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"Predictive Modeling of Estimated Age and Related Sub-surface Skin Changes Across Facial Zones in Chinese Female Population"

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1. Introduction

Skin aging, which is a universal biological process that affects individuals across all populations, is influenced by a combination of intrinsic factors, such as genetics and cellular metabolism, and extrinsic factors, including sun exposure, pollution, and life habits[1, 2]. As a result of the simultaneous influences of these factors, the structure and components of skin epidermis and dermis undergo degenerative changes like the reduction of collagen level and accumulation of solar elastosis [3, 4]. Together, these changes contribute to the clinical signs of aging, including the formation of wrinkles, loss of elasticity, pigmentation irregularities, and worsening of skin texture. Previous studies [5-7] demonstrated that skin structures differ across body sites and that the aging process differs due to uneven sun exposure. This observation has led us to investigate how these changes may differ across facial regions during the aging process.

The study of skin aging in Chinese population is of special interest due to unique genetics, environment and life habits that influence the aging process. However, much of the existing research has focused on Caucasians, leaving gaps in our understanding of aging phenotypes in Chinese females [8-11]. Additionally, the visible signs of aging are often the focus of cosmetic and dermatological interventions, while the underlying structural and compositional changes in the skin that contribute to these visible signs are less frequently explored.

Advanced non-invasive imaging techniques, such as two-photon microscopy (TPM) and ultrasound imaging, combined with the clinical evaluation of surface aging signs, offer an opportunity to bridge this gap by providing detailed insights into the subsurface changes associated with visible aging signs.

This study aims to decode the aging process of subsurface changes in the Chinese female population, focusing on four key facial zones: the cheek, the corner of the eye, the forehead, and glabella. For the analysis, we mainly used a predictive model that estimates an individual's age based on the clinical grading for wrinkles and pigmentation, which are the main indicators of visible skin aging. By comparing the predicted age with the actual age of an individual, we classified the participants into slow, normal, and fast-aging groups and investigated how the changes of subsurface structure and quality contributed to the aging rate. This approach provides insights for the understanding of aging trajectories among individuals and practical implications for the development of personalized skincare solutions and anti-aging treatments.

2. Materials and Methods

Study Design and Subjects

This is a multi-center, observational study. A total of 547 Chinese female volunteers aged 18 to 65 years old were recruited to this study from Shanghai, Guangzhou and Luoyang. The volunteers are divided into 5 age groups: 18-25 years old (y/o), 26-35 y/o, 36-45 y/o, 46-55 y/o, 56-65 y/o. All the volunteers are free of moles or other blemishes in the test areas, have no history of clinical treatments, aesthetic procedures, or use of nutricosmetics products, have not engaged in outdoor work involving prolonged sun exposure exceeding 4 hours per day and have a consistent habit of using sun protection methods, and have an average daily sleep duration no less than 6 hours over the past six months. The exclusion criteria were (a) with facial dermatological disease that may interfere with the assessment, (b) pregnancy, breastfeeding, or plans to conceive during the study, (c) with severe impairments in cardiac, hepatic, or renal function, or with significant immunodeficiency, (d) having psychiatric disorders, severe endocrine diseases, or taking oral contraceptives, (e) who have participated in a clinical drug trial or other experimental studies within the past 30 days, or those who have used systemic medications within the past week that could influence trial outcomes. All the volunteers fully understood and voluntarily signed the informed consent form prior to enrollment. The clinical measurements were performed according to the principles of the Declaration of Helsinki. The study protocol was approved by Shanghai Weipu Testing International Group Co., Ltd. Independent Ethics Committee, approval number: 202403JC03.

Clinical Gradings

The clinical gradings were performed by dermatologists who completed a consistency check based on the Asian Skin Aging Atlas, Volume 2 [12]. The indicators included crow's feet wrinkles, undereye wrinkles, forehead wrinkles, glabella wrinkles, spot density and identified pigmentary spots on the cheek.

Instrumental Measurements

The test zones include cheek, corner of the eye, forehead and glabella. Two-photon Microscopy (SUPERVISION-780, China) was used to assess the subsurface biophysical parameters including stratum corneum thickness, epidermis thickness, dermis-epidermis junction undulation, intensity of collagen and elastin fiber intensity. TPM measures collagen levels using second harmonic generation (SHG) signals and elastin fiber levels using autofluorescence (AF) signals. The SHG to AF Aging Index of dermis (SAAID) is an indicator of aging by comparing the signal of collagen and elastin fibers[13]. Dermis-epidermis junction index (DEJI) is an indicator of DEJ undulation, a higher DEJI indicates greater undulation of the DEJ.

