Skin Barrier Function in Healthy Subjects and Patients with Atopic Dermatitis in Relation to *Filaggrin* Loss-of-Function Mutations

Journal of Investigative Dermatology (2011) **131,** 540–542; doi:10.1038/jid.2010.307; published online 21 October 2010

TO THE EDITOR

Patients with atopic dermatitis (AD) have a defective skin barrier that even exists in nonlesional skin and is characterized by increased transepidermal water loss (TEWL) as well as enhanced percutaneous penetration of both lipophilic and hydrophilic compounds (Hata *et al.*, 2002; Jakasa *et al.*, 2006, 2007; Gupta *et al.*, 2008; Proksch *et al.*, 2008; Jensen *et al.*, 2009). Various causes for the impaired barrier in AD have been suggested, including an aberrant composition and structure of the intercellular lipid bilayers and an altered expression of the cornified envelope and keratinocyte structural proteins (Proksch *et al.*, 2008). Furthermore, barrier function in nonaffected skin has been shown to be dependent on disease activity (Chamlin *et al.*, 2002; Jakasa *et al.*, 2006; Gupta *et al.*, 2008). The well-established association between filaggrin gene (*FLG*) loss-of-function mutations and AD supports the hypothesis that an intrinsically impaired skin barrier may be a primary step in the development of AD (Brown and McLean, 2009; O'Regan *et al.*, 2009). This seems plausible because filaggrin is a key epidermal protein that regulates several functions critical for the structure and composition of the stratum corneum (SC) (Kezic *et al.*, 2008; Brown and McLean, 2009; O'Regan *et al.*, 2009). Previous investigators have reported contrasting findings regarding the influence of *FLG* mutations on the skin barrier in AD (Hubiche *et al.*, 2007; Nemoto-Hasebe *et al.*, 2009; Jungersted *et al.*, 2010). Furthermore, because only one-third of AD patients have *FLG* loss-of-function mutations, these mutations only explain the barrier abnormalities in a subset of AD patients. Therefore, the main objective of this study was to investigate whether the barrier reduction of nonlesional skin on AD patients was limited to carriers of *FLG* mutations.

This study was based on our previous investigations that reported an impaired skin barrier for polyethylene glycols (PEG) of different molecular weights and sodium lauryl sulfate (Jakasa *et al.*, 2006, 2007). In this investigation, we genotyped the subjects who participated in the previous studies. To obtain a sufficient sample size for the groups, we recruited more subjects from the outpatient clinic of the Academic Medical Centre and through a public advertisement. After recruitment, our study population consisted of 20 healthy individuals who were wild type for *FLG* mutations (CTRL-wt), 15 AD patients who were wild type for *FLG* (AD-wt), and 17 AD patients who were heterozygous for *FLG* (AD-Flg). All subjects were of Dutch Caucasian decent. The characteristics of all subjects are presented in Table 1. The medical ethics committee of the Academic Medical Center, University of Amsterdam, approved the experimental protocol. All subjects gave their written, informed consent. The study was conducted according to the Declaration of Helsinki Principles.

The skin barrier in the nonlesional skin was assessed by TEWL and by percutaneous penetration of PEG (PEG 370). The penetration of PEG 370 was characterized by the diffusivity (D), diffusion rate constant (D/L^2), and partition of PEG 370 into the SC ($K_{sc/v}$). For a detailed method description, please refer to our previous study (Jakasa *et al.*, 2007). Genotype analysis was performed for the four most prevalent mutations in European Caucasians: R501X, 2282del4, R2447X, and S3247X, according to the procedure described elsewhere (Sandilands *et al.*, 2007).

The results of this study showed that AD patients, irrespective of their *FLG* genotype, have an altered skin barrier. As seen in Figure 1a, a significant difference in TEWL was found between CTRL-wt and AD patients with or without *FLG* mutations. Similar results were also obtained for the percutaneous penetration of PEG 370. Compared with CTRL-wt, both AD patient subgroups had a higher apparent diffusion coefficient D and diffusion rate (D/L^2) of PEG 370 (Figure 1b and c). Increased diffusivity of PEG 370 resulted in a higher permeability coefficient (K_p) of PEG 370 in AD-Flg patients (Figure 1e). However, in AD-wt patients, the higher diffusivity of PEG was offset by a decrease in the partition coefficient of PEG 370 (Figure 1d). Lower partition of PEG in AD patients had previously been reported (Jakasa *et al.*, 2007); nevertheless, this study showed that this effect was limited to only AD-wt patients. A reduction in the partition of PEG 370 suggests an altered composition and/or structure of the SC in AD-wt patients; however, the reason for this is not clear.

