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Multi-Omics Integration Reveals the Genetic Mechanisms of Periodontitis and Predicts Therapeutic Drugs

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ABSTRACT

Background: Periodontitis is a chronic inflammatory disease driven by host immune dysregulation. However, the specific genetic regulatory mechanisms underlying this disease remain unclear. Identifying key molecular targets is crucial for precise therapeutic intervention.

Methods: This study integrated genome-wide association study (GWAS) summary statistics from the Gene-Lifestyle Interactions in Dental Endpoints and FinnGen R11 cohorts, with single-cell spatial transcriptomics and single-cell RNA sequencing profiles. The tissue-specific enrichment of these genetic signals was validated using genetically informed spatial mapping and QTL Enrichment analyses. Furthermore, this study employed methods such as single-cell pathway-based GWAS and single-cell Mendelian randomization to systematically dissect the genetic basis of periodontitis. An artificial intelligence-driven drug screening framework (DrugRefLector) and molecular docking were used to predict potential therapeutic compounds.

Results: Tissue-specific enrichment analysis revealed that periodontitis genetic signals were enriched not only in jawbone and teeth, but also significantly in tissues such as the brain and renal cortex. Multi-dimensional single-cell analysis identified monocytes and NK cells as key immune subsets and 23 genes causally associated with periodontitis. Among these, *GNLY* was prioritized as a computational lead, with evidence from multiple analytical approaches suggesting a potential role in linking innate immune recognition, cytotoxic effects, and tissue damage. The DrugReflector framework predicted 5 candidate compounds with therapeutic potential, all of which exhibited favorable binding affinities to *GNLY* in molecular docking simulations, providing a structural basis for subsequent drug optimization.

Conclusion: This study develops a multi-layered analytical framework to systematically investigate the genetic architecture and key regulators of periodontitis. It provides candidate drugs and a theoretical foundation for targeted immunomodulatory therapies.

Abbreviations: eQTL, expression quantitative trait loci; sQTL, splicing quantitative trait loci; GWAS, genome-wide association study; sc-ST, single-cell spatial transcriptomics; gsMap, spatial mapping of cells for complex traits; QTLEnrich, QTL Enrichment; scRNA-seq, single-cell RNA sequencing; UMAP, Uniform manifold approximation and projection; scPagwas, single-cell pathway-based GWAS; hdWGCNA, High-Dimensional Weighted Gene Co-Expression Network Analysis; LD, linkage disequilibrium; sc-MR, single-cell Mendelian randomization; scTenifoldKnk, Single-Cell Tenifold Knockout; DrugReflector, Artificial Intelligence - driven drug screening framework; GLIDE, Gene-Lifestyle Interactions in Dental Endpoints; MAGMA, Multi-marker Analysis of Genomic Annotation; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; MGI, Mouse Genome Informatics; CLPP, colocalization posterior probability; RCP, regional colocalization probability; TRS, Trait-Related Score; IV, Instrumental variable; IVW, inverse variance weighting; WM, Weighted Median; MR-PRESSO, Mendelian Randomization Pleiotropy Residual Sum and Outlier; FDR, False Discovery Rate; q-PCR, quantitative Real-time polymerase chain reaction; WB, Western blot

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Clinical relevance: The genetic enrichment in the brain and kidney provides a mechanistic basis for the systemic comorbidities of periodontitis, supporting the rationale for integrated oral-systemic health management.

Introduction

Periodontitis is a chronic inflammatory disorder initiated by dental plaque biofilms and driven by dysregulated host immune responses, which results in the progressive destruction of tooth-supporting tissues.[1] As one of the most prevalent non-communicable diseases worldwide, periodontitis poses a substantial public health burden. According to the Global Burden of Disease Study, approximately 89.61 million new cases of severe periodontitis were reported globally in 2021, and the global burden of this disease has continued to rise over the past 3 decades.[2,3] Periodontitis is not merely a localized oral infection; the chronic systemic inflammation it induces is associated with an increased risk of multiple systemic disorders, including diabetes mellitus, cardiovascular disease, and Alzheimer's disease.[4-6] Therefore, elucidating the pathogenesis of periodontitis and developing effective intervention strategies are critical not only for improving oral health but also for preserving overall systemic health.

The clinical management of periodontitis follows a stepwise strategy centred on mechanical plaque removal. However, for patients with severe or aggressive periodontitis, the efficacy of conventional therapy is often limited, primarily due to the difficulty in correcting the dysregulated host inflammatory response.[7,8] To address this, host modulation therapy has gained attention as a complementary strategy. Its goal is not simply to suppress immunity but to modulate the inflammatory response to limit tissue damage and promote repair. In recent years, research has expanded beyond early non-steroidal anti-inflammatory drugs to include various strategies, including pro-resolving mediators, sub-antimicrobial-dose doxycycline, and cytokine therapy. Although these approaches have demonstrated therapeutic potential in preclinical models and selected clinical trials, their widespread clinical application is hindered by several limitations, including short drug half-lives, systemic adverse effects associated with long-term use, and disease rebound following treatment discontinuation.[9,10] These limitations underscore the need for an in-depth analysis of the host immune regulatory mechanisms in periodontitis to uncover precise molecular targets.

Traditional research faces challenges in dissecting the genetic basis and fine molecular mechanisms of periodontitis. Recent advancements from the Human Genome Project, cross-trait analysis tools, and multi-omics datasets have provided new avenues for investigation.[11] Multiple genome-wide association studies of periodontitis have implicated genes involved in inflammatory signalling and DNA methylation in immune cells.[12,13] However, systematically elucidating how these loci influence specific cell types and signalling pathways through the regulation of gene expression (eg, expression quantitative trait loci (eQTL) and splicing quantitative trait loci (sQTL)) remains a critical gap. The complex interplay between genetic variation, epigenetic regulation, gene expression, and protein function across biological levels is beyond the capacity of any single omics approach. Multi-omics integration bridges this gap by systematically combining data across genomics, epigenomics, and transcriptomics to construct a comprehensive regulatory network from genetic variation to phenotype.[14,15]

Against this background, we established a multi-layered research framework spanning from macroscopic genetic mapping to microscopic functional dissection to investigate the genetic basis and key regulatory molecules of periodontitis (Figure 1). First, by leveraging genome-wide association study (GWAS) and integrating single-cell spatial transcriptomics (sc-ST) with eQTL information, we employed methods such as genetically informed spatial mapping (gsMap), QTL Enrichment (QTLEnrich), and colocalization analysis to achieve tissue-specific localization of genetic signals and map the spatial expression distribution of risk genes at the embryonic level.[16,17] Subsequently, single-cell RNA sequencing (scRNA-seq) data were used to construct a periodontitis-associated immune cell atlas. The single-cell pathway-based GWAS (scPagwas) anchored key immune subsets enriched for genetic risk, and High-Dimensional Weighted Gene Co-Expression Network Analysis (hdWGCNA) was performed to identify core gene modules.[18] Notably, single-cell Mendelian randomization (sc-MR), an emerging technique for genetic causal inference, has rarely been applied in periodontitis research. This study pioneers the application of this approach to evaluate the causal relationship between immune cell-specific gene expression and periodontitis.[19] Finally, Single-Cell Tenifold Knockout (scTenifoldKnk) dissected the biological functions of key genes, and an artificial intelligence (AI)-driven drug screening framework (DrugReflector) was used to identify potential therapeutic compounds for periodontitis.[20] Through this integrated multi-omics strategy, we aimed to uncover key molecules mediating periodontitis pathogenesis and their functional interactions, thereby providing a foundation for targeted therapy development, biomarker discovery, and early disease risk stratification.

Methods

Data sources

Genome-Wide Association Summary Statistics: GWAS summary statistics for periodontitis were obtained from 2 independent cohorts: the Gene-Lifestyle Interactions in Dental Endpoints (GLIDE) and the FinnGen R11.[21,22] A genome-wide meta-analysis was performed to enhance statistical power.

