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ORIGINAL RESEARCH

Investigation of the Causal Association Between Treg Cells and Actinic Keratosis Through Two-Sample Mendelian Randomization Analysis

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Purpose: Actinic keratosis is a highly prevalent precancerous skin condition and a major risk factor for cutaneous squamous cell carcinoma with a significant risk of malignant transformation. Despite its clinical importance, the causal role of immune factors, especially regulatory T (Treg) cells, in actinic keratosis pathogenesis remains unclear. A two-sample Mendelian Randomization (MR) approach was employed to investigate the potential causal link between distinct characteristics of Treg cells and the likelihood of developing actinic keratosis.

Patients and Methods: The analysis utilized publicly available genetic data on 167 Treg cell traits and actinic keratosis risk. The Inverse Variance Weighted (IVW) method was the primary analytical approach, supported by MR-Egger, weighted median, and weighted mode analyses. Sensitivity of the findings was assessed through Cochran's Q test, MR-Egger analysis, the MR pleiotropy residual sum and outlier (MR-PRESSO) test, and leave-one-out tests.

Results: The IVW analysis revealed a significant association between resting Treg cell activity and actinic keratosis (OR: 0.9997, p = 0.0420). Additional significant associations included CD39+ resting Treg percentage of CD4 Tregs (OR: 0.9998, p = 0.0123), CD28- double negative (DN) Treg percentage (OR: 0.9995, p = 0.0359), and CD28+ CD45RA- CD8dim Tregs (OR: 1.0002, p = 0.0312). No significant associations were found in supplementary analyses, but sensitivity tests confirmed the reliability of the results.

Conclusion: This study suggests a potential causal relationship between certain Treg cell traits and the risk of actinic keratosis, indicating that further research is needed to clarify the underlying mechanisms of this association.

Keywords: actinic keratosis, regulatory T cells, immunity, Mendelian randomization, GWAS

Introduction

Actinic keratosis represents a prevalent precancerous skin disorder marked by the appearance of rough, scaly areas on parts of the skin that have been exposed to the sun.[1] The incidence of actinic keratosis increases with age, especially in populations over the age of 50 years, affecting approximately 79% of men and 68% of women aged 60 to 69 years.[2] Clinically, actinic keratosis manifests as erythematous, rough, and scaly spots or bumps on skin areas exposed to sunlight, including the face, ears, neck, scalp, and dorsal hands.[3] This condition can be a source of aesthetic concern for many individuals, as these lesions often appear on visible areas of the skin. Of greater concern is the fact that untreated actinic keratosis may evolve into cutaneous squamous cell carcinoma (cuSCC), a type of skin cancer with significant morbidity and potential for metastasis.[4] The primary risk factor for actinic keratosis is prolonged exposure to cumulative ultraviolet (UV) radiation, which induces DNA damage and promotes the development of atypical keratinocytes.[5] Therefore, identifying the risk factors for actinic keratosis is crucial for developing strategies to reduce the occurrence and progression of cuSCC from actinic keratosis.

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The skin immune microenvironment is composed of various cells and molecular components, and this intricate network maintains the skin's immune homeostasis, ensuring the proper functioning of the immune system and protecting the body from external insults.[6] Studies have shown that immunocompromised individuals have a notably higher occurrence of actinic keratosis compared to those with a normal immune system.[7] Chronic exposure to UV radiation can induce phenotypic and functional changes in the cellular components of the skin microenvironment. UV radiation can increase the number of Regulatory T cells (Treg) cells and decrease the number of effector T cells, facilitating immune evasion by tumor cells and accelerating tumor progression and metastasis.[8] The relationship between Treg cells and actinic keratosis has garnered growing interest, especially regarding the clinical implications and underlying pathogenic mechanisms. Stravodimou et al assessed the subpopulations of tumor-infiltrating lymphocytes (TILs), including Treg cells, within actinic keratosis, in situ squamous cell carcinoma (isSCC), and invasive squamous cell carcinoma (inSCC) of the skin.[9] Their study suggested that the number of Treg cells gradually rose from normal skin to actinic keratosis and isSCC, but then decreased in cases of inSCC.[9] Few studies have sought to elucidate the relationships between Treg cells and actinic keratosis; the results could be impacted by unrecognized confounding factors or reverse causation, making it challenging to establish clear causal links.

Mendelian randomization (MR) is recognized as a reliable methodological strategy for assessing causal links between risk factors and health outcomes.[10] The core principle of MR hinges on the random allocation of alleles at conception, which guarantees that genetic variants remain unaffected by confounding factors, such as measurement bias or reverse causation.[11] MR analysis addresses the limitations inherent in traditional observational studies, thereby bolstering the validity and reliability of causal links in epidemiological research.[10]

Hence, the objective of this study was to delve deeper into the causal relationship between Treg cells and actinic keratosis through a two-sample MR analysis. The study's hypothesis suggested that Treg cells have a causal link with the risk of developing actinic keratosis. Elucidating the causal relationship between Treg cells and AK would not only deepen our understanding of the immunological mechanisms underlying skin photodamage and carcinogenesis, but also holds promise for providing scientific evidence and potential therapeutic targets for the future development of novel immune protective strategies or the identification of high-risk patients.

Materials and Methods

Study Design

Consistent with the MR-STROBE guidelines, a two-sample MR analysis was conducted to explore the causal connection between 167 Treg traits and actinic keratosis.[12] The foundation of this analysis was based on summary genetic data from pertinent genome-wide association studies (GWAS). The MR analysis hinges on three key assumptions: a robust link between genetic variants and exposure, the absence of confounding factors influencing genetic variants, and the impact of selected genetic variants on the outcome solely mediated through the risk factors (Figure 1). All MR analyses leveraged publicly accessible summary statistics, thereby negating the need for additional ethical approval or informed consent.

Data Source

Publicly available summary data provided the datasets for Treg cells and actinic keratosis used in this MR analysis. GWAS summary statistics for various Treg cell traits were sourced from the study reported by Orrù et al,[13] with the GWAS ID detailed in Supplementary Table 1. Data from 3757 European individuals were utilized in the original GWAS for immune traits, encompassing 167 Treg traits. The Treg traits were categorized into three distinct types of exposures: absolute numbers, relative numbers, and median fluorescence intensity (MFI). Data for actinic keratosis were sourced from the UK Biobank database, encompassing 1349 cases and 359,845 controls.[14] All data were derived from European populations to minimize bias related to ethnic differences.

