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Letter to the Editor

The novel adiponectin receptor agonist APN5N alleviates sensitive skin by upregulating adiponectin expression

To the Editor,

Sensitive skin is a common condition defined by unpleasant sensations, such as stinging, burning, pain, pruritus, and tingling, in response to stimuli that normally should not provoke such reactions [1]. The mechanism underlying sensitive skin remains elusive, but impaired skin barrier function, neurosensory dysfunction, and adiponectin (APN) deficiency are suggested to contribute to its development [2–4]. In our previous transcriptomic study, we demonstrated that sensitive skin is characterized by APN deficiency, and administering exogenous APN can restore disturbed homeostasis *in vitro*, suggesting APN's potential in treating sensitive skin [3,4]. However, its large size (28 kDa with 244 amino acids) makes it unsuitable for topical application. Recently, we engineered a transdermally deliverable peptide (GLYYF; P5) that reproduces the biological effect of APN by specifically binding to APN receptor 1 (AdipoR1) [5]. Here, we further enhanced its activity by developing a C-terminal amidation peptide (GLYYF-NH_2; APN5N) and validated its efficacy using *in vitro* and *in vivo* models of sensitive skin.

We initially designed multiple candidate peptides either by modifying P5 through C-terminal amidation or using D-amino acids to enhance the peptide activity. APN5N was selected based on its effect to activate AMPK (data not shown). In human RD striated muscle cells [4,6], treatment with APN5N for 24 h increased phospho-AMPK levels in a concentration-dependent manner after 24 h (Fig. 1a). *AdipoR1* small interfering (si)RNA transfection abolished APN5N- or recombinant human APN-induced upregulation of p-AMPK expression (Fig. 1b). Immunofluorescence (IF) staining revealed the colocalization of FITC-APN5N and AdipoR1 (Fig. 1c). These results indicate that APN5N, like P5, can activate the AMPK signaling pathway through AdipoR1 *in vitro*. To examine the efficacy *in vivo*, different concentrations of APN5N (0.005%, 0.05%, 0.1%, and 0.5%) or vehicle were topically applied to the buttock skin of elderly individuals (n = 3) for 24 h, and APN, AdipoR1, and p-AMPK expression was determined using IF staining (Fig. 1d) and western blotting (Fig. 1e). APN5N-treated skin showed a dose-dependent upregulation of APN, AdipoR1, and p-AMPK expression. Taken together, APN5N could bind to AdipoR, activate the AMPK signaling pathway, and substantially upregulate endogenous APN expression *in vivo*.

A sensitive skin model using RD cells was used to evaluate the efficacy of APN5N *in vitro* [4]. *APN* siRNA-transfected cells exhibited significantly elevated expression of transient receptor potential vanilloid 1 (*TRPV1*) and calcitonin gene-related peptide (*CGRP*) (Fig. 2a), and APN5N significantly downregulated their expression (Fig. 2a).

To investigate whether topical application of APN5N can alleviate sensitivity in patients with sensitive skin, we conducted a randomized, double-blind clinical study on 54 patients with sensitive skin (mean age: 46.1 ± 8.7 years; range: 24–66). The patients were identified and selected based on a 10% lactic acid stinging test (LAST) on the malar prominence [3,7]. Patients with skin disorders such as dermatitis, rosacea, or acne were excluded. Participants who experienced stinging sensation at least once at 1-min intervals for 10 min were classified as having sensitive skin. They were randomly assigned to either the APN5N group or the placebo group (n = 27 each), and a 0.01% APN5N-containing formulation and vehicle was topically applied twice daily for 8 weeks, respectively. The study was approved by the Institutional Review Board of Seoul National University Hospital and conducted according to the Declaration of Helsinki. All participants provided written informed consent and completed the study. At week 4, 29.6% and 14.8% of the APN5N- and vehicle-treated patients became non-sensitive, respectively, as determined by LAST ($P = 0.327$). At 8 weeks, APN5N significantly alleviated skin sensitivity, compared with the placebo (48.1% and 14.8%, respectively; $P = 0.018$) (Fig. 2b). Adverse events (mild and transient comedo-like skin lesions) were reported in three patients (two and one in the APN5N and vehicle groups, respectively). Biophysical measurements, such as hydration, transepidermal water loss, erythema index, and melanin index, did not significantly change during treatment (Supplementary Table S2). Histological analyses revealed that, unlike the placebo group that exhibited comparable expression levels, the APN5N-treated group showed substantial increases in APN, p-AMPK, and AdipoR1 levels but a marked decrease in TRPV1 level, compared with the baseline levels after 8 weeks of application (Fig. 2c). After 8 weeks, APN5N also significantly upregulated *APN* mRNA expression compared to both placebo treatment and pre-APN5N treatment levels (Fig. 2d). Interestingly, epidermal keratinocytes exhibited notable changes in TRPV1 expression following APN5N treatment. Further *in vitro* studies involving keratinocytes, sensory neurons, or other skin cells could help elucidate the specific effects of APN5N treatment on each cell type and their interactions.

