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Role of leptin G-2548A polymorphism in age- and gender-specific development of obesity

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Role of leptin G-2548A polymorphism in age- and gender-specific development of obesity

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Leptin is involved in the regulation of food intake and energy expenditure, and therefore, is central to adiposity-sensing pathway. We examined the relationship of the leptin G-2548A polymorphism with obesity and obesity-related anthropometric and metabolic parameters in a total of 394 (239 obese and 155 non-obese) subjects between 5 and 45 years of age. Body weight, height, waist circumference (WC), hip circumference (HC) and blood pressure (BP) were measured. Body mass index (BMI) and waist-to-hip ratio (WHR) were calculated. Levels of fasting blood glucose (FBG), insulin, leptin and leptin receptor were determined, and homeostasis model assessment of insulin resistance (HOMA-IR) was calculated. Genotyping was carried out by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The *LEP* G-2548A polymorphism showed association with obesity in children and adolescents (≤18 years of age) but not in adults. However, analysis by gender stratification revealed association with obesity in girls only. In addition, G-2548A polymorphism showed association with BMI, WC, HC, fasting blood glucose and serum leptin levels. This suggests that G-2548A polymorphism may influence the susceptibility to metabolic disturbances and obesity at an early life. Further investigation with a larger sample size is required to validate the effect of *LEP* G-2548A polymorphism in obese Pakistani girls.

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1. Introduction

Obesity and associated metabolic disorders have become a global public health problem (Stevens *et al.* 2012). Rates of adult overweight and obesity have risen globally from 30% in 1980 to 37% in 2013. South Asia has seen a steady increase in overweight and obesity since 1980, with the rate of overweight or obese adults rising from 16% to 21% in 2013. Pakistan has seen rates increase from 26% to 33% over the same period, and by gender the differences are striking. In 1980, 24% of men and 29% of women were overweight or obese, a five percentage point difference, but in 2013, the rates were 28% for men and 38% for women, the largest gap in the region (Ng *et al.* 2014a). The rapid increase in the prevalence of overweight and obesity in developing countries points towards various social, environmental and

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behavioural transitions. In South Asia, including Pakistan, these transitions are occurring rapidly, with increasing urbanization, changing lifestyles, energy-dense diets, and reduced physical activity (Popkin and Gordon-Larsen, 2004).

Leptin is an adipokine (~16 kDa protein with 167 amino acids) produced predominantly by the white adipose tissue. It is a central regulator for adiposity-sensing pathways localized to the hypothalamus. When the mass of adipose tissue increases, released leptin curtails appetite and stimulates energy expenditure. When the mass of adipose tissue decreases, a lower leptin production favours an increase in appetite and less energy expenditure (Halaas *et al.* 1995). Thus, leptin is an anti-obesity hormone and functions as a satiety signal. It crosses the blood–brain barrier and on reaching the brain, acts on receptors in the arcuate nucleus within the hypothalamus of the brain where it modifies the expression of many neuropeptides reported to be involved in the regulation of body weight (Mantzoros 1999). It decreases the production of some orexigenic (appetite-stimulating) neuropeptides such as neuropeptide Y (NPY), melanin-concentrating hormone (MCH), orexins and agouti-related peptide (AGRP), whereas it increases the production of anorexigenic (appetite-decreasing) neuropeptides such as α-melanocyte-stimulating hormone (α-MSH), cocaine and amphetamine regulated transcripts (CART), and corticotrophin releasing hormone (CRH) (Jéquier 2002).