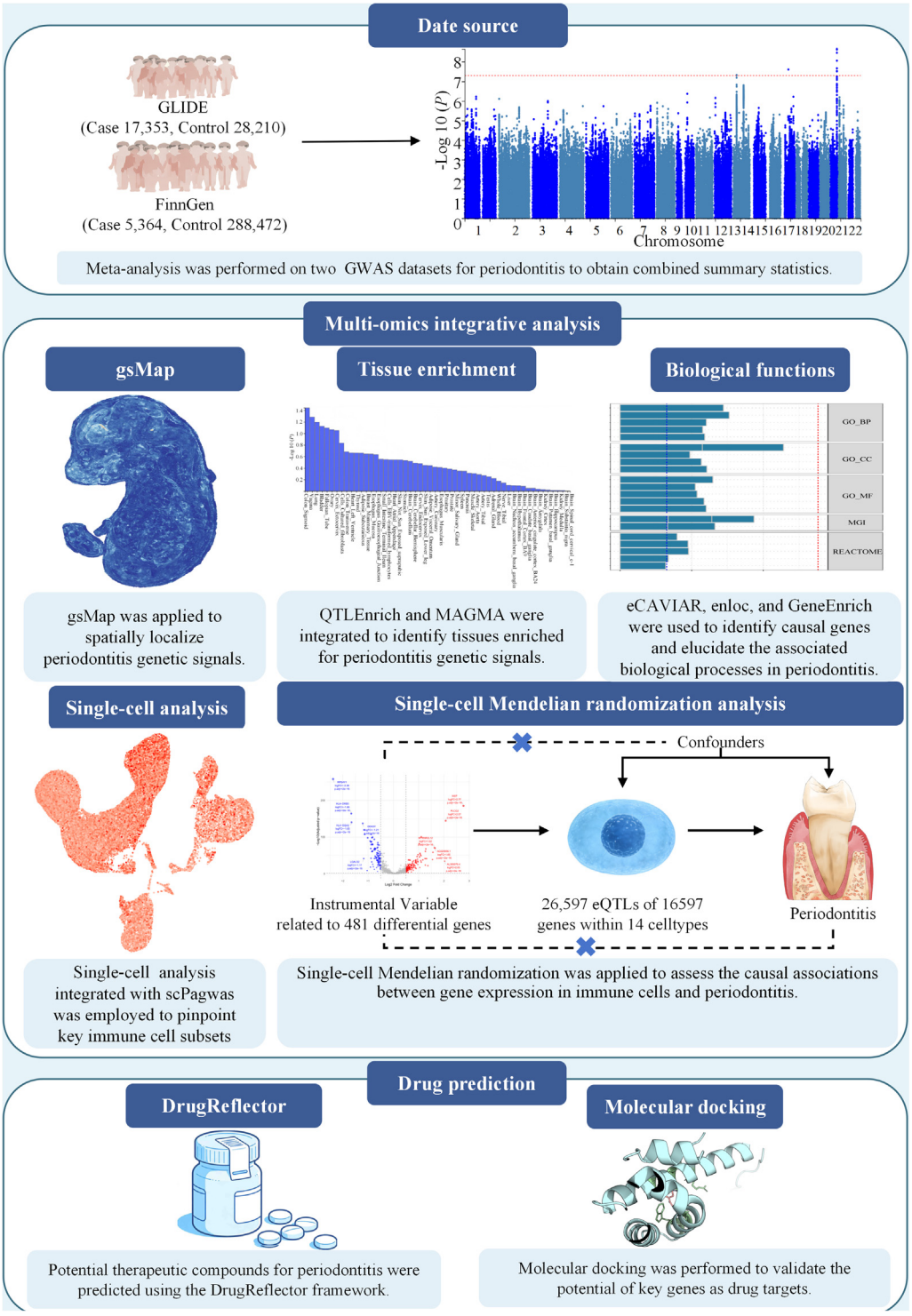

Fig. 1 – Overview of research data sources and design process. Abbreviations: GLIDE, Gene-Lifestyle Interactions in Dental Endpoints; FinnGen, FinnGen R11; GWAS, Genome-wide association study; gsMap, genetically informed spatial mapping of cells for complex traits; QTLEnrich, QTL Enrichment; MAGMA, Multi-marker Analysis of Genomic Annotation; DrugReflector, Artificial Intelligence (AI)- driven drug screening framework.

During effect size integration, the I^2 statistic was used to assess heterogeneity between studies. An $I^2 > 50\%$ was considered indicative of moderate to high heterogeneity, in which case a random-effects model was used for effect size pooling.

Single-cell RNA sequencing Data: scRNA-seq data were obtained from the Gene Expression Omnibus. The primary dataset (GSE267170) comprised gingival tissue samples from 10 healthy controls, 15 periodontitis patients, and 11 periodontitis patients with type 2 diabetes mellitus. In

accordance with the study objectives, only samples from healthy controls and patients with periodontitis without diabetes were retained for subsequent analyses. These samples were simultaneously profiled with genome-wide genotyping and EPIC methylation array data, facilitating systematic dissection of disease-associated gene expression and epigenetic alterations.[23] To further enhance the generalizability of our findings, an independent validation dataset (GSE164241) was incorporated. After excluding buccal mucosa samples, sequencing data from the gingival tissues of 13 healthy controls and 8 periodontitis patients were retained.[24]

Healthy Human Peripheral Blood Mononuclear Cell Single-Cell Multi-omics Data: Peripheral blood mononuclear cells from a healthy 25-year-old female donor were analyzed using 10x Genomics Chromium single-cell multi-omics technology.[25]

All datasets used in this study are publicly available, and no additional ethical approval was required for their secondary analysis (Supplementary Table 1).

Data quality control

Systematic quality control was implemented before data analysis to ensure data accuracy and reliability. First, all GWAS data were uniformly lifted over to the hg38 human genome build to ensure consistent genomic coordinates, and single-nucleotide polymorphisms (SNPs) with a minor allele frequency below 0.01 were excluded to minimize false-positive associations. For scRNA-seq data, the Seurat R package (version 4.4.0) was used to load and initialize the data. The percentages of mitochondrial genes and haemoglobin genes (HBA1, HBA2, HBB) were then calculated to assess cell viability.[26] Following quality control to isolate high-quality cells (UMI $\geq$ 1000, genes 200-5000, mitochondrial $\leq$ 15%, haemoglobin $\leq$ 3%), we employed principal component analysis (PCA) for initial dimensionality reduction. Based on the PCA results, t-distributed stochastic neighbour embedding (t-SNE) and uniform manifold approximation and projection (UMAP) nonlinear dimensionality reduction algorithms were used to map high-dimensional cell expression features onto a 2-dimensional plane, thereby realizing intuitive visualization of cell clusters. The assessment of this reduction step included visualization with heatmap plots. To eliminate sample-derived batch effects, the Harmony algorithm (theta = 2, lambda = 1) was applied to integrate and correct batches.[27,28]

Tissue-specific localization of periodontitis genetic signals

Risk Locus Identification and Tissue Enrichment Analysis: The FUMA platform (https://fuma.ctglab.nl/snp2gene) was used to identify and annotate genomic risk loci ($P < 5 \times 10^{-6}$).[29] Multi-marker Analysis of Genomic Annotation (MAGMA), a gene- and pathway-analysis tool based on multivariate regression models, was employed to perform gene-level tissue enrichment analysis.[30] To further explore tissue enrichment in periodontitis, the QTLEnrich method was used to assess the enrichment of periodontitis-associated signals among eQTL and Sqtl.[17,31]

Spatial Localization at Single-Cell Resolution: Integrating sc-ST data with GWAS statistics, the gsMap algorithm was employed to systematically map the spatial distribution patterns of periodontitis-associated cellular patterns at single-cell resolution. This algorithm calculates cellular trait relevance by integrating GWAS-derived candidate genes with their spatially resolved expression profiles, thereby mapping genotype-phenotype associations to specific anatomical niches at single-cell resolution. Utilizing a spatial transcriptomic atlas of E16.5 mouse embryonic tissues (spanning 25 organs), we constructed a single-cell atlas to delineate the spatial pathogenesis of periodontitis.[16]

Functional enrichment and colocalization analysis

Gene Set Functional Enrichment Analysis: The GeneEnrich method was used to evaluate the enrichment of candidate gene sets within biological pathways and background gene sets (https://github.com/segrelabgenomics/GeneEnrich).[31] Considering that the colocalized e/sQTLs originated from different GTEx tissues, all genes expressed in 49 GTEx tissues were used as the background gene set, without correction for expression levels due to expression variability across tissues. Functional gene sets were derived from databases including Gene Ontology (GO), Reactome, Kyoto Encyclopedia of Genes and Genomes (KEGG), and Mouse Genome Informatics (MGI). $P < .05$ was set as the nominal significance threshold.

