Geographical ancestry is a key determinant of epidermal morphology and dermal composition

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Summary

Background Geographical ancestry plays a key role in determining the susceptibility of human skin to external insults and dermatological disease. Despite this, studies of skin from individuals of diverse geographical ancestry focus primarily on epidermal pigmentation. Few reports characterize the gross morphology and composition of the dermis and dermal-epidermal junction (DEJ).

Objectives To characterize epidermal morphology and dermal composition in skin from individuals of diverse geographical ancestry.

Methods Immunohistochemical techniques were used to assess epidermal morphology and protein composition of the DEJ and dermal extracellular matrix in photoprotected skin from young African, Eurasian and Far East Asian individuals (n = 7 per group; age 18–30 years).

Results The epidermis of African skin was thicker, with deeper rete ridges and a more convoluted DEJ than Eurasian and Far East Asian skin. Compared with Eurasians, protein composition of the DEJ was collagen VII poor in African and Far East Asian skin (P < 0·001 and P < 0·01, respectively); the dermis of African skin was enriched in fibrillar collagens (P < 0·05), but was relatively elastin poor (P < 0·05). African dermis was abundant in fibrillin-rich microfibrils and fibulins 5 (P < 0·001 and P < 0·001, respectively) compared with Eurasian and Far East Asian skin.

Conclusions We demonstrate that fundamental differences exist in skin structure and composition in individuals of diverse geographical ancestry. Disparate environmental pressures encountered by ancestral human populations living at different latitudes may have driven adaptations in skin structure and composition. Further research into the functional significance and clinical consequences of these differences is warranted.

What’s already known about this topic?

• While the role of epidermal pigmentation in characterizing human skin of differing geographical origins is well characterized, potential differences in dermal composition and epidermal structure are less well defined.

What does this study add?

• This unique study compares dermal extracellular matrix composition and epidermal morphology of skin from three groups with diverse geographical ancestry.
• This study identifies previously unappreciated differences in the abundance and architecture of dermal fibrillar collagens, collagen VII anchoring fibrils and the elastic fibre network.
Geographical ancestry plays a key role in determining the susceptibility of skin to external insults, primarily ultraviolet radiation (UVR) and common dermatological diseases, such as keloids and nonmelanoma skin cancers. People with highly pigmented skin types have a lower incidence of skin cancer but have a greater incidence of keloid scarring than their lighter-skinned counterparts. Despite these differences in susceptibility to skin conditions, studies of human skin using individuals of diverse geographical ancestry focus primarily on epidermal pigmentation and the evolution of skin colour.

Adaptation to environmental pressures underpins diversity in human populations; skin pigmentation is, perhaps, the most obvious example of this effect. The development of lightly pigmented, melanin-poor skin in human populations has been well documented for the lineages leading to Eurasians (modern Europeans with lightly pigmented skin) and Far East Asians. In these populations, the genetic, physiological and environmental mechanisms causing loss of pigmentation were disparate, but have given rise to a similar phenotype (for a review, see Sturm). In contrast to pigmentation research, the majority of studies of skin conditions, studies of human skin using individuals of diverse geographical ancestry focus primarily on epidermal pigmentation and the evolution of skin colour.

It highlights potential functional and clinical differences in skin of diverse geographical origins, which may have arisen as a consequence of adaptations to ancestral environments.

Morphometric analysis of the epidermis

For each of the parameters measured, five different cryosections were used per individual, and data are presented as mean ± SD. Epidermal thickness was measured using only the inter-rete ridge regions of the epidermis, from the basement membrane to the top of the granular layer at 10 randomly selected points across each section. Rete ridge height was measured from the DEJ to the base of the rete ridge. The DEJ convolution index was measured using the method described by Timar et al.

