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ORIGINAL RESEARCH

Mendelian Randomization Identifies CD25+ CD4 + Tregs and Plasma Proteins in Androgenetic Alopecia Pathogenesis

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Background: Androgenetic alopecia (AGA) is a chronic form of hair loss influenced by various factors, with increasing focus on the role of immune cell-driven follicular microinflammation. However, the precise immune phenotypes involved and their causal relationship with AGA remain poorly understood. This study aims to identify and validate the causal role of immunophenotypes in AGA using Mendelian randomization (MR) integrated with multi-omics data, and to explore the mediating role of plasma proteins in this relationship.

Materials and Methods: We utilized publicly accessible Genome-Wide Association Studies (GWAS) summary statistics encompassing immune phenotypes, plasma proteins, and AGA. MR analyses using inverse variance weighted (IVW), MR-Egger regression, and weighted median methods were performed to examine the causal links between 731 immune phenotypes and AGA. Sensitivity analyses were conducted to ensure robustness and address heterogeneity and pleiotropy. Linkage disequilibrium score regression was employed to assess genetic correlations, while Steiger filtering confirmed the causal direction. A multivariable MR approach was used to estimate the direct effects of each exposure on AGA, accounting for confounding factors. Additionally, mediation analysis of 3622 plasma proteins identified potential mediators in the immune-AGA pathway.

Results: We identified elevated CD25 on secreting CD4 regulatory T cell (Treg) as an independent genetic risk factor for AGA. Mediation analysis revealed five plasma proteins, SDF-1, GPIbα, KIR3DS1, MVI, and WFDC5, as key mediators in the immune-AGA axis.

Conclusion: This study established that specific immune cell phenotypes, particularly CD25 on secreting CD4 Treg, were causally linked to AGA, with plasma proteins mediating this effect. These findings provide new insights into AGA's immunological mechanisms and suggest potential immune-targeted therapeutic strategies.

Keywords: androgenetic alopecia, causality, immune cell, plasma protein, Mendelian randomization

Introduction

Androgenetic alopecia (AGA) is the most prevalent form of non-scarring hair loss, characterized by the progressive miniaturization of hair follicles (HFs) in a distinct pattern. Clinically, male patients typically present with a receding frontal hairline accompanied by thinning at the vertex, whereas female patients usually exhibit diffuse thinning over the crown while retaining the frontal hairline. By age 70, approximately 80% of men and 50% of women are affected, with the incidence rising with age.[1] Traditionally, the pathogenesis of AGA has been attributed mainly to genetic predisposition and dysregulated androgen metabolism. Specifically, the activation of androgen receptor (AR) signaling pathways

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mediated by dihydrotestosterone (DHT) leads to transcriptional changes that result in follicular regression.[1] However, emerging evidence demonstrates immune-mediated microinflammation as a key feature of AGA.

A retrospective study of 58 AGA specimens (5 male, 53 female) revealed perifollicular lymphocytic infiltrates in 87.9% of cases, with inflammation predominantly localized to the infundibular and isthmic regions.[2] Importantly, this inflammation was frequently adjacent to miniaturized follicles, and severe inflammation surrounded highly miniaturized follicles. Follicular spongiosis, a marker of epithelial barrier disruption, was observed in 29.3% of these specimens.[2]

These findings align with earlier histological evidence: Analysis of 47 scalp samples from 23 male AGA patients showed perifollicular infiltration of mononuclear and lymphocytic cells, mast cell degranulation, and fibroblast activation in nearly half of cases.[3] Further immunohistochemical studies of progressive alopecia patients (3 male, 1 female) confirmed follicular infiltration of CD4+ T-cells specifically at the bulge region, while control tissues exhibited minimal T-cell presence.[4]

Critically, immune-mediated inflammation and resultant reactive oxygen species (ROS) can damage HF cells and their homeostasis.[5,6] They can also stabilize AR, upregulate its expression, and enhance androgen sensitivity even under normal androgen level,[7] further hindering follicle function. Despite these insights, the precise mechanisms linking immune cells to AGA pathogenesis remain unclear.

Mendelian randomization (MR) is a statistical approach that uses genetic variants as instrumental variables (IVs) to infer causal relationships between exposures and outcomes. By utilizing single-nucleotide polymorphisms (SNPs) strongly associated with modifiable exposures as IVs, MR can simulate randomized controlled trials, thus minimizing confounding bias and reverse causality.[8,9] Recent genome-wide association studies (GWAS) have identified several genetic loci associated with immune cell subsets and their functional characteristics. This opens up opportunities to explore the causal roles of immune cells in complex diseases.[10] While AGA's genetics are well-characterized, the interplay between immune cell dynamics and disease susceptibility is poorly defined. Notably, shared genetic pathways involving inflammation and autoimmunity suggest a potential immunological axis in AGA pathogenesis. However, no prior study has systematically employed MR to evaluate the causal roles of specific immune cell subsets in AGA.

