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ORIGINAL RESEARCH

DNA Methylation-Regulated ZDHHC17 Promotes the Risk of Facial Skin Aging

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Background: Emerging evidence suggests that protein palmitoylation plays a critical role in regulating cellular signaling, yet its involvement in skin aging remains largely unexplored. Additionally, the epigenetic regulation of palmitoylation-related genes in age-related dermal changes has not been systematically studied. This research aimed to investigate the causal relationship between palmitoylation genes, DNA methylation, and facial skin aging using Mendelian randomization (MR) methods.

Methods: We performed an integrated genetic analysis using two-sample MR and summary-data-based MR (SMR) to identify palmitoylation-related genes with a potential causal role in facial skin aging. For significant genes, we further conducted a two-step MR mediation analysis to investigate whether DNA methylation influences facial aging by altering these palmitoylation genes, complemented by sensitivity tests to evaluate the robustness of findings. Analyses utilized large-scale datasets, including facial aging genome-wide association study (GWAS) summary statistics (n = 423,992), gene expression data from eQTLGen, and methylation profiles from GoDMC.

Results: The initial two-sample MR identified a significant positive association between ZDHHC17 expression and facial aging risk ($p = 0.0112$). SMR analysis confirmed the link between ZDHHC17 transcription and facial aging traits. Further mediation analysis revealed that DNA methylation at cg23935522 positively regulates ZDHHC17 expression ($p = 0.00188$), indirectly increasing facial aging susceptibility ($p = 0.0227$). The mediation effect accounted for 25.29% of the total association between the methylation site and facial aging risk. Sensitivity analyses demonstrated no evidence of horizontal pleiotropy, and the MR-PRESSO global test showed no significant outliers for ZDHHC17. Heterogeneity tests showed consistent effect estimates across genetic instruments, supporting the robustness of the results.

Conclusion: Collectively, this study offers new insights into the molecular mechanisms of skin aging and highlights ZDHHC17 methylation as a potential biomarker or therapeutic target in age-related dermatological intervention.

Keywords: facial skin aging, DNA methylation, palmitoylation, ZDHHC17, Mendelian randomization

Introduction

Facial skin aging is one of the most prominent age-related external features observed globally. Its primary clinical manifestations include loss of skin elasticity, wrinkle formation, abnormal pigmentation, and impaired barrier function. With increasing environmental pollution and ultraviolet (UV) exposure, the incidence of premature skin aging is expected to rise significantly in the next 30 years. This not only affects individual appearance but is also closely associated with an increased risk of various chronic dermatological conditions.[1] Current research has revealed multiple molecular mechanisms involved in aging, including collagen degradation through excessive activation of matrix metalloproteinases (MMPs), telomere shortening, accumulation of reactive oxygen species (ROS), abnormal remodeling

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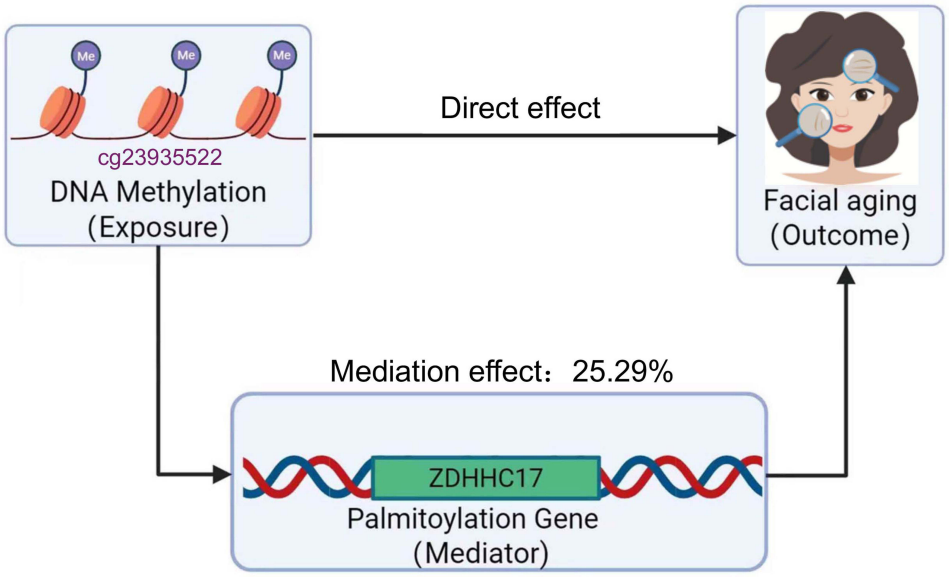
Graphical Abstract



of the extracellular matrix, and chronic photo-damage. Although these mechanisms span multiple biological processes, the molecular cascade reactions within the epidermal-dermal interface remain incompletely understood.[2]

