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Decreased ATP synthesis and lower pH may lead to abnormal muscle contraction and skin sensitivity in human skin

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SUMMARY

Background: Sensitive skin represents hyperactive sensory symptoms showing exaggerated reactions in response to internal stimulants or external irritants. Although sensitive skin is a very common condition affecting an estimated 50% of the population, its pathophysiology remains largely elusive, particularly with regard to its metabolic aspects.
Objective: The objective of our study was to investigate the pathogenesis of sensitive skin.
Methods: We recruited healthy participants with 'sensitive' or 'non-sensitive' skin based on standardized questionnaires and 10% lactic acid stinging test, and obtained skin samples for microarray analysis and subsequent experiments.
Results: Microarray transcriptome profiling revealed that genes involved in muscle contraction, carbohydrate and lipid metabolism, and ion transport and balance were significantly decreased in sensitive skin. These altered genes could account for the abnormal muscle contraction, decreased ATP amount in sensitive skin. In addition, pain-related transcripts such as TRPV1, ASIC3 and CGRP were significantly up-regulated in sensitive skin, compared with non-sensitive skin.
Conclusions: Our findings suggest that sensitive skin is closely associated with the dysfunction of muscle contraction and metabolic homeostasis.

1. Introduction

Sensitive skin is a challenging skin condition characterized by prickling, burning, itching, stinging, pain, or tingling sensation, in response to various internal and external stimulants including physical (ultraviolet irradiation, heat, cold, climate), chemical (cosmetics), psychological, or hormonal factors [1]. Sensitive skin is becoming increasingly common worldwide, and it affects about 50% of the general population based on self-assessment questionnaires [1,2]. Despite the high prevalence and significant clinical impact of sensitive skin, it represents a complex challenge due to a lack of consensus regarding its epidemiology, pathophysiology, diagnosis and treatment guidelines [2]. Previous studies suggested that sensitive skin is associated with increased neurosensory responses, impaired skin barrier function, and altered immune responsiveness [3,4]. However, the pathophysiology involved in the development of sensitive skin remains largely enigmatic.

Here, through an unbiased microarray analysis of skin samples obtained from subjects with sensitive or non-sensitive skin, we identified the unexpected gene expression signature in sensitive skin related to muscle contraction, energy metabolism, and ion balance. This altered gene signature contributed to decreased ATP synthesis, and abnormal muscle contraction, leading to skin sensitivity in human skin.

2. Materials and methods

2.1. Subjects

For microarray analysis, we recruited healthy volunteers who perceived their skin to be 'sensitive' ($n = 9$, mean age 31.1 years,

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range 23–41 years, 4 males, 5 females) or to be 'not sensitive' (n = 9, mean age 35.9 years, range 25–49 years, 2 males, 7 females). Sensitive skin individuals were identified based on self-assessment questionnaires [5] and 10% lactic acid stinging test using a Hilltop chamber, as previously described [6,7]. The stinging sensation was rated by volunteers at every 1 min for 10 min on a 4-point scale with cumulative stinging scores (0 = none, 1 = slight, 2 = moderate, and 3 = severe). Lactic acid stinging test is one of the most frequently used and reliable diagnostic test for sensitive skin [1,7]. Subjects showing positivity to the lactic acid sting test with conforming replies from the questionnaires were classified as having sensitive skin, while those showing negative lactic acid sting test results with incompatible questionnaire responses were classified as having non-sensitive skin (Supplementary Fig. 1). Skin pH was measured before and after lactic acid sting test using a pH meter (PH905, Courage + Khazaka Electronic GmbH, Germany). Then, we obtained skin samples using 2-mm punch biopsy from facial malar prominence after lactic acid challenge and normal saline application, and subcutaneous fat tissues were removed from dermis. Equal amounts of RNA samples from three individuals allocated in each group were pooled onto one microarray chip, and were subject to hybridization using Affymetrix Human Genome U133 Plus 2.0 array (Affymetrix, Santa Clara, CA, USA). Data were normalized and analyzed by the invariant set normalization algorithm and the perfect match/mismatch (PM/MM) difference model using the dChip software [8]. The microarray dataset is accessible in Gene Expression Omnibus (GEO series accession number GSE48506). Besides, another group of volunteers with sensitive and non-sensitive skin (5 subjects per group) provided facial or buttock skin samples for the other experiments. This study was approved by the Institutional Review Board at Seoul National University Hospital, and all subjects provided written informed consent. The study was conducted according to the Declaration of Helsinki.

2.2. Real-time reverse transcription polymerase chain reaction (RT-PCR)

Total RNA was prepared from facial skin samples using the Trizol method (Life Technologies, Inc. Carlsbad, CA, USA) and 1 μg of total RNA was converted to cDNA using the First Strand cDNA Synthesis Kit (MBI Fermentas, Hanover, MD, USA). To quantitatively estimate the mRNA expression of each gene, PCR was performed on a 7500 Real-time PCR System (Applied Biosystems, Life Technologies, Inc.) using SYBR® Premix Ex Taq™ (Takara Bio Inc., Shiga, Japan). The information on primers used in this study is presented in Table 1. The PCR conditions were 50 °C for 2 min, 95 °C for 2 min, followed by 40 cycles at 95 °C for 15 s and 60 °C for 1 min. The data are presented as fold changes in gene expression normalized to 36B4.

