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Original Article

Chitinase 3-Like 1 (*CHI3L1*) Polymorphism Contributes to Visceral Obesity and Obesity-related Inflammation Induces *Chi3l1* in Adipocytes

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Abdominal obesity is considered as one of the most risky factors governing the development of metabolic diseases. Here we identify that human chitinase 3-like 1 (*CHI3L1*, also called YKL-40 in human) single nucleotide polymorphism (SNP), rs883125, is associated with abdominal obesity in Korean women. Korean women subjects with the rs883125 G/G or C/G genotype present higher waist-hip ratio than subjects with C/C genotype suggesting that human subjects who G nucleotide substitution at the rs883125 tended to more accumulate intra-abdominal fat at the abdominal cavity. In addition, *Chi3l1* gene expression is increased in adipose tissue from obese mice and pro-inflammatory cytokine enhances *Chi3l1* expression in adipocytes, indicating that *Chi3l1* is greatly related with obesity and obesity-induced pro-inflammatory responses. Taken together, the minor allele of rs883125 is associated with a higher prevalence of abdominal obesity in Korean women. These findings suggest that genotype of rs883125 can be a biomarker of incident abdominal obesity and abdominal obesity-related metabolic diseases.

Key Words: Chitinase 3-like 1, CHI3L1, YKL-40, SNP, Abdominal obesity, Korean women

INTRODUCTION

For the several decades, the prevalence and severity of obesity is dramatically and continuously has increased worldwide. Obesity has become a major public health problem since it is one of the key factors for development of metabolic diseases such as insulin resistance, type 2 diabetes, atherosclerosis, and several cancers. Although obesity is considered as a lifestyle disease, it is substantially recognized that genetic factors are also involved in the pathogenesis of obesity (Herrera and Lindgren, 2010; Choquet and Meyre, 2011). Single nucleotide polymorphisms (SNPs) is represented these genetic factors that contribute to development of obesity. Many researchers have found SNPs, which are associated with obesity, and identified those SNPs are located in genes that involved in food intake, fat metabolism, adipocyte differentiation, and energy metabolism (Dina et al., 2007; Frayling et al., 2007; Loos et al., 2008; Lindgren et al., 2009; Meyre et al., 2009).

Abdominal fat, also known as visceral fat, is located in peritoneal cavity around the organs. Accumulation of excess energy as abdominal fat in adipocytes leads abdominal obesity and is a very high risk factor for cardiovascular diseases and

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type 2 diabetes (Despres et al., 2008). Increase of fat storage in visceral adipocytes changes their characteristics and functions such as production of cytokines (McArdle et al., 2013). Enlarged adipocytes produce and secret pro-inflammatory cytokines and chemo-attractant molecules, including tumor necrosis factor-α (TNF-α), interleukin (IL)-1, IL-6, and monocyte chemotactic protein 1 (MCP1), in turn, induce a state of chronic low-grade inflammation with increase of infiltration of various immune cells into adipose tissue and lead alternation of whole body energy balance (Esser et al., 2014).

Chitinase 3-like 1 (CHI3L1, also called YKL-40 in human) is a chitinase-like glycoprotein, which binds to chitin but cannot degrade it because of mutations in their active domains (Rehli et al., 1997). CHI3L1 involves in tissue remodeling and immune responses through participating in M2 macrophage differentiation, inflammasome activation, and Th1/Th2 immune balance (Rehli et al., 2003). CHI3L1 is produced by various cells including macrophage and neutrophils (Krause et al., 1996; Volck et al., 1998). Additionally, circulating levels of CHI3L1 are increased in patients with inflammation-related diseases including asthma, atrophy, liver fibrosis, atherosclerosis, and type 2 diabetes (T2DM) (Chupp et al., 2007; Lee et al., 2012). Furthermore, CHI3L1 expression is induced by inflammatory cytokines such as IL-6, INF-γ, and TNFα (Ling and Recklies, 2004). In contrast, CHI3L1 inhibits cellular responses induced by those cytokines, implying that enhancement of CHI3L1 is one of the defense mechanisms to cytokine mediated-inflammation. Although several studies are reported that serum CHI3L1 levels are higher in obese subjects (Kyrgios et al., 2012; Huang et al., 2014; Huang et al., 2016), it is still not clear whether CHI3L1 is involved in obesity especially abdominal obesity. Therefore, in this study, we investigated the contribution of CHI3L1 to development of obesity in Korean women through analysis of *CHI3L1* SNPs and found that rs883125 is associated with visceral obesity.

