

# Serum leptin levels and GHR-d3/fl gene polymorphism in acromegalic patients with thyroid nodules

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## Abstract

**Background.** Acromegaly is a rare and serious syndrome that is commonly associated with pituitary neoplasms. Thyroid multinodular disease is a common finding in acromegaly. Leptin is a polypeptide hormone, and studies have shown that it can increase cell proliferation and inhibit apoptosis.

**Objectives.** The aim of the study was to determine the relationship of serum leptin levels with certain blood parameters and determine if growth hormone receptor (GHR)-d3/fl gene polymorphism is associated with thyroid nodules in acromegalic patients.

**Material and methods.** A total of 24 acromegalic patients with or without thyroid nodules were included in the study. Gene polymorphisms and blood parameters were examined.

**Results.** A marked increase was observed in serum leptin concentration in acromegalic patients with thyroid nodules compared to patients without them ( $p < 0.05$ ). GH levels were lower in patients without nodules than in patients with nodules ( $p < 0.05$ ). Blood glucose levels were higher in patients with nodules compared to those without them ( $p < 0.05$ ), and the presence of thyroid nodules was associated with decreased blood low-density lipoprotein (LDL) levels compared to patients without nodules ( $p < 0.05$ ). A significant relationship was observed between growth hormone receptor (GHR)-d3/fl gene polymorphism and leptin levels in acromegalic patients with thyroid nodules ( $p < 0.001$ ).

**Conclusions.** These data from acromegalic patients indicate that thyroid nodules are associated with increased serum leptin, GH and blood glucose levels and with decreased LDL levels. GHR-d3/fl gene polymorphism status was strongly related to higher leptin levels.

**Key words:** thyroid, gene polymorphism, acromegaly, hormone, endocrinology

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Acromegaly is associated with excessive secretion of growth hormone (GH) and insulin-like growth factor-1 (IGF-1), resulting in bone overgrowth and an increase in soft tissue.<sup>1</sup> Thyroid nodules are a common clinical problem in females. Epidemiological studies have shown the prevalence of palpable thyroid nodules to be approximately 5% in women and 1% in men living in iodine-sufficient parts of the world.<sup>2,3</sup> In contrast, high-resolution ultrasound can detect thyroid nodules in 19–67% of randomly selected individuals, with higher frequencies in women and the elderly.<sup>4</sup> Thyroid enlargement can also be seen in acromegalic patients.<sup>5,6</sup> Long-term elevation of serum GH levels in acromegaly is associated with goiter development and is probably caused by the mitogenic effects of IGF-1.<sup>7</sup>

Changes in enzyme activity may lead to somewhat higher serum triiodothyronine (T3) and lower reverse T3 (rT3) concentrations in patients with active acromegaly, although the levels are usually within physiological ranges.<sup>8,9</sup> The prevalence rates of certain benign and malignant neoplasms are higher in acromegalic patients compared with the healthy population.<sup>10</sup>

Leptin is a potent anorexigenic hormone found in the anterior pituitary and secreted by adipose tissue in proportion to adipose content. It is a product of the obesity (Ob) gene that regulates both satiety and energy expenditure.<sup>11</sup> Leptin is secreted with diurnal fluctuations, and greater secretion is observed in subjects who are obese or who have insulin resistance.<sup>12,13</sup> While a negative influence of thyroid hormones on serum leptin concentrations has been described, some authors report no influence of thyroid hormones on leptin levels.<sup>14–17</sup>

There are many reports concerning the stimulatory effect of leptin on cell mitosis and its involvement in the carcinogenesis of breast, colon, prostate, lung, kidney and ovary cells. Studies have shown that leptin's abilities to increase cell proliferation and inhibit apoptosis are involved in creating certain types of tumors.<sup>18–24</sup> Specifically, increased expression of leptin and its receptor are well documented in papillary thyroid cancer. Similar to other tumor types, some authors reported that leptin exerts oncogenic effects on papillary thyroid carcinoma cells by stimulating cell proliferation and inhibiting apoptosis.<sup>25–27</sup>

The principal regulator of GH sensitivity is the GH receptor (GHR) and consists of an extracellular domain of 246 amino acids, a single transmembrane domain, and a cytoplasmic domain. The encoding gene has 9 exons, but there are 2 isoforms of the GHR in humans generated by the deletion of exon 3 of the gene, resulting in 3 genotypes: homozygous GHR-fl, homozygous GHR-d3, and heterozygous GHR-d3/fl.<sup>28</sup>

## Objectives

Acromegaly is frequently associated with thyroid enlargement, and the size is dependent on the duration of

GH excess.<sup>5,6</sup> Thyroid multinodular disease is a common finding in acromegaly.<sup>5,7,29,30</sup> The reported frequencies of diffuse goiter and nodular goiter in acromegalic patients are 78–92 and 63%, respectively.<sup>5</sup> Leptin, an Ob gene product, is secreted exclusively by adipocytes and regulates energy balance. Because GH modulates fat mass, it is possible that GH and chronic GH excess affects leptin. The aim of this study was to examine leptin levels in acromegalic patients, with or without thyroid nodules, and to identify any relationship with GHR gene polymorphism.

