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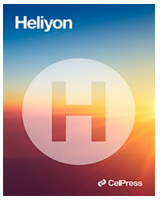

Research article

Bioinformatics reveals the pathophysiological relationship between diabetic nephropathy and periodontitis in the context of aging

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ABSTRACT

Diabetic nephropathy (DN) is one of the most common microvascular complications of diabetes mellitus. Periodontitis (PD) is a microbially-induced chronic inflammatory disease that is thought to have a bidirectional relationship with diabetes mellitus. DN and PD are recognized as models associated with accelerated aging. This study is divided into two parts, the first of which explores the bidirectional causal relationship through Mendelian randomization (MR). The second part aims to investigate the relationship between PD and DN in terms of potential crosstalk genes, aging-related genes, biological pathways, and processes using bioinformatic methods. MR analysis showed no evidence to support a causal relationship between DN and PD ($P = 0.34$) or PD and DN ($P = 0.77$). Using the GEO database, we screened 83 crosstalk genes overlapping in two diseases. Twelve paired genes identified by Pearson correlation and the four hub genes in the key cluster were jointly evaluated as key crosstalk-aging genes. Using support vector machine recursive feature elimination (SVM-RFE) and maximal clique centrality (MCC) algorithms, feature selection established five genes as the key crosstalk-aging genes. Based on five key genes, an ANN diagnostic model with reliable diagnosis of two diseases was developed. Gene enrichment analysis indicates that AGE-RAGE pathway signaling, the complement system, and multiple immune inflammatory pathways may be involved in common features of both diseases. Immune infiltration analysis reveals that most immune cells are differentially expressed in PD and DN, with dendritic cells and T cells assuming vital roles in both diseases. Overall, although there is no causal link, CSF1R, CXCL6, VCAM1, JUN and IL1B may be potential crosstalk-aging genes linking PD and DN. The common pathways and markers explored in this study could contribute to a deeper understanding of the common pathogenesis of both diseases in the context of aging and provide a theoretical basis for future research.

1. Introduction

Diabetic nephropathy (DN) is one of the most prevalent microvascular complications of type 1 and type 2 diabetes. Its diagnostic criteria encompass an elevated urinary microalbumin, diabetic retinopathy, a reduced estimated glomerular filtration rate (GFR), and distinct histological features [1]. Statistically, about 40 % of patients requiring renal replacement therapy are attributed to DN [2]. DN can rapidly progress to end-stage kidney disease without timely treatment due to the absence of symptoms in its early phase, which

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seriously threatens patients' health. Periodontitis (PD) is a chronic multifactorial inflammatory disease that leads to the destruction of periodontal supporting tissues and is usually associated with dysbiosis of the oral microflora [3]. Both DN and PD have significantly contributed to the global public health and economic burden.

Previous studies have emphasized the bidirectional effect between diabetes and PD. In this relationship, diabetes serves as a significant risk factor for the progression of PD, while PD, in turn, influences glycemic control and worsens the development of diabetic complications [4]. A recent meta-analysis indicates a connection between PD and an increased risk of diabetic microangiopathy, including DN, particularly in older patients [5]. Studies have shown that PD is an important risk factor for renal insufficiency in diabetic patients [6], and is strongly linked to the progression of chronic kidney disease (CKD) [7]. Additionally, epidemiological research has supported that PD not only predicts overt nephropathy, defined as massive albuminuria, and end-stage renal disease in type 2 diabetes in a dose-dependent manner [8], but also serves as a robust predictor of DN mortality [9]. To some extent, despite mounting evidence substantiating their independent correlation, little is known about the shared features underlying gene regulatory mechanisms.

Chronic inflammation is a well-known risk factor for DN, affecting its various stages of development. Pro-inflammatory cytokines, such as IL-6, TNF-α, IL18 and IL-1, play a significant role in promoting vascular inflammation and fibrosis, contributing to the development and progression of DN [10]. These cytokines are also implicated in PD, driving an immune response in periodontal tissues that leads to tissue destruction [11]. PD, as a chronic oral infection, triggers a systemic inflammatory response that impacts overall health. This response involves periodontal pathogenic bacteria, their byproducts, and local production of inflammatory mediators [12]. Additionally, individuals with CKD are more susceptible to or experience worsened periodontal disease due to systemic inflammatory load and immune deficiency [13]. These findings suggest that the activation of the inflammatory response may be a key link between DN and PD.

Aging is an inevitable, time-dependent decline in physiological function that affects numerous organ systems and cellular processes, often negatively impacting health [14]. Aging is usually followed by alterations in the immune system, including immune aging, deterioration of the immune response, and an accompanying chronic low-grade inflammatory state [15]. Many age-related phenotypes and diseases share common features of immune aging, namely increased susceptibility to disease and persistent systemic inflammation. Diminished immune response capacity and persistent inflammatory adverse reactions increase the risk of early age-related disease and hinder intrinsic tissue regeneration, thereby expediting disease progression [16]. Extensive research has established connections between aging and chronic age-related conditions such as cardiovascular diseases, neurodegenerative disorders, and metabolic diseases [17–19]. Aging, a common risk factor for DN and PD, affects their progression. It has been argued that the accumulated effects of prolonged exposure of periodontal tissues to microorganisms are, in part, a result of aging-related effects [20]. The aging process alters oral mucosal immunity, increases inflammation, affects tissue healing, and thus accelerates the advancement of PD [21]. Aging is more closely related to DN, and various potential mechanisms, including hyperglycemia, hypertension, chronic inflammation, oxidative stress, lipid metabolism disorders, down-regulation of anti-aging proteins, and the accumulation of advanced glycosylation end-products (AGEs), lead to nephron loss and disease progression in DN [22,23]. Therefore, it is of interest to explore the biological relationship between PD and DN in the context of aging, which can help to access the relevant pathological mechanisms and better guide clinical interdisciplinary management.

Mendelian randomization (MR) employs genetic variation as an instrument to assess the causal impact of specific risk factors on observed outcomes [24]. This study comprised two parts: first, it investigated the bidirectional causality between PD and DN using MR. The second part aimed to reveal the potential interconnections of the two diseases at the transcriptome level with aging-related genes and the linkage with immune cell action through bioinformatics analysis. In this part, dysregulated crosstalk genes in PD and DN were assessed and their correlation with aging-related gene pairs was examined by correlation analysis and PPI networks to determine the role of aging in DN and PD. Next, we analyzed the biological processes (BPs) and pathways by which the two diseases interact to gain more insight into the common genetic mechanisms between PD and DN. Finally, we utilized machine learning methods to build diagnostic models and explore the relationship between immune infiltration of the two diseases, aiming to better guide the clinic and provide new insights into pathological mechanisms.

2. Methods and materials

2.1. MR analysis

Summary statistics for periodontitis were obtained from the Gene-Lifestyle Interactions in Dentistry Endpoints (GLIDE) consortium, including 17,353 cases and 28,210 controls [25]. Periodontitis cases were classified according to the Centers for Disease Control and Prevention/American Academy of Periodontology definitions, using consistent criteria for detailed evaluation or self-reporting. Data for diabetic nephropathy (DN) included 3283 cases and 210,463 controls, and were sourced from the FinnGen consortium. These data are accessible on the GWAS project website hosted by the IEU (https://gwas.mrcieu.ac.uk) under the ID: finn-b-DM_NEPHROPATHY. All participants included in the original GWAS were of European descent.

MR analysis relies on three crucial assumptions: (1) instrumental variables (IVs) must be strongly correlated with the exposure of interest; (2) they must not be associated with confounding factors between each exposure and outcome; and (3) only pass-through exposures affect outcome [26]. We performed linkage disequilibrium (LD) aggregation to ensure the independence of SNPs (with LD R2 < 0.001, LD distance >10000 kb). Considering the limited number of SNPs that reached genome-wide significance (P-value $<5 \times 10^{-8}$) and to obtain more SNPs, we expanded the threshold to 5×10^{-6} and obtained thirteen SNPs associated with DN and eight SNPs associated with PD, respectively. The strength of the IVs was assessed using the F-statistic, calculated as $F = [(n - k - 1)/k]/[R^2/(1$

- R^2)]. SNPs with *F*-statistic thresholds less than 10 were removed. Notably, all F-statistic values for the detected SNPs in this study exceeded 10.

