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New wrinkles on wrinkling: an 8-year longitudinal study on the progression of expression lines into persistent wrinkles

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Summary

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Background While cumulative lifetime sun exposure is well recognized as having an important role in the progression of facial wrinkling, the role of facial expression has largely been overlooked, in part due to the lack of comprehensive longitudinal data on the change in both expression lines and persistent wrinkles with age.
Objectives To track the detailed pattern of facial wrinkling in the same group of people over several years and to verify that expression lines evolve into persistent wrinkles. In addition, to identify factors predictive of a faster or slower rate of wrinkling.
Methods Standardized images were captured at baseline and at 8 years of 122 women (ages 10–72 years, skin types I–VI) with and without a smiling expression. The wrinkle pattern with expression at baseline was compared with the pattern without expression at 8 years. Severity of facial wrinkling was quantified using computer-based image analysis. Skin colour, hydration, sebum and pH were measured at baseline. A structured questionnaire captured demographic and lifestyle data at baseline and at 8 years.
Results Each subject's unique pattern of persistent facial wrinkling observed without expression at year 8 was predicted by the pattern of lines observed with a smiling expression at baseline. Having a drier, more alkaline stratum corneum, a lighter complexion, being middle-aged (40s) or becoming menopausal were associated with faster persistent wrinkling.
Conclusions Repeated skin flexure during facial expression causes persistent wrinkles. The pattern of expression lines predicts the pattern of future persistent wrinkles. Certain intrinsic and extrinsic factors are not causative, but influence the rate, of facial wrinkling.

Skin wrinkling is the hallmark clinical sign of skin ageing and photoageing. There are several types of wrinkles classified with consideration to their location, pattern, histology and aetiology.[1,2] Of significant concern are the lines and grooves associated with facial expression. These kinds of wrinkles, classified as type 3 by Piérard *et al.*,[2] can be either temporary or persistent. They align perpendicular to the direction of muscle contraction as typified by the crow's feet wrinkles around the eye which radiate across the obicularis oculi. Facial muscle physiology is unique in that its primary role is to move skin, not bones. Expression lines (hereafter temporary wrinkles) form in the skin during muscle contraction and disappear when the facial muscles relax, providing the human face with the unique ability to express emotion. On the other hand, persistent wrinkles are visible at rest without muscle contraction. The age-related changes in temporary and persistent facial skin wrinkling occur very slowly over a person's lifetime.[3–6] In youth, temporary facial wrinkles are minimal. The skin of a child is simply too tight to form a groove during muscle contraction. In early adult life, as skin laxity increases, the early signs of wrinkling show as lines during facial expression, whereas persistent wrinkles are still largely absent. Persistent facial wrinkles, such as crow's feet, glabellar frown lines and forehead transverse lines, then begin to appear.[7]

Certain host and environmental factors, such as hormonal changes, sun exposure and smoking, are known to accelerate the onset and rate of skin ageing, including the acceleration of facial wrinkling.[3,4,6,8,9] The histological effects of chronic sun exposure are well known,[10] such as loss and fragmentation of dermal collagen,[11,12] deposition of large amounts of poorly

functional elastin in the upper dermis,[13] overproduction and abnormally located dermal glycosaminoglycans[14] and changes in stratum corneum keratin intermediate filaments.[15] These molecular changes result in a decline in the skin's youthful elastic properties which are believed to be fundamental in wrinkle formation.[16–21]

