

Quantitative Survey of Longitudinal Usage Benefit of the Same Anti-Aging Skincare Product Containing Galactomyces Ferment Filtrate (Pitera) for Anti-Aging and Youthful Skin in Those Initiating Use in Their 20s and Early 30s and Continuing until Their 30s, 40s, and over 50s

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Abstract

Background: Facial skin aging is a major concern, especially among women. For many, it is important to limit facial skin aging and maintain youthful healthy skin for as long as possible. However, little research has longitudinally investigated the efficacy of skincare product usage on facial skin aging. Therefore, we conducted a quantitative survey to longitudinally compare the skin of individuals with continuous long-term use of the same skincare formula to that of control participants. **Purpose:** To elucidate anti skin aging benefit by longitudinal usage of the same skin care formula, and whether skin aging-related SNPs variation influenced on the skin aging. **Method:** We measured facial skin aging parameters of texture, pore, wrinkles and tone together with skin hydration and elasticity in 141 East Asian females (mean age 47.1, SD 7.81) who had continually used the same anti-aging skincare formula (SK-II Facial Treatment Essence: FTE) containing *Galactomyces* Ferment Filtrate (Pitera) from a young age. The average duration of FTE use in this group was 13.1 ± 7.81 years. As a control, we also included 137 Asian females (mean age 47.3, SD 9.51) with no history of using the FTE product. In addition, the skin aging-related SNPs [GPX1 (rs1050450), SOD2 (rs4880), and MMP1 (rs1799750)] were determined using oral cavity swab samples and PCR to understand the

genetic potential or predisposition for skin aging. **Result:** The findings demonstrated that the Long-term FTE group had excellent skin appearance and physical properties for all measured variables, namely, skin texture, pores, wrinkles, tone, spots, hydration, TEWL, and mechanical elasticity, which were significantly better than those of the Control group. There were significant relationships between the parameters of skin aging appearance and skin aging-related SNPs in the Control group, however, not in the Long-term FTE group. Moreover, the frequencies of genotypes of skin aging-related SNPs did not differ significantly between the two groups. **Conclusion:** These results suggest that longitudinal usage of an appropriate cosmetic agent from a younger age is beneficial and decelerates facial skin aging.

Keywords

Facial Skin Aging, Texture, Pore, Wrinkle, SNPs, SK-II Pitera™ Facial Treatment Essence Formula, *Galactomyces* Ferment Filtrate

1. Introduction

Facial skin aging is a psychophysical and social concern, especially among women. It involves changes of the skin with increasing age, such as wrinkles, hyperpigmented spots, roughness/texture, and loss of elasticity [1]-[4]. Facial wrinkles are known as a sign of skin aging, which increase more rapidly and more conspicuously in Caucasian than in Chinese women. Meanwhile, hyperpigmented spots and uneven skin tone are more apparent in Chinese than in Caucasian women after their 30s. Skin barrier function also tends to be impaired by facial skin aging, as assessed by decreased skin hydration and increased trans-epidermal water loss (TEWL).

The intensity and speed of the aging process differ markedly between individuals. Therefore, some women look younger than their actual age, while others look much older. Among various parameters of skin aging, wrinkles, hyperpigmented spots, skin roughness (texture), and enlarged pores are considered to be particularly representative of an older appearance of the female face. A previous study indicated the possibility that maintaining good facial moisturization may contribute to a younger-looking facial appearance. Indeed, we demonstrated an effect of reversing facial skin aging by applying anti-aging skin products for 12 months, compared with their 11 years of longitudinal skin aging [5]. However, it remains unknown whether longer-term use of regular and daily skincare with an efficient moisturizer can maintain a youthful and healthy appearance of the skin over 10 or 20 years.

SK-II Pitera™ Facial Treatment Essence (FTE) contains *Galactomyces* ferment filtrate (GFF, Pitera™), which works as a potent antioxidative agonist for the aryl hydrocarbon receptor [6] [7]. GFF is known to increase the expression of filaggrin, caspase-14, and claudin-1, which may facilitate the production of natural moisturizing factors and strengthen the tight junction structure [8] [9]. It also

potentiates the anti-inflammatory system in epidermal keratinocytes [10]. In parallel with this, clinical studies have revealed that the daily application of Pitera™ indeed increases the skin hydration and decreases the TEWL of facial skin [11]-[13]. It also alleviates or completely restores mask-induced skin damage.

In the present study, we measured signs of facial skin aging parameters with hydration and TEWL, and determined the genotypes of SNPs potentially related to skin aging [GPX1 (rs1050450), SOD2 (rs4880), and MMP1 (rs1799750)] [14]-[18] in 141 East Asian females who were long term FTE product users compared to 137 East Asian females who were in the same age range and life style, but with no history of FTE product use.

