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ORIGINAL ARTICLE

# Study of the leptin levels and its gene polymorphisms in patients with idiopathic short stature and growth hormone deficiency

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**Abstract** Leptin levels may regulate fat metabolism, skeletal growth, and puberty. Leptin gene variants affect risk of obesity, cancer, but their effect on onset of growth hormone deficiency (GHD) and idiopathic short stature (ISS) is unknown. We tested the hypothesis that the phenotype of GHD and ISS may be associated with polymorphism in the leptin gene. The prevalence of a single nucleotide polymorphism (SNP) in the leptin gene (*LEP*) promoter at −2548 and the leptin and insulin growth factor-1 (IGF-1) concentrations in GHD and ISS were compared to those of healthy controls. IGF-1 and leptin concentrations were significantly lower in both the GHD and ISS groups than in the control group. The ISS and GHD groups had a significantly different distribution of SNP alleles at the *LEP* −2548 ($P = 0.010$). Individuals with *LEP* −2548A/G or G/G genotype in ISS group (47.5%) showed a significantly lower weight and body mass index (BMI) (but not leptin levels) than individuals carrying the A/A genotype (52.5%). *LEP* −2548A/A in GHD patients (65.8%) was associated with lower weight, BMI, leptin concentrations than those of individuals carrying the A/G or G/G genotype (34.2%). These data suggest that the *LEP* −2548A polymorphism may associate with the weight and BMI of the children with ISS and GHD.

**Keywords** Growth hormone deficiency · Idiopathic short stature · Leptin · SNP polymorphism · Insulin growth factor-1

**Abbreviations**

| | |
|---|---|
| BMI | Body mass index |
| CDGP | Constitutional delayed growth and puberty |
| GHD | Growth hormone deficiency |
| IGF-1 | Insulin growth factor-1 |
| ISS | Idiopathic short stature |
| LEP | Leptin |

## Introduction

Short stature is a common cause for referrals to endocrinologists. Short stature can arise from multiple causes [1]. Deficiency of growth hormone (GH) without effects on other pituitary hormones occurs in many short stature children and has an incidence of 1/3,480–1/10,000 live births [2, 3]. Short stature children without a definitive cause are diagnosed with idiopathic short stature (ISS). ISS is defined as a condition in which the height of an individual is more than 2 standard deviations score (SDS)

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below the corresponding mean height for a given age, sex, and population group without evidence of systemic, endocrine, nutritional, or chromosomal abnormalities [4]. Specifically, children with ISS have normal birth weight and normal levels of GH (GH sufficient). Children with constitutional delayed growth and puberty (CDGP) and familial short stature may be classified as ISS, but they may benefit from alternate treatments [5]. ISS describes a heterogeneous group of children who comprise approximately 60–80% of all short children at or below 2 SDS [6, 7].

While variants in GH gene cause GH deficiency (GHD) short stature, variants of insulin-like growth factor, its receptors and the insulin-like growth factor (IGF) signaling pathway appear to account for approximately 25% of the ISS patients [8]. Additional gene polymorphisms and mutations may account for other cases of ISS. Modifications involving the short stature homeobox (SHOX) gene region (e.g., deletion or duplication) appear to account for 2–15% of ISS [9]. A recent genome- wide single nucleotide polymorphism (SNP) study in Koreans examined 44 height-associated loci for their relationship with ISS and had identified five ISS-associated loci, SPAG17, KBTBD8, HHIP, HIST1H1D, and ACAN [10]. Although HHIP is a hedgehog protein and interacts in the IGF pathway, Yang et al. [11] did not detect SNPs in other IGF related genes previously associated with ISS. In a large SNP survey of people of European descent, height was associated with SNPs of 180 genes. These loci accounted for approx. 10% of the genetic influence [12]. A genome-wide association study which investigated extensive SNPs libraries (294,831 SNPs) can account for 40% of the genetic contributions for height, and it suggests that many genetic influences may be missed by a genome-wide association study which evaluates fewer SNPs and fewer subjects [11]. Thus, other genetic variants must also modulate height in ISS.