Kligman *et al.*[1] studied the histology of persistent wrinkles. While the skin showed many of the common histological features appropriate for the subjects' age and body site, they did not observe any remarkable differences in the epidermal or dermal structure between the wrinkle and the surrounding skin, a finding confirmed by Bosset *et al.*[22] and Piérard and Lapière[23] who did find differences in the hypodermal septa. For extremely deep facial wrinkles which have persisted for decades, the fold can chronically shadow the underlying skin, protecting it from further photodamage and lessening the degree of elastosis in the fold relative to the surrounding skin,[24,25] a phenomenon modelled in hairless mice.[26] Kligman *et al.* explained a wrinkle as a simple configurational change and proposed an 'old glove' model for wrinkle formation: 'Smooth when new, the fabric develops grooves at sites of long-sustained stress'. Muscle contraction, like that during everyday facial expression, forces the skin to fold repetitively along the same groove. With time, this repeated mechanical stress causes that groove or temporary wrinkle to etch in as a permanent or persistent wrinkle.[1] Persistent wrinkle formation would then have an obligatory mechanical component as borne out by the lack of persistent wrinkles on the dorsal forearms, which can show severe signs of skin photodamage yet no wrinkles. In these areas, muscle physiology is for moving bones, not skin, and therefore the prerequisite temporary wrinkling never occurs. Ultraviolet (UV)-induced skin damage, then, is not the cause, but an accelerator of facial wrinkling when combined with repeated mechanical stress. Damage to the epidermal and dermal molecular architecture reduces the skin's ability to accommodate that mechanical stress. Based on this model, the age at onset and rate of persistent facial wrinkling would be dependent on both the cumulative amount of mechanical stress (frequency of temporary wrinkling) in combination with the decline in skin elasticity caused by, for example, cumulative sun exposure.

There are no long-term longitudinal studies tracking the progression of both temporary and persistent facial wrinkling. The present study addresses this topic and is unique for its use of standardized facial imaging, with and without facial expression, of the same individuals over nearly a decade. In addition, a comprehensive set of skin biophysical measurements and demographic/lifestyle information was collected to better understand factors associated with the rate of wrinkle formation.

Materials and methods

The baseline survey was conducted at an indoor shopping mall in Redondo Beach, CA, a suburb of Los Angeles, from October 1999 to February 2000. Women who were shopping in the mall were randomly invited to participate in the survey if they were between the ages of 10 and 70 years, in good health and not pregnant.[3,27] In total, 1437 women were enrolled. During February to May of 2008, at the same study location, 122 of these same women participated in a follow-up survey (mean ± SD age 40·4 ± 15·7 years at baseline and 48·8 ± 15·7 years at 8 years). They were of Caucasian ($n = 60$), African-American ($n = 20$), Asian ($n = 22$) or Latino ($n = 20$) heritage. None of the subjects had moved away from the Los Angeles area nor reported having had surgical, laser, filler, botulinum toxin or other cosmetic procedures deemed to affect facial wrinkling between their baseline and follow-up visits. The study was conducted under a protocol approved by the authors' institutional review committee and written informed consent was obtained from all study subjects.

Imaging and image analysis

Each subject cleansed her face with a commercial facial cleanser and sat quietly for 15 min prior to imaging. Left oblique view facial images were captured using a Fuji DS330 camera equipped with a close-up lens mounted into a standardized illumination rig fitted with chin and forehead rests.[28] This system was used at both baseline and 8 years, thereby minimizing camera and lighting variance between visits; colour standards captured at baseline and follow-up confirmed consistency of lighting conditions. Two images were collected at each visit, one with a neutral relaxed expression (to measure persistent wrinkling) and one with a smiling expression (to measure temporary wrinkling), a total of four images per subject.

For image analysis, the perimeter of the region of interest (ROI) on each subject's baseline neutral image was defined, i.e. 'masked', using predefined facial landmarks (e.g. left and right corners of eye, bridge of nose, and corner of mouth). This mask was then applied to the subject's other three images. Corrections for slight differences in head repositioning were made by registering the subject's mask to facial landmarks without changing the mask shape or size. In this way, the same region of the face was analysed for all four of the subject's images. Computer-based image analysis was used to identify and quantify facial wrinkles in the ROI automatically, based on shape and pixel contrast. Wrinkles were defined as being > 5 mm in length, having a perimeter/length ratio $< 2{\cdot}5$ and circularity ($\text{perimeter}^2/\text{area}$) > 34. The overall wrinkle severity was expressed as the fraction of the ROI occupied by wrinkles: wrinkle area fraction (WAF) = total wrinkle pixel area/total ROI pixel area.

