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"Integrated Analysis of the Age-related Microbiome and Metabolites in Korean Women's Skin"

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1. Introduction

The skin microbiome changes in response to various host health conditions, including skin diseases and immune deficiencies [1]. Aging also affects the skin microbiome, with a positive association found between aging and skin microbial diversity [2], as well as between crow's feet wrinkles and microbiome diversity [3]. Several studies have reported an age-related decrease in the relative abundance of *Cutibacterium* in older age groups [4, 5]. More comprehensive studies, including microbial composition, skin parameter, metabolites, and metabolic pathways, have been attempted. Zhou *et al.* found a relationship between collagen diffusion coefficient, distribution of *Staphylococcus epidermidis*, and abundance of anti-bacterial resistance genes in the skin microbiome in Caucasian women [6]. The skin microbial profile is also significantly associated with host facial hydration levels and aging and also plays a role in collagen metabolism and reactive oxygen species damage protection in Caucasian women [7]. However, research encompassing aging, skin conditions, the microbiome, and metabolites as well as data on Asian skin, is still lacking.

Our previous study characterized skin physiological parameters and microbial communities in healthy Korean women of two age groups [5]. The younger group had more sebum on the cheeks and abundant *Cutibacterium* and *Lactobacillus*. Phylogenetic investigation of communities by reconstruction of unobserved states (PICRUSt2) analysis revealed that energy metabolism, such as glycolysis/gluconeogenesis and the citrate cycle, was dominant in the microbiome of the young group. Another study confirmed that the skin microbiome of subjects in their 20s-30s could be classified into two subtypes (younger-type; unique to group 20s-30s, aged-type; similar functional type to group 50-60s) using PICRUSt2 analysis. Therefore, we suggest that an individual's skin condition, biological age, taxonomic composition, and especially functional microbiome analysis should be integrated [8]. This study aimed to comprehensively analyze the skin microbiome of Korean women using shotgun sequencing and find correlations between the microbial functional groups and biological age groups in skin physiological parameters, microbial communities, and metabolite profiles.

2. Materials and Methods

Participants and sample collection

A single-center, non-interventional study was conducted with 23 Korean women (60–66 years, n = 12; 20–27 years, n = 11, IEC Korea) to compare age-related skin microbiome functions and metabolomes. Participants with skin disorders or recent antibiotic used were excluded. On the sampling day, subjects washed only with water and avoided cosmetics. Skin hydration, pH, and transepidermal water loss (TEWL) were measured on the right cheek, and sebum was measured on the forehead. Microbial samples were collected from the left cheek using sterile swabs, and metabolites samples from the lower right cheek using Sebutape. All samples were stored at -80 ℃. The study was approved by the Institutional Review Board of IEC Korea [approval: IECK(2)-IRB-202207-116-01] and conducted under the controlled temperature and humidity.

Bacterial DNA extraction and shotgun sequencing

Metagenomic DNA was extracted using a FastDNA® Spin Kit for soil. DNA was quantified using a Qubit 2.0 fluorometer and prepared for sequencing using a NEBNext Ultra II FS DNA Library Prep Kit with less than 100 ng input. Paired-end sequencing (2 × 150 bp) was performed on a Next Seq 1000 system, generating an average of 39.6 million paired-end reads per library.

Taxonomy profiling and whole-genome metagenomic data analysis

Metagenomic analysis by CJ Bioscience employed the UBCG pipeline [9]. Taxonomic profiling used Kraken2 [10] with the EzBioCloud database (k-mer option of 35) [11], followed by a custom Bowtie2 core gene database [12]. Reads were mapped (a quality threshold of phred33), converted to BAM [13], and filtered (>25% core gene coverage). Species abundance was normalized by total core gene length. Functional profiling utilized DIAMOND with BLASTX against KEGG [14, 15], selecting top hits, with pathways predicted via MinPath program [16].