Instrumental Variables (IVs) Selection

Specific criteria were used to select IVs for investigating the causal relationship between Treg cells and actinic keratosis. Initially, SNPs were chosen as instrumental variables (IVs) if they had a P-value below the genome-wide significance

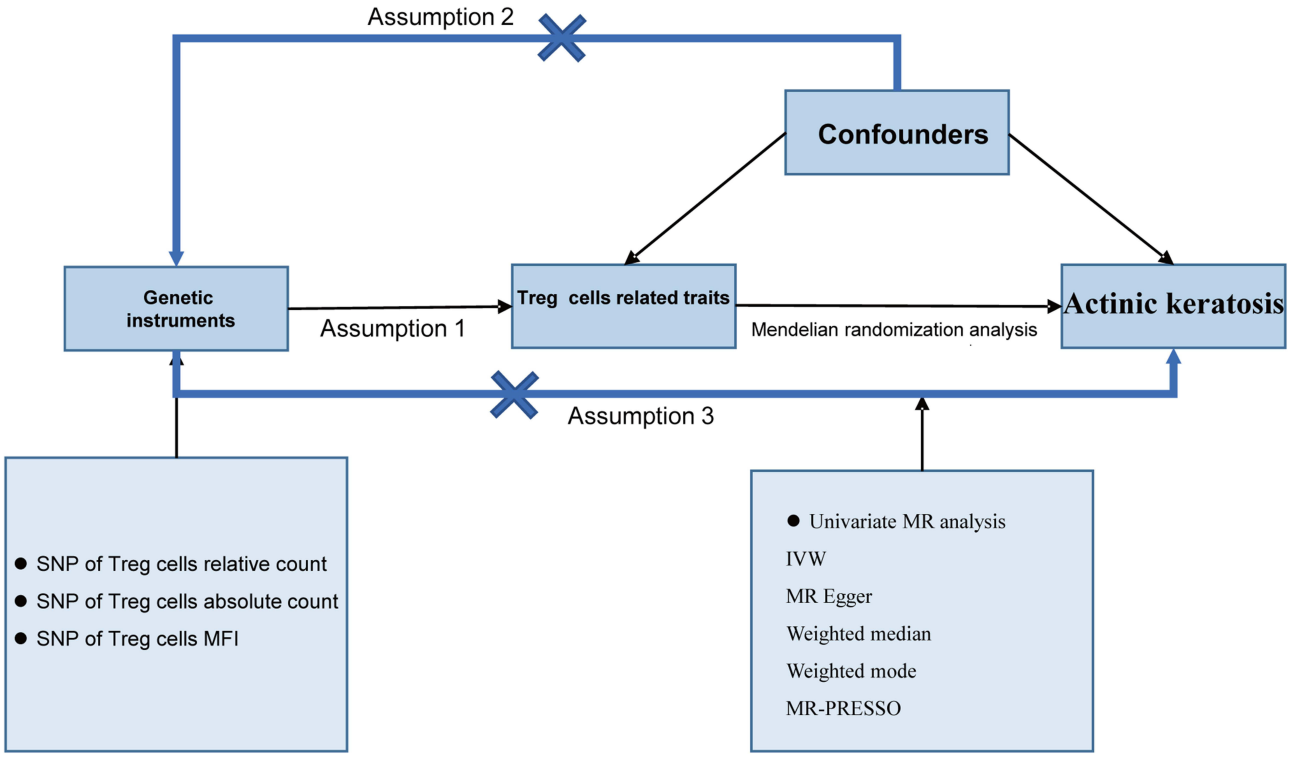

Figure 1 Flowchart of the Mendelian Randomization (MR) study. Assumption 1 posits that Treg cells have a significant relationship with genetic IVs. Assumption 2 indicates that these IVs do not influence outcomes through confounding variables. Assumption 3 asserts that genetic IVs do not directly impact outcomes but exert influence solely through indirect exposure.
Abbreviations: MR, Mendelian Randomization; IVs, instrumental variables.

threshold of $P < 5\times10^{-6}$.[15] Secondly, SNPs with a minor allele frequency (MAF) exceeding 0.01 were incorporated to guarantee adequate genetic diversity. Thirdly, SNPs with $R^2 < 0.001$ within a 10,000 kb window were excluded to minimize linkage disequilibrium (LD) effects. Fourthly, proxy SNPs with high LD ($R^2 > 0.8$) were identified and used as substitutes when selected IVs were unavailable in the summary outcome data to maintain analysis integrity.[15] Fifthly, to ensure consistency, the exposure and outcome datasets were aligned by eliminating ambiguous SNPs with discordant alleles and intermediate allele frequencies. Subsequently, the harmonized SNPs were chosen as the definitive genetic IVs for the MR analysis. Lastly, the robustness of each SNP as an IV was evaluated using the F-statistic, calculated with the formula $F = R^2 * (N-2) / (1-R^2)$, where R^2 reflects the proportion of exposure variance accounted for by the SNP, and N denotes the sample size. SNPs with an F-statistic exceeding 10 were considered sufficiently potent and included in the analysis.

MR Analysis

The primary analysis employed the Inverse Variance Weighted (IVW) method to explore the causal link between Treg cells and the risk of actinic keratosis.[16] For each exposure-outcome relationship, odds ratios (OR) and 95% confidence intervals (CI) were calculated. The IVW method operates on the principle that IVs with less variance contribute more significantly to the overall effect estimate compared to those with greater variance.[16] The weighted average of effect sizes was calculated using the IVW approach, with weights derived from the inverse of each SNP's variance.[16] Additional methods such as MR-Egger, simple mode, weighted median, and weighted mode analyses were employed to further strengthen the reliability of the findings.[17] Specifically, the MR-Egger method incorporated an intercept term to assess causal effects, accounting for pleiotropic biases where genetic variants have effects on multiple traits.[17] Similarly, the weighted median method offered an alternative approach to estimating the causal link between exposure and outcome, relying on the validity of at least 50% of the IVs, thereby enhancing the study's robustness against flawed instruments. The Weighted Mode method was used to identify the primary causal effect among the genetic instruments in the analysis,

assuming that the true causal effect aligns with the most common value of the individual effects stemming from each genetic variant. Statistical analyses were conducted using R software (version 4.3.2) with the "Two-sample MR" package. Scatter plots and sensitivity analysis diagrams were generated for data visualization, facilitating clear interpretation of the findings.[18]

Sensitivity Analysis

Sensitivity analyses play a pivotal role in this MR study by providing insights into the reliability and accuracy of the estimated causal effects. These analyses evaluate the potential influence of pleiotropy and heterogeneity among the IVs. To assess heterogeneity, Cochran's Q test was utilized. A P-value greater than 0.05 from Cochran's Q test suggests minimal heterogeneity, implying that the observed variations among IV estimates may be due to random chance. To detect horizontal pleiotropy, MR-Egger regression was employed. An intercept close to zero and a P-value above 0.05 in MR-Egger regression indicate the absence of pleiotropic effects.[19] Horizontal pleiotropy was tackled using the MR pleiotropy residual sum and outlier (MR-PRESSO) test, which detects and adjusts for outliers. SNPs identified as outliers ($P < 0.05$) were excluded from the analysis, and the causal relationship was reassessed subsequent to their removal. Additionally, the leave-one-out test was conducted to assess the influence of each individual instrument on the overall causal estimate, ensuring that no single instrument disproportionately affected the results.