2. Materials and Methods

2.1. Subjects and Study Protocol

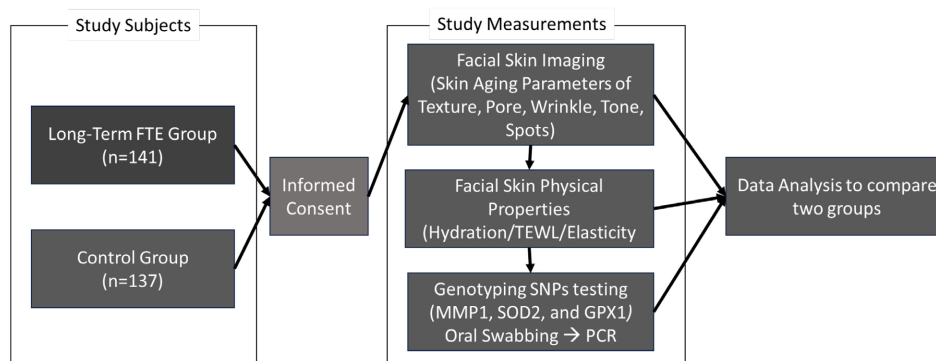


Figure 1. Illustration of the study procedure. After study subjects recruiting of two groups (Long-term FTE group and Control group), informed consent was obtained from all participants followed by series of the measurements from facial skin imaging, skin physical properties and genotype SNPs testing. All the collected data was then analyzed to compare Long-term FTE group to Control group.

The study procedure was illustrated in **Figure 1**. In short, after the subject recruiting for two groups by meeting the inclusion criteria, informed consent was obtained from all participants. Subjects were then measured their skin aging parameters, skin physical properties and skin-aging related SNPs testing. After the data collection, data was analyzed to compare in two groups. A total of 278 healthy East Asian females were participated in the study. The participants were divided into two groups. The first group consisted of 141 Asian females (age range 28 to 76, mean age 47.1, SD 7.81) who were longitudinal FTE skincare product users with a written usage record (named Long-term FTE group, hereafter), having started regular daily use of FTE, twice a day in the morning and evening after cleaning their face, in their 20s or early 30s and continued this until the time of the present study. The second group consisted of 137 control females (age range 26 to 77, mean age 47.3, SD 9.51) who were in the same age range and had the same skincare habits and practices but no history of FTE product use (named Control group, hereafter), ensuring a consistent age distribution between the two

groups. This research was performed in Tokyo, Nagoya, and Osaka, Japan, in May 2013. The examination room was maintained at a constant temperature and humidity (room temperature $20^{\circ}\text{C} \pm 2^{\circ}\text{C}$, relative humidity $50\% \pm 5\%$).

The average duration of FTE use in the Long-term FTE group was 13.1 years (SD 7.81) and the average age of starting FTE use was 28.9 (SD 7.49). The age of the subjects and the number of years of longitudinal FTE use are presented in **Figure 2** and **Table 1**. The number with history of longitudinal FTE use of more than 10 years was 88, more than 20 years was 22, and more than 30 years was 12 (**Table 2**). The earliest age of starting FTE use was 6 years old; this subject had continued using FTE for 32 years since then. The longest duration of FTE use was 34 years.

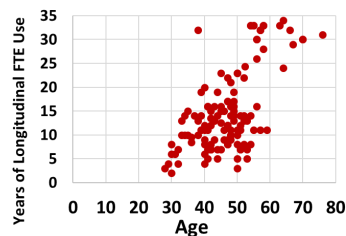


Figure 2. Chronological age and years of longitudinal FTE use among the subjects in the Long-term FTE group.

Table 1. Number, age distribution and years of FTE use of the subjects in the long-term FTE group and the Control group.

Age Group	Long-term FTE group			Control Group	
	n	Age	Years of FTE Use	n	Age
20s/30s	28	34.8 ± 3.47	9.8 ± 6.08	27	34.7 ± 3.41
40s	62	44.7 ± 3.09	11.9 ± 4.68	59	44.6 ± 2.88
50s	37	53.6 ± 2.87	14.0 ± 9.53	36	53.7 ± 2.83
60s/70s	14	65.6 ± 4.12	21.7 ± 10.76	15	65.4 ± 5.65
All	141	47.1 ± 7.81	13.1 ± 7.81	137	47.3 ± 9.51

Table 2. Years of longitudinal FTE use and associated numbers in the long-term FTE group.

Years of Longitudinal FTE Use	Number of Longitudinal FTE Users
>3	141
>10	88
>20	22
>30	12

None of the subjects underwent any type of esthetic treatment such as laser cosmetic procedures during the study period. This study was conducted in accordance with the tenets of the Declaration of Helsinki and approved by P&G

Ethics Committee. Data acquisition and analysis were performed in compliance with protocols approved by the Ethical Committee of Global Product Stewardship in P&G Innovation Godo Kaisya (ethics approval number CT13-006). Written informed consent was obtained from all subjects prior to enrollment in the study.

