Natural moisturizing factor components in the stratum corneum as biomarkers of filaggrin genotype: evaluation of minimally invasive methods

S. Kezic, A. Kammeyer,* F. Calkoen, J.W. Fluhr†‡ and J.D. Bos*

Coronel Institute of Occupational Health, Academic Medical Center, University of Amsterdam, PO Box 22 700, 1100 DE Amsterdam, the Netherlands
**Department of Dermatology, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands*
†Bioskin, Berlin, Germany
‡Center of Experimental Applied Cutaneous Physiology, Department of Dermatology and Allergology, Charité-Universitätsmedizin Berlin, Berlin, Germany

Correspondence
Sanja Kezic.
E-mail: s.kezic@amc.uva.nl

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Conflicts of interest
None declared.

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Summary

Background The carriers of loss-of-function mutations in the filaggrin gene (*FLG*) have reduced levels of natural moisturizing factor (NMF) in the stratum corneum. The concentration of NMF components which are formed by filaggrin protein breakdown in the stratum corneum might therefore be useful as a biomarker of the *FLG* genotype.

Objectives To investigate the feasibility of different sampling methods for the determination of two NMF components, 2-pyrrolidone-5-carboxylic acid (PCA) and urocanic acid (UCA), in the stratum corneum as biomarkers for the *FLG* genotype.

Methods PCA and UCA from the stratum corneum were sampled by using a tape stripping technique and an extraction technique using skin patches containing potassium hydroxide (KOH). The concentrations of PCA and UCA were measured by high-performance liquid chromatography. Eleven carriers of an *FLG* mutation and 10 individuals wild type for the two most common *FLG* mutations (R501X and 2282del4) were included in the study.

Results The most significant difference between the *FLG* genotypes was found for PCA sampled by the tape stripping technique. The mean values of PCA obtained by the tape stripping technique were, respectively, 0·18, 0·50 and 1·64 mmol g^{-1} protein in homozygous (or compound heterozygous), heterozygous and wild-type genotypes ($P < 0·005$ homozygous vs. heterozygous; $P < 0·0001$ heterozygous vs. wild type). The tape stripping technique showed less intrasubject variation compared with the KOH patches, in particular when the concentrations of UCA and PCA on the tape strips were normalized for protein amount.

Conclusions The concentration of PCA in the stratum corneum collected by tape stripping showed it to be a feasible biomarker of the *FLG* genotype.

Filaggrin (filament-aggregating protein) is a key structural protein required for fully competent epidermal barrier function. By aggregating keratin filaments into keratin fibrils within the cytoskeleton of corneocytes, filaggrin is responsible for the mechanical strength and integrity of the stratum corneum.[1] Filaggrin is proteolytically degraded into a pool of free amino acids including histidine and glutamine which are further converted to, respectively, urocanic acid (UCA) and 2-pyrrolidone-5-carboxylic acid (PCA).[2] Free amino acids and their derivatives are major components (approximately 50%) of natural moisturizing factor (NMF), which is retained within the mature corneocytes of the stratum corneum.[2–5] NMF is highly hygroscopic and plays a key role in the maintenance of epidermal hydration and is reduced in dry skin.[6] Expression of the human gene encoding for filaggrin (*FLG*) and subsequent activation of hydrolysis of filaggrin peptides into NMF are influenced by the properties of the microenvironment, including local pH, external humidity and transepidermal water loss.[6]

FLG is located within the epidermal differentiation complex on chromosome 1 at locus 1q21.[7] *FLG* is highly polymorphic and to date 20 mutations in the European white population

have been detected from which six are prevalent at varying frequencies.[1] Two of the most common *FLG* loss-of-function mutations (R501X, 2282del4) are present in almost 10% of the white European population.[8] Recent studies demonstrated that loss-of-function mutations in *FLG* underlie ichthyosis vulgaris and strongly predispose to atopic eczema.[9] Between 8% and 48% of individuals with atopic eczema carry one or more *FLG*-null alleles.[9] In a large German study *FLG* variants increased the risk for eczema threefold (odds ratio 3·12; 95% confidence interval 2·33–4·17; $P < 0·001$) with a population-attributable risk of 13·5%.[10] Furthermore, *FLG* has been shown to increase the risk for irritant contact dermatitis.[11]

Recently, we showed that individuals who are carriers of an *FLG* loss-of-function mutation have significantly reduced levels of NMF compared with noncarriers.[12] As filaggrin is the main precursor protein of the amino acid-derived components of NMF, *FLG*-null mutations may be one of the reasons why excessively dry skin is a common feature in atopic eczema. Knowing the *FLG* genotype status might improve classification of atopic eczema phenotypes and identification of susceptible individuals.