Results

SNPs Selection

SNPs were extracted from GWAS summary statistics for each exposure under a well-recognized threshold. For each Treg cell, the number of instrumental variables (IVs) ranged between 2 and 704, with all F-statistic values surpassing 10, indicating that the potential for weak instrument bias is insignificant (see Supplemental Table 2 and 3 for details). Proxy SNPs were utilized to replace unmatched SNPs in the summary data, as shown in Supplemental Table 4.

Causal Effects of Treg Cells on Actinic Keratosis

The IVW analysis showed a significant association between resting Treg AC (absolute count) and actinic keratosis (OR: 0.9997, 95% CI: 0.9995–1.0000, p = 0.0420, Table 1). The scatter plot for effect sizes of SNPs for resting Treg AC and actinic keratosis is shown in Figure 2A, and the forest plot is displayed in Figure 2B, further illustrating the causal

Table 1 Treg Cells That Genetically Associated with Actinic Keratosis

Exposure	Outcome	nsnp	Method	or_ci	pval
Resting Treg AC -Absolute count	Actinic keratosis	20	MR Egger	0.9997 (0.9994–1.0000)	0.0953
Resting Treg AC -Absolute count	Actinic keratosis	20	Weighted median	0.9997 (0.9994–1.0000)	0.0892
Resting Treg AC -Absolute count	Actinic keratosis	20	Inverse variance weighted	0.9997 (0.9995–1.0000)	0.0420
Resting Treg AC -Absolute count	Actinic keratosis	20	Simple mode	0.9998 (0.9993–1.0004)	0.5479
Resting Treg AC -Absolute count	Actinic keratosis	20	Weighted mode	0.9997 (0.9994–1.0001)	0.1144
CD39+ resting Treg % CD4 Treg -Relative count	Actinic keratosis	20	MR Egger	0.9998 (0.9995–1.0000)	0.0682
CD39+ resting Treg % CD4 Treg -Relative count	Actinic keratosis	20	Weighted median	0.9997 (0.9994–1.0000)	0.0224
CD39+ resting Treg % CD4 Treg -Relative count	Actinic keratosis	20	Inverse variance weighted	0.9998 (0.9996–0.9999)	0.0123
CD39+ resting Treg % CD4 Treg -Relative count	Actinic keratosis	20	Simple mode	0.9998 (0.9994–1.0002)	0.4324
CD39+ resting Treg % CD4 Treg -Relative count	Actinic keratosis	20	Weighted mode	0.9997 (0.9995–1.0000)	0.0418
CD28- DN (CD4-CD8-) %DN -Relative count	Actinic keratosis	11	MR Egger	0.9995 (0.9989–1.0001)	0.1092
CD28- DN (CD4-CD8-) %DN -Relative count	Actinic keratosis	11	Weighted median	0.9996 (0.9989–1.0002)	0.1974
CD28- DN (CD4-CD8-) %DN -Relative count	Actinic keratosis	11	Inverse variance weighted	0.9995 (0.9991–1.0000)	0.0359
CD28- DN (CD4-CD8-) %DN -Relative count	Actinic keratosis	11	Simple mode	0.9995 (0.9985–1.0006)	0.4151
CD28- DN (CD4-CD8-) %DN -Relative count	Actinic keratosis	11	Weighted mode	0.9996 (0.9990–1.0002)	0.1815
CD28+ DN (CD4-CD8-) %DN -Relative count	Actinic keratosis	11	MR Egger	1.0005 (0.9999–1.0011)	0.1092
CD28+ DN (CD4-CD8-) %DN -Relative count	Actinic keratosis	11	Weighted median	1.0004 (0.9999–1.0010)	0.1417
CD28+ DN (CD4-CD8-) %DN -Relative count	Actinic keratosis	11	Inverse variance weighted	1.0005 (1.0000–1.0009)	0.0359

(*Continued*)

Table 1 (Continued).

Exposure	Outcome	nsnp	Method	or_ci	pval
CD28+ DN (CD4-CD8-) %DN -Relative count	Actinic keratosis	11	Simple mode	1.0005 (0.9994–1.0015)	0.4060
CD28+ DN (CD4-CD8-) %DN -Relative count	Actinic keratosis	11	Weighted mode	1.0004 (0.9998–1.0010)	0.1972
CD28+ CD45RA- CD8dim %CD8dim -Relative count	Actinic keratosis	17	MR Egger	1.0001 (0.9999–1.0004)	0.1937
CD28+ CD45RA- CD8dim %CD8dim -Relative count	Actinic keratosis	17	Weighted median	1.0001 (0.9999–1.0003)	0.3874
CD28+ CD45RA- CD8dim %CD8dim -Relative count	Actinic keratosis	17	Inverse variance weighted	1.0002 (1.0000–1.0004)	0.0312
CD28+ CD45RA- CD8dim %CD8dim -Relative count	Actinic keratosis	17	Simple mode	1.0001 (0.9998–1.0004)	0.3636
CD28+ CD45RA- CD8dim %CD8dim -Relative count	Actinic keratosis	17	Weighted mode	1.0002 (1.0000–1.0004)	0.1222
CD3 on CD39+ CD8br -MFI	Actinic keratosis	11	MR Egger	1.0005 (0.9994–1.0016)	0.4089
CD3 on CD39+ CD8br -MFI	Actinic keratosis	11	Weighted median	1.0004 (0.9998–1.0011)	0.2046
CD3 on CD39+ CD8br -MFI	Actinic keratosis	11	Inverse variance weighted	1.0008 (1.0002–1.0013)	0.0053
CD3 on CD39+ CD8br -MFI	Actinic keratosis	11	Simple mode	1.0004 (0.9994–1.0015)	0.4159
CD3 on CD39+ CD8br -MFI	Actinic keratosis	11	Weighted mode	1.0004 (0.9997–1.0011)	0.2575
CD28 on CD28+ CD45RA+ CD8br -MFI	Actinic keratosis	9	MR Egger	0.9996 (0.9991–1.0001)	0.1578
CD28 on CD28+ CD45RA+ CD8br -MFI	Actinic keratosis	9	Weighted median	0.9996 (0.9992–1.0000)	0.0725
CD28 on CD28+ CD45RA+ CD8br -MFI	Actinic keratosis	9	Inverse variance weighted	0.9996 (0.9992–0.9999)	0.0222
CD28 on CD28+ CD45RA+ CD8br -MFI	Actinic keratosis	9	Simple mode	0.9995 (0.9988–1.0003)	0.2424
CD28 on CD28+ CD45RA+ CD8br -MFI	Actinic keratosis	9	Weighted mode	0.9996 (0.9992–1.0000)	0.1130
CD25 on CD39+ CD4+ -MFI	Actinic keratosis	11	MR Egger	1.0006 (1.0000–1.0012)	0.0724
CD25 on CD39+ CD4+ -MFI	Actinic keratosis	11	Weighted median	1.0004 (1.0001–1.0008)	0.0205
CD25 on CD39+ CD4+ -MFI	Actinic keratosis	11	Inverse variance weighted	1.0003 (1.0000–1.0006)	0.0294
CD25 on CD39+ CD4+ -MFI	Actinic keratosis	11	Simple mode	1.0004 (0.9997–1.0010)	0.2861
CD25 on CD39+ CD4+ -MFI	Actinic keratosis	11	Weighted mode	1.0004 (1.0001–1.0008)	0.0464
CD39 on CD39+ CD4+ -MFI	Actinic keratosis	14	MR Egger	0.9997 (0.9993–1.0000)	0.1085
CD39 on CD39+ CD4+ -MFI	Actinic keratosis	14	Weighted median	0.9997 (0.9994–1.0000)	0.0389
CD39 on CD39+ CD4+ -MFI	Actinic keratosis	14	Inverse variance weighted	0.9997 (0.9995–1.0000)	0.0360
CD39 on CD39+ CD4+ -MFI	Actinic keratosis	14	Simple mode	0.9999 (0.9992–1.0006)	0.8048
CD39 on CD39+ CD4+ -MFI	Actinic keratosis	14	Weighted mode	0.9997 (0.9994–1.0000)	0.0617
CD4 on CD39+ secreting Treg -MFI	Actinic keratosis	14	MR Egger	0.9990 (0.9977–1.0003)	0.1643
CD4 on CD39+ secreting Treg -MFI	Actinic keratosis	14	Weighted median	0.9995 (0.9989–1.0002)	0.1632
CD4 on CD39+ secreting Treg -MFI	Actinic keratosis	14	Inverse variance weighted	0.9994 (0.9989–0.9999)	0.0187
CD4 on CD39+ secreting Treg -MFI	Actinic keratosis	14	Simple mode	0.9999 (0.9988–1.0010)	0.8367
CD4 on CD39+ secreting Treg -MFI	Actinic keratosis	14	Weighted mode	0.9999 (0.9990–1.0008)	0.7911
CD45RA on CD39+ resting Treg -MFI	Actinic keratosis	6	MR Egger	0.9996 (0.9990–1.0002)	0.2310
CD45RA on CD39+ resting Treg -MFI	Actinic keratosis	6	Weighted median	0.9996 (0.9991–1.0001)	0.1306
CD45RA on CD39+ resting Treg -MFI	Actinic keratosis	6	Inverse variance weighted	0.9995 (0.9991–0.9999)	0.0250
CD45RA on CD39+ resting Treg -MFI	Actinic keratosis	6	Simple mode	0.9992 (0.9984–1.0000)	0.0978
CD45RA on CD39+ resting Treg -MFI	Actinic keratosis	6	Weighted mode	0.9996 (0.9991–1.0001)	0.2111