Untargeted metabolome analysis

Metabolomic analysis compared 10 participants per age group (selected by Sebutape weight). Samples were homogenized in 50% acetonitrile with internal standards, centrifuged (2,300 × g, 4°C, 5 min), and analyzed via CE-FTMS by Human Metabolome Technologies. An Agilent CE system coupled with a Q Exactive Plus MS operated in cation/anion modes scanned m/z 60–900 (positive) and 70–1,050 (negative). Peaks were extracted using MasterHands software (v2.19.0.2) and matched against a 1,000-standard library. Relative metabolite intensities were calculated from spectral data.

$$\text{Relative intensity} = \text{Metabolite Peak Area} / (\text{Internal Standard Peak Area} \times \text{Sample Amount})$$

Statistical analysis

Statistical analyses used R ver. 4.3.0. Microbiome composition/functional differences were assessed via PCoA, hierarchical clustering (Bray–Curtis), and PERMANOVA. LEfSe identified group-specific microbial/functional features. [17]. α-Diversity (Shannon/Simpson) and metabolome differences were tested using Wilcoxon (two groups) or Kruskal–Wallis (three groups), with significance at $P < 0.05$.

3. Results

3.1. Comparison between microbial taxonomic and functional divisions

Our previous study showed that the functional profile of the skin microbiome can be divided, even within the same age group [8]. Therefore, we analyzed the skin microbiomes of 23 participants using shotgun sequencing. To determine intergroup dissimilarity (β-diversity), we used the Bray–Curtis dissimilarity distances and unweighted pair group method with arithmetic mean (UPGMA) hierarchical clustering. UPGMA hierarchical clustering revealed that the microbiome samples did not cluster according to age when considering either taxonomic or functional analysis. Instead, they were categorized into three distinct groups based on their functional profiles (Figure 1a, b). We defined the functional age (FA) groups as follows: young group (Y, large number of participants in their 20s), old group (O, large number of participants in their 60s), and aged group (A, intermediate group). In the principal coordinate analysis (PCoA) of β-diversity, the FA groups were classified both taxonomically and functionally (Figure 1c, d). We investigated the commonalities between a biologically younger group in their 20s and the Y group among the FA groups.

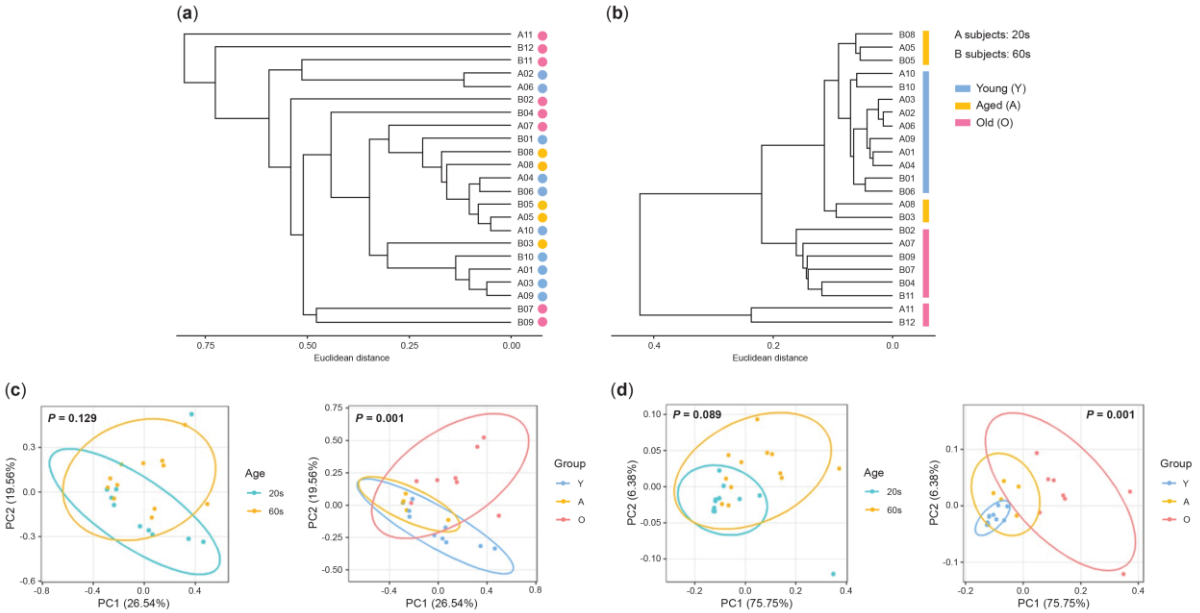

Figure 1. β-diversity analyses of the skin microbiomes of different age groups. Bray–Curtis dissimilarity distances were calculated based on taxonomic (a and c) or functional (b and d) profiles. The unweighted pair group method with arithmetic mean hierarchical clustering showed that the microbiome samples were not clustered by age in both taxonomic (a) or functional (b) analyses but could be categorized into three groups depending on their functional profiles. PCoA analyses did not clearly distinguish between age groups but did so for the three groups in taxonomic (c) and functional (d) analyses. Left panel, age group; right panel, the three groups. The significance among groups was calculated using permutational multivariate analysis of variance.