Levels of NMF in the study by Kezic *et al.*[12] were measured using *in vivo* Raman microspectroscopy. Although *in vivo* Raman microspectroscopy is a reliable technique for measuring the NMF components in the skin simultaneously at different depths *in vivo*,[13] this technique has certain disadvantages that hamper its routine use in research and clinical practice. The Raman instrumentation is expensive and poorly accessible and the recording and interpretation of the spectra need trained personnel.

The aim of this study was to evaluate other more feasible approaches for research and clinical practice for the determination of NMF or its components as biomarkers of the *FLG* genotype. For that purpose we evaluated two techniques for sampling two breakdown products of the filaggrin protein: UCA and PCA. PCA is one of the major components of NMF (12%)[4] and is often used as an indicator to determine the amount of NMF present in the stratum corneum. PCA is formed nonenzymatically from the glutamine protein.[14–17] In contrast to PCA, UCA is formed from histidine breakdown by an enzymatic reaction catalysed by the enzyme histidase.[16,17] Thus, the amount of UCA will be dependent on the filaggrin levels in the stratum corneum as well as on the histidase activity. In this study we chose to determine UCA in addition to PCA because of its distant biological functions. UCA is a major chromophore in the skin and is of ultimate importance because of the immunoregulatory role of its *cis* isomer.[18]

For sampling of PCA and UCA we evaluated two sampling techniques: sequential tape stripping of the stratum corneum by using adhesive tape and potassium hydroxide (KOH) extraction by using skin patches. Several methods based on these techniques followed by high-performance liquid chromatography (HPLC) or high-performance capillary electrophoresis detection have been developed for the determination of the stratum corneum free amino acids, PCA and UCA.[6,19–23] For the analysis of UCA and PCA in the present study we used HPLC. The levels of UCA and PCA were determined in individuals with different *FLG* genotypes.

Materials and methods

Subjects

Measurements were carried out on a panel of subjects consisting of 10 individuals without known loss-of-function mutations in *FLG*, seven individuals with one loss-of-function mutation, either R501X or 2282del4, and four individuals who were either homozygous for one mutation or compound heterozygous. As not all individuals wanted to be exposed to KOH, sampling with KOH patches was performed in only nine subjects with the wild-type *FLG* genotype and five heterozygous carriers. None of the subjects had visible skin abnormalities on the investigated skin sites. Written informed consent was obtained from all subjects prior to the study. The experimental protocol was approved by the Medical Ethical Committee of the Academic Medical Centre, Amsterdam.

Genotyping

Genomic DNA was extracted from buccal swab samples obtained from all subjects using a Puregene® DNA isolation kit (Gentra Systems, Minneapolis, MN, U.S.A.). The polymorphisms R501X and 2282del4 were genotyped following the published methods of Palmer *et al.*[24] which are described in more detail elsewhere.[11] Specifically, genotyping for R501X was performed by means of a fluorogenic 5′ nuclease polymerase chain reaction (PCR) TaqMan® assay (Applied Biosystems, Foster City, CA, U.S.A.). Genotyping for 2282del4 was performed by sizing a fluorescently labelled PCR fragment of DNA.

Chemicals

All reagents were purchased from Merck/VWR International BV (Amsterdam, the Netherlands). PCA and *trans*-UCA were supplied by Sigma Aldrich (Zwijndrecht, the Netherlands). *cis*-UCA was prepared by ultraviolet A irradiation of an aqueous *trans*-UCA solution, yielding a mixture of approximately 60% *cis*-UCA and 40% *trans*-UCA. Enrichment of *cis*-UCA was achieved by repeated recrystallization of *trans*-UCA at 0 °C. The final supernatant contained 97% *cis*-UCA. After vacuum drying the residue was used as *cis*-UCA.

Sampling procedures

Sequential tape stripping

Round adhesive tape discs (3·8 cm^2, D-Squame; CuDerm, Dallas, TX, U.S.A.) were attached to the skin of the forearm on four adjacent locations. Each tape was pressed on to the volar aspect of the forearm for 10 s with standardized force, using a disc pressure applicator (CuDerm).[25] The tape strip

was gently removed with tweezers and stored in a closed vial at 4 °C until use. The first strip was discarded as it may have contained dirt and remnants of cosmetic products; the second and third tape strips were applied on the same skin spot and pooled for the analysis. The amount of stratum corneum proteins on the tape was determined by measuring the absorption of the disc at about 850 nm (infrared radiation) using the D-Squame Scan 850A Instrument (CuDerm).[25] The absorption value is displayed as a percentage and is translated into protein content by using a simple transfer function.[26] The levels of PCA and UCA were corrected for this protein value. Before HPLC analysis, 500 μL KOH solution, 0·1 mol L^{-1}, was added to the tape strips, followed by 2 h of continuous shaking. The alkaline extracts were neutralized with 3 μL perchloric acid, 12 mol L^{-1}, and the vials were shaken again for 2 h. After filtration through a 0·2-μm membrane filter, the samples were introduced into the HPLC system.

