

Development and validation of a minimally invasive and image-guided tape stripping method to sample atopic skin in children

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Abstract

Background. Molecular skin profiling techniques, typically performed on skin samples taken by punch biopsy, have enhanced the understanding of the pathophysiology of atopic dermatitis (AD), thereby enabling the development of novel targeted therapeutics. However, punch biopsies are not always feasible or desirable, and novel minimally invasive methods such as skin tape stripping have been developed.

Aim. To develop, optimize and validate a novel tape stripping method guided by noninvasive *in vivo* skin imaging to sample atopic skin in children.

Methods. Skin tape stripping-based procedures were compared and optimized using data from 30 healthy controls (HCs: 5 adults, 25 children) and 39 atopic children. Evaluations were guided by high-resolution photography, reflectance confocal microscopy, optical coherence tomography and transepidermal water loss measurements. We assessed and compared adverse events (AEs), the time needed to perform the sampling and the cDNA levels obtained from the tapes.

Results. Tape stripping methods based on previously described protocols resulted in erosions in all participants and required a median time of 65 min to perform (range 60–70 min), but provided good cDNA yield. Shorter durations appeared less invasive but provided lower cDNA yield. The final optimized tape stripping protocol, using 11 tapes of 22 mm in diameter, each applied twice for 5 s with 90° rotation, did not produce significant AEs, was completed within a median time of 7 min (range 5–15 min) and provided good cDNA yield both in HCs and atopic children.

Conclusion. Our minimally invasive method is safe and reliable, and provides reproducible acquisition of cDNA in atopic children. In addition, it enables rapid sample collection, a crucial factor in clinical practice.

Introduction

Molecular profiling techniques, typically performed on skin samples obtained by punch biopsy, have enhanced the pathophysiological understanding of major skin diseases such as psoriasis and atopic dermatitis (AD), thus allowing the development of novel targeted therapeutics. AD is a highly prevalent, relapsing, chronic inflammatory skin disease, which is more frequent in children than in adults.¹ However, punch biopsies are not always feasible nor desirable and minimally invasive or noninvasive techniques allowing skin molecular phenotyping are required to assess AD severity and treatment effectiveness.

Previously, adhesive tape stripping of the stratum corneum (SC) has been used to study cutaneous drug pharmacokinetics,^{2–4} melanocytic lesions,⁵ skin cancers,⁶ psoriasis⁷ and AD,^{8–17} among others. However, in AD, a standardized procedure has not been validated, resulting in variability and potential harm, given the sensitivity of atopic skin especially in children. Hence, standardization of tape stripping procedures in AD is required to achieve adequate high-quality material for molecular studies while not harming the skin.

We describe a novel SC sampling method using minimally invasive, adhesive tape stripping, guided by *in vivo* imaging.

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Methods

Study design

To determine the optimal SC sampling protocol, we first sampled 30 healthy controls (HCs) comprising 5 adults (Phase 1a) and 25 children (Phases 1b and 1c; Figure 1). Subsequently, the optimized method was used for a cohort of 39 paediatric patients with AD (Phase 2) to: (i) assess the safety of the method safety in paediatric patients with AD, (ii) determine the reliability of the method in paediatric patients with AD and (iii) obtain biological material for molecular analysis in subsequent studies.¹⁸ In addition, in Phase 2 we sampled lesional and nonlesional skin (the latter taken approximately 10 cm from the target lesion) and later mometasone furoate cream 1 mg g⁻¹ (Elocom; Merck Sharp & Dohme, Madrid, Spain) was applied twice daily for 2 weeks to AD lesions to assess the molecular response to topical corticosteroids. The study ran from October 2017 to April 2019.

Clinical evaluation

Participants were evaluated at baseline against the inclusion and exclusion criteria (Supplementary Tables S1–S3). High resolution, standardized pictures (Powershot G12; Canon Inc., Tokyo, Japan) were taken at the skin sampling sites at the baseline visit and at follow-up 7 days later. Any adverse events (AEs), such as erythema, oedema, erosion, infection, pruritus or postinflammatory hyperpigmentation,

were recorded during the week after the procedure and graded using a semiquantitative scale (0 = absent, 1 = mild, 2 = moderate, 3 = severe). The time needed to perform the entire SC sampling process using the different protocols was also recorded.

Tape stripping procedure

Before initiating the procedure and to avoid contamination, sterile gloves and gowns were used, and all surfaces and instruments were cleaned using RNaseZAP (Invitrogen/Thermo Fisher Scientific, Waltham, MA, USA). Four different protocols were evaluated (Table 1) and the final optimized protocol is presented in Supplementary Data S1. Sampling locations were the cubital and popliteal fossae. Circular adhesive tapes (D-Squame; CuDerm, Dallas, TX, USA) were placed on the skin, and 225 g cm⁻² of pressure was applied using a standardized pressure device (D500 D-Squame Pressure Instrument; CuDerm). Each tape was then gently removed with a quick uniform hand movement, following the longitudinal axis of the arm. In the protocols where the same tape was applied twice or four times at the same skin site, the orientation was changed (90° rotation) to maximize the collection of biological material per tape (Supplementary Figure 1, Supplementary Video S1). Tapes were immediately pooled and placed in RNase-free 1.5-mL tubes for subsequent analyses (Figure 2). For transcriptomic analyses, tapes were placed in tubes containing RNA stabilizing lysis solution (RNAqueous Micro Kit; Ambion/Thermo