3.2 Skin physiological parameters between age and FA groups

Among hydration, sebum content, pH, and TEWL, only sebum and pH showed significant differences between the two age groups. (Table 1, sebum P=0.0034; pH P=0.016). No significant differences were found in hydration and TEWL. Meanwhile, differences in physiological parameters between the FA groups were also confirmed for sebum and pH. The amount of sebum in the Y group was high, low in the O group, and intermediate in the A group. Sebum levels between the Y and O groups significantly differed (P = 0.0009). Similarly, pH significantly

also differed between the Y and O groups (P = 0.0207), with the A group showing an intermediate value. Hydration and TEWL did not differ among the three groups.

Table 1. Clinical properties

Group		Hydration (AU)		Sebum (μg cm^{-2})		TEWL (gm^{-2} h^{-1})		pH	
		Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
Age	20s	51.32	11.77	76.59	46.64	13.20	3.77	5.15	0.17
	60s	53.09	9.60	28.63	22.13	11.94	2.76	5.34	0.30
FA group	Y	51.35	10.00	78.10	40.79	13.74	3.55	5.14	0.22
	A	56.86	9.95	46.00	50.03	10.83	2.73	5.23	0.36
	O	50.48	11.86	21.88	12.59	12.11	2.96	5.40	0.17

SD, standard deviation.

3.3 Microbial composition between age and FA groups

The 20s and 60s groups showed averages of 37,859,490 and 41,274,447 total reads, with 802,744 (bacteria) and 247,998 (archaea) classified. Top genera included *Cutibacterium*, *Corynebacterium*, and *Staphylococcus* (Figure 2a). α-Diversity increased significantly in the 60s group and functionally older (O) groups (Figure 2b). The functionally young (Y) group exhibited dominance of *Propionibacteriaceae* (family), *Propionibaceteriales* (order), *Actinomycetia* (class), *Actinobacteria* (phylum), and *Cutibacterium* (genus; Figure 2c). Microbial community in the aged (A) group resembled the Y group, while the O group showed enrichment of *Ralstonia* (previously included in the genus *Pseudomonas*) and related classes (*Betaproteobacteria*) and orders (*Burkhoderiales*).

3.4 Comparison of the enriched metabolic pathways in FA groups

We performed shotgun sequencing to identify potential functional differences in the skin microbiomes (Figure 3). Linear discriminant analysis (LDA) effect size (LEfSe) showed that the skin microbiome of the Y group was enriched in pathways involved in energy metabolism and

pathways that generate cellular structures (Figure 3a). The heatmap showed that the Y and A groups were similar, but the O group had different enriched pathways (Figure 3b). Group O mainly showed pathways related to bacterial growth and virulence, such as fatty acid degradation, quorum sensing, biofilm formation, and lipopolysaccharide biosynthesis (Figure 3a). These results are similar to our previous results using PICRUSt2, which showed that pathways involved in energy metabolism were predominant in the young group, whereas those related to degradation were predominant in the old group [5].