Patch test sample

The procedure followed was a modification of the so-called 'chamber sampling'.[22] Patch testers (Silverpatch™; van der Bend, Brielle, the Netherlands), consisting of a filter paper (1 cm^2) that was covered with a plastic sheet, were moistened with 20 μL KOH solution, 0·1 mol L^{-1}, and fixed on to the volar aspect of the forearm with elastic adhesive tape for 1 h. To investigate intraindividual variation, a skin patch was placed on four adjacent locations. The filter papers were subsequently collected and put into 477 μL KOH solution and vortexed for 30 s. The medium was neutralized with 3 μL perchloric acid, 12 mol L^{-1}, and vortexed again for 30 s. The epidermal extracts were passed through a 0·2-μm membrane filter, prior to injection into the HPLC system.

High-performance liquid chromatography analyses

PCA, *trans*-UCA and *cis*-UCA were separated from each other on a 250 × 3 mm reversed-phase Prevail column (Grace/Alltech, Breda, the Netherlands) with a flow rate of 0·4 mL min^{-1}, delivered by HPLC pumps (model PU 1850; Jasco, Tokyo, Japan). Isocratic elution was performed with 20 mmol L^{-1} ammonium formate, containing 1·5 mmol L^{-1} tetrabutylammonium hydroxide and 1% acetonitrile at pH 7·3. The effluent was monitored at 210 nm for PCA and at 270 nm for both UCA isomers, using two sequential UV/Vis detectors, model 759A (Applied Biosystems) and model UV-975 (Jasco). Ten-microlitre samples were injected into the system with a Promis II autosampler (Spark Holland, Emmen, the Netherlands). Data recording was done with Jasco-Borwin chromatography software (JMBS Development, Le Fontanil, France).

Although in all samples both UCA isomers were measured, for the purpose of this methodological study we used only the total amount of UCA calculated as the sum of the concentration of both isomers.

Statistics

For comparison of the groups in respect to genotypes, we used Student's one-sided t-test. All calculations, including linear regression analysis, were performed using Prism 5 software (Graph-Pad, San Diego, CA, U.S.A.).

Results

Figure 1a–c shows the levels of PCA, UCA and the sum of UCA and PCA in the stratum corneum in relation to the *FLG* genotype determined by the tape stripping technique. The levels of both filaggrin breakdown products were significantly

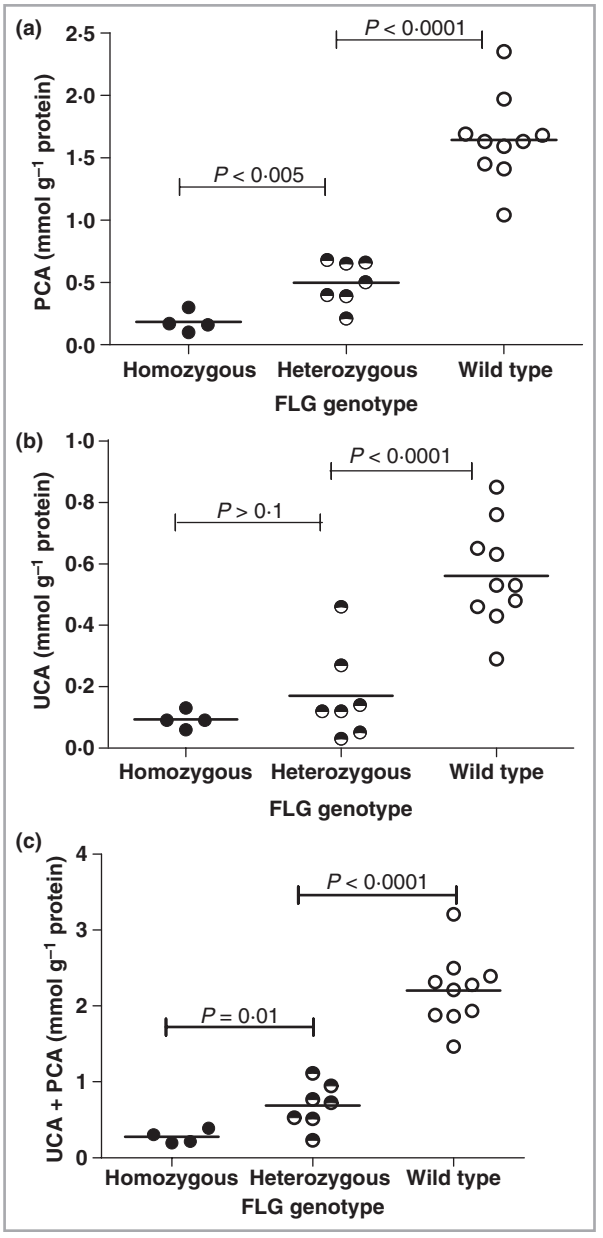

Fig 1. The levels of (a) 2-pyrrolidone-5-carboxylic acid (PCA), (b) urocanic acid (UCA) and (c) the sum of UCA and PCA levels obtained by tape stripping normalized for protein amount.