## Material and methods

### Study design

The study was performed according to the Helsinki Declaration and was approved by the Ethical Committee of Pamukkale University (09/198). All the patients and volunteers provided written acknowledgement of informed consent for participation. The study group included 24 patients with acromegaly (13 males, 11 females; mean age, 52.04 ± standard deviation [SD] 9.46; range, 30–68 years), including 17 patients with thyroid nodules (9 males and 8 females; mean age, 51.05 ± SD 9.66; range, 19–84 years) and 7 patients without thyroid nodules (4 males, 3 females; mean age, 46 ± SD 17.6; range, 30–68 years). The participants' anthropometric measurements and blood parameters were evaluated.

The diagnosis of acromegaly was established on the basis of criteria proposed by Freda and confirmed by pathological examination of surgically resected tissues<sup>31</sup>. Clinical signs, magnetic resonance imaging (MRI), and GH and IGF-1 levels were assessed to support the diagnosis. Active acromegaly was associated with increased serum levels of GH and IGF-1, and modified sellar morphology visible on MRI, indicating the presence of a pituitary adenoma.

The main inclusion criteria were age over 18 years and the presence of acromegaly.

The patients included in the study were not surgically or medically treated for acromegaly (e.g. with somatostatin, dopamine analogs or GH receptor antagonists) at the time of inclusion in the study. Exclusion criteria included pregnancy, the use of any drugs at the time of the study or current surgical treatment for thyroid nodules. On this basis 11 patients were excluded from the study.

### Clinical and laboratory assessment

Height and weight were measured in light clothing without shoes. Body height and weight were measured by a stameter and digital electronic scale, respectively. Body mass index (BMI) was calculated as the patient's weight in kilograms divided by the square of his/her height in meters. The average value of systolic and dia-

stolic blood pressure measurements were taken with the subject in a sitting position at 2- to 3-min intervals after resting for at least 15 min.

Serum levels of glucose, total cholesterol, triglyceride, high- and low-density lipoprotein (HDL and LDL) cholesterol, alkaline phosphatase (ALP), calcium and phosphorus were analyzed with commercial kits (Beckman-Coulter Inc., Brea, USA) in an LX-20 autoanalyzer (Beckman-Coulter Inc., Brea, USA). Levels of serum insulin, free T3, free thyroxine (T4), thyroid-stimulating hormone, prolactin, GH, leptin, IGF-1 and insulin-like growth factor-binding protein 3 (IGFB3) were measured in an Immulite 2000 immunoassay analyzer (Siemens Healthcare, Erlangen, Germany) using the chemiluminescence method.

## Genetic analysis

DNA was isolated from peripheral blood with a standard phenol/chloroform extraction method. Genotyping for the d3 gene was performed by the polymerase chain reaction (PCR) method using a personal thermal cycler (Techgene, USA). PCR was conducted in 50  $\mu$ L reaction mixture containing about 1  $\mu$ g of DNA sample, 5  $\mu$ L reaction buffer (x10) include 160 mM  $(\text{NH}_4)_2\text{SO}_4$ , 670 mM Tris-HCl (pH 8.8), 0.1% Tween-20, 5  $\mu$ L dNTP (2 mM), 3  $\mu$ L  $\text{MgCl}_2$  (25 mM), 1 U platinum Taq-polymerase and 100 pmol of each primer. The following primers were used: G1 5'-TGT GCT GGT CTG TTG GTC TG-3', G2 5'-AGT CGT TCC TGG GAC AGA GA-3' and G3 5'-CCT GGA TTA ACA CTT TGC AGA CTC-3' (32). Amplification was performed for 39 cycles comprised of denaturation, extension and annealing at temperatures of 94°C for 30 s, 57°C for 30 s, and 68°C for 30 s, respectively. The final extension time was carried out at 68°C for 10 min. The initial denaturation stage was carried out at 95°C for 2 min. The fragments obtained were electrophoresed in a 1% agarose gel and visualized by ethidium bromide staining under ultraviolet (UV) light. The polymorphism detected by PCR was evident as a 935-bp fragment in the presence of the full-length (fl/fl) fragment and as a 592-bp product in the presence of the deletion (d3/d3). Each sample was described as d3/d3, d3/fl, or fl/fl.