Ultrasound device DermaScan (Cortex Technology, Hadsund, Denmark) was performed for the assessment of dermis quality including dermis density and the distribution of low echogenic pixels (LEP), medium echogenic pixels (MEP) and high echogenic pixels (HEP). The images from DermaScan were applied with a 0-255 scale associated with pixels and divided them into certain intervals with 0-30 as LEP, 50-150 as MEP and 200-255 as HEP. A line was chosen to divide the epidermis into two parts of equal thickness as upper dermis and lower dermis, corresponding with LEPs in the upper dermis and LEPi in the lower dermis. LEP quantifies the quality of dermis like solar elastosis and collagen degeneration, while MEP and HEP quantify the fiber structure including collagen and elastin fibers. The ratio of LEPs to LEPi (LEPs/LEPi), which represents the proportion of LEP in the upper dermis to that in the lower dermis, is considered to reflect the integrity of the extracellular matrix [14].

Modelling and Statistical Analysis

Data modeling was conducted using R's [15] *lm* procedure followed by clustering using the *hclust* procedure.

Group comparisons were conducted using IBM SPSS Statistics version 27.0 (IBM Corp., Armonk, NY, USA). A two-sample independent t-test was used to compare the means for the first age group. For the remaining groups, Welch's ANOVA was performed for the comparison of biophysical parameters among different aging clusters due to their unbalanced sample size. Games-Howell post-hoc test was applied for multiple comparisons. For all analysis work, the significance level for testing was set at $p < 0.05$.

3. Results

Predicted Age

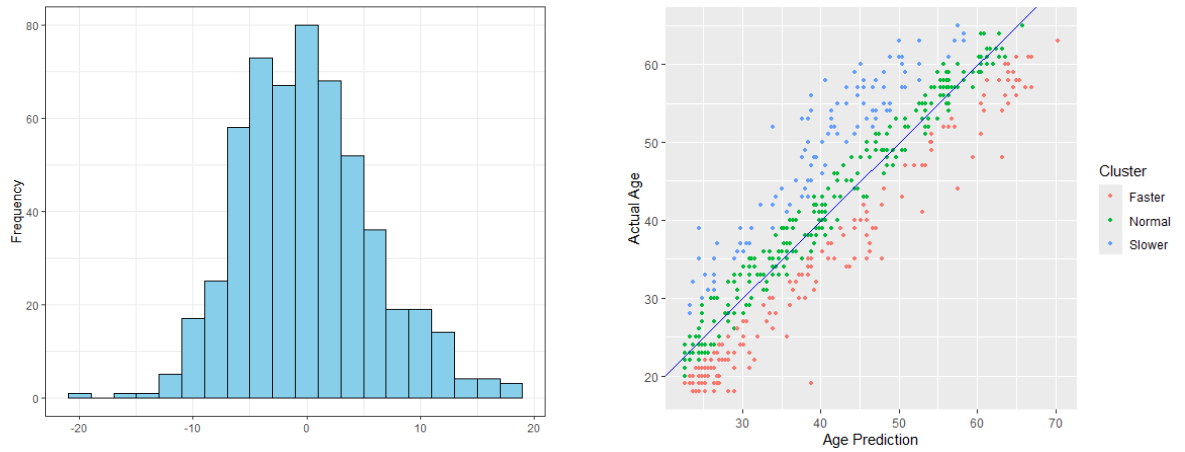
In order to understand the rate of apparent aging among individuals a score was calculated by summing the normalized ratings for six measures of skin aging: 1) wrinkles on the forehead 2) wrinkles under the eye 3) the glabella wrinkles 4) wrinkles at the corner of the eye 5) identified pigmentary spots on the cheek and 6) spot density. This aging score was then regressed against the individuals' age to estimate an age prediction.

The difference between the age predicted from the aging scores and actual age was used to cluster individuals. Negative scores indicated that individuals had fewer wrinkles than the average for their age. Positive scores indicated that individuals had more wrinkles than average for their age. Heirarchical clustering resulted in a normal aging range of –5.1 to +3.5 years for individuals. Those with differences above +3.5 years were classified as slow aging group and those with differences below –5.1 years were classified as fast aging group.