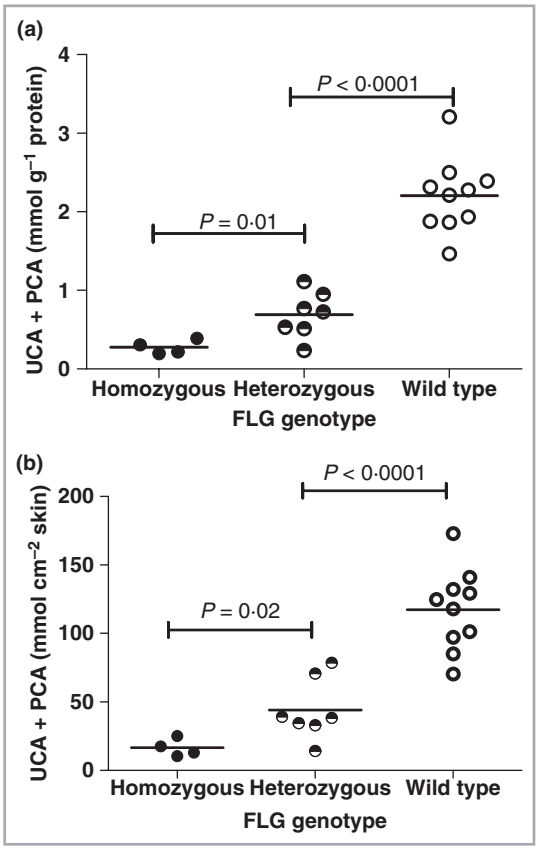

Fig 2. The levels of total urocanic acid (UCA) and 2-pyrrolidone-5-carboxylic acid (PCA) obtained by tape stripping normalized for protein amount (a) or expressed as absolute amount per cm^2 of skin (b).

lower in the carriers of the *FLG* mutations. The lowest value for PCA was found in homozygous or compound heterozygous carriers (mean ± SEM 0·18 ± 0·04 mmol g^{-1} protein) increasing to 0·50 ± 0·07 in heterozygous carriers and 1·64 ± 0·11 mmol g^{-1} protein in the wild type. The difference between carriers and noncarriers showed a higher significance level for PCA and the PCA/UCA sum compared with UCA alone (Fig. 1). Furthermore, the difference between homozygous and heterozygous genotypes revealed a higher significance level when the concentrations of UCA and PCA were normalized for the amount of protein determined on the tape strips (P = 0·01 vs. P = 0·02, respectively) (Fig. 2). Normalization with the protein amount also improved the interindividual variation in the levels of UCA and PCA within a group with the same genotype (Fig. 2) as well as the variation in the values obtained from different skin sites on the forearm (Table 1).

In contrast to the tape stripping technique, only heterozygous (n = 5) and wild-type (n = 9) subjects participated in the study with KOH patches. Despite the lower numbers of individuals, it is clear that the differences in the UCA and PCA levels in relation to genotype were less pronounced for patches (Fig. 3) compared with tape stripping. Furthermore, the variation in the values obtained on four adjacent skin sites of the forearm was larger with the patch technique compared with tape stripping, in particular when tape stripping values for PCA and UCA were normalized for proteins (Table 1).

When comparing the levels of PCA and UCA obtained by the two sampling techniques a better correlation was detectable for UCA (r^2 = 0·58) (Fig. 4b) compared with PCA (r^2 = 0·40) (Fig. 4a).

As seen from Figure 5a, a good correlation between UCA and PCA could be calculated for the tape stripping technique (r^2 = 0·83). Lower correlation values were calculated for the KOH patches (r^2 = 0·41) (Fig. 5b).

Discussion

Recently, we showed by *in vivo* Raman spectroscopy that carriers of *FLG* mutations have a reduced amount of NMF in the stratum corneum.[12] As the filaggrin protein is the main source of several major NMF components in the stratum corneum, the measurement of NMF components might provide valuable information for identifying patients who are carriers of *FLG* loss-of-function mutations. As discussed earlier, *in vivo* Raman spectroscopy has been shown to be a powerful tool for measuring several NMF components noninvasively, simultaneously at different stratum corneum depths.[12,13] Another advantage of Raman spectroscopy is that it can determine at the same time the hydration gradient across the skin, thus monitoring the consequence of reduced NMF.[27] However, Raman spectrophotometry cannot distinguish *cis* and *trans* isomers of UCA which might be of relevance in research studies.