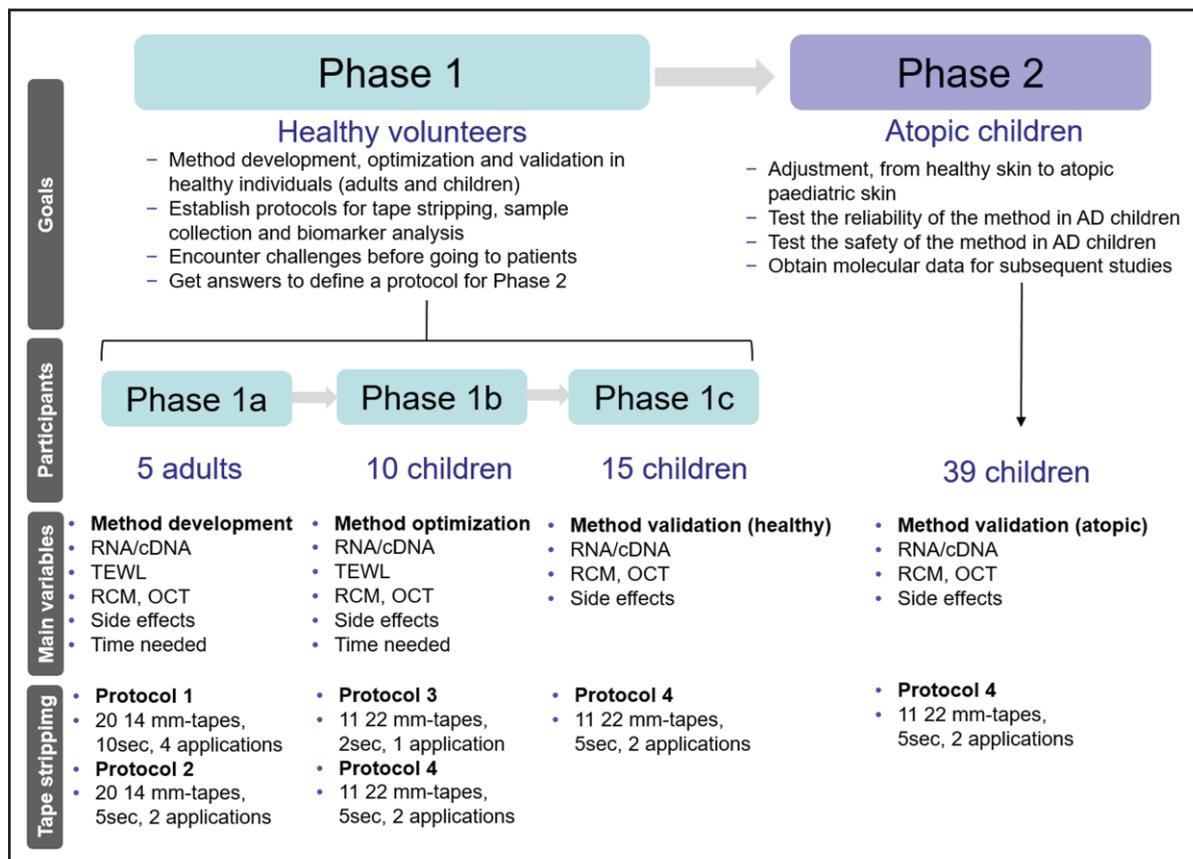


Figure 1 Workflow and summary of the different study phases. AD, atopic dermatitis; OCT, optical coherence tomography; RCM, reflectance confocal microscopy; TEWL, transepidermal water loss.

Table 1 Protocols used during the tape stripping method development in healthy controls and atopic paediatric patients

Parameter	Protocol			
	1 (long)	2 (short)	3 (ultrashort)	4 (optimized)
Population	Adults	Adults	Children	Children
Disease state	Healthy	Healthy	Healthy	Healthy and atopic
Skin site	Cubital	Cubital	Cubital	Cubital and/or popliteal
Tape diameter, mm	14	14	22	22
Number of tapes	20	20	11	11
Tape pressure time, s	10	5	2	5
Applications, <i>n</i> /tape	4	2	1	2

Fisher Scientific, Waltham, MA, USA). All tubes were stored at -80°C until further processing. No tapes were discarded at any phases of the study.

Noninvasive *in vivo* imaging and transepidermal water loss assessment

A reflectance confocal microscopy (RCM) device (Vivascope 3000; Caliber ID/MAVIG, Rochester, NY, USA) and an optical coherence tomography (OCT) device (NITID; DermaLumics, Madrid, Spain) were used to confirm sufficient corneocyte detachment by tape stripping and that the integrity of the epidermis was preserved. Additionally, in Phase 2 we also used a dynamic OCT device (Vivosight; Michelson Diagnostics, Kent, UK) to obtain data on skin vascularization for use in further studies.

RCM uses an 830-nm laser light source to enable horizontal optical step sectioning of $\sim 3\mu\text{m}$ with a lateral resolution of $\sim 1\mu\text{m}$; this provides cellular resolution up to a depth of

$\sim 250\mu\text{m}$ within a field of view (FOV) of $0.75 \times 0.75\text{ mm}$.^{19,20} OCT employs a 1305-nm laser source that allows vertical optical step sections of $\sim 7\mu\text{m}$, with a lateral resolution of $\sim 10\mu\text{m}$ and provides skin architectural information up to 1 mm depth within a FOV of $1 \times 2\text{ mm}$.^{21,22}

The handheld RCM and OCT devices were applied directly to the sampling areas just after the first tape strip(s) to avoid microorganism contamination [the first tape strip(s) were stored for future microbiome analysis] and after the final tape strip (Figure 2). At least one RCM vertical stack of horizontal images and at least one OCT raster of vertical images were acquired. In these regions of interest, we evaluated corneocyte detachment and calculated the number of microerosions.