Colocalization Analysis: To identify potential causal genes and regulatory mechanisms that may mediate the association between risk loci and periodontitis, 2 Bayesian-based colocalization methods were applied: eCAVIAR (https://github.com/fhormoz/ caviar) and enloc (https://github.com/xqwen/fastenloc). These methods assess whether coincident GWAS signals and e/sQTL signals tag the same causal variant or haplotype, accounting for local linkage disequilibrium (LD) and allelic heterogeneity. Input for the analysis consisted of Z-scores for each variant from GWAS and e/sQTL data. Initially, the core LD region for each lead variant was derived from the 1000 Genomes Project Phase 3 reference panel, comprising variants with an $r^2 > 0.1$. Subsequently, this core region was symmetrically expanded by 50 kb at both ends to form the final LD window. In eCAVIAR analysis, a maximum of 2 independent causal variants per locus was assumed, with a colocalization posterior probability (CLPP) > 0.01 set as the significance threshold.[32] In enloc analysis, the DAP-G algorithm was used to perform fine-mapping of GWAS and e/sQTL loci separately, without presetting an upper limit on the number of independent causal variants, and a regional colocalization probability (RCP) > 0.1 was considered evidence of significant colocalization.[33]

Single-cell data analysis

Cell Clustering and Annotation: Based on the dimensionality reduction results following harmony correction, principal component heatmaps, t-SNE, and UMAP projection visualizations were generated, and cell cycle distribution (S phase and G2M phase scores) was evaluated. A multi-resolution clustering strategy (0.01 to 3.0) was employed for cell neighborhood construction, and the optimal resolution (0.05) was determined using a clustering tree to obtain stable cell subpopulations. The FindAllMarkers function (minimum expression

percentage 25%, log fold change threshold 0.25) was used to identify genes specifically highly expressed in each cluster, and heatmaps were generated based on marker genes. Cell type annotation was performed using the SingleR algorithm.

High-dimensional Weighted Gene Co-expression Network Analysis: For monocytes, the hdWGCNA method was employed to elucidate the transcriptional characteristics. First, genes expressed in at least 5% of cells were selected as the candidate gene set. Metacells were constructed using the MetacellsByGroups function based on cell type and sample origin (k = 25) to reduce the sparsity of single-cell data. After normalization and scaling of metacell expression data, principal component analysis was applied to reduce dimensionality, and the Harmony algorithm was again used to correct for batch effects arising from sample origin. The TestSoftPowers function was used to test soft thresholding powers, and an appropriate soft threshold was selected to construct a signed topological overlap matrix. The dynamic tree-cutting algorithm was then applied to identify co-expression modules. Eigengene values for each module were calculated, and the association between modules and cell types was analyzed.[34,35]

Exon Expression Analysis: Exon expression data from the GTEx project were used to analyze the transcript structure characteristics of prioritized genes and their differential expression distribution across various human tissues (https://www.gtexportal.org/home /transcriptPage).[17]

scPagwas Analysis: The scPagwas computational framework was employed to integrate scRNA-seq with GWAS data, revealing the enrichment characteristics of periodontitis-associated genetic variants at single-cell resolution (https://github.com/ dengchunyu/scpagwas). By correlating cell type-specific gene expression patterns with periodontitis-associated genetic loci, a Trait-Related Score (TRS) was calculated at the single-cell level to identify key immune cell populations enriched for genetic risk.[18]

Chromatin Accessibility and Gene Expression Association Analysis: The Open4Gene method was used to integrate scRNA-seq and scATAC-seq data to explore stably expressed genes in normal immune cells. This method is based on a Hurdle model that addresses the zero-inflated characteristics commonly observed in single-cell data, comprising 2 components: the zero part uses a binomial distribution to model the association between chromatin accessibility status and the probability of zero gene expression; the count part uses a truncated negative binomial distribution to model the association between chromatin accessibility signal intensity and gene expression level.[25]

sc-MR analysis

To evaluate the causal relationship between immune cell-specific gene expression and periodontitis, single-cell eQTL data were integrated with a Mendelian randomization framework.[36] *Cis*-eQTL data were obtained from the OneK1K single-cell dataset, comprising scRNA-seq data of 1,267,758 peripheral blood mononuclear cells from 982 healthy donors. Across 14 cell types, a total of 26,597 independent *cis*-eQTLs were identified (Supplementary Table 1).[19] Instrumental variable (IV) selection followed the core assumptions of MR: (1) Relevance assumption: independent *cis*-eQTLs associated with immune cell types were selected at a threshold of $P < 5 \times 10^{-6}$; (2) Independence assumption: linkage disequilibrium clumping was performed ($r^2 = 0.001$, clumping window 10,000 kb) to remove correlated variants; (3) Exclusion restriction assumption: IVs associated with known confounders of periodontitis were removed using PhenoScanner V2 (http://www.phenoscanner.medschl.cam.ac.uk/) and literature review. Palindromic SNPs were also excluded to avoid strand orientation ambiguity. The F-statistic was calculated using the formula $beta^2/se^2$ to assess the strength of each SNP. Weak SNPs ($F < 10$) were removed to mitigate weak-instrument bias.[37] MR analysis employed methods appropriate for the number of IVs available: the Wald ratio method for single instrumental variables[38]; inverse variance weighting (IVW) for 2 instrumental variables[39]; for 3 or more IVs, IVW was used as the primary method, supplemented by sensitivity analyses including Weighted Median (WM), MR-Egger, and Mendelian Randomization Pleiotropy Residual Sum and Outlier (MR-PRESSO).[40] False Discovery Rate (FDR) correction was applied to control for the risk of false positives due to multiple comparisons.[41]

Gene functional validation and drug prediction

Virtual Knockout Simulation: The scTenifoldKnk was used to simulate target gene knockout at the single-cell level and identify downstream genes perturbed by the knockout. Genes with an adjusted $P < 0.05$ were considered significantly differentially expressed. Functional enrichment analysis was performed on the differentially expressed genes, and gene set enrichment analysis was applied to the full gene set to evaluate potential biological functional changes induced by target gene knockout.[42]

AI-Driven Drug Screening and Molecular Docking: The DrugReflector framework was employed to identify regulators associated with disease phenotypes and to screen potential therapeutic compounds using an active-learning approach based on transcriptomic data. The model was trained on the Connectivity Map (CMap/LINCS L1000) dataset and uses an ensemble architecture comprising 3 feeds-forward neural networks, with 3-fold cross-validation, to predict the transcriptional regulatory effects of small-molecule perturbations on 978 landmark genes. The input data consisted of a list of whole-tissue differentially expressed genes (DEGs) derived from scRNA-seq analysis. Before inference, the input gene set was mapped to the landmark gene space, and missing genes were imputed by mean substitution. The model calculates the reversal probability for each candidate compound against the input transcriptomic signature by applying a Softmax transformation to the ensemble Logit scores. Based on these probabilities, 9,597 compounds from the CMap library were ranked in descending order, with a higher rank (ie, a smaller Rank value) indicating greater potential to reverse the disease-associated transcriptional profile.[20,43]

To translate the model-output compound identifiers (BRD IDs) into clinically meaningful information, deep annotation was performed using the Broad Institute Drug Repurposing Hub database.[44] Exact matches of the BRD IDs, corresponding generic names, PubChem CIDs, highest clinical development phase, mechanism of action, and molecular target

information were retrieved. Clinical phases were strictly classified into 6 categories: Launched, Phase 3, Phase 2, Phase 1, Preclinical, and Withdrawn. Molecules not recorded in the database were uniformly labelled as "Other/Unknown."

To evaluate the binding affinity between candidate drugs and the target protein, molecular docking studies were performed.[45] The target protein structure was obtained from the RCSB PDB database, and ligand structures were obtained from the PubChem database. Preprocessing of the protein structure was performed in PyMOL (version 2.6.0) to remove water molecules and ligands, retaining only the backbone. The docking procedure was then conducted using AutoDock Vina (version 1.2.7) to identify putative binding sites and perform flexible docking, with binding affinities quantified by Vina scores (kcal/mol). These poses were ranked, and the top 5 with the highest scores were selected for further analysis. The conformation exhibiting the most favourable binding energy was visualized in PyMOL to analyse hydrogen bonding and interaction patterns with the ligand.[46]

Western blot and quantitative real-time polymerase chain reaction

ITGB6 expression was analysed by Western blotting. Briefly, Human Gingival Fibroblasts-1 (HGF-1) cells were plated in 6-well plates at a density of 5×10^5 cells per well and cultured for 24 hours. To simulate periodontal inflammation, the treatment group was exposed to 200 ng/mL LPS. Total protein was extracted on ice using RIPA lysis buffer. The protein lysates were then separated by electrophoresis on a 10% SDS-PAGE gel and electrotransferred onto PVDF membranes. The membranes were blocked with 5% bovine serum albumin (BSA) in Tris-buffered saline with Tween 20 (TBST) for 1 hour at room temperature. Subsequently, the membranes were probed with a primary antibody specific for ITGB6 (1:3000 dilution, Cell Signaling Technology) overnight at 4°C. Following primary antibody incubation, the membranes were incubated with a horseradish peroxidase (HRP)-conjugated secondary antibody for 1 hour at room temperature. Protein bands were visualized using the BeyoECL Moon chemiluminescence kit (Beyotime, China). Three independent biological replicates were performed. Band intensities were quantified using ImageJ software, and ITGB6 protein levels were normalized to GAPDH.