MR estimates were calculated using the inverse variance weighting (IVW) method (random effects) [27] and supplemented by the weighted median [28] and MR-Egger methods [29], which are relatively robust to horizontal pleiotropy. To test for horizontal pleiotropy, we calculated the intercept of the MR Egger regression line. We tested for heterogeneity between MR estimates using the Cochran's Q statistic for IVW and MR Egger methods. MR-PRESSO (Multiplicity Residuals and Outliers) to detect any level of multiplicity abnormality [30]. MR estimates are reported as an odds ratio (OR). MR statistical analyses were performed in R (version 4.3.0), including the software packages "TwoSampleMR" (version 0.5.7), and "MRPRESSO" (version 1.0).

2.2. Data collection of GEO database

The study cohorts for publicly available datasets related to DN and PD were derived from the Gene Expression Omnibus (GEO) database. We searched for relevant data included in the GEO database until September 9, 2022. For the DN dataset, the keywords used in the search included "diabetic nephropathy," "human genome," and "glomerulus." Meanwhile, for the PD dataset, the search terms consisted of "periodontitis," "gingival tissue," and "human genome. The sample tissues were consistent between the two datasets and contained case and control groups. Raw data were obtained from the GEO database, and the experiment type was microarray. The datasets selected for PD included GSE10334 (platform: GPL570) and GSE23586 (GPL570), with 67 controls and 186 cases. For DN, the chosen datasets were GSE30528 (GPL571) and GSE96804 (GPL17586), comprising 33 controls and 50 cases.

Based on the dataset information, we identified common genes among the disease datasets. Specifically, for DN, we determined overlapping genes between the GSE30528 and GSE96804 datasets. The same approach was applied to PD datasets. These intersecting

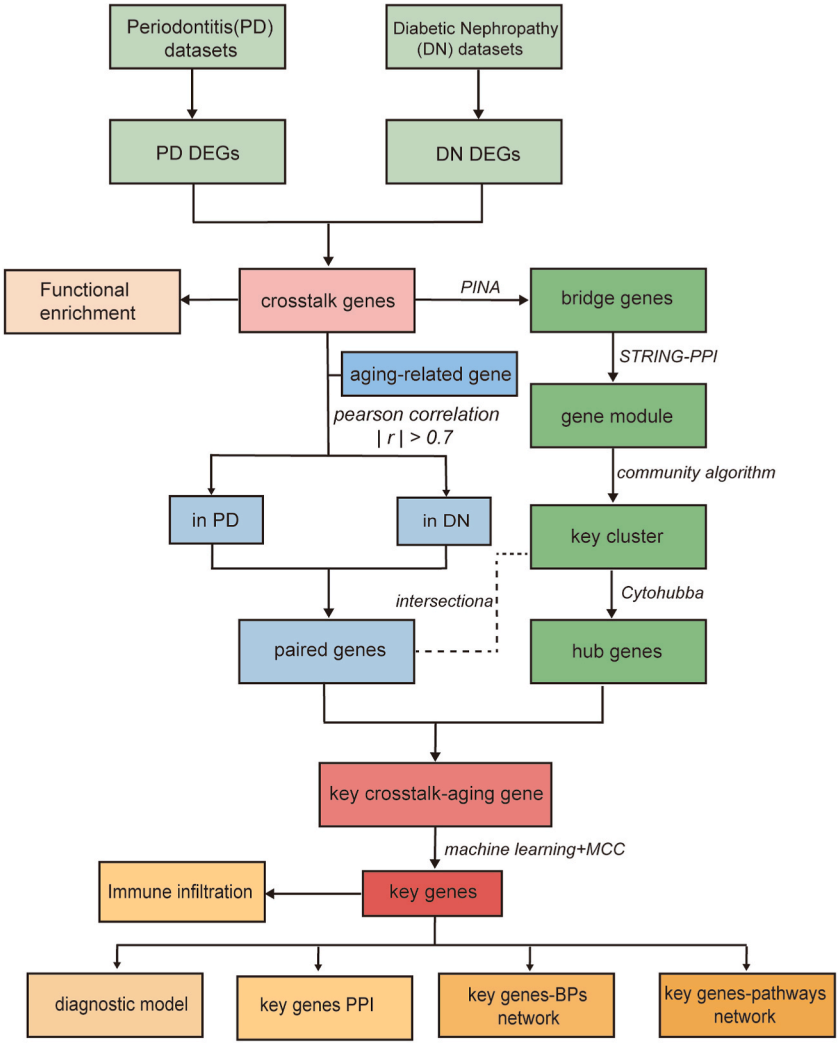

Fig. 1. The work flowchart of the study.

genes' expression profiles from each dataset were separately obtained and subsequently integrated with the clinical data. We utilized the ComBat method in the R package "sva" (version 3.36.0) to remove batch bias, which was then normalized by the "limma" package (version 3.44.3) before further analysis. PCA analysis was applied to determine the effect of ComBat before and after correction. The general workflow graph of our study is presented in Fig. 1.

2.3. Identification of DEGs

The "limma" package was used to perform differential expression analysis on the batch-corrected DN and PD datasets. We determined differentially expressed genes (DEGs) in both the DN and PD datasets as those with *P*-values <0.05 and $|\log 2(\text{fold change})| > 0.6$. To visualize the expression of these DEGs, volcano plots and heatmaps were generated using the "heatmaps" package (version 1.0.12) and the "ggplot2" package (version 3.3.2), respectively.

2.4. Functional enrichment analysis and crosstalk gene

GO annotation analysis and KEGG pathway enrichment analysis of DEGs and crosstalk genes were conducted using the "ClusterProfiler" package (version 4.4.4) for R, and a false discovery rate threshold of <0.05 was considered statistically significant. Bar and bubble charts are utilized to visualize the results. The crossover between DN-associated DEGs and PD-associated DEGs was identified as potential crosstalk genes. These crosstalk genes are likely to play a crucial role in bridging the pathogenesis of DN and PD.

2.5. Correlation between crosstalk and aging-related genes

The literature search was carried out on PubMed for articles related to aging-related genes, resulting in the discovery of several pertinent datasets. Notably, due to variances in the composition of "senescence gene sets" in current literature, a recent paper proposes the existence of "SenMayo" as a 125-gene aging genome validated in human datasets and experiments. Its performance encompasses adaptability to aging in various tissues and species, along with superior responsiveness for clearing senescent cells compared to the existing six genomes [31] (Table S1). To explore the potential role of aging in linking DN and PD, the expression values of crosstalk and aging-related genes were acquired in DN and PD datasets, separately. Using the "corrplot" R package (version 0.92), the Pearson correlation coefficient was computed to determine the correlation between crosstalk genes and age-related genes. We treated *P*-values <0.05 and $|r| > 0.7$ as thresholds for screening paired genes in DN and PD. These shared paired genes in both diseases may be worth investigating.

2.6. Pathway relationships between crosstalk genes and aging genes

By applying the Cytoscape tool (version 3.9.0), the crosstalk genes and aging-related genes were displayed as a gene-pathway network. In addition, we applied the ggplot2 tool to create a circular bar chart to show the number of shared pathways found in each category (R code in Table S5).

2.7. Identification of key modules and genes

The genes associated with crosstalk genes and their interaction pairs were extracted from the PINA database (v3.0). We refer to those extracted genes associated with aging genes as bridging genes, since some crosstalk genes can be indirectly linked to aging genes, and studying gene interactions allows for a more accurate and profound investigation of the mechanisms. The Louvain algorithm, an iterative community identification algorithm that maximizes the modularity of the network by combining different modules while clustering the vertices, is one of the best ways to explore modules in human PPI networks [32]. Here, we use the Louvain detection algorithm in the "igraph" R package (version 1.3.5) to extract key modules from all these associated gene PPI networks. The weight was set to $|r|$, and modules with fewer than 30 genes were removed.

To understand the biological relationships involved in key cluster genes, GO and KEGG enrichment analyses were carried out using the Metascape reference library. 3 for minimal overlap, 1.5 for minimum enrichment, and *P* value < 0.01 were used as threshold conditions. Subsequently, we introduced all key clusters of genes into the Cytoscape program and selected the top 15 key genes using the cytohubba plugin.