Biophysical measurements

Biophysical skin measurements were made at baseline on the parent population of subjects.[3,27] Both facial and nonfacial body sites were chosen based on the skin parameter being measured. For skin colour, the Chromameter CR300 (Minolta,

Osaka, Japan) was used to measure CIE L*, a*, b* on the sun-exposed forehead and left upper cheek and the sun-protected left inner upper arm (three measurements per site). The Corneometer CM 825PC (Courage + Khazaka, Cologne, Germany) was used to measure stratum corneum capacitance, a measure of stratum corneum hydration, on the left upper cheek, the left ventral forearm and the left outer calf (three measurements per site). The Skin pH Meter pH 900 (Courage + Khazaka) was used to measure skin surface pH on the left upper cheek, left ventral forearm and left outer calf (two measurements per site). The Sebumeter (Courage + Khazaka) was used to measure sebum secretion rate on the central forehead, a site of high sebum secretion. After cleansing the face with the commercial facial cleanser, any residual surface sebum on the forehead was removed with 70% ethanol swabs. Thirty minutes after cleansing, the amount of sebum secreted on the forehead was measured.

Demographic and lifestyle data

Each subject completed a structured questionnaire at both baseline and 8 years to collect data on host and environmental factors that might be associated with the rate of facial wrinkling. Variables collected included age, race, place of residence, height, weight, daily hours of sleep, history of cosmetic procedures, daily water consumption, daily caffeinated beverage consumption, daily facial cleansing, frequency and duration of physical exercise, history of skin disease, skin care product usage, use of sun protection, menstrual status, hormone replacement therapy status, pregnancy history, smoking history, alcohol consumption history, type of diet, vitamin supplementation, tanning booth history, stress level, outdoor or indoor employment history, education and income level.

Statistical analysis

Descriptive statistics (mean ± SEM) were used to describe the population. A Pearson Product Moment Correlation was used to analyse the association of baseline temporary wrinkling with 8-year persistent wrinkling as well as the association of the temporary/persistent wrinkling ratio at baseline with the change in persistent wrinkling over 8 years. Analysis of covariance (ANCOVA) was used to compare persistent wrinkling at baseline with that at 8 years by age group (stratified in decades), with stratum corneum capacitance and cheek L*-value as covariates. Analysis of variance was used to identify host and lifestyle factors significantly associated with the change in persistent wrinkling. Persistent WAF change from baseline was the dependent variable and either host factors (biophysical measures at each body site, change in body mass index and change in menstrual status) or lifestyle factors (daily hours of sleep, daily water consumption, daily caffeinated beverage consumption, daily facial cleansing, skin care product usage, smoking history, vitamin supplementation, tanning booth history, stress level and use of sun protection) were potential predictors. ANCOVA was used to compare the persistent WAF mean change from baseline between ethnic groups, high vs. low cheek hydration, change vs. no change in menstrual status, dark vs. light cheek skin colour, and high vs. low cheek skin pH, all with age as covariate. Multiple linear regression was used to model the persistent WAF change from baseline as a function of host and environmental factors. The software programs Microsoft SPSS or SigmaStat were used to run data analyses, with $P < 0{\cdot}05$ considered statistically significant.

Results

For the 122 women in the study, persistent wrinkling, i.e. wrinkling measured with a relaxed expression, increased by an average of 39% over the 8-year period. Temporary wrinkling, i.e. wrinkling measured with a smiling expression, increased by an average of 22% over the 8-year period (Table 1). To track the progression of individual lines and wrinkles on each person, we inspected each subject's pattern of wrinkling around the eyes and on the cheek with and without facial expression at baseline and compared it with the pattern at 8 years. We consistently found that the subjects' unique pattern of persistent facial wrinkling observed with a neutral expression at 8 years was predicted by the pattern of temporary wrinkling observed with a smiling expression at baseline (Fig. 1). Quantitatively, there was a strong correlation ($r = 0{\cdot}77$, $P < 0{\cdot}001$) between each subject's severity of temporary wrinkling at baseline and subsequent persistent wrinkling at 8 years (Fig. 2). We were interested in knowing if having high severity of smiling-induced temporary wrinkles relative to persistent wrinkles at baseline increased the likelihood of having a greater increase in persistent wrinkles over the 8-year period. For subjects over 25 years of age (those who showed wrinkling), the increase in persistent wrinkling from baseline to 8 years tended to be greater for those subjects with higher baseline ratios of temporary to persistent wrinkling ($r = 0{\cdot}50$, $P < 0{\cdot}001$).