2.2. Facial Optical Imaging and Objective Image Analysis

The subjects washed their faces using the prescribed cleansing foam and then spent 20 min becoming accustomed to the environment of the measurement room at a constant temperature and humidity. Each subject's face was photographed using an image capture system, which we named "Magic Ring" (Figure 3), consisting of a portable image-capturing module that can be positioned on either the left-side or the right-side of the subject's face [13]. Illumination is provided by a number of 5600-K light-emitting diodes (LEDs) mounted in the imaging module. A high-resolution complementary-symmetry metal-oxide-semiconductor (CMOS) digital camera, capable of generating 2592 (vertical) \times 1944 (horizontal) effective picture elements (pixels), is also mounted in the imaging module, and the images that it collects are digitally transferred to a computer. The casing of the module encloses the area of skin under study and the mount containing the illumination source and the camera, so that the intensity of illumination and the distance between the camera head and skin surface are controlled to ensure the reproducible collection of images. A rectangular capturing guide appears on the monitor of the digital camera during image acquisition. A neutral 8.0 gray color board (Gretag-Macbeth GmbH, Munich, Germany) was used for white balancing of the camera. The region of interest (ROI) of the images was from the outer edge of the eyes to the cheek, and the following characteristic objects were extracted by measuring the contrast in the shape and pixels using an image analysis algorithm [1]. Wrinkles were defined as ≥ 5 mm in length, perimeter/length ratio ≤ 2.5 , and circularity (perimeter²/area) ≥ 34 . Total wrinkle area fraction was quantified as follows: total wrinkle area (pixels)/ROI (pixels). Hyperpigmented spots were defined as ≥ 5 mm² in area, color contrast $\Delta E \geq 3$ compared with the surrounding skin region, and circularity (perimeter²/area) ≥ 20 . Total spots area fraction was also quantified as follows: total hyperpigmented area (pixels)/ROI (pixels). As an index of skin surface roughness, total texture area fraction [total texture area (pixels)/ROI (pixels)] was quantified as ≤ 3 mm² in area, aspect ratio ranging from 0.5 to 2, and color contrast $\Delta E \geq 1.5$, while pores were defined as ≤ 4 mm² in area, color contrast $\Delta E \geq 2$, and circularity (perimeter²/area) ≥ 20 , which differ from hyperpigmented spots in terms of size and circularity. Total pore area fraction was quantified as follows: a histogram-equalized image was filtered by fast Fourier transformation to remove one or more skin features (e.g., pigmented spots and moles). Then, skin feature segmentation was performed to extract images of follicles with one or more of the following features: threshold follicle area major diameter (200 - 1000 μ m), width/length aspect ratio (0.3 - 1), and redness (a-value) contrast of more than 1.5 units compared with the surrounding skin. Facial skin

color (lightness, redness, yellowness) was also measured in the region of interest. The mean values of the resulting data obtained by these evaluations were analyzed.



Figure 3. Magic Ring facial imaging system and image analysis overlays of facial skin texture, pores, wrinkles, skin tone, and hyperpigmented spots.

2.3. Biophysical Measurements

Skin hydration (water content), TEWL, and skin elasticity of the cheek were measured using Corneometer1 (Courage + Khazaka Electronic GmbH, Cologne, Germany), Vapometer™ (Delfine Technologies), and Cutometer (Courage + Khazaka), respectively.

2.4. Genotyping of Single-Nucleotide Polymorphisms (SNPs) in MMP1, SOD2, and GPX1

Using oral cavity swab samples (**Figure 4**) and routine PCR sequencing, genotyping was performed on SNPs related to skin aging in Glutathione Peroxidase 1 (GPX1, SNP ID: rs1050450; CATCGAAG CCCTGCTGTCTCAAGGGC[C/T]CAGCTGTGCCTAGGGCGCCCCTCCT; Seq. ID Nos. 5 and 6), Superoxide Dismutase 2 (SOD2, SNP ID: rs4880; CAG-CACCAGCAGGCAGCTGGCTCCGG[C/T]TTTGGGG-TATCTGGGCTCCAGGCAG (Seq. ID Nos. 3 and 4), and Matrix Metalloproteinase 1 (MMP1, SNP ID: rs1799750; GAATTGTAGTTAAATAATTAGAAAG[-G] ATATGACTTATCTCAAATCAATCCA (Seq. ID Nos. 1 and 2). These were SNPs reported to potentially confer a risk of skin aging, such as skin hyperpigmentation, anti-oxidation, or wrinkling. In brief, the genotyping was performed as follows: DNA extraction was performed using a Gentra Puregen Buccal Cell Kit following the manufacturer's instructions (Qiagen, Valencia, CA, USA). The genotypes of the three SNPs (GPX1 rs1050450, SOD2 rs4880, MMP1 rs1799750) were determined by PCR-based TaqMan assays in the presence of two differently fluorescently labeled probes, which allow the detection of both alleles in a single reaction (Applied Biosystems Inc., Foster City, CA, USA). The specificity of the TaqMan assays was confirmed by Topo-cloning and capillary sequencing. Under optimized PCR conditions [volume 25 µl; using the TaqMan Universal PCR Master Mix as indicated by the manufacturer (Applied Biosystems Inc.), PCR: 95°C for 10 min and then 50 cycles of 60°C for 60 s and 92°C for 15 s, performed on RotorGene 6000 (Qiagen)], reliable accurate results were obtained with at least 100