Antibodies

Mouse monoclonal antibodies were used to detect elastin (clone BA-4, dilution 1 : 200; Sigma Aldrich, Gillingham, U.K.), fibrillin-rich microfibrils (clone 11C1.3, dilution 1 : 1000; Neomarkers, Runcorn, U.K.), collagen I (clone SD8, dilution 1 : 500; Abcam, Cambridge, U.K.), collagen III (clone 1E7-D7/Col3,
Immunohistochemical staining

Cryosections were fixed in ice-cold acetone or 4% paraformaldehyde (PFA), and hydrated in Tris-buffered saline (TBS). Primary antibody was applied for 1 h at room temperature. Sections were washed in TBS prior to incubation in rabbit antimouse Alexa Fluor® 488 secondary antibody (Life Technologies, Paisley, U.K.).

Alcian blue staining

Cryosections were fixed in 4% PFA and hydrated in distilled water. Sections were stained in 1% Alcian blue (pH 2.5) for 30 min at room temperature and washed in distilled water. Following light counterstaining with nuclear fast red, sections were dehydrated and permanently mounted.

Microscopy, image analysis and statistical testing

Brightfield and fluorescence images were captured using the Biozero-8000 all-in-one fluorescence microscope (Keyence, Osaka, Japan). Image analysis was performed using ImageJ software.\(^1\) The distribution and immunoreactivity of the basement membrane components were analysed by measuring fluorescence intensity across a standardized line of 10 × 25 μm positioned across the DEJ. Resultant data were analysed using area under the curve (AUC). The distribution and immunoreactivity of the fibrillar collagens was analysed by measuring fluorescence intensity across a standardized line of 20 × 200 μm positioned perpendicular to the DEJ. Resultant data were analysed using AUC. Elastic fibre abundance was analysed by calculating the percentage of positive immunohistochemical staining in the area of the papillary dermis, which reached a maximal depth of 200 μm from the DEJ. All resultant data are presented as mean ± SEM. Statistical significance was determined using ANOVA and Tukey’s post hoc test (IBM SPSS Statistics 20; IBM United Kingdom, Portsmouth, U.K.). Results were considered significant if \( P < 0.05 \) (95% confidence level). Box plots represent the median and interquartile range (IQR), while the whiskers represent the lowest datum within 1.5 × IQR of the lower quartile, and the highest datum within 1.5 × IQR of the upper quartile.

Results

The epidermis of African skin is thicker, with deeper rete ridge projections and a more convoluted dermal-epidermal junction than Eurasian and Far East Asian skin

The morphology of the epidermis and DEJ was compared across the three groups by analysis of epidermal thickness, rete ridge projection and DEJ convolution. Epidermal thickness was significantly reduced in Eurasian (39.9 ± 3.3 μm; \( P < 0.001 \)) and Far East Asian (37.2 ± 4.0 μm; \( P < 0.001 \)) skin compared with African skin (51.3 ± 4.1 μm; Fig. 1a–d). Similarly, rete ridge projection depth was significantly decreased in Eurasian (73.8 ± 8.0 μm; \( P < 0.05 \)) and Far East Asian (70.6 ± 13.8 μm; \( P < 0.05 \)) skin compared with African skin (89.2 ± 10.6 μm; Fig. 1e). As a consequence of this reduction in rete ridge depth, the convolution of the DEJ was significantly decreased in Eurasian (1.6 ± 0.1 arbitrary units (a.u.; \( P < 0.01 \)) and Far East Asian (1.8 ± 0.4 a.u.; \( P < 0.05 \)) skin compared with African skin (2.4 ± 0.7 a.u.; Fig. 1f). Interestingly, epidermal morphology and DEJ convolution were not significantly different when Eurasian and Far East Asian skin types were compared (Fig. 1d–f).