Leptin is a product of the leptin gene that is located on chromosome 7q31.3 and spans approximately 20 kb. The gene contains 3 exons separated by 2 introns (Green *et al.* 1995). Mutations in coding sequence or at splice site of *LEP* that renders the leptin protein unable to signal through its receptor produce severe early-onset obesity in humans (Montague *et al.* 1997; Strobel *et al.* 1998). However, such coding sequence variation in *LEP* is extremely rare in general populations (Maffei *et al.* 1996; Carlsson *et al.* 1997). Evidence for association between common leptin polymorphisms and obesity has been sought in several studies. One of these polymorphisms includes G-2548A (rs7799039) in the 5′ promoter region. This variant may affect the expression of *LEP* at the transcriptional level by the adipose tissue, and is thought to be involved in the regulation of plasma leptin levels and BMI (Strobel *et al*. 1998). However, most findings to date regarding the association of *LEP* G-2548A with obesity and obesity-related anthropometric and metabolic traits have remained incongruous and less clear (Li *et al*. 1999; Mammes *et al.* 2000; Poitou *et al*. 2005; Wang *et al*. 2006; Duarte *et al*. 2007; Constantin *et al*. 2010). On one hand, this variant has been associated with obesity and overweight in various populations (Li *et al*. 1999; Mammes *et al.* 2000; Wang *et al*. 2006; Hinuy *et al*. 2008). On the other hand, many studies failed to find any association and differences in genotype and allele frequencies of this variant between lean and obese subjects (Portolés *et al.* 2006; Duarte *et al*. 2007; Constantin *et al*. 2010; Furusawa *et al*. 2010). No association of *LEP* G-2548A was reported with obesity-related metabolic phenotypes such as hypertension, hyperglycemia, dyslipidemia and coronary artery disease in Brazilian women (Hinuy *et al*. 2008). A recent study reported the association of *LEP* G-2548A with obesity and metabolic syndrome in Tunisian subjects (Boumaiza *et al*. 2012). Furthermore, it is important to note that most literature to date regarding the association between obesity and *LEP* G-2548A have remained scarce in non-Caucasian populations. Particularly, status and association of this polymorphism in relation to obesity and obesity-related anthropometric and metabolic traits have never been sought in the Pakistani population. Thus, conflicting data on this subject from other countries and the lack of data on this subject with reference to the Pakistani population emphasizes the need for studies among Pakistanis. The current study was, therefore, carried out to observe whether G-2548A polymorphism of *LEP* is associated with obesity and obesity-related anthropometric and metabolic parameters in a sample of Pakistani population.

2. Material and methods

2.1 Subjects

The study was conducted at University of Health Sciences Lahore, Pakistan. A total of 394 subjects including 239 obese (BMI ≥30 kg/m^2 or 95th percentile) and 155 non-obese (BMI<25 kg/m^2 or 5th–85th percentile) individuals between 5 and 45 years of age were recruited from local public and private hospitals, schools, colleges and universities after obtaining informed consent. Simple random sampling without replacement technique was used to collect samples. Subjects with the history of endocrinopathies (pituitary dysfunction, Cushing's syndrome and hypothyroidism) and history of medication such as phenothiazines, tricyclic antidepressant, anticonvulsants and steroids were excluded from the study. Complete demographic information (name, age, sex, address, education, socioeconomic status) was collected on a questionnaire. Information regarding family history of obesity was obtained by drawing three-generation pedigrees and confirmation by two independent sources. Complete general physical examination was performed.

Subjects were further categorized into 2 groups: Less than or equal to 18 years (group 1) and greater than 18 years (group 2). In subjects >18 years, obesity was defined as BMI ≥ 30 and those with BMI<25 were categorized as non-obese (WHO 2013). Children and adolescents ≤18 years of age were divided into obese (>95th percentile) and non-obese (5th–85th percentile) groups according to Center for Disease Control and Prevention (CDC) BMI for age growth charts.

2.2 Measurement of anthropometric parameters

Body weight, height, waist and hip circumference, systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured according to the standard procedures (WHO 1989). Body height was measured using a wall-mounted stadiometer and body weight was measured using a digital scale. The body mass index (BMI, also called Quetelet's Index) of each subject was calculated as weight (in kilograms) divided by height (in meters squared). Waist circumference (WC) was measured just above navel midway between the lower margin of the last rib and iliac crest to the nearest 0.1 cm. Hip circumference (HC) was taken as the maximal circumference over the buttocks. Waist-to-hip ratio (WHR) was calculated from the values of waist and HC. Blood pressure (BP) was measured twice from the right arm of the subject in a sitting position using a standard mercury sphygmomanometer.

2.3 Measurement of metabolic parameters

Blood samples were drawn after an overnight fast of 8–12 h. Fasting blood glucose (FBG) levels were determined by the glucose oxidase method using Huma Star 180 chemistry analyser (Human, Wiesbaden, Germany). Corresponding commercial kits (according to respective manufacturers' instructions) were employed to determine the blood concentrations of insulin (BIO Source, Europe SA, Nivelles, Belgium), leptin (BIO Source Europe SA, Nivelles, Belgium) and leptin receptor (LEPR) (SPI BIO, Bertin Pharma, Montigny Le Bretonneux, France) by enzyme-linked immunosorbent assay (ELISA) and an automated EIA analyser (Bio-Rad Laboratories, Hercules, CA, USA). FBG and fasting insulin levels were used to measure homeostasis model assessment of insulin resistance (HOMA-IR) calculated by the formula (Matthews *et al.* 1985):

$$\text{HOMA}-\text{IR} = \text{Fasting insulin } (\mu\text{IU/mL}) \times \text{Fasting glucose } (\text{mmol/L})/22.5$$

