

## INVESTIGATIVE REPORT

# Pathophysiological Study of Sensitive Skin

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**Sensitive skin is a clinical syndrome characterized by the occurrence of unpleasant sensations, such as pruritus, burning or pain, in response to various factors, including skincare products, water, cold, heat, or other physical and/or chemical factors. Although these symptoms suggest inflammation and the activation of peripheral innervation, the pathophysiology of sensitive skin remains unknown. We systematically analysed cutaneous biopsies from 50 healthy women with non-sensitive or sensitive skin and demonstrated that the intraepidermal nerve fibre density, especially that of peptidergic C-fibres, was lower in the sensitive skin group. These fibres are involved in pain, itching and temperature perception, and their degeneration may promote allodynia and similar symptoms. These results suggest that the pathophysiology of skin sensitivity resembles that of neuropathic pruritus within the context of small fibre neuropathy, and that environmental factors may alter skin innervation. Key words: sensitive skin; questionnaire; pruritus; innervation; neuropathy; C-fibres.**

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Sensitive skin (1, 2) is a frequent clinical syndrome, first discussed in 1947 (3), and described more precisely by Thiers in the 1980s (4). It is characterized by the occurrence of unpleasant sensations, such as pruritus, burning, prickling, tickling, and stinging, after contact with various factors (e.g. water, cosmetics, soaps, detergents, cold, heat, wind, ultraviolet (UV) light). The high incidence of sensitive skin indicates its importance to issues of public health (5); approximately 50% of individuals (60% of women and 40% of men) report having sensitive skin (2, 5, 6). Skin sensitivity is largely known as a facial condition, but is not restricted to this area; extrafacial sites, mainly the hands, can also be involved (7, 8).

Although the pathophysiology of sensitive skin remains unclear, the underlying mechanism is neither immunological nor allergic (5, 9, 10). For example, the increased prevalence in summer (11) suggests a role

for UV exposure, and sensitive skin is more frequent in people with pale skin (12). An alteration of the skin barrier, secondary to the frequent use of cosmetic products or to other factors, has been evoked (13), but is not observed in all subjects. This skin barrier alteration may promote skin inflammation, either via the penetration of irritating substances into the skin or by abnormal bacterial colonization (as in atopic dermatitis (14)) or both; however, this link between skin sensitivity and cutaneous microbiota has not been confirmed (15). A defect in the mechanisms controlling inflammation could also be cited. In addition, abnormal sensations, vasodilation and abnormal skin reactions to rapid temperature changes are highly suggestive of involvement of the cutaneous nervous system, particularly epidermal transient receptor potential (TRP) channels. These receptors are expressed on cutaneous nerve endings, and it is known that the activation of these channels may consequently promote the release of neuropeptides, inducing cutaneous neurogenic inflammation (5, 9).

To address these questions, we measured sensory nerve fibre densities in pale non-sensitive skin and sensitive skin and focused on inflammation markers that had not previously been studied in the context of sensitive skin. One such marker is protease-activated receptor 2 (PAR2), a receptor that is activated by proteases, such as tryptase (16) and kallikrein, released during inflammation and is suspected to be involved in a histamine-independent itch-signalling pathway (17, 18). In the skin, this receptor is expressed on keratinocytes, endothelial cells (19) and afferent nerve fibres (16), and its activation induces the release of inflammatory neuropeptides, such as calcitonin gene-related peptide (CGRP) and substance P (SP) (20), supporting neurogenic inflammation. PAR2 is also involved in inflammatory immune responses, as it increases the expression of cell adhesion molecules on keratinocytes, secondary to the activation of nuclear factor  $\kappa$ B (NF $\kappa$ B) (21). Moreover, activated PAR2 promotes inflammatory hyperalgesia through sensitization of transient receptor potential vanilloid-1 (TRPV-1) (22), which is proposed to participate in skin sensitivity (9). Furthermore, TRPV-1 and acid-sensing ion channel 1 (ASIC-1) are activated by numerous factors (23, 24). Indeed, the TRPV-1 and ASIC-1a genes may be overexpressed in particular patterns of inflammation (25, 26). In a blinded histological study comparing sensitive and

non-sensitive skin, we first searched for these markers to detect possible epidermal inflammation and a crucial role of PAR2. We also assessed the involvement of G protein-coupled receptor 32, which contributes to the resolution of acute inflammation with its ligand resolvin D1 (27).