In most studies related to AD, skin barrier function was assessed by measuring TEWL. It is still unclear whether TEWL is also a good parameter of the skin barrier for the ingress of compounds (Proksch *et al.*, 2008; Jensen *et al.*, 2009). Scharschmidt *et al.* (2009) reported increased penetration of a water-soluble compound in filaggrin-deficient flaky tail (*ft/ft*) mice when compared with *wt/wt* mice, however basal TEWL values in *ft/ft* mice were in

Abbreviations: AD, atopic dermatitis; FLG, filaggrin; PEG, polyethylene glycol; SC, stratum corneum; TEWL, transepidermal water loss

Table 1. Characteristics of the subjects and SC thickness

	CTRL-wt (*n*=20)	AD-wt (*n*=15)	AD-Flg (*n*=17)
Age	27 (21–56)	24 (18–52)	26 (19–60)
Female gender (%)	11 (55)	11 (73)	10 (59)
EASI score*	—	1.2 (0.4–22.8)	0.9 (0.0–3.0)
SC thickness/μm	7.9 (5.1–13.8)	9.3 (7.2–10.8)	8.2 (5.1–12.0)

Abbreviations: AD-Flg, AD patients with *FLG* mutations; AD-wt, AD patients without *FLG* mutations; CTRL-wt, control subjects without *FLG* mutations; EASI, eczema area and severity index; SC, stratum corneum.
The results are shown as the median value (range). *EASI (maximum 72 points) (Hanifin *et al.*, 2001).

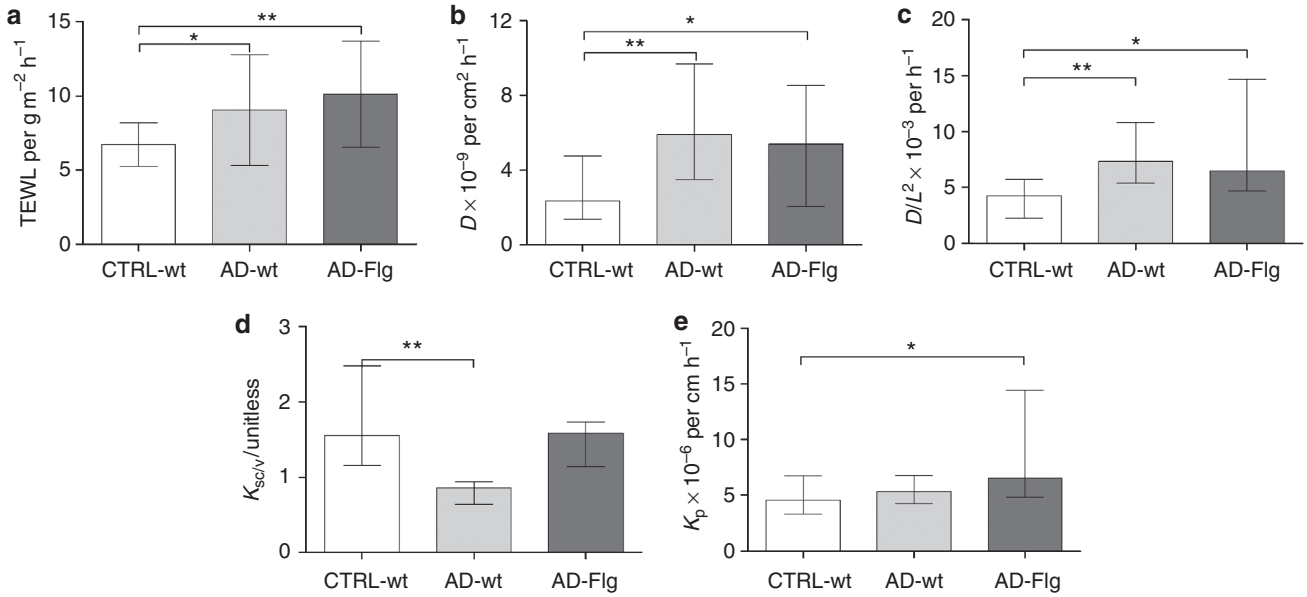

Figure 1. Skin barrier parameters in healthy subjects and AD patients. (**a**) Transepidermal water loss (TEWL), (**b**) diffusion coefficient (D), (**c**) diffusion rate constant (D/L^2), (**d**) SC/vehicle partition coefficient ($K_{sc/v}$), and (**e**) permeability coefficient (K_p). AD-Flg, AD patients with *FLG* mutations; AD-wt, AD patients without *FLG* mutations; CTRL-wt, control subjects without *FLG* mutations. The results are shown as mean values ± SD (**a**) or as median with interquartile range (**b**-**e**). Analysis of variance (ANOVA) followed by a *post hoc* Bonferroni's multiple comparison test was used to compare means between the two AD groups and controls. For other parameters (**b**-**e**), we applied a nonparametric Kruskal–Wallis test followed by Dunn's *post hoc* multiple comparison tests. *$P<0.05$, **$P<0.01$.