In this study, we conducted a two-sample MR (TSMR) analysis, integrating large-scale GWAS datasets to systematically assess the causal relationships between 731 immune cell traits and AGA risk. Additionally, we performed mediation analysis to investigate the potential mediating roles of plasma proteins in the relationship between immune cells and AGA. By elucidating the causal pathways, our study aims to provide novel insights into the immunogenetic mechanisms underlying AGA and advance targeted immune-based therapeutic strategies.

Materials and Methods

Study Framework

The study design is illustrated in Figure 1. MR employs genetic variants as IVs to infer causal relationships. For valid causal inference, IVs must satisfy three core assumptions: (1) a strong association with immune cell traits, (2) independence from confounding factors, and (3) exclusivity of their effect on AGA through immune cells.

GWAS Data Source

Immunophenotype data were derived from IEU OpenGWAS project (https://gwas.mrcieu.ac.uk/) conducted by Orrù et al[11] which analyzed 3757 Sardinian individuals without cohort overlap. The dataset included 731 immune traits, categorized into absolute cell counts (118), median fluorescence intensities (389), morphological parameters (32), and relative cell counts (192). Genotyping involved 20,143,392 SNPs across these traits, utilizing high-density arrays or imputation based on a Sardinian sequence-based reference panel.[12] Covariates such as sex, age, and age^2 were adjusted for in subsequent correlation analyses.

Human plasma proteome data, encompassing measurements of 3622 plasma proteins, were sourced from IEU OpenGWAS Project (https://gwas.mrcieu.ac.uk/). This project was conducted in 3301 healthy individuals of European descent from the INTERVAL study, as described by Sun et al.[13] Plasma protein concentrations were quantified using SomaLogic Inc. (Boulder, CO, USA), via a multiplexed, aptamer (SOMAmer)-based assay.[14] Samples were genotyped

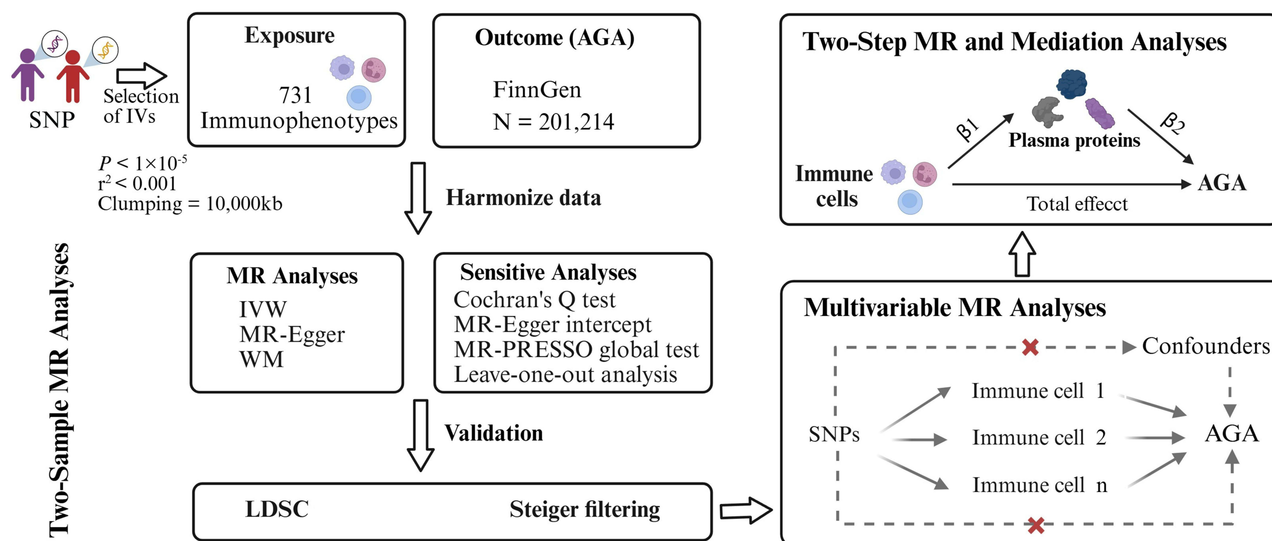

Figure 1 The schematic illustration of this research. Created in BioRender. Yamay, (L) (2025) https://BioRender.com/dtu0xvj.

with the Affymetrix Axiom UK Biobank array, followed by imputation using the 1000 Genomes Phase 3-UK10K reference panel. Protein abundances were adjusted for age, sex, processing time, and the first three genetic principal components. The AGA GWAS dataset, which was obtained from the FinnGen Biobank (Round 9, https://www.finngen.fi/en), included 201,214 individuals of European descent, minimizing population stratification bias. Importantly, the three GWAS datasets were entirely independent, with no overlap, ensuring data independence.