## METHODS (for full details see Appendix S1<sup>1</sup>)

### Recruitment

Fifty healthy, 30–50-year-old women were recruited according to skin types I to III. Their skin sensitivity was assessed according to a new questionnaire (Table S1<sup>1</sup>) associated with a stinging test performed on the nasolabial folds, as described by Frosch & Kligman in 1977 (28). Twenty-six subjects were non-sensitive skin subjects and 24 were sensitive skin subjects. All subjects gave their informed, written consent.

### Skin biopsy processing

A punch biopsy was removed from the neck of each subject, just below the ear. Each skin sample was identified by a code number to allow for further blinded histological analyses. Immediately after excision, the biopsies were fixed overnight in a 4% paraformaldehyde bath and then, preserved in a phosphate-buffered saline (PBS) – 10% sucrose bath for an additional 24 h prior to being frozen and stored at –80°C. The biopsies were cut into 7- $\mu$ m- or 30- $\mu$ m-thick sections, which were spaced at least at 98  $\mu$ m apart.

*ASIC-1, GPR32, NF $\kappa$ B, PAR2, TRPV-1, NGF and Sema3A evaluation.* The evaluations were performed on 7- $\mu$ m-thick sections. For ASIC-1, NF $\kappa$ B, PAR2, TRPV-1, NGF and Sema3A, the overall epidermal fluorescence intensity was scored from 0 (no immunoreactivity) to 3 (high immunoreactivity), and the result was expressed in arbitrary units. GPR32 immunoreactivity was scored on epidermal basal cells from 0 to 3, and the result was expressed as the percentage of highly immunoreactive epidermal basal cells (scoring from 2 to 3).

*Determination of the linear nerve fibre densities.* Immunostainings of PGP9.5, NF200 or CGRP were performed on 30- $\mu$ m-thick sections. The NF200- and CGRP-positive fibres were counted until 300- $\mu$ m depth in the dermis. For intraepidermal nerve fibres, we counted PGP9.5-immunoreactive fibres or branches that crossed the dermo-epidermal junction or arose from it. Secondary branches or fragments occurring in the epidermis were not counted, as described by Lauria and colleagues (29). In order to have comparable results between the subjects, we determined, for each fibre type, a linear density using the corresponding dermo-epidermal junction length, as previously described (30). The number of counted fibres was divided by this length to obtain a linear density, expressed as number of nerve fibres per mm of dermo-epidermal junction.

### Epidermal thickness evaluation

The epidermal thickness was determined on a portion of each section after NF $\kappa$ B staining because this staining highlighted each epidermal cell. The selected portion was the more representative of the entire epidermis on the section and was devoid of hair follicles. We used photographs and the ImageJ software to measure the length of the dermo-epidermal junction and the epidermal area. Using these data, we calculated the corresponding epidermal thickness, which was expressed in  $\mu$ m.

### Statistical analysis

The relevance of the double recruitment procedure was determined using descriptive statistics and a correlational study between the 2 score sets (Spearman's correlation method). It was performed using the SAS<sup>®</sup> 9.2 software.

Comparison of the mean ages of both groups was performed using the Student *t*-test after validation of the normality using the Agostino-Pearson normality test (GraphPad software).

Data of the immunostainings are means of the triplicates for each subject. Data for each group are the mean of the means of the corresponding subjects. Data are expressed as the mean  $\pm$  standard error of the mean (SEM), except for the linear densities of PGP9.5-, NF200- and CGRP-immunoreactive fibres, which are expressed as the mean  $\pm$  standard deviation (SD). Each statistical analysis was performed using the Mann-Whitney test with the GraphPad software. A *p*-value  $\leq$  0.05 was considered to be statistically significant.