Abbreviations: MR, Mendelian Randomization; SNPs, single nucleotide polymorphisms; OR, Odds Ratio; IVW, Inverse-variance weighted.

association. However, supplementary analyses suggested no significant association between resting Treg AC and actinic keratosis (Table 1). Significant associations were also identified for CD39+ resting Treg % CD4 Treg (OR: 0.9998, 95% CI: 0.9996–0.9999, p = 0.0123, Figure 3A–D), CD28- DN %DN (OR: 0.9995, 95% CI: 0.9991–1.0000, p = 0.0359, Figure 4A–D), CD28+ DN %DN (OR: 1.0005, 95% CI: 1.0000–1.0009, p = 0.0359, Figure 5), CD28+ CD45RA- CD8dim %CD8dim (OR: 1.0002, 95% CI: 1.0000–1.0004, p = 0.0312, Figure 6), CD3 on CD39+ CD8br (MFI) (OR: 1.0008, 95% CI: 1.0002–1.0013, p = 0.0053, Figure S1), CD28 on CD28+ CD45RA+ CD8br (MFI) (OR: 0.9996, 95% CI: 0.9992–0.9999, p = 0.0222, Figure S2), CD25 on CD39+ CD4+ (MFI) (OR: 1.0003, 95% CI: 1.0000–1.0006, p = 0.0294, Figure S3), CD39 on CD39+ CD4+ (MFI) (OR: 0.9997, 95% CI: 0.9995–1.0000, p = 0.0360, Figure S4), CD4 on CD39+ secreting Treg (MFI) (OR: 0.9994, 95% CI: 0.9989–0.9999, p = 0.0187, Figure S5), and CD45RA on CD39+ resting Treg (MFI) (OR: 0.9995, 95% CI: 0.9991–0.9999, p = 0.0250, Figure S6). However, no significant associations were found in supplementary analyses. The complete MR analysis results were presented in Supplemental Table 5.

Heterogeneity, directional pleiotropy, and horizontal pleiotropy among the genetic instruments were assessed using Cochran's Q test, MR-Egger, and MR-PRESSO. These analyses demonstrated the absence of significant heterogeneity or horizontal pleiotropy among the examined SNPs, confirming the reliability of the results (All P > 0.05, Supplemental