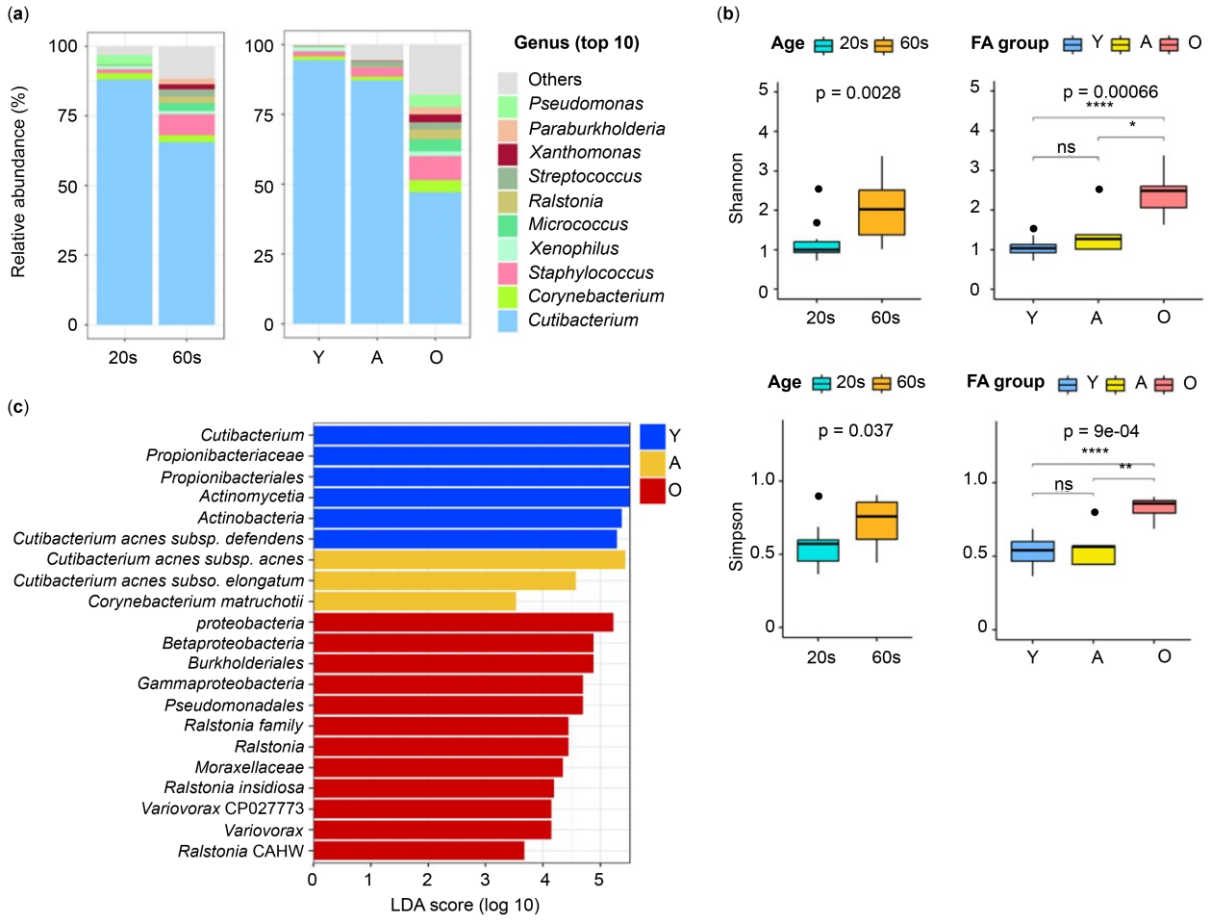

Figure 2. Taxonomic analyses of skin microbiome depending on functional aging. (a) The relative abundances of the top 10 genera were represented as bar plots for age (left panel) or functional age (right panel) groups. (b) α-diversity analyses of skin microbiome. Shannon (upper) and Simpson (lower) diversity indices were calculated. Significance was determined by the Wilcoxon rank-sum or Kruskal–Wallis test between two or three groups, respectively. (c) The enriched microbiome in Y (blue), A (yellow), and O (red) groups were analyzed using linear discriminant analysis (LDA) effect size. LDA score ≥ 3 and $P < 0.05$ in the Kruskal–Wallis and pairwise Wilcoxon tests were considered significant. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$; ns, not significant. Y, young group; O, older group; A, aged group.