## Statistical analysis

Descriptive statistics, proportions for categorical variables, and means and SDs for continuous variables were used to describe the study groups. Comparisons between acromegalic patients with and without thyroid nodules were made with Student's *t*-tests. The results were expressed as means  $\pm$  standard error. Mann-Whitney *U* tests were performed to analyze between-group differences in continuous variables. Values were considered to be statistically significant when  $p < 0.05$ . Comparisons of gene polymorphisms and leptin levels were made by one-

way analysis of variance (ANOVA). Regression analysis was used to tease out the impact of the genotype. All statistical analyses were carried out using SPSS 10.0 software (SPSS Inc., Chicago, USA).

## Results

A total of 24 acromegalic patients were included: 13 males (9 with nodules and 4 without) and 11 females (8 with nodules and 3 without). The mean ages of the participants with and without nodules were  $51.05 \pm 9.66$  and  $54.57 \pm 9.18$ , respectively ( $p > 0.05$ ). The participants' anthropometric measurements and blood parameters are shown in Table 1. In the patients with nodules, statistically significant increases were observed in IGFB3, blood glucose and leptin levels, and decreases were noted for GH, BMI, homeostasis model assessment (HOMA), LDL and phosphorus (P) levels.

In this study, the frequencies of the GHR-fl/fl, d3/d3, and d3/fl genotypes were 62.5, 29.1, and 8.34%, respectively. There was a strong relationship between gene polymorphism status and leptin levels in acromegalic patients (Table 2). A marked increase was observed in serum leptin levels in patients with the GHR-d3/fl genotype ( $p < 0.01$ ).

## Discussion

Acromegaly is an acquired disorder related to excessive production of GH and IGF-1 and characterized by progressive somatic disfigurement and systemic manifestations.<sup>33</sup> Leptin, a neuroregulatory peptide, is secreted by adipose tissue in proportion to adipose content. Because GH modulates fat mass, the study was undertaken to investigate the possible effects of acromegaly on leptin in patients with and without thyroid nodules.

The growth-promoting effects of GH on thyroid follicular cells or a concurrent TSH excess are the conventional hypotheses linking acromegaly to thyrotoxicosis.<sup>30</sup> Current evidence favors a TSH-independent mechanism in most cases.<sup>34</sup> In addition, G protein abnormalities can constitutively activate GH-releasing hormone (GHRH) receptors leading to acromegaly, as well as cause constitutive TSH receptor activation leading to thyrotoxicosis.<sup>35</sup> This may also be a possible mechanism underlying the combination of acromegaly and thyroid nodules studied here.

In this study, serum leptin levels in acromegalic patients with different GHR genotypes (fl/fl, d3/d3, or d3/fl) were examined, and a statistically significant relationship between gene polymorphism status and nodule formation was observed. While no differences were found in homozygous fl and d3 patients, a marked predisposition for thyroid nodule development was observed in patients that were heterozygous (d3/fl). Specifically, patients with the GHR-d3/fl genotype exhibited both increased leptin

**Table 1.** Mann Whitney U test results of clinical and laboratory characteristics of acromegalic patients with or without thyroid nodule

Parameters	Acromegalic patient with thyroid nodule (n = 17)		Acromegalic patient without thyroid nodule (n = 7)		U	p-value
	mean rank	sum of ranks	mean rank	sum of ranks		
Age (years)	14.07	201.50	11.85	98.50	48.500	ns
BMI (kg/m <sup>2</sup> )	14.00	238.00	8.86	62.00	34.000	< 0.05*
Weight (kg)	13.38	227.50	10.36	72.50	44.500	ns
Height (cm)	12.29	209.00	13.00	91.00	56.000	ns
Fasting glucose (mg/dL)	14.41	245.00	7.86	55.00	27.000	< 0.05*
Insulin (μIU/mL)	13.53	203.00	10.00	70.00	42.000	ns
HOMA	13.97	237.50	8.93	62.50	34.500	< 0.05*
GH (ng/L)	11.97	203.50	15.79	96.50	50.500	< 0.05*
IGF1 (ng/L)	12.76	217.00	11.86	83.00	55.000	ns
IGFB3 (μg/L)	11.76	200.00	14.29	100.00	47.000	< 0.05*
Total cholesterol (mg/dL)	13.41	228.00	10.29	72.00	44.000	ns
Triglyceride (mg/dL)	13.35	227.00	10.43	73.00	45.000	ns
LDL (mg/dL)	12.12	206.00	13.43	94.00	53.000	ns
HDL (mg/dL)	12.26	208.50	13.07	91.50	55.000	ns
TSH (μIU/mL)	12.94	220.00	11.43	80.00	52.000	ns
Leptin (ng/mL)	13.24	225.00	9.71	75.00	47.000	< 0.05*
FT3 (pg/mL)	12.15	206.50	13.36	93.50	53.500	ns
FT4 (ng/dL)	11.97	203.50	13.79	96.50	50.500	ns
COR (μg/dL)	13.26	225.50	10.64	74.50	46.000	ns
PRL (ng/mL)	12.88	219.00	11.57	81.00	53.000	ns
CA (mg/dL)	12.29	209.00	13.00	91.00	56.000	ns
P (mg/dL)	13.85	235.50	9.21	64.50	36.500	< 0.05*
ALP (IU/L)	13.16	210.50	9.36	65.50	37.500	ns
Right thyroid lobe (mm)	12.38	210.50	12.79	89.50	57.500	ns
Left thyroid lobe (mm)	13.21	224.50	10.79	75.50	47.500	ns
Isthmus (mm)	10.22	163.50	14.92	89.50	27.000	ns