## RESULTS

### Subject recruitment and validation of the procedure

Caucasian women aged from 30 to 50 years presenting with phototypes I–III were enrolled according to a sensitive skin self-assessment questionnaire (Table S1<sup>1</sup>) and their stinging test score. Twenty-six women comprised the non-sensitive skin group and 24 the sensitive skin group. The mean ages of both groups were similar, as were the mean ages in the 2 following age ranges: 30–40 and 41–50 years (Table I). A correlational study demonstrated that the questionnaire score was fully consistent with the stinging test score (*p*-value < 0.0001, correlation coefficient –0.761). This reflected the reliability of the recruitment in each group.

### Epidermal thickness is not modified in sensitive skin

To our knowledge, no previous study has determined whether a decrease in epidermal thickness is associated with skin sensitivity. Thus, we measured epidermal thickness on non-sensitive and sensitive skin biopsies from the recruited female subjects. The epidermis was 40.4  $\mu$ m thick ( $\pm$  1.7) in the non-sensitive skin group and 42.4  $\mu$ m thick ( $\pm$  1.5) in the sensitive skin group, showing no significant difference between the 2 groups (*p* = 0.357). This indicated that sensitive skin could not be related to decreased epidermal thickness.

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Table I. General characteristics of the non-sensitive skin and sensitive skin groups

	Age 30–40 years		Age 41–50 years		Total	Mean age, years	Student's <i>t</i> -test
	Subjects <i>n</i>	Mean age, years	Subjects <i>n</i>	Mean age, years			
Non-sensitive skin	16	34.8	10	44.5	26	39.7	0.979
Sensitive skin	15	34.6	9	45.2	24	39.9	

### Markers of epidermal inflammation were not increased in sensitive skin

Skin inflammation is clinically observed in sensitive skin, and we investigated certain markers not previously studied within the context of sensitive skin. We searched for epidermal over-expression of PAR2, TRPV-1, ASIC-1 and activated NFκB and possible epidermal down-regulation of GPR32 to determine whether skin sensitivity could be related to unresolved inflammation. Blinded immunohistological analyses revealed that these receptors and transcription factor were all expressed by all epidermal layers from both non-sensitive and sensitive types of skin, except for the GPR32, which was only produced by some epidermal basal cells. However, the expression levels of PAR2, TRPV-1, NFκB, ASIC-1 and GPR32 were not significantly altered in the sensitive skin group compared with the non-sensitive skin group (Table II). These data must be interpreted cautiously, as non-significant results do not mean negative results and an immunohistochemical study does not reveal molecular mechanisms. Nevertheless, we did not find any evidence to implicate these inflammatory factors in sensitive skin.

### Epidermal innervation is modified in sensitive skin

Because sensitive skin is predominantly characterized by unpleasant sensations reminiscent of those of small-fibre neuropathies (31), we evaluated sensory innervation in both groups. We first scored epidermal immunostainings for nerve growth factor (NGF) and semaphorin 3A (Sema3A), which are known to enhance and inhibit innervation, respectively. No significant difference was found between the 2 groups (Table II); however, these non-significant results were obtained using fluorescent immunostaining and did not indicate that innervation could not be modified. Thus, we wanted to evaluate the density of different sub-types of sensory nerve fibres. The linear densities of the Aβ fibres were 14.92 (±3.28) and 14.94 (±4.20) fibres per mm of dermo-epidermal junction (DEJ) for the non-sensitive skin group and sensitive skin group,

Table II. Summary of the results of the immunohistological studies comparing non-sensitive skin and sensitive skin

	Non-sensitive skin group Mean ± SEM	Sensitive skin group Mean ± SEM	<i>p</i> -value
PAR2 <sup>a</sup>	0.423 ± 0.118	0.417 ± 0.149	0.646
NFκB <sup>a</sup>	3.212 ± 0.091	3.104 ± 0.121	0.494
TRPV-1 <sup>a</sup>	2.173 ± 0.081	2.229 ± 0.110	0.607
ASIC-1 <sup>a</sup>	2.269 ± 0.177	2.583 ± 0.192	0.221
GPR32 <sup>b</sup>	32.315 ± 4.292	30.816 ± 5.145	0.911
NGF <sup>a</sup>	1.212 ± 0.082	1.271 ± 0.101	0.328
Sema3A <sup>a</sup>	1.538 ± 0.094	1.573 ± 0.115	0.766