As expected, persistent wrinkling age-group means increased with each advancing decade at both baseline and 8 years (Table 1). By tracking the subjects in each age group over the 8-year period, we examined the relationship between age at baseline and change in persistent wrinkling. Persistent wrinkling increased most for those who were in their 40s at baseline (+73%) and least for those who were in their teens or 60s at baseline. Figure 3 shows neutral expression images at baseline and 8 years of a mother (age 52 years at baseline) and her daughter (age 16 years at baseline).

Change in menstrual status (i.e. becoming menopausal) was found to be significantly associated with an increased change, i.e. rate of wrinkling. After correcting for differences in age, subjects who entered menopause during the 8-year interval ($n = 28$) showed a significantly faster rate of wrinkling (+95%), compared with the group of women whose menstrual status was unchanged, i.e. who either remained menopausal or remained regular (+28%, $P = 0{\cdot}005$).

Table 1 Wrinkle area fraction (WAF) group means in 2000 and 2008

All subjects	n	WAF 2000 (mean ± SEM)	WAF 2008 (mean ± SEM)
Persistent wrinkling	122	0·045 ± 0·04	0·063 ± 0·05
Temporary wrinkling	122	0·078 ± 0·05	0·095 ± 0·06
Persistent wrinkling by subgroups			
Baseline age group (years)			
10s	16	0·014 ± 0·008	0·017 ± 0·010
20s	14	0·016 ± 0·009	0·025 ± 0·011
30s	24	0·036 ± 0·007	0·050 ± 0·009
40s	30	0·047 ± 0·006	0·082 ± 0·008*
50s	20	0·074 ± 0·007	0·091 ± 0·009
60s	17	0·075 ± 0·008	0·090 ± 0·010
Change in menstrual status			
No change	94	0·047 ± 0·004	0·060 ± 0·005
Entered menopause	28	0·037 ± 0·003	0·072 ± 0·009*
Baseline cheek hydration			
> 50	59	0·040 ± 0·004	0·049 ± 0·006
< 50	63	0·050 ± 0·004	0·076 ± 0·005*
Baseline cheek pH			
< 5·3	41	0·055 ± 0·005	0·063 ± 0·007
> 5·3	81	0·040 ± 0·004	0·062 ± 0·005*
< 5·6	87	0·044 ± 0·004	0·058 ± 0·005
> 5·6	35	0·048 ± 0·006	0·075 ± 0·007
Baseline cheek skin lightness			
L* > 55	101	0·047 ± 0·003	0·068 ± 0·004*
L* < 55	20	0·035 ± 0·008	0·036 ± 0·010
Ethnicity			
African-American	20	0·036 ± 0·007	0·036 ± 0·009*
Caucasian	60	0·059 ± 0·004	0·081 ± 0·005
East Asian	22	0·029 ± 0·007	0·047 ± 0·009
Latino	20	0·032 ± 0·007	0·053 ± 0·009

*Change from 2000 to 2008 is significantly different ($P < 0·05$) compared with the corresponding subgroups' change from 2000 to 2008.

Having a drier and more alkaline stratum corneum at baseline was predictive of an increased rate of persistent wrinkling. Compared with the group with above-average cheek hydration at baseline, the group with below-average cheek skin hydration showed significantly higher rates of persistent wrinkling. Compared with the group with a more acidic surface pH (< 5·3), the group with more alkaline pH (> 5·3) showed significantly higher rates of persistent wrinkling. A similar result was observed for subgroups above or below pH 5·6.

Subjects with lighter skin at baseline (L*-value > 55) showed a significantly ($P = 0·015$) faster rate of persistent wrinkling compared with subjects with darker skin at baseline (L*-value < 55). In addition, the variance in WAF change from baseline was significantly higher for the lighter vs. darker subjects (Fig. 4, $P = 0·03$). Most of the subjects with L* < 55 were African-American. When grouped by ethnic heritage, African-Americans showed the least change in persistent wrinkling over the 8-year span, significantly less than their age-matched Caucasian counterparts ($P = 0·01$). An African-American subject with cheek L* = 55 and showing remarkably little change in facial wrinkling over 8 years is shown in Figure 5.