2.3. Western blot analysis and immunofluorescence staining

Buttock skin samples were obtained and homogenized, and proteins were extracted using RIPA buffer (Millipore, Billerica, MA, USA) containing complete protease, phosphatase inhibitor (Roche Applied Science, Indianapolis, IN, USA), 5 mM PMSF, and 1 mM DTT. The protein content was determined using the Bradford reagent (Bio-Rad, Hercules, CA, USA). Equal amounts (50 μg) of protein were loaded, transferred and analyzed by Western blot analysis using specific antibodies, i.e., a mouse monoclonal myosin II (Pan) IgM (Covance, Princeton, NJ, USA), rabbit monoclonal antibody against actin α smooth muscle (Abcam, Cambridge, MA, USA), rabbit monoclonal antibody against myoglobin (Epitomics, Abcam), rabbit polyclonal antibody against actin alpha 1 skeletal muscle (Novus Biologicals, Littleton, CO, USA), rabbit polyclonal antibody against carbonic anhydrase III (Novus Biologicals), rabbit polyclonal antibody against TRPV1 (Millipore), rabbit monoclonal antibody against CGRP (Epitomics), and a goat polyclonal antibody

Table 1
Information on primers.

Gene symbol	Forward sequence	Reverse sequence	Size (bp)	Entrez gene ID
ACTR2	TTCCTGGGTGGTGCAGTT	ACGGGTATGACGGGAACA	152	10097
CDH1	GCCCCGCCTTATGATTCT	TCTGCATTTCCCAGCACA	228	999
FKBP5	AAGGCCCTTGGACTGGAC	TTCCTTGGCATCCTGCTC	246	2289
GM2A	AAAGCCATCCCAGCTCAGTA	GAGAACTCAGGGGGACACTG	158	2760
HLA-C	TCATCTCAGTGGGCTACGTG	TCCTCTGGAGGGTGTGAGAC	228	3107
PI3	AGCAGCTTCTTGATCGTGGT	GACCTTTGACTGGCTCTTGC	193	5266
S100A8	ATGCCGTCTACAGGGATGAC	ACGCCCATCTTTATCACCAG	160	6279
SERPINB13	TGGTGCTGGTGAACATGG	TTTGGCCTGCAAGTCCTC	170	5275
TFRC	GGAGAATCCTGGGGGTTATG	GGTGATTTTCCCTGCTCTGA	151	7037
YWHAZ	AGCAGGCTGAGCGATATGAT	TCTCAGCACCTTCCGTCTTT	180	7534
TTN	ACGCAGCCGTTACAAAGC	CCATTGGTGGCTTTCAGG	230	7273
MYBPC1	CGAGAAGATCGCCTTCCA	TCTGCCAGCTCCACAACA	168	4604
MYOZ1	CCAAACGCATGACCTTCC	ATCCAAGGGGATGCCAAT	176	58529
TPM1	TGAGTTTGCGGAGAGGTCAG	GTGTAAGCAGGCAGAGTGGA	166	7168
NEB	GTTGCCGACTCTCCGATCAA	CTGACTGATCTGGTCGCCTG	162	4703
ENO3	CTACCTGGGGAAAGCCAAGTT	CACATTGAAGGCTGGCACTG	160	2027
GYG2	GACTGAAACCATCTTGCCAGC	TTCCTCCTCTCTTCCTCGGG	201	8908
PYGM	TCACACTCGTAAAGGACCGC	TCTGTAGCGTCCGTCCCATA	180	5837
PCK1	GCATCGAGCTGACGGATT	AGGGCCAGTTGTTGACCA	165	5105
LIPE	CTTCCTCCGGGAGTATGTCA	CCTGTCTCGTTGCGTTTGTA	150	3991
PLIN	CTCTCGATACACCGTGCAGA	TGGTCCTCATGATCCTCCTC	207	5346
GPAM	TGAATCTGCACTGACCCTTG	GGGGAGTGCAGGAGTAACAA	212	57678
LPL	GTCCGTGGCTACCTGTCATT	TGTCCCACCAGTTTGGTGTA	213	4023
MB	TGGCACCTGCCCTAAAATAGC	CGCAGTCTTCTGGGATGCTT	151	4151
CASQ1	GCCAACCCAGATCCCACTAC	CGGTCCACACCATCGTACTC	167	844
CA3	GGAAGACCTGCCGAGTTGTA	CCGCTGCATACTTGACTCCA	162	761
ATP6V1B1	GGCTGGTGAAGAAGTCCAAG	TTCAGGAAGAGGCAGACGTT	154	525
TRPV1	TGTGCCTGCGTCTAGCTGGTTG	CCGGGAAAGCCTCCTCCGAGT	232	7442
CGRP	TGCCCAGAAGAGAGCCT	TGAAGGTCCCTGCGGC	146	796
ASIC3	GATGGGGCTGTTCATCGGGGC	GGAGGGGTGGGAGGTCTTCGG	230	9311
ACTB (beta-actin)	ACAGAGCCTCGCCTTTGC	ACGATGGAGGGGAAGACG	159	60
RPLP0 (36B4)	TCGACAATGGCAGCATCTAC	TGATGCAACAGTTGGGTAGC	130	6175

against ASIC3 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). As a control, the level of β-actin was determined in each cell lysate using a goat polyclonal antibody for β-actin (Santa Cruz Biotechnology, Inc.).