The mRNA expression levels of *IL-6*, *TNF-α*, and *ITGB6* were assessed via quantitative real-time PCR (q-PCR). Initially, HGF-1 cells (5×10^5 per well) were seeded in 6-well plates and cultured for 24 hours. The treatment group was exposed to 200 ng/mL LPS for 6 hours. Afterward, cells were collected for q-PCR analysis of mRNA expression. Relative mRNA expression levels were normalized to GAPDH and calculated using the $2^{-\Delta\Delta Ct}$ method. Primer sequences are presented in Supplementary Table 2.

Results

Multi-cohort meta-analysis identifies genome-wide significant risk loci for periodontitis

To comprehensively identify genetic variants associated with periodontitis, a meta-analysis was performed integrating GWAS summary data from 2 independent European-ancestry cohorts: FinnGen R11 and GLIDE, comprising a total of 339,399 samples. To control for heterogeneity between studies, a random-effects model was employed, identifying 10 risk loci that reached genome-wide significance ($P < 5 \times 10^{-8}$) (Supplementary Table 3). These findings provide a reliable genetic foundation for subsequent functional dissection.

Tissue specificity and functional enrichment analysis of periodontitis genetic associations

To explore the potential tissue sites where periodontitis-associated genetic variants may exert their effects, the gsMap algorithm was employed to map GWAS signals onto the E16.5 mouse embryo spatial transcriptome atlas. Figure 2A displays genome-wide spatial association signals of periodontitis-associated genes identified by the gsMap algorithm. The results revealed that periodontitis genetic signals were significantly enriched not only in the target organs—the jawbone and teeth ($P = 2.099 \times 10^{-2}$)—but also in central nervous system-related regions (choroid plexus, $P = 4.139 \times 10^{-3}$; brain, $P = 4.181 \times 10^{-3}$) and the adrenal gland ($P = 2.965 \times 10^{-2}$) (Figure 2B and Supplementary Table 4), providing a cellular-level spatial perspective for understanding its pathological mechanisms. The gsMap-derived gene-specific scores (GSS) are provided in Supplementary Table 5.

To further validate the specificity of the above tissue enrichment, the QTLEnrich method (adjusting for confounders and tissue sample size) was used to evaluate the enrichment of cis-e/sQTLs from 49 tissues in the GTEx v8 database among periodontitis-associated signals ($P < .05$). The results showed significant enrichment of periodontitis genetic signals in eQTLs from multiple GTEx tissues, with the highest enrichment observed in the renal cortex ($P = 1.89 \times 10^{-3}$), and the hypothalamus also showed significant enrichment ($P = 8.76 \times 10^{-3}$) (Supplementary Tables 6 and 7). The kidneys play a central role in maintaining systemic homeostasis, while the hypothalamus serves as a key integrative hub for the neuroendocrine-immune network. These enrichment findings corroborate the spatial localization results from gsMap, collectively revealing the potential impact of the periodontitis genetic basis on systemic organs. Meanwhile, MAGMA enrichment analysis did not observe significant tissue enrichment signals, suggesting that different analytical methods may offer complementary perspectives in detecting tissue-specific genetic signals (Supplementary Figure 1).

In this study, 2 Bayesian colocalization methods, eCAVIAR and enloc, were employed in conjunction with e/sQTL data from 49 GTEx tissues to infer potential causal genes underlying periodontitis risk loci. eCAVIAR identified 3 genes whose expression or splicing regulation signals colocalized with periodontitis risk loci (CLPP > 0.01). Among these, the eQTL signal for *ITGB6* in subcutaneous adipose tissue showed significant colocalization with the periodontitis risk locus (CLPP = 0.14) and was negatively correlated with periodontitis risk (Supplementary Table 8). The enloc analysis similarly confirmed significant colocalization between *ITGB6* in subcutaneous adipose tissue and periodontitis phenotype (RCP = 0.50) (Supplementary Table 9). Exon-level analysis revealed that *ITGB6* exhibited overall high expression levels

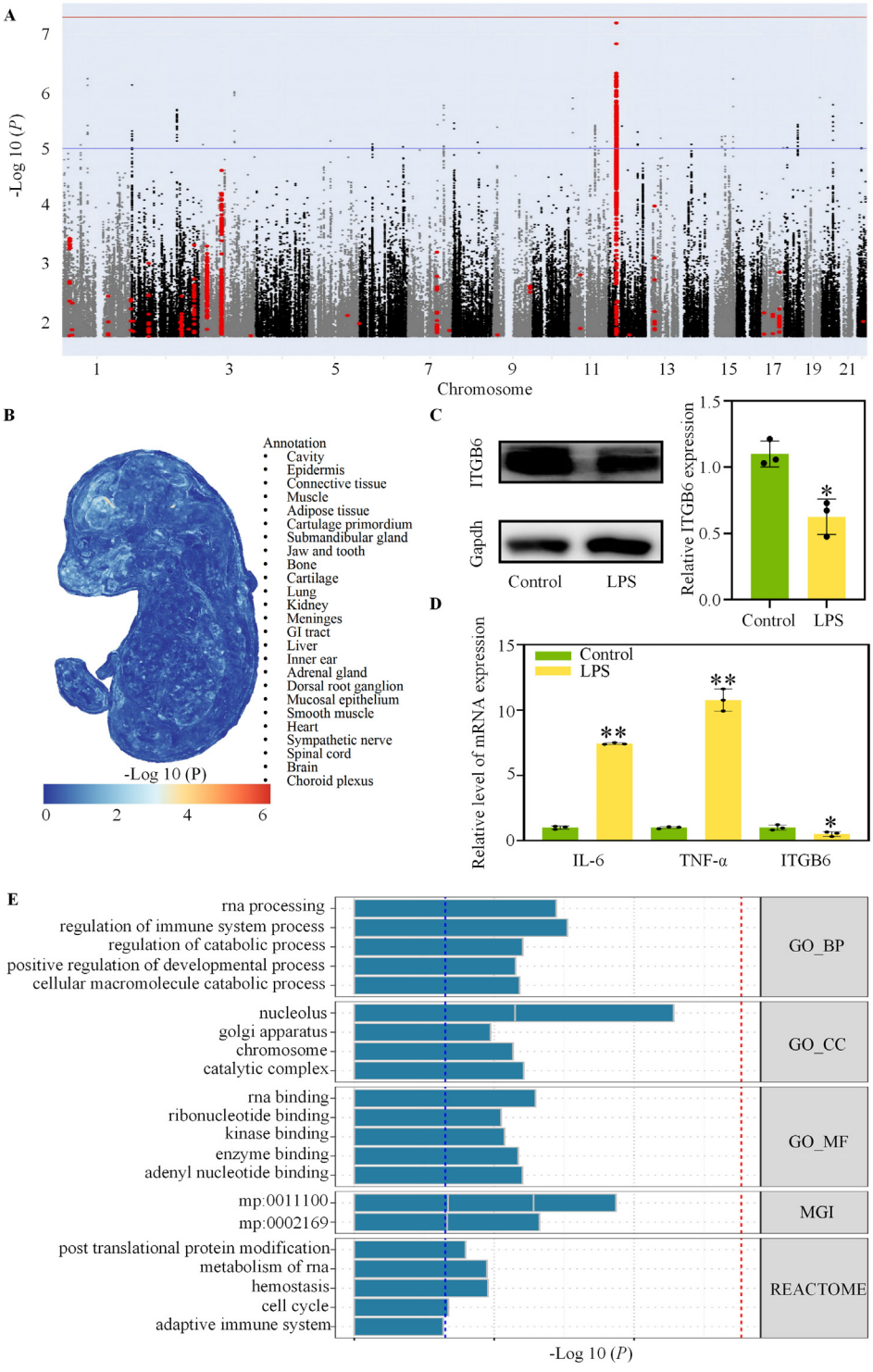

Fig. 2 – Analysis of Tissue Enrichment and Biological Functions of Genetic Signals in Periodontitis. A Genome-wide spatial association signal of periodontitis-associated genes identified by the gsMap algorithm. B Spatial mapping of periodontitis-associated cellular patterns, generated by gsMap from E16.5 mouse embryonic single-cell spatial transcriptomics data across 25 organs. C Western blot analysis for ITGB6 protein expression (n = 3, *P < .05). D Relative mRNA expression levels of the genes TNF-α, IL-6, and ITGB6, as measured by qPCR (n = 3, *P < .05, **P < .01 vs. control). E Functional enrichment analysis of all target genes was performed using GeneEnrich, covering signalling and metabolic pathways (Reactome, KEGG), gene ontologies, and mouse phenotype ontologies. Abbreviations: gsMap, genetically informed spatial mapping of cells for complex traits; KEGG, Kyoto Encyclopedia of Genes and Genomes; GO, Gene Ontology; MGI, Mouse Genome Informatics. The data are analysed using Student's t-test and presented as mean ± SD.

in kidney tissue, potentially related to tissue-specific alternative splicing of Exon 15 (Supplementary Figure 2). Previous studies have reported that the *ITGB6* gene functions in cell adhesion and regulation of the *TGF-β1* pathway. To validate *ITGB6* expression changes in periodontal tissue, mRNA and protein levels of *ITGB6* in HGF-1 under healthy and inflammatory conditions were examined. As shown in Figure 2C, compared with the control group, ITGB6 expression was significantly downregulated in HGF-1 cells under inflammatory conditions, consistent with the q-PCR results (Figure 2D). This aligns with the negative regulatory direction suggested by colocalization analysis, supporting that *ITGB6* may participate in periodontitis protection by maintaining epithelial barrier function.