In recent years, cross-disciplinary studies of epigenetic regulation and post-translational protein modifications have uncovered novel mechanisms underlying skin aging. Among these, protein palmitoylation, a dynamic and reversible lipid modification, may participate in the aging process by regulating the function of skin homeostasis-related proteins. Protein palmitoylation involves the covalent attachment of palmitic acid to cysteine residues, which modulates target protein membrane localization, protein-protein interactions, and signaling activity.[3,4] This process is catalyzed by the DHHC (Asp-His-His-Cys) family of transferases and is dynamically balanced by depalmitoylation enzymes such as acyl-protein thioesterases (APTs). Emerging evidence highlights the functional significance of zinc finger DHHC-type palmitoyl-transferase (ZDHHC) enzymes in skin biology, where they regulate key processes such as epidermal differentiation, inflammatory responses, and extracellular matrix organization.[5] For instance, ZDHHC13-mediated palmitoylation is essential for maintaining skin barrier integrity through the stabilization of proteins like peptidyl arginine deiminase 3 (PADI3) and transglutaminase 1 (TGM1), while its deficiency leads to cyclic alopecia and aberrant cornification.[6] Studies have also shown that mice lacking the ZDHHC13 gene exhibit increased skin fragility and impaired wound healing, while palmitoylation of β-catenin enhances Wnt signaling activity during hair follicle regeneration.[6,7] Similarly, loss of ZDHHC21 results in defects across all three epidermal lineages, including hyperplasia of the interfollicular epidermis and sebaceous glands, along with delays in hair follicle differentiation.[8] Notably, oxidative stress and UV exposure, key drivers of skin aging, have been shown to alter the expression and activity of several ZDHHC enzymes,[9–11] further implicating this protein family in age-related dermal dysfunction. However, the spatiotemporal roles of palmitoylation in photoaging and intrinsic aging remain to be systematically elucidated.

Complementing dynamic protein modifications such as DNA methylation, a stable epigenetic mark, also plays a multi-level regulatory role in skin aging.[12] DNA methylation refers to the addition of a methyl group at the 5′ position of cytosine within CpG islands, which can repress gene transcription by altering chromatin structure or recruiting methyl-binding proteins. This epigenetic memory system contributes to critical physiological processes such as epidermal barrier formation, antioxidant defense, and regulation of the inflammatory microenvironment.[13] Whole-genome methylation analyses have revealed characteristic “methylation drift” in aged skin, including global hypomethylation accompanied by

hypermethylation and silencing of specific genes such as collagen type I alpha 1 (COL1A1).[14] Notably, UV exposure may induce abnormal DNA methyltransferase (DNMT) activity, leading to hypermethylation of antioxidant gene promoters like superoxide dismutase 2 (SOD2) and persistent activation of the nuclear factor-κB (NF-κB) pathway, resulting in increased inflammation and accelerated skin aging.[15] While these findings underscore the pivotal role of epigenetic dysregulation in skin aging, the intricate interplay between DNA methylation and palmitoylation modifications remains largely unexplored.

Given the regulatory role of DNA methylation in gene expression, it is plausible that it may influence the molecular progression of facial aging by modulating the activity and substrate specificity of palmitoylation-related enzymes. Mendelian randomization (MR) is an effective method for inferring causal associations between genetic variants and complex traits.[16] The application of single-nucleotide polymorphisms (SNPs) as IVs can address the inherent challenges of confounding and reverse causation, leading to more unbiased estimate of causal associations. Using large-scale genome-wide association study (GWAS) summary-level data, this study aims to uncover the mechanistic link between DNA methylation and facial aging through a two-stage analytical strategy. In the first stage, we conducted two-sample MR analyses to systematically screen for causal relationships between palmitoylation-related genes and skin aging phenotypes. Subsequently, we employed summary-data-based MR (SMR) and heterogeneity in dependent instruments (HEIDI) tests, integrating data from large-scale GWAS and expression quantitative trait locus (eQTL) resources, to identify putatively functional genes.[17] In the second stage, for the significant genes identified, we performed a two-step MR procedure for mediation analysis to investigate whether DNA methylation influences facial aging by altering the expression of these palmitoylation genes. This procedure involved separately estimating the effect of DNA methylation on the gene expression (step one) and the effect of the gene expression on facial aging (step two). We anticipate that these findings will reveal the interaction network between epigenetic and protein modifications in facial skin aging.

Materials and Methods

Data Source

The GWAS summary statistics for facial skin aging were derived from the UK Biobank (dataset ukb-b-2148).[18] The UK Biobank cohort, recruited during 2006–2010, received ethical approval from the North West Multicentre Research Ethics Committee (REC reference: 16/NW/0274). Facial skin aging has been measured on a questionnaire (field code 1757; details available at https://biobank.ctsu.ox.ac.uk/ukb/field.cgi?id=1757). Participants responded to the question "Do people say that you look", with options including "Younger than you are", "Older than you are", "About your age", "Don't know", or "Prefer not to answer." Participants were coded as follows: 1 for those reporting they looked younger, 0 for those who looked older, and 0.5 for those who looked their age. Individuals who responded "Do not know" or "Prefer not to answer" were excluded from further analyses. Observations were made by third parties, including non-participants and non-researchers, who did not know the actual ages of the participants. Researchers then coded the participants' facial aging based on their perceived age relative to their actual age.