Genetic variants of gene(s) associated with satiety may associate with the phenotypes of ISS patients. Children with ISS are poor eaters and have a lower body mass index (BMI) than age-matched controls [7]. Leptin (LEP) levels are associated with the satiety response, onset of puberty, and differentiation of chondrocytes in the epiphyseal growth plate [13]. A significant correlation between changes in leptin and fat and endogenous GH secretion in short children has been documented [14, 15]. Polymorphisms in the leptin gene or its promoter are associated with severe obesity [16], hypertension [17], and higher risk of oral and breast cancer in some studies [18–20]. Abnormal levels of insulin growth factor-1 (IGF-1) and leptin have been reported in both GHD and ISS groups [21, 22]. Furthermore, children and adults with GHD exhibit elevated leptin concentrations in some studies [23, 24], but these high leptin levels were associated with fat-free mass and not to differences in fat mass of the GHD patients [24]. In other studies, leptin concentrations in the serum were significantly associated with fat mass, but were not elevated in patients with GHD [25]. Similarly, Marzullo et al. [26] reported that circulating leptin levels showed no significant difference between GHD patients (9.8 ± 1.6 g/L) and control group (8.7 ± 1.4 g/L). However, leptin levels were significantly higher in homozygous GH insensitivity patients (20.7 ± 4.2 g/L) than in normal controls [26].

One possible mechanism for the disparate leptin level associations with different GHD patients is polymorphisms of the leptin gene or the leptin receptor in GHD patients. The polymorphisms of leptin receptor, and leptin promoter mainly associate with modulation of leptin levels and BMI in obese adults [27–29]. The leptin receptor Q223R polymorphism is associated with obesity [27–29] and the LEP promoter polymorphism at G −2548A influences leptin levels in obese adults [30]. These associations of specific alleles at LEP SNPs or LEP receptor SNPs with obesity raise the possibility that they may be associated with the characteristic of short stature. Comparison between different genotypes (SNP polymorphism) for their leptin expression level may aid in early stage diagnosis of short stature patients. The primary aim of this study was to investigate the contribution of the leptin gene promoter polymorphism (G −2548A) to phenotypic characteristics of GHD and ISS patients.

## Materials and methods

### Subjects

We retrospectively collected and analyzed data from 320 patients who were diagnosed with short stature and followed-up in our pediatric endocrinology clinic during the last 10 years (2000–2010). The inclusion criteria were stature below the 10th centile and growth rate <25th centile during the 12 months following the first observation. All children were pre-puberty, Tanner I stage. Children with constitutional delay of growth and puberty (CDGP), short children with Turner syndrome, chronic renal failure, Prader–Willi syndrome; and short children born small for gestational age (SGA) were excluded from the study. The subjects were screened for GH deficiency: 158 showed GH deficiency and 162 subjects were diagnosed with ISS. Patients who were assigned to the GHD group exhibited the following diagnostic criteria: (i) 2 or more GH levels ≤10 ng/mL or 20 mU/L in the provocative tests [31]; (ii) bone age (BA) diagnosed by a radiologist was at least 2 years lower than chronological age (CA); and (iii) height was third percentile of the average for their age; (iv) growth rate was ≤4 centimeters per year. Children suspected of having brain tumors or central nervous system

(CNS) lesions were examined with X-ray of skull or brain MRI. Patients diagnosed with ISS and nutritional issues, undernourishment, chromosomal diseases, chronic diseases, precocious puberty, brain tumor or irradiation were excluded. A total of 146 children aged 9.28 ± 2.66 years old were recruited as normal controls. Normal controls were children who received a routine examination for growth and development at the out-patient clinic of Chung Shan Medical University Hospital from January 2009 to January 2010. The 89 boys (61%) and 57 girls (39%) in the normal control group were in Tanner Stage I (pre-puberty stage) and had no congenital or chronic disease. GH levels of control individuals were measured by using a physiological test (exercise test, cycle for 10–15 min). The peak GH levels of the tests were recorded. This retrospective analysis was approved by the ethics review committee of our university hospital, and written parental consent was obtained for all participants. The study abided by the ethical standards of the Declaration of Helsinki.

*Evaluation of the short child*

Evaluations included a medical history, family medical history, and a comprehensive physical examination for phenotypic characteristics, body proportions, and pubertal staging. Then patients with or without a particular diagnosis for short stature underwent initial diagnostic testing which included BA, BA/CA ratio, height, weight, BMI and the growth-related hormones, such as IGF-1, GH, free thyroxine (T4), and leptin levels. BMI was calculated as weight (kg)/height (m).