Transepidermal water loss (TEWL) was also measured (Tewameter C+K Multiprobe; Courage + Khazaka Electronic GmbH, Köln, Germany) before, during and at the end of tape stripping in Phases 1a and 1b (Figure 2) to quantify the epidermal barrier disruption associated with

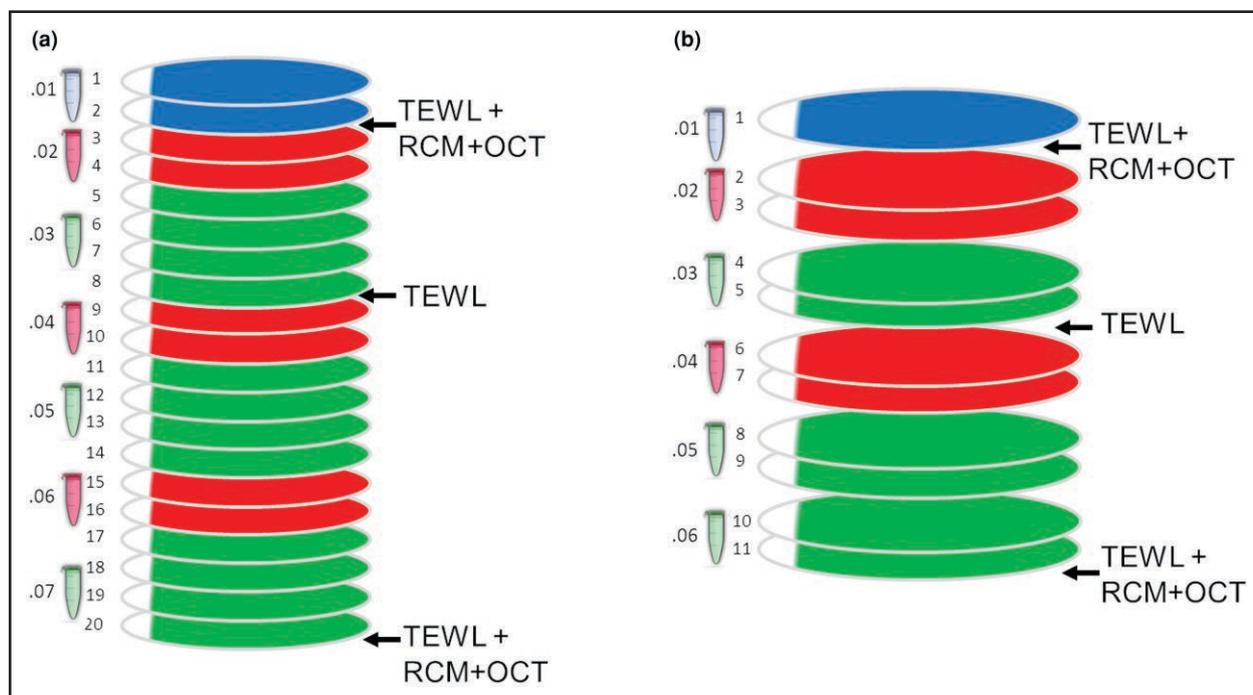


Figure 2 (a, b) Stratum corneum sampling experiments guided by noninvasive skin imaging (OCT, RCM) and TEWL quantification. Blue indicates tapes used for future microbiome studies, red indicates tapes used for protein analysis and green indicates tapes used for transcriptomic analysis. (a) In Phase 1a, 14-mm diameter tapes ($n=20$) were used, with different pressure times and rotations. (b) In subsequent phases, 22-mm diameter tapes ($n=11$) were used. Imaging was conducted between tape applications as indicated by the arrow. OCT, optical coherence tomography; RCM, reflectance confocal microscopy; TEWL, transepidermal water loss.

the different approaches before the study validation phases were performed.

RNA extraction and cDNA library preparation

Total RNA was extracted using (RNAqueous Micro Kit; cat. no. AM9780; Ambion/Thermo Fisher Scientific) according to the manufacturer's instructions, but with an initial incubation of the tapes in the lysis solution of the kit for 30 min at 42° C. Following DNase I digestion and elution of RNA, cDNA was immediately synthesized using Smart-seq2 based library formation.²³ Generated cDNA was preamplified by mixing with 50 µL KAPA HiFi HotStart Ready Mix (Roche, Basel, Switzerland) and 2 µL of 10 µmol L⁻¹ cDNA preamplification primer (IDT). The quantity and quality of all cDNA libraries were checked using a Qubit HD dsDNA assay (Life Technologies, Carlsbad, CA, USA) and a high-sensitivity DNA chip (Agilent, Santa Clara, CA, USA). All cDNA libraries had a minimum cDNA concentration of 4 ng µL⁻¹ and an average transcript length of 500–600 bp.

Statistical analysis

Statistical analyses were performed using SPSS Statistics (V22; IBM SPSS Corp., Armonk, NY, USA) and GraphPad Prism (V7.04; GraphPad, La Jolla, CA, USA). Descriptive variables such as mean, median, range and relative frequency were used to describe the cohort characteristics and clinical results. Fisher exact test was used to compare qualitative variables, χ^2 test was used to compare quantitative variables that followed a normal distribution and Mann–Whitney *U* test was used to compare quantitative variables that did not follow a normal distribution. Comparisons of the cDNA concentrations in different groups were made using ANOVA followed by Tukey *post hoc* test. Two-sided $P < 0.05$ were considered statistically significant.

Results

Phase 1a: stratum corneum sampling in adult healthy volunteers

Demographic and clinical results

In this phase, we had five adult HCs (four women, one man; median age 33 years, range 23–41 years). SC sampling was performed at the cubital fossae using 14-mm diameter tapes and two different methods: namely, Protocol 1 (long) and Protocol 2 (short), which differed in the time for which pressure was applied to the tape and in the number of times each tape was applied to the skin site (Table 1). Immediately after tape stripping, all participants developed mild to moderate erythema and oedema (Supplementary Table S4). No statistically significant differences in these effects were found ($P = 0.21$). All participants reported moderate to severe pruritus immediately after the procedure.