the range of normal values. The enhanced TEWL and increased diffusivity of PEG 370 observed in the present study suggests impairment of the skin barrier in both directions. This was further supported by a positive correlation between TEWL and D/L^2 ($r=0.34$; $P=0.006$).

In summary, this study demonstrated a reduced skin barrier in AD patients, irrespective of *FLG* genotype, implying that other factors besides *FLG* loss-of-function mutations modulate skin barrier integrity. Enhanced TEWL and diffusivity in AD suggests defects in the intercellular lipid bilayers of the SC. The mechanisms underlying the disturbance of lipid organization in the SC might be different in AD patients with and without *FLG* mutations. Therefore, an investigation that examined the composition and the organization of intercellular lipids of the SC in AD patients in relation to *FLG* genotype and state of disease would be interesting.

CONFLICT OF INTEREST

The authors state no conflict of interest. The study was carried out in Amsterdam, the Netherlands. The genotype analysis was performed by the Epithelial Genetics Group, Division of Molecular Medicine, Colleges of Life Sciences and Medicine, Dentistry and Nursing, University of Dundee, UK.

***Ivone Jakasa*[1,2], *Ellen S. Koster*[2], *F. Calkoen*[2], *WH Irwin McLean*[3], *Linda E. Campbell*[3], *Jan D. Bos*[4], *Maarten M. Verberk*[2] *and Sanja Kezic*[2]**

[1]Laboratory for Analytical Chemistry, Department of Chemistry and Biochemistry, Faculty of Food Technology and Biotechnology, University of Zagreb, Zagreb, Croatia; [2]Coronel Institute of Occupational Health, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands; [3]Epithelial Genetics Group, Division of Molecular Medicine, Colleges of Life Sciences and Medicine, Dentistry and Nursing, University of Dundee, Dundee, UK and [4]Department of Dermatology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands
E-mail: s.kezic@amc.uva.nl or ijakasa@pbf.hr

REFERENCES

Brown SJ, McLean WH (2009) Eczema genetics: current state of knowledge and future goals. *J Invest Dermatol* 129:543–52

Chamlin SL, Kao J, Freiden IJ *et al.* (2002) Ceramide-dominant barrier repair lipids alleviate childhood atopic dermatitis: changes in barrier function provide a sensitive indicator of disease activity. *J Am Acad Dermatol* 47:198–208

Gupta J, Grube E, Ericksen MB *et al.* (2008) Intrinsically defective skin barrier function in children with atopic dermatitis correlates with disease severity. *J Allergy Clin Immunol* 121:725–30

Hanifin JM, Thurston M, Omoto M, *et al.*, the EASI Evaluator Group (2001) The eczema area and severity index (EASI): assessment of reliability in atopic dermatitis. *Exp Dermatol* 10:11–8

Hata M, Tokura Y, Takigawa M *et al.* (2002) Assessment of epidermal barrier function by photoacoustic spectrometry in relation to its importance in the pathogenesis of atopic dermatitis. *Lab Invest* 82:1451–61

Hubiche T, Ged C, Benard A *et al.* (2007) Analysis of SPINK 5, KLK 7 and FLG genotypes in a French atopic dermatitis cohort. *Acta Derm Venereol* 87:499–505

Jakasa I, de Jongh CM, Verberk MM *et al.* (2006) Percutaneous penetration of sodium lauryl sulphate is increased in uninvolved skin of atopic dermatitis patients compared to control subjects. *Br J Dermatol* 155:104–9

Jakasa I, Verberk MM, Esposito M *et al.* (2007) Altered penetration of polyethylene glycols into uninvolved skin of atopic dermatitis patients. *J invest Dermatol* 127:129–34

Jensen JM, Pfeiffer S, Witt M *et al.* (2009) Different effects of pimecrolimus and betamethasone on the skin barrier in patients with atopic dermatitis. *J Allergy Clin Immunol* 124(3 Suppl 2):R19–28