2.8. In-depth investigation of key genes

Based on machine learning algorithms, we further explored the potential key genes of crosstalk and aging. SVM-RFE is a sequential backward selection method that removes superfluous features by ranking each feature with a score according to the maximum interval law of SVM [33]. The SVM-RFE analysis based on the R package "e1071" was used to filter the DN and PD datasets individually, and the thresholds were set as follows: halve. above $= 100$ and $k = 10$. The MCC plugin of the Cytoscape program was also used for screening. Ultimately, genes that overlapped between these algorithms were defined as key genes.

Additionally, by employing the "neuralnet" (version 1.44.2) R package, we built artificial neural network (ANN) models in DN and PD, respectively, for the key genes acquired from the mentioned methods. ANN is an essential section of deep learning, which can infer a collection of classification rules from a jumble of data to achieve accurate classification and build an efficient and reliable diagnostic

model [34]. The ANN model is based on the input features, hidden layer, and output layer as a framework, and set the maximum number of iterations to 200. The test set and training set are divided based on 1:4 ratio. The receiver operating characteristic (ROC) curve was applied to assess the ANN model's precision in both the training and testing sets. Finally, the expression patterns of these five genes in the DN and PD datasets were evaluated. Moreover, to assess the predictive efficacy of these key genes, ROC curves were created with "pROC" (version 1.18.0) in the R program.

2.9. Functional annotation of key genes

We imported key genes into the PINA database to access the interaction context of key genes. Based on the gene expression data of DN and PD case samples, the "GENIE3" package (version 1.18.0) of the R program was used to rank the weight values of the acquired genes and key genes in descending order in DN and PD, and the interaction pairs with the top 5 % weight were selected as the construction of subsequent PPI networks. The GO and KEGG databases are used as reference libraries for key genes to explore their functions and identify the pathogenic mechanisms common to both diseases. ClueGO is employed to fuse and visually present networks by function. The shared pathway diagrams were investigated using the KEGG database to identify relationships between key genes and pathways. The kappa score for GO term fusion was set to 0.6, and the minimum GO tree and minimum clustered genes were set to 3 and 2, respectively.

2.10. Immune infiltration analysis

Based on 28 immune cell-associated genomes, we performed quantitative analysis with ssGSEA (version 3.15 of the R package "GSVA") to calculate the abundance of immune cells in two diseases [35]. The results are presented using heatmaps. Using the "corrplot" package (version 0.92), we observed the correlation between abundant immune cells and various immune cells in both diseases. Spearman's correlation analysis was then applied to understand the connections between immune cells and key genes, utilizing the gene expression matrix and immune cell abundance data. Finally, we used the "ggplot2" software package to visualize the differences in immune cell infiltration between the two disease groups. These approach helps us understand the relationship between immune cells, genes, and the diseases studied.

3. Result

3.1. Results of a two-sample MR analysis

Using an IVW model, we found no evidence of a potential causal effect between DN and PD risk: OR = 0.96, 95 % confidence interval (CI): 0.89–1.04, $P = 0.34$ (Fig. 2). This was confirmed by additional analyses of the MR-Egger model and the weighted median model. The MRPRESSO did not detect an outlier. No evidence of directed horizontal pleiotropy was found in the MR-Egger intercept test (intercept −0.007, $P = 0.77$). Heterogeneity and horizontal pleiotropy were not observed using Cochran's Q test ($P = 0.14$).

In addition, there was little evidence to support reverse causality of DN on PD risk: OR = 1.02, 95 % CI: 0.91–1.14, $P = 0.77$ (Fig. 2). Sensitivity analyses supported this. No evidence of directed horizontal pleiotropy was found in the MR-Egger intercept test (intercept −0.006, $P = 0.76$). Tests for heterogeneity and pleiotropy were negative. Again, no outliers were detected in MRPRESSO.

Despite the absence of a causal relationship, it is still necessary to explore the underlying biological mechanisms between the two, given their close association.

3.2. Data pre-processing

The DN dataset consists of two datasets, GSE30528 and GSE96804, totaling 50 cases and 33 control samples. The PD dataset includes datasets GSE10334 and GSE23586, with 186 cases and 67 controls. Following the application of the ComBat method to mitigate batch bias, the variance between data sets was markedly reduced (Fig. S1).

Exposure	Outcome	Method	nsnp	OR(95%CI)	pval	Q statistic / pval
DN	PD	IVW	10	0.96(0.89 to 1.04)	0.34	13.64 / 0.14
		Weighted median	10	0.98(0.91 to 1.06)	0.60	
		MR Egger	10	0.99(0.83 to 1.17)	0.87	13.50 / 0.09
		MR PRESSO	10	0.96(0.89 to 1.04)	0.34	
PD	DN	IVW	6	1.02(0.91 to 1.14)	0.77	3.01 / 0.70
		MR Egger	6	1.03(0.90 to 1.18)	0.70	
		Weighted median	6	1.01(0.89 to 1.14)	0.91	2.91 / 0.57
		MR PRESSO	6	1.02(0.91 to 1.14)	0.77	

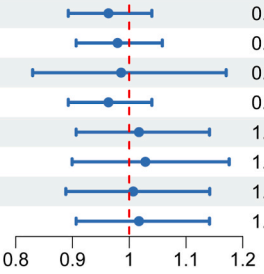

Fig. 2. Forest plot representing the association between bidirectional MR estimation of periodontitis and diabetic nephropathy.

3.3. Identification of DEGs

By performing differential analysis, we obtained 240 up-regulated genes and 352 down-regulated genes in DN and 464 up-regulated genes and 244 down-regulated genes in PD, respectively. Volcano map and heat map were applied to visualize the expression patterns of DEGs in both diseases (Fig. 3A–D). The DEGs of DN are mainly participated in several BPs of GO analysis, such as system development and cell chemotaxis (Fig. 4A), while the DEGs of PD are mainly engaged in several BPs of GO analysis, such as immune response, B-cell activation (Fig. 4C). Additionally, DN and PD DEGs are jointly involved in the "cytokine-cytokine receptor interaction" and "viral protein interaction with cytokines and cytokine receptors" pathways (Fig. 4B–D). One of the hallmarks of aging is the acquisition of a secretory phenotype associated with aging, characterized by the release of pro-inflammatory cytokines, chemokines [36]. Therefore, aging may potentially underlie the common developmental mechanisms observed in both DN and PD.

3.4. Identification of crosstalk genes

After intersecting the DEGs of the two datasets, we eventually obtained 83 crosstalk genes. The Venn diagram of the overlapping DEGs is shown in Fig. 5A, of which 46 are jointly up-regulated and 8 are jointly down-regulated in DN and PD. GO analysis results showed that crosstalk genes were mainly intimately related to cytokine−mediated signaling pathway, leukocyte migration, humoral immune response, response to lipopolysaccharide, and cell chemotaxis (Fig. 5B). Consistent with the previous study, Fig. 5C displays that crosstalk genes are primarily enriched in several pathways, including cytokine-cytokine receptor interaction, *Staphylococcus* aureus infection, and viral protein interaction with cytokine and cytokine receptor.

3.5. Correlation between crosstalk genes and aging-related genes

Aging-related genes are an important articulated part in our study. The expression profiles of aging-related genes in DN and PD data are portrayed through heatmaps (Fig. 6A and B). In the DN group, 62 aging-related genes were significantly differentially expressed

