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Progranulin is increased in human and murine lipodystrophy

Konstanze Miehle [a,1,*], Thomas Ebert [a,b,1], Susan Kralisch [a,b], Annett Hoffmann [a], Jürgen Kratzsch [c], Haiko Schlögl [a], Michael Stumvoll [a], Mathias Fasshauer [a,b]

[a] Department of Internal Medicine (Endocrinology and Nephrology), University of Leipzig, Liebigstr. 18, 04103 Leipzig, Germany
[b] Leipzig University Medical Center, IFB Adiposity Diseases, Philipp-Rosenthal-Str. 27, 04103 Leipzig, Germany
[c] Institute for Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, University of Leipzig, Liebigstr. 27, 04103 Leipzig, Germany

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ABSTRACT

Aims: Lipodystrophies (LD) are genetic or acquired disorders sharing the symptom of partial or complete adipose tissue deficiency and a dysregulation of adipokines including leptin and adiponectin. Progranulin, an adipokine with proinflammatory and insulin resistance-inducing characteristics, has not been investigated in LD so far.

Methods: Circulating progranulin was determined in LD patients ($N = 37$) and in age-, gender-, and body mass index-matched healthy control subjects ($N = 37$). Additionally, we investigated progranulin expression in an LD mouse model as compared to wild-type mice. Moreover, we elucidated circulating progranulin before and during metreleptin supplementation in 10 patients with LD.

Results: Median [interquartile range] circulating progranulin was increased in patients with LD (82.9 [25.9] μg/l) as compared to controls (73.6 [22.8] μg/l) ($p = 0.005$). C-reactive protein (CRP) remained an independent and positive predictor of progranulin in multivariate analysis. Progranulin mRNA was significantly upregulated in all adipose tissue depots, i.e. visceral, subcutaneous, and brown adipose tissue, and in muscle of LD animals versus wild-type mice. Progranulin levels did not significantly change during metreleptin supplementation.

Conclusions: Progranulin serum concentration is increased in patients with LD, and shows an independent and positive correlation with CRP. Different adipose tissue depots and muscle might be potential origins of elevated progranulin.

1. Introduction

The term lipodystrophy (LD) summarizes a group of genetic or acquired disorders sharing the symptom of partial or complete adipose tissue deficiency [1,2]. Patients with LD are predisposed to severe metabolic derangement including dyslipidemia, hepatic steatosis, insulin resistance/diabetes mellitus, and polycystic ovary syndrome (PCOS) [1]. As a consequence, these patients have an increased risk for severe complications, such as liver cirrhosis, pancreatitis, and coronary heart disease [1,3].

* *Corresponding author at:* Department of Endocrinology and Nephrology, Liebigstr. 18, 04103 Leipzig, Germany. Fax: +49 341 9713389.
E-mail address: konstanze.miehle@medizin.uni-leipzig.de (K. Miehle).
[1] These authors equally contributed to this work.
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Since the late 90s, dysregulation of adipokines, i.e. adipocyte-secreted factors, has been suggested to influence LD [4]. The adipokines leptin and adiponectin are significantly downregulated in LD in humans [5], as well as in murine LD models [6]. Most interestingly, administration of leptin, adiponectin, and a combination of both improved significantly lipid and glycemic control profiles in mice with congenital LD [4], as well as in lipoatrophic peroxisome proliferator-activated receptor (PPAR) $\gamma^{+/-}$ mice treated with the PPARγ/retinoid X receptor inhibitor HX531 [7]. Furthermore and in accordance with the rodent data, Oral and co-workers elegantly demonstrated that leptin therapy improved glycemic control and decreased triglyceride levels in patients with LD [8].

Progranulin (also referred to as proepithelin, pc-cell derived growth factor, acrogranin, and granulin-epithelin precursor) has been described by Matsubara and co-workers as a novel adipokine inducing insulin resistance [9]. Progranulin is upregulated by tumor necrosis factor (TNF) α and dexamethasone in cellular models of insulin resistance [9]. Furthermore, progranulin knockout mice are resistant to high fat diet-induced insulin resistance, adipocyte hypertrophy, and obesity [9]. Moreover, the authors demonstrated that progranulin levels in blood and adipose tissue were markedly increased in obese mice [9]. In agreement with these findings, Youn and co-workers revealed that serum progranulin concentrations are also elevated in patients with obesity and diabetes mellitus type 2 as compared to individuals with normal glucose tolerance [10]. Thus, progranulin appears to be a novel insulin resistance-inducing adipokine which is associated with adipose tissue mass.