At the 7-day follow-up visit, skin erosions were observed in all participants (Figure 3a,b) with no statistical differences between the two protocols ($P = 0.20$). No signs of skin infection or postinflammatory hyperpigmentation were noted in any participant.

With respect to the time needed to perform tape stripping, Protocol 1 required a significantly ($P < 0.01$) longer time (median 65 min, range 60–70 min) than Protocol 2 (median 35 min, range 30–40 min).

Noninvasive *in vivo* assessment

In both protocols and in all cases, corneocyte detachment was clearly seen with RCM (Figure 3g). However, OCT revealed significantly ($P = 0.03$) more microerosions with the longer Protocol 1 (median of 12 erosions; Figure 3c) than the shorter Protocol 2 (median 8; Figure 3d).

TEWL increased with subsequent tape stripping in both protocols; however, there were no statistically significant differences in TEWL between Protocol 1 and Protocol 2 at any of the time points evaluated (Figure 3e).

Molecular results

When using ultra low-abundance tissue samples for molecular-level analysis, total mRNA levels are preferred over protein to enhance the assessment of quantity and quality. The mRNA yield from the SC samples was expressed as cDNA concentration, as it is not quantifiable before amplification. The levels of cDNA from sets of four tapes varied between participants, but were all sufficient for RNA-sequencing analysis [mean (SD) level was 295.5 (138.8) ng µL⁻¹]. No statistically significant differences between the three sets of tapes or between Protocols 1 and 2 were observed, suggesting that SC sampling was performed consistently (Figure 4a).

Phase 1b: method optimization in healthy children

Demographic and clinical results

To minimize the AEs previously observed in adults, the methods adopted for children were modified by decreasing the number of tapes (from 20 to 11) and reducing the pressure applied. In addition, larger tapes (22 mm diameter) were used to maximize the material collection for molecular analyses. In this way, Protocol 3 (ultrashort; 11 tapes applied once for 2 s each) and Protocol 4 (optimized; 11 tapes applied twice for 5 s each) (Table 1) were tested in 10 healthy children (6 girls, 4 boys; median age 7 years, range 4–16 years).

Immediately after tape stripping, all participants developed mild erythema (Figure 5a,b; Supplementary Table S4). Mild oedema was seen in one participant using Protocol 3 and in four participants with Protocol 4. Mild pruritus was reported by two participants: one after Protocol 3 and one after Protocol 4. At the 7-day follow-up visit, there were no skin erosions, oedema, erythema, skin infection or post-inflammatory hyperpigmentation observed in any of the participants. With respect to the time needed to perform SC sampling, there was no significant difference between Protocol 3 (median 5 min, range 3–7 min) and Protocol 4 (median 7 min, range 5–15 min) ($P < 0.099$).

Noninvasive *in vivo* assessment

Corneocyte detachment was observed with RCM following Protocols 3 and 4, but no impact deeper into the skin (papillary dermis) was evident (Figure 5f). With OCT, significantly fewer microerosions were detected for Protocol 3 (median 9; Figure 5c) than for Protocol 4 (median 13; Figure 5d) ($P < 0.001$).

Assessment of TEWL in the paediatric participants was highly variable and consequently, the results were impossible