Pharmacological tests of GH secretion

GH deficiency was defined as peak GH ≤10 ng/mL after two of three different pharmacological stimuli (Insulin, clonidine, and levodopa) [31]. Subjects underwent an overnight fast that allowed water. After insertion of cannula and acclimation period, pre-baseline blood sample was drawn at 15–30 min before the application of the stimulus. The insulin-induced hypoglycemia (ITT) (0.1 U/kg soluble insulin) was performed to test the hypothalamic–pituitary–adrenal axis and the GH axis [32]. GH level was measured in blood sample drawn 120 min after the insulin stimulation. Children with a recent history of epilepsy or hypoglycemic convulsions were not tested and children suspected of having hypopituitarism received extra precautions. If the child was symptomatic, blood glucose that was <2.2 mmol/L or reduced by 50% stimulated GH production [9]. As precautions, lunch was provided and children were observed for 90 min. Clonidine stimulation (0.15 mg/m$^2$ orally) was performed and GH level was measured as described [10]. Side effects included drowsiness, pallor, and sometimes a marked fall in blood pressure and children were observed all day. Levodopa was administered orally to stimulate GH release. The dose for Levodopa was 125 mg for the children with body weight (BW) <15 kg, 250 mg for BW 15–30 kg, and 500 mg for BW >30 kg.

Radioimmunoassay (RIA)

Serum total IGF-I, GH, and leptin levels were measured using commercial RIA kits (Diagnostic Systems Laboratories, Inc., Webster, TX). The mean detection level for IGF-I and leptin assays were 0.9 nmol/L and 0.01 ng/mL, respectively. The intra- and inter-assay coefficients of variation (CV) were 7.2 and 9.8% for IGF-I assay and 5.5 and 6.3% for leptin assay, respectively.

Thyroxine (T4) measurement

Serum T4, was measured using an automated chemiluminescent immunoassay (Immulite 2000: Diagnostic Products Corp, Los Angeles, CA). Its sensitivity was 0.3 g/dL for T4. Intra- and interassay CV were 4.6 and 5.6%, respectively.

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)

Blood samples from the ISS group and the GHD group were collected in EDTA-containing tubes. Genomic DNA was extracted by QIAamp DNA blood mini kits (Qiagen, Valencia, CA, USA) according to the instructions of manufacturer. DNA was dissolved in TE buffer [10 mM Tris (pH 7.8), 1 mM EDTA] and then quantitated by optical density at 260 nm. The final preparation was stored at −20°C and used as templates for PCR. PCR-RFLP was used to analyze gene polymorphisms of *LEP* −2548. Sequences of primers used and PCR cycling conditions for analysis of the *LEP* −2548 genotypes are given in Supplemental Table 1. A PCR was performed in a 10 μL volume containing 100 ng of the DNA template, 1.0 μL of 10 μL PCR buffer (Invitrogen, Carlsbad, CA, USA), 0.25 U of Taq DNA polymerase (Invitrogen), 0.2 mM dNTPs (Promega, Madison, WI, USA), and 200 nM of each primer. Digested products were separated on a 2.5% agarose gel and then stained with ethidium bromide. Furthermore, the selected samples showing polymorphisms of *LEP* −2548 by PCR-RFLP were confirmed by DNA sequencing analysis.

Statistical analysis

The demographic and clinical pathological features were presented as mean ± standard deviation (SD) for the

normal subjects, ISS, and GHD groups, and were compared by analysis of variance (ANOVA) test. When a significant difference between groups was apparent, multiple comparisons of means were performed by using the Bonferroni procedure with type-I error adjustment. Age dependent IGF-I SDS means and SD were illustrated with fractional polynomial fit after smoothing the means and SD in each age class. Linear regression models were used to assess the influence of different groups on IGF-I SDS. A Pearson correlation coefficient determined the correlation of leptin levels relative to GH. Genotype frequencies were tabulated as numbers with percentage (%) and analyzed with $X^2$ test. The demographics and clinical pathological features were compared using independent two-sample *t* test between genotypes. In addition, Hardy–Weinberg equilibrium was assessed using goodness-of-fit $X^2$ tests for biallelic markers. The statistical significance was at the *P* value of <0.05. Statistical analyses were performed using SPSS 15.0 statistics software (SPSS Inc, Chicago, IL, USA).

