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Cover Letter

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Dear Biochemical and Biophysical Research Communications Editor,

Patients with severe chronic obstructive pulmonary disease (COPD) are known to have comorbidities. Therefore, treatment for COPD needs to focus on these comorbidities as well as the lungs. We previously reported a new IL-18 transgenic (Tg) COPD mice model. We analyzed comorbidities in IL-18 Tg mice for this paper. We found (1) Aging-related decrease of body weight in IL-18 Tg mice. (2) Decrease in the weight of the quadriceps femoris and gastrocnemius muscles in IL-18 Tg mice. (3) Decrease of bone mineral density in aged IL-18 Tg mice. (4) Impaired glucose tolerance in aged IL-18 Tg mice. We believe that our findings are one of the first reports the progression of comorbidity in IL-18 transgenic COPD mice model, may be of clinical benefit in the treatment of patients with severe COPD who have comorbidities and a poor clinical prognosis and the data will be of interest to the readers of Biochemical and Biophysical Research Communications.

We look forward to hearing from you regarding its possible publication in Biochemical and Biophysical Research Communications.

Yours sincerely,

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*Highlights (for review)

Aging-related decrease of body weight in IL-18 Tg mice.

Decrease in the weight of the muscles in IL-18 Tg mice.

Decrease of bone mineral density in aged IL-18 Tg mice.

Impaired glucose tolerance in aged IL-18 Tg mice.

The progression of comorbidity in IL-18 transgenic chronic obstructive pulmonary disease mice model

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Abstract

Patients with severe COPD are known to have comorbidities such as emaciation, cor pulmonale and right heart failure, muscle weakness, hyperlipemia, diabetes mellitus, osteoporosis, muscle atrophy, arterial sclerosis, hypertension, and depression. Therefore, treatment for COPD needs to focus on these comorbidities as well as the lungs. We previously reported a new mouse model of COPD utilizing the human surfactant protein C promoter SP-C to drive the expression of mature mouse IL-18 cDNA; constitutive IL-18 overproduction in the lungs of transgenic (Tg) mice induces severe emphysematous change, dilatation of the right ventricle, and mild pulmonary hypertension with aging. In the present study, we evaluated the progression of comorbidity in our COPD model. In female Tg mice, significant weight loss was observed at 16 weeks and beyond, when compared with control wild-type (WT) mice. This weight loss was suppressed in IL-13-deficient (knockout; KO) Tg mice. Muscle weight and bone mineral density were significantly decreased in aged Tg mice relative to control WT and IL-13 KO Tg mice. The aged Tg mice also showed impaired glucose tolerance. IL-18 and IL-13 may play important roles in the pathogenesis of comorbidity in COPD patients.

Keywords: IL-18; IL-13; COPD; Transgenic mouse

Introduction

Chronic obstructive pulmonary disease (COPD) is an important pulmonary inflammatory disease whose prevalence and associated mortality rates have been increasing [1,2]. COPD often coexists with other diseases (comorbidities) that may have a significant impact on prognosis. Comorbidities include cardiovascular disease (CVD) (such as ischemic heart disease, heart failure, hypertension, and pulmonary hypertension), osteoporosis, diabetes, infections, and lung cancer, and are common at any severity of COPD, so that differential diagnosis can often be difficult (see review [3]). It is thought that several common genetic or constitutional factors may predispose individuals with COPD to both pulmonary and systemic inflammation [4].

The proinflammatory cytokines IL-1, IL-18, IL-33, IL-36, IL-37, and IL-38 belong to the IL-1 family [5]. IL-18 is well known to play an important role in Th1 polarization, and can also act as a co-factor for Th2 cell development and IgE production [6,7,8,9]. IL-18 has been reported to take part in the differentiation of Th17 cells by amplifying IL-17 production by polarized Th17 cells in synergy with IL-23 [10]. IL-18 plays important roles in the pathogenesis of inflammatory diseases such as atopic dermatitis [11], rheumatoid arthritis (RA), adult-onset Still's disease, Sjögren's syndrome, and inflammatory bowel diseases including Crohn's disease [see review [6]].