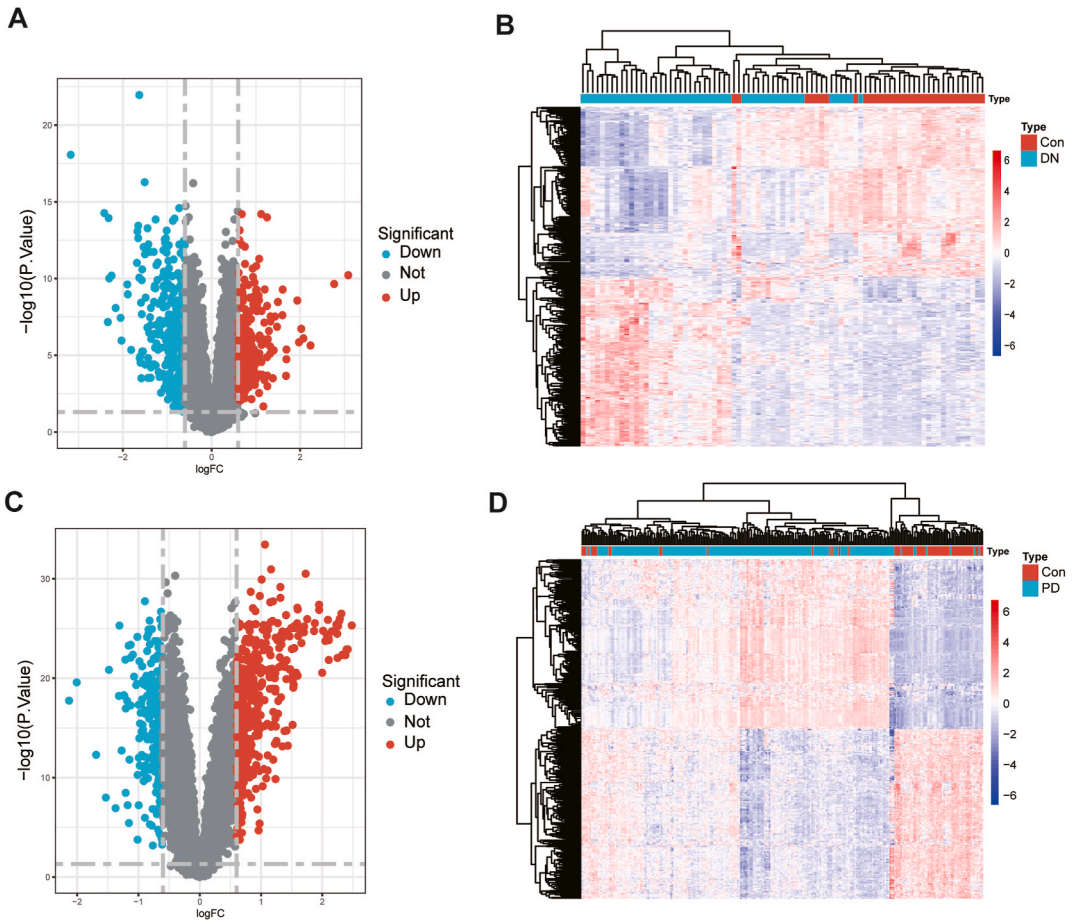

Fig. 3. Characterization of DEGs (A) Volcano diagram of DN's DEGs. (B) Heat map of DN's DEGs. (C) Volcano diagram of PD's DEGs. (D) Heat map of PD's DEGs.

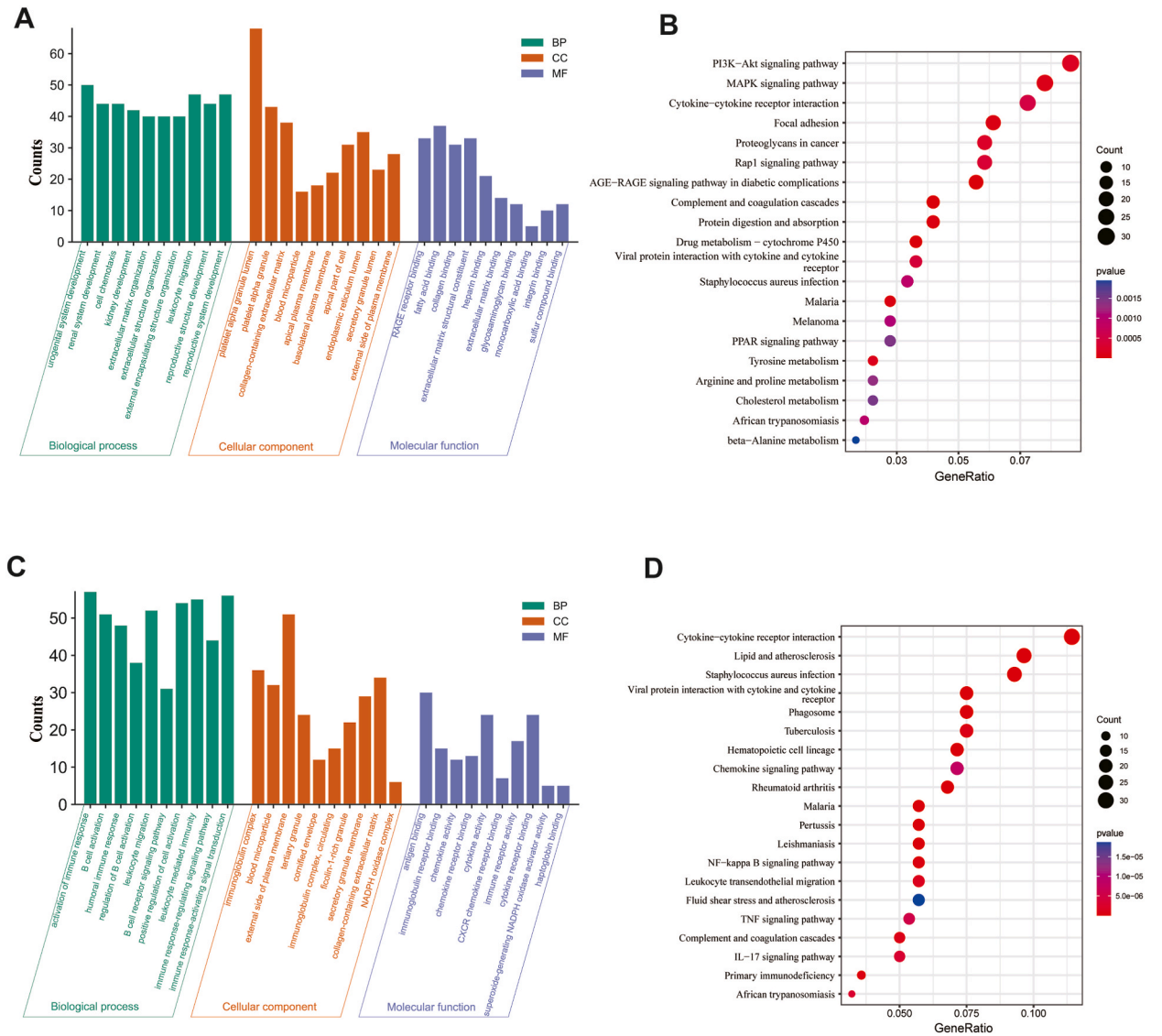

Fig. 4. Enrichment analysis of DN and PD. (A) GO analysis terms of DN DEGs. (B) KEGG analysis terms for DN DEGs. (C) GO analysis terms of PD DEGs. (D) KEGG analysis terms for PD DEGs.

among control and case samples ($P < 0.05$), 19 of which were identified as DEGs for DN. 88 aging-related genes were significantly differentially expressed in the PD group ($P < 0.05$), 24 of which were PD DEGs. These results suggest that aging plays an important role in the pathogenesis of both diseases. Using Pearson correlation coefficients, we investigated the correlation between 83 crosstalk genes and 125 aging-associated genes in the DN dataset (Fig. 6C), and the same for the PD dataset (Fig. S2). Together 270 pairs of related crosstalk-aging genes were explored in DN, and 212 pairs in PD ($P < 0.05$, $|r| > 0.7$). By analysis, 14 pairs of related genes were common in both diseases, including 12 crosstalk genes and 4 aging-related genes (Table S2).

To better understand the relationship between these two groups of genes, the KEGG database was used to determine the pathways through which crosstalk genes interact with aging-related genes. Using Cytoscape software, we built a gene-common pathway network composed of 49 crosstalk genes, 98 aging-related genes, 111 common pathways, and 1088 edges linking pathways and genes (Fig. 7A). The cytokine-cytokine receptor interaction pathway is the most enriched pathway for genes. According to Fig. 7B, "signal transduction" and "immune system" are the main areas implicated in aggregation pathways, with additional infectious disease-related pathways also occupying a part.