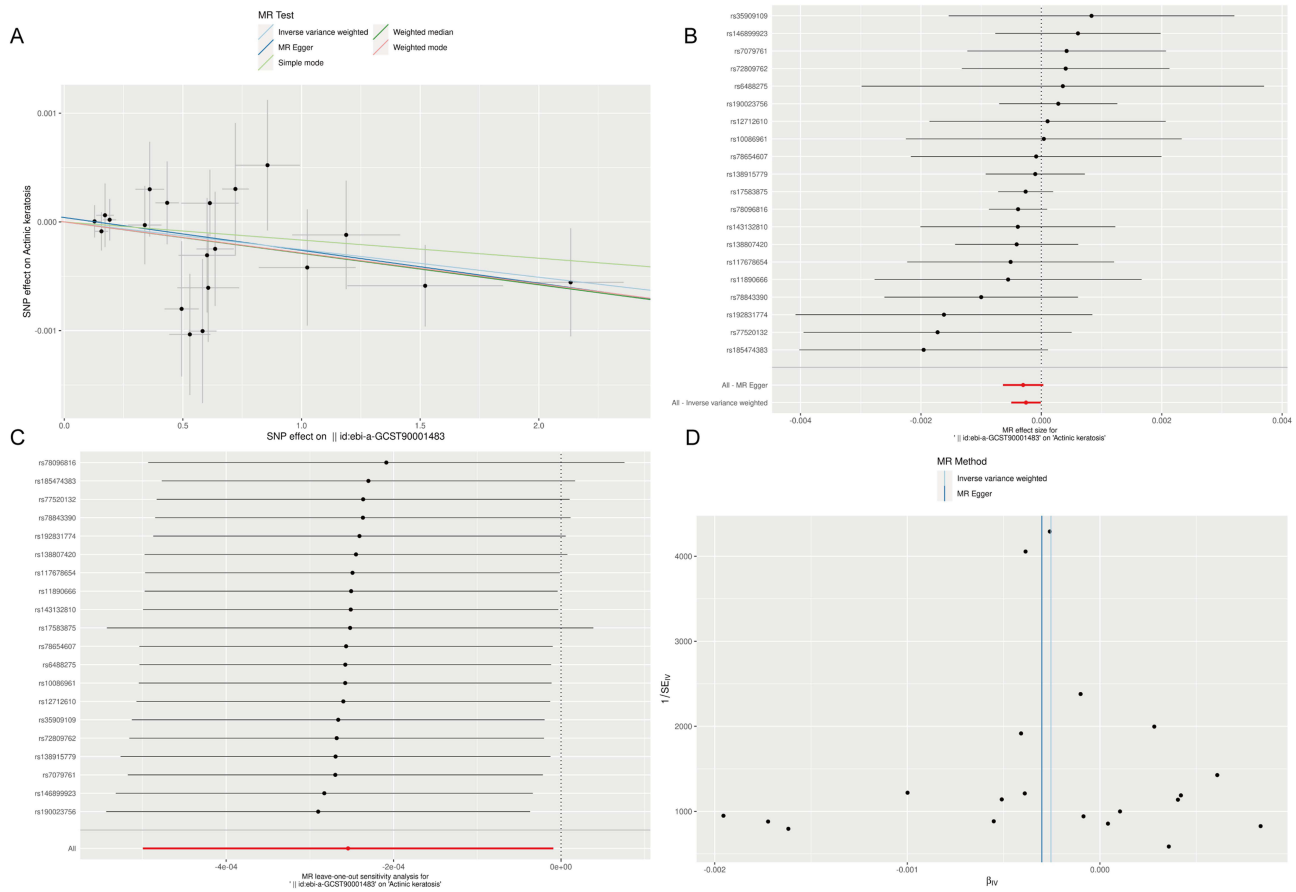

Figure 2 Analysis of the casual effect of Resting Treg AC -Absolute count on actinic keratosis through MR. (**A**) A scatter plot illustrating the association between Resting Treg AC -Absolute count and risk of actinic keratosis. (**B**) The MR estimate is visualized in a forest plot. (**C**) Leave-one-out analysis validates the robustness of the result. (**D**) A funnel plot evaluates consistency and potential heterogeneity.
Abbreviation: MR, Mendelian Randomization.

Tables 6 and 7). The robustness of the findings was confirmed through leave-one-out analyses, demonstrating that the removal of any single SNP did not significantly alter the observed associations (as shown in Figures 2C–6C and S1C–S6C). Funnel plots indicated balanced pleiotropy, further supporting the robustness of the results (Figures 2D–6D and S1D–S6D).

Discussion

The MR analysis suggested significant associations between several Treg cell traits and actinic keratosis. Specifically, resting Treg AC was significantly associated with actinic keratosis. Similarly, significant associations were identified for CD39+ resting Treg % CD4 Treg, CD28- DN %DN, CD28+ DN %DN, CD28+ CD45RA- CD8dim %CD8dim, CD3 on CD39+ CD8br (MFI), CD28 on CD28+ CD45RA+ CD8br (MFI), CD25 on CD39+ CD4+ (MFI), CD39 on CD39+ CD4 + (MFI), CD4 on CD39+ secreting Treg (MFI), and CD45RA on CD39+ resting Treg (MFI). These findings highlight the potential role of Treg cells in influencing actinic keratosis. Additionally, sensitivity analyses, including MR-Egger and MR-PRESSO, further confirmed the reliability of the finding.

Studies based on observations have hinted at a possible link between Treg cells and actinic keratosis. Treg cells, particularly the Foxp3+ subset, are key regulators of immune responses, maintaining tolerance and preventing autoimmune reactions. Their suppressive functions can influence the local immune environment, potentially affecting the development or progression of pre-malignant lesions such as actinic keratosis.[20] Despite limited reports on the association between Treg cells and actinic keratosis, it is important to note that actinic keratosis is a precancerous lesion for cuSCC. Certain research has indicated a connection between Treg cells and cuSCC. Rollison et al found that elevated