2.4 DNA extraction and genotyping

Genomic DNA was extracted from whole blood using genomic DNA purification kit (Fermentas, USA). Genotyping of G-2548A polymorphism was carried out by polymerase chain reaction-restriction fragment length polymorphism assay (PCR-RFLP). A DNA fragment containing G-2548A polymorphism was amplified using specific primers (Forward primer sequence: 5′TAAGCCAAGGCAAAATTG AG3′ and reverse primer sequence: 5′CTTCAAAATTTA TGTTCCTCTGC3′). The PCR was carried out using thermocycler (Icycler 5, BioRad, USA) according to the optimized conditions. In a 25 μL volume reaction, PCR components comprised of 100 ng DNA, 1X Taq buffer, 2 mM $MgCl_2$, 200 μM of each dNTP, 10 pmol of each primer and 0.5U Taq DNA polymerase. Thermal cycling was performed as follows: Initial denaturation at 95°C for 4 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 53°C for 30 s and extension at 72°C for 1 min, and then a final extension step at 72°C for 10 min.

Amplified PCR products (281 bp) were digested with Hha I restriction enzyme (Favorgen, Taiwan) to check polymorphism by RFLP assay. The digested PCR fragments along with DNA ladder (100 bp, Fermentas, Germany) were resolved by electrophoresis using 2% agarose gel. The gel was run at 120 V for 1 h. After that, the gel was placed in a Gel Documentation System (GDS) to visualize the digested PCR fragments under UV light. Presence of G allele revealed two fragments of 172 bp and 109 bp while the presence of A allele produced a single fragment of 281 bp.

2.5 Statistical analysis

The data were analysed using Statistical Package for Social Sciences (SPSS Inc. Chicago, IL, USA, version 17.0). Quantitative variables were expressed as mean ± standard error of mean (SEM). Student's *t*-test was applied to observe the differences between case and control groups. The whole data were stratified according to age and sex in sub-groups. Hardy Weinberg equilibrium test (HWE) was applied to determine the variation in distribution of alleles and genotypes within the concerned population. Allelic frequencies were calculated by gene counting. Chi-square (χ^2) test was used to determine the significant differences of genotype and allelic frequencies between obese and non-obese groups. Association of G-2548A polymorphism with obesity was determined by Pearson Chi-square using co-dominant, dominant and recessive models. The association of G-2548A polymorphism with anthropometric and metabolic traits was determined by using General Linear Model (GLM) assuming a recessive genetic model. The genotypes were coded as (0, 1, 2) in co-dominant model, (0, 1) in dominant model and (1, 0) in recessive model corresponding to the number of copies of risk allele. Differences of obesity-related anthropometric and metabolic traits adjusted for age and sex across genotypes of G-2548A polymorphism were analysed by *t*-test. A *p*-value of <0.05 was considered statistically significant.

3. Results

Figure 1 represents the characteristics of the obese subjects whereas anthropometric and biochemical parameters of both

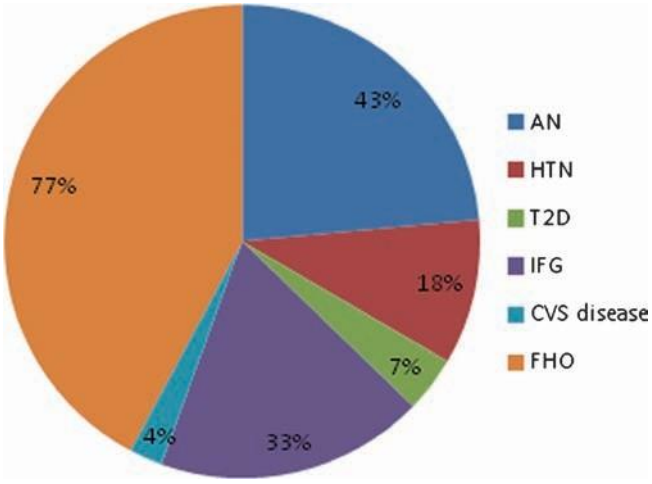

Figure 1. Characteristics of obese subjects. AN stands for acanthosis nigricans, HTN for hypertension, T2D for type 2 diabetes, IFG for impaired fasting glucose, CVD for cardiovascular disease and FHO for family history of obesity.

obese and non-obese subjects are shown in Figure 2. BMI, waist circumference, leptin levels and HOMA-IR are significantly higher and leptin receptor levels are significantly lower (p<0.05) in obese as compared to non-obese subjects (table 1; figure 2). Analysis by gender revealed significantly higher leptin levels of obese females as compared to obese males (p<0.05). Stratification of data by age revealed significantly higher leptin levels of obese females both in >18 year and ≤18 year old population (p<0.05, table 1). There was no significant difference (p>0.05) in the age, BMI, WC, LEPR