After genotype imputation and quality control, genome-wide association analyses were performed using linear mixed models (LMMs) implemented in the BOLT-LMM software (v2.5). As described in the original study, BOLT-LMM tests the relationship between genotype and phenotype while accounting for covariates (age, sex, and study participation center).[18] This method employs a Bayesian mixed-model approach that accounts for both population structure and cryptic relatedness among participants, effectively controlling for inflation of test statistics and reducing false positive associations. The BOLT-LMM framework is particularly advantageous in large cohorts like the UK Biobank, as it provides accurate association testing while accommodating the complex genetic relationships present in real-world population data.[19] To ensure population homogeneity, only individuals of European ancestry were included, comprising a total sample size of 423,992 participants.

Data related to palmitoylation-associated genes were compiled from the previous studies,[3,4,20] with gene expression profiles retrieved from the eQTLGen consortium (https://eqtlgen.org). In total, 22 palmitoylation genes were identified, including PPT2, PPT1, and several members of the DHHC gene family. Detailed list of these genes was provided in Supplementary Table S1. DNA methylation profiles were obtained from the Genetics of DNA Methylation Consortium

(GoDMC) database, which provides results from large-scale cis- and trans-eQTL meta-analyses covering 420,509 methylation sites across the genome. These datasets are available at: http://mqtldb.godmc.org.uk/downloads. For palmitoylation-related genes, specific DNA methylation site data can also be accessed through the epigenome-wide association study (EWAS) DataHub at: https://ngdc.cncb.ac.cn/ewas/datahub/exploration. All data utilized in this study were accessed in accordance with ethical guidelines and participant-informed consent protocols from the original research cohorts.

MR Analysis of the Relationship Between Palmitoylation and Facial Skin Aging

In this study, MR analysis was conducted to investigate the potential causal relationship between genetically predicted expression of palmitoylation-related genes and facial skin aging. The analysis was performed using the R package "TwoSampleMR" (version 0.5.7). Single nucleotide polymorphisms (SNPs) significantly associated with gene expression ($p < 5 \times 10^{-8}$) were selected as instrumental variables (IVs). To avoid weak instrument bias, only SNPs with an F-statistic greater than 10 were included. Furthermore, SNPs in linkage disequilibrium ($R^2 < 0.001$ within a 10,000 kb window) were excluded to ensure independence among instruments.

For palmitoylation genes with a single eligible SNP, the Wald ratio method was used to estimate causal effects. In cases where multiple SNPs were available for a gene, several complementary MR methods were applied, including inverse-variance weighted (IVW), MR Egger regression, weighted median, simple mode, and weighted mode. Among these, IVW was used as the primary approach to assess causality. The effect size was reported as the odds ratio (OR) of facial skin aging risk per one standard deviation (SD) increase in genetically predicted gene expression levels.

To validate the direction of causality, Steiger filtering was applied. This method ensures that the variance explained by the genetic variants is greater for the exposure (gene expression) than for the outcome. SNPs passing this criterion were labeled as "TRUE", indicating reliable directionality, while those failing were marked as "FALSE". All SNPs identified as "FALSE" were excluded, and MR analyses were repeated using the IVW method on the remaining valid instruments to ensure the robustness of the causal inference.

Sensitivity Analysis

To assess the reliability of the findings, a series of sensitivity analyses were conducted. Heterogeneity among the genetic instruments was evaluated using Cochran's Q test, where a p-value exceeding 0.05 indicates no significant heterogeneity across the variants. To detect potential horizontal pleiotropy, the intercept term from MR-Egger regression was examined. A p-value above 0.05 suggests no substantial evidence of unbalanced pleiotropic effects.

We also applied the MR-PRESSO method to identify outlier SNPs that may bias causal estimates due to horizontal pleiotropy. The non-significant global test result ($p > 0.05$) was interpreted as an absence of pleiotropic outliers. Additionally, leave-one-out analysis was performed to evaluate the influence of individual IVs. In this procedure, MR analyses were iteratively repeated after excluding one SNP at a time, allowing us to determine whether any single IV disproportionately affected the overall causal estimate.

SMR Analysis

To investigate the potential causal relationship between palmitoylation-related genes and facial skin aging, we performed SMR analysis using publicly available summary statistics on gene expression, genotypes, and genotype-phenotype associations. The analysis was carried out using SMR software (version 1.3.1), which enables the assessment of whether gene expression impacts a phenotype through underlying genetic variation.