Selection Criteria for IVs

To identify SNPs as IVs from the GWAS datasets, stringent quality control measures were applied. For immune traits with limited SNP availability, a relaxed threshold ($P < 1\times10^{-5}$) was applied to expand the candidate IV pool, enhancing MR statistical power and preventing analytical failure due to insufficient instruments.[10,15,16] Linkage disequilibrium (LD) clumping ($r^2 < 0.001$; clumping window = 10,000 kb) was performed to ensure genetic independence and minimize correlations among SNPs.[17] Weak instrument bias was assessed using the F statistic, calculated as $F = R^2(n-k-1)/(1-R^2)k$, where R^2 represents the proportion of variance explained by the SNPs, n is the sample size, and k denotes the number of SNPs.[18] SNPs with an F statistic greater than 10 were retained for analysis, while those below this threshold were excluded.[19]

TSMR Analyses

The causal relationships between immunophenotypes and AGA were evaluated using three MR methods: inverse variance weighted (IVW), MR-Egger regression, and weighted median (WM). The IVW approach combines Wald ratios of individual IVs within a meta-analysis framework. This method assumes that valid MR assumptions hold and that there is an absence of horizontal pleiotropy.[20] MR-Egger regression accounts for pleiotropic effects under the assumption that these effects are independent of genetic associations with immunophenotypes, improving causal estimation accuracy.[21] In contrast, the WM method provides valid causal estimates even if up to 50% of the IVs are invalid.[22]

To ensure the robustness of our findings, sensitivity analyses were conducted. Heterogeneity among IVs was assessed using Cochran's Q test within the IVW framework, with significance defined as $P < 0.05$.[23] Pleiotropy, where a single genetic variant influences multiple traits, was evaluated using the MR-Egger intercept and the MR pleiotropy residual sum and outlier (MR-PRESSO) global test, with $P > 0.05$ indicating negligible pleiotropy.[24] Additionally, a leave-one-out analysis was performed to confirm the stability of causal estimates by sequentially excluding individual SNPs.

The results from all methods (IVW, MR-Egger, and WM) demonstrated consistent directionality and statistical significance ($P < 0.05$). No significant heterogeneity or pleiotropy was detected in the IVW analysis, supporting a potential causal relationship between immunophenotypes and AGA.

LD Score Regression and Steiger Filtering

To address the potential confounding effects from shared genetic architecture between immunophenotypes and AGA, we employed LD score regression (LDSC). This method assesses genetic linkage to determine whether the observed causal relationships are influenced by overlapping genetic factors.[25,26] Additionally, Steiger filtering was applied to evaluate the directionality of causality. Results marked as "true" indicated unidirectional causality from immunophenotypes to AGA, with no evidence of bidirectional effects.[27]

Multivariable MR Analyses

Multivariable MR (MVMR) analysis was employed to assess the combined effects of multiple risk factors while isolating the direct impact of each exposure on the outcome, thereby controlling for potential confounding from other exposures.[28] In this study, MVMR was applied to selected immune cell types to account for their interactive effects, providing a more precise estimation of each immune cell's unique contribution to AGA. The primary analytical method utilized was the MVMR-IVW approach, supplemented by Cochran's Q test to evaluate heterogeneity.

Two-Step MR and Mediation Analyses

Mediation analysis was employed to dissect the total effect of immune cell traits on AGA into two components: a direct effect and an indirect (mediated) effect transmitted through specific plasma proteins.[29] This approach was designed to determine whether plasma proteins act as causal intermediaries in the pathway linking immune dysregulation to AGA. Initially, TSMR was used to estimate the causal effect of immune cells on candidate plasma proteins (yielding coefficient β_1). A separate MR analysis was then performed to assess the effect of these plasma proteins on AGA (yielding coefficient β_2). The indirect (mediated) effect was calculated as $\beta_1 \times \beta_2$. The total effect of immune cells on AGA, previously derived from MR analysis, enabled the computation of the direct effect by subtraction (direct effect = total effect - mediation effect). The mediation proportion, representing the percentage of the total effect mediated through a given plasma protein, was calculated as (mediation effect / total effect) × 100%. Confidence intervals were estimated using the delta method.[30] A mediation effect was considered significant if both steps showed statistically meaningful results and a direct causal relationship was established between the exposure and outcome.

Statistical Analysis

All statistical analyses were conducted using R (version 4.2.3) with the packages TwoSampleMR (version 0.5.7), MRPRESSO (version 1.0), and MendelianRandomization (version 0.8.0).