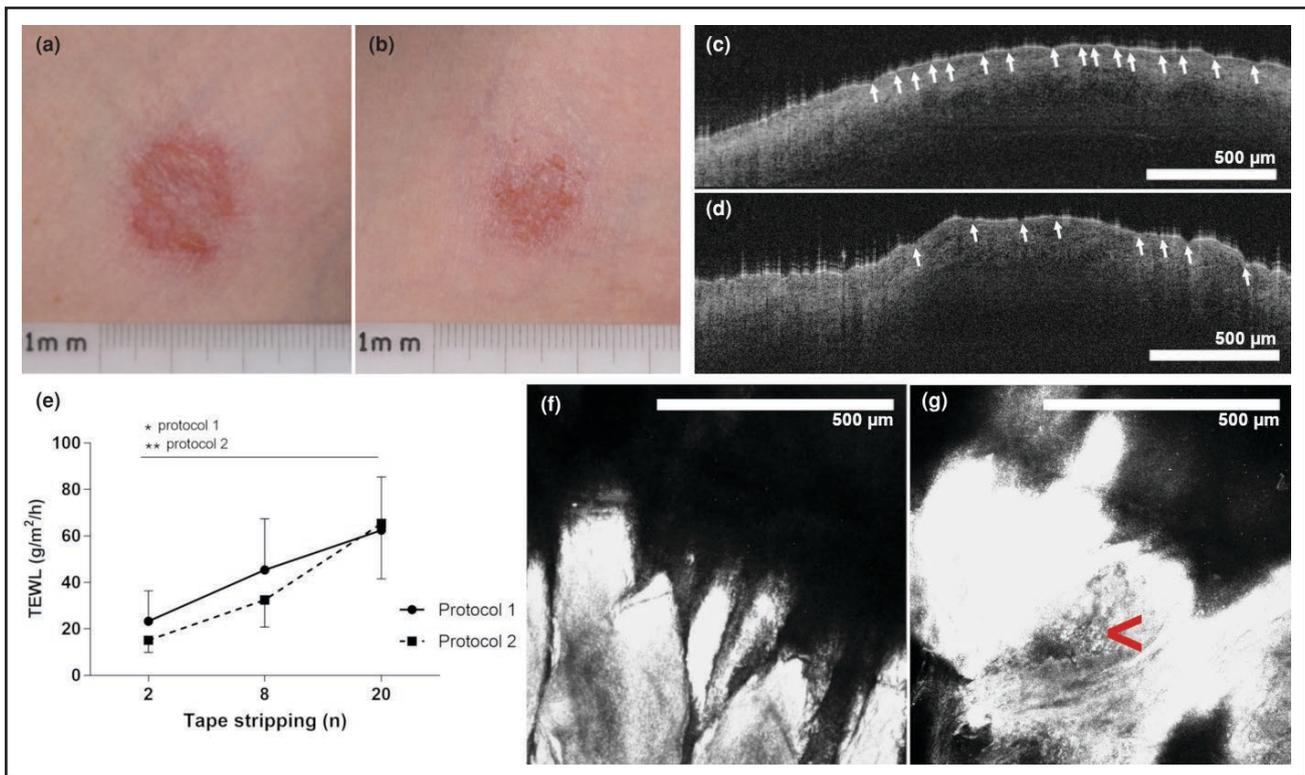


Figure 3 (a–g) Clinical and noninvasive assessments performed in adult volunteers after different tape stripping methods in Phase 1a. (a,b) Illustrative clinical images at the 7-day follow-up after stratum corneum sampling; erosions were seen in all participants: (a) Protocol 1 (long) at the right cubital fossa; (b) Protocol 2 (short) at the left cubital fossa. (c,d) Optical coherence tomography images showing more erosions with (c) the longer Protocol 1 compared with (d) the shorter Protocol 2. (e) Transepidermal water loss (TEWL) data showing differences between tape stripping number 2 and 20, but no differences between the long (Protocol 1) or short (Protocol 2) approaches. Data shown are mean (SD). Statistically significant differences are indicated by ** $P < 0.001$ and * $P < 0.05$. (f,g) Reflectance confocal microscopy image at the stratum corneum (depth 10 μm) (f) before tape stripping and (g) after Protocol 2, showing the presence of detached corneocytes (asterisk).

to interpret. This was likely caused by the children moving and/or perspiring during measurement acquisition. Owing to the imprecise nature of these data, which undermined the possibility of finding any significant differences between protocols used, it was decided to forego the evaluation of TEWL in subsequent experiments.

Molecular results

The cDNA levels [mean (SD)] in the set of tapes collected from children ranged from $6.0 \pm 6.4 \text{ ng } \mu\text{L}^{-1}$ to $13.3 \pm 7.1 \text{ ng } \mu\text{L}^{-1}$. No differences were observed between protocols or between sets of tapes (Figure 4b).

Phase 1c: Method validation on healthy paediatric participants

Demographic and clinical results

Based on the results reported above, Protocol 4 was selected for the next phases of the study. Before applying this optimized method in patients with AD, the method was validated in healthy children. Two skin locations typically affected in atopic skin were sampled: the cubital fossa and the popliteal fossa. The cohort included 15 healthy children (10 boys, 5 girls; median age 6 years, range 1–14 years). Immediately after tape stripping, all participants developed mild, transient erythema in both locations (Supplementary Table S4). At the 7-day follow-up visit, none of the participants had erosions,

oedema, erythema, skin infection or postinflammatory hyperpigmentation.

Noninvasive *in vivo* assessment

In all participants, RCM revealed corneocyte detachment and OCT revealed microerosions, similar to findings in Phase 1b. As mentioned earlier, TEWL measurements were not performed.

Molecular results

Based on the results from Phase 1b, it was decided to pool tapes 8–11 for the RNA analysis collected from Phases 1c and 2. In healthy children, the mean (SD) cDNA concentration in the tapes was $5.2 (9.1) \text{ ng } \mu\text{L}^{-1}$, with no differences seen between popliteal and cubital fossa sites.

Phase 2: Method validation on atopic dermatitis paediatric patients

In Phase 2, the optimized SC sampling method (Protocol 4) was used in 39 children with AD (20 boys, 19 girls; mean age 5.5 years, range 1 month to 16 years) to assess its acceptability (tolerance and safety) and its ability to provide an adequate yield of tissue for molecular analysis. One patient from the original 40-participant cohort was withdrawn as they did not fulfil the inclusion criteria on the day of sampling (they had no active lesions on the cubital fossae). As previously