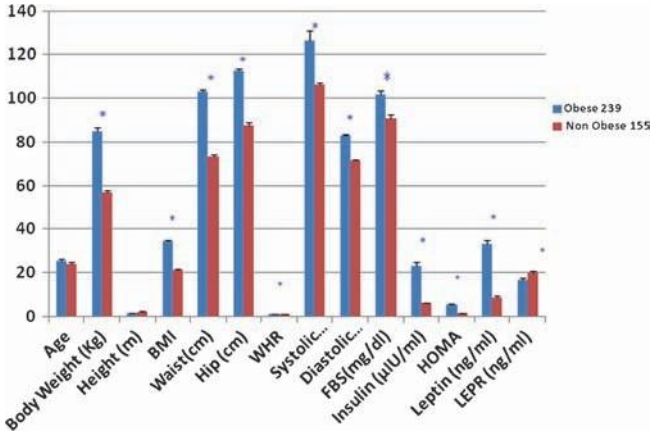

Figure 2. Anthropometric and biochemical parameters of obese (n=239) and control (n=155) subjects. WHR stands for waist–hip ratio, SBP for systolic blood pressure, DBP for diastolic blood pressure, FBG for fasting blood glucose and HOMA-IR for homeostasis model assessment of insulin resistance.

levels and HOMA-IR of obese female and obese male subjects (table 1).

Significantly higher levels of leptin and HOMA-IR were found in non-obese females as compared to non-obese males (p<0.05) (table 2). Stratification of data by age revealed significantly high leptin levels and HOMA-IR values of non-obese adult females (p<0.05) (table 2). In the group with ≤18 years non-obese subjects, BMI and waist circumference of females were significantly higher as compared to males (p>0.05) (table 2).

3.1 *Allele and genotype frequencies of G-2548A variant*

The genotypes of G-2548A polymorphism of *LEP* were in Hardy-Weinberg equilibrium (p>0.05) in both obese and non-obese groups. Genotype and allele frequencies for G-2548A polymorphism were compared using Chi-square test in obese and non-obese subjects. There was no signifi- cant difference in the allele frequency of LEP G-2548A polymorphism (p>0.05) in the entire sample. Analysis of data after stratification by gender revealed no significant (p>0.05) difference in genotype and allele frequencies between obese and non-obese male and female subjects (table 3).

The whole data was stratified according to age in two groups: ≤18 years and >18 years. The frequency of G allele was 0.53 in obese and 0.32 in non-obese children and adolescents. There were significant differences in genotype and allele frequencies (p<0.05) between obese and non-obese children and adolescents (table 4), whereas no significant difference was observed between obese and non-obese >18 years old subjects (table 5).

When the group with ≤18 years of subjects was further analysed according to gender, differences in genotype and allele frequencies remained significant (p<0.05) only in girls, whereas no significant difference was observed in boys (table 4). The above results revealed major age and gender related differences in genotype and allele frequencies of G-2548A polymorphism of *LEP*.

3.2 *Association of G-2548A polymorphism with obesity*

We found significant association of G-2548A polymorphism of *LEP* with obesity in a recessive genetic model (p<0.05) (table 3). Study subjects were stratified according to age in >18 years and ≤18 years groups; significant association (p=0.0001) was found only in children and adolescents (≤18 years of age) (table 4). The data were further analysed for gender differences in the group with subjects ≤18 years of age, association was found with obesity only in girls. *LEP* G-2548A polymorphism increased the risk of obesity in girls (p<0.05) (table 4).

Table 1. Anthropometric and metabolic parameters of obese subjects

Groups	Age	BMI (kg/m^2)	Waist circumference (cm)	Leptin (ng/mL)	Leptin receptor (ng/mL)	HOMA-IR
Obese	25.24 ± 0.76	34.27±0.45*	102.86±1.10*	33.21 ± 1.6*	16.38 ± 0.99*	5.28 ± 0.5*
Non-obese	23.90 ±0.74	21.18±0.26	73.13±1.11	8.33 ± 0.72	19.74 ± 0.71	1.30 ± 0.08
Obese						
Females	24.45±0.5	35.01±0.59	103.3±1.37	37.24±2.04*	17.12±1.68	4.51±0.41
Males	24.08±0.65	33.42±0.68	102.3±1.77	28.47±2.46	15.51±0.83	6.18±0.96
>18 years, obese						
Female	35.41±0.35	37.15±.60	108±1.30	37.20±2.51*	16.34±.73	4.49±.47
Males	34.58±0.41	36.76±.81	109±2.22	28.81±3.75	16.22±1.21	7.19±1.58
≤18 years, obese						
Females	11.27±0.76	28.33±0.79	86.98±2.11	37.36±3.07*	20.02±7.56	4.57±0.81
Males	11.91± 0.54	28.88±0.79	92.29±2.16	28.62±2.62	14.34±0.91	4.80±0.71

Data are presented as mean ± SEM and were compared by *t*-test. *$p<0.05$ was considered as significant.