IL-18 is also involved in the development of lung diseases including lung injury [12,13] and idiopathic pulmonary fibrosis (IPF) [14]. It has been shown that IL-18 and its receptor are involved in the pathogenesis of COPD [15,16,17]. Previously, we established a new animal model of COPD in which constitutive overproduction of mature IL-18 protein in the lungs of transgenic (Tg) mice resulted in severe emphysema accompanied by pulmonary inflammation. IL-13 gene deletion resulted in suppression of emphysema and inflammation in the IL-18 Tg mice [18]. In the present study, we evaluated comorbidity (in terms of body and muscle weight, bone mineral density, and glucose tolerance) and the roles of IL-13 in our COPD mouse model.

Materials and Methods

Lung-specific IL-18-transgenic (Tg) mice

We used female IL-18 Tg mice with a C57BL/6N (B6) background in which mature mouse IL-18 was overproduced in the lungs under the control of the human surfactant protein (SP) C promoter [18]. We established B6 background IL-13 deficient (knockout; KO) IL-18 Tg (IL-13KO/IL-18 Tg) mice by backcrossing IL-18 Tg mouse line A with B6 IL-13 KO mice, as reported previously [19]. Age-matched female B6 wild-type (WT) mice, purchased from Charles River Japan (Yokohama, Japan), were used as controls. All procedures were approved by the Committee on the Ethics of Animal Experiments, Kurume University (Approval No. H22-079-084). Animal care was provided in accordance with the procedures outlined in the "Principle of laboratory animal care" (National Institutes of Health Publication No.86-23, revised 1985).

Histological examinations

For the histological analysis, mice were sacrificed with an intraperitoneal injection of sodium pentobarbital (2.5–5 mg per mouse). After gross examination, the extracted tissues were placed in 10% buffered formalin and further fixed for at least 24 hours. Sections (4 μm thick) were cut from paraffin-embedded tissues, placed on poly-l-lysine-coated slides, and then incubated overnight at 55-60°C. Deparaffinized sections were stained with hematoxylin and eosin (HE), as reported previously [12,20,21].

Measurement of bone mineral density

Mice aged 24 weeks were sacrificed, and the right thighbone of each was extirpated and cut into 20 slices 1 mm thick. Bone mineral density was analyzed by the DEXA (dual-energy X-ray absorptiometry) method using a DCS-600EX-ⅢR instrument (Aloka Corporation, Tokyo, Japan). The weight and surface area of each slice was measured, and the bone density (weight/surface area) calculated [22].

Glucose tolerance test

Glucose tolerance tests were performed as reported previously [23]. Briefly, a dose of glucose (1 g/kg) was administered by intraperitoneal (i.p.) injection, and the blood glucose level was measured at 0, 30, 60 and 120 minutes after the injection.

Statistical analyses

Results are expressed as means ± standard error of the mean (SEM). ANOVA was used to compare differences between groups. The SAS 9.1.3 software package, Japanese edition (SAS Institute, Cary, NC, USA), was used for statistical analysis. $P < 0.05$ was considered to represent statistical significance.

Results

Aging-related body weight loss in IL-18 Tg mice

We examined the body weight of female WT, IL-13 KO/IL-18 Tg, and IL-18 Tg mice every week from 7 to 24 weeks after birth (n=4 to 5 in each group). Representative results are shown in Figure 1. In WT, IL-13 KO/IL-18 Tg, and IL-18 Tg mice, body weight increased until 15 weeks of age. There was no significant difference in body weight among the groups until that time. From 16 to 24 weeks, body weight decreased significantly in IL-13 KO/IL-18 Tg and IL-18 Tg mice, when compared to WT mice. Interestingly, from 22 to 24 weeks, IL-18 Tg mice were significantly lighter than IL-13KO/IL-18 Tg mice. These results showed that weight loss was suppressed in IL-13KO/IL-18 Tg mice.

Decrease of quadriceps femoris and gastrocnemius muscle weight in IL-18 Tg mice

At 7 week of age, the quadriceps femoris muscle of WT mice was significantly heavier than that in IL-18 Tg and IL-13 KO/IL-18 Tg mice, although body weight did not differ significantly among the three groups (Fig. 1). There was no significant difference in muscle weight between IL-18 Tg and IL-13 KO/IL-18 Tg mice. At 16 weeks, the quadriceps femoris in WT mice was also significantly heavier than in IL-18 Tg and

IL-13KO/IL-18 Tg mice, and was significantly heavier in IL-13KO/IL-18 Tg mice than in IL-18 Tg mice. Interestingly, at 25 weeks, the quadriceps femoris was significantly heavier in WT and IL-13KO/ IL-18 Tg mice than in IL-18 Tg mice. There was no significant difference in quadriceps femoris weight between WT and IL-13KO/IL-18 Tg mice (Fig. 2).