## Results

The demographics and clinical pathological features were compared between the normal, ISS, and GHD groups for significant differences (Table 1). There were significant differences in BA, BA/CA ratio, height, weight, BMI and the growth-related hormones, IGF-1, IGF-1 SDS, GH, T4, and leptin levels among the three groups ($P < 0.05$). Patients in the GHD group displayed the lowest BA, BA/CA, height, weight, and BMI. Moreover, concentrations of GH in the GHD group were significantly lower than the normal control and ISS groups. Figure 1 shows the blotted IGF-I SDS in the ISS and GHD group. Mean values and first and second SDS are shown as smoothed lines. The levels of IGF-1 SDS and leptin were significantly lower in both GHD and ISS groups than those in the control group. Pearson correlation coefficients revealed that leptin levels were significantly correlated with IGF-1 in the control group ($\gamma = 0.184$, $P = 0.027$; Fig. 2a), ISS group ($\gamma = 0.414$, $P < 0.001$; Fig. 2b) and the GHD group ($\gamma = 0.317$, $P < 0.001$; Fig. 2c). IGF-1 levels was also strongly associated with BMI in normal group ($r = 0.265$, $P = 0.0008$), ISS ($r = 0.293$, $P = 0.0002$), and GHD ($r = 0.323$, $P < 0.0001$). However, patients in GHD group exhibited significantly higher T4 levels than normal controls.

No significant differences were observed in the percent distribution of alleles (A/A, AG, and G/G) at *LEP* −2548 among the three groups (Table 1). The percent distribution of the *LEP* −2548 stratified into A/A, AG, and G/G genotypes was 57.5, 35.6, 6.9% in the normal group, versus 52.5, 40.7, and 6.8% in the ISS group, and 65.8, 26.6, and 7.6% in the GHD group, respectively.

The distribution of alleles at *LEP* −2548 SNP was compared between groups (Table 2). The distribution of the genotypes at the *LEP* −2548 SNP stratified into two sets as A/A, and [AG/or G/G] was 84 patients (57.5%), 62 patients (42.5%) in the normal group, respectively. Similarly, the distribution of A/A and [AG/or G/G] was 85 (52.5%) and 77 (47.5%) in the ISS group, and 104 (65.8%) and 54 (34.2%) in the GHD group, respectively. Only ISS and GHD groups showed a significant difference in the distribution of the alleles at the *LEP* −2548 ($P = 0.010$); the two groups also exhibited a significant difference between the incidence of the A and G alleles at the *LEP* −2548 ($P = 0.039$). Furthermore, only boys had significantly different frequencies of alleles at LEP −2548 SNP between the ISS and GHD groups ($P = 0.043$). The control group exhibited an incidence of 75.3% for A, and 24.7% for G at *LEP* −2548, and the ISS group were 72.8% A and 27.2% G. The GHD group had the A allele 79.1%, and the G allele 20.9%, respectively.

The potential role of the genotypes of *LEP* −2548 in the demographics and pathological features were examined and compared for the three groups. The normal control group did not show any significant association between the [A/G or G/G] alleles at *LEP* −2548 with demographics and pathological features ($P > 0.05$). In the ISS group, higher weight and BMI were associated with specific alleles at *LEP* −2548 ($P < 0.05$). Moreover, the GHD group exhibited a significant association between the [A/G or G/G] alleles at *LEP* −2548 with weight, BMI, and leptin levels ($P < 0.05$) (Table 3).

## Discussion

Comparison of clinical features in our study revealed that the GHD patients had lower BA, BA/CA, height, weight, and BMI than the ISS group and controls. Leptin in short stature subjects has been postulated to be the messenger of adipose tissue for hypothalamic regulation of GH production [15]. The levels of IGF-1 and leptin in both the GHD and ISS groups were significantly lower than those in the control group, in agreement with previous report [21]. Pearson correlation coefficients revealed that leptin levels were significantly correlated with IGF-1 in the three groups, similar to Zotter et al. [21]. Taken together, the findings indicated that relatively low levels of leptin in GHD and ISS patients might contribute to regulation of children's growth by affecting IGF-1 levels.