Data are presented as: <sup>a</sup>the means of epidermal fluorescence intensity (arbitrary unit) or <sup>b</sup>as a percentage of highly fluorescent epidermal basal cells (scores 2–3). SEM: mean ± standard error of the mean.

respectively, without any significant difference. This non-involvement of these fibres in skin sensitivity is rather consistent, as these nerve endings are mainly involved in tactile perceptions. They are not known as pruriceptors or nociceptors, contrary to small fibres (Aδ and C), which are involved in the perception of noxious stimuli such as hot or cold temperature (32). Concerning these small fibres, we evaluated the intraepidermal nerve fibre density (IENFD) by counting intraepidermal PGP9.5 immunoreactive nerve fibres (Fig. 1). The linear density was 16.58 (±3.28) fibres per mm of DEJ for the non-sensitive skin group and 14.56 (±3.93) fibres per mm of DEJ for the sensitive skin group; the difference was significant (*p* = 0.027), showing that the Aδ or C fibre population was altered. Furthermore, CGRP immunostaining revealed that the CGRP-immunoreactive nerve fibre density was 7.51 (±3.07) fibres per mm of DEJ for the non-sensitive skin group and 5.26 (±2.17) fibres per mm of DEJ for the sensitive skin group (*p* = 0.008).

## DISCUSSION

To our knowledge, this work is the first to explore most of the pathophysiogenic hypotheses regarding sensitive skin, which is a very frequent and multifactorial syndrome. Our comparison between a non-sensitive skin group and a sensitive skin group was performed using several criteria for defining these groups. The allocation of subjects into either group is sometimes performed according to their self-perceived skin sensitivity (33,

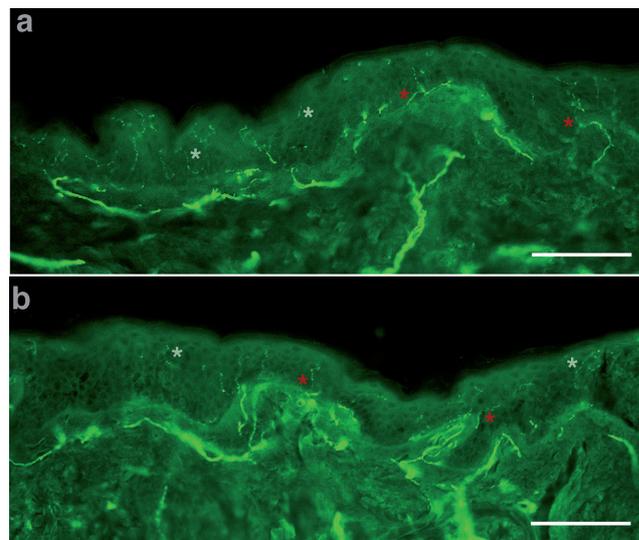


Fig. 1. Intraepidermal nerve fibre density was significantly lower in patients with sensitive skin. PGP9.5 immunostaining revealed cutaneous innervation in subjects with (a) non-sensitive skin and (b) sensitive skin. These images show (a) 14 and (b) 8 intraepidermal nerve fibres, respectively. Red asterisks indicate fibres or branches that crossed the dermo-epidermal junction or arose from it. White asterisks indicate some epidermal fibre fragments as examples of fibres that were not considered for the determination of the linear intraepidermal nerve fibre density. Scale bars: 100 μm.

34) or using a self-assessment questionnaire (35). Other authors preferred to use the stinging test, which consists of the application of lactic acid or physiological serum at the nasolabial folds and the determination of a sensitive skin score (34, 36–38). For the first time, we implemented both a self-assessment questionnaire to ascertain the subjects' self-perceived sensitivity and the stinging test, which helped to establish the diagnosis and measure the severity of sensitivity. The analysis of the allocation of these subjects according to either one or the other recruitment procedure revealed the consistency of these 2 methods, validating our new questionnaire and the accuracy of our recruitment.