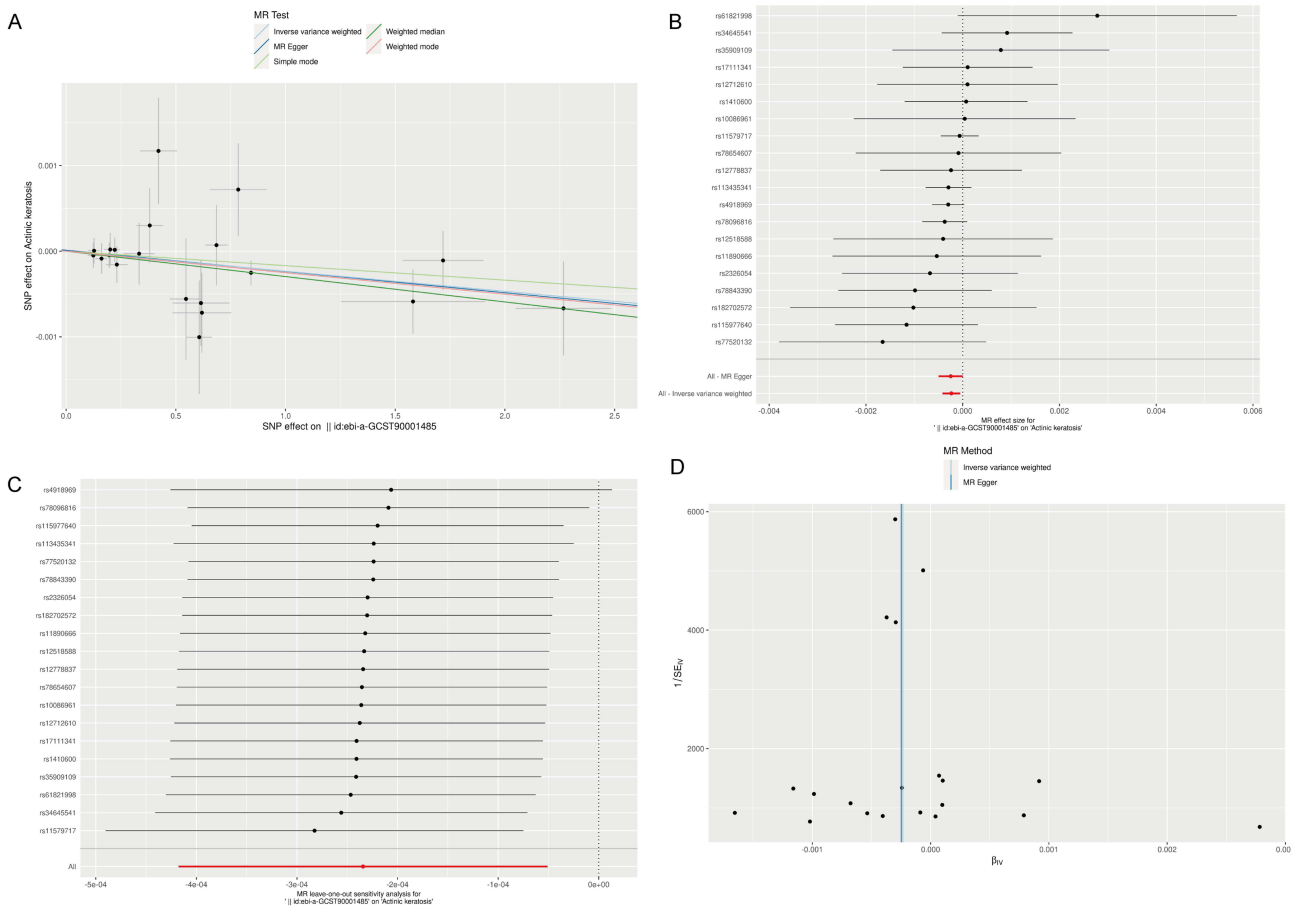

Figure 3 Analysis of the casual effect of CD39+ resting Treg % CD4 Treg -Relative count on actinic keratosis through MR. (**A**) A scatter plot illustrating the association between CD39+ resting Treg % CD4 Treg -Relative count and risk of actinic keratosis. (**B**) The MR estimate is visualized in a forest plot. (**C**) Leave-one-out analysis validates the robustness of the result. (**D**) A funnel plot evaluates consistency and potential heterogeneity.
Abbreviation: MR, Mendelian Randomization.

baseline levels of circulating CCR4hi Tregs were notably linked to a higher risk of developing cuSCC. People with high levels of both CCR4hi Tregs and UV radiation exposure had four times the likelihood of developing cuSCC compared to those with lower levels.[21] Azzimonti et al concluded that the intratumoral presence of Tregs, along with low CD8+/Foxp3+ CD25+ ratios and elevated TGF-β1 and IL-10, may contribute to cuSCC aggressiveness.[22] Therefore, analyzing the potential relationship between Treg cells and actinic keratosis is meaningful. Investigating this potential association could provide valuable insights into the mechanisms underlying skin carcinogenesis and help identify novel therapeutic strategies to prevent the development of cuSCC from actinic keratosis. Foxp3 is a transcription factor that is essential for the development, functionality, and stability of Treg cells.[23] Treg cells marked by Foxp3 display immunosuppressive characteristics that directly hinder effector cells from attacking tumor cells. Foxp3+ Tregs induce immunosuppression via multiple mechanisms, including secreting IL-10 and TGF-β, inhibiting the activation and proliferation of CD4+ T-cells, and suppressing the activation of dendritic cell activation and cytokine production.[24] The immunosuppressive environment created by Foxp3+ Tregs facilitates tumor tolerance and persistence by suppressing effective immune responses, thus enabling further proliferative modifications. Previous studies have reported a relationship between Foxp3+ Tregs and actinic keratosis. However, the trait used in this study did not include information on Foxp3. Therefore, further research incorporating more GWAS data is necessary to comprehensively investigate this relationship.

This MR study identified several positive traits of resting Treg cells, including resting Treg AC, CD39+ resting Treg % CD4 Treg, and CD45RA on CD39+ resting Treg. Treg cells are a crucial subset of T cells responsible for maintaining

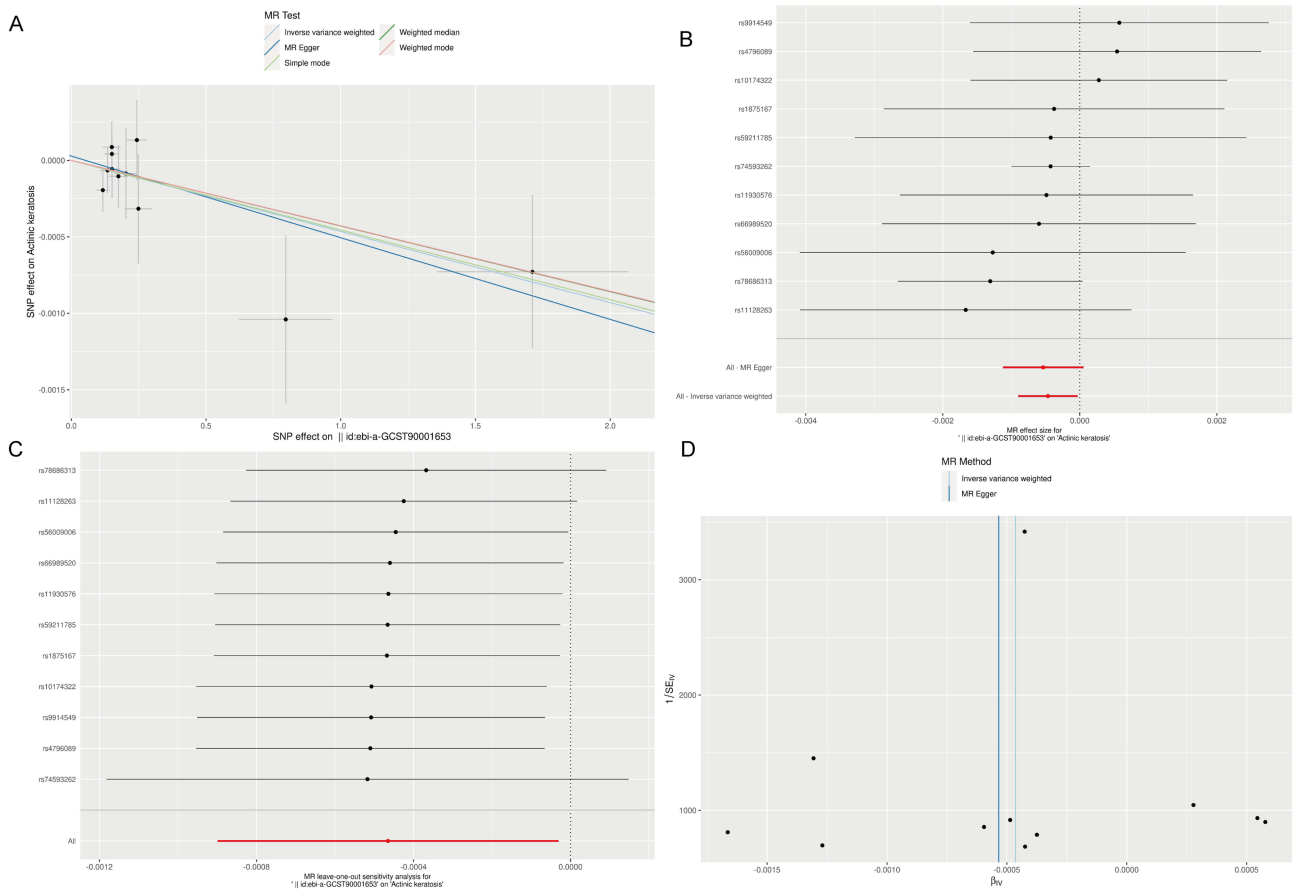

Figure 4 Analysis of the casual effect of CD28- DN (CD4-CD8-) %DN -Relative count on actinic keratosis through MR. (**A**) A scatter plot illustrating the association between CD28- DN (CD4-CD8-) %DN -Relative count and risk of actinic keratosis. (**B**) The MR estimate is visualized in a forest plot. (**C**) Leave-one-out analysis validates the robustness of the result. (**D**) A funnel plot evaluates consistency and potential heterogeneity.
Abbreviation: MR, Mendelian Randomization.