3.6. Identification of key crosstalk-aging networks and genes

By importing crosstalk genes into the PINA database, 2551 bridge genes closely related to crosstalk genes were obtained. According

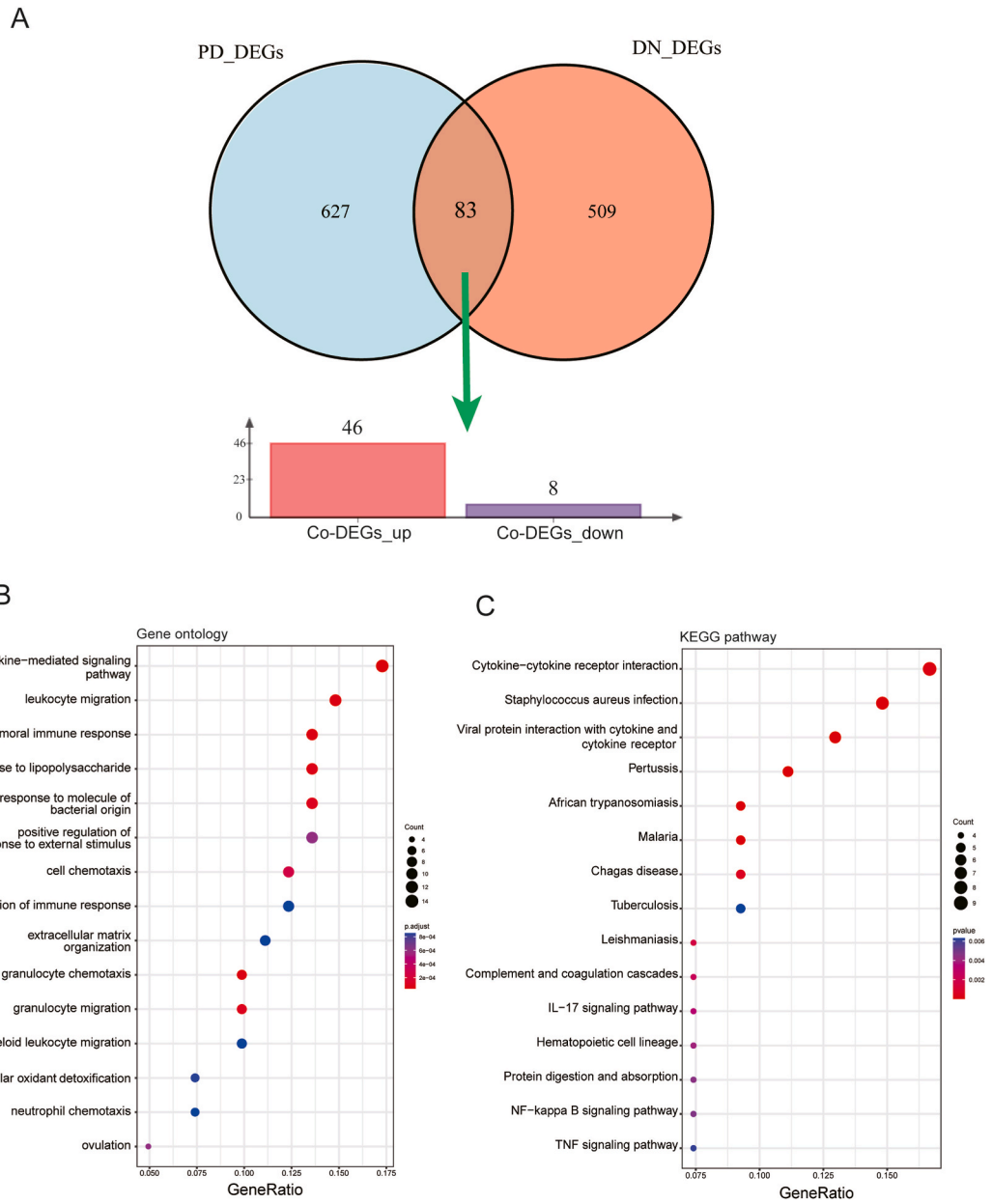

Fig. 5. Analysis of crosstalk genes. (A) Interaction Venn diagram of PD DEGs and DN DEGs and the number of co-expressed genes. (B) Top 15 BPs of crosstalk genes. (C) Top 15 KEGG of crosstalk genes.

to the STRING database, a PPI network of crosstalk, bridge and aging-related genes was developed, consisting of 2576 nodes and 40855 edges. Subsequently, we divided all genes into 11 clusters using the Louvain community algorithm, and after removing gene modules with fewer than 10 genes, the PPI network was finally classified into six clusters. The different densities have a measured significance for the assessment of connectivity in the network. The densities of the different modules are recorded in Table S3 and it is observed that the densities of all six clusters are higher than the density before clustering (0.02636438), demonstrating the reliability of this clustering algorithm. In addition, it can be clearly observed that cluster 4 has the highest number of crosstalk and aging related genes, suggesting that the genes in this cluster are important modular genes in the disease (Fig. 8A and B).

Enrichment analysis revealed that the BPs of cluster 4 were mainly enriched in the inflammatory response, enzyme-linked receptor protein signaling pathway, positive regulation of cell migration, regulation of MAPK cascade, and chemotaxis. The KEGG pathway is mainly concentrated in the chemokine signaling pathway, pathways in cancer, Rap 1 signaling pathway, AGE-RAGE signaling pathway in diabetic complications, and human cytomegalovirus infection (Fig. S3). The top 15 hub genes in cluster 4 were found by using the cytohubba algorithm (Fig. 8C). We selected the crosstalk gene VCAM1 and the top 3 aging-related genes IL6, IL1B, and IL10 as hub

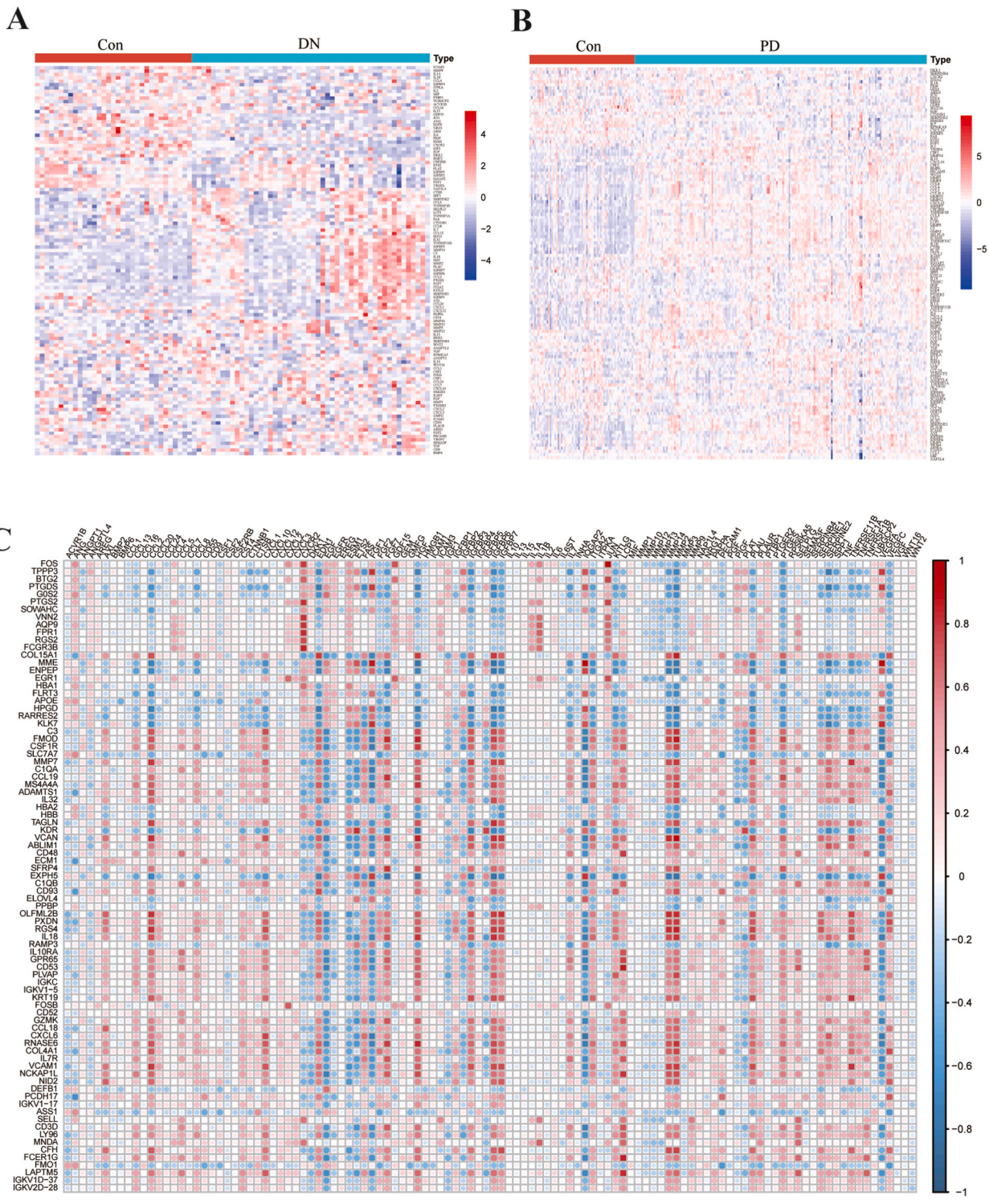

Fig. 6. Expression and correlation of aging-related genes in DN and PD. (A and B) Heatmaps of aging-related gene expression in DN (A) and PD (B). (C) Correlation between crosstalk genes and aging-related genes in DN samples.