3.3 Association of LEP G-2548A polymorphism with anthropometric and metabolic parameters

Children and adolescent having G allele (both GG and GA) had significantly higher BMI, WC, HC, SBP and DBP as compared to subjects homozygous for A allele (AA) in the present study. G allele carrying children and adolescents had significantly high leptin (25.25±1.16 ng/mL) and FBG levels (94.5±1.81 mg/dL) as compared to subjects homozygous for A allele (leptin = 20.33±1.21 ng/mL and FBG = 86.3±1.21 mg/dL) (table 6).

Girls having G allele (both GG and GA) had significantly high leptin levels as compared to girls homozygous for A allele (table 5). There was no significant difference in the leptin receptor levels between G allele (GG and GA) and A allele (AA) carriers (tables 6 and 7). Fasting insulin levels although not statistically significant but was high in G allele carriers as compared to individuals homozygous for A allele (tables 6 and 7). General Linear Model (GLM) multivariate analysis applied on the subgroup with female children and adolescents using age as a covariate in a recessive model also revealed significant association of G-2548A polymorphism with BMI, WC, HC, FBG and leptin levels (table 7).

3.4 Association of G-2548A polymorphism with clinical conditions

There was no significant association ($p>0.05$) of G-2548A polymorphism of *LEP* with acanthosis nigricans, type 2 diabetes, hypertension and impaired fasting glucose (table 8)

4. Discussion

Obesity represents a major pandemic throughout the world as its prevalence is increasing both in developed and

Table 2. Anthropometric and metabolic parameters of non-obese subjects

Groups	Age	BMI (kg/m^2)	Waist circumference (cm)	Leptin (ng/mL)	Leptin receptor (ng/mL)	HOMA-IR
Non-obese						
Females	23.32±1.05	21.37±0.26	74.35±1.12	12.47±0.78*	18.20±0.86	1.51±0.13*
Males	24.60±1.04	20.95±0.49	71.65±2.06	3.33±0.99	21.56±1.14	1.04±0.85
>18 years, non-obese						
Female	30.97±1.02	22.02±0.37	75.17±1.58	12.39±1.08*	20.81±1.34	1.56±0.20*
Males	28.50± 0.83	21.95±0.54	73.55±2.49	2.20±0.33	21.93±1.27	0.92±0.08
≤18 years, non-obese						
Females	14.71±0.41	20.64±0.33*	73.42±1.15*	12.57±1.15	17.67±1.71	1.45±0.163
Males	13.33±0.97	18.09±0.73	66.16±3.34	6.72±3.82	20.40±2.55	1.40±0.207

Data are presented as mean ± SEM and were compared by *t*-test. *$p<0.05$ was considered as significant.

Table 3. Genotype and allele frequencies of *LEP* gene G-2548A polymorphism according to BMI status and gender

Genotype	Obesity Status			Females			Males		
	Obese n=236	Non-Obese n=131	x^2/P	Obese n=127	Non-Obese n=71	x^2/P	Obese n=109	Non-Obese n=60	x^2/P
Co-dominant Model									
AA	64 (27%)	50 (38%)		33 (26%)	27 (39%)		30 (28%)	21 (35%)	
GA	118 (49%)	54 (41%)	4.89/0.08	69 (54%)	31 (43%)	3.25/0.19	49 (45%)	26 (43%)	1.26/0.53
GG	54 (23%)	27 (21%)		25 (20%)	13 (18%)		30 (28%)	13 (22%)	
Dominant Model									
A/A + G/A	182(76%)	104 (79%)	0.25/0.69	102 (80%)	58 (82%)	0.06/0.81	79 (72%)	47 (78%)	0.7/0.4
G/G	54 (23%)	27 (21%)		25 (20%)	13 (18%)		30 (28%)	13 (22%)	
Recessive Model									
A/A	64 (27%)	50(38%)	4.8/0.02*	33(26%)	27(38%)	3.13/0.07	30 (28%)	21 (35%)	1.03/0.31
G/A +G/G	172 (73%)	81(62%)		94(74%)	44(62%)		79 (72%)	39 (65%)	
Alleles									
A	246 (52%)	154 (58%)	3.01/0.08	135(53%)	85 (60%)	1.66/0.19	109 (50%)	68 (57%)	1.38/0.24
G	226 (48%)	108 (42%)		119(47%)	57 (57%)		109(50%)	52 (43%)	