We first examined the thickness of the epidermis, as alterations in the cutaneous tissue structure have been suggested to be related to sensitive skin (34, 39). In particular, some measurements of trans-epidermal water loss have suggested that sensitive skin may be associated with alterations in the skin barrier (34). A previous study also reported a decrease in ceramides, the major constituents of stratum corneum lipids, in sensitive skin (35). In our study, we did not find any modification of the epidermal thickness. Hence, our results do not exclude or confirm any relationship between skin sensitivity and an increased cutaneous permeability; however, alterations in the skin barrier do appear to be more functional than anatomical, if they in fact exist.

Sensitivity is thought to be related to skin inflammation, with clinical or subclinical consequences (10). Thus, we addressed whether sensitive skin is associated with the absence of a resolution of physiological epidermal inflammation. However, our examination of GPR32 did not confirm this hypothesis. We also studied certain important markers of inflammation, but did not find any variations in PAR2, TRPV-1, NF $\kappa$ B, or ASIC-1 expression between the subjects from the sensitive and non-sensitive skin groups.

We hypothesized that innervation could be altered in sensitive skin because many subjects complain of unpleasant sensations, such as stinging, burning or prickling. Several nerve fibre populations constitute the sensory innervation in the skin: A $\beta$  nerve fibres and the small nerve fibres A $\delta$  and C (weakly or not myelinated, respectively). We first determined the A $\beta$ -fibre density via the immunostaining of neurofilament 200 (NF200), a marker of A fibres and the majority of A $\beta$  fibres (40). In the 2 groups, the NF200-immunoreactive nerve fibres were found to be dermal fibres, and not epidermal fibres, supporting that the identified nerve fibres were A $\beta$  fibres. Our results showed no difference of their density between the 2 groups. We further showed that the small fibre population linear density was decreased in the sensitive skin group, without any imbalance between Sema3A and NGF. The number of peptidergic C-fibres, which are involved in the perception of pain, temperature and itching, was especially decreased, sug-

gesting that this sub-type of nerve endings is altered or undergoes degeneration following contact with environmental factors, which are thought to be responsible for the occurrence of skin sensitivity. Specific nociceptive channels on these nerve endings, such as TRP channels, could be over-stimulated, leading to the release of neuropeptides including CGRP. As a consequence, this may promote cutaneous neurogenic inflammation locally around nerve endings, dysaesthesia and even allodynia. Although further studies are obviously needed to confirm these hypotheses, our results are consistent with a recent clinical study revealing that a severe skin sensitivity can be associated with neuropathic pain (41).

The mechanisms of skin sensitivity resemble those of neuropathic pruritus or neuropathic pain within the context of small-fibre neuropathy (42). Similar to patients with small-fibre neuropathies (43), subjects with sensitive skin exhibit decreased IENFD and frequent pruritus. In spite of these similarities, classic small-fibre neuropathy shows major differences: the frequent occurrence of erythema; no known sensory deficit; no extracutaneous involvement; and no known internal cause, but a relationship with contact with environmental factors (5, 31).

The clinical consequences of the reduction in IENFD in both sensitive skin and small-fibre neuropathies are surprising. Indeed, the selective loss of small sensory fibres should inherently generate a sensory deficit, and assigning the cause of a sensory syndrome to a loss of small nerve fibres makes no pathophysiological sense (44). Indeed, the fibres lacking are known to be involved in the perception of pain and temperature changes as well as in the mediation of pruritus. In fact, it is difficult to univocally explain peripheral neuropathic pain, pruritus, paraesthesia or dysaesthesia, which reflects their complex and diverse mechanisms involving different types of nerve fibres (44).

To conclude, we provide here the first evidence of the involvement of sensory nerve endings by showing a decrease of IENFD in patients with sensitive skin and, in particular, a decrease in the peptidergic C-fibre density. To date, the treatment of sensitive skin was mainly to avoid irritants and to protect keratinocytes (5, 45). Our results suggest that prevention and treatment of sensitive skin may be aided by protecting the intraepidermal nerve fibres and by promoting their development; sensitive skin appears to be a more complex condition than a cosmetic syndrome.

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