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Adiponectin Deficiency Contributes to Sensitivity in Human Skin

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TO THE EDITOR

Sensitive skin is defined as an exaggerated reaction to environmental factors, characterized by prickling, burning, itching, stinging, pain, or tingling sensation (Berardesca *et al.*, 2006; Farage and Maibach, 2010). Recently, in addition to a previous notion that sensitive skin is associated with increased neurosensory responses, impaired skin barrier function, and altered immune responsiveness (Ständer *et al.*, 2009), we discovered that sensitive skin is closely associated with the dysfunction of muscle contraction, carbohydrate and lipid metabolism, and ion balance, by using an unbiased microarray analysis of skin samples obtained from subjects with sensitive or non-sensitive skin (Kim *et al.*, 2014). However, the critical regulator implicated in the pathogenesis of sensitive skin remains still elusive.

Previous microarray findings suggest that adiponectin (ADIPOQ) is one of the downregulated genes in sensitive skin (Kim *et al.*, 2014). ADIPOQ is an adipocyte-derived adipokine with multiple salutary effects such as anti-apoptotic, anti-inflammatory, and anti-oxidative activities, as well as anti-obesity and anti-insulin resistance roles in diverse organs and cells including skeletal muscles, liver, and adipose tissues (Goldstein and Scalia, 2004). Particularly, ADIPOQ is known to regulate muscle phenotypes and functions (Krause *et al.*, 2008). ADIPOQ induces vascular smooth muscle cell differentiation, suppresses triglyceride accumulation, and stimulates fatty acid oxidation and glucose uptake in myocytes (Yamauchi *et al.*, 2002).

Healthy volunteers who perceived their skin to be 'sensitive' or 'non-sensitive' were recruited. Sensitive skin individuals were identified based on questionnaires and a 10% lactic acid stinging test as previously described (Farage and Maibach, 2010; Kim *et al.*, 2014). Those with underlying skin diseases such as atopic dermatitis, contact dermatitis, and rosacea were excluded. This study was approved by the Institutional Review Board at Seoul National University Hospital and conducted according to the Declaration of Helsinki. All subjects provided written informed consent. We found that the expression of ADIPOQ mRNA (Figure 1a) and protein (Figures 1b and c, as shown by Western blot and immunofluorescence staining, respectively) was markedly downregulated in sensitive skin tissues, in comparison with non-sensitive skin tissues. ADIPOQ binds to ADIPOQ receptor 1 (ADIPOR1) and ADIPOQ receptor 2 (ADIPOR2) and activates 5′-AMP-activated protein kinase (AMPK). AMPK is a master regulator of glucose and lipid metabolism and is activated by various cellular stresses, physiologic stimuli such as muscle contraction, and hormones such as ADIPOQ (Hardie *et al.*, 2006; Ruderman *et al.*, 2013). ADIPOQ regulates peripheral and whole-body energy metabolism via controlling AMPK activity (Sattar and Sattar, 2012). Expression of ADIPOR1/2 was reduced in sensitive skin (Figure 1d). In addition, the level of phospho-AMPK was markedly decreased in sensitive skin tissues, whereas the total AMPK level remained unchanged (Figure 1e). These results suggest that decreased expression of ADIPOQ and its receptors, accompanied by low AMPK activity, is implicated in the development of sensitive skin.

To examine the contributory role of ADIPOQ in the pathogenesis of sensitive skin, we performed small interfering RNA (siRNA)-mediated knockdown of the *ADIPOQ* gene *in vitro*. Our previous *in vivo* results obtained in sensitive skin tissues suggest that decreased synthesis of ATP and lower pH cause abnormal muscle contraction and skin sensitivity (Kim *et al.*, 2014). Thus, we examined transcript and physiologic changes in human rhabdomyosarcoma (RD) cells, as an *in vitro* model of human skeletal muscle, transfected with ADIPOQ siRNA. Intriguingly, transient transfection of ADIPOQ siRNA into RD cells recapitulated the distinct gene expression signature related to muscle composition, carbohydrate and lipid metabolism, and ion balance (Figure 2a), as previously described in human sensitive skin *in vivo* (Kim *et al.*, 2014). ATP amount was also significantly decreased in ADIPOQ siRNA–treated RD cells as measured using the ENLITEN ATP Assay System Bioluminescence Detection Kit (Promega, Madison, WI; Figure 2b). To measure intracellular pH, cells were incubated with 4 μM BCECF AM

Abbreviations: ADIPOQ, adiponectin; ADIPOR, adiponectin receptor; AMPK, 5′-AMP-activated protein kinase; ASIC3, acid sensing ion channel 3; CGRP, calcitonin gene–related peptide; RD, rhabdomyosarcoma; siRNA, small interfering RNA; TRPV1, transient receptor potential cation channel subfamily V member 1