Results

Causality of Immunophenotypes on AGA

Using the IVW method, 35 immunophenotypes were identified as potentially associated with AGA development (Table S1 and Figure 2). Among these, four immunophenotypes demonstrated a statistically significant causal relationship with AGA ($P < 0.05$) when validated across MR-Egger regression and the WM methods. The results were consistent across all three methods, with no contradictory trends observed (Figure 3). Specifically, CD25 on secreting CD4 regulatory T cell (Treg) ($OR_{IVW} = 1.201$, 95% CI: 1.039–1.388, $P = 0.013$) and CD64 on CD14+ CD16- monocyte ($OR_{IVW} = 1.088$, 95% CI: 1.012–1.169, $P = 0.022$) were identified as risk factors for AGA. Conversely, CD3 on CD45RA+ CD4+ T cell ($OR_{IVW} = 0.822$, 95% CI: 0.686–0.985, $P = 0.034$) and CD28- CD8dim T cell Absolute Count ($OR_{IVW} = 0.824$, 95% CI: 0.681–0.997, $P = 0.046$) demonstrated protective effects against AGA.

Cochran's Q test revealed no significant heterogeneity among the four immunophenotypes ($P > 0.05$). Additionally, both the MR-Egger intercept and MR-PRESSO global test detected no outliers ($P > 0.05$) (Table S2). The leave-one-out

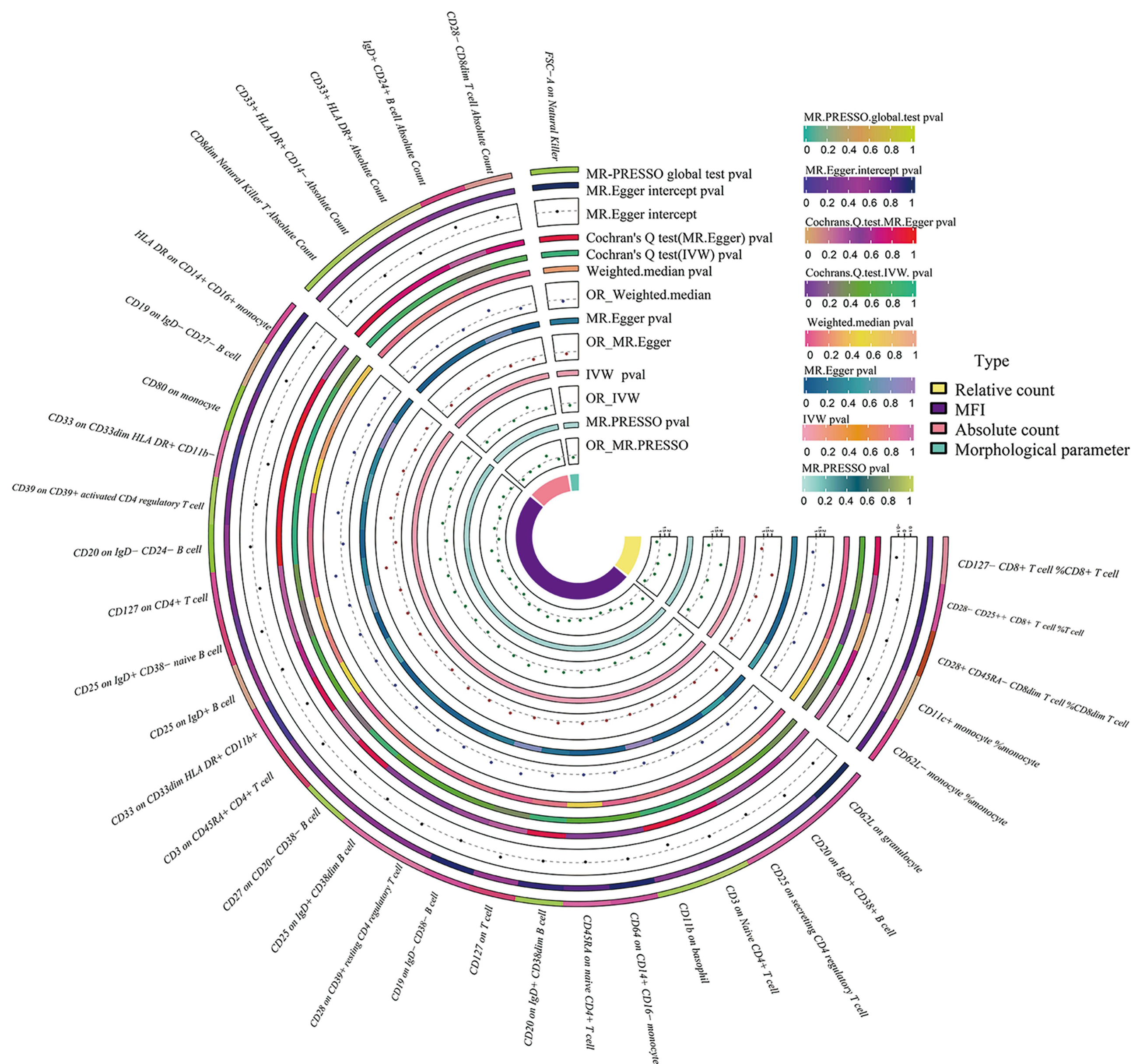

Figure 2 Causal effects of Immunophenotypes on AGA. The causality between 731 immune cells and AGA was analyzed by IVW method.
Abbreviations: IVW, Inverse variance weighted; WM, weighted median.

analysis confirmed the robustness of the results, demonstrating that no single SNP disproportionately influenced the causal estimates (Figure S1).

