

Skin pH, Atopic Dermatitis, and Filaggrin Mutations

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Background: The acidic pH of the skin plays a role in antimicrobial defense by regulating the bacterial skin flora and aspects of barrier. Filaggrin is a co-factor in maintaining a low skin pH because of its degradation into acidic amino acids. Accordingly, lack of filaggrin due to filaggrin mutations may influence skin pH.

Objective: We aimed to determine the epidermal pH in different groups stratified by filaggrin mutations and atopic dermatitis. Further, we investigated the changes in pH according to severity of mutational status among patients with dermatitis, irrespective of skin condition.

Methods: pH was measured with a multiprobe system pH probe (PH 905), and the study population was composed of 67 individuals, who had all been genotyped for 3 filaggrin mutations (R501X, 2282del4, R2447X).

Results: We found no clear pattern in relation to filaggrin mutation carrier status. Individuals with wild-type filaggrin displayed both the most acidic and most alkaline values independent of concomitant skin disease; however, no statistical differences between the groups were found.

Conclusions: The lack of significant diversity in skin pH in relation to filaggrin mutation carrier status suggests that the effect of filaggrin mutations on skin pH is not pronounced.

An acidic skin pH is important for proper functioning of antimicrobial skin defense as well as for skin cohesion, skin immune defense, and skin lipid bilayer formation by means of pH-regulated enzyme activity.¹ Filaggrin is a key structural protein in the epidermis, serving a number of complex interrelated functions, concerning properties of mechanical strength of the corneocytes, maintaining the extracellular lipid bilayer, as well as regulating epidermal homeostasis regarding skin hydration, skin pH, antimicrobial skin defense, immunomodulation, and UV protection. Importantly, it plays a key role in the regulation of skin pH.^{2,3}

The filaggrin breakdown products urocanic acid and 2-pyrrolidone-5-carboxylic acids are acidic amino acids. Hence, a lack of filaggrin results in decreased amounts of these amino acids, and thus, filaggrin mutations may potentially change skin pH.^{2,4}

The past reports on skin pH and filaggrin mutation carrier status are inconsistent.

An experimental study showed significant decrease in pH among filaggrin-deficient mice, suggesting that the acidification of stratum corneum is dependent on the amount of filaggrin in the skin.⁵ Two human studies have reported a more alkaline skin pH among filaggrin mutation carriers compared with wild-type individuals; the difference was more pronounced in individuals with concomitant atopic dermatitis (AD).^{6,7} Contrary to subjects with ichthyosis vulgaris, one study did not detect significant difference in skin pH between filaggrin-deficient and wild-type individuals⁸ and another study only found significance between controls and FLG homozygotes with AD.⁹

The objective of this study was to investigate the relationship between skin pH and filaggrin mutation carrier status on non-lesional skin sites in healthy individuals and in individuals with AD.

MATERIALS AND METHODS

The study participants were recruited from patients of the Department of Dermato-Allergology at Gentofte Hospital Denmark and through advertisement. The eligible study participants were assessed for AD by means of the UK Working Party's Diagnostic Criteria¹⁰ and filaggrin mutation carrier status (R501X, 2282del4, and 2447X), determined by a multiplex analysis described previously.¹¹ The study was carried out from the end October of 2011 to mid March 2012 and enrolled 67 subjects. The participants were divided into 4 groups on the basis of mutation status and assessed by the presence or absence of AD: (1) 20 healthy controls with no prior skin diseases and no mutations (FLGwt), (2) 10 subjects with mutations (FLGnull), no AD but with contact dermatitis (irritant or allergic according to International Contact Dermatitis Research

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TABLE 1. Exclusion Criteria and Precautions for Participation