Chi-square analysis of genotypes and alleles. *p<0.05 was considered statistically significant.

developing countries. The rising prevalence of obesity is an issue of great concern both in children and adults due to adverse health problems associated with it (Hossain *et al.* 2007). It is a multifactorial and heterogeneous condition due to complex interaction of genetic, environmental, behavioural and developmental factors (Herbert 2008). A number of genetic variants are involved in the development of an obese phenotype. The results of previous studies investigating the association of *LEP* G-2548A variant with obesity in different populations have been contentious (Mammes *et al.* 2000; Portolés *et al.* 2006; Wang *et al.* 2006; Duarte *et al.* 2007; Hinuy *et al*. 2008; Constantin *et al*. 2010; Şahın *et al*. 2013; Fan and Say 2014). In the present study, we analysed the association of *LEP* G-2548A polymorphism with obesity and obesity-related anthropometric and metabolic parameters in a sample of Pakistani population. The frequency of G

Table 4. Genotype and allele frequencies of *LEP* gene G-2548A polymorphism according to age (≤18 years) and gender

Genotype	Obese n=75	Non-obese n=45	x^2/P	Females			Males		
				Obese n=30	Non-obese n=31	x^2/P	Obese n=45	Non-obese n=14	x^2/P
Co-dominant Model									
AA	16 (21%)	25 (56%)		7 (23%)	17 (54%)		10 (22%)	5 (36%)	
GA	34 (46%)	11 (24%)	14.67/0.0006*	14 (47%)	7 (23%)	6.73/ 0.034	20 (44%)	4 (28%)	1.44/0.48
GG	25 (33%)	9 (20%)		9 (30%)	7 (23%)		15(33%)	5 (36%)	
Dominant Model									
A/A + G/A	50 (67%)	36 (80%)	2.46/0.11	21 (70%)	24 (77%)		30 (67%)	9 (64%)	0/1
G/G	25 (32%)	9 (20%)		9 (30%)	7 (23%)	2.46/0.11	15 (33%)	5(36%)	
Recessive Model									
A/A	16 (22%)	25 (56%)	14.6/0.0001*	7(23%)	17 (55%)	14.6/0.0001*	10 (22%)	5(36%)	0/ 0.48
G/A +G/G	59 (78%)	20 (44%)		23 (76%)	14 (46%)		35 (78%)	9 (64%)	
Alleles									
A	66 (47%)	61 (68%)	12.76/0.0003*	28 (47%)	41 (66%)	4.7/0.03*	40 (44%)	14 (50%)	0.27/0.60
G	84 (53%)	29 (32%)		32 (53%)	21 (34%)		50 (56%)	14 (50%)	

Chi-square analysis of genotypes and alleles. *p<0.05 was considered statistically significant

Table 5. Genotype and allele frequencies of *LEP* gene G-2548A polymorphism according to age (>18 years) and gender

Genotype	Subjects			Females			Males		
	Obese n=161	Non-obese n=86	x^2/P	Obese n=97	Non-obese n=41	x^2/P	Obese n=64	Non-obese n=45	x^2/P
Co-dominant Model									
AA	46 (29%)	27 (31%)		26 (27%)	11 (27%)		20 (31%)	16 (36%)	
GA	84 (52%)	42 (49%)	0.28/0.86	55 (57%)	24 (58%)	0.08/0.96	29 (45%)	18 (40%)	0.33/0.84
GG	31 (19%)	17 (20%)		16 (16%)	6 (15%)		15(23%)	11 (24%)	
Dominant Model									
A/A + G/A	130 (81%)	69 (80%)	0.01/0.92	81 (84%)	35 (85%)	0.07/0.79	49 (77%)	34 (76%)	0.01/0.90
G/G	31(19%)	17 (20%)		16 (16%)	6 (15%)		15 (23%)	11(24%)	
Recessive Model									
A/A	46 (29%)	27 (31%)	0.21/0.64	26 (27%)	11 (27%)	0/1/0.9	20 (31%)	16 (36%)	0.22/0.63
G/A +G/G	115 (71%)	59 (68%)		71 (73%)	30 (73%)		44 (69%)	29 (64%)	
Alleles									
A	176 (55%)	96 (56%)	0.06/0.80	107 (55%)	46 (56%)	0.02/0.88	69 (54%)	50 (56%)	0.05/0.8
G	146 (45%)	76 (44%)		87 (45%)	36 (44%)		59 (46%)	40 (44%)	

allele was higher in obese subjects compared to non-obese children and adolescents; previous studies have also reported high frequency of G allele in European and Brazilian obese women (Mammes *et al*. 2000; Hinuy *et al*. 2008). We also observed higher G allele frequency in girls in the present study; similar results have been reported by Le Stunff *et al.* (2000) in Caucasian obese girls. Contrariwise, Şahın *et al.* (2013) reported no difference in genotype and allele frequencies of *LEP* G-2548A polymorphism between obese and non-obese Turkish subjects. These differences in the genotype and allele distribution in various studies might be due to ethnic and geographical variation.