Noninvasive *ex vivo* analysis of NMF components in the stratum corneum seemed therefore a feasible alternative to *in vivo*

Table 1 Variation in the values of 2-pyrrolidone-5-carboxylic acid (PCA) and urocanic acid (UCA) obtained by measurements on four adjacent skin sites by tape stripping and patch techniques

	Tape stripping						Patch		
	PCA		UCA		PCA+UCA		PCA	UCA	PCA+UCA
	mmol cm^{-2} skin	mmol g^{-1} protein	mmol cm^{-2} skin	mmol g^{-1} protein	mmol cm^{-2} skin	mmol g^{-1} protein	mmol cm^{-2} fskin	mmol cm^{-2} skin	mmol cm^{-2} skin
CV (%)	25	20	34	24	25	20	34	29	31

CV, coefficient of variation.

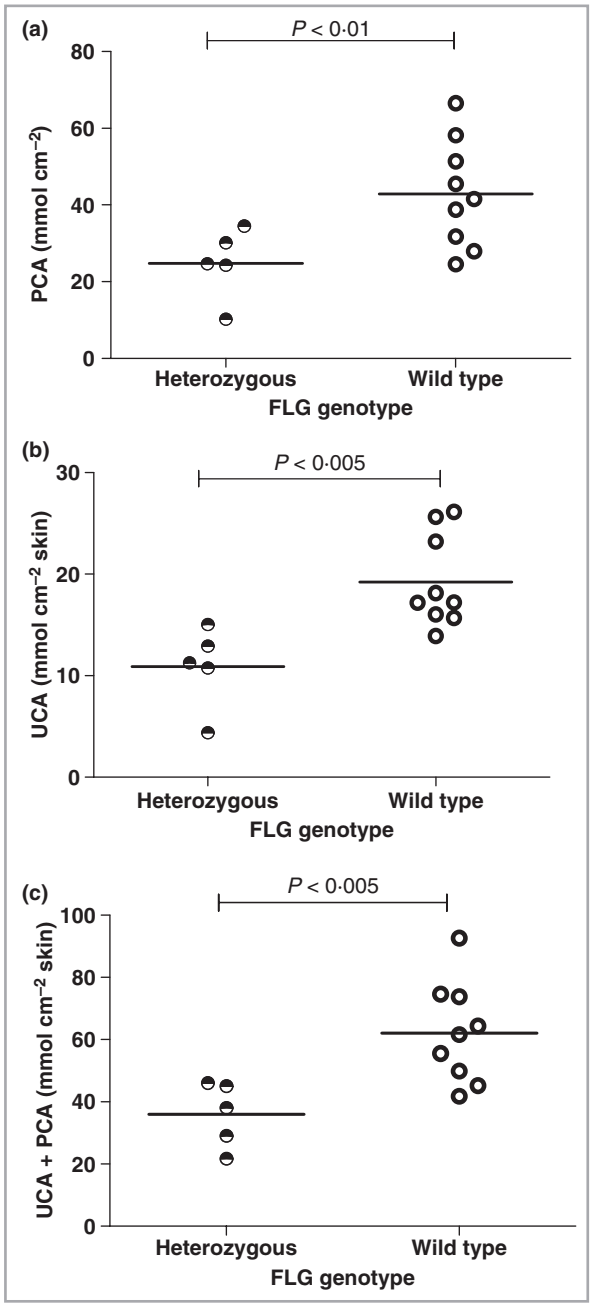

Fig 3. Levels of (a) 2-pyrrolidone-5-carboxylic acid (PCA), (b) urocanic acid (UCA) and (c) the sum of UCA and PCA levels in the stratum corneum in relation to the *FLG* genotype determined by potassium hydroxide patches.

Raman spectroscopy. Sampling of NMF components from the stratum corneum by sequential tape stripping or by extraction with KOH using skin patches offered the possibility of noninvasive sampling. Both sampling techniques have previously been used for the determination of stratum corneum levels of UCA or amino acids, which were subsequently analysed by an appropriate technique, mainly by HPLC.[6,19–23] In the present study we were able to show that the tape stripping technique followed by HPLC is a suitable and feasible method for