Given that SMR assumes that observed associations are primarily driven by a single causal variant, we incorporated the heterogeneity in dependent instruments (HEIDI) test to verify the validity of the results. The HEIDI test outcomes suggested that the associations between palmitoylation gene expression and facial skin aging were unlikely to be confounded by linkage disequilibrium ($p > 0.05$), thereby strengthening the evidence for a causal connection, and reducing the likelihood of genetic confounding effects.

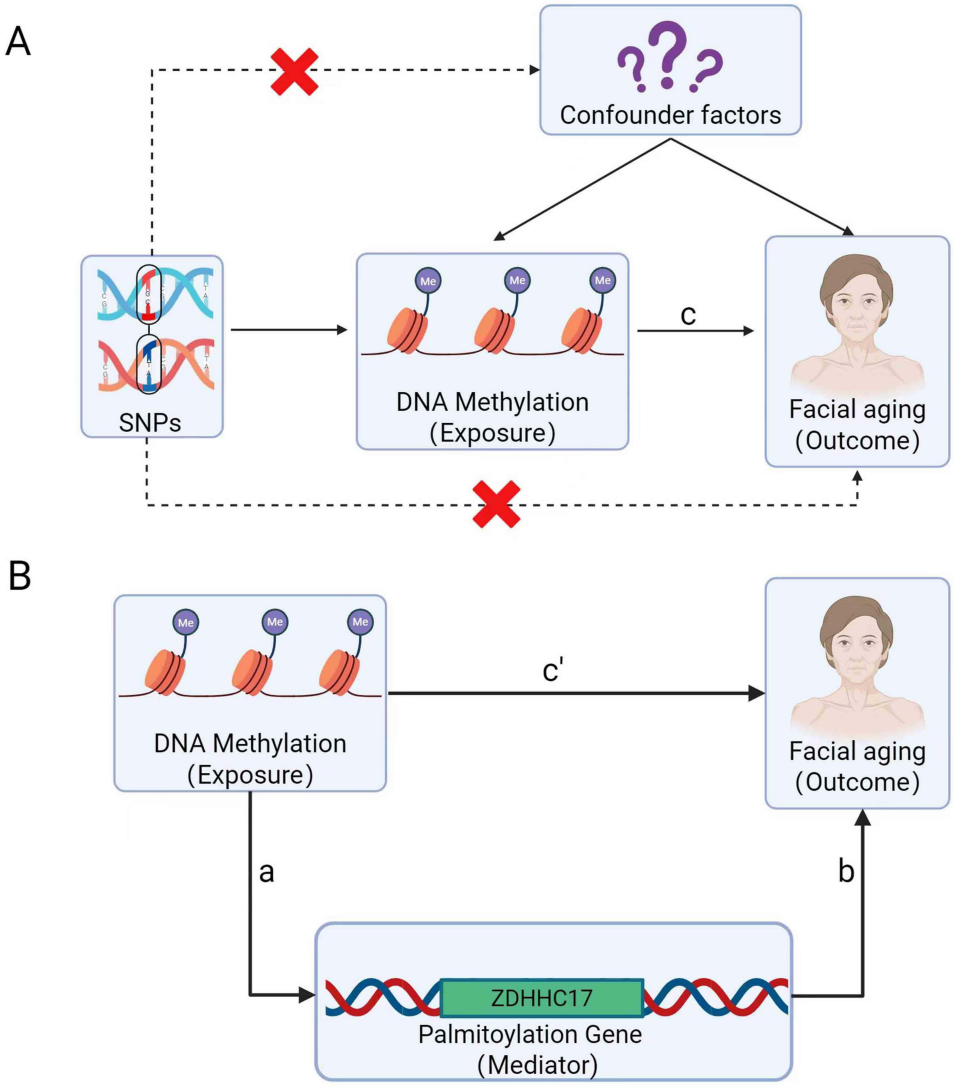


Figure 1 Study design for the 2-step Mendelian randomization (MR) mediation analysis. (**A**) Two-sample MR analysis was used to assess the causal effect of genetically predicted DNA methylation on facial skin aging, which corresponds to the total effect (c) in the mediation model. (**B**) Mediation analysis model decomposes the total effect (c) into a direct effect (c') and an indirect effect mediated by ZDHHC17 gene expression. Path a represents the effect of DNA methylation on the mediator, and path b represents the effect of the mediator on facial skin aging. The indirect effect is quantified as the product a × b.

Mediation Analysis of the DNA Methylation–Palmitoylation–Facial Skin Aging Pathway

To investigate the potential mediating role of palmitoylation in the relationship between DNA methylation and facial skin aging, we employed a two-step MR approach, as outlined in Figure 1. First, a two-sample MR analysis was conducted to assess the overall causal effect of DNA methylation on facial skin aging (Figure 1A). Subsequently, to dissect this overall effect, we performed a mediation MR analysis (Figure 1B). This framework allowed us to divide the total effect of DNA methylation on facial skin aging (path c) into two components: a direct effect (path c') of DNA methylation on facial aging that is independent of the mediator, and an indirect effect mediated through palmitoylation gene expression. The indirect effect is quantified as the product of the effect of DNA methylation on the mediator (path a) and the effect of the mediator on facial skin aging (path b). The proportion mediated, calculated as (a × b) / c, thus estimates the fraction of the total effect of DNA methylation on facial skin aging that operates by altering palmitoylation gene expression.