Table 1. Summary of predicted age

		18~25 y/o	*26~35 y/o*	*36~45 y/o*	*46~55 y/o*	*56~65 y/o*	*total N*
Slow aging	**N**	0	10	14	33	33	90
	Mean predicted age, y.o.	N/A	25.7	32.8	42.6	50.1	
Normal aging	**N**	58	63	64	55	57	297
	Mean predicted age, y.o.	22.7	30.3	39.2	49.3	57.8	
Fast aging	**N**	52	36	34	21	17	160
	Mean predicted age, y.o.	27.1	38.5	46.4	56.6	63.0	
Total N		110	109	112	109	107	547

Charts comparing aging-signs-based age prediction versus actual reported age



(**a**) (**b**)

Figure 1. (a) The distribution of the difference between predicted age and actual age; (b) The regression line for predicted age versus actual age with colors indicating cluster membership.

The differences across clusters were compared within each age group to analyze the aging patterns of the four facial zones specified below.

Cheek

In the 18-25 y/o group, dermis density of normal aging group is significantly higher than that of fast aging groups ($p = 0.017$), and the DEJI is significantly higher in normal aging group than fast aging group ($p = 0.028$). In the 26-35 y/o group, the amount of degenerated elastin fibers in slow aging group is significantly lower than that in the normal aging group ($p = 0.031$) and fast aging group ($p = 0.003$) group. In 36-45 y/o group, the stratum corneum (SC) thickness in fast aging group is significantly higher than slow aging group ($p = 0.009$). In the

46-55 y/o group, the SC thickness in the fast aging group is significantly higher than that in the slow aging group (p = 0.004). In the 56-65 y/o group, the DEJI is significantly higher in normal aging group than fast aging group (p = 0.017).

Corner of the eye

In the 18-25 y/o group, dermis density of normal aging groups is significantly higher than that of fast aging groups (p = 0.027). In the 36-45 y/o group, the LEPs/LEPi is significantly higher in fast aging group than normal aging group (p = 0.006), and the SC thickness in fast aging group is significantly higher than normal aging group (p = 0.047). In the 46-55 y/o group, the SC thickness in fast aging group is significantly higher than slow aging group (p = 0.014).

Forehead

In the 26-35 y/o group, the amount of degenerated elastin fibers in fast aging group is significantly higher than that in slow aging group (p = 0.023). In the 36-45 y/o group, the DEJI is significantly higher in normal aging group than that in fast aging group (p=0.039), and the amount of collagen is significantly higher in slow aging group than that in normal aging group (p = 0.013). In the 46-55 y/o group, the SC thickness in fast aging group is significantly higher than that in slow aging group (p = 0.036). In the 55-65 y/o group, the SC thickness in fast aging group is significantly higher than that in slow aging group (p = 0.036).

Glabella

In the 18-25 y/o group, dermis density of normal aging group is significantly higher than that of fast aging group (p = 0.041). In the 26-35 y/o, the amount of degenerated elastin fibers in slow aging group is significantly lower than that in the normal aging group (p = 0.045) and fast aging group (p = 0.007). In the 46-55 y/o group, the SC thickness in the fast aging group is significantly higher than that in the slow (p = 0.031) and normal (p = 0.030) aging group. The DEJI is significantly higher in the slow aging group than that in the fast aging group (p=0.027). In the 56-65 y/o group, the SC thickness in the fast aging group is significantly higher than that in the slow aging group (p = 0.036), and the collagen is significantly higher in the slow aging group than that in the normal aging group (p = 0.008).