ns – non-significant, \* significant differences between the groups (p < 0.05).

**Table 2.** GHR gene polymorphisms; leptin, IGF-1, and GH levels; and BMI in acromegalic patients with or without thyroid nodule

Patients	n	Leptin level	BMI	IGF-1	GH
fl/fl genotypes with nodule	11	6.44 ± 9.09 <sup>a</sup>	28.70 ± 4.61	740.54 ± 326.89	15.77 ± 11.96
fl/fl genotypes without nodule	4	3.25 ± 4.74 <sup>a</sup>	25.72 ± 2.99	436.50 ± 300.98	9.86 ± 7.07
d3/d3 genotypes with nodule	4	7.57 ± 8.60 <sup>a</sup>	28.60 ± 2.65	393.50 ± 272.94	5.36 ± 3.37
d3/d3 genotypes without nodule	3	4.79 ± 2.80 <sup>a</sup>	26.93 ± 1.66	1045.00 ± 677.62	31.43 ± 25.93
d3/fl genotypes with nodule	2	28.19 ± 3.46 <sup>b</sup>	35.15 ± 0.63	563.00 ± 216.37	4.96 ± 9.19
p-value		< 0.01**	ns	ns	ns

\*\* The differences between the means of groups carrying different letters in the same column are statistically significant (p < 0.01), ns – non-significant.

levels and a predisposition to nodule formation. Notably, nodules were found in all patients with the GHR-d3/fl genotype (Table 2).

GH plays an important role in the regulation of adiposity and modulates fat deposition and accumulation via regulatory molecules in preadipocytes and adipocytes.<sup>36</sup> Some authors have reported that GH excess is associated with decreased leptin levels and decreased fat mass.<sup>37</sup> In con-

trast, the present study found that increased leptin levels were associated with decreased GH levels in acromegalic patients with thyroid nodules. Lower leptin levels have been reported in acromegalic patients.<sup>1,38</sup> Different treatments for acromegaly lead to increased leptin levels.<sup>39–41</sup> Collectively, the evidence indicates that leptin secretion is reduced in active acromegaly, presumably reflecting reduced body fat stores. Restoration of GH secretion is as-

sociated with an increase in leptin levels, probably due to an increase in fat stores.<sup>1</sup> Leonhardt et al. reported a significant increase in serum leptin levels in a hypothyroid patient compared to hyperthyroid and normal controls.<sup>42</sup> Similar results were reported by Yoshida et al.<sup>43</sup> Leptin is also related to the progression of some tumors, including thyroid cancers.<sup>18–22,24–27</sup>

In the present study, a marked increase in leptin levels was observed in acromegalic patients with thyroid nodules compared to those without. These findings indicate that the thyroid may have a greater effect on leptin levels than the pituitary. The mechanism underlying the link between leptin and LDL levels is not clear. However, some authors have reported that leptin has a pro-oxidant effect. Previous reports have described leptin having a direct effect on endothelial cell generation of reactive oxygen species that have been shown to play a role in LDL oxidation.<sup>44–46</sup> In this study, decreased LDL levels may be related to higher leptin levels in acromegalic patients with thyroid nodules.

GHR polymorphism has been reported in acromegaly, but to the best of the authors' knowledge, this is the first study investigating anthropometric measurements, blood parameters and gene polymorphism in acromegalic patients with or without thyroid nodules.<sup>32</sup> Although the study included only a small group of patients because of the rarity of the combination of the 2 conditions and the unusual inclusion criteria, the results indicate that thyroid nodules are associated with changes in some parameters in acromegalic patients. Practitioners need to take these results into consideration when treating acromegalic patients. At the same time, although the GHR-d3/fl genotype was a predisposing factor in acromegalic patients in this study, there were no differences in IGF-1 levels in these patients. The possible cause of this may be related to other growth factors, and higher leptin levels may contribute to thyroid nodule development. This is a preliminary result, and further studies are needed.

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