Sensitive skin is a challenging neuropathic disorder with limited treatment options [2]. APN deficiency in sensitive skin disrupts metabolic, energy, and acidic homeostasis, subsequently inducing dysfunction of muscle contraction and pain sensation [4]. APN is a pleiotropic adipokine that regulates numerous physiological functions. Reduced APN levels are associated with several diseases and conditions, such

Abbreviations: AdipoR1, Adiponectin receptor 1; APN, adiponectin; AMPK, 5'-AMP-activated protein kinase; CGRP, calcitonin gene-related peptide; TRPV1, transient receptor potential cation channel subfamily V member 1

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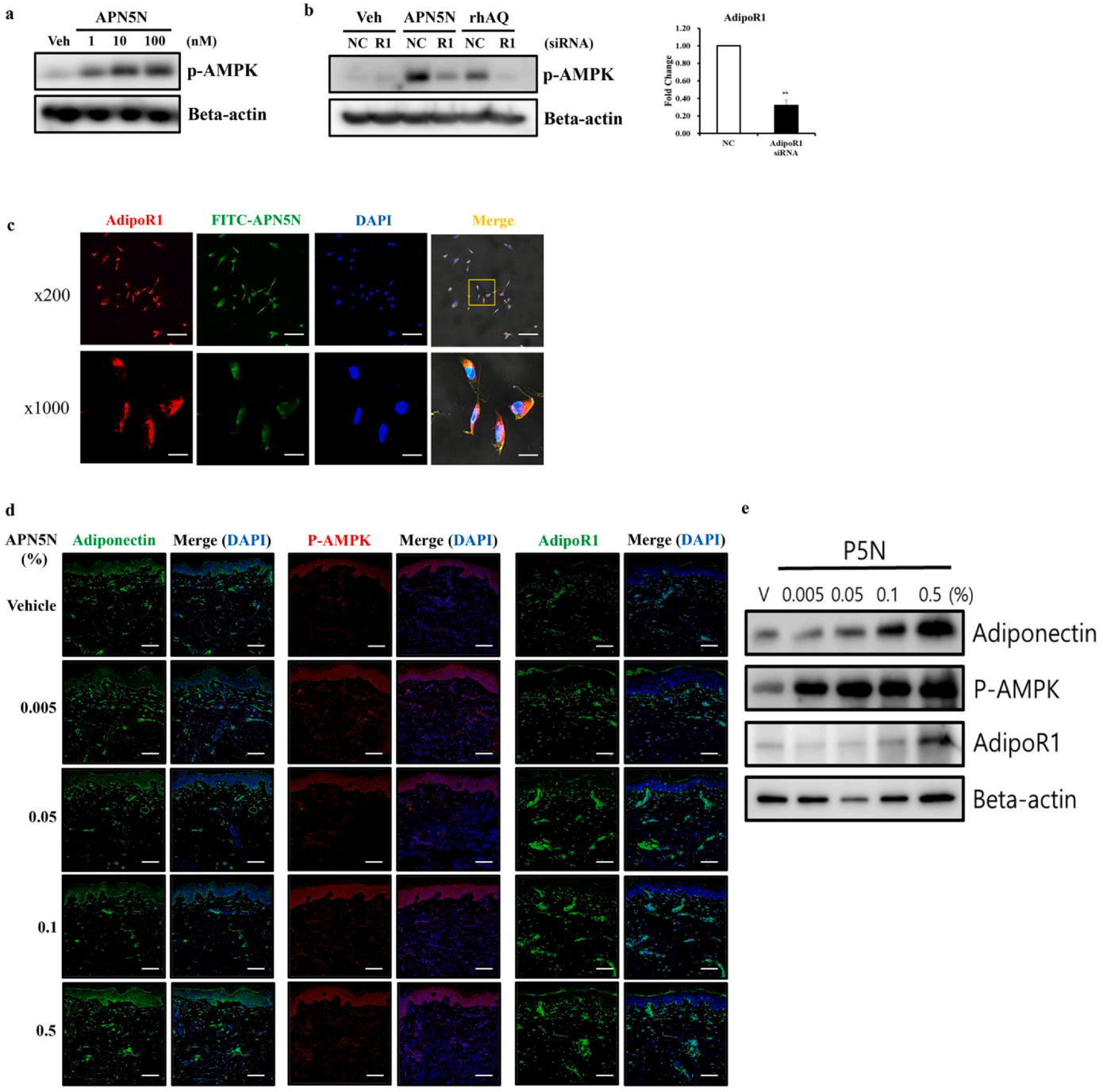