At 7 weeks of age, the gastrocnemius muscle was significantly heavier in WT mice than in IL-18 Tg and IL-13KO/IL-18 Tg mice, but there was no significant difference in the weight of this muscle between IL-18 Tg and IL-13KO/IL-18 Tg mice. At 16 weeks and 25 weeks, the gastrocnemius was significantly heavier in WT and IL-13KO/IL-18 Tg mice than in IL-18 Tg mice, but showed no significant difference between WT and IL-13KO/IL-18 Tg mice (Fig. 2).

Decrease of bone mineral density in aged IL-18 Tg mice

Next, we examined bone mineral density in mice at 24 weeks of age. That in IL-18 Tg mice was significantly decreased in comparison with WT and IL-13KO/IL-18 Tg mice. However, there was no significant difference in bone mineral density between WT and IL-13KO/IL-18 Tg mice (Fig. 3).

Impaired glucose tolerance in aged IL-18 Tg mice

We examined glucose tolerance in mice at 20 weeks of age. At 30 and 60 minutes after glucose administration, blood glucose levels showed no significant difference in IL-18 Tg and WT mice. However, at 120 minutes after glucose administration, blood glucose levels were significantly higher in IL-18 Tg mice than in WT mice (Fig. 4).

Discussion

COPD is characterized by an intense inflammatory process in the airways, parenchyma, and pulmonary vasculature. COPD is also associated with systemic inflammation [3,24]. For instance, the presence of systemic inflammation in COPD has been linked with a variety of complications including weight loss [25][26] [27], cachexia [28] [24], osteoporosis [29] [30] [31], cardiovascular disease [32] [33] [34], diabetes mellitus [35] [36], sleep disorder and depression [37,38]. It is has been reported that inflammatory cytokines including TNF-α and IFN-γ may be involved in systemic inflammation in COPD [3]. However, the precise mechanisms of systemic inflammation in severe COPD are still uncertain. We showed previously that IL-18 was overexpressed in the lungs and serum of patients with very severe COPD [15]. Therefore, inflammatory cytokines including IL-18, TNF-α, and IFN-γ overexpressed in lung tissues may ‘‘spill’’ over into the systemic circulation, promoting a generalized inflammatory reaction in COPD.

We previously reported that constitutive overproduction of IL-18 in the lungs resulted in increased production of both Th1 and Th2 cytokines (including IFN-γ and IL-13), emphysematous changes, and severe pulmonary inflammation in the lungs of mice [18]. It has been reported that IL-18 produced by osteoblasts is a powerful osteoclast-inhibitor [39]. Both IL-18 and IL-12 reduce the absorptive activity of osteoclasts through the production of IFN-γ [40]. Our present results showed that bone mineral density in IL-18 Tg mice was significantly decreased in comparison with WT and IL-13-deficient Tg mice, suggesting that IL-18 may inhibit the activities of osteoclasts partly through the production of IL-13 in IL-18 Tg mice.

It has been reported that IL-18 mRNA was highly expressed in biopsy samples of skeletal muscle from COPD patients, relative to those from healthy controls [41]. There is some evidence that patients with COPD have increased skeletal muscle apoptosis [42]. Previous studies have shown that IL-18 causes apoptosis in various cell types including muscle cells and lymphocytes [43]. Here we showed that the weight of the quadriceps and gastrocnemius muscles was significantly decreased in aged IL-18 Tg mice, when compared with control WT and IL-13-deficient Tg mice, suggesting that overexpression of IL-18 may induce apoptosis in muscle cells via IL-13. However, we were unable to detect apoptotic muscle cells to any any significant degree in either the quadriceps of the gastrocnemius (data not shown). Further analysis will be needed to verify this issue.

It has been reported that 47% of COPD patients present 3 or more determinants of metabolic syndrome, including cardiovascular disease, diabetes mellitus, hyperlipidemia, and hypertension [35]. Expression of IL-18 is increased in the retinas of diabetic OLETF rats, a model of type 2 diabetes mellitus, and chronic hyperglycemia accelerates the release of IL-18 and IFN-γ from inflammatory cells [44]. It is widely believed that IL-18 can exacerbate type 2 diabetes mellitus and cardiovascular disease [45]. Therefore, overexpression of IL-18 may induce hyperglycemia in patients with very severe COPD.