Zotter et al. [21] investigated the effect of standard insulin tolerance test on plasma leptin levels in children with ISS and in children with GHD. Plasma leptin levels were significantly lower in all patients at 60 min ($P < 0.001$) and 120 min ($P < 0.001$) after insulin

**Table 1** Demographics and clinical pathological features of subjects in normal, ISS, and GHD groups

| Variable | Normal ($n = 146$) | ISS ($n = 162$) | GHD ($n = 158$) | $P$ value |
|---|---|---|---|---|
| Gender, $n$ (%)[a] | | | | |
| Male | 89 (61.0) | 79 (48.8) | 91 (57.6) | 0.081 |
| Female | 57 (39.0) | 83 (51.2) | 67 (42.4) | |
| CA (year)[b] | 9.28 ± 2.66 | 8.81 ± 3.67 | 8.40 ± 3.25 | 0.059 |
| BA (year)[b] | 8.56 ± 3.22 | 7.18 ± 3.89[c] | 6.04 ± 3.40[c,d] | <0.001* |
| BA/CA[b] | 0.91 ± 0.21 | 0.79 ± 0.21[c] | 0.69 ± 0.19[c,d] | <0.001* |
| Height (cm)[b] | 133.88 ± 15.92 | 119.98 ± 19.89[c] | 115.22 ± 16.53[c,d] | <0.001* |
| Weight (kg)[b] | 31.84 ± 11.73 | 25.08 ± 10.44[c] | 22.35 ± 9.39[c,d] | <0.001* |
| BMI (kg/m$^2$)[b] | 17.17 ± 3.47 | 16.62 ± 3.00 | 16.07 ± 2.60[c] | 0.007* |
| IGF-1 (ng/mL)[b] | 216.40 ± 123.52 | 171.36 ± 111.17[c] | 149.03 ± 118.59[c] | <0.001* |
| IGF-1 SDS[†] | 0.00 ± 0.91 | −0.31 ± 0.87[c] | −0.37 ± 1.17[c] | 0.003 |
| GH (ng/mL)[b] | 10.82 ± 12.31 | 9.58 ± 8.68 | 3.86 ± 3.70[c,d] | <0.001* |
| T4 (μg/dL)[b] | 8.39 ± 2.06 | 9.10 ± 4.27 | 9.15 ± 1.96 | 0.056 |
| Leptin (ng/ml)[b] | 4.47 ± 4.81 | 3.31 ± 3.62[c] | 3.18 ± 3.88[c] | 0.012* |
| *LEP* −2548, $n$ (%)[a] | | | | |
| A/A | 84 (57.5) | 85 (52.5) | 104 (65.8) | 0.119 |
| A/G | 52 (35.6) | 66 (40.7) | 42 (26.6) | |
| G/G | 10 (6.9) | 11 (6.8) | 12 (7.6) | |

$N = 466$

Pair-wise multiple comparisons between groups were determined using Bonferroni's test with $\alpha = 0.017$ adjustment

* Significant difference among the three groups, $P$ value $< 0.05$

[†] Age dependent IGF-I SDS

[a] Data were presented as number (percentage) with $X^2$ test/Fisher exact test; and [b] mean ± SD with ANOVA test. Mean peak GH was listed

[c] Indicated a statistically significant difference between the indicated group and the control group, $P < 0.05$

[d] Indicated a statistically significant difference between ISS group and GHD group $P < 0.05$

administration. This effect was independent of GHD: similar leptin declines were detected in ISS and GHD patients. A correlation was found when comparing plasma leptin levels of all patients to BMI SDS ($r = 0.43$; $P < 0.001$) and plasma IGF-1 levels ($r = 0.31$; $P < 0.01$) [16]. Although women have higher leptin concentrations than men [33] and regulation can differ by gender in children [34], the gender difference between groups in our study was not significant. Diverse BMIs and modest variation in gender between controls and ISS patients may contribute to differences in their leptin levels. However, it is intriguing that individuals with LEP −2548A/G or G/G genotype in the ISS group showed a significantly lower weight and BMI but not leptin levels. The mechanism is elusive and studies on larger groups are necessary to confirm the observation.

The ISS and GHD groups showed a significant difference in the distribution of the SNPs at the *LEP* −2548. The SNP at *LEP* −2548 was significantly associated with the demographics and pathological features among the patients with short stature and it differed between the ISS and GHD groups. Individuals with ISS had almost equal genotype distribution between A/A genotype and A/G or G/G genotype, and ISS children carrying an A/G or G/G genotype showed lower weight and BMI than individuals carrying A/A genotype. In contrast, approximate two-thirds of GHD patients carried A/A genotype, and the GHD patients carrying an A/A genotype showed lower weight and BMI than individuals carrying A/G or G/G genotype. This observation suggested that the same genotype might have distinct phenotype between ISS and GHD children, and the distribution of genotypes had correlated well with short status phenotype. However, the retrospective study design may hinder the detection of an association between GHD, ISS, and leptin gene polymorphism.