For immunofluorescence staining, sections of facial skin specimens (4 μm) were stained with the same above mentioned antibodies in a humidified chamber at 4 °C for 18 h. After washing in PBS, the sections were incubated with a secondary Alexa 488- or Alexa 594-conjugated goat anti-mouse IgG (Invitrogen, Life Technologies, Inc.) antibody for 1 h at room temperature. The nuclei were counterstained with DAPI staining.

2.4. ATP assay

Buttock skin samples were obtained and homogenized, and ATP was extracted with 0.5% TCA solution for 15 min at 4 °C, and was measured using the ENLITEN® ATP Assay System Bioluminescence Detection Kit (Promega, Madison, WI, USA). Total protein concentration was measured using the Bradford reagent, and the ATP generated was normalized to total protein.

2.5. Cell studies

Human RD striated muscle cells derived from rhabdomyosarcoma were obtained from Korean Cell Line Bank (Seoul, Korea). RD cells are frequently used as an *in vitro* skeletal muscle model [9–11]. The cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 units/mL penicillin G, and 100 μg/mL streptomycin. The medium was changed every 2 days, and cells were subcultured at least once a week.

A7r5 smooth muscle cells derived from embryonic rat aorta and having the ability to contract in response to phorbol esters were obtained from American Type Culture Collection (Manasass, VA, USA). Phorbol ester-induced muscle contraction model is a well-established model using A7r5 cells [12–14]. The cells were maintained in DMEM supplemented with 10% FBS, 100 units/mL penicillin G, and 100 μg/mL streptomycin. The medium was changed every 2 days, and cells were subcultured at least once a week.

2.6. Confocal microscopy

A7r5 cells were cultured on chamber slides. After treatment with 10^{-7} M phorbol-12,13-dibutyrate (PDBu) for 30 min, the cells were fixed and permeabilized by the addition of paraformaldehyde for 5 min. The cells were then washed with phosphate-buffered saline (PBS), and incubated for 5 min in blocking solution (Ultra V blocking solution, Thermo Scientific). Cells were stained for α-actin by incubation in a 1:500 dilution of rabbit monoclonal anti-α-smooth muscle actin antibody (Abcam) overnight at 4 °C followed by incubation with an Alexa 594-labeled secondary antibody (Invitrogen). Myosin was visualized using a mouse monoclonal anti-myosin smooth muscle antibody (Covance) followed by Alexa

Table 2
Significantly down-regulated genes in human sensitive skin selected by microarray analysis.

Gene title	Gene symbol	Entrez gene ID	S/NS(−)	S/NS(+)
Structural constituent of muscle & muscle contraction				
Titin	TTN	7273	0.08	0.08
Actin, alpha 1, skeletal muscle	ACTA1	58	0.18	0.09
Myosin binding protein C, slow type	MYBPC1	4604	0.22	0.10
Myozenin 1	MYOZ1	58,529	0.26	0.14
Tropomyosin 1 (alpha)	TPM1	7168	0.28	0.16
Nebulin	NEB	4703	0.33	0.29
Kelch repeat and BTB (POZ) domain containing 10	KBTBD10	10,324	0.10	0.08
Carbohydrate metabolism				
Enolase 3 (beta, muscle)	ENO3	2027	0.29	0.25
Glycogenin 2	GYG2	8908	0.31	0.27
Protein phosphatase 1, regulatory (inhibitor) subunit 1A	PPP1R1A	5502	0.42	0.28
Phosphorylase, glycogen; muscle (McArdle syndrome, glycogen storage disease type V)	PYGM	5837	0.24	0.28
Phosphoenolpyruvate carboxykinase 1 (soluble)	PCK1	5105	0.58	0.35
Lipid metabolism				
Lipase, hormone-sensitive	LIPE	3991	0.41	0.31
Perilipin1	PLIN1	5346	0.34	0.32
Glycerol-3-phosphate acyltransferase, mitochondrial	GPAM	57,678	0.52	0.34
Fatty acid binding protein 4, adipocyte	FABP4	2167	0.43	0.34
Lipoprotein lipase	LPL	4023	0.43	0.35
Ion transport & Ionic balance				
Calsequestrin 1 (fast-twitch, skeletal muscle)	CASQ1	844	0.45	0.23
Myoglobin	MB	4151	0.16	0.14
Carbonic anhydrase III, muscle specific	CA3	761	0.42	0.18
ATPase, H+ transporting, lysosomal 56/58 kDa, V1 subunit B1 (Renal tubular acidosis with deafness)	ATP6V1B1	525	0.58	0.51
Signaling pathway				
Adiponectin, C1Q and collagen domain containing	ADIPOQ	9370	0.42	0.39
Phosphodiesterase 3B, cGMP-inhibited	PDE3B	5140	0.48	0.40
Activin A receptor, type IC	ACVR1C	130,399	0.43	0.42
Others				
G0/G1switch 2	G0S2	50,486	0.36	0.28
Cell death-inducing DFFA-like effector c	CIDEC	63,924	0.45	0.35
Tenomodulin	TNMD	64,102	0.59	0.46
Retinol binding protein 4, plasma	RBP4	5950	0.35	0.26
Cysteine-rich secretory protein 3	CRISP3	10,321	0.33	0.58

S: Sensitive skin, NS: Non-sensitive skin, (−): normal saline application, (+): lactic acid application.