pg of chromosomal DNA. The presence or absence of SNPs in the particular genes was reflected by the following weighting: wild-type genotype = 1, heterozygous genotype = 2, and homozygous genotype = 3. The genetic skin score was determined, using the weightings corresponding to each polymorphism's presence or absence in the biological samples of the subjects (e.g., MMP = 1, SOD2 = 2, GPX = 1).



Figure 4. Oral cavity swabbing DNA sampling kit.

2.5. Statistical Analysis

SPSS 22 (IBM® SPSS® Statistics) for Windows 2010 was used for statistical analyses. Data were analyzed by nonparametric, paired, and unpaired tests including, if appropriate, analysis of (co-)variance and Welch's *t*-test. Differences were considered to be significant at $p < 0.05$. In accordance with the above-described procedures, the parameters of texture, pores, wrinkles, skin tone, and spots were reverse-fitted on a scale from 0 to 1 (a higher score representing less texture, fewer pores and wrinkles, and a brighter tone), and the fitted scores of texture, pores, wrinkles, and skin tone were summed and defined as Total Aging Score. A higher Total Aging Score indicates more youthful-looking skin. In addition to these image analysis parameters, hydration, TEWL, and elasticity in the Long-term FTE group were compared to those in the Control group. Moreover, correlation coefficients (*r*) of the relationships of the skin imaging parameters of Total Aging Score, texture, pores, wrinkles, tone, and spots with the genotypes of the GPX1, MMP1, and SOD2 SNPs were determined.

3. Results

3.1. Comparison of Skin Aging Parameters between Long-Term FTE Group and Control Group

We measured various facial skin optical and physical parameters in both the Long-term FTE group of 141 female volunteers and the Control group of 137 female volunteers. The facial skin hydration in the Long-term FTE group was significantly higher while TEWL was correspondingly lower than those in the Control group (**Table 3**). In parallel with this, the values of six fit-scaled skin aging-related parameters (a higher score representing more youthful-looking skin), namely, texture, pores, wrinkles, tone (lightness), spots, and the total aging score, were also significantly higher than those in the Control group (**Table 4**). In addition, facial skin in the Long-term FTE group had significantly lower levels of

redness and yellowness than that in the Control group (Table 3). Moreover, elasticity in the Long-term FTE group was significantly higher than that in the Control group (Table 3). This trend of youthful-looking skin in the Long-term FTE group was observed in all age groups, including those in their 20 s/30 s, 40 s, 50 s, and 60 s/70 s (Table 3, Table 4).

Table 3. Facial skin measurements of hydration, elasticity, skin tone (a: redness, b: yellowness) in the long-term FTE group (above) and Control group (below).

Long-term FTE group						
Age Group	Age	Hydration	Elasticity (Ur/Uf)	a	b	TEWL
20s/30s	34.8 ± 3.47	60.3 ± 9.04†	0.513 ± 0.039†	10.0 ± 1.06†	16.6 ± 1.65	16.2 ± 2.99†
40s	44.7 ± 3.09	58.8 ± 9.21†	0.498 ± 0.041†	10.1 ± 1.11	16.8 ± 1.71†	15.6 ± 2.58†
50s	53.6 ± 2.87	59.0 ± 8.84†	0.485 ± 0.034†	9.97 ± 0.94	17.2 ± 1.77†	15.4 ± 2.79†
60s/70s	65.6 ± 4.12	58.7 ± 9.33†	0.482 ± 0.036†	9.91 ± 1.02†	17.9 ± 1.69	15.2 ± 2.44†
All	47.1 ± 7.81	59.2 ± 9.84†	0.499 ± 0.038†	9.96 ± 1.43†	16.9 ± 1.94†	15.5 ± 3.13†
Control Group						
Age Group	Age	Hydration	Elasticity (Ur/Uf)	a	b	TEWL
20s/30s	34.6 ± 3.41	54.6 ± 9.33	0.493 ± 0.043	10.6 ± 1.05	16.5 ± 1.63	16.8 ± 2.81
40s	44.6 ± 2.88	53.4 ± 9.55	0.469 ± 0.037	10.3 ± 1.09	17.5 ± 1.69	17.3 ± 2.88
50s	53.7 ± 2.83	55.6 ± 9.92	0.468 ± 0.043	10.1 ± 1.13	17.7 ± 1.74	16.4 ± 2.93
60s/70s	65.4 ± 5.65	51.1 ± 8.82	0.458 ± 0.036	10.0 ± 1.18	17.8 ± 1.77	15.9 ± 3.01
All	47.3 ± 9.51	53.9 ± 9.66	0.472 ± 0.039	10.2 ± 1.49	17.3 ± 2.01	16.7 ± 3.32