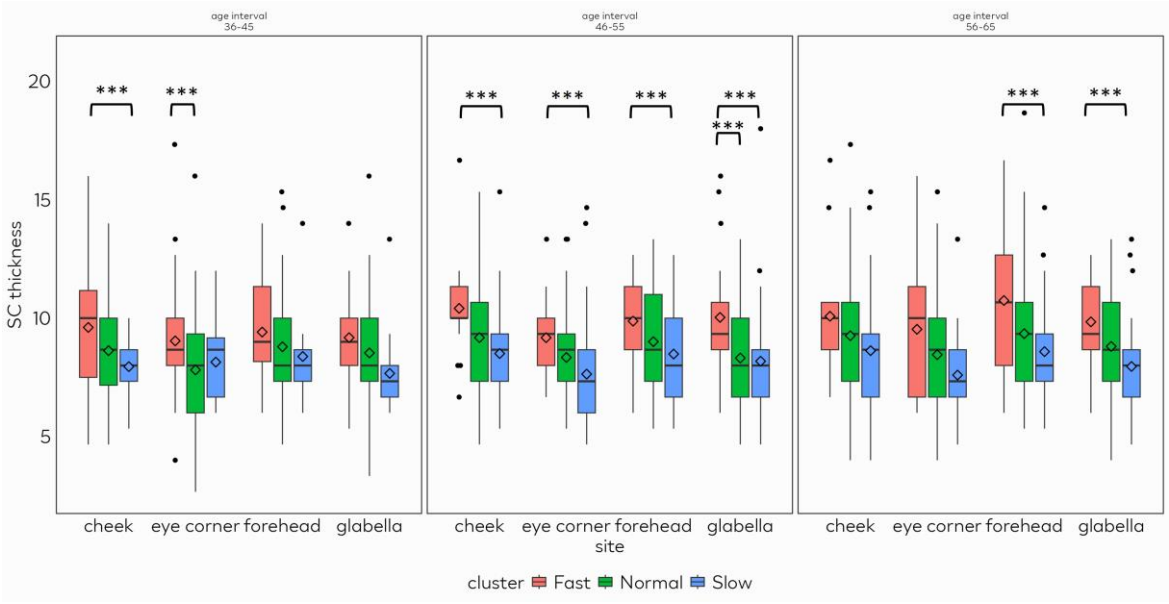
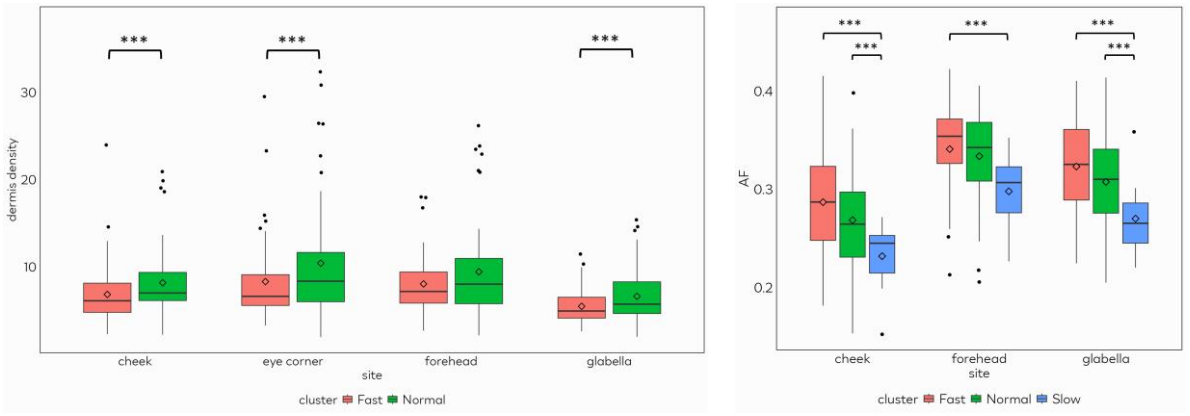


Figure 2. The comparison of SC thickness in four facial zones among three clusters in the age group of 36-45, 46-55 and 56-65 y/o.



(**a**) (**b**)

Figure 3. (a) The comparison of dermis density among three clusters in the age group of 18-25 y/o. (b) The comparison of AF, refering to the degenerated elastin fiber, among three clusters in the age group of 26-35 y/o.

4. Discussion

The present analysis leveraging an age prediction model reveals distinct aging patterns across different facial zones, driven by both structural and compositional changes. Previous studies [6, 8, 11, 16] have shown that along aging, the subsurface structure and components undergo significant changes, especially the change in elastin fiber and collagen level. The collagen intensity decreases with increasing age, while the degenerative elastin fiber increase, resulting in a decreasing SAAID. A comparative analysis among aging clusters provides evidence on how these changes influence skin aging at different time points and offers insights for the development of targeted anti-aging treatments across different age stages.