To further dissect the biological functions of periodontitis-associated genes, GeneEnrich enrichment analysis was performed to assess their enrichment in specific biological pathways. The results showed that these genes were significantly enriched in regulation of immune system processes (GO: BP), molecular functions concentrated in RNA binding and kinase binding (GO: MF), and cellular components localized to the nucleolus and Golgi apparatus, regions active in synthesis and secretion (GO: CC). REACTOME pathway analysis further validated their core roles in adaptive immunity and protein post-translational modification (Figure 2E). These enrichment characteristics align closely with the physiological functions of the brain and kidneys described above, suggesting that periodontitis-associated genetic variants may exert regulatory effects across multiple organ systems by modulating fundamental processes, including immune responses and protein metabolism.

Periodontitis-associated immune cell subsets and key molecules

Unsupervised clustering was performed on scRNA-seq data from gingival tissue samples of healthy controls and patients with periodontitis in the GSE267170 dataset (after batch-effect removal with Harmony), identifying 12 cell clusters using UMAP. These clusters were annotated as 5 major cell types: monocytes, B cells, NK cells, T cells, and HSC-G-CSF cells (Figure 3A and B). Supplementary Figure 3 presents the annotation results obtained using the t-SNE method.

To finely map genetic risk signals to specific immune cell types, the scPagwas method was employed to calculate trait-associated polygenic risk scores for each cell, which were visualized in UMAP space (Figure 3C). Monocytes (2.325×10^{-8}) and NK cells (3.405×10^{-12}) were identified as the key cell populations most strongly associated with periodontitis (Figure 3D). Pathway enrichment analysis revealed that the risk pathways commonly involved in these 2 cell types included immune-related pathways (hsa05330, hsa05320) and lipid metabolism pathways (hsa00564, hsa00561, hsa04975), suggesting that innate immunity and metabolic reprogramming may participate in periodontitis pathogenesis (Figure 3E). Through gene correlation ranking, key genes including *IL-1β, RPL23*, and *GNLY* were identified (Supplementary Figure 4). To validate the robustness of the above findings, an independent dataset (GSE164241) was introduced for parallel analysis. UMAP clustering identified 9 distinct cell types, including monocytes, T cells, and B cells (Supplementary Figure 5A and B). *GNLY* exhibited predominant expression in $CD8^+$ T cells (Supplementary Figure 5C). The scPagwas analysis further revealed that plasma cells, $CD8^+$ T cells, and monocytes were among the cell populations significantly associated with periodontitis, reinforcing the close association between immune cells and periodontitis (Supplementary Figure 5D-F). Furthermore, in both datasets, substantial numbers of differentially expressed genes were detected across cell subsets in periodontitis patients compared to healthy controls, reflecting widespread remodeling of the immune microenvironment under disease conditions (Supplementary Figures 6 and 7).

The hdWGCNA method was applied to uncover gene expression programs associated with monocytes. To overcome the inherent sparsity of single-cell data, a soft-thresholding power of 6 was selected to construct a signed topological overlap matrix, thereby approximating a scale-free distribution (Figure 3F). Based on the dynamic tree-cutting algorithm, 11 co-expression modules were identified, among which the M1 and M9 modules were specifically highly expressed in monocytes (Figure 3G and H). Module eigengene analysis revealed key genes within each module (Supplementary Figure 8), with *CST7, GZMB*, and *GNLY* showing close association with cytotoxic granule function and substantial overlap with high-risk genes identified by scPagwas. Figure 3I illustrates the distribution of these modules across different cell types. Correlation analysis between modules revealed moderate co-expression relationships, with the M4 module showing weaker connectivity with other modules (Figure 3J). The genes contained within these modules are presented in Supplementary Table 10. Additionally, Open4-Gene Hurdle analysis revealed that these genes are expressed in normal immune cells (Supplementary Table 11).

Single-cell Mendelian randomization validates causal associations

To further explore the causal relationship between specific gene expression in immune cells and periodontitis, this study integrated *cis*-eQTL data from 14 immune cell types derived from 982 healthy donors. This study targeted 481 DEGs identified between the periodontitis and healthy groups; among these, 196 genes passed stringent IV screening, yielding 12,282 SNPs as valid IVs (Supplementary Table 12). The F-statistics for these IVs were robust, all exceeding a value of 10. Following sc-MR analysis with FDR correction, 23 genes were identified as having significant causal associations with periodontitis (Figure 4). These genes were primarily enriched in $CD4^+$ T cells ($CD4^+$ NC 15.94%), $CD8^+$ T cells ($CD8^+$ ET 13.04%), and NK cells (NK 11.59%), with no evidence of significant heterogeneity or horizontal pleiotropy (Supplementary Table 13). Notably, increased expression of the *GNLY* gene in $CD8^+$ ET cells exhibited a protective effect against periodontitis (OR = 0.987, 95% CI = 0.976-0.999, $P_{FDR} = 0.044$). This result suggests that, within the polygenic architecture of periodontitis, the basal function of *GNLY* in this specific cell population may act as a subtle modulatory or protective factor.