Regression analysis showed that baseline values of cheek hydration, cheek pH, cheek L*-value and age as well as entering menopause were most predictive of the change in persistent wrinkling from baseline to 8 years.

Discussion

By following the change in pattern of facial wrinkling on the same individuals with and without facial expression over a long period of time, we have shown that temporary wrinkles observed with expression eventually evolve into persistent wrinkles observed at rest. Quantitatively, the increase in persistent wrinkling over 8 years was proportional to the amount of temporary wrinkling at baseline, suggesting that those who show more wrinkles with expression at any given age will tend to show more persistent wrinkles at a later age. While our study focused on the area around the eye and on the cheek, it seems certain that glabellar frown lines, transverse forehead wrinkles and similar wrinkles classified as type 3 follow the same progression although with differing ages at onset and rates of change unique to those facial regions. Thus, repeated mechanical flexure along the same skin groove causes

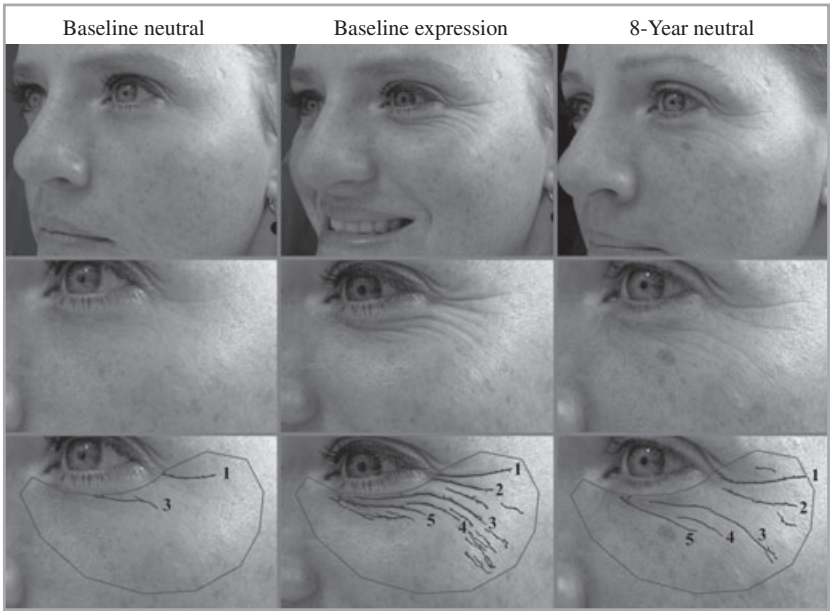

Fig 1. Progression of temporary into persistent wrinkling. At baseline at age 28 years (left column), only a few shallow wrinkles are evident in this subject's neutral image which are better observed in the zoomed images without (middle row) and with (bottom row) wrinkle image analysis overlays. The baseline expression image (middle column) shows substantial temporary wrinkles around the eye which are not evident in the baseline neutral image. Eight years later at age 36 years (right column), the pattern of persistent wrinkles (individually numbered) in the neutral image can be traced back to the pattern of temporary wrinkles in the baseline expression image.

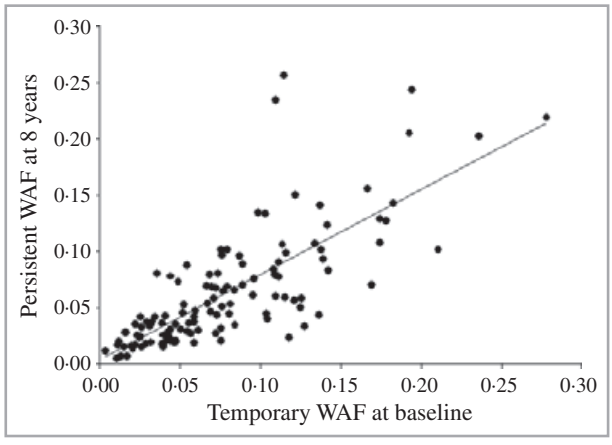

Fig 2. Correlation between baseline temporary and 8-year persistent wrinkling. Each point represents a subject in the study ($r = 0{\cdot}77$, $P < 0{\cdot}001$). WAF, wrinkle area fraction.