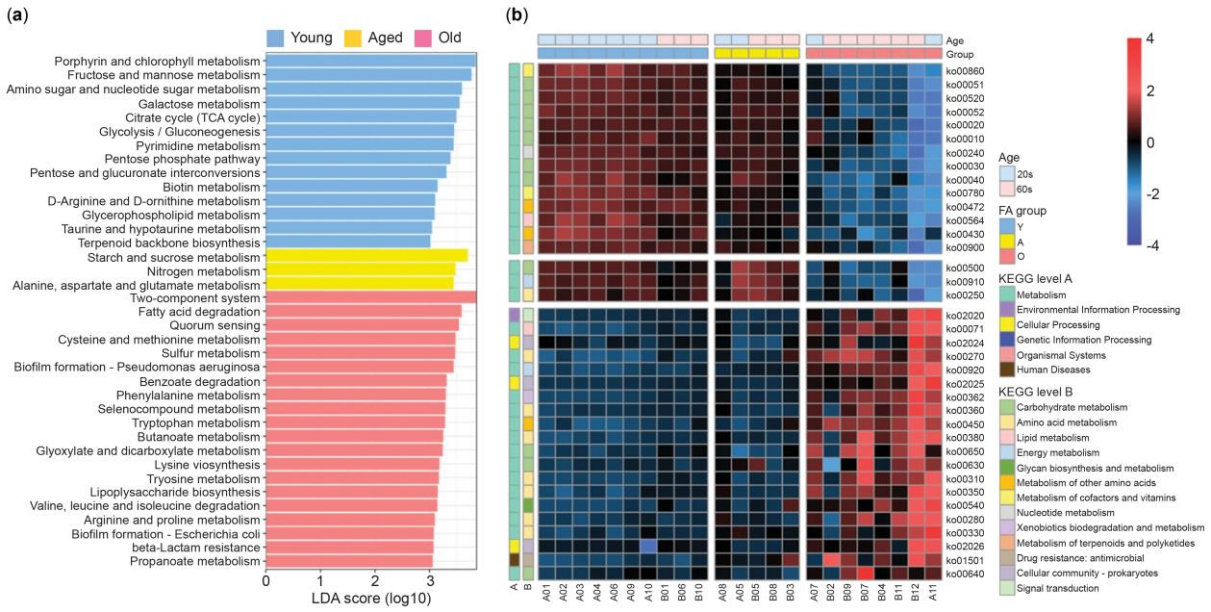

Figure 3. Significantly enriched metabolic pathways depending on skin aging. (a) Linear discriminant analysis (LDA) effect size (LEfSe) analysis of KEGG pathways was conducted. The LEfSe showed significantly enriched KEGG pathways among the Y (blue), A (yellow), and O (red) groups. LDA score ≥ 3 and P < 0.01 in the Kruskal–Wallis and pairwise Wilcoxon tests were considered significant. (b) The abundances of significantly different pathways analyzed by LEfSe were represented in a heatmap. The blue color in the heatmap indicates a lower frequency, whereas the red color indicates a higher frequency. The relative frequency of each KEGG pathway was normalized to a Z-score. Y, young group; O, older group; A, aged group.

3.5 Metabolomics between age and FA groups

The substances abundantly present on the skin surface of individuals in their 20s were 1H-imidazole-4-propionic acid, 3-phenyllactic acid (PLA), tridecanoic acid, 2-hydroxy-4-methylvaleric acid, and hydroxyphenyllactic acid (hydroxyPLA) (Table 2).Commonly abundant substances in the 20s and Y groups were PLA, hydroxyPLA, and 1H-imidazole-4-propinoic acid. PLA and hydroxyPLA were of great interest because of their association with phenylalanine (Phe) metabolism, which was abundant in the O group.

3.6 Analysis of phenyllactic acid-related pathways

Phe metabolized into phenylpyruvate (PPA), phenyllactic acid (PLA), or phenylacetic acid (PAA) [18]. Skin Phe levels were higher in the 60s group versus 20s, while PLA was elevated in the 20s group (Table 2, P = 0.002). PAA was undetected and hydroxyPAA showd no age difference. In the Y group had higher PLA, while O group showed more hydroxyPAA. Metagenomic analysis revealed Y group had higher aminotransferase (K00817; Phe→PPA), and hydroxycarboxylate/lactate dehydrogenase (K00016, K00782, K18928, K18929, and K18930; PPA→PLA). Conversely, phenylacetaldehyde dehydrogenase was upregulated in O groups.

Table 2. Significantly enriched metabolites in the 20s group.

Metabolite	20s		60s		*P*-value
	Relative intensity				
	Mean	*SD*	*Mean*	*SD*	
1H-Imidazole-4-propionic acid	2.7E-02	2.2E-02	7.4E-03	3.2E-03	0.025
2-Hydroxy-4-methylvaleric acid H2-Hydroxy-3-methylvaleric acid	8.4E-03	3.9E-03	5.1E-03	2.2E-03	0.037
3-Phenyllactic acid p-Methoxyphenylacetic acid m-Ethoxybenzoic acid	3.8E-03	1.9E-0.3	1.4E-03	5.3E-04	0.002
4-(b-Acetylaminoethyl)imidazole	3.4E-04	1.8E-04	9.8E-05	3.2E-05	0.024
Hydroxyphenyllactic acid Homovallic acid	1.8E-03	6.7E-04	1.1E-03	5.9E-04	0.037
Tridecanoic acid	2.01E-01	1.5E-01	7.8E-02	7.4E-02	0.037

SD, standard deviation.