MATERIALS AND METHODS

Study population

This study approved by Ethical Committee and the Institutional Review Board at Dermapro (IRB no. 1-220777-B-N-02-DICN14002), and all participants provided informed written consent before participation. A total of 106 healthy volunteers (Korean females, aged 20~60 years) were enrolled.

Biological parameters

BMI was calculated as weight (kg)/height^2 (m^2). Body fat contents (kg), body fat rate (%) were obtained by bioelectrical impedance analysis using InBody 3.0 (Biospace, Seoul, Korea).

SNP genotyping

The experimental determination of SNP genotypes were conducted by the Theragen Etex Co., Ltd. For genotyping analysis, DNA was extracted from peripheral blood leukocytes by using Exgene^{TM} Blood SV (GeneAll, Seoul, Korea). 125 ng DNA was mixed with 2.5 μL of TaqMan OA GT Master Miz and used for the real-time quantitative PCR by TaqMan assay (Applied Biosystems, Calsbug, CA). The PCR mixture was loaded on the Open Array by using Accufill automated machine (Applied Biosystems, Calsbug, CA). The reaction ready Open Array chip was used in Quant Studio 12K (Applied Biosystems, Calsbug, CA). The real-time PCR reactions were performed at 95℃ for 10 min, 40 cycles at 92℃ for 15 sec, at 60℃ for 1 sec. The genotyping was determined by the Vic and/or Fam fluorescent dye intensity.

Animal experiments

All animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Aestura Corporation and performed in accordance with their guidelines. 7-week-old C57BL/6J mice were purchased from the Central Laboratory Animal Inc. and maintained in a 12 h dark- 12 h light cycle chamber with controlled temperature of 22~25℃ and 40~50% humidity. For the HFD study, 8-week-old C57BL/6J male mice were fed a NCD or 60% HFD (Research Diet, Inc., D12492) for 16 weeks.

Cell culture

3T3-L1 cells were obtained from ATCC (CL-173). 3T3-L1 cells were grown to confluence in Dulbecco's modified

Eagle medium (DMEM; Hyclone, SH30243.01) supplemented with 10% bovine calf serum (Gibco, 26010-074). To induce adipocyte differentiation, at 2 days post-confluence, 3T3-L1 cells were incubated with DMEM containing 10% fetal bovine serum (FBS; Hyclone, SH30919.03), 0.52 mM 3-isobutyl-1-methylxanthine (Sigma Aldrich, I5879), 1 μM dexamethasone (Sigma Aldrich, D1756), and 1 μg/mL insulin (Roche, 11 376 497 001) for 2 days. Then, the culture medium was replaced with DMEM containing 10% FBS and 1 μg/mL insulin and the cells were cultured for 2 additional days. The culture medium was changed every two days with DMEM containing 10% FBS.

For the inflammatory environmental mimicking experiments, differentiated 3T3-L1 adipocytes were incubated with or without 10 ng/mL TNFα (R&D Systems, 210-TA) for 24 h.

RNA isolation and quantitative real-time PCR (qPCR)

The RNA isolation and cDNA synthesis procedure were performed as described previously. Briefly, total RNA was isolated from mouse cells or cell lines with TRIzol Reagent (Ambion, 15596-018) and subjected to cDNA synthesis using RevertAid^{TM} First Strand cDNA Synthesis (Thermo Scientific). mRNA relative amounts were measured using the CFX96^{TM} Real-Time System (Bio-Rad Laboratories Inc.) and calculated by normalization to the level of cyclophilin mRNA. The primer sequences that were used for quantitative real-time PCR analyses are provided in Supplementary Table 1.

Statistical analysis

The genetic association analysis used SPSS 15.0 and linear regression analysis.