An association between the *LEP* −2548G/A variant of the leptin gene and pathogenesis, severity of chronic obstructive pulmonary disease in the Chinese population was found [35]. A significant association between the variation of *LEP* −2548G/A allele with BMI, serum leptin levels, and FPG was observed in Type 2 diabetes mellitus patients [36]. The leptin promoter polymorphism *LEP* −2548G/A is associated with increased leptin secretion and elevated cancer risk, such as colon cancer and breast carcinoma [19, 37]. These studies suggest that this

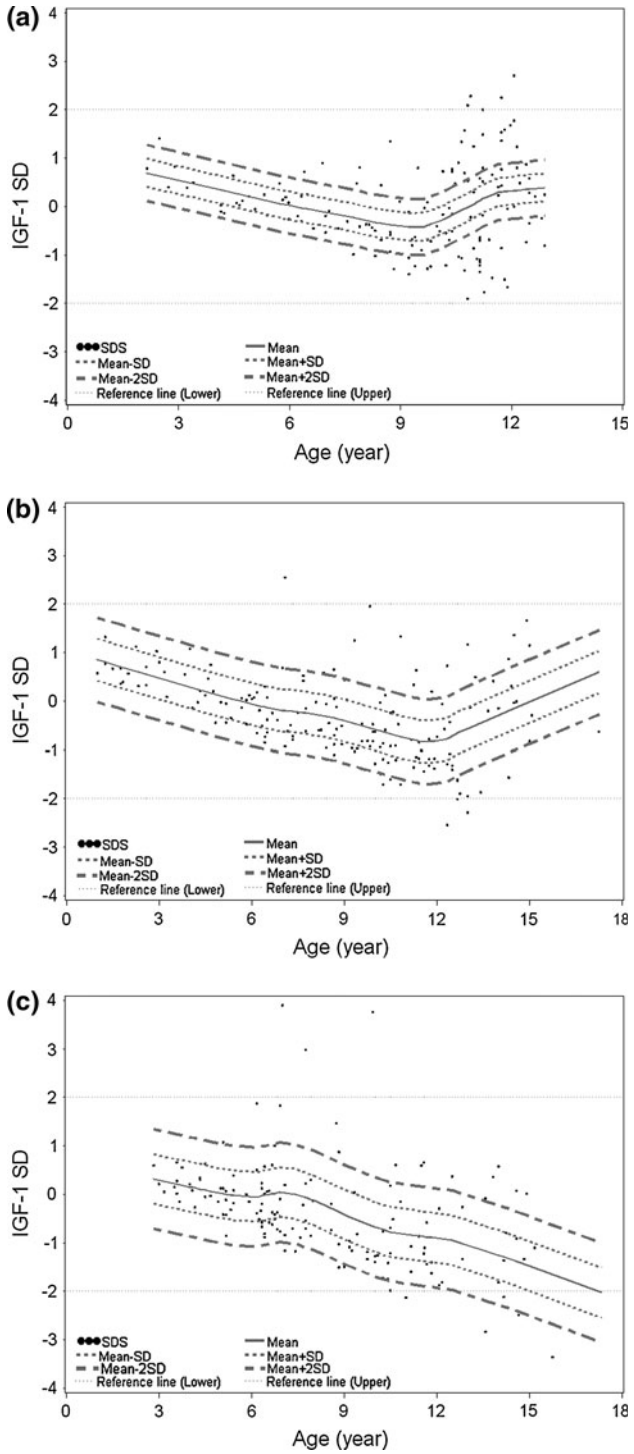


**Fig. 1** IGF-I SDS blotted over age with smoothed lines indicating mean values and first and second S.D. **a** Control group. **b** ISS group. **c** GHD group