Fig. 1. APN5N increases phospo-AMPK levels via binding to AdipoR1 and induces the expression of APN, adipoR1, and phospho-AMPK. (a) Human RD striated muscle cells were treated with APN5N for 24 h. Western blot analysis was performed using antibodies against phospho-AMPKα (Thr172). As a loading control, β-actin levels were determined using an anti-β-actin antibody. (b) RD cells were transfected with *AdipoR1* siRNA or scrambled siRNA (NC). Then, the transfected cells were treated with APN5N (100 nM), recombinant human APN (100 ng/ml), or vehicle (DMSO). Western blot analysis was performed using antibodies against phospho-AMPKα (Thr172). As a loading control, the level of β-actin was determined using an anti-β-actin antibody. The siRNA knockdown efficiency for AdipoR1 was quantified by real-time PCR (n = 3). $^{**}P < 0.01$ vs. the NC group. Data are presented as the mean ± SEM of the ratio between each gene and *36B4*. (c) Double-labelled immunofluorescence staining with AdipoR1 and FITC-APN5N. Scale bar, 100 μm (upper), 20 μm (lower). (d–e) Human buttock skin samples were obtained from subjects (range: 80.3 ± 0.6 years; n = 3) after APN5N (0.005%, 0.05%, 0.1%, and 0.5%) or vehicle (EtOH:PG=3:7) application for 24 h. Determination of APN, p-AMPK, and AdipoR1 expression using (d) immunofluorescence staining (Scale bar, 100 μm) and (e) western blotting. Abbreviations: adipoR1, adiponectin receptor 1; AMPK, 5'-AMP-activated protein kinase; APN, adiponectin; siRNA, small interfering RNA, Veh, vehicle.

as obesity, diabetes, cardiovascular diseases, inflammation, atopic dermatitis, hair loss, sensitive skin, and skin aging [4,5,8,9]. Although APN is an excellent therapeutic target, it is challenging to develop APN-based therapeutic modulators, including peptides and small molecules, which are currently unavailable. Using P5 as a base [5], we developed C-terminal amidated APN5N, which could activate the AMPK signaling pathway in a dose-dependent manner through binding to AdipoR1 *in vitro* and *in vivo* human skin. APN5N treatment significantly reduced