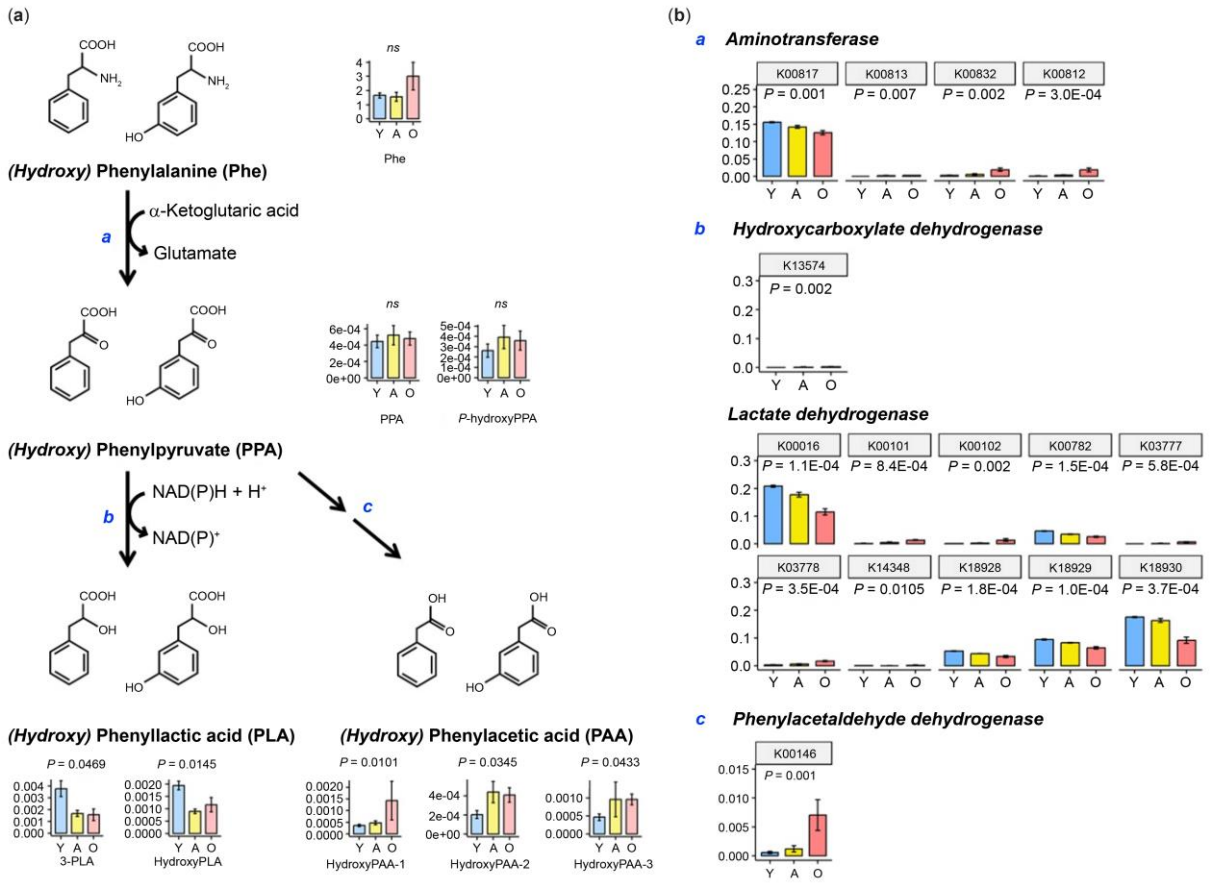

Figure 4. Functional and metabolic differences in phenylalanine metabolism among functional age groups. (a) The metabolic pathways of phenylalanine (Phe) to phenyllactic acid (PLA) or phenylacetic acid (PAA). The relative peak areas of phenylpyruvate (PPA), PLA, and PAA detected using CE-FTMS are indicated. (b) The significantly different KEGG orthologs (KOs) in Phe metabolism. The relative frequencies of KOs related to Phe metabolism were analyzed among functional age groups, and only significantly different KOs were represented. The significance of metabolite and functional genes was calculated using the Kruskal–Wallis test.