†: Significant difference from the corresponding Control group ($p < 0.05$).

Table 4. Facial skin imaging measurements of texture, pores, wrinkles, skin tone (lightness), spots, and total aging score in the Long-term FTE group (above) and the Control group (below).

Long-term FTE group						
Age Group	Texture	Pores	Wrinkles	Tone	Spots	Total Aging Score
20s/30s	0.865 ± 0.129	0.857 ± 0.088	0.849 ± 0.085	0.860 ± 0.103	0.867 ± 0.145	3.433 ± 0.368
40s	0.706 ± 0.161*†	0.792 ± 0.127*†	0.761 ± 0.132*†	0.721 ± 0.136*†	0.697 ± 0.172*†	3.082 ± 0.494*†
50s	0.600 ± 0.152*†	0.682 ± 0.159*†	0.672 ± 0.116*†	0.626 ± 0.116*†	0.607 ± 0.144*†	2.582 ± 0.461*†
60s	0.552 ± 0.143†	0.746 ± 0.129†	0.664 ± 0.163†	0.590 ± 0.124†	0.552 ± 0.115†	2.554 ± 0.520†
70s	0.604 ± 0.106†	0.733 ± 0.113†	0.727 ± 0.116†	0.598 ± 0.116†	0.562 ± 0.114†	2.664 ± 0.418†
Overall	0.696 ± 0.179†	0.772 ± 0.141†	0.747 ± 0.136†	0.712 ± 0.150†	0.694 ± 0.182†	3.029 ± 0.550†
Control Group						
Age Group	Texture	Pores	Wrinkles	Tone	Spots	Total Aging Score
20s/30s	0.762 ± 0.137	0.780 ± 0.132	0.721 ± 0.146	0.728 ± 0.144	0.701 ± 0.178	2.993 ± 0.517
40s	0.517 ± 0.173*	0.612 ± 0.167*	0.530 ± 0.140*	0.510 ± 0.153*	0.483 ± 0.199*	2.171 ± 0.584*

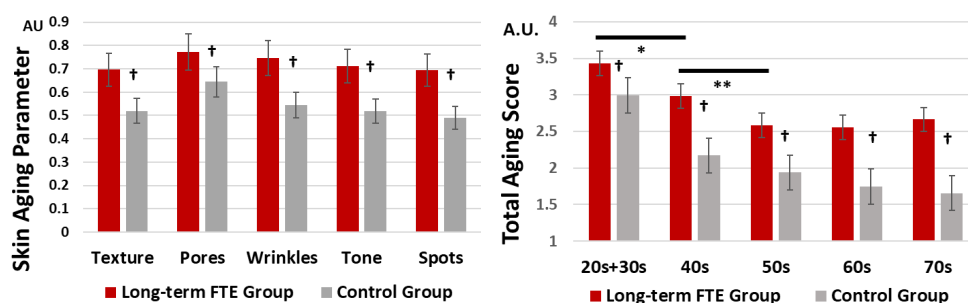
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50s	0.415 ± 0.162‡	0.598 ± 0.143‡	0.485 ± 0.122‡	0.437 ± 0.126‡	0.412 ± 0.158‡	1.937 ± 0.474‡
60s	0.353 ± 0.136‡*	0.610 ± 0.191‡*	0.417 ± 0.190‡*	0.362 ± 0.412‡*	0.315 ± 0.100‡*	1.744 ± 0.568‡*
70s	0.275 ± 0.171‡‡	0.636 ± 0.130‡‡	0.428 ± 0.187‡‡	0.316 ± 0.130‡‡	0.244 ± 0.112‡‡	1.657 ± 0.563‡‡
Overall	0.520 ± 0.205	0.645 ± 0.167	0.545 ± 0.166	0.518 ± 0.179	0.489 ± 0.210	2.230 ± 0.663

: Significant difference from those in their 20s/30s ($p < 0.05$). ‡: Significant difference from those in their 40s ($p < 0.05$). ‡: Significant difference from those in their 50s ($p < 0.05$). ‡‡: Significant difference from those in their 60s ($p < 0.05$). †: Significant difference from the corresponding Control group ($p < 0.05$).