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Results

MR Analysis of the Relationship Between Palmitoylation and Facial Skin Aging

In this study, the IVW method was employed to evaluate the causal effects of palmitoylation-related genes on facial skin aging. A total of 41 SNPs were selected as IVs, as listed in Supplementary Table S2. The estimated effects of each SNP on facial skin aging are presented in Supplementary Table S3 and Supplementary Figures S1a–S3a (which correspond to ZDHHC5, ZDHHC17, and ZDHHC20, respectively). Importantly, all included SNPs had F-statistics exceeding 10, suggesting strong instrument strength and minimal risk of weak instrument bias.

The IVW analysis identified three palmitoylation genes, ZDHHC5, ZDHHC17, and ZDHHC20, as significantly associated with facial skin aging (Figure 2). Additional causal estimates obtained from MR-Egger, weighted median, simple mode, and weighted mode methods are provided in Supplementary Table S4 and Supplementary Figures S1b–S3b (which correspond to ZDHHC5, ZDHHC17, and ZDHHC20, respectively), supporting the robustness of the findings across multiple analytical frameworks.

Sensitivity Analysis

To evaluate the potential impact of instrument bias on the IVW results, we performed a series of sensitivity analyses. Cochran's Q test showed that ZDHHC17 and ZDHHC20 had no significant heterogeneity ($p > 0.05$), while ZDHHC5 exhibited evidence of heterogeneity ($p < 0.05$). Funnel plot analysis did not reveal marked asymmetry or dispersion among SNPs for any of the genes, supporting overall consistency in the instrument effects (Supplementary Figures S1c–S3c, which correspond to ZDHHC5, ZDHHC17, and ZDHHC20, respectively). Given the absence of substantial heterogeneity, a fixed-effect IVW model was applied to estimate causal associations.

The MR-Egger regression intercepts for all three genes were close to zero, with non-significant p-values ($p > 0.05$), suggesting no indication of horizontal pleiotropy. In the MR-PRESSO global test, p-values for ZDHHC17 and ZDHHC20 were greater than 0.05, while ZDHHC5 showed a marginal signal ($p < 0.05$), indicating the potential presence of pleiotropic influence. Additionally, leave-one-out analysis confirmed that the removal of any individual SNP did not significantly alter the overall MR estimates (Supplementary Figures S1d–S3d, which correspond to ZDHHC5, ZDHHC17, and ZDHHC20, respectively), supporting the robustness of the causal inference across all tested genes (Supplementary Table S5).

SMR Analysis

The SMR analysis revealed that among the tested palmitoylation-related genes, only ZDHHC17 and ZDHHC20 exhibited effect directions consistent with those observed in the IVW results for facial skin aging. Notably, ZDHHC17 showed a more pronounced significance, with a b-SMR of 0.00828 and a p-SMR of 0.0588, while ZDHHC20 demonstrated a weaker association (b-SMR = 0.00578, p-SMR = 0.225).

To mitigate concerns about potential pleiotropy influencing the results, we further conducted the HEIDI test. The test indicated that both ZDHHC17 and ZDHHC20 had p-values greater than 0.05, suggesting that the observed associations were unlikely to be confounded by linkage or pleiotropic effects, thereby reinforcing the robustness of our findings. Comprehensive SMR and HEIDI results are provided in Supplementary Table S6.

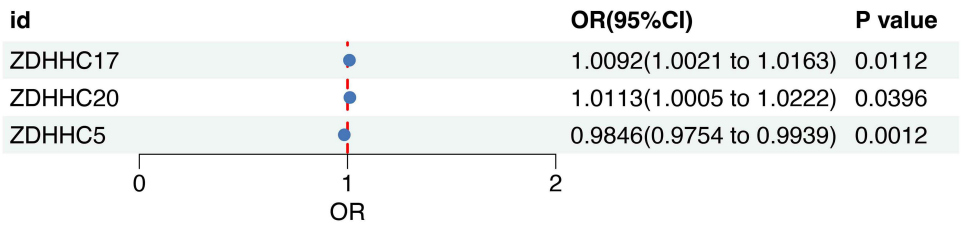

Figure 2 Significant causal effects of palmitoylation genes on facial skin aging risk. Odds ratios (OR) and 95% confidence intervals (CI) were analyzed from the inverse-variance weighted (IVW) Mendelian randomization (MR) analysis for the three palmitoylation genes (ZDHHC5, ZDHHC17, ZDHHC20).