488-labeled IgM secondary antibody (Invitrogen). Images were acquired using a confocal laser-scanning microscope (LSM 510 META, Zeiss, Germany).

2.7. Statistical analysis

Data are presented as the means ± SEM. Significance was analyzed by the paired *t*-test or Student's *t*-test. Differences were considered statistically significant when $P < 0.05$.

3. Results and discussion

3.1. Decreased expression of muscle contraction-related genes in human sensitive skin

To elucidate the changes of the gene expression and signaling pathways involved in the pathogenesis of sensitive skin, a microarray analysis was performed using paired skin samples after either 10% lactic acid or normal saline application from subjects with sensitive or non-sensitive skin. Among 17 up-regulated and 29 down-regulated genes differentially expressed in sensitive skin, up-regulated genes represented the inflammatory and immune responses (Supplementary Table 1 and Supplementary Fig. 2). On the other hand, we found an unexpected genetic signature related to muscle composition/contraction, carbohydrate/lipid metabolism, and ion transport/ionic balance from down-regulated genes in sensitive skin after 10% lactic acid challenge compared with those of non-sensitive control skin (Table 2 and Fig. 1).

Many down-regulated genes such as titin (TTN), skeletal muscle alpha-1-actin (ACTA1), slow-type myosin binding protein C (MYBPC1), myozenin 1 (MYOZ1), alpha tropomyosin 1 (TPM1), nebulin (NEB) were muscle-specific, and associated with the muscle contraction and relaxation process as well as muscle structure (Table 2 and Fig. 1A). In human facial skin, striated muscle fibers are found in the reticular dermis and subcutis [15], along with smooth muscles accompanying the hair follicles (arrector pili muscle). The sarcomere, a functional unit of muscle, is composed mainly of thick filaments (myosin), thin filaments (actin), and elastic components (TTN). The type of myosin present in muscle (myosin II) is a 520 kD hexamer consisting of two identical heavy chains and four light chains. The thin filaments comprise actin and two regulatory proteins (troponin and TPM). When the muscle contraction is triggered by nerve impulses, Ca^{2+} released from the sarcoplasmic reticulum binds to troponin and myosin binding site on actin is exposed by TPM. In the presence of ATP, the myosin head binds to actin that enables the thin filament to slide along the thick filament, allowing for the shortening of the sarcomere. This actomyosin-based process is called the cross-bridge cycling [16,17]. The extremely large TTN protein (3000 kD) is a molecular spring that provides the structural integrity and contractile stability to sarcomeres, and maintains the calcium-sensitive tension if sarcomeres are stretched beyond thin and thick filament overlap [18]. NEB is an integral component of the thin filament, and it plays a major role in the regulation of muscle