4. Discussion

Skin microbiome structure varies with age. Previous PICRUSt-based functional profiling suggested some individuals in their 20s exhibit microbiome features resembling older groups [8]. This study, the first Korean women's shotgun metagenomic analysis, identified three functional clusters via UPGMA hierarchical clustering. The 20s group showed functional similarities to the "functionally young" (Y) group, despite taxonomic differences, underscoring functional profiling's relevance in age-related microbiome studies.

The 20s and Y groups showed higher sebum and lower pH, with abundant *Cutibacterium*, a lipid lobing bacterium linked to youthful skin [19]. These groups also had more carbohydrate metabolism pathways, supporting *Cutibacterium* growth, which ferments carbohydrates into propionic acid with antimicrobial effects [20, 21]. In contrast, the O group had higher α-diversity and more pathways for biofilm formation and quorumsensing, likely related to reduced Cutibacterium abundance. However, propionic acid levels did not differ between groups.

Metabolites analysis found 1H-imidazole-4-propionic acid, PLA, and hydroxyPLA were higher in the 20s and Y group, while the O and 60s groups had more Phe on the skin surface, with Phe metabolism enhanced in the O group. PLA and hydroxyPLA, produced by bacteria like *Cutibacterium* and *Lactobacillus*, had broad antimicrobial activity and were more abyndant in younger skin [5, 22, 23]. In older skin, Phe tended to persist or convert to PAA, and higher serum Phe is linked to aging markers such as leukocyte telomere length [24]. While previous studies connect serum Phe and aing, the link to skin aging remains unclear.

The study was limited by the small number of participants. Many metabolites were not found in most participants or showed very large variations. Additionally, the metabolome analysis results demonstrated the difficulty in distinguishing between multiple substances with the same molecular weight. Therefore, further experiments are required to confirm whether PLA and hydroxyPLA are key metabolites of the microbiome in young skin. In the future, we will conduct skin microbiome and metabolite analyses on more participants and examine the effects of PLA and hydroxyPLA on the skin using in vitro tests and clinical trials.

5. Conclusion

This first shotgun sequencing study of Korean skin microbiomes links functional profiles (more critical than taxonomy) to clinical/metabolomic data. Age/function-based comparisons reveal skin aging-microbiome connections, guiding future research on youth-associated metabolites influencing aging mechanisms.

References

[1] X.E. Zhang, P. Zheng, S.Z. Ye *et al*., Microbiome: role in inflammatory skin diseases, J. Inflamm. Res. 17 (2024) 1057–1082. https://doi.org/10.2147/JIR.S441100.
[2] Y.R. Woo, H.S. Kim, Interaction between the microbiota and the skin barrier in aging skin: a comprehensive review, Front. Physiol. 15 (2024) 1322205. https://doi.org/10.3389/fphys.2024.1322205.
[3] T. Myers, A. Bouslimani, S. Huang *et al*., A multi-study analysis enables identification of potential microbial features associated with skin aging signs, Front. Aging. 4 (2023) 1304705. https://doi.org/10.3389/fragi.2023.1304705.
[4] B. Howard, C.C. Bascom, P. Hu *et al*., Aging-associated changes in the adult human skin microbiome and the host factors that affect skin microbiome composition, J. Invest. Dermatol. 142 (2022) 1934–1946.e21. https://doi.org/10.1016/j.jid.2021.11.029.
[5] H.J. Kim, H.N. Oh, T. Park *et al*., Aged related human skin microbiome and mycobiome in Korean women, Sci. Rep. 12 (2022) 2351. https://doi.org/10.1038/s41598-022-06189-5.
[6] W. Zhou, E. Fleming, G. Legendre *et al*., Skin microbiome attributes associate with bio-physical skin ageing, Exp. Dermatol. 32 (2023) 1546–1556. https://doi.org/10.1111/exd.14863.
[7] E. Russo, L. Di Gloria, M. Cerboneschi, *et al*., Facial skin microbiome: aging-related changes and exploratory functional associations with host genetic factors, a pilot study, Bio-medicines. 11 (2023) 684. https://doi.org/10.3390/biomedicines11030684.
[8] H.J. Kim, J.J. Kim, N.R. Myeongz *et al*., Segregation of age-related skin microbiome char-acteristics by functionality, Sci. Rep. 9 (2019) 16748. https://doi.org/10.1038/s41598-019-53266-3.
[9] S.I. Na, Y.O. Kim, S.H. Yoon *et al*., UBCG: up-to-date bacterial core gene set and pipeline for phylogenomic tree reconstruction, J. Microbiol. 56 (2018) 280–285. https://doi.org/10.1007/s12275-018-8014-6.
[10] D.E. Wood, J. Lu, B. Langmead, Improved metagenomic analysis with Kraken 2, Genome Biol. 20 (2019) 257. https://doi.org/10.1186/s13059-019-1891-0.