Our present findings suggest that IL-18 and IL-13 may play important roles in the pathogenesis of comorbidity in COPD patients, and raise the possibility that blockade of IL-18 may be a feasible treatment for COPD. Caspase-1 inhibitors, antibodies against IL-18 and its receptor, IL-18 binding protein, or inhibitors of genes downstream of the IL-18 signal transduction pathway, such as those encoding MyD88, IL-1 receptor associated kinase, tumor necrosis factor receptor-associated factor 6, nuclear factor-kB, C-jun N-terminal kinase, and p38 mitogen-activated protein kinase, as well as IL-13 inhibitors, may be of clinical benefit in the treatment of patients with severe COPD who have comorbidities and a poor clinical prognosis.

Figure legends

Figure 1. Aging-related decrease of body weight in IL-18-transgenic (Tg) mice.

We examined the body weight of female WT, IL-13 KO/IL-18 Tg, and IL-18 Tg mice every week from 7 to 24 weeks from birth (n=4 to 5 in each group).

*P<0.05: WT mice vs. IL-18 Tg mice.

◊ P<0.05: WT mice vs. IL-13KO/IL-18 Tg mice.

†P<0.05: IL-18 Tg mice vs. IL-13KO/IL-18 Tg mice.

Figure 2. Decrease in the weight of the quadriceps femoris and gastrocnemius muscles in IL-18 Tg mice.

Mice (n=12 each group) were sacrificed at 7, 16, and 25 week after birth, and the weights of the quadriceps femoris and gastrocnemius muscles were measured.

A) quadriceps femoris; B) gastrocnemius

*P<0.05: WT mice vs. IL-18 Tg mice.

◊ P<0.05: WT mice vs. IL-13KO/IL-18 Tg mice.

†P<0.05: IL-18 Tg mice vs. IL-13KO/IL-18 Tg mice.

Figure 3. Decrease of bone mineral density in aged IL-18 Tg mice.

Mice were sacrificed at 24 weeks of age (WT: n=7, IL-18 Tg: n=7, IL-13 KO/IL-18 Tg: n=8). Bone mineral density was measured as described in the Methods section.

*P<0.05

Figure 4. Impaired glucose tolerance in aged IL-18 Tg mice.

Glucose tolerance tests were performed in mice at 20 weeks of age (WT: n=5, IL-18 Tg: n=5), as described in the Methods section.

*P<0.05 vs. WT mice.