Mediation Analysis of the DNA Methylation–Palmitoylation–Facial Skin Aging Pathway

In this study, we conducted a mediation analysis to investigate the causal relationship between DNA methylation, ZDHHC17 gene expression, and facial skin aging. Methylation site information for ZDHHC17 was obtained from the NGDC website, and corresponding summary statistics were retrieved from the GoDMC database (Supplementary Table S7). As illustrated in Figure 1A, the first step involved estimating the effect of DNA methylation on ZDHHC17 expression (pathway a, β_a). To maintain methodological consistency and reduce potential bias, methylation sites associated with only one SNP (cg00515312 and cg14226131) were excluded from the analysis. After filtering, a total of 13 SNPs were used as IVs (Supplementary Table S8).

All included SNPs had F-statistics greater than 10, confirming their strength as valid instruments. The IVW method identified one methylation site, cg23935522, as significantly associated with ZDHHC17 expression (OR = 1.2854, 95% CI: 1.0972–1.5060, $p = 0.00188$), indicating a positive regulatory relationship (Table 1). Using the same site, we further estimated its causal effect on facial aging via IVW (pathway c, β_c), which also showed statistical significance (OR = 1.0091, 95% CI: 1.0013–1.0170, $p = 0.0227$) (Table 2). Detailed SNP-specific information can be found in Supplementary Tables S9–S11.

As shown in Figure 1B, in the second step, we employed instruments related to ZDHHC17 expression to evaluate its mediating effect on facial aging risk (pathway b, β_b). By integrating results from both analytical stages, we estimated the total effect (β_c), the indirect (mediated) effect (β_{ab}), and the direct effect ($\beta_{c'}$). The proportion of the mediated effect, as shown in Table 3, indicated that cg23935522 influenced facial aging indirectly through ZDHHC17 expression, accounting for approximately 25.29% of the total effect.

Table 1 MR Effect of DNA Methylation Site cg23935522 on ZDHHC17

Site	MR Method	SNP (n)	Beta	SE	OR	95% CI	*P* value
cg23935522	IVW	13	0.2511	0.0808	1.2854	1.0972, 1.5060	1.88E-03
	WM		0.1768	0.0186	1.1934	1.1507, 1.2378	2.13E-21
	MR Egger		0.1068	0.1745	1.1127	0.7904, 1.5665	5.53E-01
	Weighted mode		0.1763	0.0198	1.1927	1.1473, 1.2400	1.26E-06
	Simple mode		0.2780	0.0586	1.3205	1.1773, 1.4812	4.76E-04

Abbreviations: CI, confidence interval; IVW, inverse-variance weighted; MR, Mendelian randomization; OR, odds ratio; SE, standard error; SNP, single nucleotide polymorphism; WM, weighted median.

Table 2 MR Effect of DNA Methylation Site cg23935522 on Facial Skin Aging

Site	MR Method	SNP (n)	Beta	SE	OR	95% CI	*P* value
cg23935522	IVW	5	0.0091	0.0040	1.0091	1.0013, 1.0170	0.0227
	WM		0.0054	0.0046	1.0054	0.9964, 1.0145	0.2380
	MR Egger		0.0007	0.0102	1.0007	0.9809, 1.0210	0.9467
	Weighted mode		0.0049	0.0054	1.0049	0.9943, 1.0157	0.4134
	Simple mode		0.0195	0.0082	1.0197	1.003, 1.0362	0.0758

Abbreviations: CI, confidence interval; IVW, inverse-variance weighted; MR, Mendelian randomization; OR, odds ratio; SE, standard error; SNP, single nucleotide polymorphism; WM, weighted median.

Table 3 Mediation Analysis of DNA Methylation Site cg23935522, ZDHHC17 Gene Expression, and Facial Skin Aging Risk

CpG Site	Path a β_a	Path b β_b	Total Effect β_c	Direct Effect $\beta_{c'}$	Indirect Effect β_{ab}	Proportion Mediated (%)
cg23935522	0.2511	0.0091	0.0091	0.0068	0.0023	25.29

Sensitivity analyses further supported the reliability of these findings. Neither Cochran's Q test nor MR-Egger regression indicated significant heterogeneity or horizontal pleiotropy. Additionally, the MR-PRESSO global test did not detect any outliers, and leave-one-out analysis confirmed that no single SNP had a disproportionate effect on the overall results (Supplementary Tables S5 and S6).

Discussion

In this study, we investigated the causal relationship between palmitoylation-related genes and facial skin aging using a combination of two-sample MR, SMR, and mediation analyses. Our findings revealed a significant association between ZDHHC17 gene expression and facial aging risk, with the observed effect being modulated by DNA methylation patterns. These results offer novel insights into the molecular mechanisms underlying facial skin aging, emphasizing the role of epigenetic regulation in aging-related phenotypes. Moreover, the identification of ZDHHC17 as a potential contributor highlights its promise as a biomarker or therapeutic target for early diagnosis and intervention in age-associated skin conditions.