In cell and mouse experiments, the results are presented as mean ± SEM. Statistical significance was assessed by the two-tailed Student's *t*-test using GraphPad Prism 5.0 (GraphPad Software). When cells were used for experiments, three replicates per group were chosen. Differences were considered statistically significant at $P < 0.05$.

Table 1. Demographic characteristics of the study subjects

Variables	Values
Age (y)	44.51±6.22
Height (cm)	158.36±5.21
Weight (kg)	59.1±8.96
BMI (kg/m^2)	23.58±3.55
Fat mass (kg)	19.87±6.28
Skeletal muscle mass (kg)	21.2±2.43
Percent body fat (%)	32.98±5.72
Waist-hip ratio	0.9±0.04

RESULTS

Baseline characteristics of this study are presented in Table 1.

CHI3L1 SNP rs883125 is associated with abdominal obesity in Korean Women

To identify polymorphisms in *CHI3L1* that are associated with obesity, we analyzed the subjects using a dominant model. The SNP rs883125 is associated with obesity, especially abdominal obesity (Table 2), whereas other *CHI3L1* SNPs, rs2275353 and rs10399805, had no relation with obesity (data not shown). We found that rs883125 G-allele was correlated with increased waist-hip ratio in Korean women, indicating that rs883125 contribute to visceral obesity.

Inflammatory cytokines promotes *Chi3l1* expression in adipocytes

To determine whether CHI3L1 contributes in visceral obesity, we analyzed the mRNA levels of *Chi3l1* in epididymal adipose tissue of diet-induced obese mice model. Consistent with previous reports (Ahangari et al., 2015), the transcripts of *Chi3l1* were elevated in adipose tissue of high fat diet (HFD)-induced obese mice (Fig. 1A).

To understand molecular mechanisms of obesity-mediated *Chi3l1* enhancement, we evaluated which factors influence to induction of *Chi3l1* in adipocytes. Several evidences indicate that the levels of CHI3L1 are enhanced in inflammatory environments (Recklies et al., 2005; Di Rosa and Malaguarnera, 2016). Notably, TNFα significantly increased

Table 2. Association of rs883125 of *CHI3L1* and obesity-related quantitative traits among BMI, body fat contents, body fat rate and waist-hip ratio in collected Korean women

	Genotype				Dominant model		
	CC	CG	GG	CG + GG	β	SE	*P* value
n	62	43	3	46			
Age (years)	45±6	44±6	43±8	44±6			
BMI (kg/m^2)	23.1±3.1	24.2±3.9	25.0±5.7	24.3±4.0	1.24	0.70	0.08
Body fat content (kg)	19.0±5.4	20.9±7.3	23.8±7.4	21.1±7.3	2.06	1.24	0.10
Body fat rate (%)	32.2±5.2	34.0±6.5	34.5±3.6	34.0±6.3	1.72	1.13	0.13
Waist-hip ratio	0.89±0.04	0.90±0.05	0.88±0.06	0.9±0.05	0.02	0.01	0.02

the *Chi3l1* gene expression in 3T3-L1 adipocytes suggesting that obesity-induced inflammatory responses could be a one of causing factor for enhancement of *Chi3l1* in adipocytes (Fig. 1B).