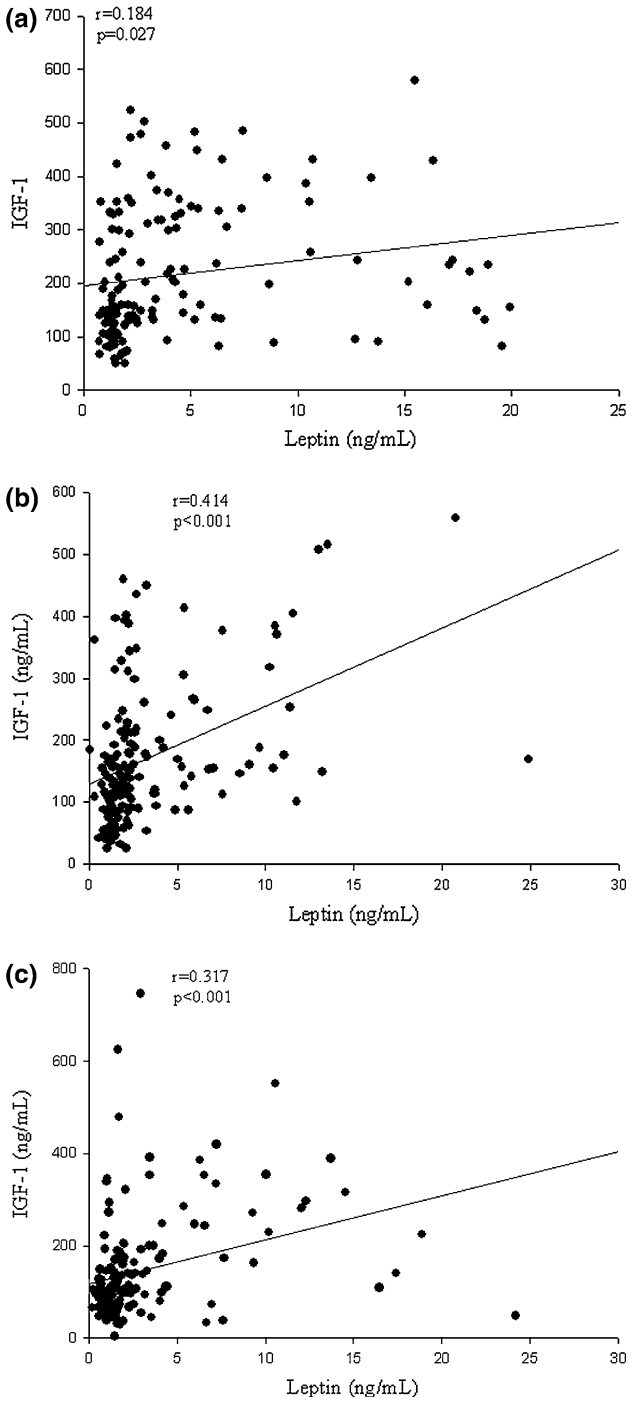


**Fig. 2** Correlation between leptin levels and IGF-1 levels. **a** Control group. **b** ISS group. **c** GHD group

polymorphism affects growth, weight gain, and cellular growth and is consistent with a potential role of leptin in the ISS and GHD short statue phenotypes. *LEP* −2548G/A is proximal to a binding site for the transcriptional factor Sp1. Furthermore nucleolin, a transcriptional repressor, can bind Sp1 or its consensus site [38]. In obese patients, serum leptin concentration shows an independent inverse correlation with bone mineral density and male sex, but a positive correlation with total fat mass and the presence of *LEP* −2548A allele of leptin gene. These parameters are responsible for 83% of leptin concentration variability. No significant correlations have been reported between the

**Table 2** The distribution of SNPs at *LEP* −2548 among normal, ISS and GHD groups

| Variable | Normal ($n = 146$) | ISS ($n = 162$) | GHD ($n = 158$) | *P* value N:ISS | *P* value N:GHD | *P* value ISS:GHD |
|---|---|---|---|---|---|---|
| *LEP −2548 genotypic frequencies* | | | | | | |
| Total (%) | | | | | | |
| A/A | 84 (57.5) | 85 (52.5) | 104 (65.8) | 0.219 | 0.086 | 0.010* |
| A/G or G/G | 62 (42.5) | 77 (47.5) | 54 (34.2) | | | |
| Male (%) | | | | | | |
| A/A | 54 (56.2) | 40 (50.6) | 60 (65.9) | 0.458 | 0.175 | 0.043* |
| A/G or G/G | 42 (43.8) | 39 (49.4) | 31 (34.1) | | | |
| Female (%) | | | | | | |
| A/A | 38 (60.3) | 45 (54.2) | 44 (65.7) | 0.461 | 0.527 | 0.156 |
| A/G or G/G | 25 (39.7) | 38 (45.8) | 23 (34.3) | | | |
| *Prevalence of allele* | | | | | | |
| Total (%) | | | | | | |
| A | 220 (75.3) | 236 (72.8) | 250 (79.1) | 0.269 | 0.156 | 0.039* |
| G | 72 (24.7) | 88 (27.2) | 66 (20.9) | | | |
| Male (%) | | | | | | |
| A | 146 (76.0) | 114 (72.2) | 144 (79.1) | 0.407 | 0.476 | 0.134 |
| G | 46 (24.0) | 44 (27.8) | 38 (20.9) | | | |
| Female (%) | | | | | | |
| A | 94 (74.6) | 122 (73.5) | 106 (79.1) | 0.831 | 0.389 | 0.258 |
| G | 32 (25.4) | 44 (26.5) | 28 (20.9) | | | |