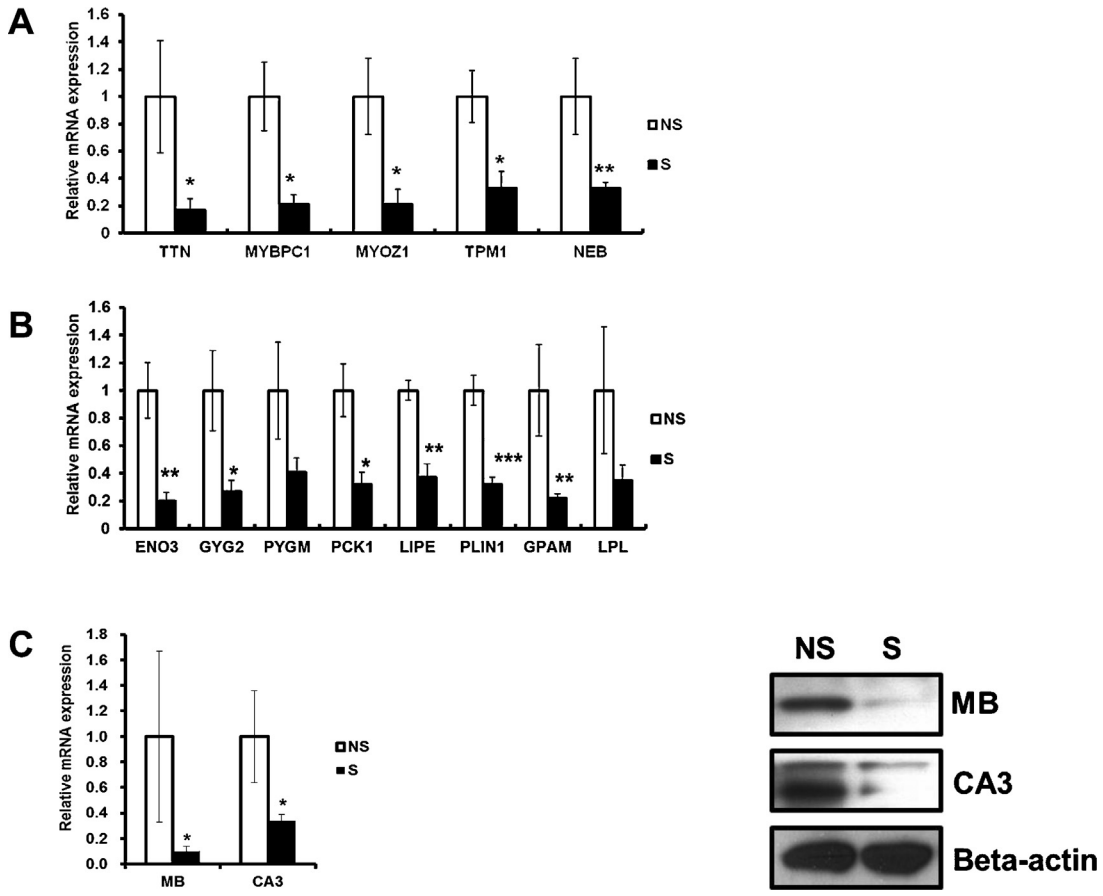

Fig. 1. Confirmation of characteristic gene expressions in sensitive skin. Sensitive (S) and non-sensitive (NS) human skin samples were obtained from facial malar prominence or buttock after 10% lactic acid or normal saline (vehicle) application. A microarray analysis was performed and differentially down-regulated genes in sensitive skin were confirmed by real-time PCR or Western blot analysis. (A) Down-regulated genes related to muscle contraction. (B) Down-regulated genes related to carbohydrate and lipid metabolism. (C) Down-regulated genes related to ionic transport & ionic balance. All data represent mean ± SEM. ($n = 5$). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