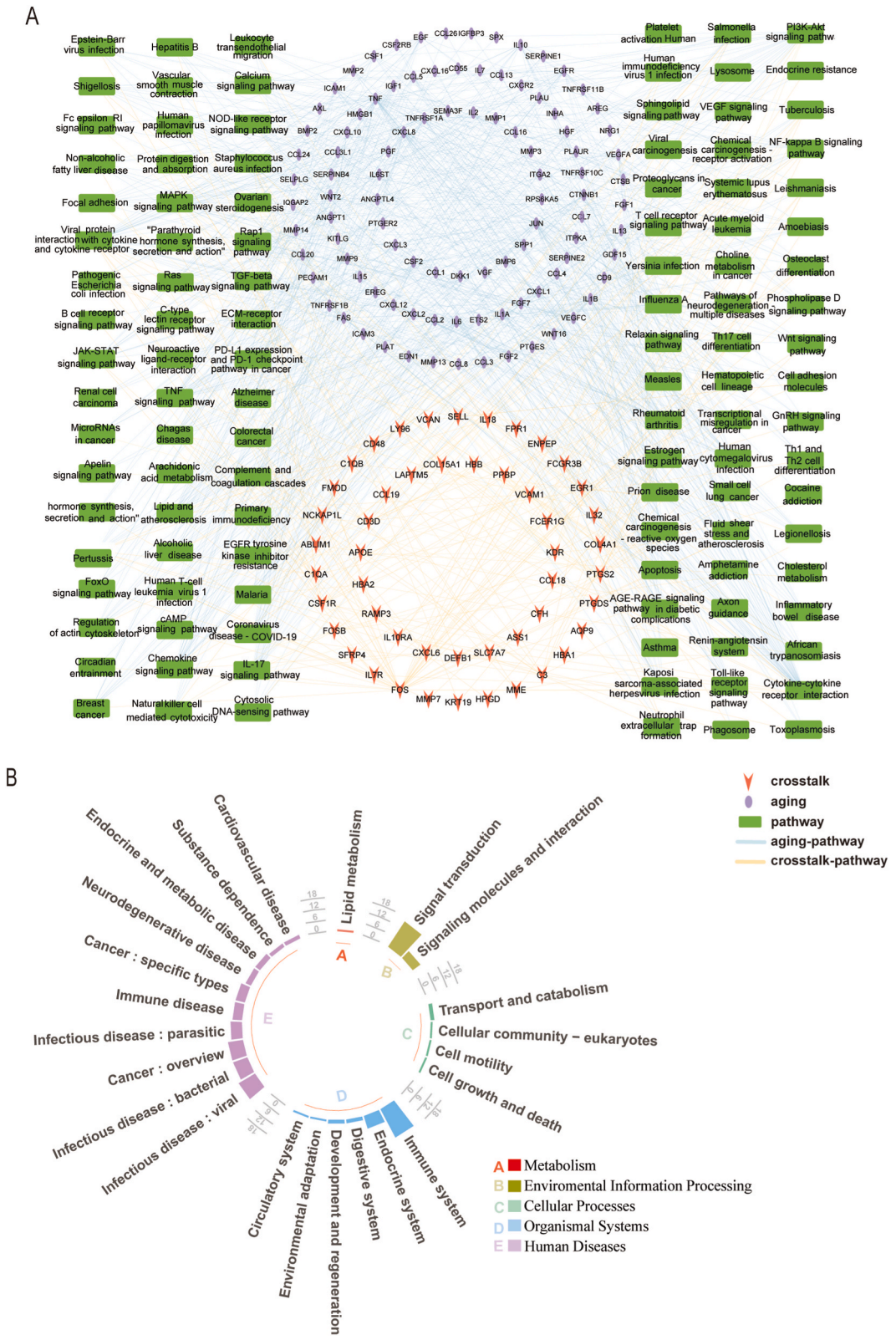

Fig. 7. Gene-pathway analysis (A) Pathway gene network (B) Round bar diagram of secondary classification of common pathways.

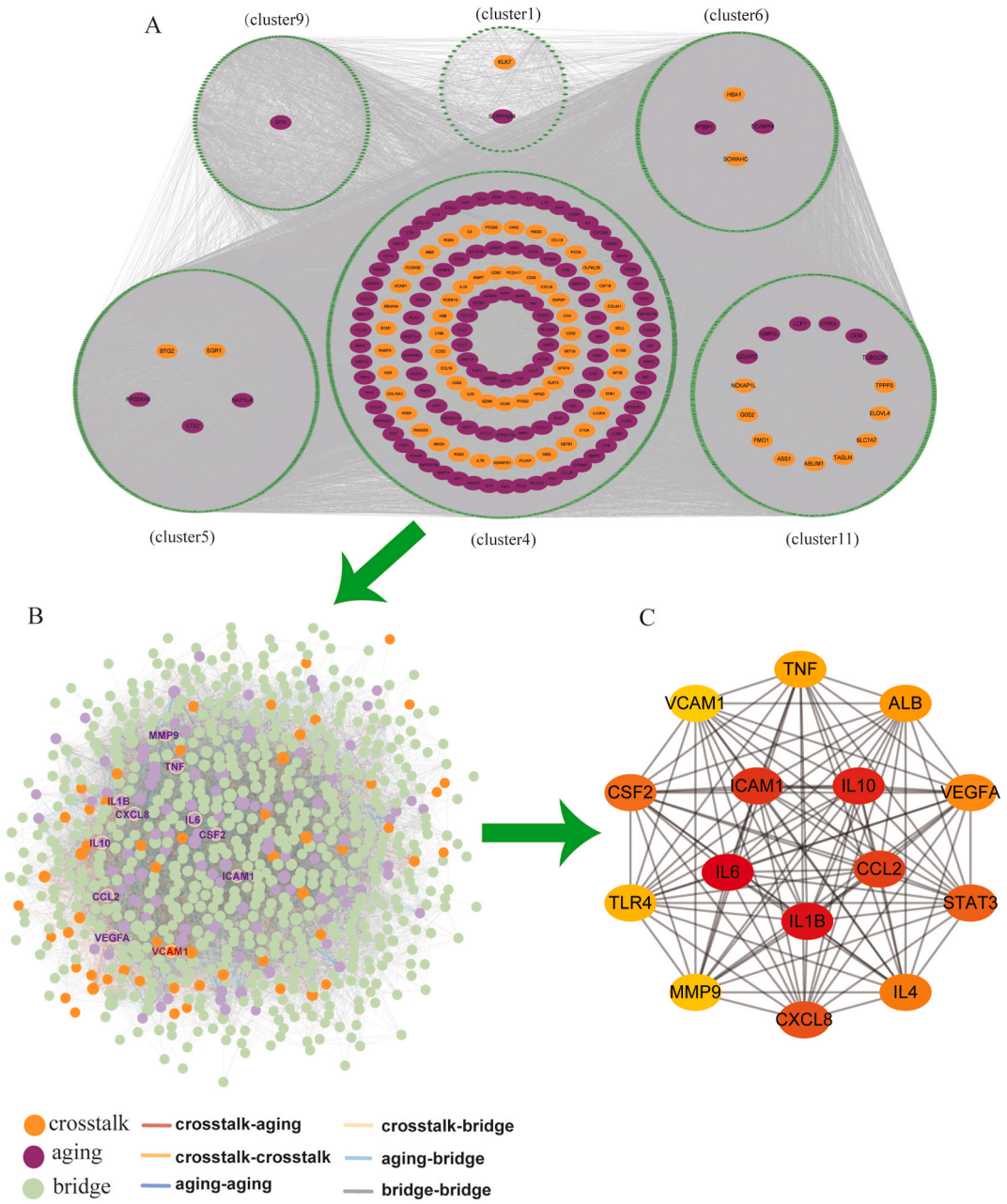

Fig. 8. Identification of key cluster and genes. (A) Network module for applying community algorithm detection. (B) PPI network for key modules (cluster 4). (C) Hub genes determined by cytoHubba in the key modules.

genes, which may play important roles in the key cluster.