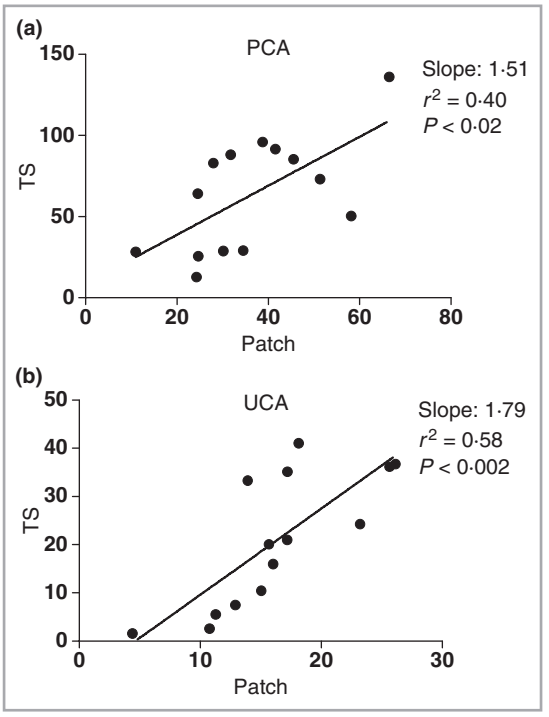

Fig 4. The correlation between the values of (a) 2-pyrrolidone-5-carboxylic acid (PCA) and (b) urocanic acid (UCA) as determined by tape stripping (TS) and patch techniques.

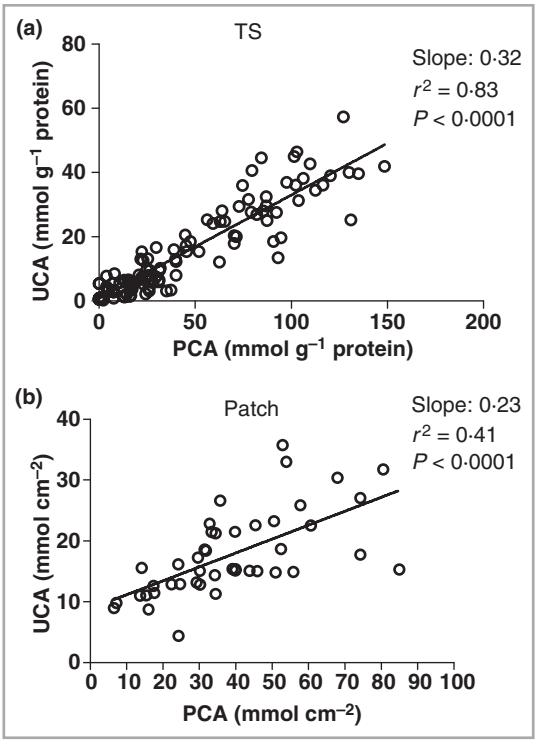

Fig 5. Correlation between the values of urocanic acid (UCA) and 2-pyrrolidone-5-carboxylic acid (PCA) as determined by (a) tape stripping (TS) and (b) patch techniques.

the determination of UCA and PCA in the stratum corneum as biomarkers of the *FLG* genotype. The tape stripping technique showed a higher discrimination potential with respect to *FLG* genotypes than the KOH patches. As a biomarker of the *FLG* genotype, PCA showed a better predictive value than UCA. This can be explained by the fact that the levels of PCA are directly related to the amount of filaggrin. In contrast to PCA which is formed nonenzymatically from glutamine, the conversion of UCA from its precursor histidine is an enzymatic process; thus the concentration of UCA is additionally influenced by the activity of the catalysing enzyme histidase.[14–17] Furthermore, the concentration of UCA may be influenced by endogenous histidine concentrations. It has been shown that the total amount of UCA in the skin is significantly higher after a histidine-rich diet compared with a normal diet.[28] Even though a limited number of subjects was included in the present study, by using tape stripping a significant difference in the amount of PCA and the sum of PCA and UCA levels was detectable for the homozygous vs. heterozygous and wild-type *FLG* genotypes. This is consistent with the findings from our previous study in which the levels of NMF obtained by Raman spectroscopy were significantly reduced (factor 2) in the carriers of *FLG* mutations compared with the noncarriers.[12] Direct comparison of the concentrations of PCA and UCA obtained in the present study with the levels of NMF reported in the Raman study[12] is not possible for several reasons. In the Raman study, the reference spectrum of NMF was constructed from a superposition of the spectra of PCA, ornithine, serine, proline, glycine, histidine and alanine and no individual data on the levels of PCA and UCA were reported. Furthermore, in the Raman study the NMF levels were expressed in arbitrary units as an NMF to keratin ratio, whereas in the present study absolute concentrations in mmol g^{-1} protein are used.

This analysis method delivers a measure of the relative NMF content, expressed in arbitrary units. The differences between the *FLG* genotypes have been further accentuated by normalization of the concentrations of UCA and PCA on the strip for protein amount. Normalization for protein, which compensates for different amounts of stripped stratum corneum, also improved intraindividual variability, reducing the coefficient of variance, for example for PCA from 25% to 20%. The KOH patch technique induced more subjective discomfort in the volunteers than tape stripping. Furthermore, the KOH sampling procedure is more time-consuming. In contrast to tape stripping where the concentrations were normalized for proteins, the KOH patch technique cannot be normalized for possible differences in extraction recovery. This might be one of the possible explanations for the larger variations in the concentration of UCA and PCA which were obtained from adjacent forearm skin sites when using KOH patches (e.g. for PCA the coefficient of variation amounted to 34% and 20% for patches and tape stripping, respectively). Furthermore, considering the predictive value of UCA and PCA for the *FLG* genotype, the differences in UCA and PCA levels between carriers and noncarriers of *FLG* mutations were less pronounced when using patches than with tape stripping.