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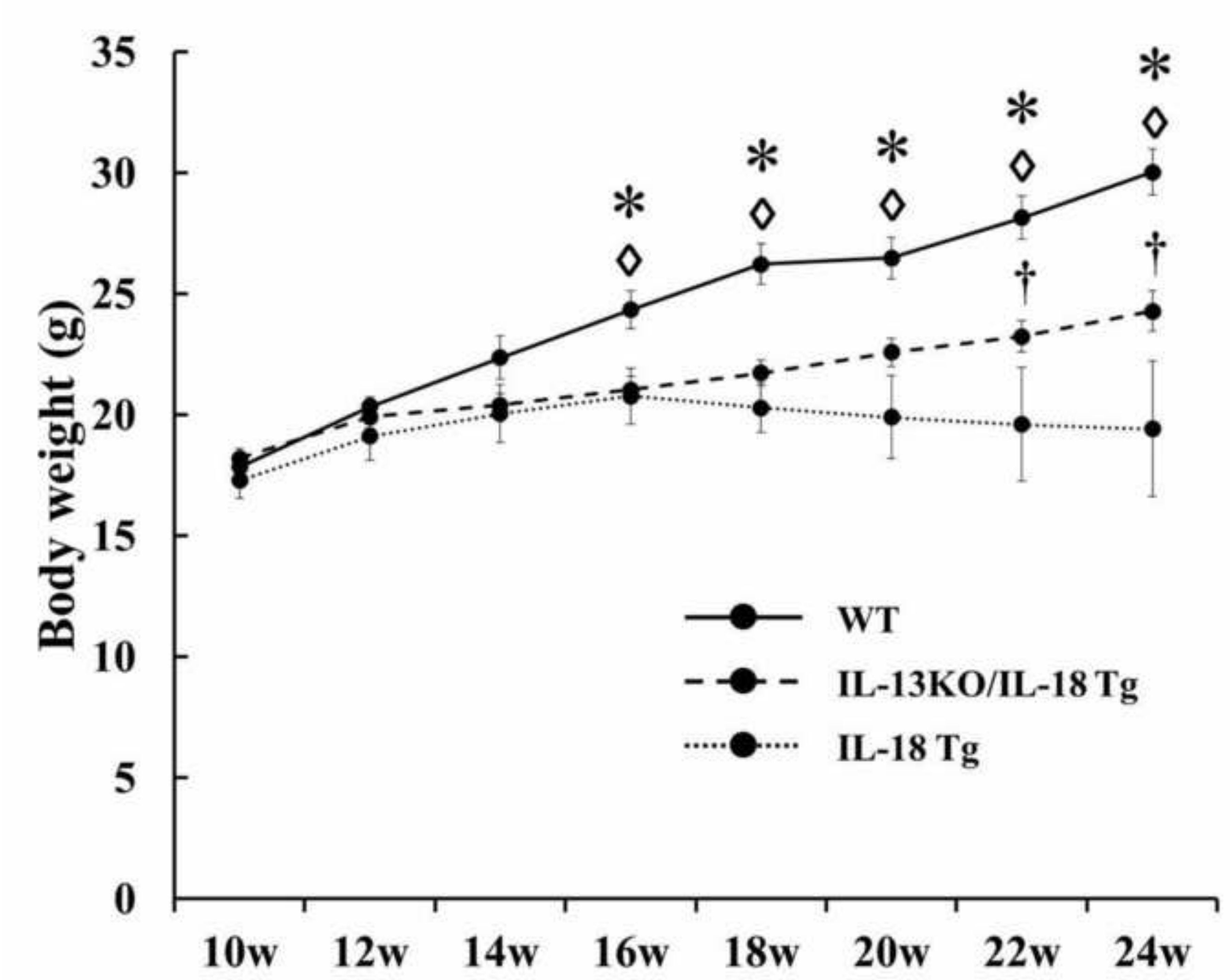

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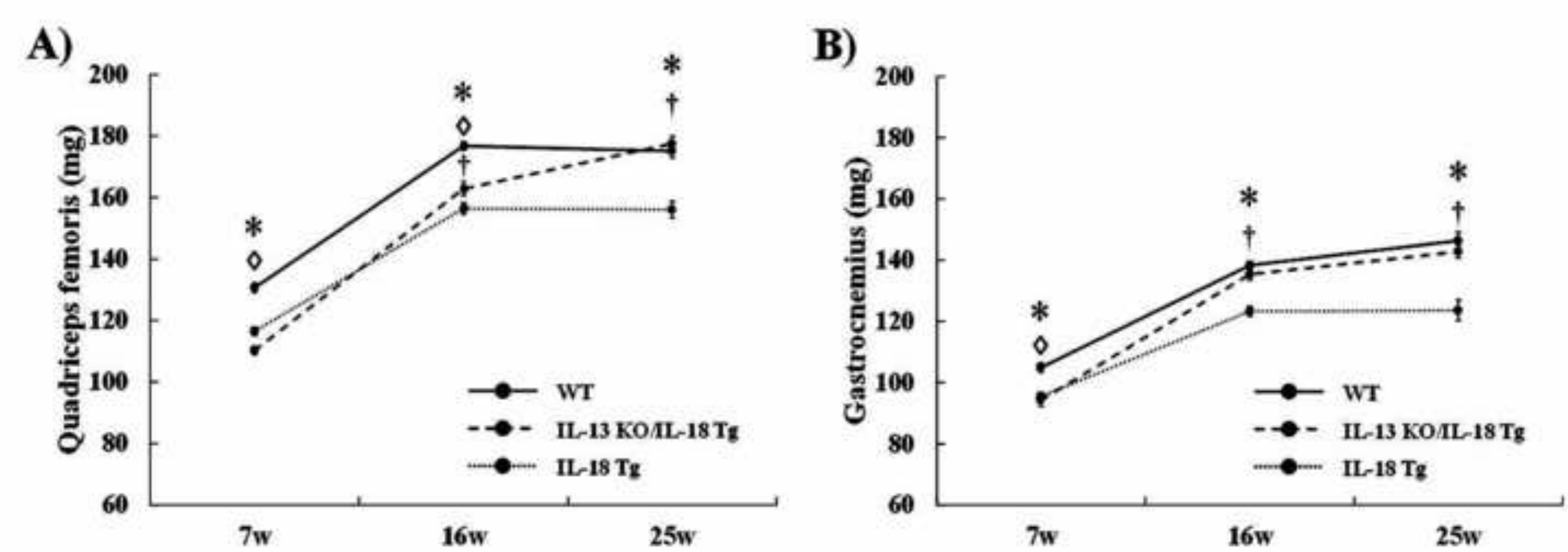