Examination of Genetic Correlation and Causal Direction

LDSC revealed minimal genetic correlation for three immunophenotypes: CD25 on secreting CD4 Treg (Rg = −0.514, Se = 0.564, P = 0.361), CD64 on CD14+ CD16- monocyte (Rg = −0.467, Se = 0.323, P = 0.148), and CD3 on CD45RA+ CD4+ T cell (Rg = −0.274, Se = 0.392, P = 0.484). These results suggest no significant genetic overlap between AGA and these immune cell traits, supporting the independence of their genetic associations and validating the causal inferences (Table S3). Due to insufficient SNP heritability, genetic correlation could not be estimated for CD28- CD8dim T cell absolute count. Steiger filtering further confirmed the directionality of causality, indicating no evidence of reverse causality between the identified immunophenotypes and AGA (Table S4).

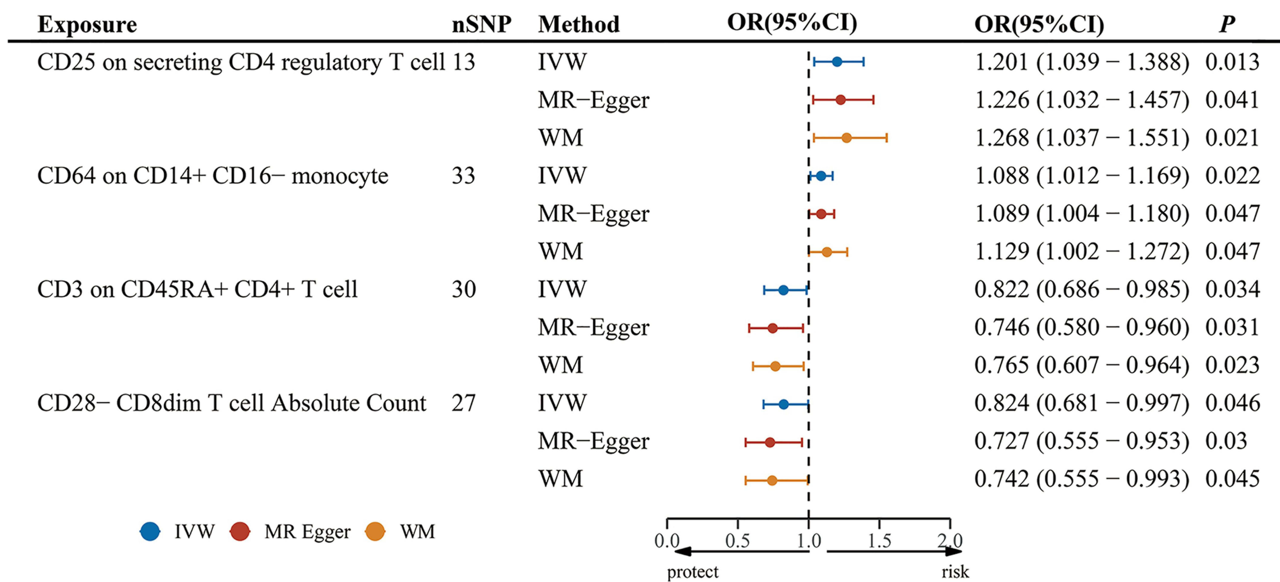

Figure 3 Causal estimation of significant immunophenotypes. Forest plots depicted the potential causal relationships of immunophenotypes with AGA by IVW, MR-Egger regression and WM.
Abbreviations: IVW, Inverse variance weighted; WM, weighted median.

Independent Causal Effect of Immunophenotypes in AGA

MVMR analysis was conducted on the identified three immunophenotypes without significant genetic overlap between AGA. Only CD25 on secreting CD4 Treg showed an independent causal effect on AGA (OR = 1.172, 95% CI: 1.011–1.359, $P = 0.035$), while the associations for CD64 on CD14+ CD16- monocyte and CD3 on CD45RA+ CD4+ T cell became non-significant after multivariable adjustment (Figure 4). Heterogeneity testing in the MVMR-IVW analysis indicated no significant variability across genetic instruments ($P > 0.05$), suggesting that Tregs may play a key role in AGA pathogenesis.