3.2. Longitudinal Usage of FTE Product Mitigated and Paused Facial Skin Aging

All skin aging parameters of texture, pores, wrinkles, skin tone (lightness), spots, and Total Aging Score in those in their 20s/30s, 40s, and 50s/60s/70s were significantly larger and better in the Long-term FTE group than in the Control group. Notably, based on the Total Aging Score, no aggravation of skin aging was observed in the long-term FTE group after reaching their 40s, and there were no significant differences in Total Aging Score in those in their 50s, 60s, and 70s (Figure 5).



*: Significant difference between the age groups of 20s/30s and 40s in the Long-term FTE group ($p < 0.05$). **: Significant difference between the age groups of 40s and 50s in the Long-term FTE group ($p < 0.05$). †: Significant difference from the corresponding Control group ($p < 0.05$).

Figure 5. Facial skin imaging parameters of Texture, Pores, Wrinkles, Tone and Spots (left) and Total Aging Score [sum of texture, pores, wrinkles, and skin tone (lightness)] in age range (right) in Long-term FTE group and Control group.

3.3. Genotyping of SNPs in GPX1, MMP1, and SOD2 and Determining their Relationships with Skin Aging

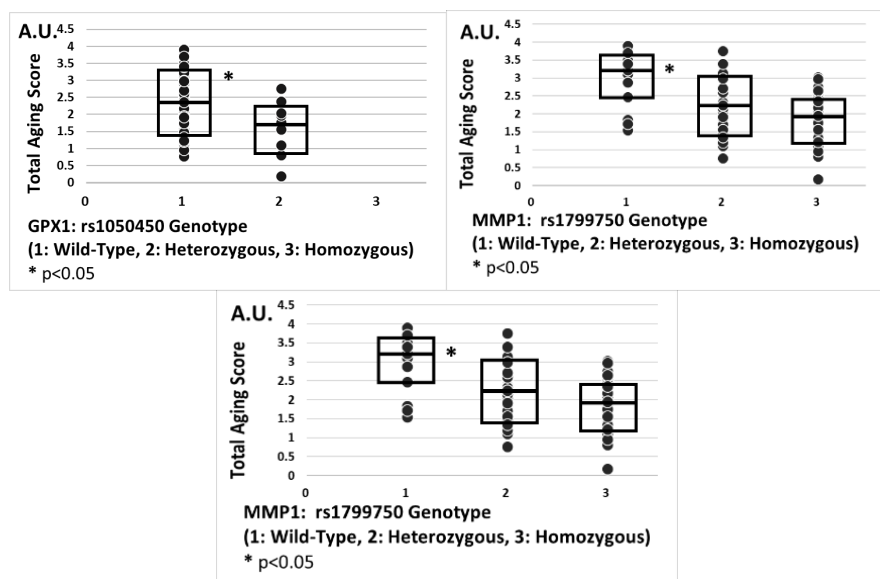
The genotypes of three representative SNPs in GPX1, MMP1, and SOD2, believed to be related to skin aging, were determined in the two groups. First, the associations of these three genotypes with skin aging-related parameters were determined in the Control group (Table 5).

All three tested genotypes of GPX1, MMP1, and SOD2 showed significant associations with skin aging parameters and Total Aging Score in the Control Group (Figure 6). Meanwhile, the GPX1 genotype was associated with the individual variables of spots and pores, and the MMP1 genotype was significantly associated

with wrinkles, texture, and tone. Furthermore, the SOD2 genotype was associated with pores. These findings suggest that these three genotypes affect skin aging chronically. In contrast, no significant associations of these genotypes with skin aging-related parameters were observed in the Long-term FTE group. Notably, there were no significant differences in genotype frequencies of the SNPs in GPX1, MMP1, and SOD2 between the Long-term FTE group and the Control group (**Figure 7**). The above findings suggested that most of the skin aging-related parameters measured in this study were significantly better in the Long-term FTE group than in the Control group; however, no differences were observed in the frequency of skin aging-related DNA genotypes between the two groups. Representative facial photos of the Long-term FTE group and the Control group in age 30s, 40s, 50s and 60s were shown in **Figures 8-11**.

Table 5. Correlation coefficients (r) of age, Total Aging Score, texture, pores, wrinkles, skin tone (lightness), and spots with the three genotypes of SNPs in GPX1, MMP1, and SOD2 in the Control group. Bold text indicates a significant correlation ($p < 0.05$).