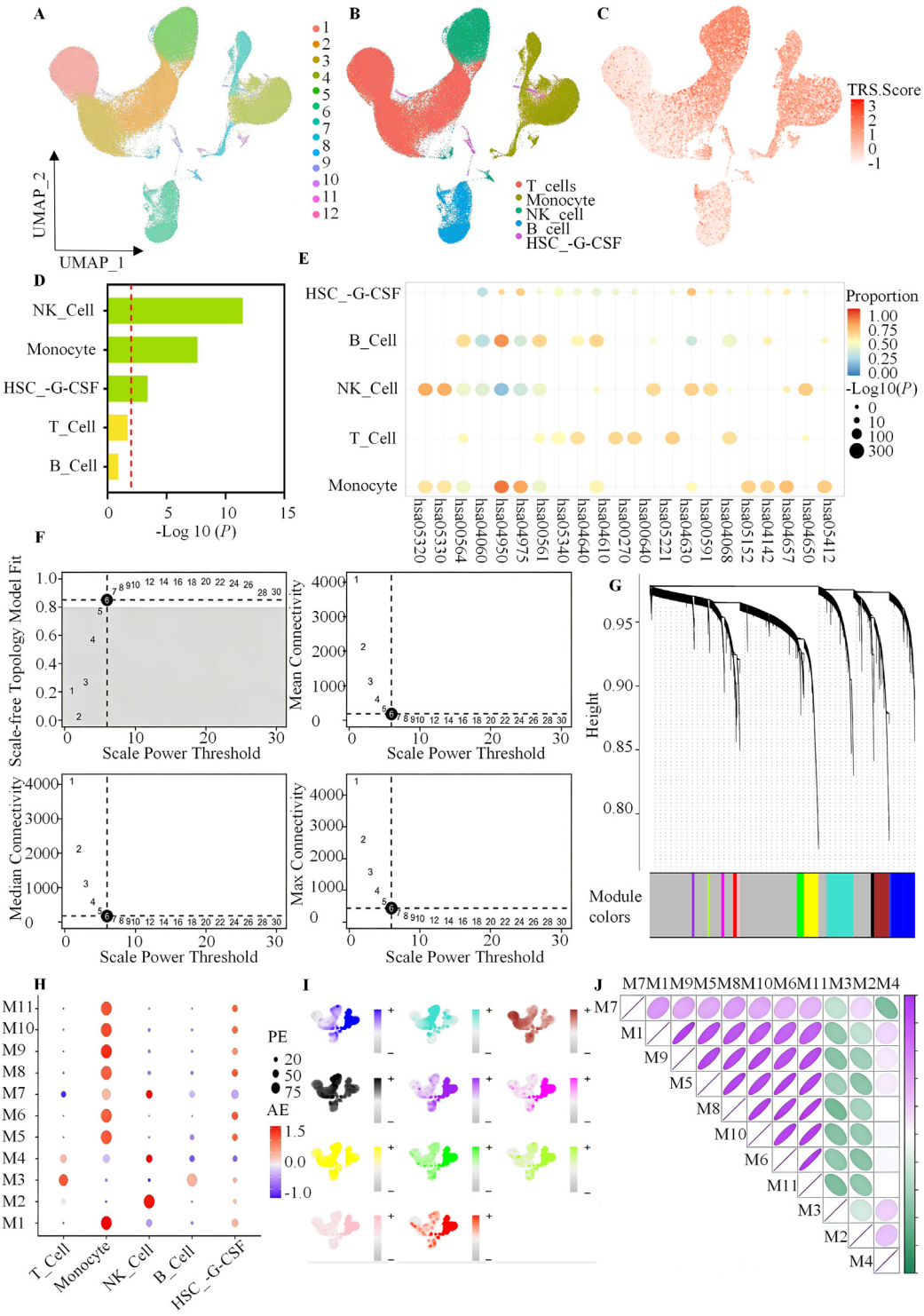

Fig. 3 – Single-cell data analysis. A UMAP visualization of 12 transcriptionally distinct cell clusters identified via Seurat. B Cell type annotation using canonical marker genes. C UMAP projection of the scPagwas-derived TRS. D Cell types associated with periodontitis. E Top 5 enriched KEGG pathways for each cell type in periodontitis. F Determination of the optimal soft-thresholding power based on scale-free topology and mean connectivity. G Dendrogram identifying 11 co-expressed gene modules. H Bubble chart depicting the functional enrichment of different modules. I UMAP showing the expression patterns of each module across cells. (J) Correlation matrix illustrating relationships among modules. Abbreviations: TRS, Trait-Related Score; PE, Percent expressed; AE, Average expression; scPagwas, single-cell pathway-based GWAS.

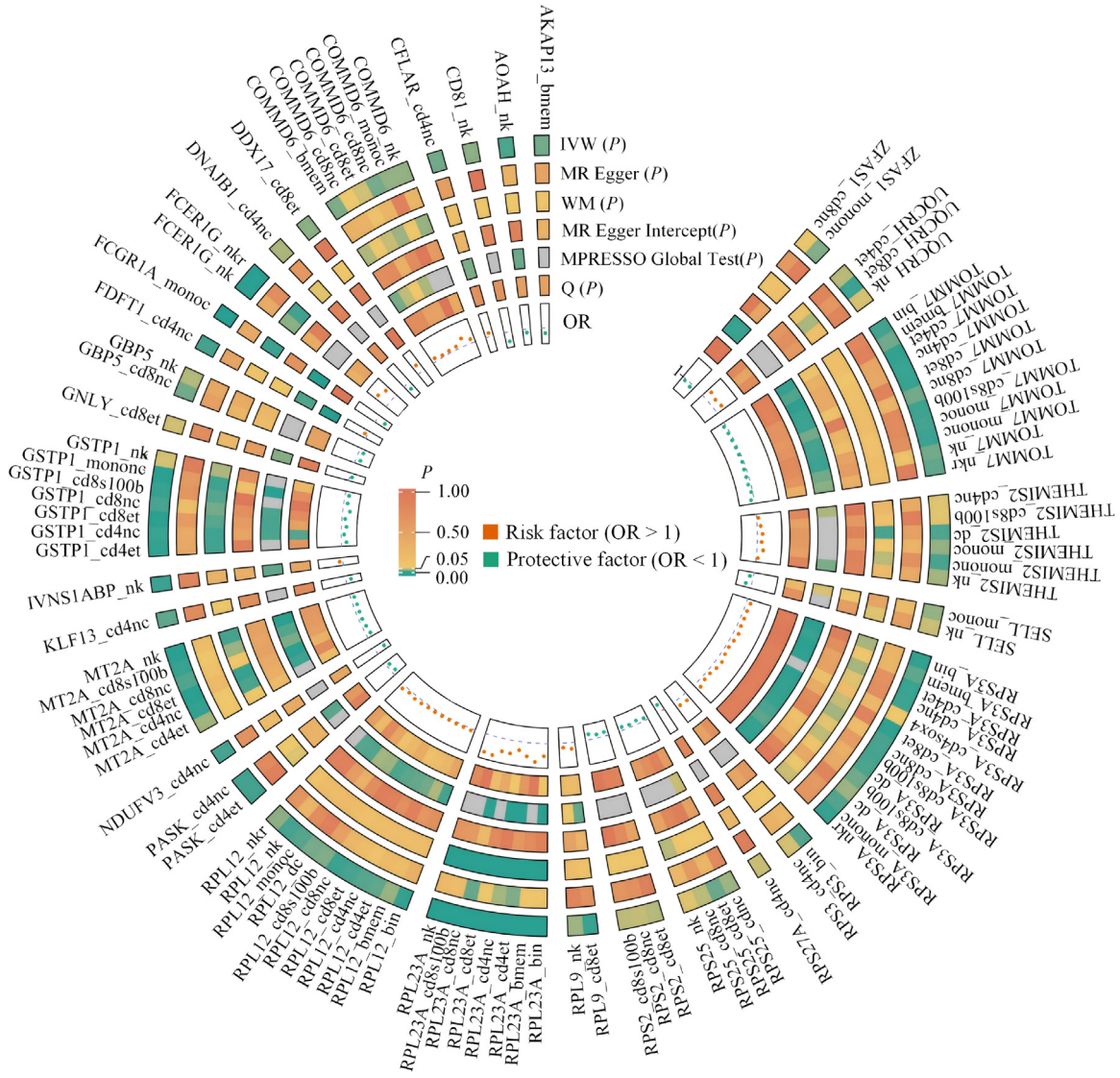

Fig. 4 – Single-cell Mendelian randomization results for causal associations between immune cell-specific gene expression and periodontitis. Guide to the circular plot: Outer rings (concentric tracks): Each concentric ring represents a distinct statistical metric or sensitivity analysis, including the primary IVW, MR-Egger, WM, MR-Egger intercept (for pleiotropy assessment), MR-PRESSO global test, and Cochran's Q (for heterogeneity assessment). Innermost ring: The innermost track displays the OR for each gene–cell type pair. Red indicates OR > 1 (risk effect), while green indicates OR < 1 (protective effect). Gene–cell type labels: Each radial sector corresponds to a single gene–cell type pair tested in the sc-MR analysis. Labels follow the format Gene_celltype. Abbreviations: P, P-value; IVW, inverse variance weighting; WM, Weighted Median; MR-PRESSO, Mendelian Randomization Pleiotropy Residual Sum and Outlier; Q, Cochran Q; OR, Odds Ratio.

Virtual knockout simulation analysis

To assess the potential biological functions of the *GNLY* gene at the single-cell level, this study simulated its knockout effect using the scTenifoldKnk method and identified 11 significantly perturbed downstream genes (Figure 5A). Functional enrichment analysis revealed that these perturbed genes were significantly enriched in pathways including Toll-like receptor signaling, *IL-17* signalling, apoptosis, lipid metabolism, and atherosclerosis (Figure 5 B and C). Notably, these pathways play critical roles in both immune response and tissue destruction during periodontitis. The broad perturbation of related gene networks following *GNLY* knockout further underscores the necessity of *GNLY* in maintaining immune homeostasis.