temporary lines eventually to etch in as permanent wrinkles. An identical twin case study showing that long-term prevention of forehead muscle contraction, via regular treatment with botulinum toxin A, prevents the imprinting of forehead and glabellar frown lines further demonstrates the key role skin flexure and repetitive mechanical stress plays in the formation and progression of facial wrinkling.[29]

Intrinsic factors (e.g. age, skin type, hormonal status) or extrinsic factors (e.g. smoking, sun exposure) are frequently cited as causative in skin wrinkling. However, these factors should be considered as modulators of the rate of wrinkling, at least for type 3 wrinkles. With respect to age, we found that subjects who were in their 40s at baseline showed a significantly faster rate of wrinkling compared with other age groups. Others have also found an increased rate of skin wrinkling during middle age for women,[30,31] but not necessarily men.[32] We examined the relationship between menopausal status and rate of wrinkling as a possible explanation for the observed faster rate of wrinkling for the middle-aged women in our study. Being menopausal was not associated with a higher rate of wrinkling. In fact, the postmenopausal women in this study (who tended to be in their 50s and 60s) had a very low rate of wrinkling. It was the women who had entered menopause between baseline and 8 years who showed the highest rate of wrinkling (+95% increase). This suggests that the change in hormonal status, rather than hormonal status *per se*, is the important determinant in accelerating skin wrinkling. The observation that hormone replacement therapy did not improve skin wrinkling in women who were 5 years postmenopausal is consistent with this view.[33]

We examined the subjects' baseline biophysical skin properties as predictors of the rate of persistent wrinkling. Of the 16 biophysical variables analysed, cheek stratum corneum hydration, cheek skin lightness (L*-value) and cheek skin surface pH were most predictive; subjects with drier, fairer, and more alkaline skin were more likely to have a higher rate of wrinkling. We were surprised to find that having drier cheek skin at baseline was predictive of more persistent wrinkling 8 years later. This suggests an important role for the paper-thin