Age	<18 and >69 y
Reproductive health	Pregnant or nursing women, lack of contraceptives
Skin diseases	Active or widespread eczema
Chronic inflammatory diseases besides eczema	Rheumatoid arthritis, Crohn disease, ulcerative colitis, systemic lupus erythematosus, or psoriasis
Treatment	UV-treatment of test area within 3 wks of trial, treatment with topical steroids 2 wks before trial, application of emollients on the test areas throughout the trial period, immune-modulating medical treatment
Precautions	Smoking and coffee consumption within 2 h before measurements, consumption of food within 4 h of measurements, analgesics 24 hours before measurements

Group guidelines¹²), (3) 19 subjects with FLGwt and AD, and (4) 18 subjects with FLGnull and AD. Changes in pH in relation to heterozygosity and homozygosity/compound heterozygosity status were also assessed irrespective of skin condition among the individuals from the hospital population (not healthy controls) (n = 47): (1) 19 individuals were FLGwt (no filaggrin mutation), (2) 20 FLGhet (heterozygote single mutation), and (3) 8 FLGhom/comp (homozygote or compound heterozygote mutations). Exclusion criteria are shown in Table 1.

The study was approved by the local ethical committee (H-1-2011-011); also, informed consent from all participants was obtained.

A multiprobe system pH probe was used (PH 905; Courage and Khazaka electronic GmbH, Köln, Germany). The probe was placed 9 cm distal from the center of the axillary fossa on the non-lesional

skin. Consecutive measurements were taken, 3 from each test area generating a calculated average.

The pH values were not normally distributed and assessed using the Shapiro-Wilk test ($P > 0.05$); thus, non-parametrical tests (Kruskal-Wallis and Mann-Whitney) were used to analyze the pH data. The level of significance was set at $\alpha = 5\%$.

RESULTS

The study comprised 67 subjects: 28 men and 39 women, with a median age of 38 years (range, 18–68 years). Distribution of age and sex was equal in the 4 groups.

Overall median pH was 5.61 (range 5.26–6.03). The median results of the respective groups were as follows: healthy controls, 5.53; FLGnull-AD, 5.71; FLGwt+AD, 5.98; and FLGnull+AD = 5.63; as well as among the hospital population FLGwt, 5.98; FLGhet, 5.66; and the FLGhom/comp, 5.44 (Fig. 1). The most acidic and alkaline values were detected among the wild-type individuals independent of concomitant skin disease. Statistical testing failed to reveal reliable difference between pH values. Although a tendency toward a more acidic pH going from the wild-type to homozygous filaggrin mutation carrier status was apparent, this difference did not reach statistical significance ($P = 0.632$).

DISCUSSION

This study showed no association between the filaggrin genotype and skin pH. Furthermore, all pH values measured were within the pH range of 4 to 6 as in normal intact skin.¹³

Surface pH is sensitive to both endogenous and exogenous factors influencing the pH gradient. The 3 factors thought to contribute as proton sources in normal stratum corneum are as follows: (1) Natural moisturizing factor, including filaggrin breakdown products, (2) α -hydroxy acids naturally present in