AI-driven drug screening and molecular docking predicts candidate compounds

Based on the DrugReflector framework, 5 compounds with potential therapeutic activity were identified: BRD-K14693417, BRD-K15574380, osimertinib, arecaidine-propargyl-ester, and doxofylline (Figure 5D & Supplementary Table 14). Mechanism of action enrichment analysis revealed

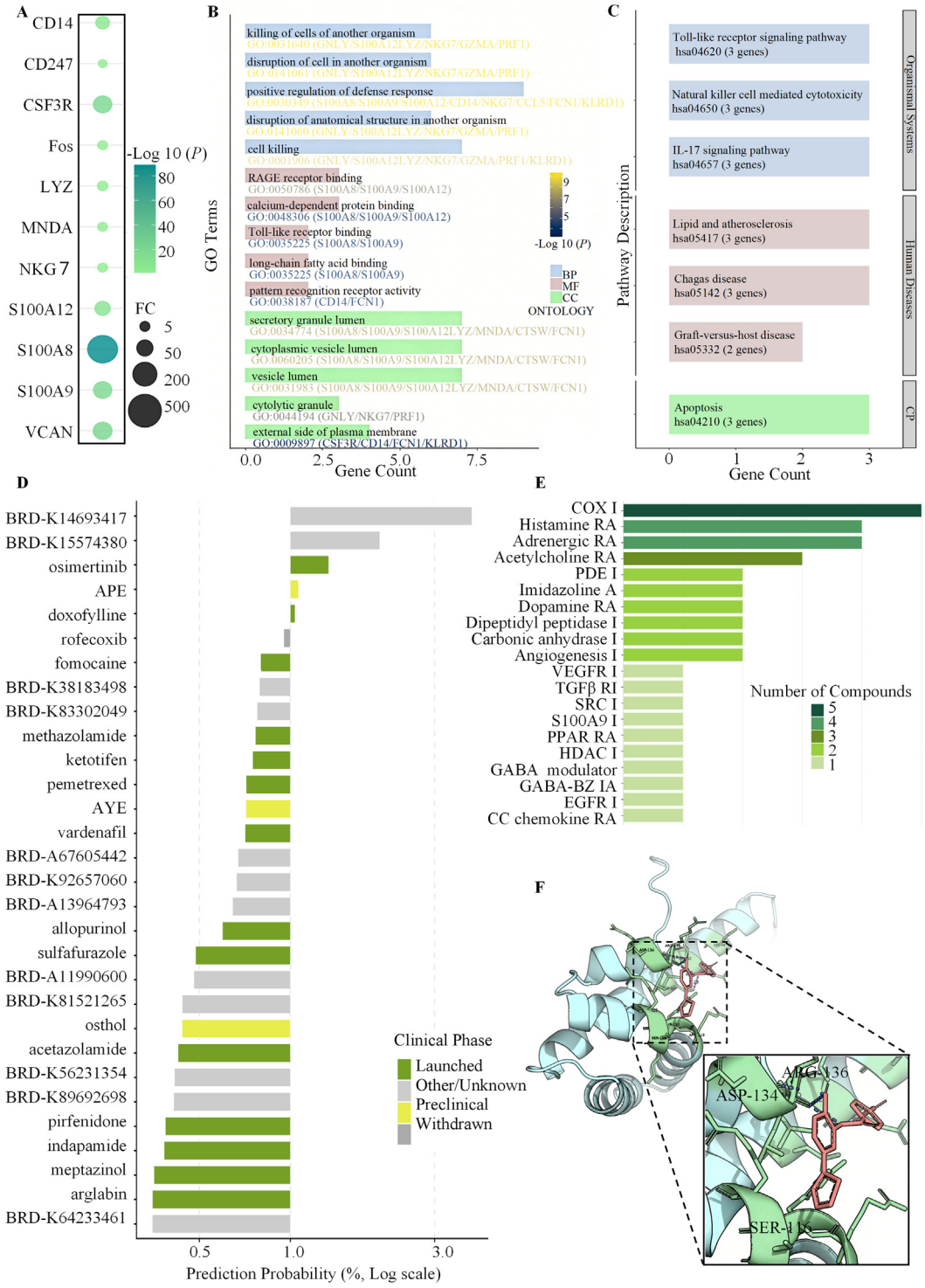

Fig. 5 – Identification of potential therapeutic agents for the treatment of Periodontitis. A Virtual knockout experiment. B GO enrichment. C KEGG enrichment. D Candidate drug screening results from DrugRefLector prediction. E Mechanisms of action of potential drugs. F Molecular docking.

that these compounds primarily target functional categories, including cyclooxygenase inhibitors, histamine receptor antagonists, and adrenergic receptor antagonists—all mechanisms closely related to inflammation regulation and neuroimmune modulation (Figure 5E). To evaluate whether *GNLY* could serve as a potential target for the candidate compounds, molecular docking validation was performed (Figure 5F). The results showed that BRD-K15574380 exhibited the strongest binding energy with GNLY (-6.7 kcal/mol). In comparison, osimertinib and BRD-K14693417 also

demonstrated moderate binding affinity (binding energies of -6.2 kcal/mol and -5.9 kcal/mol, respectively), providing a structural basis for subsequent drug optimization and experimental validation (Supplementary Figure 9).

Discussion

Periodontitis is a chronic inflammatory disease triggered by dental plaque biofilms and driven by host immune dysregulation, with its initiation and progression involving complex interactions among genetic susceptibility, immune regulation, and environmental factors. While genome-wide association studies have identified multiple periodontitis susceptibility loci, single-omics approaches are insufficient to elucidate how these genetic variants influence disease progression by regulating gene expression in specific cell types. This creates a critical translational gap between genetic association and functional mechanism that remains to be addressed.[47,48] To address this research gap, this study developed a multi-layered, integrated analysis framework spanning from macroscopic genetic mapping to the dissection of microscopic functional mechanisms. Through a large-scale GWAS meta-analysis, we identified periodontitis risk loci; integrating sc-ST and eQTL data enabled tissue-specific localization of genetic signals; utilizing scRNA-seq data allowed us to pinpoint key immune cell subsets; subsequently, sc-MR was employed to evaluate causal relationships between gene and disease; and finally, scTenifoldKnk were performed to dissect the potential biological functions of core genes. Through this strategy, we systematically revealed the genetic architecture of periodontitis, identified and experimentally validated key causal genes. An in-depth discussion of these findings is presented below.

In this study, we used E16.5 mouse embryonic sc-ST data for cross-species mapping, guided by 3 considerations. At the data level, this dataset uniquely covers 25 organs at single-cell resolution, whereas no publicly available human sc-ST data of comparable breadth currently exists. At the theoretical level, gene expression between mouse and human shows a correlation exceeding 80% at the tissue level and over 75% at the cell-type level, and this cross-species conservation provides the scientific basis for mapping genetic signals across species.[49] At the empirical validation level, the original gsMap study successfully recapitulated known tissue–trait associations using this dataset, demonstrating that this strategy can accurately map human GWAS signals to the correct target tissues and cell types.[16] This study found that the genetic risk for periodontitis exhibits distinct tissue-specific spatial patterns, with significant enrichment detected in brain and kidney tissues by both the gsMap and QTLEnrich methods. This finding provides a novel perspective for understanding the association between periodontitis and various systemic diseases. As the centre of the neuroendocrine-immune regulatory network, the brain plays a central role in integrating signals from the autonomic and immune systems. The enrichment of genetic signals in this region suggests that the pathological process of periodontitis may be subject to remote regulation by the central nervous system, influencing peripheral immune responses and inflammatory levels through the hypothalamic-pituitary-adrenal axis. This provides a potential mechanism underlying the association between periodontitis and various systemic diseases (Alzheimer's disease, cardiovascular disease, rheumatoid arthritis, etc.). Previous studies have reported that dysfunction of the hypothalamic-pituitary-adrenal axis is often accompanied by elevated cortisol levels, which can exacerbate periodontal tissue damage.[50] Notably, the prevalence of moderate periodontitis is significantly higher in patients with chronic kidney disease (14.6%) compared to controls (8.6%).[51] The enrichment of periodontitis genetic signals in the renal cortex provides a genetic-level explanation for this clinical association. The kidneys play a crucial role in maintaining the homeostasis of the internal environment and electrolyte balance. At the same time, the systemic low-grade inflammation induced by periodontitis may increase renal burden by affecting kidney function. Conversely, renal dysfunction may also influence the inflammatory repair capacity of periodontal tissues by altering the immune microenvironment. A bidirectional promotional relationship may exist between these 2 conditions. MAGMA analysis computes gene-level association strength (Z-score) using a multiple linear principal component regression model and subsequently regresses these scores against tissue-specific gene expression data from 54 tissues in GTEx v8 to identify trait-relevant tissues.[52] Its analytical framework is fundamentally different from those of QTLEnrich and gsMap—MAGMA tests whether trait-associated genes are preferentially expressed in a given tissue. Therefore, the absence of a significant tissue enrichment signal in MAGMA does not negate the results obtained from gsMap and QTLEnrich. Combined with the functional characteristics identified by GeneEnrich pathway enrichment analysis—including "regulation of immune system process," "RNA binding," and "protein post-translational modification"—it can be seen that the genetic basis of periodontitis exerts effects on both distant organs and local target tissues by influencing fundamental biological processes such as immune regulation and protein metabolism.

Through multi-dimensional single-cell analysis, this study systematically dissected periodontitis-associated immune cell subsets and their core regulatory molecules. First, scPagwas's analysis finely mapped periodontitis genetic risk signals to specific immune cell types: monocytes and NK cells. These 2 cell types serve as core effector cells of innate immunity and bridges connecting innate and adaptive immunity, respectively. Their enrichment suggests that the immunopathology of periodontitis involves hyperactivation of innate immunity and dysregulation of cytotoxic effects. The hdWGCNA co-expression network analysis further confirmed that *GNLY*, along with cytotoxic granule-associated genes such as *CST7* and *GZMB*, were co-enriched in monocyte-specific modules, suggesting that these 3 genes may form a functionally synergistic regulatory module collectively involved in regulating immune cell killing function.