The present study reported no significant association of G-2548A polymorphism with obesity in adults; likewise, numerous studies have reported no association of this variant with obesity in Tunisian, Romanian and multi-ethnic adult

Table 6. Anthropometric and metabolic variables of children and adolescents according to G-2548A recessive genetic model

	GG/GA 75	AA 31	*p*-Value
Body weight (kg)	60.6±2.38	51.7±2.98	0.02*
Height (m)	1.49±0.023	1.47±0.04	0.79
BMI (kg/m^2)	26.7±0.68	22.8±0.79	0.001*
Waist (cm)	86.5±1.71	78.1±2.3	0.005*
Hip (cm)	96.1±1.62	86.9±2.11	0.002*
WHR	0.90±0.007	0.90±0.019	0.83
SBP (mmHg)	117±1.98	106±2.68	0.04*
DBP (mmHg)	75.1±1.24	70.5±1.94	0.04*
FBG (mg/dL)	94.5±1.81	86.3±1.21	0.04*
Insulin (μIU/mL)	19.3±2.01	16.2±3.06	0.38
HOMA	3.51±0.42	3.63±0.72	0.87
Leptin(ng/mL)	25.2±1.16	20.3±1.21	0.04*
Leptin receptor(ng/mL)	17.3±2.73	16.2 ±1.43	0.77

Data are presented as mean ± SEM and were analysed by *t*-test. *p<0.05 was considered as significant.

Table 7. Anthropometric and metabolic variables of girls according to G-2548A recessive genetic model

	GG/GA 37	AA 25	*p*-Value
Body weight (kg)	56.9±2.66	49.5±2.61	0.05
Body weight (kg)	56.9±2.66	49.5±2.61	0.05
BMI (kg/m^2)	25.7±0.86	22.5±0.81	0.009*
Waist (cm)	83.4±2.06	76.7±2.41	0.04*
Hip (cm)	95.1±2.25	86.2±2.67	0.01*
WHR	0.87±0.009	0.89±0.02	0.48
SBP (mmHg)	113±2.13	109±4.01	0.33
DBP (mmHg)	73.9±1.35	70.7±2.67	0.29
FBS (mg/dL)	97.5±2.19	88.5±1.99	0.04*
Insulin (μIU/mL)	17.6±3.05	11.9±2.65	0.16
HOMA	3.45±.631	2.59±.630	0.33
Leptin (ng/mL)	28.9±3.15	19.3±2.89	0.02*
Leptin receptor (ng/mL)	18.4±5.39	15.5 ±1.74	0.60

Data are presented as mean ± SEM and were analysed by GLM. *p<0.05 was considered as significant.

Table 8. Genotype and allele frequencies of *LEP* gene G-2548A polymorphism according to clinical conditions

Clinical Conditions	Genotypes			x^2/P	Alleles		x^2/P
	AA	AG	GG		A	G	
Acanthosis nigricans							
Present (n=103)	33(32%)	51(49%)	19(18%)		117(57%)	89(43%)	
Absent (n=127)	37(35%)	34(32%)	34(34%)	1.7/0.42	137(51%)	131(49%)	1.5/0.21
Type 2 diabetes							
Present (n=25)	5(20%)	12(48%)	8(32%)		22(44%)	28(56%)	
Absent (n=237)	59(28%)	106(50%)	47(22%)	1.4/0.48	224(53%)	200(47%)	1.4/0.23
Hypertension							
Present (n=45)	14(31%)	22(49%)	9(20%)		50(56%)	40(44%)	
Absent (n=192)	52(27%)	94(49%)	46(23%)	0.4/0.79	198(52%)	186(48%)	0.4/0.49
Fasting glucose							
Impaired fasting glucose	22(30%)	39(53%)	12(16%)		83(57%)	63(43%)	
Normal fasting glucose	38(28%)	65(48%)	32(24%)	1.51/0.47	141(52%)	129(48%)	0.8/0.36

Chi-square analysis of genotypes and alleles.