[11] S.H. Yoon, S.M. Ha, S. Kwon *et al*., Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies, Int. J. Syst. Evol. Microbiol. 67 (2017) 1613–1617. https://doi.org/10.1099/ijsem.0.001755.
[12] B. Langmead, S.L. Salzberg, Fast gapped-read alignment with Bowtie 2, Nat. Methods. 9 (2012) 357–359. https://doi.org/10.1038/nmeth.1923.
[13] H. Li, B. Handsaker, A. Wysoker *et al*., 1000 Genome Project Data Processing Subgroup, The Sequence Alignment/Map format and SAMtools, Bioinformatics. 25 (2009) 2078–2079. https://doi.org/10.1093/bioinformatics/btp352.
[14] M. Kanehisa, M. Furumichi, M. Tanabe, *et al*., KEGG: new perspectives on genomes, pathways, diseases and drugs, Nucleic Acids Res. 45 (2017) D353–D361. https://doi.org/10.1093/nar/gkw1092.
[15] B. Buchfink, C. Xie, D.H. Huson, Fast and sensitive protein alignment using DIAMOND, Nat. Methods. 12 (2015) 59–60. https://doi.org/10.1038/nmeth.3176.
[16] Y. Ye, T.G. Doak, A parsimony approach to biological pathway reconstruction/inference for genomes and metagenomes, PLOS Comput. Biol. 5 (2009) e1000465. https://doi.org/10.1371/journal.pcbi.1000465.
[17] N. Segata, J. Izard, L. Waldron, *et al*., Metagenomic biomarker discovery and explanation, Genome Biol. 12 (2011) R60. https://doi.org/10.1186/gb-2011-12-6-r60.
[18] S. Kaufman, A model of human phenylalanine metabolism in normal subjects and in phenylketonuric patients, Proc. Natl Acad. Sci. U. S. A. 96 (1999) 3160–3164. https://doi.org/10.1073/pnas.96.6.3160.
[19] C. Mayslich, P.A. Grange, N. Dupin, Cutibacterium acnes as an opportunistic pathogen: an update of its virulence-associated factors, Microorganisms. 9 (2021) 303. https://doi.org/10.3390/microorganisms9020303.
[20] M. Shu, Y. Wang, J. Yu, *et al*., Fermentation of Propionibacterium acnes, a commensal bacterium in the human skin microbiome, as skin probiotics against methicillin-resistant Staphylococcus aureus, PLOS ONE. 8 (2013) e55380. https://doi.org/10.1371/journal.pone.0055380.
[21] K. Nakamura, A.M. O'Neill, M.R. Williams, *et al*., Short chain fatty acids produced by Cutibacterium acnes inhibit biofilm formation by Staphylococcus epidermidis, Sci. Rep. 10 (2020) 21237. https://doi.org/10.1038/s41598-020-77790-9.
[22] H. Kawtharani, S.P. Snini, S. Heang, *et al*., Phenyllactic acid produced by Geotrichum candidum reduces Fusarium sporotrichioides and F. langsethiae growth and T-2 toxin concentration, Toxins (Basel). 12 (2020) 209. https://doi.org/10.3390/toxins12040209.
[23] M. Lee, D. Kim, J.Y. Chang, Metabolites of kimchi lactic acid bacteria, indole-3-lactic acid, phenyllactic acid, and Leucic acid, inhibit obesity-related inflammation in human mesenchymal stem cells, J. Microbiol. Biotechnol. 34 (2024) 306–313. https://doi.org/10.4014/jmb.2308.08015.
[24] J.G. Eriksson, M.A. Guzzardi, P. Iozzo, *et al*., Higher serum phenylalanine concentration is associated with more rapid telomere shortening in men, Am. J. Clin. Nutr. 105 (2017) 144–150. https://doi.org/10.3945/ajcn.116.130468.
[25] R. Salekeen, M.H.B. Siam, D.I. Sharif, *et al*., In silico insights into potential gut microbial modulation of NAD+ metabolism and longevity, J. Biochem. Mol. Toxicol. 35 (2021) e22925. https://doi.org/10.1002/jbt.22925.