	GPX1	MMP1	SOD2
Age	-0.051	0.022	0.099
Total Aging Score	-0.260	-0.340	-0.226
Texture	-0.147	-0.379	-0.127
Pores	-0.238	-0.133	-0.244
Wrinkles	-0.171	-0.259	-0.201
Skin Tone (Lightness)	-0.225	-0.272	-0.157
Spots	-0.254	-0.058	-0.080



*: Significant difference of Total Aging Score among the genotypes ($p < 0.05$).

Figure 6. Associations of genotypes of SNPs in GPX1, MMP1, and SOD2 with total aging score in the control group.

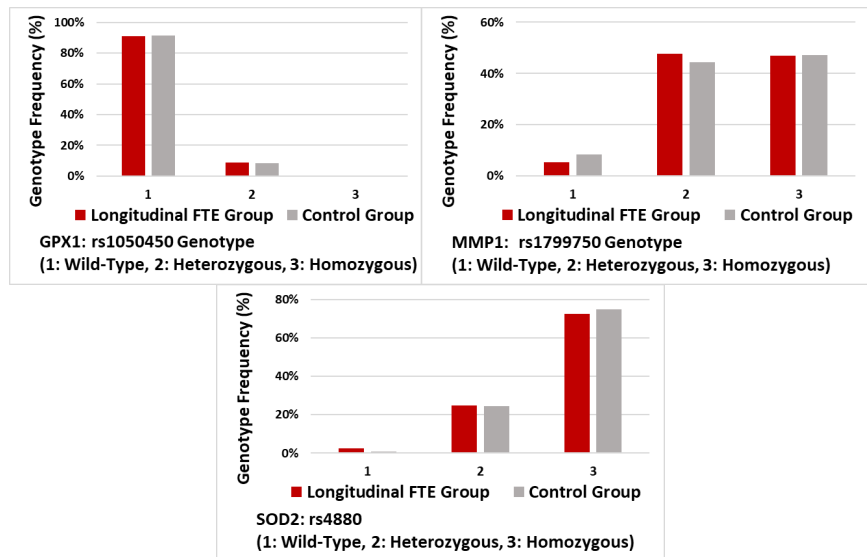


Figure 7. Distribution of frequencies of genotypes of SNPs in GPX1, MMP1, and SOD2 between the long-term FTE group and control group.

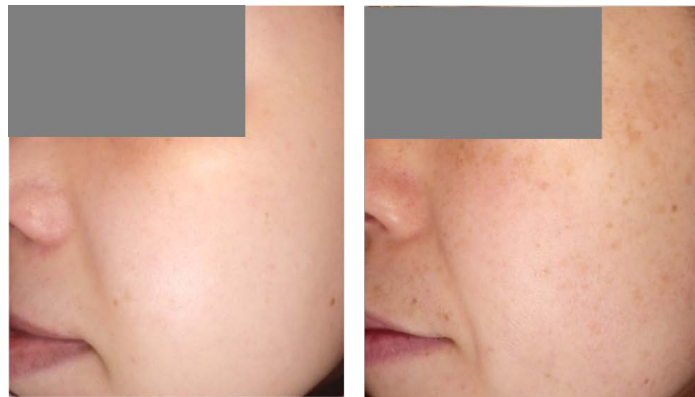


Figure 8. Facial images of 36/37-year-old subjects. Left: Subject in THE Long-term FTE group (age 36, after 10 years of FTE use). Right: Subject in the control group (age 37). These two subjects had the same genotypes of SNPs in GPX1, MMP1, and SOD2.



Figure 9. Facial images of 45-year-old subjects. Left: Subject in the long-term FTE group (age 45, after 16 years of FTE use). Right: Subject in the control group (age 45). These two subjects had the same genotypes of SNPs in GPX1, MMP1, and SOD2.



Figure 10. Facial images of 55/56-year-old subjects. Left: Subject in the long-term FTE group (age 56, after 26 years of FTE use). Right: Subject in the control group (age 55). The two subjects had the same genotypes of SNPs in GPX1, MMP1, and SOD2.



Figure 11. Facial images of 65/66-year-old subjects. Left: Subject in the long-term FTE group (age 66, after 32 years of FTE use). Right: Subject in the control group (age 65). The two subjects had the same genotypes of SNPs in GPX1, MMP1, and SOD2.