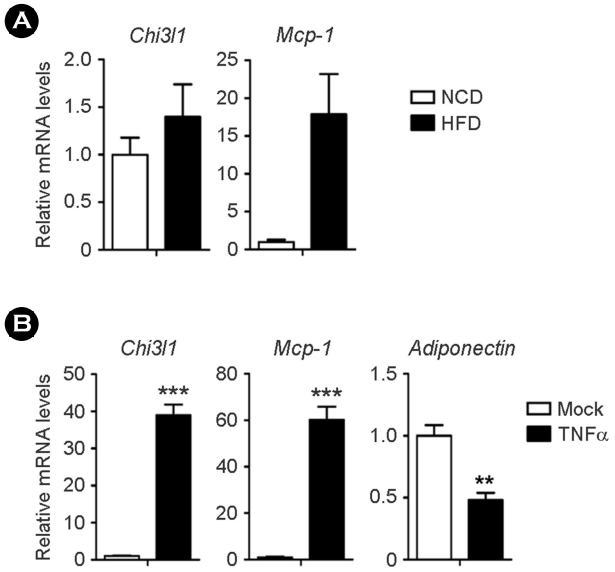

Fig. 1. *Chi3l1* is increased in adipose tissue of obese subjects and induced by inflammation in adipocytes. (A) C57BL/6J male mice were fed a NCD or 60% HFD for 16 weeks. *Chi3l1* and *Mcp-1* mRNA levels were measured by qPCR. (B) Differentiated 3T3-L1 adipocytes were incubated with or without TNFα (10 ng/mL) for 24 h. The mRNA levels of *Chi3l1*, *Mcp-1*, and adiponectin were measured by qPCR. All graphs show mean value ± SEM. $^{**}P < 0.01$; $^{***}P < 0.001$ in two-tailed Student's *t*-test.

DISCUSSION

Characteristics of fat tissues are completely different according to its location (Ibrahim, 2010). Among various fat tissues, abdominal adipose tissue is closely involved in development of metabolic diseases due to its spatial position, closed to other organs, and its own properties, more pro-inflammatory than subcutaneous fat (Lee et al., 2013). Consequently, it is important to control the intra-abdominal adiposity. In this study, we identify that *CHI3L1* rs883125 G-allele associates with higher waist-hip ratio, indicating rs883125 could be a risk factor for abdominal obesity in Korean women.

Previous studies have approached that mouse CHI3L1 is involved in obesity, especially abdominal obesity (Ahangari et al., 2015). In visceral adipose tissue of high fat diet-induced obese mice, the mRNA levels of *Chi3l1* are enhanced than visceral adipose tissue of normal chow diet-fed mice. In addition, *Chi3l1* null mice have reduced visceral adipose tissue, which is composed with small adipocytes, compared with WT mice. On the other hand, *Chi3l1* overexpressed mice have increased visceral fat pad. Furthermore, serum CHI3L1 levels have positive correlation with obesity, especially abdominal obesity in human (Thomsen et al., 2015). Therefore, these results support our finding, which is CHI3L1 contributes accumulation of fat in abdominal adipose tissue.

During weight gain, excess energy is accumulated in adipocytes as a fat resulting formation of enlarged adipocytes. Enlarged adipocytes secret various chemokines and cytokines to adapt the changed environment (Tilg and Moschen, 2006). Several immune cells are recruited into the visceral adipose tissue upon these signals from adipocytes and

actively participate adipose tissue remodeling event (Sun et al., 2011). Even though the functions of CHI3L1 are not fully elucidated yet, it is known to have a closed relationship with inflammation and tissue remodeling via modulation of extracellular matrix (Kim et al., 2012; Di Rosa and Malaguarnera, 2016). Thus, obesity-induced enhancement of CHI3L1 might be involved in adipose tissue remodeling through regulation of inflammatory responses and adipocytes microenvironments.

A limitation of this study is the absence of serum CHI3L1 data. Therefore, it is difficult to conclude that rs883125 involved in abdominal obesity through regulating CHI3L1 levels. Accordingly, it is clearly of importance to clarify whether rs883125 influences the expression, synthesis, and circulating levels of CHI3L1.

Collectively, our study suggests rs883125, one of SNPs of *CHI3L1*, is significantly associated with abdominal obesity in Korean women. And these genetic variants could be a biomarker for development of abdominal obesity.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to disclose.

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Supplementary

Table 1. Primer sequences used for quantitative real-time PCR

Gene	Sequence (5' to 3')	Direction
Chi3l1	GTACAAGCTGGTCTGCTACTTC	Forward
	ATGTGCTAAGCATGTTGTCGC	Reverse
Mcp-1	AGGTCCCTGTCATGCTTCTG	Forward
	TCTGGACCCATTCCTTCTTG	Reverse
Adiponectin	GGCAGGAAAGGAGAACCTGG	Forward
	AGCCTTGTCCTTCTTGAAGAG	Reverse
Cyclophilin	CAGACGCCACTGTCGCTTT	Forward
	TGTCTTTGGAACTTTGTCTGCAA	Reverse