Our two-sample MR and SMR analyses demonstrated that elevated ZDHHC17 expression is positively associated with an increased risk of facial skin aging. Accumulating evidence suggests that palmitoylation represents a pivotal regulatory mechanism in skin biology, maintaining cell membrane dynamics, signal transduction, and protein-protein interactions in dermal and epidermal cells.[5] ZDHHC17, also known as HIP14 (huntingtin-interacting protein 14), belongs to the ZDHHC family of palmitoyltransferases characterized by a conserved DHHC motif (Asp-His-His-Cys). This enzyme plays a critical role in protein palmitoylation, particularly modifying key neuronal and cytoskeletal proteins.[21] The mechanisms through which ZDHHC17 may promote facial skin aging likely involve disruption of multiple cellular processes essential for skin maintenance.[22] It may mediate the palmitoylation of key cytoskeletal proteins, such as MAP6 and SNAP25,[23] vital for maintaining cytoskeletal integrity and cellular mechanics in keratinocytes and dermal fibroblasts. Moreover, ZDHHC17 regulates the palmitoylation of various ion channels, including BK channels and TRPM7, influencing cellular ion homeostasis.[24] Given the importance of ion balance in skin barrier function and cellular signaling, dysregulation of these pathways could contribute to aging-related skin changes. Additionally, ZDHHC17 interacts with key signaling pathways, such as JNK and p38 MAPK,[21] which are known to be involved in stress responses and inflammatory signaling in skin cells. Its aberrant activity has also been linked to inflammatory responses involving SNARE and the NLRP3 inflammasome,[25,26] which are recognized as significant hallmarks of intrinsic skin aging. Furthermore, ZDHHC17 participates in CALCOCO1-mediated Golgiphagy,[27] potentially leading to cellular dysfunction in aged skin. These insights suggest that ZDHHC17 may modulate molecular pathways related to skin aging, though further research is needed to elucidate its specific roles in skin biology.

Our initial IVW analysis also identified ZDHHC5 and ZDHHC20 as significantly associated with facial aging risk. ZDHHC5 is a unique member of the palmitoyltransferase family, characterized by an extended cytoplasmic C-terminal tail that contains multiple regulatory elements for protein-protein interactions and substrate recruitment.[28] ZDHHC5 plays a crucial role in cell adhesion by regulating the palmitoylation of desmosomal proteins, such as desmoglein-2 (DSG2) and plakophilin-3 (PKP3), which are essential for maintaining cell-cell adhesion in epithelial tissues.[28] Depletion of ZDHHC5 leads to mislocalization of these proteins and a significant reduction in overall cell adhesion,[29] suggesting its importance in maintaining the structural integrity of skin tissues. Additionally, ZDHHC5 is critical in regulating fatty acid uptake through the palmitoylation of CD36, which stabilizes CD36 at the plasma membrane for effective fatty acid capture.[30] This dysregulation of lipid uptake mechanisms may contribute to altered lipid metabolism in aged skin. Similarly, ZDHHC20 has emerged as a key regulator of lipid metabolism through the palmitoylation of fatty acid synthase (FASN), enhancing its stability by competing with ubiquitination and preventing proteasomal degradation.[31] This modulation of FASN not only influences lipid synthesis but may also affect cellular responses to oxidative stress,[32] which is particularly relevant in skin aging. Additionally, ZDHHC20's role in the regulation of lipid metabolism may have broader implications for maintaining skin barrier function and integrity. While the specific role of ZDHHC5 and ZDHHC20 in skin aging remains underexplored, their established functions in lipid metabolic regulation and cell adhesion suggests potential implications for age-related skin changes that warrant further investigation.

Our findings establish a causal role for DNA methylation at cg23935522 in regulating ZDHHC17 expression and influencing facial aging risk. This mechanistic insight allows for a comparison with the important results reported by Marioni et al, who found no association between facial aging and blood-based DNA methylation measures.[33] The distinction between these findings reflects differences in research objectives and methodological approaches rather than a genuine contradiction. Marioni et al primarily examined whether blood-based epigenetic clocks or agnostic EWAS signatures serve as biomarkers for perceived facial age, concluding that they do not. In contrast, our study employs a hypothesis-driven causal inference framework to investigate specific biological processes, particularly protein palmitoylation mediated by ZDHHC family genes, in facial skin aging. This distinction is crucial; their unbiased search for biomarkers contrasts with our focus on a predefined set of biologically relevant genes. The absence of association in global epigenetic measures does not rule out causal, gene-specific mechanisms. MR is well-suited for identifying such causal pathways even in the absence of broad correlative signals, as it mitigates confounding and reverse causation. Thus, establishing causality through MR provides evidence at a different hierarchical level than correlational studies, offering more direct insight into biological mechanisms. Looking forward, future research incorporating skin-specific molecular QTL data would be valuable to further investigate these tissue-specific epigenetic mechanisms in facial skin aging.