Data were presented as numbers (%) with $X^2$ test

* Significance *P* value $< 0.05$

**Table 3** Comparison of demographics and pathological features between two genotypes of *LEP* −2548 for normal, GHD, and ISS groups

| | Normal group | | ISS group | | GHD group | |
|---|---|---|---|---|---|---|
| | A/A ($n = 84$) | A/G or G/G ($n = 62$) | A/A ($n = 85$) | A/G or G/G ($n = 77$) | A/A ($n = 104$) | A/G or G/G ($n = 54$) |
| Chronologic age (year) | 9.07 ± 2.83 | 9.57 ± 2.40 | 9.28 ± 3.64 | 8.29 ± 3.65 | 8.13 ± 3.19 | 8.90 ± 3.33 |
| BA (year) | 8.42 ± 3.32 | 8.75 ± 3.10 | 7.70 ± 3.96 | 6.61 ± 3.76 | 5.84 ± 3.28 | 6.43 ± 3.62 |
| BA/CA | 0.91 ± 0.18 | 0.91 ± 0.24 | 0.80 ± 0.19 | 0.80 ± 0.23 | 0.69 ± 0.19 | 0.69 ± 0.20 |
| Height (cm) | 133.14 ± 17.20 | 134.88 ± 14.07 | 121.83 ± 19.89 | 117.95 ± 19.83 | 113.67 ± 16.57 | 118.19 ± 16.19 |
| Weight (kg) | 31.55 ± 12.01 | 32.23 ± 11.42 | 26.78 ± 11.05 | 23.21 ± 9.46* | 21.11 ± 8.45 | 24.75 ± 10.66* |
| BMI ($kg/m^2$) | 17.15 ± 3.46 | 17.20 ± 3.53 | 17.22 ± 3.51 | 15.95 ± 2.13* | 15.67 ± 2.03 | 16.85 ± 3.31* |
| IGF-1 (ng/mL) | 217.89 ± 137.64 | 214.37 ± 104.30 | 179.07 ± 113.25 | 162.85 ± 108.94 | 145.02 ± 127.42 | 156.97 ± 99.57 |
| IGF-1 SDS | 0.07 ± 0.98 | −0.09 ± 0.80 | −0.39 ± 0.86 | −0.21 ± 0.87 | −0.34 ± 1.23 | −0.43 ± 1.03 |
| GH (ng/mL) | 11.40 ± 13.04 | 10.04 ± 11.31 | 10.18 ± 9.55 | 8.89 ± 7.59 | 3.77 ± 3.48 | 4.03 ± 4.12 |
| T4 (μg/dL) | 8.60 ± 1.69 | 8.11 ± 2.47 | 8.85 ± 1.82 | 9.38 ± 5.92 | 9.18 ± 1.89 | 9.10 ± 2.12 |
| Leptin (ng/ml) | 4.27 ± 4.69 | 4.75 ± 5.00 | 3.82 ± 4.36 | 2.74 ± 2.49 | 2.64 ± 2.83 | 4.24 ± 5.24* |

[a] Data were presented as mean ± SD with independent two-sample *t* test

* Significance *P* value $< 0.05$

examined leptin gene polymorphisms and bone mineral density or bone mineral content [39]. The *LEP* −2548 G/G homozygote plays a genetic recessive role in the development of extreme obesity in Taiwanese aborigines [16].

## Conclusions

In summary, these studies show that IGF-1 and leptin levels in both the GHD and ISS groups were significantly

lower than those in the control group. The ISS and GHD groups had a significantly different distribution of SNP alleles at the *LEP* −2548, especially in male. In ISS group, individuals with *LEP* −2548A/G or G/G genotype showed a significantly lower weight and BMI than individuals carrying the A/A genotype. However, in GHD patients, individuals with *LEP* −2548A/G or G/G genotype showed a significantly higher weight, BMI, and leptin concentrations than individuals carrying the A/A genotype.

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**Conflict of interest** The authors declare that they have no conflict of interest.

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