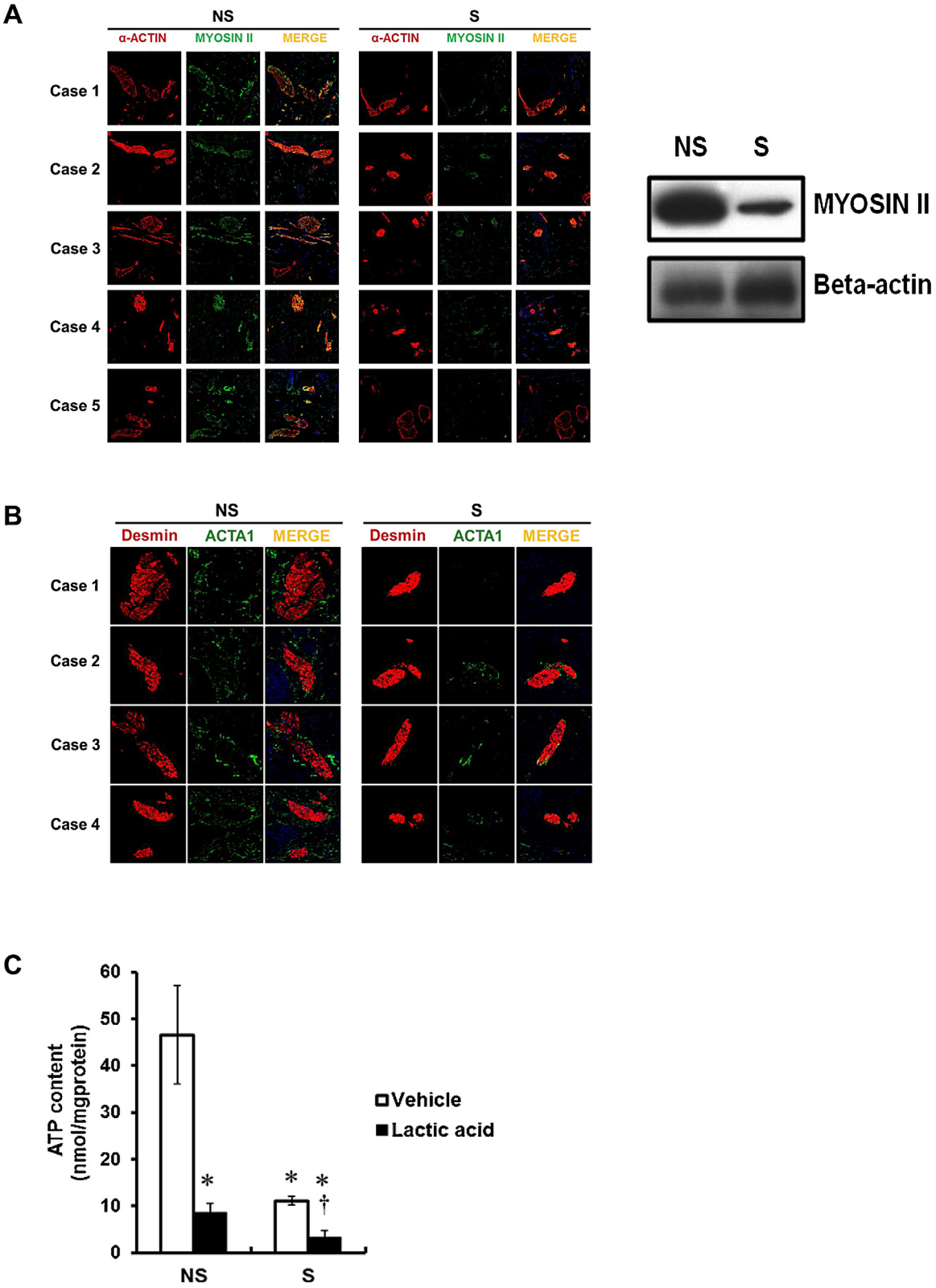

Fig. 2. Sensitive skin showed more compacted actin-bound myosin cross-bridges and lower ATP level, compared with those of non-sensitive skin. Sensitive (S) and non-sensitive (NS) human skin samples were obtained from facial malar prominence or buttock after 10% lactic acid challenge. (A) Muscle contraction visualized by actin-bound myosin cross-bridges. ($n = 5$). Immunofluorescence staining was performed using antibody against myosin II (Pan) and antibody against alpha smooth muscle actin (α-ACTIN). (B) Desmin expression does not differ, but actin alpha 1 skeletal muscle (ACTA1) expression is decreased in sensitive skin. Immunofluorescence staining was performed using antibody against desmin and antibody against ACTA1. (C) ATP contents. All data represent mean ± SEM. ($n = 3$). $^{*}P < 0.05$, vs. the NS vehicle group, $^{\dagger}P < 0.05$ vs. the S vehicle group.