4. Discussion

Aging is the natural fate of all living organisms. However, facial skin aging is a major concern, especially among women. Physiological or chronological aging of the skin is accelerated by various external stresses such as ultraviolet (UV) radiation, environmental pollutants, and mechanical stress [19] [20]. These exogenous stresses stimulate keratinocytes to produce proinflammatory cyto/chemokines [21]-[25], which may facilitate the aging process (referred to as inflammaging). Over the course of natural aging, facial skin gradually acquires signs of aging including wrinkles, hyperpigmented spots, roughness (texture), enlarged pores, and decreased elasticity [26]-[28]. We previously conducted longitudinal research on facial skin aging by tracking the same individuals over 10 years. However, in terms of studies evaluating the efficacy of products against their chronological skin aging, these have only examined the effects of using the same skincare product for a few months to a year at most. Therefore, the current study is very meaningful as we were able to investigate the facial skin aging of subjects who had the same

skincare product usage routine for many years. In this study, we examined a total 278 East Asian Female skin aging research divided into two groups of the Long-term FTE group (n = 141) who had long-term FTE product usage containing GFF in their skin care, and the Control group (n = 137). Various facial skin aging parameters, skin physical properties and skin-aging related SNPs genotypes were measured in order to compare these two groups. The results showed that the skin conditions of the Long-term FTE group, members of which had been using the same skincare, FTE product for an average of 13 years, maintained a high level of youthfulness. It is also noteworthy that no differences in visible skin aging parameters were observed among subjects in their 50s to 70s in the Long-term FTE group, who had been using the same products for an average of more than 20 years, starting in their 20s or 30s. One possible reason for this is that they started using the skincare product when they were young, before the visible signs of skin aging had appeared, and they continued using the same skincare product to maintain high levels of moisturization and strong barrier function of the skin. In addition, these Long-term FTE users received regular skincare consultations to check their skin condition and be reminded of the appropriate usage of the product. We believe that understanding the condition of the skin and confirming the right usage of the product in this way is also important for maintaining youthful-looking skin.

We also investigated the genotypes of three skin aging-related SNPs in GPX1, MMP1, and SOD2. We found that these three genotypes were associated with skin aging parameters in the Control group. However, there were no such association with skin aging related genotypes and skin aging parameters in the Long-term FTE group, and there were no differences in the frequency of any of these genotypes between the Long-term FTE group and the Control group. The maintenance of youthful skin in the Long-term FTE group was thus suggested not to be an innate characteristic, but rather to be due to continued, long-term use of the same skincare product in the correct manner from a young age.

GFF, *Galactomyces* Ferment Filtrate (Pitera™), is the main skincare component of the FTE product. GFF is known to activate the aryl hydrocarbon receptor and enhance the expression of filaggrin, which is an essential source of natural moisturizing factors. GFF also accelerates the production of the anti-inflammatory cytokine IL-37 in epidermal keratinocytes and acts against inflammaging [29]. Moreover, GFF is capable of inhibiting the expression of cyclin-dependent kinase inhibitor 2A (CDKN2A, also known as p16INK4A), which is a critical biomarker for facial senescence in epidermal keratinocytes [30]. We cannot rule out the possibility that these biological functions of GFF contribute long-term anti-aging benefits and enable the maintenance of youthful-looking and healthy skin. Furthermore, it has been reported that FTE suppresses fluctuations in major daily skin conditions such as the skin aging-related parameters of texture, pores, wrinkles and skin tone, moisturization, and barrier function, and keeps them stable. It is considered that this daily skin-stabilizing effect also contributes to maintaining

youthful-looking skin for such a long period of time.

A limitation of the current study is that measurement of the skin condition in the Long-term FTE group prior to FTE use could not be performed. Moreover, the genotypes of only three SNPs were determined in this study. Analyzing more SNPs should provide a more detailed understanding of the effect of individuals' genetic background on the anti-aging effects of skincare products. It is hoped that it will become possible to characterize the state of skin aging before starting the use of skincare products, along with the performance of follow-up surveys of a longer period of time.

In conclusion, these results suggested that longitudinal usage of an appropriate cosmetic agent containing GFF from a younger age is beneficial by changing the skin's destiny to maintain healthier and younger-looking facial skin for many years.

Author Contributions

Conceptualization and clinical investigation were performed by Kukizo Miyamoto, Shiomi Yagi, Wang Summer, Yasuko Inoue, Sudarsana Suda, and Masutaka Furue. All authors approved submission of the final version of the manuscript.

Institutional Review Board Statement

This study was conducted in accordance with the tenets of the Declaration of Helsinki and approved by the P&G Ethics Committee.

Ethical Statement

Data acquisition and analysis were performed in compliance with the tenets of the Declaration of Helsinki. This study protocols were approved by the Ethical Committee of Global Product Stewardship at P&G Innovation Godo Kaisya (approval number CT13-006). Written informed consent was obtained from all participants prior to inclusion in the study. Written informed consent was obtained from all subjects prior to enrollment in the study.

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

Data Availability Statement

The data used in this study are available on request from the corresponding author. The data are not publicly available because of privacy restrictions.

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Conflicts of Interest

Masutaka Furue is a consultant of P&G Innovation GK. The other authors are employees of P&G Innovation GK. The authors declare no conflicts of interest regarding the publication of this paper.

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