Malaysian population (Ben *et al.* 2009; Constantin *et al.* 2010; Fan and Say 2014). However, stratification of data in the present study revealed significant association of this polymorphism with obesity in children and adolescents, and further analysis revealed significant association of this variant with obesity in the subgroup with girls only. The evolutionary theory of aging assumes that the effect of a gene could change over an individual's life span as genetic mutation functioning at late ages are subject to weaker selection than early-acting mutations (Zwaan 1999). Very few studies have been carried out to explore the role of genetic factors in determining changes in adiposity with age. Framingham Heart Study provided the evidence for age-specific linkage effects on BMI of chromosomal regions with a tendency for stronger linkage in younger individuals extended to the sex-specific subsets (Atwood *et al*. 2006). Results from twin studies have presented evidence that changes in BMI over time are influenced by genetic factors (Dina *et al.* 2007; Scuteri *et al*. 2007). Similarly, Bouchard *et al.* (2007) reported that different gene polymorphisms act at different ages and have a role in development of adiposity over a period of time. Riestra *et al*. (2010), in line with our study, reported association of G allele of *LEP* polymorphism with overweight in Spanish pubertal girls, suggesting a gender-specific effect of this polymorphism. On the other hand, a former study carried out in Taiwanese aborigines analysing the association of G-2548A variant with obesity did not report any gender differences (Wang *et al.* 2006). The current study did not report any association of G-2548A variant with obesity in males, whereas Jiang *et al.* (2004) reported the association of this genetic variant with increased BMI in men. It has been reported recently that there is variation in distribution of genotype and the association of *LEP* G-2548A variant with obesity in different ethnic groups and genders (Ng *et al.* 2014b).

Carriers of G allele had significantly higher BMI, WC, HC, FBG and serum leptin levels compared to homozygous A allele carrier children and adolescents in the present study. The study by Wang *et al*. (2006) is in line with ours, reporting significantly higher waist circumference in G allele carriers, suggestive of G-2548A variant's role in central fat deposition. In contrast to these findings, Constantin *et al.* (2010) found no association of this genetic variant with BMI, WC, WHR and other anthropometric parameters in Romanian subjects. Furthermore, a study in Tunisian subjects reported the association of G-2548A polymorphism with obesity, higher BMI, WC and insulin levels in subjects homozygous for AA genotype (Boumaiza *et al.* 2012). Fasting insulin levels, although not statistically significant, were high in G allele carriers as compared to A allele carriers in the present study. Moderately high fasting blood glucose, insulin and leptin levels observed in G allele carriers might serve as a preclinical sign of disturbed carbohydrate and lipid metabolism associated with G-2548A gene polymorphism. The present study reported high leptin levels in G allele carrier children and adolescents. Hyperleptinemia observed in G allele carriers suggests that G-2548A variant might be involved in inducing leptin resistance. Similarly, previous studies reported the association of G allele with high plasma leptin levels in obese Brazilian women and Asian diabetics (Ren *et al.* 2004; Hinuy *et al.* 2008). Contrary to our findings, Mammes *et al*. (1998, 2000) in his first study on French obese subjects reported high leptin levels in A allele carriers,

and subsequently in another study, he reported low leptin levels in G allele carriers. Ben *et al.* (2009) also reported association of G allele with low leptin levels in Tunisian obese women. The present study reported no effect of G-2548A variant on plasma leptin levels in adult obese subjects; likewise, Wang *et al.* (2006) reported no significant effect of this variant on plasma leptin levels in obese adults.

Despite the abundance of data on adults, relatively few studies have been carried out in children regarding the impact of G-2548A on BMI and metabolic traits associated with obesity. In our study, the group with children and adolescents revealed significant differences in genotype and allele frequencies and association of G-2548A gene variant with BMI, WC, HC, FBG and plasma leptin levels. Further analysis of subgroup revealed association of G allele with increased BMI and leptin levels in girls only but not in boys. Subdivision of the total sample in the present study resulted in a decrease in statistical power. Thus, further studies with a larger sample size can be performed to validate the effect of *LEP* G-2548A polymorphism in obese Pakistani girls.

5. Conclusion

The present study found significantly high BMI, WC, HC, WHR, SBP, DBP, FBG, insulin, HOMA-IR and leptin concentration in obese subjects as compared to non-obese subjects. However, leptin receptor levels of obese subjects were significantly lower as compared to non-obese. G allele-carrying female children and adolescents are at high risk of obesity as shown by significant association of G-2548A variant with BMI, WC, HC, FBG and serum leptin levels. However, the above-mentioned findings were not seen in adults. This suggests that G-2548A polymorphism may influence the susceptibility to metabolic disturbances and predisposes to obesity at a younger age. The age- and gender-related differences observed in comparison to previous reports might be due to study population, ethnic differences, sample size and models used for genetic analysis. However, from the results of our current study it can be concluded that *LEP* G-2548A polymorphism may have a role in age- and gender-specific development of obesity in the studied population.

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