The applied HPLC method allowed us to determine both isomers of UCA simultaneously with PCA. Because the present study was primarily focused on UCA as a biomarker of the *FLG* genotype we used for the calculations only the total amount of both UCA isomers. However, for research purposes the UCA isomer composition might be relevant. In the skin, UCA, which is a major UV-absorbing skin molecule, is formed from histidine as the *trans* isomer. Upon exposure to UV radiation, *trans*-UCA is converted to its *cis* isomer which plays an important role in UV-induced immune suppression.[18] Recently, it was shown that *cis*-UCA induces UV-inducible genes associated with apoptosis, cell growth arrest, cytokines and oxidative stress.[29]

In summary, the tape stripping technique followed by HPLC determination was shown to be a valuable approach for the determination of UCA and PCA in the stratum corneum. As a biomarker of the *FLG* genotype, PCA can be used alone or as a combined amount of UCA and PCA. The samples (second and third tape strips only) are simple and fast to take by using commercially available adhesive tape and the procedure is minimally invasive and not time consuming. Tape stripping samples with UCA and PCA are stable and can be stored over a long period of time. The HPLC technique is a common tool in clinical laboratories making this method very suitable and feasible for use in research and clinical practice.

As polymorphisms in the *FLG* gene lead to a reduced amount of filaggrin and consequently to a decreased level of its breakdown products, the measurement of PCA and/or UCA could improve identification of *FLG*-mutation carriers, which might enable more targeted prevention in susceptible individuals. Due to ethical considerations, the measurement of the levels of UCA and PCA would be less demanding then *FLG* genotyping. However, before introducing this test as a standard it should be realized that there are several intrinsic and extrinsic factors which might influence the predictive value of UCA and PCA for the *FLG* genotype. Thus, low humidity influences the function of hydrolytic enzymes responsible for the proteolysis of filaggrin and the generation of its breakdown products.[4] As possible genetic modifiers of the effect of the *FLG*-null allele, polymorphisms in the serine protease inhibitor *SPINK5* have been suggested.[8] In addition, a heterozygote for a loss-of-function mutation might carry an expanded exon 3 on the other allele, lessening the overall effect of the mutation.[1,8] Furthermore, it has recently been reported that filaggrin skin expression could be modulated by the atopic inflammatory response mediated by the cytokines interleukin (IL)-4 and IL-13.[30] Also, as discussed previously, both UCA and PCA levels might originate from sources other than filaggrin, e.g. from food. Thus, before using it as a predictive test, the specificity and sensitivity (i.e. the number of false-positive and false-negative outcomes) should be investigated in a larger group of subjects.

References

1 O'Regan GM, Sandilands A, McLean WH *et al.* Filaggrin in atopic dermatitis. *J Allergy Clin Immunol* 2008; **122**:689–93.

2 Scott IR, Harding CR. Filaggrin breakdown to water binding compounds during development of the rat stratum corneum is controlled by the water activity of the environment. *Dev Biol* 1986; **115**:84–92.

3 Rawlings AV, Scott IR, Harding CR *et al.* Stratum corneum moisturization at the molecular level. *J Invest Dermatol* 1994; **103**:731–40.

4 Rawlings AV, Harding CR. Moisturization and skin barrier function. *Dermatol Ther* 2004; **17**:43–8.

5 Harding CR, Scott IR. Stratum corneum moisturizing factors. In: *Skin Moisturization* (Leyden JJ, Rawlings AV, eds). London: Informa Health Care, 2002; 61–80.

6 Horii I, Nakayama Y, Obata M *et al.* Stratum corneum hydration and amino acid content in xerotic skin. *Br J Dermatol* 1989; **121**:587–92.

7 Mischke D, Korge BP, Marenholz I *et al.* Genes encoding structural proteins of epidermal cornification and S100 calcium-binding proteins form a gene complex ('epidermal differentiation complex') on human chromosome 1q21. *J Invest Dermatol* 1996; **106**:989–92.

8 Sandilands A, Terron-Kwiatkowski A, Hull PR *et al.* Comprehensive analysis of the gene encoding filaggrin uncovers prevalent and rare mutations in ichthyosis vulgaris and atopic eczema. *Nat Genet* 2007; **39**:650–4.