Jungersted JM, Scheer H, Mempel M *et al.* (2010) Stratum corneum lipids, skin barrier function and filaggrin mutations in patients with atopic eczema. *Allergy* 65:911–8

Kezic S, Kemperman PM, Koster ES *et al.* (2008) Loss-of-function mutations in the filaggrin gene lead to reduced level of natural moisturizing factor in the stratum corneum. *J Invest Dermatol* 128:2117–9

Nemoto-Hasebe I, Akiyama M, Nomura T *et al.* (2009) Clinical severity correlates with impaired barrier in filaggrin-related eczema. *J Invest Dermatol* 129:682–9

O'Regan GM, Sandilands A, McLean WH *et al.* (2009) Filaggrin in atopic dermatitis. *J Allergy Clin Immunol* 124:R2–6

Proksch E, Brandner JM, Jensen JM (2008) The skin—an indispensable barrier. *Exp Dermatol* 17:1063–72

Sandilands A, Smith FJ, Irvine AD *et al.* (2007) Filaggrin's fuller figure: a glimpse into the genetic architecture of atopic dermatitis. *J Invest Dermatol* 127:1282–4

Scharschmidt TC, Man MQ, Hatano Y *et al.* (2009) Filaggrin deficiency confers a paracellular barrier abnormality that reduces inflammatory thresholds to irritants and haptens. *J Allergy Clin Immunol* 124:496–506

The Dermoscopical and Histopathological Patterns of Nevi Correlate with the Frequency of BRAF Mutations

Journal of Investigative Dermatology (2011) **131,** 542–545; doi:10.1038/jid.2010.332; published online 11 November 2010

TO THE EDITOR

The frequency of BRAF mutations among nevi is variable (Thomas, 2006), but little is known about the frequency among nevi, stratified by dermoscopy and by the detection method.

In this study, we evaluated BRAF status in 45 nevi from 43 patients (23 women, mean age 39.9 years) using the Sanger method (Loewe *et al.*, 2004); a random subset of 24 of these nevi was tested by the more sensitive ultradeep pyrosequencing (UDPS, Huse *et al.*, 2007; Tan *et al.*, 2008; See Supplementary Data: Material and Methods S1, Supplementary Tables S1 and S2 online).

RESULTS

Patient demographics, anatomic location of nevi, dermoscopic patterns, histopathological diagnosis, and results of the genetic tests using Sanger and UDPS are summarized in Table 1. Dermoscopically, the 45 nevi consisted of 17 (37.7%) reticular nevi (R), 5 (11.1%) globular nevi (G), 12 (26.6%) mixed reticular nevi with central globules/homogeneous areas (MC), and 11 (24.4%) mixed reticular/homogeneous nevi with peripheral globules (MP).

Using Sanger method, 6 of the 45 nevi (13.3%) contained $BRAF^{V600E}$; their dermoscopic patterns were G ($n=2$), MC ($n=2$), and MP ($n=2$), and all ($n=6$) were compound nevi on histopathology. None of the nevi classified as R on dermoscopy or as junctional nevi on histopathology showed BRAF mutations. Patients with nevi harboring $BRAF^{V600E}$ were younger than those with wild-type BRAF (30 versus 41 years, respectively; $P=0.03$).

Using UDPS, 19 (79.2%) of the 24 randomly selected nevi were $BRAF^{V600E}$ positive, including G nevi (100%), MP nevi (90.9%), R nevi (75%), and MC nevi (57%). Six nevi showed heterogeneity of mutations (patients 3, 9, 14 and 16–18) and two nevi (patients 1 and 3), which were negative for $BRAF^{V600E}$, revealed p.K601E variant. Representative electropherogramms are depicted in Figure 1 (lanes 3 and 4). There was no significant age difference between patients with $BRAF^{V600E}$ nevi and patient whose nevi showed wild-type BRAF (37 versus 44 years, respectively; $P=0.20$).

COMMENT

The frequency of BRAF mutations among nevi was highly dependent on the sensitivity of the employed method; only 13.3% of nevi showed BRAF mutation using Sanger method, whereas 79.2% showed mutation with UDPS. The finding of BRAF mutation by UDPS and not by Sanger method probably attests to clonal heterogeneity within the nevus, whereby clones of BRAF mutations and clones with wild-type BRAF coexist (Lin *et al.*, 2009). The age difference seen between patients

Abbreviations: G, globular nevi; MC, mixed reticular nevi with central globules/homogeneous areas; MP, mixed reticular/homogeneous nevi with peripheral globules; R, reticular nevi; UDPS, ultradeep pyrosequencing