Our mediation analysis not only establishes ZDHHC17 as a mediator but also reveals that the majority (74.71%) of cg23935522's total effect on facial aging operates through direct pathways independent of ZDHHC17 expression. To our knowledge, this specific CpG site (chr12:77,162,317) has not been previously reported in epigenetic studies of facial skin aging. Interestingly, cg23935522 is situated within the gene body of ZDHHC17, a genomic context where DNA methylation often exhibits more complex regulatory roles compared to promoter regions, potentially influencing transcriptional elongation, alternative splicing, or higher-order chromatin architecture.[34,35] The substantial direct effect observed suggests this locus may function as a regulatory hub, where its methylation status could influence ZDHHC17 expression while also affecting other aging-related pathways through mechanisms such as disruption of intronic enhancer activity or modulation of long-range chromatin interactions. This potential regulatory characteristic positions cg23935522 as a promising biomarker, capturing broader epigenetic aging signatures in skin and providing a strategic approach to modulating aging-related pathways. Future applications may utilize technologies like CRISPR-based epigenome editing to manipulate methylation at this site,[36] altering ZDHHC17 activity in a tissue-specific manner. Additionally, the methylation status of cg23935522 may serve as a promising biomarker for early detection of age-related skin changes in personalized dermatology.[37]

This study possesses several notable strengths. First, we employed a two-sample MR framework to infer causality between ZDHHC17 expression and facial skin aging, thereby minimizing confounding and addressing the limitations of reverse causality inherent in observational designs. In addition to MR, the integration of SMR and mediation analyses allowed us to further explore potential epigenetic mechanisms, enhancing the interpretability and robustness of our findings. Second, the genetic data used for facial skin aging were obtained from a large, well-characterized cohort of 423,999 individuals of European ancestry, providing substantial statistical power of our conclusions.

This study has certain limitations that warrant consideration. First, the facial aging phenotype was based on a subjective, self-reported measure from the UK Biobank, which inherently lacks the precision of quantitative tools like VISIA skin image analysis. The subjective nature of our outcome measure may introduce non-differential misclassification, potentially leading to attenuation of the observed effect sizes toward the null. Importantly, our MR framework helps mitigate concerns about measurement error in the outcome variable, as genetic instruments are fixed and unaffected by subjective reporting biases, thereby reducing the risk of spurious causal inferences. Future studies employing objective skin measurements will be invaluable for refining our findings, potentially uncovering more precise causal pathways linking specific skin aging features to their underlying biology. Second, our genetic instruments and outcome data were derived from individuals of European ancestry. This homogenous population was chosen to reduce bias from population stratification, strengthening the internal validity of our causal estimates. However, this limits the generalizability of our findings to other ethnic groups, whose genetic architecture, environmental exposures, and skin aging phenotypes may differ. Future investigations in large-scale, multi-ethnic cohorts are necessary to confirm the broader applicability of our results and to explore potential ancestry-specific effects. Third, the original GWAS for this phenotype acknowledged that direct reliability metrics were unavailable. However, its utility in genetic studies is

supported by indirect validations, including a significant heritability estimate, the successful replication of known skin aging loci (eg, IRF4, MC1R), and genetic correlations with relevant traits like adiposity.[18] Future studies that obtain direct reliability metrics with objective clinical assessments will be crucial to further strengthen confidence in these findings. Fourth, the absence of experimental validation, such as in vitro assays or in vivo models, to confirm the functional relevance of cg23935522 methylation and ZDHHC17 expression represents a key gap. Future functional studies are warranted to investigate the mechanistic impact of this epigenetic mark on gene expression and cellular aging phenotypes. Fifth, environmental influences such as UV exposure, pollution, or lifestyle factors, which may interact with epigenetic modifications and influence skin aging, were not incorporated into this study. Further research should integrate detailed environmental exposure data to elucidate the complex interplay between genetics, epigenetics, and environment in skin aging. Finally, although our findings suggest that ZDHHC17 methylation may hold potential as a biomarker, its clinical utility in early detection or intervention remains to be determined through longitudinal and translational research.

Conclusion

This study elucidates the regulatory role of DNA methylation at cg23935522 on ZDHHC17 gene expression and its downstream impact on facial skin aging using integrated two-sample MR, SMR, and mediation analyses. Our findings provide novel insights into the molecular mechanisms underlying age-related skin changes and underscore the importance of epigenetic regulation in facial skin aging. By identifying ZDHHC17 as a potential mediator between methylation patterns and facial aging risk, this research highlights the broader relevance of palmitoylation and transcriptional control in dermatological aging processes and suggests possible future applications in biomarker development or targeted intervention.

Data Sharing Statement

Publicly available data sets were analyzed in this study. The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

Ethics Statement

According to Article 32 of the Ethical Review Measures for Life Science and Medical Research Involving Human Beings of the People's Republic of China, the data used in this study will not cause any form of harm to human beings, nor will it touch sensitive personal privacy or trade secrets, so the ethical review can be exempted. The database used in this study was publicly available and legally available.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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