immune homeostasis. Resting Tregs can suppress the activation and proliferation of effector T cells, dendritic cells, and other immune cells that are crucial for mounting an effective anti-tumor response.[23] Resting Tregs secrete anti-inflammatory cytokines such as IL-10 and TGF-β.[25] These cytokines could help reduce the chronic inflammation induced by UV radiation, potentially slowing the progression of actinic keratosis to cuSCC. Previous observational research has failed to document a direct link between resting Treg cells and actinic keratosis. However, our MR study has identified a genetic relationship between resting Treg cells and actinic keratosis. This discovery indicates that resting Treg cells might play a role in the development and advancement of actinic keratosis. Therefore, further research is necessary to explore the molecular mechanisms through which resting Treg cells influence the pathogenesis of actinic keratosis.

Our study's findings provide important insights into the potential clinical relevance of Treg cell subsets in the pathogenesis of actinic keratosis. Specifically, the identified protective Treg subsets, such as resting Treg AC, suggest that immune status may play a critical role in actinic keratosis development and progression. For clinicians, this underscores the importance of considering patients' immune profiles when managing actinic keratosis, as these Treg subsets could serve as promising targets for novel immunomodulatory interventions aimed at preventing progression to cutaneous squamous cell carcinoma. From a patient perspective, our research sheds light on why susceptibility to actinic keratosis varies despite similar ultraviolet exposure, highlighting the influence of genetic factors related to Treg function. In the long term, these genetic insights may enable the creation of personalized risk assessment models that identify individuals at higher risk, facilitating proactive skin surveillance and earlier intervention. Overall, these findings emphasize the value

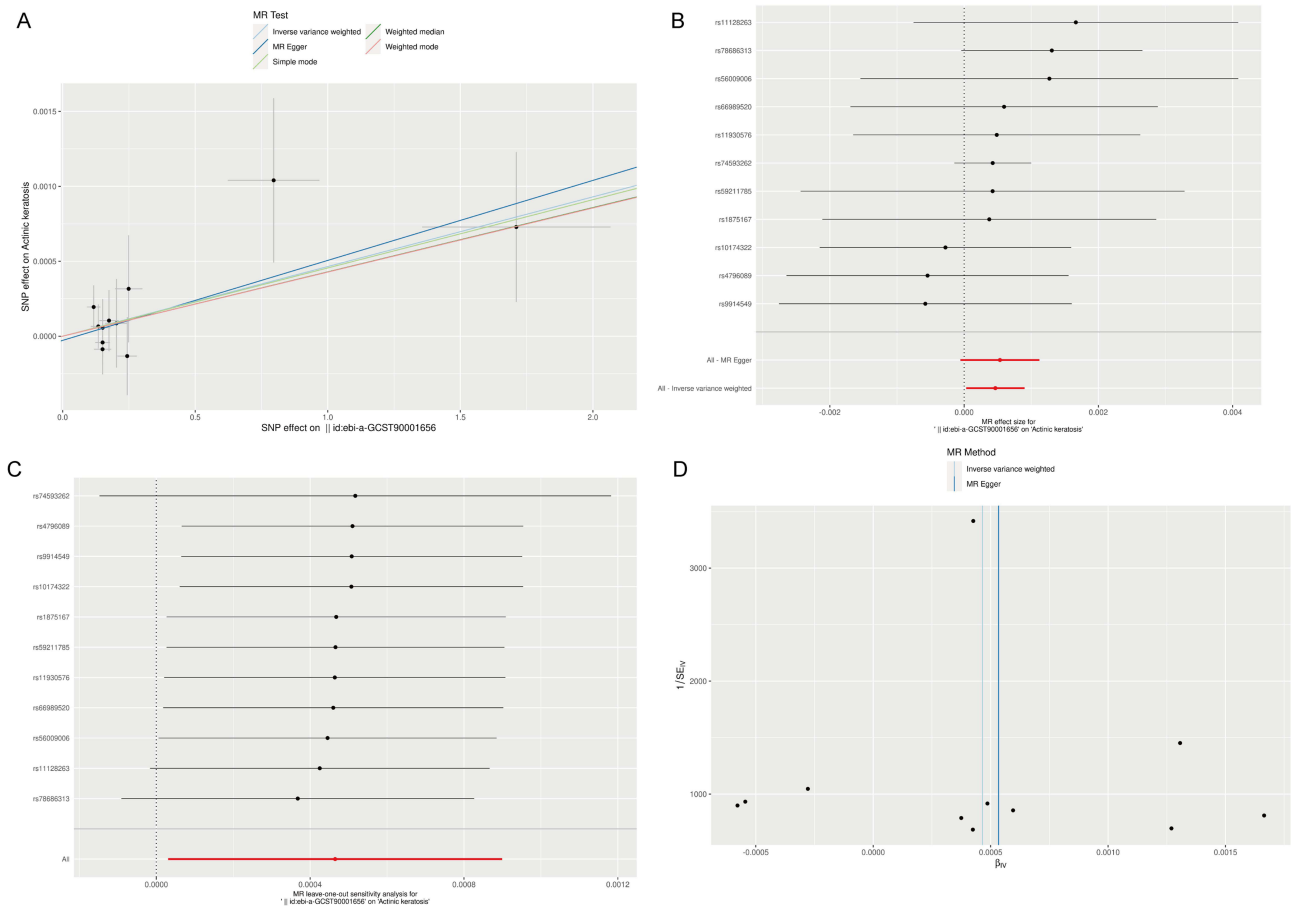

Figure 5 Analysis of the casual effect of CD28+ DN (CD4-CD8-) %DN -Relative count on actinic keratosis through MR. (**A**) A scatter plot illustrating the association between CD28+ DN (CD4-CD8-) %DN -Relative count and risk of actinic keratosis. (**B**) The MR estimate is visualized in a forest plot. (**C**) Leave-one-out analysis validates the robustness of the result. (**D**) A funnel plot evaluates consistency and potential heterogeneity.
Abbreviation: MR, Mendelian Randomization.

of integrating immunological evaluation into comprehensive actinic keratosis management strategies to improve prevention and clinical outcomes.