Mediation Analysis: Linking Immune Cells to AGA Through Plasma Proteins

To investigate whether specific plasma proteins mediate the causal relationship between CD25 on secreting CD4 Treg and AGA, we conducted a mediation analysis using data on 3622 plasma proteins. Among the tested proteins, five were identified as statistically significant mediators, suggesting that they may partially transmit the effect of immune dysregulation to HF pathology (Table 1). Stromal cell-derived factor 1 (SDF-1) was found to mediate 13.7% of the total effect (β = 0.025). Similarly, platelet glycoprotein Ib alpha chain (GPIbα) accounted for 14.4% of the mediation effect (β = 0.026). Killer cell immunoglobulin-like receptor 3DS1 (KIR3DS1) contributed 10.0% to the total mediation (β

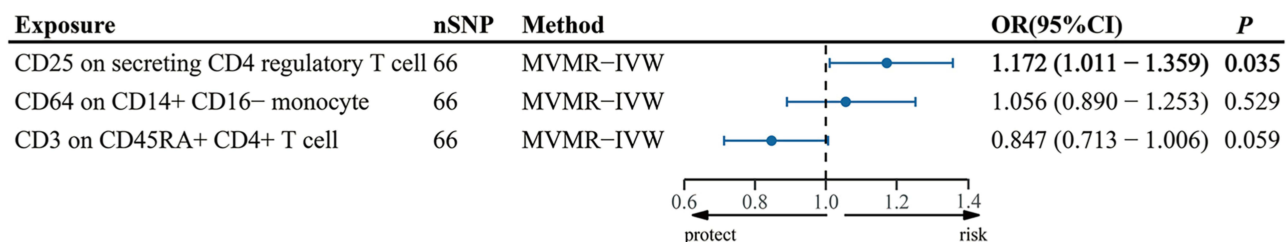

Figure 4 Independent causal effect of immunophenotypes in AGA. Forest plots depicted the potential causal relationships of three immunophenotypes with AGA by MVMR-IVW method. Bold font for OR and *P*-values indicates statistically significant effects ($P < 0.05$). The MVMR analysis revealed that genetically predicted levels of CD25 on secretory CD4 Tregs were significantly associated with an increased risk of AGA.
Abbreviation: MVMR-IVW, multivariable Mendelian randomization- inverse variance weighted.

Table 1 Mediation Analysis of CD25 on Secreting CD4 Tregs with AGA

Mediator	Direct Effect β_1(95% CI)	P_1	Direct Effect β_2(95% CI)	P_2	Direct Effect β	Mediation Effect β	Mediation Proportion
SDF-1	0.061(0.002–0.120)	0.042	0.411(0.020–0.803)	0.039	0.158	0.025	13.7%
GPIbα	0.068(0.013–0.122)	0.015	0.390(0.089–0.691)	0.011	0.157	0.026	14.4%
KIR3DS1	0.050(0.004–0.096)	0.034	0.367(0.002–0.732)	0.049	0.165	0.018	10.0%
Unconventional MVI	0.054(0.004–0.105)	0.035	0.453(0.090–0.817)	0.015	0.159	0.025	13.4%
WFDC5	0.050(0.004–0.096)	0.035	0.397(0.060–0.735)	0.021	0.164	0.020	10.8%

= 0.018). Furthermore, unconventional myosin-VI (MVI) mediated 13.4% of the effect (β = 0.025), while WAP four-disulfide core domain protein 5 (WFDC5) explained 10.8% of the total effect (β = 0.020).

Discussion

Our MR analysis supported a causal immunological contribution to the pathogenesis of AGA, identifying elevated expression of CD25 on secreting CD4 Tregs as a potential risk factor independent of major confounders. This finding implicates dysregulation of the IL-2/CD25 axis in Treg biology as a mechanism directly disrupting HF homeostasis. Importantly, our results align with evidence that AGA, traditionally regarded as a non-inflammatory and androgen-driven condition, exhibits significant inflammatory components.[2–4]

Tregs play a pivotal role in maintaining immune tolerance and homeostasis within the skin and HF microenvironment. Under physiological conditions, they prevent excessive immune responses that could otherwise damage HFSCs or disrupt the hair cycle. Ali et al[31] demonstrated that Treg-derived Jag1 activates Notch signaling in HFSCs to promote proliferation and anagen entry. Similarly, Liu et al[32] showed that glucocorticoid-induced TGF-β3 secretion by Tregs activates Smad2/3 signaling in HFSCs, further supporting Treg-mediated regulation of hair growth. Central to Treg biology is CD25 (IL-2RA), the alpha subunit of the high-affinity IL-2 receptor. CD25+ CD4+ Tregs maintain immune homeostasis through CTLA-4/B7 interactions, secretion of inhibitory cytokines IL-10 and TGF-β, and competitive sequestration of IL-2.[33,34] Upon IL-2 binding, CD25 activates the JAK-STAT pathway, where STAT5 phosphorylation promotes Treg survival and function.[32]