The dermal-epidermal junction of African and Far East Asian skin is collagen VII poor compared with Eurasian skin

Given the observed differences in epidermal morphology and DEJ convolution between the three groups, we characterized the protein composition of the basement membrane using immunofluorescence staining for collagen VII, laminin-332 and integrin β4. Collagen VII distribution and immunoreactivity varied across all groups (Fig. 2a–c), with a significantly more widespread distribution at the DEJ in Eurasian (1153.4 ± 64.8 a.u.; \( P < 0.001 \)) and Far East Asian (719.2 ± 93.5 a.u.; \( P < 0.05 \)) skin compared with the discrete distribution observed in African skin (461.0 ± 31.4 a.u.; Fig. 2d). In contrast, there was no significant difference in the distribution of laminin-332 (Fig. 2e–h) or integrin β4 (Fig. 2i–l) between the three groups.

African skin is enriched in fibrillar collagens compared with Eurasian and Far East Asian skin

Analysis of the fibrillar collagens was performed using antibodies raised against mature collagens I (Fig. 3a–c) and III (Fig. 3f–h). The overall intensity of collagen I immunostaining was significantly reduced in the papillary dermis of Eurasian skin compared with African skin (7532.6 ± 924.3 a.u.;
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P < 0.05), whereas the results for Far East Asian skin were not significantly different from either (Fig. 3e). Similarly, collagen III immunostaining was significantly reduced in the papillary dermis of Eurasian (10 471.9 ± 1036.9 a.u.) skin compared with African skin (15 198.0 ± 1203.1 a.u.; P < 0.05); the results for Far East Asian skin were, again, intermediate between the two (13 766.0 ± 1175.1 a.u.; Fig. 3f).

African skin is elastin poor, but enriched in microfibril-associated proteins compared with Eurasian and Far East Asian skin

While fibrillar collagens confer tensile strength, skin also relies on an extensive elastic fibre system to confer resilience (or passive recoil). Immunohistochemical analysis of the major elastic fibre network components of the papillary dermis was performed using antibodies raised against elastin (tropoelastin and processed, insoluble elastin), fibrillin-rich microfibrils and the microfibril-associated protein, fibulin-5.22

Elastin-positive fibres were sparsely distributed throughout the papillary dermis in both African (11.0 ± 0.5%) and Far East Asian skin (11.8 ± 1.3%). However, in Eurasian skin, a small, yet significant, enrichment of elastin-positive fibres was identified (15.4 ± 1.7%) compared with the skin of African (P < 0.05), but not Far East Asian, participants (Fig. 4a–d). In each of the different skin types, fibrillin-rich microfibrils are arranged in characteristic candelabra-like structures, with arborizing fibres connecting the oxytalan elastic fibres of the DEJ to the elaunin elastic fibres of the superficial papillary dermis (Fig. 4e–g). However, the abundance of fibrillin-rich microfibrils is significantly reduced in Eurasian (26.2 ± 2.4%; P < 0.001) and Far East Asian (24.2 ± 1.9%; P < 0.001) skin compared with African skin (43.1 ± 2.2%; Fig. 4h). Similarly, the distribution of the microfibril-associated protein, fibulin-5, which decorates the oxytalan and elaunin elastic fibres of the papillary dermis, was considerably less abundant in Eurasian (6.8 ± 0.7%; P < 0.001) and Far East Asian (10.7 ± 0.6%; P < 0.001) skin than in African skin (15.4 ± 0.8%; Fig. 4i–l).

Dermal glycosaminoglycan content does not change as a function of geographical ancestry

Glycosaminoglycan content of the dermal ECM was assessed using the histological stain, Alcian blue (Fig. 5a–c). Alcian blue staining was uniformly distributed throughout the dermis, with no significant difference identified between African (85.0 ± 0.8 a.u.), Eurasian (85.0 ± 0.9 a.u.) and Far East Asian (86.4 ± 0.8 a.u.) skin (Fig. 5d).

Discussion

In this comparative study we have established that photoprotected skin of Eurasian and Far East Asian individuals has a significantly decreased epidermal thickness and a less convoluted, albeit collagen VII-enriched, DEJ compared with African skin. Furthermore, the dermal ECM of both Eurasian and Far East Asian skin is relatively deficient in fibrillar collagens I and III. The distribution and organization of elastic fibres within the dermal matrix is also subject to ethnicity, with African skin being enriched in fibrillin-rich microfibrils, but with a comparatively poor elastin content.