To date, regulation of the insulin resistance-inducing adipokine progranulin has not been studied in patients with LD. Thus, we determined progranulin in patients with LD and in age-, gender-, and body mass index (BMI)-matched healthy control subjects. To detect relevant changes in progranulin plasma levels and mRNA expression in insulin-sensitive tissues, we have investigated progranulin in an LD mouse model as compared to control mice. Moreover, we investigated circulating progranulin before and during metreleptin supplementation in 10 patients with LD to reveal a possible impact of metreleptin supplementation on progranulin.

2. Material and methods

2.1. LD patients and controls

The study cohort and metreleptin treatment criteria have been described recently [11,12]. In brief, 37 patients with generalized ($n = 4$) and partial ($n = 33$) LD (32 female, 5 male) and 37 age-, gender-, and BMI-matched healthy controls were included in the study (entire study population: age 16–75 years, BMI 17.4–46.1 kg/m^2). All LD patients fulfilled the consensus statement criteria 2013 of the American Association of Clinical Endocrinologists (AACE) for LD [2]. At the time of recruitment, none of the LD patients was pregnant or suffered from cancer or acute infection. None of the 37 LD patients was on metreleptin treatment at the time of characterization. In 24 LD patients, a genetic diagnosis had been established. Of those, 18 showed lamin A, 5 PPARγ, and one polymerase I and transcript release factor mutations. Of the remaining 13 LD patients, 7 were suspected to have a genetic origin since a positive family history was present, 2 patients were suffering from autoimmune LD, and 4 patients had idiopathic LD. Thirty-two partial LD patients had femorogluteal LD and one patient was affected by a localized form of the disease. Of the 32 female LD patients, 17 were pre- and 15 were postmenopausal. Ten LD patients (8 female, 2 male) were treated with metreleptin for 1 year. Laboratory measurement has been carried out prior to and during metreleptin supplementation (at 1 week, and 1, 3, 6, and 12 months, respectively). The 6 months data were used for calculation because glycemic and lipid profiles at this time were superior to other time points. The Ethics Committee of the Leipzig University approved this study. Moreover, all patients and controls gave written informed consent.

2.2. Laboratory measurement

In all subjects, venous blood withdrawal was performed after an overnight fast. Adipokines were analyzed in serum by enzyme-linked immunosorbent assay (ELISA) technique using commercially available kits purchased from R&D Systems, Minneapolis, MN for progranulin, from Mediagnost, Reutlingen, Germany, for adiponectin and leptin, and from R&D Systems, Minneapolis, MN for interleukin (IL)-6 and TNFα. Standard laboratory methods were used for determination of routine blood biochemistry markers, i.e. fasting insulin (FI), fasting glucose (FG), glycosylated hemoglobin A1c (HbA1c), creatinine, triglycerides (TG), total, high density lipoprotein (HDL), and low density lipoprotein (LDL) cholesterol, free fatty acids (FFA), as well as C-reactive protein (CRP). Homeostasis model assessment of insulin resistance (HOMA-IR) calculation has been performed referring to Matthew et al. [13]. Renal function was assessed by measuring creatinine and calculating eGFR by the CKD-EPI equation [14].

2.3. Mouse study

Mouse experiments were performed as described recently [12]. In brief, Tg(aP2-SREBF1c)9884Reh/0 mice (Jackson Laboratory, Bar Harbor, ME) were used as an animal model of lipodystrophy and C57BL/6NTac mice (F15; Jackson Laboratory, Bar Harbor, ME) served as controls. Both mouse models were on a low-density lipoprotein receptor knockout background, maintained in pathogen-free facilities under controlled temperature (21 ± 1 °C) and darkness occurring between 6 AM and 6 PM, and maintained on a cholesterol-enriched semisynthetic Clinton/Cybulsky diet (V1534, Sniff, Soest, Germany). All animal experiments were conducted according to accepted standards of humane animal care, and were approved by the local ethics committee (approval No. TVV37/12). After 20 weeks, animals were killed. Immediately thereafter, samples of visceral (VAT), brown (BAT), as well as subcutaneous (SAT) adipose tissue, muscle, and liver were taken and deep-frozen in liquid nitrogen.

We performed progranulin (*PGRN*) mRNA amplification relative to *36B4* by quantitative real-time RT-PCR on a LightCycler 480 real-time PCR 96-well thermocycler using