Granulysin is a multifunctional effector molecule capable of directly killing pathogens and limiting their spread by modulating the cytotoxic activity of immune cells.[53,54] sc-MR analysis revealed that increased *GNLY* expression in $CD8^+$ ET cells is associated with a modest reduction in the risk of periodontitis. Although the effect size is small, it aligns with the

general characteristics of polygenic diseases, in which individual genetic loci typically contribute only modestly to overall risk.[55] This finding is biologically plausible, as it is consistent with GNLY's known functions in directly clearing periodontal pathogens and enhancing innate immune surveillance. Furthermore, virtual knockout simulation indicated that *GNLY* deletion perturbs key inflammatory pathways, including Toll-like receptor and *IL-17* signaling, underscoring its necessity in maintaining immune homeostasis. Interestingly, *GNLY* function exhibits a notable concentration dependency. Early studies demonstrated that *GNLY* can act as an immunologic alarmin by activating antigen-presenting cells via TLR4.[56] At lower concentrations (nanomolar range), *GNLY* primarily functions as a chemoattractant and immune activator, recruiting immune cells such as monocytes and memory T cells, and inducing the production of pro-inflammatory cytokines (eg, *MCP-1 and IL-6*). In contrast, at higher micromolar concentrations, GNLY exerts direct cytotoxic effects against both pathogens and host cells.[57] Integrating the sc-MR analysis and virtual knockout results from this study, we hypothesize that in the baseline state or early stages of periodontitis—as reflected by genetic predisposition—*GNLY* expression in $CD8^+$ ET cells is more likely to exert regulatory and protective functions, maintaining immune homeostasis through moderate antimicrobial activity. However, within active periodontal lesions, massive infiltration and activation of immune cells may lead to a sharp increase in local *GNLY* concentration, shifting its role from "antimicrobial defense" toward a "pro-inflammatory mediator"—where its chemotactic properties could amplify local inflammatory cascades and result in collateral tissue damage. Future studies are needed to elucidate further the precise concentration-dependent and microenvironment-specific effects of *GNLY* in periodontal tissues.

In this study, 5 compounds with potential therapeutic activity were predicted. Mechanism of action enrichment analysis revealed that these compounds primarily target functional categories, including cyclooxygenase (COX) inhibitors, histamine receptor antagonists, adrenergic receptor antagonists, and acetylcholine receptor agonists. Notably, COX is a key enzyme in prostaglandin E2 (PGE2) synthesis, and PGE2, as a critical inflammatory mediator in periodontitis, promotes periodontal tissue degradation and alveolar bone resorption by regulating the RANKL/OPG ratio.[58] Furthermore, PGE2 levels can reflect periodontitis status, with studies reporting that PGE2 levels in patients with chronic periodontitis decreased significantly from approximately 326.62 ng/mL at baseline to 107.92 ng/mL 8 weeks after non-surgical treatment, suggesting that intervention strategies targeting this pathway hold potential for clinical translation.[59] Adrenergic and acetylcholine receptors mediate neuroimmune regulation, echoing the earlier finding of genetic signal enrichment in the brain and hypothalamus and further supporting the potential role of the neuroimmune network in periodontitis.[60] Molecular docking analysis revealed that BRD-K15574380, BRD-K14693417, and osimertinib exhibited moderate to strong binding affinity with *GNLY*, suggesting that they may exert partial therapeutic effects through direct interaction with *GNLY*—modulating its killing activity or chemotactic function, thereby appropriately enhancing immune clearance while avoiding excessive tissue damage.

Interestingly, osimertinib, a third-generation *EGFR* tyrosine kinase inhibitor, is primarily used clinically to treat *EGFR* mutation-positive non-small cell lung cancer.[61] At the theoretical level, inhibition of the *EGFR* signalling pathway may exert a potential protective effect against periodontitis. Exposure of gingival epithelial cells to periodontal pathogenic biofilms leads to phosphorylation of *EGFR* and its downstream MAPKs (*ERK, p38, JNK*), which subsequently suppresses the expression of $\alpha v\beta 6$ integrin—a key molecule for maintaining anti-inflammatory TGF-β1 signalling in the junctional epithelium.[62] Furthermore, inhibition of the *EGFR* pathway can reduce M1 macrophage polarization by suppressing *PI3K/AKT* phosphorylation, thereby lowering inflammatory cytokine levels and decreasing alveolar bone resorption.[63] Additionally, Liu et al. demonstrated that extracellular vesicles enriched with miR-5107-5p, obtained from EPO-stimulated macrophages (EPO-EVs) and loaded into a chitosan-based thermosensitive hydrogel, could inhibit the *EGFR* pathway upon local injection into a mouse model of periodontitis.[64] This inhibition restored the osteogenic potential of bone marrow mesenchymal stem cells and activated RhoA to promote cytoskeletal reorganization and osteogenic differentiation, thereby mitigating inflammatory bone loss in periodontitis. These findings provide a biological rationale for considering *EGFR* inhibition as a potential therapeutic strategy for periodontitis. However, it must be clearly stated that the systemic administration of osimertinib in clinical practice is associated with a significant toxicity burden. The most common adverse reactions to osimertinib include diarrhoea, rash, dry skin, and nail toxicity, among others. Oral mucositis/stomatitis is also a frequently reported adverse event.[65] Therefore, the systemic use of osimertinib for periodontitis treatment is clinically unfeasible and is not advocated in this study. It is important to emphasize that all the aforementioned drug predictions remain based on computational simulations at this stage. Their actual mechanisms of action within the complex microenvironment of periodontitis require rigorous validation through in vitro and in vivo experiments.

This study has several limitations that should be addressed in future research. First, limited population representativeness. The GWAS data used in this study were derived exclusively from European-ancestry populations, which limits the generalizability of our findings. Second, the GTEx v8 database primarily comprises tissue samples from post-mortem donors, and its gene expression regulatory patterns may differ from those in living tissues. Additionally, the immune cell eQTLs used for sc-MR analysis were derived from peripheral blood, whereas immune cells localized within periodontal tissues may exhibit distinct transcriptional regulatory characteristics. Third, the regulatory network of *GNLY* remains incompletely characterized. Although evidence from multiple dimensions in this study points to *GNLY*, a systematic delineation of its upstream regulatory mechanisms (eg, key transcription factors and epigenetic modifications) and downstream effector networks remain lacking. Future research could further integrate multi-omics data, such as ChIP-seq and ATAC-seq, to construct a comprehensive regulatory map covering both upstream and downstream pathways of *GNLY*, thereby addressing the current knowledge gap. Moreover, functional validation in

disease-relevant models will be indispensable for confirming its therapeutic value. Finally, limitations of computational predictions. The inferences regarding gene function and drug-target predictions in this study are based on bioinformatics algorithms and molecular docking simulations and have not yet been experimentally validated at the cellular level or in animal models.

Conclusions

In summary, this multi-omics integrative study reveals that genetic risk signals for periodontitis are enriched not only in oral tissues but also in the brain and kidney, providing a genetic perspective on the systemic comorbidities of periodontitis. *ITGB6* was identified and validated as a potential causal gene involved in epithelial barrier maintenance. Through convergent computational evidence, *GNLY* was prioritized as a candidate molecule warranting further functional investigation. AI-driven drug screening and molecular docking further identified several compounds exhibiting potential binding affinity for *GNLY*, providing a foundation for subsequent drug optimization. However, these predictions remain computational and require experimental validation. The analytical framework established here may also serve as a reference for multi-omics integrative studies of other complex diseases. Future research will focus on experimental validation and clinical translation, aiming to transform these computational predictions into interventions that ultimately improve patient outcomes.

Author contributions

Ruoyan Cao: Writing – original draft, Formal analysis, Data curation, Conceptualization. Junhao Xiang: Methodology. Beilei Qu: Formal analysis. Zichao Zhuang: Methodology. Shuangshuang Xu: Writing – original draft, Writing – review & editing. Tengda Chu: Writing – review & editing, Conceptualization. All authors have approved the final draft and accepted the decision to submit the manuscript for publication.

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Ethical approval and consent

The original study obtained all necessary ethical approvals and participant consent. Therefore, no additional approval was needed for this study of data.

Data availability

Data are available in public, open-access repositories corresponding to the original studies (FinnGen https://www.finngen.fi/en/access_results; GLIDE https://data.bris.ac.uk/data/dataset). The scRNA-seq datasets are available from GEO under accession numbers GSE267170 and GSE164241. The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of interest

None disclosed.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.identj.2026.109678.

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