3.7. Investigation of key crosstalk-aging genes

By taking the intersection of the genes in cluster 4 with the paired genes, we obtained 12 key crosstalk-aging genes (IL10RA, CD3D, RGS4, CSF1R, CXCL6, CD53, CD48, SELPLG, JUN, CXCL1). Combined with the previous 4 hub genes (VCAM1, IL6, IL1B, and IL10), these 16 key crosstalk-aging genes have significant relevance to the disease. By applying the SVM-RFE machine algorithm, we obtained 9 and 15 key crosstalk-aging genes in DN and PD, respectively (Fig. 9A and B). Since the PD dataset has an objective sample imbalance, in order to evaluate the SVM-RFE model performance, we found that the model has an accuracy of 0.94, a sensitivity of 0.8125, a specificity of 1, and an F1_score of 0.8965517 after 10-fold cross-validation of the PD data. As we used a database expanded with networks of key-crosstalk genes, cytoHubba was able to discover the key targets and central elements of complex networks, where MCC

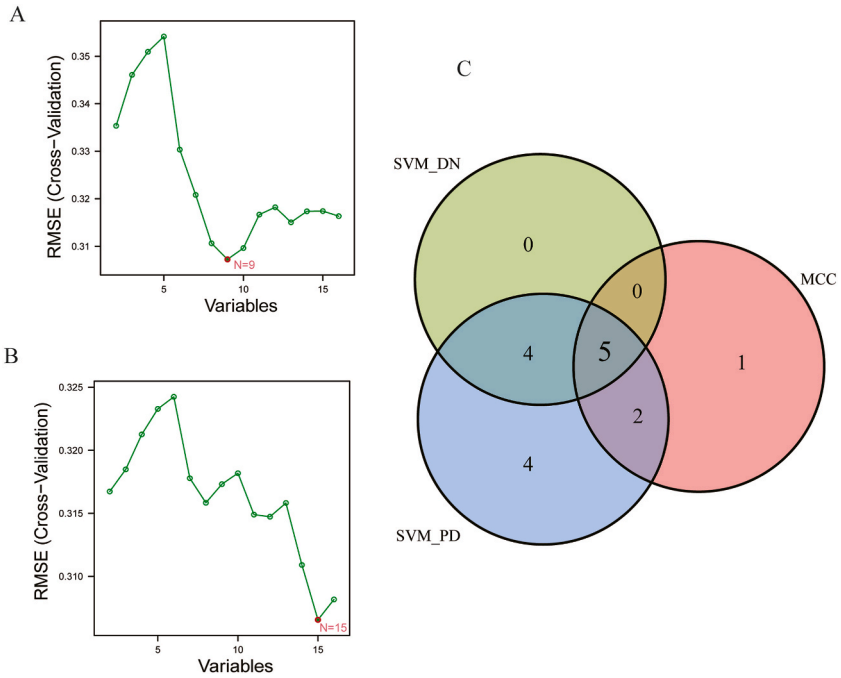

Fig. 9. Selection of features. (A–C) Key crosstalk-aging genes selection by SVM-RFE (A and B) and Cytoscape MCC algorithm (C).

was better at predicting key markers in PPI networks [37]. We selected the top 8 genes using the MCC algorithm. The two algorithms were taken to intersect and we finally obtained 5 key genes, of which CSF1R, CXCL6, VCAM1 are crosstalk genes and JUN, IL1B are aging genes (Fig. 9C).

Subsequently, according to key features, we built ANN models and classified gene expression data for both cases and controls (Fig. 10A and B). The high reliability of our prediction model based on key genes was demonstrated by ROC curves with an AUC of 0.9991 in the training cohort and 0.9167 in the test cohort for DN, and an AUC of 0.9684 for the PD training cohort and 0.79 in the test cohort (Fig. 10C and D). In addition, ROC curves were applied to display the diagnostic properties of five key genes in DN and PD, respectively. The results indicated that the five genes had high diagnostic accuracy in predicting both diseases, with AUCs greater than 0.6 (Fig. 10E and F). To further test the reliability and accuracy of the ANN model, we also performed ANN on the external DN dataset GSE104948 (GPL22945, containing 18 controls and 7 cases) and the PD dataset GSE16134 (GPL570, containing 69 controls and 241 cases), respectively. The results of the tests remain robust (Fig. S4). Fig. S5 displays the expression patterns of the 5 key genes. All five of these genes were upregulated in PD and all three crosstalk genes were upregulated in DN.

3.8. Connection of key genes

To gain a deeper understanding of the interactions among key genes, we focused on genes that are known to interact with these key genes. Using the expression profile data from both DN and PD cases, we predicted the weighted relationships of these genes with rhe key genes and constructed PPI networks involving top 5 % weighted genes (Fig. 11). We observed that CSF1R, CXCL6, and VCAM1 can interact through EIF4A1, VCAM1 and IL1B can affect each other through MAF, and CSF1R and JUN can affect each other through ETS2.

To better recognize the potential functions of key genes, we incorporated key genes into the Gene Ontology database. As depicted in Fig. 12A, we identified 301 BPs involving at least two key genes, and fused these BPs into 64 BPs based on GO terminology by ClueGO (Table S4), with regulation of chemokine production and leukocyte proliferation accounting for the vast majority (Fig. 12B). In order to investigate the potential mechanisms of key genes in DN and PD, we introduced key genes into the KEGG database and screened 30 pathways comprising at least two key genes. Among them, 14 of these pathways were associated with aging-related genes alone, and the remaining 16 pathways including AGE-RAGE signaling pathway in diabetic complications, NF-kappa B, IL-17, MAPK, and TNF signaling pathway were associated with crosstalk genes and crosstalk-aging genes (Fig. 12C). These may be significant pathways for our research, offering insights into crucial mechanisms in DN and PD.

3.9. Analysis of immune characteristics of DN and PD

After pathway exploration analysis, the immune system appears to have a profound influence on DN and PD. To gain a quantitative understanding of immune cell infiltration, we employed the ssGSEA algorithm to calculate the enrichment scores of immune cells in

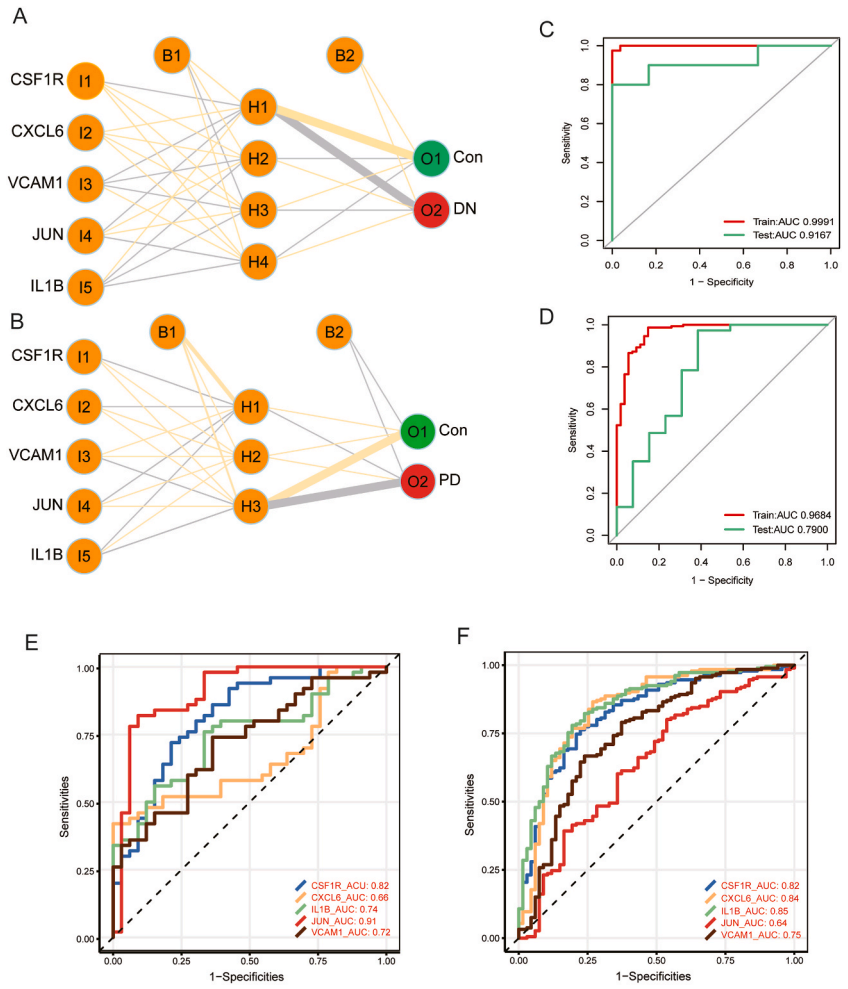

Fig. 10. Model building. (A–B) ANN models for DN (A) and PD (B). (C and D) ROC curves of ANN models in the DN (C) and PD (D) training and testing cohorts. (E and F) Evaluation of the diagnostic effect of DN (E) and PD (F) datasets based on ROC curves.