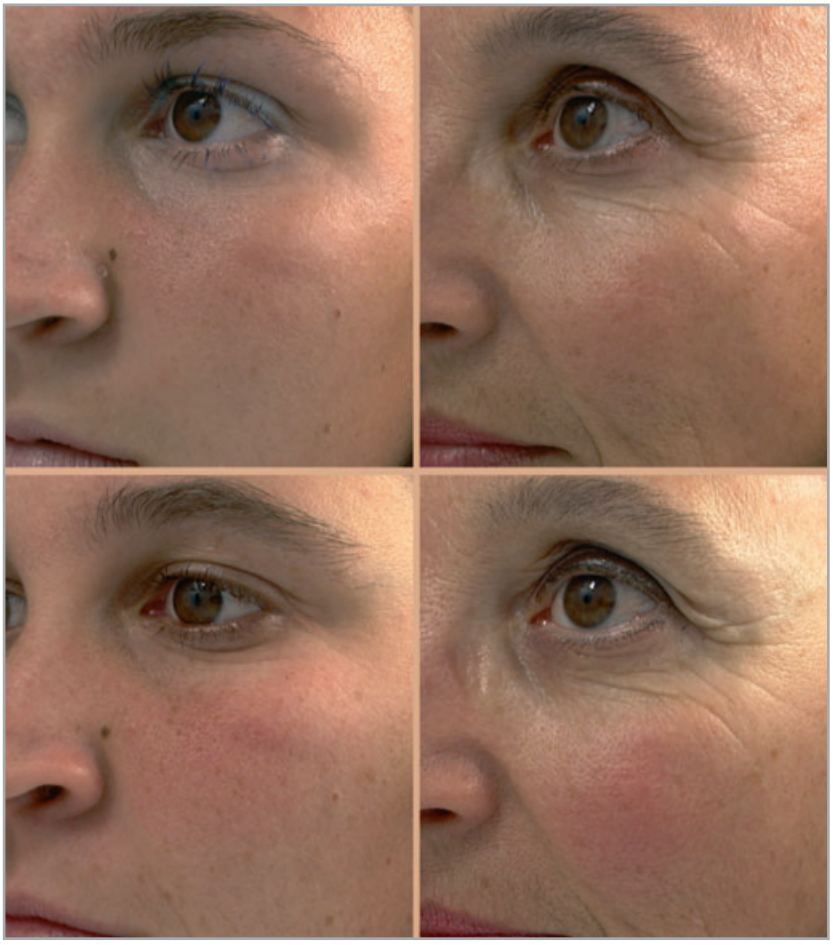

Fig 3. Neutral expression images of a mother and her daughter. Upper left: daughter's baseline image, age 16 years. Upper right: mother's baseline image, age 52 years. Lower left: daughter's 8-year image, age 24 years. Lower right: mother's 8-year image, age 60 years.

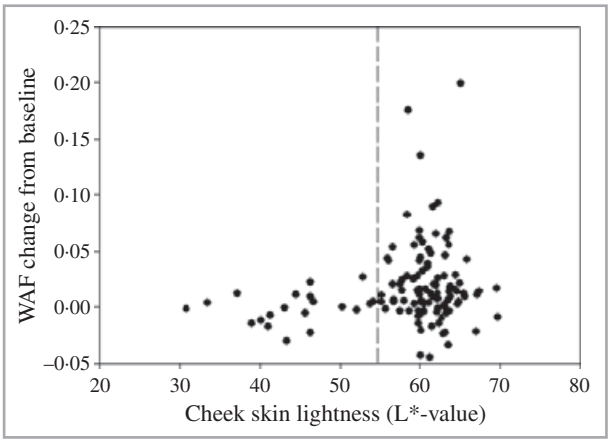

Fig 4. Change from baseline in persistent wrinkling by baseline cheek skin lightness (L*-value). The threshold at L* < 55 was drawn arbitrarily. WAF, wrinkle area fraction.

stratum corneum in wrinkle formation as also suggested using computational modelling.[31] Imokawa and Takema[34] reported that fine wrinkle formation can be linked to the dryness of the stratum corneum. The water holding capacity[35] and elasticity[36] of the stratum corneum decrease after repetitive UV exposure, making it more prone to wrinkle formation, although there is a long delay between loss of elasticity and the onset of visible wrinkling.[21] Magnenat-Thalmann *et al.*[37] used a three-layer computational model to help better understand the pivotal role of stratum corneum mechanical properties in the development of fine wrinkles. A decrease in the stratum corneum modulus by 50% (less stiff), as might be expected after using a cosmetic moisturizer, markedly lowered the amplitude (i.e. decreased wrinkle depth) and increased the frequency (i.e. decreased wrinkle width) of skin folding in old skin, to be more like that of younger skin. Together, these results suggest that regular use of skin moisturizers should delay or slow down the rate of persistent wrinkling, perhaps by plasticizing the stratum corneum, thereby diminishing the formation of temporary wrinkles during facial expression and thus the potential for persistent wrinkles. Beyond prevention, lessening the repeated mechanical stress may even allow repair of persistent wrinkles that have already formed. Case reports of the total effacement of persistent forehead wrinkles after long-term treatment with botulinum toxin A suggest that reducing everyday repeated mechanical stress on the skin may allow for dermal and epidermal remodelling.[38]

The influence of skin colour tone on the rate of wrinkling was not unexpected. It has been estimated that epidermal melanin in darker skin types can confer protection against UV-induced skin damage up to 15 times that of their lighter-