Aging on the cheek begins with a reduction of dermis density and the flattening of DEJI at an early age, after which the elastin fiber starts to be more degenerative with slow aging groups showing better preservation of skin elasticity. The thickening of SC starts to be predominant and continous to thicken along aging, followed by the further flattening of DEJ. The aging on the corner of the eye also starts on the worsening of dermis density, followed by a decline in ECM integrity and thickening of the SC. Notably, no significant cluster differences were observed in the 56–65-year-old group, suggesting that subcutaneous changes in this region occur relatively early and rapidly. Despite the subtlety of these subcutaneous changes, their impact on visible aging signs, such as wrinkles, remains persistent and pronounced.

The skin on the forehead exhibits a slower onset of aging compared to that on the cheek and the corner of the eye. Degeneration of elastin fibers becomes apparent after 26 years of age, followed by DEJ flattening and a reduction in collagen content. A higher collagen level showed in the slow aging group, which likely delays visible aging sign as forehead wrinkles. Subsequently, the SC begins to thicken, while the slow aging group, characterized by thinner SC, likely retain better skin structure and cellular turnover. In the glabella region, aging begins with a decline in dermal density, followed by increased elastin fiber degeneration. After

46 years of age, SC thickening becomes more pronounced, while slow aging group maintain higher collagen levels, which may contribute to delayed wrinkle formation.

In early adulthood (18-25 y/o), dermal density emerges as a critical factor influencing skin aging across multiple facial zones, including the glabella, cheek, and forehead. Normal aging groups consistently exhibit higher dermal density compared to fast aging groups, reflecting better collagen organization and dermal structural integrity. Reduced dermal density in fast aging groups suggests early collagen degradation or impaired synthesis, which accelerates visible aging. Early differences in dermal density set the foundation for aging trajectories, with lower density predisposing fast aging groups to premature loss of firmness. During early adulthood transitioning into midlife (26-35 y/o), elastin fiber degeneration becomes a prominent contributor to skin aging, particularly in the forehead and glabella. Slow aging groups consistently show better preservation of elastin fibers compared to normal and fast aging groups, which may likely to maintain skin elasticity and delay the wrinkles formation in the dynamic zones. Midlife (36-45 y/o) marks a turning point in the aging process, with significant changes observed across multiple facial zones. The DEJ, as an undulated structure, supports the adhesion of the epidermis to the dermis, providing mechanical stability and structural integrit[17]. Fast aging groups show reduced DEJ undulation in areas like the forehead and eye corner, reflecting weakened cohesion between the epidermis and dermis. At the same time, slow aging groups demonstrate higher collagen content in regions such as the forehead and glabella, providing superior structural support and delaying the formation of wrinkles. However, fast aging groups across all zones exhibit increased SC thickness, indicating impaired epidermal turnover. In later life (46-65 y/o) , SC thickening becomes the dominant feature in fast aging groups across all zones. Impaired epidermal turnover results in cumulative textural changes, such as roughness, dullness, and uneven skin tone. Conversely, slow aging groups consistently demonstrate thinner SC, reflecting healthier epidermal renewal. Additionally, slow aging groups maintain higher collagen levels, particularly in the glabella and forehead, which likely helps mitigate the formation of deep wrinkles.

5. Conclusion

In this study, we employed a predictive model to estimate individuals' age and categorized them into slow-, normal-, and fast-aging groups. This classification allowed us to investigate how changes in subsurface skin structure and quality contribute to variations in aging speed. The results reveal that the aging process varies significantly across facial zones and age groups. Key attributes, including dermis density, ECM integrity (LEPs/LEPi), elastin fiber degeneration, collagen levels, and stratum corneum (SC) thickness, were identified as critical contributors to visible aging signs. The 36–55 y/o age range emerges as the most critical period for aging across facial zones. Importantly, the study highlights that aging is not a uniform process across the face, instead, each facial zone exhibits unique characteristics and critical age ranges for structural and compositional changes.

It also underscores the importance of understanding the interplay between subsurface structural changes and visible aging indicators, such as wrinkles and pigmentation. By linking these structural changes to aging rate, we gain deeper insights into the underlying mechanisms of skin aging. These findings have practical implications for the development of personalized skincare solutions and targeted anti-aging treatments. For instance, interventions targeting ECM integrity and elastin fiber preservation in early to mid-life (18–45 years) may

help slow the aging process, while strategies focused on collagen support and SC turnover in later stages (36–65 years) could mitigate visible signs of aging. A large range of interventions may be beneficial in achieving such anti-aging goals, such as topical collagen-containing skincare products or collagen injectables.