However, chronic immune activation may paradoxically erode these suppressive capacity of Tregs. Elevated CD25 expression, a canonical marker of Treg activation, may signify impaired suppressive function. Similar observations have been reported in autoimmune diseases like systemic lupus erythematosus, where high CD25 expression coexists with reduced FoxP3 expression and diminished inhibitory capacity of Tregs, contributing to disease progression.[35] Such dysregulation likely stems from persistent IL-2/CD25 signaling, which compromises Treg stability in inflammatory environments. Murine studies have shown that overactive STAT5 suppresses HF growth, whereas STAT5 deletion enhances HFSC proliferation and anagen entry.[36] Moreover, excessive IL-2/STAT5 signaling has been implicated in perifollicular fibrosis in scarring alopecia (SA) and inflamed stroma near HF bulbs in alopecia areata (AA).[37] AA, a prototypical autoimmune disorder, features CD8+ cytotoxic T cell-mediated follicular destruction and acute hair loss, often accompanied by reduced or dysfunctional Tregs in lesional sites.[38] Histological analyses of SA and AA biopsies revealed increased perifollicular infiltrates of CD8+ T cells, effector T cells (Teffs), and Tregs,[37] supporting an IL-2-driven CD8+ T cell-Teff-Treg regulatory circuit in lymphocyte-mediated alopecia. Notably, while JAK inhibitors effectively treat AA, Yale et al[39] reported hair regrowth in an AGA pattern in four AA patients treated with oral JAK1/2 inhibitors. This further suggests shared mechanisms of Treg dysfunction in AA and AGA.

Skin-resident Tregs were reported declining during homeostasis but expanding after inflammation.[37] In AGA, this dynamic may be disrupted, with chronic inflammation driving Treg expansion functionally defective due to sustained CD25 signaling. Elevated pro-inflammatory cytokines, including IL-6, have been detected in affected scalp regions.[40] Under inflammatory conditions, Tregs may adopt a pro-inflammatory phenotype, acquiring Th1/Th17-like characteristics and secreting IFN-γ and IL-17A during immune dysregulation.[41] IL-6, in collaboration with TGF-β or via STAT3/transcription factors RORγt and RORα, can induce CD4+ Tregs to produce IL-17A.[41] Such inflammatory Tregs may act

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as pathogenic Teffs, amplifying local inflammation and tissue injury as seen in psoriasis and inflammatory bowel disease.[41] Thus, unresolved perifollicular microinflammation in AGA may establish a pathological loop: inflammatory mediators perpetuate inflammation, while fibrotic remodeling, excessive collagen deposition and thickening around HFs, alters the niche and impairs regeneration. Additionally, inflammatory and immune responses release cytokines such as IL-6, TNF-α, and IFN-γ, activating JAK/STAT, MAPK, and PI3K/AKT pathways, phosphorylating AR at specific residues.[7] This alters AR conformation, enhances nuclear translocation, and increases transcriptional activity even at low androgen levels.[7] ROS associated with inflammation further activate stress-responsive kinases (eg, p38 MAPK, JNK) and inhibit ubiquitin-mediated AR degradation, prolonging AR stability and transcriptional activity, lowering androgen response thresholds.[7] Collectively, elevated CD25 may amplify cytokine-rich HF microenvironments, exacerbate androgen hypersensitivity, and disrupt the HF niche through inflammatory and fibrotic pathways, thereby driving AGA progression.

Beyond Tregs, our analysis identified CXCL12 (SDF-1) as a potential mediator of CD25+ CD4+ Tregs contributing to AGA pathogenesis. An et al[42] reported that CXCL12 activates TGF-β pathways in androgen-exposed dermal fibroblasts, promoting extracellular matrix deposition. CXCL12-CXCR4 paracrine signaling reprogramed dermal papilla cells toward a pro-fibrotic state and recruited Trem2+ macrophages, impairing HF regeneration.[42] Therapeutic neutralization of CXCL12 has been shown to promote hair regrowth in AGA models, highlighting it as a promising treatment target.[43] Other identified proteins, including platelet glycoprotein GPIbα, the immunoregulatory receptor KIR3DS1, the unconventional MVI, and WFDC5, may also contribute to follicular inflammation or niche remodeling. These factors warrant further investigation as potential biomarkers of immune dysregulation or therapeutic targets.