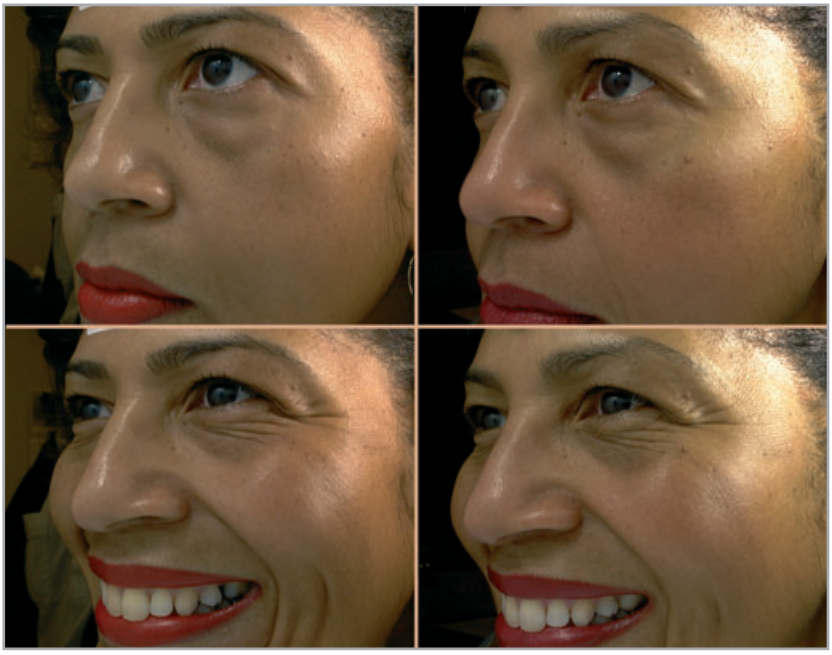

Fig 5. African-American showing remarkably little change in persistent and temporary facial wrinkling over the 8-year period. Upper left: baseline neutral image, age 49 years. Upper right: 8-year neutral image, age 57 years. Lower left: baseline expression image. Lower right: 8-year expression image.

skinned counterparts.[39,40] Cross-sectional surveys have also shown that individuals with darker skin have less facial wrinkling than those with lighter skin,[3,41] perhaps due to higher skin elasticity.[42]

Having a more alkaline skin surface pH was associated with a faster rate of wrinkling. Stratum corneum stiffness is known to correlate positively with surface pH.[43] The use of lactate to treat dry skin in xerosis and the use of topical alpha- and beta-hydroxy acids (both lower stratum corneum pH) to treat facial wrinkling are consistent with our finding.

It may seem inconsequential to demonstrate experimentally that temporary wrinkles are predictive of future persistent wrinkles, but there is practical value in appreciating this fundamental aspect of wrinkle formation. For example, in the clinic or at home, treatments can be targeted not only at currently visible persistent wrinkles but also at future persistent wrinkles by noting the exact location and severity of a patient's wrinkle pattern during facial expression. Ageing simulation is another area of application. By capturing images of a person with a neutral and, for example, a smiling expression and then morphing the temporary wrinkles from the smiling expression image on to the neutral image, the resulting image will simulate future persistent wrinkling unique in pattern and severity to that particular individual.[44] Finally, any clinical study aimed at evaluating facial wrinkling should consider measures of both persistent and temporary wrinkles in protocol design.

In summary, the present study has confirmed the temporal and morphological relationship between temporary and persistent wrinkling. Skin photodamage and the resulting loss of skin elasticity are necessary for premature type 3 wrinkle formation, but are not sufficient. Repeated mechanical stress is a required element for type 3 persistent wrinkle formation. Protecting the skin from acute and chronic sun damage and using moisturizers regularly can preserve the skin's youthful elasticity so as better to withstand mechanical stress, thereby delaying the age at onset and slowing down the rate of wrinkling over a lifetime.

What's already known about this topic?

Twenty-five years ago, the *BJD* published a seminal paper by Dr Kligman and colleagues[1] on skin wrinkling. They suggested that skin wrinkling does not have a histological basis and was merely a configurational change resulting from repeated flexure of the skin over many years. The work we present in our manuscript builds on that early work with direct longitudinal data and adds some new and interesting insights around the factors associated with skin wrinkling. A more detailed review of the literature concerning this topic is found in the introductory section.

What does this study add?

Among our findings, we observed that the subjects' unique pattern of persistent facial wrinkling observed with a neutral expression at 8 years was predicted by the pattern of expression lines observed with smiling at baseline. Having lighter skin colour or having a drier, more alkaline stratum corneum at baseline was predictive of more wrinkling at year 8. We also found that wrinkling was associated with becoming menopausal, but not necessarily with being menopausal.

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