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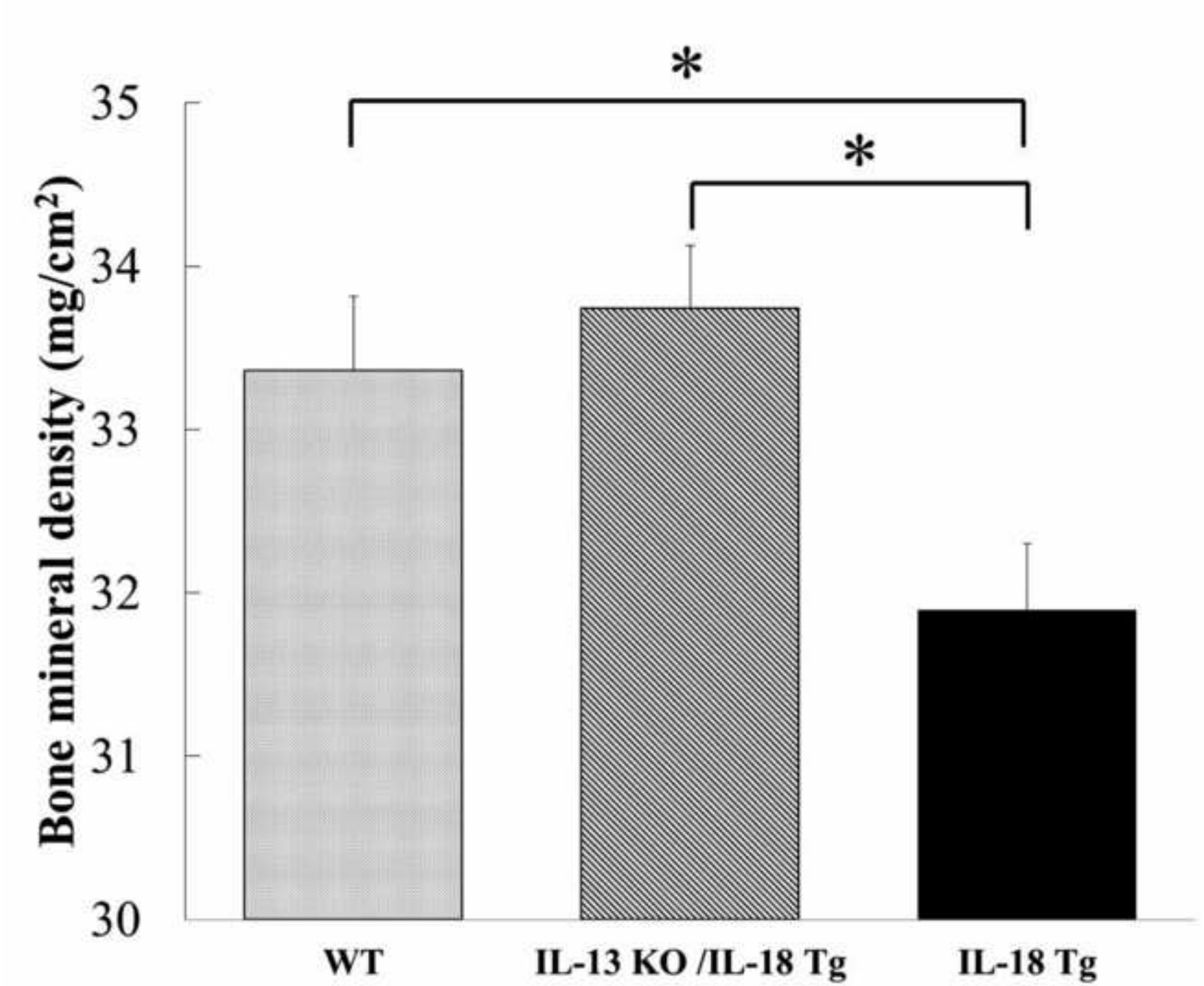

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