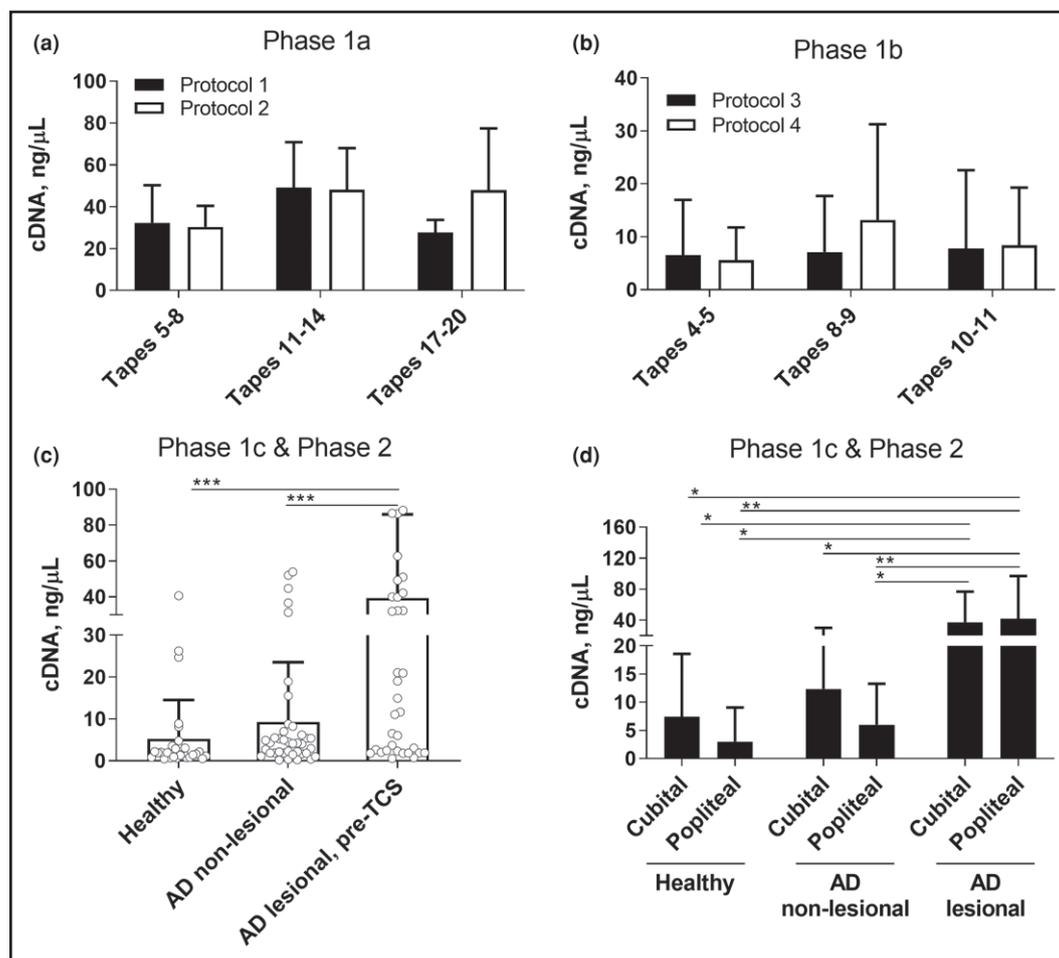


Figure 4 (a–d) cDNA levels determined in different phases of the study. (a) cDNA concentrations in Phase 1a obtained from the 14-mm diameter tape strips collected from five healthy adult participants following Protocol 1 and Protocol 2 [mean(SD)]. (b) cDNA levels in Phase 1b obtained from the 22-mm diameter tape strips collected from healthy children ($n=10$) using Protocol 3 and Protocol 4 [mean(SD)]. (c) cDNA concentrations in Phases 1c and 2 obtained from tape strips collected from atopic dermatitis (AD) ($n=39$) and healthy ($n=15$) children using the optimized protocol. For the AD children, data from lesional, nonlesional and lesional skin after topical mometasone treatment are shown [mean(SD) and individual values are plotted]. Statistically significant differences are indicated, $***P<0.005$. (d) Comparison of cDNA levels in tape strips collected from popliteal and cubital fossae from healthy ($n=15$) and atopic children ($n=39$) using the optimized Protocol 4 [mean(SD)] in Phases 1c and 2. Statistically significant differences are indicated by $*P<0.05$ and $**P<0.01$.

described, skin was sampled from both the cubital and popliteal fossae, choosing the side with the most severe AD for sampling. Nonlesional skin located approximately 10cm from the target lesion was also sampled. The baseline median Eczema Area and Severity Index (EASI) was 6.2 and median SCORing Atopic Dermatitis (SCORAD) was 35.2.

Immediately after tape stripping, all participants exhibited mild erythema and oedema at both lesional and nonlesional locations (Figure 6b, Supplementary Table S4). Mometasone furoate 1 mg g^{-1} was then applied for 2 weeks to the AD lesions according to the protocol and the same areas were then resampled with the same tape stripping protocol. No skin erosions, signs of infection or postinflammatory hyperpigmentation were noted after the initial or post-treatment tape stripping procedures.

Noninvasive *in vivo* assessment

Both RCM and OCT identified corneocyte detachment in all samples (Figure 6c–h), an observation that was significantly greater in lesional skin than in nonlesional skin ($P<0.01$).

Molecular results

The cDNA levels in lesional skin were significantly higher than those in both AD nonlesional skin and unaffected skin samples (Figure 4c), whereas the concentration of cDNA in the HCs was not significantly different from levels in the nonlesional AD samples. When the cDNA data from different skin sites were compared, no difference was found between cDNA levels acquired from cubital and popliteal fossae samples at any of the skin sites considered (lesional, nonlesional, healthy) (Figure 4d). The cDNA concentration in lesional skin samples after the 2-week mometasone treatment was significantly reduced compared with that before steroid application [mean (SD), $39.4(46.6)\text{ ng }\mu\text{L}^{-1}$ before treatment reduced to $11.0(15.9)\text{ ng }\mu\text{L}^{-1}$ after treatment; $P<0.01$].

Discussion

Through range-finding experiments, we have developed an optimized skin tape stripping protocol that is safe and

