (SNPs) and their haplotypes, we noted that the combination of rs7799039A and rs2167270G haplotypes was associated with a sixfold increase in the risk of obesity compared to other haplotype combinations. Concurrently, during the construction of haplotypes for *MC4R* gene polymorphisms, we identified that the rs571312A–rs12970134A haplotype exhibited a twofold positive association with obesity risk in this population. All four *LEP* polymorphisms, as well as the *MC4R* gene, demonstrated slight linkage disequilibrium in the cases group compared to the control group ($D' = 0.231$, $r^2 = 0.052$; $D' = 0.441$, $r^2 = 0.034$; $D' = 0.469$, $r^2 = 0.059$; $D' = 0.668$, $r^2 = 0.07$) respectively.

Consistent with our findings, Dasgupta et al. [25] demonstrated a significant association between the AAC haplotype combination involving rs7799039, rs2167270, and rs4731426 with obesity in the South Indian population, characterized by strong linkage disequilibrium (LD) ($D' > 0.8$). Shamsuddin et al. (2018) reported that the AAG haplotype combination, harboring risk alleles of rs7799039G/A, H1328080, and 2167270A/G, conferred

an eightfold risk for obesity development [31]. Additionally, Wei et al. [18] identified that the CCA haplotype, comprising rs17782313, rs476828, and rs12970134, was associated with increased morbid obesity in the Chinese population. However, in contrast, Bouafi et al. [50] found no association between *LEP* gene polymorphism haplotypes and obesity risk in the Moroccan population. Moreover, Apalasamy et al. [16] and Chehadeh et al. [36] did not find any association between *MC4R* haplotypes in the Malaysian and United Arab Emirates (UAE) populations, respectively. The findings of interaction association between the diplotypes of the studied polymorphisms also point toward a possible role of *MC4R* (rs571312C/A- rs12970134G/A) and *LEP* (rs2167270A/G- rs7799039G/A polymorphisms in obesity and metabolic changes. Upon analysis of the genetic-environmental interaction, it was observed that lifestyle factors independently contributed the highest entropy of 19.70% to the progression of obesity. Furthermore, it was revealed that lifestyle factors exhibited strong interactions with all genetic polymorphisms of the *MC4R* and *LEP* genes in the 5-locus model. This interaction demonstrated an 82.1% testing balance accuracy, indicating a higher likelihood of predicting obesity development. Several studies, including the present one, have shown that lifestyle choices can alter the impact of genetic variations that predispose individuals to obesity. For instance, research conducted by Qi et al. [56] on nurses' health or health professionals in the United States found that excessive television viewing amplified the influence of a genetic risk score comprising 32 genetic variations on BMI. Therefore, conducting a thorough assessment of genetic influence and its interaction with the gene-environment interface could enhance our ability to identify specific genetic variations associated with diseases.

Limitations

The present research's sample size, especially when stratified by BMI, was not sufficient to draw definitive conclusions. Hence, it is crucial to replicate these results in future investigations with larger sample sizes to confirm their validity. Serum levels of *MC4R* and *LEP* were not assessed in this study, consequently precluding the ability to conduct genotype–phenotype correlation studies. Another constraint was the absence of data on dietary intake or caloric consumption, which could serve as a significant confounding factor. The sample lacks national representation, with notable implications primarily relevant to northwest Indian ethnic groups. This raises doubts about the findings' relevance to other ethnicities. Further research involving diverse groups is necessary.

Conclusion

The current study unveils a distinct correlation between four polymorphisms of the *MC4R* and *LEP* genes and obesity risk in the northwest Indian population. Notably, carrying risk alleles for both *MC4R* and *LEP* variants increased the risk of obesity by twofold and sixfold, respectively. Furthermore, the research highlights how various combinations of diplotypes in *MC4R* and *LEP* genes collectively influence obesity predisposition. Associations were also observed between *MC4R* rs12970134 and *LEP* rs7799039 variants with elevated lipid profiles and waist-related traits. Recognizing these genetic links aids in predicting metabolic risks, guiding early interventions to manage lipids and central adiposity, thereby reducing the risk of metabolic syndrome and cardiovascular diseases. The interplay between genetic polymorphisms and environmental factors accentuates their combined impact on predicting obesity risk. Lifestyle factors were particularly potent in shaping the effects of genetic variants on obesity within the studied population. These findings are significant as obesity rates continue to rise steadily in India, particularly in Punjab. Therefore, evaluating the collective influence of genetic polymorphisms can expedite the identification of individuals at risk, empowering them to improve their lifestyles and fostering a healthier, disease-free existence. Thus, uncovering and understanding the mechanisms involving genes in the leptin–melanocortin pathway and obesity will facilitate the development of rational strategies and personalized disease management.

Abbreviations

MC4R	Melanocortin 4 receptor
LEP	Leptin
PCR	Polymerase chain reaction
RFLP	Restriction fragment length polymorphism
OR	Odds ratio
CI	Confidence interval
WHO	World Health Organization
GWAS	Genome-wide association studies
GPCR	G protein-coupled receptor
PCA	Phenol chloroform isoamyl
DNA	Deoxyribose nucleic acid
EtBR	Ethidium bromide
SNP	Single-nucleotide polymorphism
ANOVA	Analysis of variance
LD	Linkage disequilibrium
χ^2	Chi-square
SPSS	Statistical Package for Social Sciences
MDR	Multifactor dimensionality reduction
BMI	Body mass index
WC	Waist circumference
HC	Hip circumference
AC	Arm circumference
CC	Calf circumference
WHR	Waist-to-hip ratio
WHtR	Waist-to-height ratio
T-skf	Triceps skinfold
B-skf	Bisceps skinfold
Sub-skf	Subscapular skinfold
Sup-skf	Supra-iliac skinfold
Sum-skf	Sum of skinfold
SBP	Systolic blood pressure
DBP	Diastolic blood pressure
MBP	Mean arterial blood pressure
PP	Pulse pressure
PR	Pulse rate
TC	Total cholesterol
TG	Triglycerides
LDL-C	Low-density lipoprotein-cholesterol
VLDL-C	Very low-density lipoprotein-cholesterol
HDL-C	High-density lipoprotein-cholesterol
HWE	Hardy Weinberg equilibrium
CVC	Cross validation consistency
TBA	Testing balance accuracy
TrBA	Training balance accuracy

Availability of data and materials
The raw data will be made available by the corresponding author on request.

Declarations

Ethical approval and consent to participate
The present study protocol was performed according to the Declaration of Helsinki (1964). The current study was also agreed by the institutional ethics committee (ethics no.1609/HG) constituted by Guru Nanak Dev University, Amritsar, Punjab, India.

Consent for publication
All participants provided written consent for publication after being duly informed.

Competing interests
The authors declare no competing interests.

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