Several advantages were demonstrated in the study investigating the causal association between Treg cells and actinic keratosis through two-sample MR analysis. Firstly, stringent criteria were employed for selecting IVs to ensure robust and reliable findings. Secondly, multiple analytical methods, including Cochran's Q test, MR-Egger regression and MR-PRESSO, were utilized to address potential biases and pleiotropy. Thirdly, a total of 167 diverse Treg traits were analyzed to capture different aspects of Treg biology. These strengths provided a thorough and reliable investigation into the potential causal role of Treg cells in actinic keratosis. The MR study had several limitations. Firstly, the study relied on genetic variants sourced from GWAS predominantly conducted in populations with specific ancestral backgrounds, posing questions about the broad applicability and generalizability of the results across genetically diverse populations. Secondly, although this study examined the relationship between Treg cells and actinic keratosis, it's crucial to acknowledge that the underlying causes and development of actinic keratosis are complex, involving an interplay of genetic, environmental, and lifestyle factors. Thirdly, factors such as lifestyle, environmental exposures, and genetic elements not captured by IVs that influence both Treg cells and actinic keratosis could still bias the estimates. Additional experimental research is required to uncover the fundamental biological processes driving the outcomes of this study.

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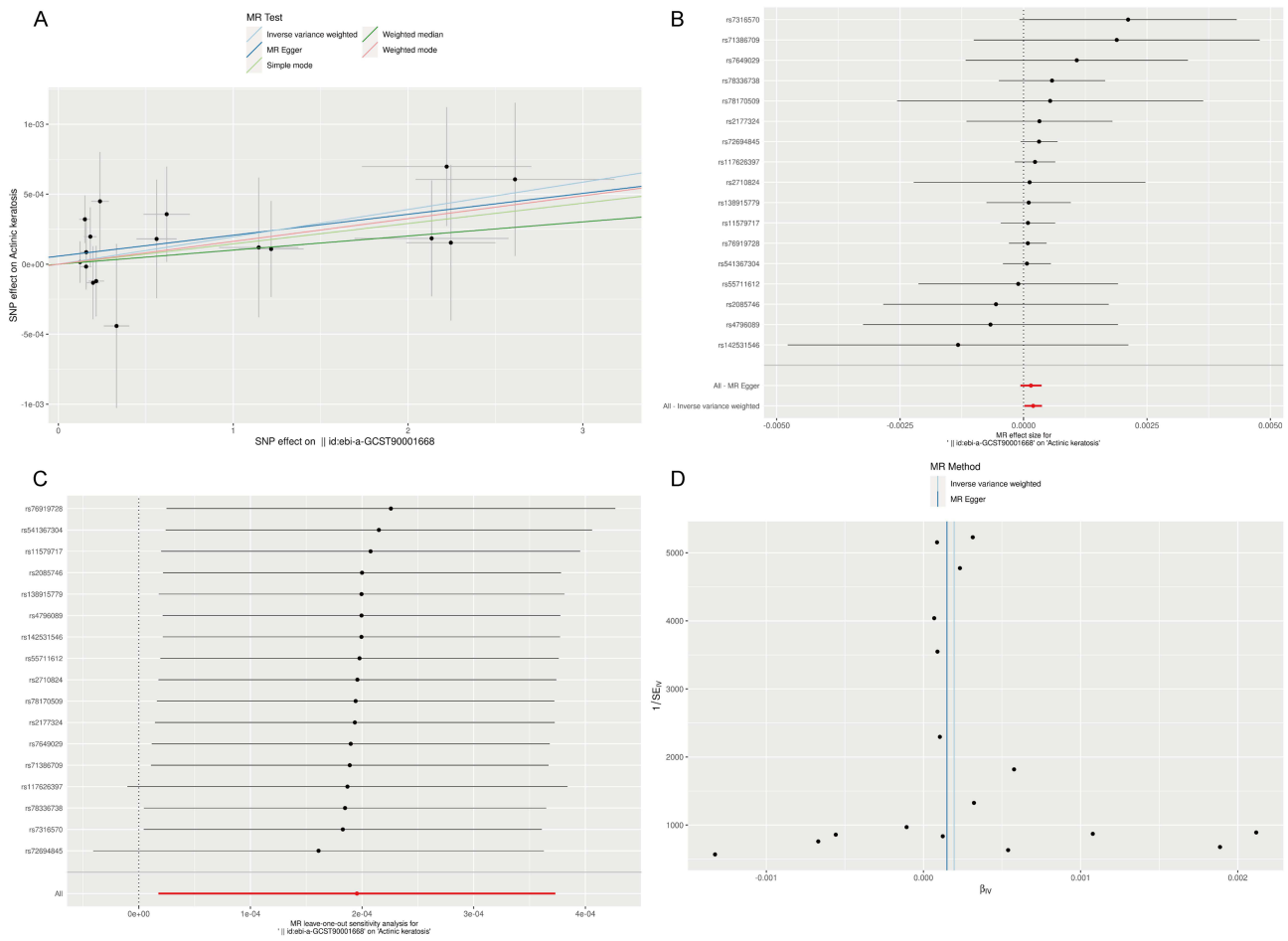

Figure 6 Analysis of the casual effect of CD28+ CD45RA- CD8dim %CD8dim -Relative count on actinic keratosis through MR. (**A**) A scatter plot illustrating the association between CD28+ CD45RA- CD8dim %CD8dim -Relative count and risk of actinic keratosis. (**B**) The MR estimate is visualized in a forest plot. (**C**) Leave-one-out analysis validates the robustness of the result. (**D**) A funnel plot evaluates consistency and potential heterogeneity.
Abbreviation: MR, Mendelian Randomization.

Conclusion

The MR analysis suggested a potential causal relationship between specific Treg cell traits and the development of actinic keratosis. These results highlight the importance of Tregs in the pathogenesis of actinic keratosis and underscore the need for further research to explore the molecular mechanisms underlying this association. Understanding the role of Tregs in actinic keratosis could provide new insights into preventive and therapeutic strategies for this common precancerous skin condition.

Data Sharing Statement

All data generated or analysed during this study are included in this published article.

Ethics Approval and Consent to Participate

This Mendelian randomization study was conducted exclusively using publicly available, anonymized, summary-level statistics from previously published GWAS data. Therefore, this secondary analysis did not require specific ethical approval or informed consent. Our study adheres to the exemption criteria outlined in the "Measures for Ethical Review of Life Science and Medical Research Involving Human Subjects" (China, February 18, 2023), specifically under Article 32, item 1 (research utilizing legally obtained public data) and item 2 (research utilizing anonymized information data).

All original studies that provided the summary data had received approval from their respective institutional review boards and obtained informed consent from all participants.

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

All authors declare that they have no any conflict of interests.

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