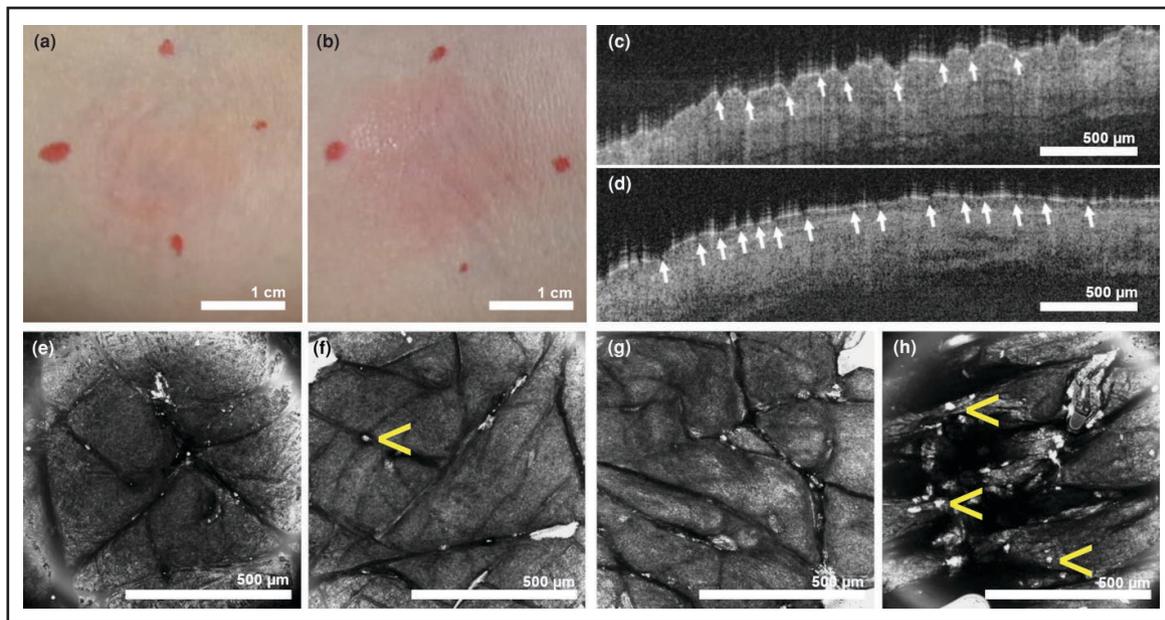


Figure 5 (a–h) Clinical and noninvasive assessments performed in healthy children with atopic dermatitis after different tape stripping methods in Phases 1b and 1c. (a,b) Illustrative clinical images just after stratum corneum (SC) sampling. Mild erythema was seen in most cases, while oedema was rare, and if it did occur was mild. (a) Protocol 3 (ultrashort) at the right cubital fossa; (b) Protocol 4 (optimized) at the left cubital fossa. (c,d) Optical coherence tomography images showed fewer erosions with (c) Protocol 3 compared with (d) Protocol 4. (e–h) Reflectance confocal microscopy images at the SC (depth 10 μm) before (e,g) and after (f,h) tape stripping with (e,f) Protocol 3 and (g,h) Protocol 4. Note that the amount of detached corneocyte tissue (arrowheads) was larger with (h) Protocol 4 than (f) Protocol 3.

reliable for sampling atopic skin. Initially, we performed an exploratory study sampling healthy adults ($n=5$) using two tape stripping methods based on previously described protocols.^{24–26} Following application of 20 tapes and independent of the pressure applied, we identified that the levels of cDNA yielded enough material for transcriptomic analysis. However, for use in children, the protocols had two important limitations: (i) all participants presented with clear skin erosions, which were more invasive than deemed acceptable, and (ii) the protocols were too long to be practically useful (35–65 min). Based on these findings, a shorter protocol was tested on healthy children. This improved approach used just 11 tapes of a larger area, each applied with 5 s of pressure and with the direction of stripping rotated each time through 90°. This protocol was well tolerated by the paediatric participants, was less time-consuming (7 min in total) and still allowed collection of sufficient biological material to perform transcriptomic analysis. Finally, the method was validated in a cohort of paediatric patients with AD with similar outcomes.

Concomitant *in vivo* imaging of skin post-stripping by RCM and OCT demonstrated partial removal of the SC and minimal effect on the viable epidermal layers below (Figures 3, 5 and 6), in agreement with previous reports.^{12,27} Therefore, it is reasonable to conclude that our improved, final protocol minimizes AEs while still providing enough material for further molecular analysis (Figure 4).

SC sampling in different participant populations (adults, healthy children and children with AD) and at different skin sites (healthy, AD lesional and AD nonlesional skin) revealed key differences in the amount of cDNA acquired. In adults, the cDNA concentration sampled [295.5 (138.8) ng μL^{-1} , 14-mm diameter tapes] was much

higher than that from healthy children [5.20 (9.11) ng μL^{-1} , 22-mm diameter tapes]. This could be due to structural differences between children and adult skin (more hydrated and cohesive corneocytes in children²⁸) as well as difficulty in sampling children.

Notably, in children with AD, the cDNA content in lesional skin was significantly higher than that of nonlesional or healthy skin. The higher RNA yield in lesional samples may be due to the less compact structure of the SC, enabling an enhanced collection of biologic material in lesional skin.²⁹ In addition, inflammatory responses in atopic skin lead to increased transcriptional activity^{13,18} and subsequent upregulated protein expression of inflammatory mediators.¹⁴

Our approach has several advantages: it is safe and reliable and it provides reproducible acquisition of cDNA across tissue sites in atopic children. In addition, it enables sample collection to be performed faster and more comfortably. The resulting minimally invasive and expedited protocol is essential for adoption into clinical practice. It is important to note that sampling the skin of atopic children presents two major challenges: (i) the skin of children with AD is more sensitive due to its intrinsically weaker barrier function,³⁰ and (ii) acquisition of skin samples from children is typically complicated because children move around, become easily distracted and are intimidated by clinical procedures. Thus, it is crucial that the method used must not only provide sufficient material for analysis, but must also be safe, accepted and well tolerated by patients, their parents/guardians and clinicians alike.

As well as method validation, the data obtained in this study are being analysed to assess the AD paediatric molecular profile and its treatment response (submitted, under review). Of particular note is that the cDNA concentration in