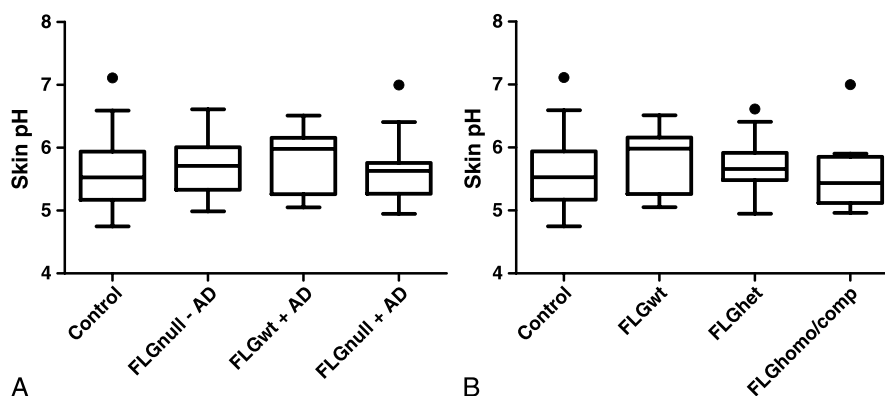


Figure 1. Baseline skin pH as a function of filaggrin mutation status, skin condition, and severity of mutational status. Data are presented as box and whiskers plot (Tukey variation). The box represents the interquartile range (IQR), the horizontal line within the box shows the median, and the whiskers illustrate the 75th percentile plus 1.5 times IQR and the 25th percentile minus 1.5 times IQR. Values outside these limits are shown as dots (outliers). A, Stratified by skin disease and genotype. N = 67. Control group: healthy subject (without filaggrin mutations), n = 20; FLGnull-AD (patients with contact dermatitis and mutations), n = 10; FLGwt+AD (patients with AD without mutations), n = 19; FLGnull+AD (patients with AD and mutations), n = 18. B, Stratified by genotype among the patient group. N = 47. FLGwt group (patients without mutations), n = 19; FLGhet (patients with a single mutation), n = 20; FLGhom/comp (homozygote patients or patients with compound heterozygote mutations), n = 8.

sweat, and (3) acidic lipids.¹³ If one of these parameters is affected, it is possible that compensatory regulatory mechanisms are activated, upregulating other parameters, so normal skin pH level is maintained. Our results are in line with the results of Fluhr et al,⁵ suggesting that a lack of filaggrin may not play an essential role for maintaining a normal pH level of the skin. A dose-response relationship with respect to pH and FLG mutation carrier status (heterozygosity vs homozygosity) as seen in prior studies^{9,14} would be expected. However, neither the study of Perusquia-Ortiz et al⁸ nor this study confirms this. The fact that no significant changes in skin pH could be detected in relation to filaggrin mutation carrier status could very well be explained by a hypothesis of compensatory mechanisms.

There is also a possibility that filaggrin mutations could have been missed in the FLGwt groups because we only tested for 3 filaggrin polymorphisms, which account for 83% of the European specific filaggrin mutations.¹⁵

In our study, individuals with FLGwt+AD had higher median pH, suggesting that skin AD and not filaggrin mutations leads to a more alkaline pH. Notably, other studies have reported significantly higher pH among individuals with AD than in healthy subjects.¹⁶ When non-positive findings or surprising findings occur, the validity of measurements must be considered. Comparing pH data from an ongoing preliminary study, a decrease was seen among healthy controls from median baseline pH to measurements after detergent provocation on day 7 (from 5.53 to 4.81). These data confirm the ability of the pH probe to measure differences in pH (data not yet published).

The limitations of our study may be the number of participants and the anatomy of the measuring site. The discrepancy of our measurements with other human studies could be caused by the higher moisture in our trial because the measurements were made 9 cm from the axilla. This area is known to have a higher pH than that of other bodily areas.¹⁷ Also, use of soaps or toiletries could have an impact on the pH¹³ and no restriction was given with respect to soaps and deodorants. The subjects were asked not to use moisturizers on the day of measuring, which should exclude the possible effect of moisturizers on pH. Finally, ideally, 6 groups should have been included, with the additional groups being (1) healthy subjects without any kind of dermatitis but with filaggrin mutations and (2) subjects with ACD and ICD and no filaggrin mutations. This would have allowed a more specific overview of the isolated effect of filaggrin mutations on skin pH and the isolated effect of non-AD dermatitis on skin pH, although recruiting a sufficient number of healthy subjects with filaggrin mutations proved to be difficult.

CONCLUSIONS

The skin pH was found within the reference range in all groups. The lack of pattern in pH changes in relation to filaggrin mutation status with wild-type individuals showing both the most acidic and most alkaline values depending on concomitant skin

disease, although not significant, indicates that skin pH depends on factors other than filaggrin in terms of regulation.

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