This study is limited to four facial zones, without the other key aging zones like nasolabial, neck or jawline due to the limitation of the instrument probes. Additionally, except for the wrinkle and spots, other aging signs like sagging, skin tone changes or loss of volume, can also contribute to the aging speed classification. Furthermore, we here only investigated the subsurface changes in different clusters and did not include the surface changes like elasticity, hydration or skintone change, further analysis on these factors may provide us more comprehensive understanding on the overall aging patterns across facial zones.

6. Conflict of interest

Y.W., A.P., J.W., V.M., Y.T., H.W., A.S., F.L., F.F. are all L'Oréal employees. C.L. was affiliated with L'Oréal China during the preparation of this manuscript but has since left the organization. The author declares no conflict of interest related to this work.

7. References

1. Warren, R., et al., *Age, sunlight, and facial skin: a histologic and quantitative study.* J Am Acad Dermatol, 1991. **25**(5 Pt 1): p. 751-60.
2. Krutmann, J., et al., *The skin aging exposome.* J Dermatol Sci, 2017. **85**(3): p. 152-161.
3. Sherris, D.A., C.C. Otley, and G.B. Bartley, *Comprehensive treatment of the aging face--cutaneous and structural rejuvenation.* Mayo Clin Proc, 1998. **73**(2): p. 139-46.
4. Shin, J.W., et al., *Molecular Mechanisms of Dermal Aging and Antiaging Approaches.* Int J Mol Sci, 2019. **20**(9).
5. Czekalla, C., et al., *Body regions have an impact on the collagen/elastin index of the skin measured by non-invasive in vivo vertical two-photon microscopy.* Exp Dermatol, 2017. **26**(9): p. 822-824.
6. Chen, K.J., et al., *In vivo detection of healthy skin: Comparison of multiphoton microscopy and reflectance confocal microscopy.* Skin Res Technol, 2023. **29**(5): p. e13340.
7. Lynch, B., et al., *A mechanistic view on the aging human skin through ex vivo layer-by-layer analysis of mechanics and microstructure of facial and mammary dermis.* Sci Rep, 2022. **12**(1): p. 849.
8. Baroni Edo, R., et al., *Influence of aging on the quality of the skin of white women: the role of collagen.* Acta Cir Bras, 2012. **27**(10): p. 736-40.
9. El-Domyati, M., et al., *Intrinsic aging vs. photoaging: a comparative histopathological, immunohistochemical, and ultrastructural study of skin.* Exp Dermatol, 2002. **11**(5): p. 398-405.
10. Cinotti, E., et al., *Structural skin changes in elderly people investigated by reflectance confocal microscopy.* J Eur Acad Dermatol Venereol, 2020. **34**(11): p. 2652-2658.
11. Wang, H., et al., *Age-related morphological changes of the dermal matrix in human skin documented in vivo by multiphoton microscopy.* J Biomed Opt, 2018. **23**(3): p. 1-4.

12. Flament, F. and R. Bazin, *Skin Aging Atlas Volume 2: Asian Type*. 2010.
13. Lin, S.J., et al., *Evaluating cutaneous photoaging by use of multiphoton fluorescence and second-harmonic generation microscopy.* Optics Letters, 2005. **30 (17)**: p. 2275-2277.
14. Crisan, D., et al., *The role of vitamin C in pushing back the boundaries of skin aging: an ultrasonographic approach.* Clin Cosmet Investig Dermatol, 2015. **8**: p. 463-70.
15. R Core Team, *R: A Language and Environment for Statistical Computing.* 2013.
16. Goh, C.F., N. Mohamed Faisal, and F.N. Ismail, *Facial Skin Biophysical Profile of Women in Malaysia: Significance of Facial Skincare Product Use.* Skin Pharmacol Physiol, 2021. **34**(6): p. 351-362.
17. Jeong, S., et al., *Anti-Wrinkle Benefits of Peptides Complex Stimulating Skin Basement Membrane Proteins Expression.* Int J Mol Sci, 2019. **21**(1).