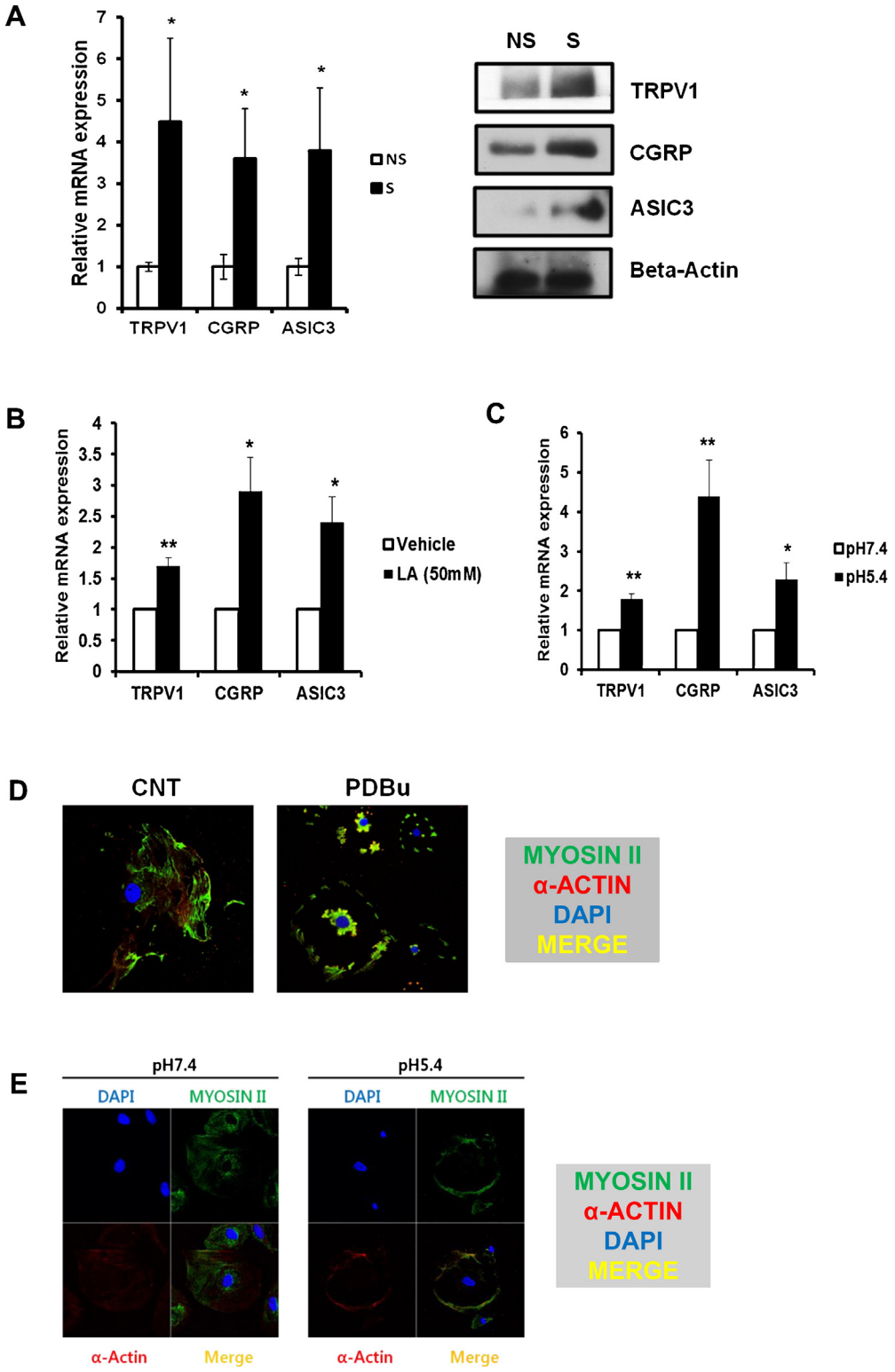

Fig. 3. Enhanced acidity in sensitive skin can mediate pain via up-regulation of pain sensors and elicitation of abnormal muscle contraction. The expressions of TRPV1, ASIC3, and CGRP mRNA were measured in (A) sensitive skin tissues and in human RD muscle cells cultured with (B) 50 mM lactic acid (LA, pH 4.2) or (C) low pH condition (pH 5.4) for 24 h. Protein expressions of TRPV1, ASIC3, and CGRP were also measured in human sensitive skin. Data represent mean ± SEM of the ratio between each gene and 36B4. (n = 3–5). $^{*}P < 0.05$, $^{**}P < 0.01$. In Western blot analyses, the level of β-actin was used as a control. (D) Phorbol-12,13-dibutyrate (PDBu)-induced muscle contraction model. (E) Effect of H^{+} on muscle contraction (pH 7.4 or 5.4).

contraction and calcium homeostasis as well as in the determination of the thin-filament length [19]. MYBPC, a major component of the thick filament, contributes to the sarcomeric structure and efficient regulation of muscle contractility as an adaptor to connect the ATP-regenerating muscle-type creatine kinase and myosin [20]. As these muscle-related genes were down-regulated in sensitive skin, we hypothesized that the development of sensitive skin may be related to the dysregulation of muscle contraction and relaxation process. To elucidate our hypothesis, we visualized actin-bound myosin cross-bridges using immunofluorescence

Table 3
Subject characteristics and skin pH.

	Age (years)	Height (cm)	Weight (kg)	BMI	Sex (M:F)	Surface pH	
						Before*	After*
Non-sensitive skin	37.8 ± 2.9	168.3 ± 3.3	66.9 ± 4.2	23.5 ± 0.9	4:5	5.27 ± 0.3	4.09 ± 0.5
Sensitive skin	37.4 ± 3.4	163.9 ± 2.9	59.5 ± 2.7	22.1 ± 0.7	4:6	5.51 ± 0.2	3.86 ± 0.4**

* LAST, lactic acid sting test.
** $P < 0.01$ vs. pH before LAST.

staining and observed that actin-bound myosin cross-bridges in sensitive skin had more compacted shape than those in non-sensitive skin, indicating more contracted cross-bridge state in sensitive skin tissues (Fig. 2A). Besides, sensitive skin showed a substantially weaker expression of myosin II, a crucial component of functional integrity, than non-sensitive skin (Fig. 2A). To rule out a possible sampling bias due to the varying inclusion of muscle tissues, we examined the expression of another muscle-specific marker, desmin, and found no significant differences between sensitive skin and non-sensitive skin (Fig. 2B). Moreover, our data showed no significant differences in gene expression between skin samples from face and buttock. Therefore, these results indicate that decreased expressions of muscle-related genes in sensitive skin was not due to either a sampling bias or differences in anatomical sites, but they were related to the pathogenesis of sensitive skin. Also, sensitive skin showed more compacted shape of cross-bridges than non-sensitive skin both in smooth muscle (ACTA2, Fig. 2A) and striated muscle (ACTA1, Fig. 2B). Therefore, both types of muscles may be involved in the pathogenesis of sensitive skin. Our results suggest that sensitive skin may be associated with abnormal muscle contraction/relaxation process.

3.2. Decreased energy metabolism and ATP may cause abnormal muscle contraction in sensitive skin

Muscle contraction and relaxation process requires a continuous energy supply in the form of ATP through metabolic pathways such as phosphocreatine, anaerobic glycolysis, or oxidative metabolism. Carbohydrate and fat are the principal substrates for oxidative metabolism, which generates most of the energy for muscle contraction [21]. The genes related to carbohydrate (ENO3, GYG2, PYGM, PCK1) and fat metabolism (LIPE, PLIN1, GPAM, FABP4, LPL) were down-regulated in sensitive skin, compared with those of non-sensitive skin (Table 2 and Fig. 1B). Moreover, expression of myoglobin, which supports ATP generation by mediating oxygen transport to muscle tissues [22], was decreased in sensitive skin (Table 2 and Fig. 1C). Also, carbonic anhydrase III (CA3) (muscle-specific), which is known to be involved in mitochondrial ATP synthesis [23], was decreased in sensitive skin (Table 2 and Fig. 1C). These findings led us to investigate whether the amount of ATP is decreased in sensitive skin or not. Indeed, sensitive skin stored significantly less ATP than non-sensitive skin in its basal state, and lactic acid stimulation resulted in significantly more decreased ATP contents in sensitive skin, compared with non-sensitive skin (Fig. 2C). Interestingly, difference of ATP contents was more apparent in basal state than in lactic acid stimulated state, suggesting that ATP synthesis might be disrupted much even in the basal state of sensitive skin, and be further compromised by external stimuli such as lactic acid. In muscle tissues, lack of ATP is known to be associated with abnormal muscle contraction/relaxation, muscle fatigue, and pain [24]. Our results suggest that lower expression of genes involved in carbohydrate and lipid metabolic pathways could result in lower level of ATP in basal condition and after lactic acid stimulation, which may result in abnormal muscle contraction in sensitive skin.