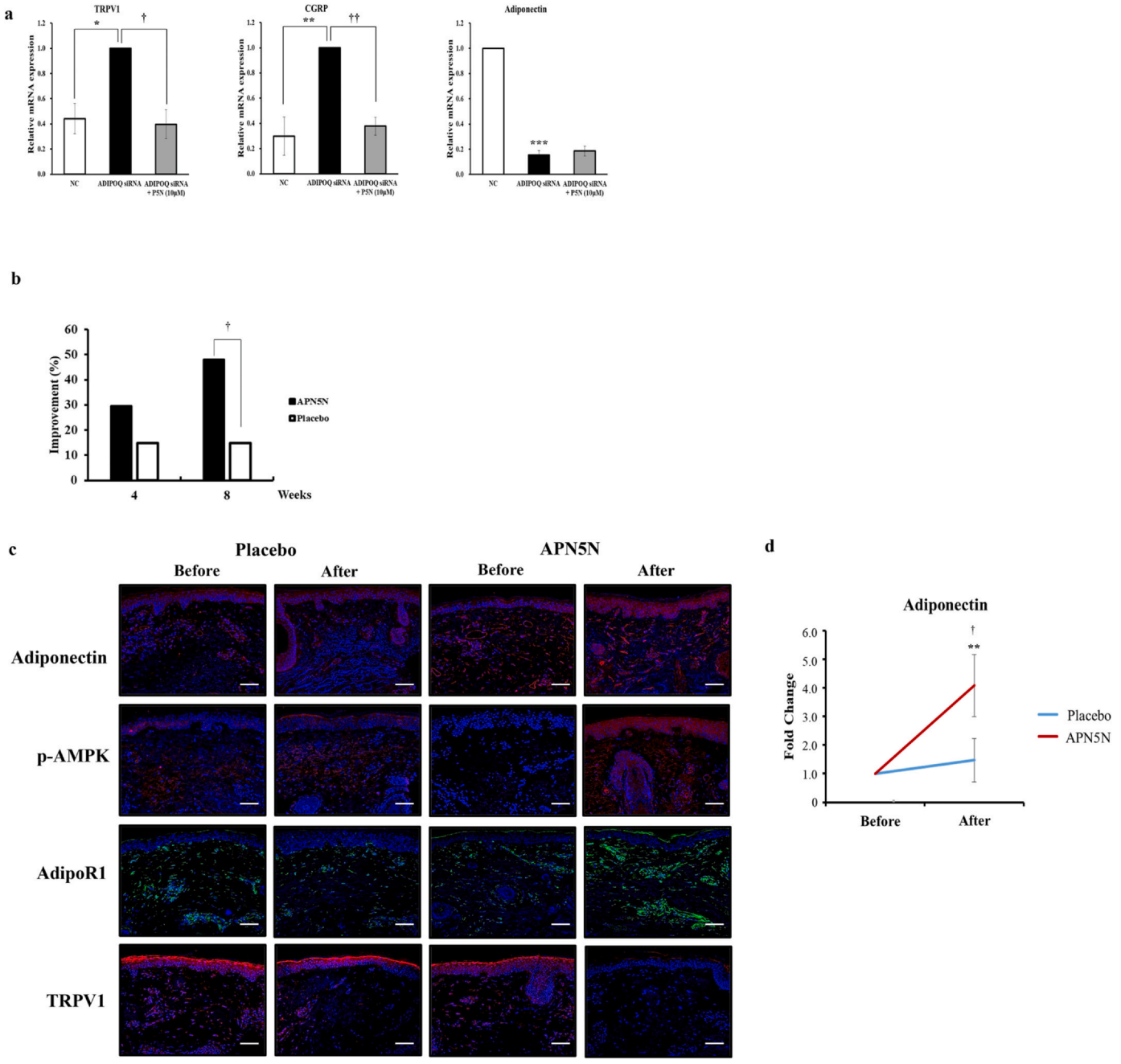

Fig. 2. Topical application of APN5N alleviates sensitive skin by upregulating APN and AdipoR1 expression and downregulating TRPV1 expression. (a) RD cells were first transfected with *APN* siRNA or scrambled siRNA (NC) and then treated with APN5N (100 nM). *TRPV1, CGRP,* and *APN* mRNA levels were determined using real-time PCR (n = 3). $^{*}P < 0.05$, $^{***}P < 0.001$ vs. the NC group; †$P < 0.05$ vs. the *APN* siRNA-treated vehicle group. Data are presented as the mean ± SEM of the ratio between each gene and *36B4*. (b–d) Fifty-four patients with sensitive skin were identified based on 10% lactic acid stinging test and topically applied a 0.01% APN5N-containing formulation or placebo twice a day for 8 weeks. (b) Improvement of sensitivity in patients with sensitive skin. Significance was determined using Fisher's exact test. (c–d) Skin samples were obtained from the malar area at baseline and 8 weeks using 2-mm punch biopsy (n = 8/group). (c) APN, p-AMPK, AdipoR1, and TRPV1 protein levels were measured using immunofluorescence staining. Scale bar, 100 μm. (d) *APN* mRNA levels were measured using real-time PCR. Data are presented as the mean ± SEM of the ratio between each gene and *36B4*. $^{**}P < 0.01$, before vs. after APN5N treatment. †$P < 0.05$, post-placebo vs. post-APN5N-treatment. Abbreviations: adipoR1, adiponectin receptor 1; AMPK, AMP-activated protein kinase; APN, adiponectin; siRNA, small interfering RNA; CGRP, calcitonin gene-related peptide; TRPV1, transient receptor potential cation channel subfamily V member 1.

the elevated *TRPV1* and *CGRP* expression in *APN*-knockdown cells. About half of the patients with sensitive skin (48.1%) that were treated with topical APN5N became non-sensitive after 8 weeks; this result significantly differed from that obtained for patients treated with the vehicle (14.8%). Consistent with the clinical results, APN5N treatment was associated with downregulated TRPV1 expression *in vivo*; it also upregulated endogenous APN and AdipoR1 expression and increased AMPK activation, as suggested previously in strategies to recover reduced APN [10]. Therefore, our findings strongly support the position that APN5N is a novel topical agent for the treatment of sensitive skin.

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CRediT authorship contribution statement

Conceptualization: EJK, DHL, JHC; Methodology: EJK, SIC; Investigation: EJK, QQL, YKK; Writing – Original Draft Preparation: EJK, QQL, SIC; Writing - Review and Editing: EJK, QQL, DHL, JHC.

Conflict of Interest

Jin Ho Chung is a CEO of JUNG JINHO Effect, Inc. Other authors state no conflict of interest.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jdermsci.2023.12.002.

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