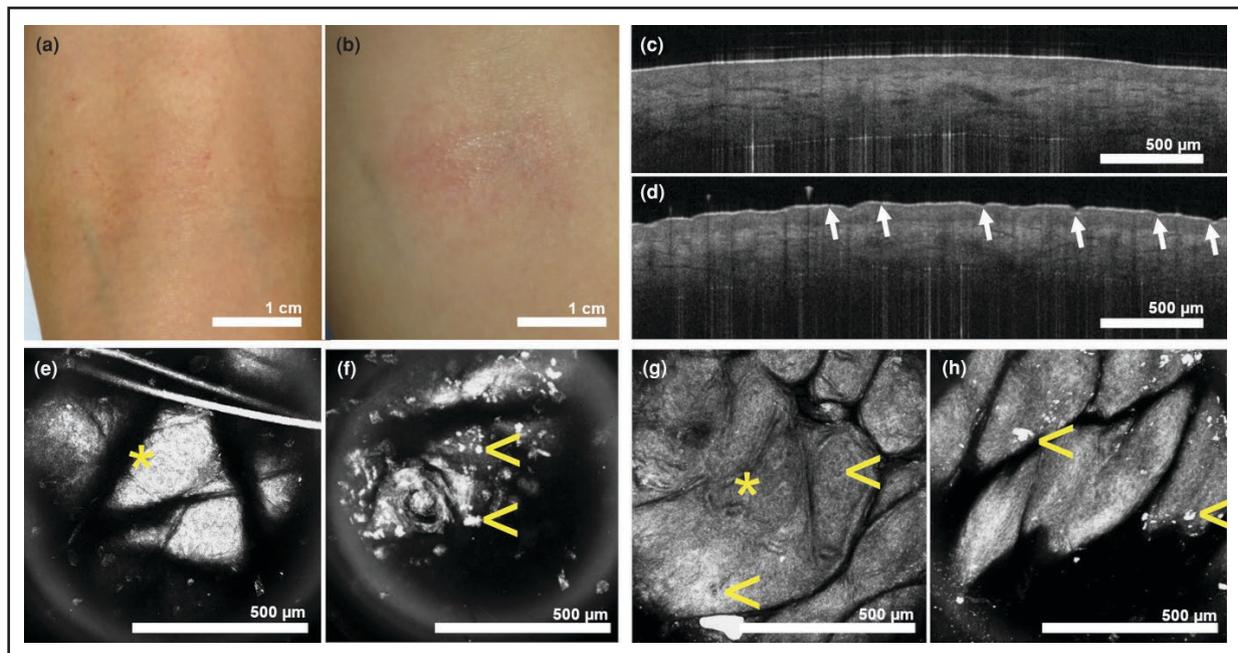


Figure 6 (a–h) Clinical and noninvasive assessments performed in an atopic child after using tape stripping Protocol 4 in Phase 2. (a) Clinical image before stratum corneum (SC) sampling at the popliteal fossa, showing mild atopic dermatitis. (b) Clinical image after SC sampling showing mild, transient erythema and oedema. (c,d) Optical coherence tomography images obtained before (c) and after Protocol 4 on lesional skin (d) showing erosions (arrows) after SC sampling. (e,f) Reflectance confocal microscopy images performed on healthy skin. (e) Before tape stripping, the SC (depth 10 μm) shows regular hyper-refractile corneocytes; (f) after tape stripping, detached corneocytes (asterisk) can be seen (g,h) Reflectance confocal microscopy images performed on lesional skin. (g) Before tape stripping, parakeratosis (arrowheads, depth 15 μm) and spongiosis (asterisk) are visible; (h) after tape stripping, detached corneocytes (asterisk) are present.

lesional skin after a 2-week topical mometasone treatment was significantly reduced ($P < 0.01$) from 39.4 (46.6) $\text{ng } \mu\text{L}^{-1}$ to 11.0 (15.9) $\text{ng } \mu\text{L}^{-1}$. This suggests that inflammation down-regulation is accompanied by a decrease in skin mRNA levels. Therefore, identification of novel molecular biomarkers is key to the assessment of treatment response and the identification of potential responders prior to the initiation of targeted treatments.

Conclusion

As children are particularly vulnerable and skin biopsies are not always possible/desirable, the use of a minimally invasive method such as the one described here may catalyse progress towards the application of precision medicine in paediatric AD.

What is already known about this topic?

- Tape stripping of the skin has been used recently to study AD as a minimally invasive sampling method.
- There is currently no standard tape stripping method to sample the skin of atopic children.

What does this study add?

- We have developed a new tape stripping protocol guided by noninvasive skin imaging to sample atopic skin in children.

- An optimized tape stripping protocol using 11 tapes of 22-mm diameter, each applied for 5 s twice with 90° rotation, is fast, safe and reliable, and provides reproducible acquisition of biomaterial in atopic children.
- Standardizing and optimizing non-invasive skin sampling methods is required to translate these methods in a time-efficient manner into clinical practice.
- Visualizing the skin while sampling helps to identify AEs quickly and enable objective quantification of the skin removed.

Acknowledgements

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Conflict of interest

OY has received consultancy honoraria from Almirall, Leo Pharma and Isispharma and speakers' honoraria from Isispharma, Pierre Fabre, Leo Pharma, BMS and MSD. MP and MR are employees from Almirall and Leo Pharma, respectively. SP has received consultancy honoraria from Almirall, Leo Pharma, Pfizer, Novartis, Sanofi, BMS, ISDIN, La Roche Posay, Regeneron, Sun Pharma, Roche and speakers' honoraria from Almirall, Leo Pharma, Pfizer, BMS,

ISDIN, La Roche Posay, Regeneron, Sanofi, Sun Pharma, Roche. JM has received consultancy honoraria from Almirall, AMGEN, Canfield, Leo Pharma, BMS, ISDIN, Sun Pharma, Roche and speakers' honoraria from Almirall, BMS, ISDIN, Novartis, Sun Pharma, Roche, Pierre Fabre. MPy has received advisory honoraria from ISDIN. RHG has, in the past, acted as a paid consultant to Almirall and Leo Pharma and has received a research grant from Leo Pharma. The other authors declare that they have no conflict of interest.

Funding

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Ethics statement

Ethics approval: the study was approved by the Ethics Committee of Hospital Clínic de Barcelona (protocols HCB/2017/0010 and HCB/2018/0363). Participants and/or guardians provided informed consent for participation and publication of their case details and images.

Data availability

Data are available on request from the corresponding author.

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