3.3. Enhanced acidity may cause skin sensitivity and abnormal muscle contraction in human sensitive skin

Muscle exposed to anaerobic state triggers the overproduction of carbon dioxide and H^+, leading to the enhanced acidity and corollary pain by irritation of nerve endings [25,26]. Normal aerobic ATP synthesis process related genes including CA3 were decreased, as well as ATP levels, in sensitive skin. Therefore, these changes can cause increased acidity. Enhanced protons (acidity) is known to elicit pain via transient receptor potential cation channel subfamily V member 1 (TRPV1) and acid sensing ion channel 3 (ASIC3) [27,28]. TRPV1 is a Ca^{2+}-permeable, nonselective cation channel expressed in nociceptive neurons of the dorsal root ganglia, corresponding C- and Aδ-sensory fibers, and other cells such as epidermal keratinocytes and skeletal muscle cells [28–30]. TRPV1 acts as a primary biological sensor regulated by membrane depolarization, noxious heat, vanilloid and endocannabinoid compounds, extracellular protons, and inflammatory mediators [28]. ASIC3, an essential sensor of acidic and primary inflammatory pain, is the most sensitive biological detector of extracellular pH. It is notable that ASIC3 becomes even more sensitive in the presence of lactate [31]. Activation of TRPV1 triggers the release of neurotransmitters such as calcitonin gene-related peptide (CGRP) and substance P, resulting in pain and neurogenic inflammation [32]. Here, we found that the expressions of TRPV1, ASIC3 and CGRP were significantly induced in human sensitive skin, compared with those in non-sensitive skin *in vivo* (Fig. 3A). Moreover, we measured the skin pH in basal condition and after lactic acid stimulation in sensitive and non-sensitive skin. While lactic acid treatment produced a substantial decrease of pH in both sensitive and non-sensitive skin tissues, sensitive skin exhibited a significantly greater pH derangement than non-sensitive skin (Table 3). However, basal skin pH without lactic acid stimulation both in sensitive and non-sensitive skin did not differ significantly (Table 3). In addition, we measured a pH of skin lysate from sensitive and non-sensitive individuals. Because of the buffering effects, basal pH values measured in non-sensitive and sensitive skin showed slight difference, but there was significant decrease in tissue pH after LAST both in non-sensitive skin and sensitive skin (Supplementary Fig. 3). These data demonstrated that subjects with sensitive skin showed impaired pH homeostasis after lactic acid stimulation and increase of detection ability for pH upon internal or external stimuli such as lactic acid. To elucidate the possible role of acidic stimulants in sensitive skin, RD cells were treated with 50 mM lactic acid (pH 4.2) and low pH culture medium (pH 5.4). These treatments significantly induced the expressions of TRPV1, ASIC3, and CGRP mRNA (Fig. 3B and C). Therefore, our results suggest that enhanced acidity might induce pain via stimulation of TRPV1, ASIC3, and CGRP in the human sensitive skin. Consistent with our findings, a TRPV1 inhibitor was demonstrated to have the potential as a bioactive molecule against sensitive skin [27]. Taken together, our results suggest that sensitive skin may be associated with pain provocation through TRPV1, ASIC3, and CGRP due to impaired acidic homeostasis.

To elucidate the effects of lower pH on muscle contraction in sensitive skin, a smooth muscle cell line A7r5, which exhibits a representative adult smooth muscle phenotype [33], were employed and treated with low pH culture medium (pH 5.4). In a muscle contraction model using A7r5 cells, phorbol 12,13-dibutyrate (PDBu) induced significant remodeling of α-actin and myosin II, characterized by loss of fiber structure and the formation of peripheral bodies during contraction (Fig. 3D) [13]. A lower pH condition provoked the contraction of muscle cells compared with normal pH condition (pH 7.4) (Fig. 3E). Our data suggest that lower pH in sensitive skin may cause abnormal muscle contraction, which may contribute to the development of sensory symptoms in sensitive skin. Future studies using various approaches such as loss- or gain-of-function studies in model systems or clinical trial involving human subjects are warranted to elucidate functional relevance of our findings.

In conclusion, we uncovered a novel, unexpected gene expression changes in sensitive skin that are related to metabolic homeostasis, namely muscle contraction, carbohydrate and lipid metabolism and ion transport/balance process, which may result in decreased synthesis of ATP and enhanced proton, leading to skin sensitivity.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jdermsci.2014.09.008.

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