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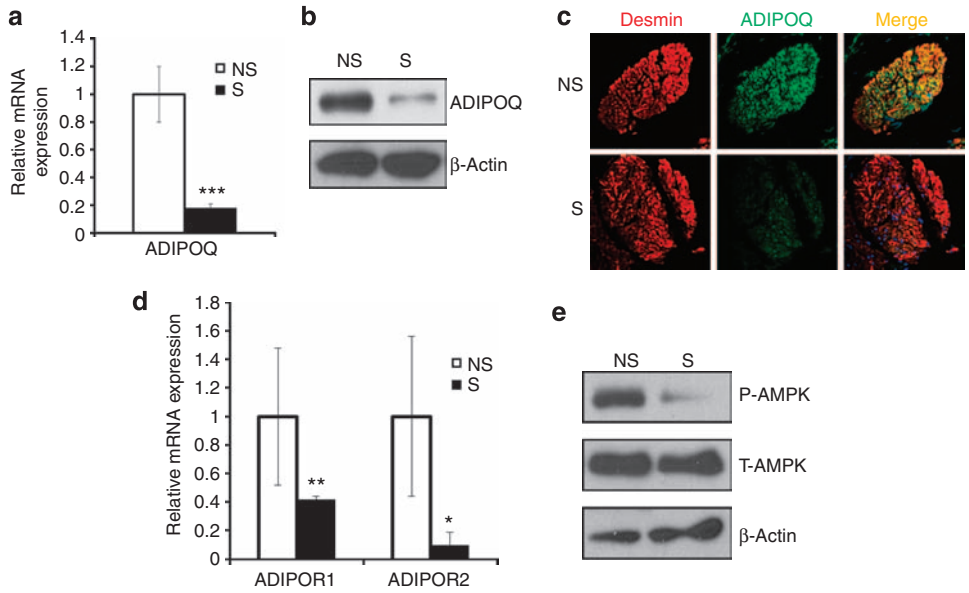

Figure 1. Confirmation of adiponectin (ADIPOQ) gene expressions in sensitive skin. Sensitive (S) and non-sensitive (NS) human skin samples were obtained from facial malar prominence or buttock after 10% lactic acid or normal saline (vehicle) application. *ADIPOQ* genes in sensitive skin were confirmed by (**a**) real-time PCR ($n=5$), (**b**) western blot analysis ($n=3$), or (**c**) immunofluorescent staining ($n=3$). (**d**) Expression of adiponectin receptor 1 or 2 (ADIPOR1/2). All real-time PCR data represent mean ± SEM of the ratio between each gene and 36B4. ADIPOR1 ($n=3$) and ADIPOR2 ($n=5$); $^{*}P<0.05$, $^{**}P<0.01$, and $^{***}P<0.001$. (**e**) Activation of 5′-AMP-activated protein kinase (AMPK). Another set of sensitive human skin samples and non-sensitive human skin samples was obtained from the buttock skin, and proteins were isolated. Western blot analysis was performed using antibodies against phosphor-AMPKα (Thr172) and total-AMPK. As a control, the level of β-actin was determined using an antibody for β-actin ($n=3$).

(Invitrogen, Life Technologies, Carlsbad, CA) or 5 μM SNARF-1 AM (Invitrogen), intracellular pH indicators. In comparison with control siRNA–transfected cells, ADIPOQ siRNA–transfected RD cells showed a much lower pH after the lactic acid challenge (Figure 2c) but higher expression of pain-related transcripts such as transient receptor potential cation channel subfamily V member 1 (TRPV1), acid sensing ion channel 3 (ASIC3), and calcitonin gene–related peptide (CGRP; Figure 2d), which is consistent with human sensitive skin *in vivo* (Kim *et al.*, 2014). More specifically, we demonstrated that sensitive skin is closely associated with impaired acidic homeostasis and pain provocation via TRPV1, ASIC3, and CGRP. Tissue acidosis elicits pain via TRPV1, ASIC3, and CGRP (Holzer, 2009). Although ASICs are the main mediators of pain caused by moderate acidity, TRPV1, along with ASICs, contributes to pain elicited by more severe acidification (Holzer, 2009; Deval *et al.*, 2010). When these nociceptors are activated, several neuropeptides such as substance P and CGRP are released, and pain develops (Reeh and Kress, 2001). Next, we examined whether exogenous administration of ADIPOQ could rescue these alterations. Indeed, treatment of RD cells with ADIPOQ induced a substantial reduction in expressions of TRPV1, ASIC3, and CGRP, which were increased by knockdown of ADIPOQ (Figure 2d), suggesting a potential therapeutic role of ADIPOQ supplementation for sensitive skin. Moreover, in A7r5 muscle cells, which can contract in response to phorbol esters (Dykes *et al.*, 2003), ADIPOQ knockdown induced aberrant muscle contraction, characterized by loss of fiber structure and the formation of peripheral bodies throughout the interval of contraction, even in the vehicle-treated state, as well as following phorbol-12,13-dibutyrate stimulation (Figure 2e).

Disruption of metabolic homeostasis can cause variable diseases such as obesity, diabetes, and the metabolic syndrome, which are closely associated with reduced ADIPOQ production (Kadowaki *et al.*, 2006). Here, we demonstrated that sensitive skin is also linked to ADIPOQ deficiency and ensuing metabolic alterations. The reduced expression of ADIPOQ and corresponding receptors and the mitigation of AMPK activity in sensitive skin might be responsible for the downregulation of genes involved in carbohydrate and fat metabolism, and decreased ATP content and pH, which could induce dysfunction of muscle contraction, and pain sensation in sensitive skin (Figure 2f).

Little is known about the relationship between sensitive skin and metabolic disorders such as obesity, diabetes, and the metabolic syndrome. Reduced ADIPOQ production in sensitive skin may influence metabolic disorders or *vice versa*. If and how obesity, diabetes, and the metabolic syndrome are associated with sensitive skin need to be elucidated in future studies. To better understand the implications of ADIPOQ in sensitive skin, further genetic or prospective studies are needed to address whether reduced ADIPOQ production is an inherited or acquired abnormality. Moreover, as the cellular location of ADIPOQ and its receptors has not been