9 Irvine AD. Fleshing out filaggrin phenotypes. *J Invest Dermatol* 2007; **127**:504–7.

10 Weidinger S, O'Sullivan M, Illig T *et al.* Filaggrin mutations, atopic eczema, hay fever, and asthma in children. *J Allergy Clin Immunol* 2008; **121**:1203–9.

11 de Jongh CM, Khrenova L, Verberk MM *et al.* Loss-of-function polymorphisms in the filaggrin gene are associated with an increased susceptibility to chronic irritant contact dermatitis: a case–control study. *Br J Dermatol* 2008; **159**:621–7.

12 Kezic S, Kemperman PM, Koster ES *et al.* Loss-of-function mutations in the filaggrin gene lead to reduced level of natural moisturizing factor in the stratum corneum. *J Invest Dermatol* 2008; **128**:2117–19.

13 Caspers PJ, Lucassen GW, Carter EA *et al.* *In vivo* confocal Raman microspectroscopy of the skin: noninvasive determination of molecular concentration profiles. *J Invest Dermatol* 2001; **116**:434–42.

14 Scott IR, Harding CR, Barrett JG. Histidine-rich protein of the keratohyalin granules. Source of the free amino acids, urocanic acid and pyrrolidone carboxylic acid in the stratum corneum. *Biochim Biophys Acta* 1982; **719**:110–17.

15 Barrett JG, Scott IR. Pyrrolidone carboxylic acid synthesis in guinea pig epidermis. *J Invest Dermatol* 1983; **81**:122–4.

16 Harding CR, Watkinson A, Rawlings AV *et al.* Dry skin, moisturization and corneodesmolysis. *Int J Cosmet Sci* 2000; **22**:21–52.

17 Scott IR. Factors controlling the expressed activity of histidine ammonia lyase in the epidermis and the resulting accumulation of urocanic acid. *Biochem J* 1981; **194**:829–38.

18 Gibbs NK, Tye J, Norval M. Recent advances in urocanic acid photochemistry, photobiology and photoimmunology. *Photochem Photobiol Sci* 2008; **7**:655–67.

19 Tateda N, Matsuhisa K, Hasebe K *et al.* Simultaneous determination of urocanic acid isomers and histidine in human stratum corneum by high-performance liquid chromatography. *Anal Sci* 2001; **17**:775–8.

20 Hermann K, Abeck D. Analysis of histidine and urocanic acid isomers by reversed-phase high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 2001; **750**:71–80.

21 Kammeyer A, Pavel S, Asghar SS *et al.* Prolonged increase of *cis*-UCA levels in human skin and urine after single total-body ultraviolet exposures. *Photochem Photobiol* 1997; **65**:593–8.

22 Jansén CT, Lammintausta K, Pasanen P *et al.* A non-invasive chamber sampling technique for HPLC analysis of human epidermal urocanic acid isomers. *Acta Derm Venereol* (Stockh) 1991; **71**:143–80.

23 Kikuchi K, Tagami H. Japanese Cosmetic Scientist Task Force for Skin Care of Atopic Dermatitis. Noninvasive biophysical assessments of the efficacy of a moisturizing cosmetic cream base for patients with atopic dermatitis during different seasons. *Br J Dermatol* 2008; **158**:969–78.

24 Palmer CN, Irvine AD, Terron-Kwiatkowski A *et al.* Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. *Nat Genet* 2006; **38**:441–4.

25 Breternitz M, Flach M, Prassler J *et al.* Acute barrier disruption by adhesive tapes is influenced by pressure, time and anatomical location: integrity and cohesion assessed by sequential tape stripping. A randomized, controlled study. *Br J Dermatol* 2007; **156**:231–40.

26 Voegeli R, Heiland J, Doppler S *et al.* Efficient and simple quantification of stratum corneum proteins on tape strippings by infrared densitometry. *Skin Res Technol* 2007; **13**:242–51.

27 Egawa M, Tagami H. Comparison of the depth profiles of water and water-binding substances in the stratum corneum determined *in vivo* by Raman spectroscopy between the cheek and volar forearm skin: effects of age, seasonal changes and artificial forced hydration. *Br J Dermatol* 2008; **158**:251–60.

28 Shibata K, Fukuwatari T, Zushi S *et al.* Effect of dietary histidine content on the change in content of skin urocanic acid isomers in hairless mice irradiated with ultraviolet B. *Biosci Biotechnol Biochem* 2001; **65**:1415–18.

29 Kaneko K, Smetana-Just U, Matsui M *et al.* *cis*-Urocanic acid initiates gene transcription in primary human keratinocytes. *J Immunol* 2008; **181**:217–24.

30 Howell MD, Kim BE, Gao P *et al.* Cytokine modulation of atopic dermatitis filaggrin skin expression. *J Allergy Clin Immunol* 2007; **120**:150–5.