Fig. 1. Morphometric analysis of epidermal thickness, rete ridge depth and dermal-epidermal junction (DEJ) convolution in African, Eurasian and Far East Asian skin. Morphometric measurements of epidermal thickness, rete ridge height and DEJ convolution index were analysed using histological cryosections from (a) African, (b) Eurasian and (c) Far East Asian skin. African skin has (d) a significantly thicker epidermis, (e) deeper rete ridges and (f) a more convoluted DEJ than Eurasian and Far East Asian skin. Scale bar = 100 μm. *P < 0.05, **P < 0.01, ***P < 0.001. a.u., arbitrary units.
Previous comparative studies have focused on the differences between Eurasian and African skin types only, and our data on epidermal morphometrics and collagen VII distribution at the DEJ are consistent with their findings. However, currently there is no consensus regarding dermal ECM composition in these different groups. Previous studies have employed disparate methods to characterize the histology of Eurasian and African skin using biopsies of photoprotected and photoexposed skin from young and old participants, resulting in poorly controlled datasets. In this study, we controlled for these confounding variables by undertaking detailed immunohistochemical comparisons of African, Eurasian and Far East Asian skin using biopsies from identical photoprotected anatomical sites of age- and sex-matched young people. However, in attempting to control for confounders, we were limited to a small sample size; therefore, it would be preferable for the findings presented here to be further validated using a larger, more diverse cohort.

From the data presented here, it is apparent that photoprotected skin of diverse geographical origins differs not only in pigmentation, but also in gross morphology and dermal composition. These architectural and compositional differences are likely to affect the primary functions of skin, which are to resist the environmental pressures of UVR, mechanical stress and the subsequent propensity for injury, and infection. Given that adaptation to an ecological niche underpins diversity in nature, we hypothesize that ancestral human populations living at different geographical locations may have encountered disparate environmental pressures, which may have driven unique adaptations in skin structure and composition that are, ultimately, retained in modern humans.

Fig 2. Immunostaining of the basement membrane components, collagen VII, laminin-332 and integrin β4 in skin of diverse origin. Immunostaining of the basement membrane proteins (a–c) collagen VII, (e–g) laminin-332 and (i–k) integrin β4 was performed on cryosections from African, Eurasian and Far East Asian skin. (d) In Eurasian skin, the distribution of collagen VII at the dermal-epidermal junction was significantly more widespread than in Far East Asian and African skin. No significant difference in the immunostaining for (h) laminin-332 and (l) integrin β4 was detected between the three groups. Scale bar = 50 μm. *P < 0.05, **P < 0.01, ***P < 0.001. a.u., arbitrary units.
Fig 3. African skin is enriched in fibrillar collagens compared with Eurasian and Far East Asian skin. Immunostaining for the fibrillar collagens was performed using antibodies raised against (a–c) mature collagens I and (f–h) III. (d) In the papillary dermis of African skin, collagen I immunoreactivity is positively correlated with increasing distance from the dermal-epidermal junction (DEJ) and (e) immunoreactivity is significantly enhanced compared with Eurasian skin. (i, j) Collagen III immunoreactivity is significantly enhanced in African skin compared with Eurasian skin. Scale bar = 100 μm. *P < 0.05. a.u., arbitrary units.
Compared with Africa, continental Europe experiences both a lower ambient temperature and incident UVR exposure. The adaptive pigmentation response of human skin to reduced UVR exposure – and hence vitamin D synthesis – is already well documented; however, in this study we observed a significant decrease in fibrillin-rich microfibrils in Eurasian skin compared with African skin. We have previously demonstrated that, as a consequence of their high ultraviolet chromophore content, fibrillin-rich microfibrils are readily degraded by physiological doses of UVR. In addition, other research groups have shown that these microfibrils mediate tissue homeostasis via sequestration of transforming growth factor (TGF)-β and bone morphogenic protein. Hence, we suggest that the relative paucity of fibrillin-rich microfibrils, as identified by antibody localization techniques, may be indicative of a selective pressure to reduce aberrant cytokine-mediated remodelling in melanin-poor, yet UVR-exposed, Eurasian skin.