The growing evidence for immune involvement in AGA opens avenues for targeted therapy. JAK inhibitors, for instance, have demonstrated hair regrowth potential in AGA mouse models,[44] though human studies remain limited. Monoclonal antibodies targeting CD25, such as daclizumab and basiliximab, have been used clinically to modulate Treg activity, primarily in transplant rejection settings.[45] Their established mechanism in modulating T-cell immunity[46] provides a rationale for exploring their potential in immune-related hair disorders. Additionally, investigational agents targeting IL-6R and CXCL12 further underscore the promise of immune modulation as a therapeutic strategy in AGA.[47] Given the central role of CD25+ CD4+ Tregs in disease pathogenesis, therapeutic modulation of CD25 could offer a novel and precise approach. However, considering the indispensable function of Tregs in preserving systemic immune tolerance, therapeutic strategies must be carefully optimized to avoid broad immune suppression.

In summary, our study establishes a causal role for CD25+ Treg dysregulation in AGA, mediated through IL-2 signaling perturbations and impaired immune-follicular crosstalk. However, three limitations warrant consideration. First, the use of summary-level cohort data precluded subgroup analyses, such as sex-specific effects. Future studies incorporating individual-level data are necessary to address demographic heterogeneity. Second, the European ancestry of our genetic cohort may limit generalizability; replication in diverse populations is needed to confirm the translational relevance of our findings. Third, the lenient P-value threshold ($P < 1\times10^{-5}$), dictated by limited instrument availability, raises the risk of false positives. Nonetheless, we mitigated this risk through robust sensitivity analyses, including F-statistics >10 to exclude weak instruments and LDSC to confirm genetic correlations. These limitations, common in MR designs, highlight the need for cautious interpretation and further experimental validation.

Biological validation remains essential to confirm our findings. While MR provides genetic evidence, in vitro (like Treg-HFSC co-cultures) and in vivo (like Treg-specific CD25-modified mice) models should address three key questions: (1) whether CD25 overexpression directly impairs Treg suppression and HFSC function; (2) if CD25 modulation reverses AGA-like phenotypes such as fibrosis or miniaturization; and (3) the therapeutic safety margins for avoiding systemic immunosuppression. However, human AGA develops under prolonged androgen exposure and chronic microinflammation, conditions that are challenging to recapitulate in animal models, while in vitro systems cannot fully capture systemic and niche-level regulatory networks. Nonetheless, these constraints must be considered when interpreting experimental outcomes. Future work should focus on directly evaluating the effects of CD25+ CD4+ Tregs on AGA through cell-based systems and animal model. Furthermore, the integration of multi-omics strategies (genomics, transcriptomics, proteomics, and epigenomics) will provide deeper insight into molecular pathways that bridge immune

regulation and HF biology. Such approaches will enable comprehensive mapping of the molecular networks underlying AGA pathogenesis.

Conclusion

Our study identifies dysregulation of CD25 on secreting CD4 Tregs as a causal contributor to AGA via IL-2 signaling defects and impaired immune-follicular crosstalk. These findings offer a foundation for future experimental validation and immune-targeted therapies, advancing precision medicine approaches for AGA treatment.

Abbreviations

AGA, androgenetic alopecia; GPIbα, platelet glycoprotein Ib alpha chain; HF, hair follicle; HFSC, hair follicle stem cell; IV, instrumental variable; IVW, inverse variance weighted; KIR3DS1, killer cell immunoglobulin-like receptor 3DS1; LDSC, linkage disequilibrium score regression; MR, Mendelian randomization; MR-PRESSO, MR Pleiotropy Residual Sum and Outlier; MVMR, multivariable MR; MVI, myosin-VI; SDF-1, stromal cell-derived factor 1; SLE, systemic lupus erythematosus; SNP, single-nucleotide polymorphism; TSMR, two-sample MR; Treg, regulatory T cell; WFDC5, WAP four-disulfide core domain protein 5; WM, weighted median.

Data Sharing Statement

All datasets mentioned in this manuscript can be downloaded online. Immune cells and plasma proteins data are publicly accessible via the IEU OpenGWAS Project (https://gwas.mrcieu.ac.uk/). Androgenetic alopecia datasets were obtained from the FinnGen Biobank (R9, https://www.finngen.fi/).

Ethics Approval and Consent to Participate

All GWAS summary data came from a public database; no raw data was used for this study. The respective institutional review board (IRB) of The First Affiliated Hospital with Nanjing Medical University approved the protocol for this study, and in accordance with their guidelines, this study only used publicly available data and did not use any individual-level data. Therefore, no additional IRB approval was required.

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Author Contributions

All authors made a significant contribution to the work reported. Yimei Du and Yongkun Du contributed to the conception, execution, acquisition of data, and analysis and interpretation. Yutao Yu contributed to the study design. Lei Wang, Yuanbo Huang and Weixin Fan contributed to the interpretation. All author participated in drafting and revising or critically reviewing the article, gave final approval of the version to be published, 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 author(s) report no conflicts of interest in this work.

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