In addition to the environmental pressure exerted by UVR, many of the architectural and compositional changes that we observed would be predicted to reduce the mechanical strength of skin. Skin thickness is not uniform across body sites; instead, it is positively correlated with exposure to mechanical stress. It has been demonstrated previously that adaptive changes to repetitive mechanical stress can occur rapidly in skin, with subsequent epidermal thickening and increased rete ridge depth. This suggests that the synthesis of structural skin proteins may be a metabolic burden, which is not only modulated within an individual but may also be affected by adaptive pressures to increase flexibility and sensory perception. It is unlikely that the skin of ancestral Eurasian and Far East Asian populations was subjected to less mechanical stress than their counterparts in Africa, but two key environmental factors (low temperature and, potentially, the subsequent adoption of clothing) may have obviated the
need for a thickened and convoluted epidermis, and a collagen III-rich dermis. The compliance of biological polymers, ECM-rich tissues and human stratum corneum is mediated, in part, by ambient temperature. The period from approximately 50,000 to 10,000 years ago comprises the Last Glacial Maximum; during this time the habitable areas of Europe were characterized by extreme cold (the mean air temperature was approximately 8°C colder than at present). Therefore, the stiffening of exposed skin in colder environments may have reduced the adaptive advantage of synthesizing abundant structural components. In addition, anthropologists consider the advent of clothing as a biological necessity for ancestral human survival in these harsher environmental conditions. As a consequence, the clothed and photoprotected skin of ancestral Europeans and Far East Asians may have experienced reduced shear stress and, hence, fewer mechanical injuries.

It is likely that ancestral populations would have faced a variety of challenges to health, for example parasitic illnesses, infections and nutritional disease. Highly pigmented skin is more resistant to the bite or sting of arthropods than melanin-poor skin (owing primarily to its increased epidermal thickness), and it has been suggested that response to infectious disease may have affected natural selection more than an individual’s response to climate (for a review, see Cooke and Hill). Although the rate of wound healing is not thought to be different between different skin types, highly pigmented skin appears to be between three and 18 times more likely to develop keloid scars. TGF-β1 is strongly implicated in the pathogenesis of keloids, and an increased abundance of fibrillin-rich microfibrils in highly pigmented skin suggests a greater ability to sequester and release this cytokine. Furthermore, TGF-β1 mRNA expression is elevated in African American skin compared with Eurasian skin. Exposure to high environmental UVR levels at or close to the equator is thought to have driven the melanization of skin in ancestral African populations, and it is often assumed that increased pigmentation developed primarily as a protective mechanism against skin cancer (see the review by Jablonski). However, the short lifespan of early humans (25–40 years) when compared with the relatively late onset of skin cancer (age > 60 years for squamous cell carcinoma) suggests that photocarcinogenesis may have played, at most, a minor role in driving skin remodelling.

Our unique analysis of three distinct populations has also enabled the identification of a number of features for which Far East Asian skin represents an intermediate between Eurasian and African skin types. Collectively, these observations suggest that differences in dermal composition between Eurasian and Far East Asian populations occurred independently after the divergence of Eurasians and Far East Asians from the original proto-Eurasian population. Disparate environmental pressures at different geographical locations may have driven unique adaptations in skin composition; for example, during the Last Glacial Maximum, East Asia remained largely unglaciated, except at high elevations, with mean air temperatures only 3°C colder than at present, which is in contrast to the extreme cold experienced in Northern Europe.

Further research into the functional significance of the observed differences between these diverse groups is warranted. The results will provide us with the necessary knowledge to ensure that optimal skin health is achieved for all individuals, regardless of ancestral background.

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References

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