DN and PD samples, which was shown in a heat map(Fig. 13A). The results revealed that central memory CD4 T cell, plasmacytoid dendritic cell, effector memeory CD8 T cell, and immature dendritic cell were highly expressed and clustered together in DN and PD. Fig. 13B displays the correlation between high-level immune cells and various immune cells in both diseases. It can be observed that central memory CD4 T cells and effector memory CD8 T cells are highly correlated with most immune cells in DN, and in PD, four cells are positively correlated with most immune cells. Furthermore, we observed significant differences in most immune cell subsets between cases and normal samples in DN and PD (Fig. 13C and D). The correlation of the five key genes with individual immune cells can be found in Fig. S6.

4. Discussion

In this study, we first investigated the bidirectional causal relationship between DN and PD using mendelian randomization. Unfortunately, we did not find a bidirectional causal relationship between them. PD is associated with diabetes mellitus, and extensive evidence supports PD being placed as the sixth complication of diabetes [38]. As a result, PD and DN are frequently comorbid, sharing a common pathogenesis that may mutually influence each other despite significant tissue differences. Therefore, our study explores the common mechanisms and correlations between the two diseases in the context of aging through bioinformatics, community algorithms, and machine algorithms with reference to some hypotheses of the periodontal-renal axis. Five genes, namely CSF1R, CXCL6, VCAM1, JUN and IL1B, were selected as key genes explaining the link between DN and PD in the context of aging. It was hypothesized that pathways such as the AGE-RAGE signaling pathway, the complement system, and multiple immune-inflammatory pathways may be the underlying common mechanisms of DN and PD.

The current discussion is that immune dysregulation may be one of the most prevalent underlying features of both diseases [39,40]. Innate immune changes are coupled with increased pro-inflammatory cytokine expression, monocyte infiltration, and decreased

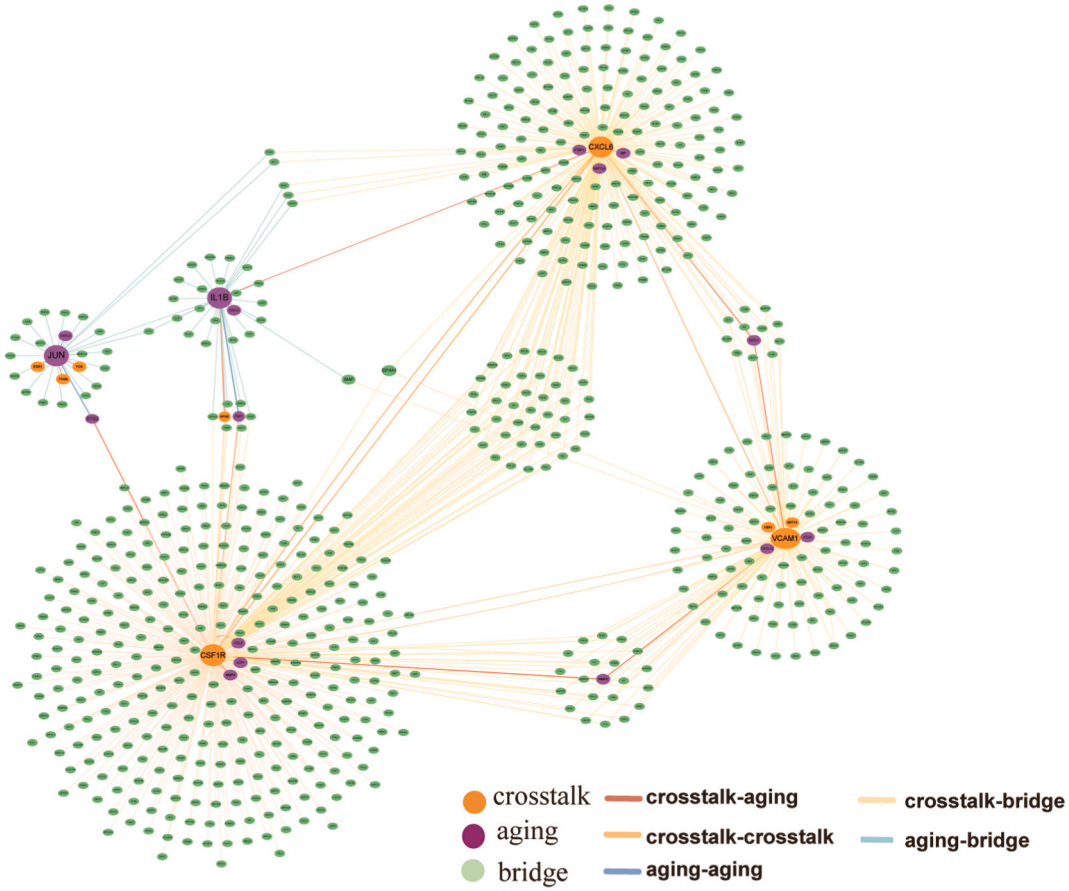

Fig. 11. PPI networks of important cluster genes associated with key genes (CSF1R, CXCL6, VCAM1,JUN, and IL1B).

bacterial killing; each of these factors may play a role in aggravated periodontal disease. For instance, elevated cytokine levels and inflammation may enhance bone resorption and hinder repair, and reduced bacterial killing may facilitate pathogen growth [41]. Furthermore, the activation of the innate immune system contributes to the development of DN by inducing the production of inflammatory cytokines that cause tissue damage [42], suggesting that this similar element may lead to its association with both diseases is plausible. Our study on DN and PD crosstalk genes confirmed their biological functions primarily revolve around cytokine pathways, leukocyte migration, and immune response activation, underscoring a shared basis of immune-inflammatory mechanisms in both conditions.

Recent evidence underscores the pivotal role of aging in the pathogenesis of chronic inflammatory diseases, including PD and DN [20,43,44]. There is no doubt that aging contributes to the enhancement of pathological processes that lead to the dysregulation of the immune system in response to the harmful challenges of chronic inflammatory states and accelerated bone loss processes [45,46]. It exacerbates the morbidity and severity of PD, causing alterations in oral microbial composition, potentially leading to weakened immunity and health deterioration [47]. Additionally, aging kidneys are concerned with physiological changes and significantly increase susceptibility to nephrotoxic injury [48]. The relatively high proportion of aging-related genes among the DEGs of PD and DN in our study suggests an important link between aging and these two diseases.

To minimize the effects of overfitting and to improve the quality of performance metrics, as many samples as possible should be selected for clinical biomarker discovery experiments [49]. In our study, the PD dataset included a total of 186 gingival tissue samples and the DN dataset included 50 kidney tissue samples. Despite the objective sample imbalance in the PD dataset, SVM-RFE achieved the best performance after using cross-validation, allowing for reduced intervention of methods such as resampling, cost-sensitive learning, and threshold adjustment [50]. The SVM-RFE machine algorithm is able to construct optimal classifiers for each subtype while minimizing structural risk, and is well suited as a way to improve learning performance for the differential selection of the two diseases [51]. Usually, the root mean square error (RMSE) is evaluated for the difference between the observed and predicted values to predict the performance of the model and compare the fitting results of different models [52]. The smaller the RMSE, the better the model performance. In our study, we found that the SVM residual distribution (RMSE) was minimal among numerous machine learning methods such as random forest, generalized linear model (GLM), gradient boosting machine (GBM) and SVM by the DALEX R package (results not attached), indicating the good performance of SVM-RFE model.

This study focuses on identifying five key genes CSF1R, CXCL6, VCAM1, JUN and IL1B to elucidate the association between DN and

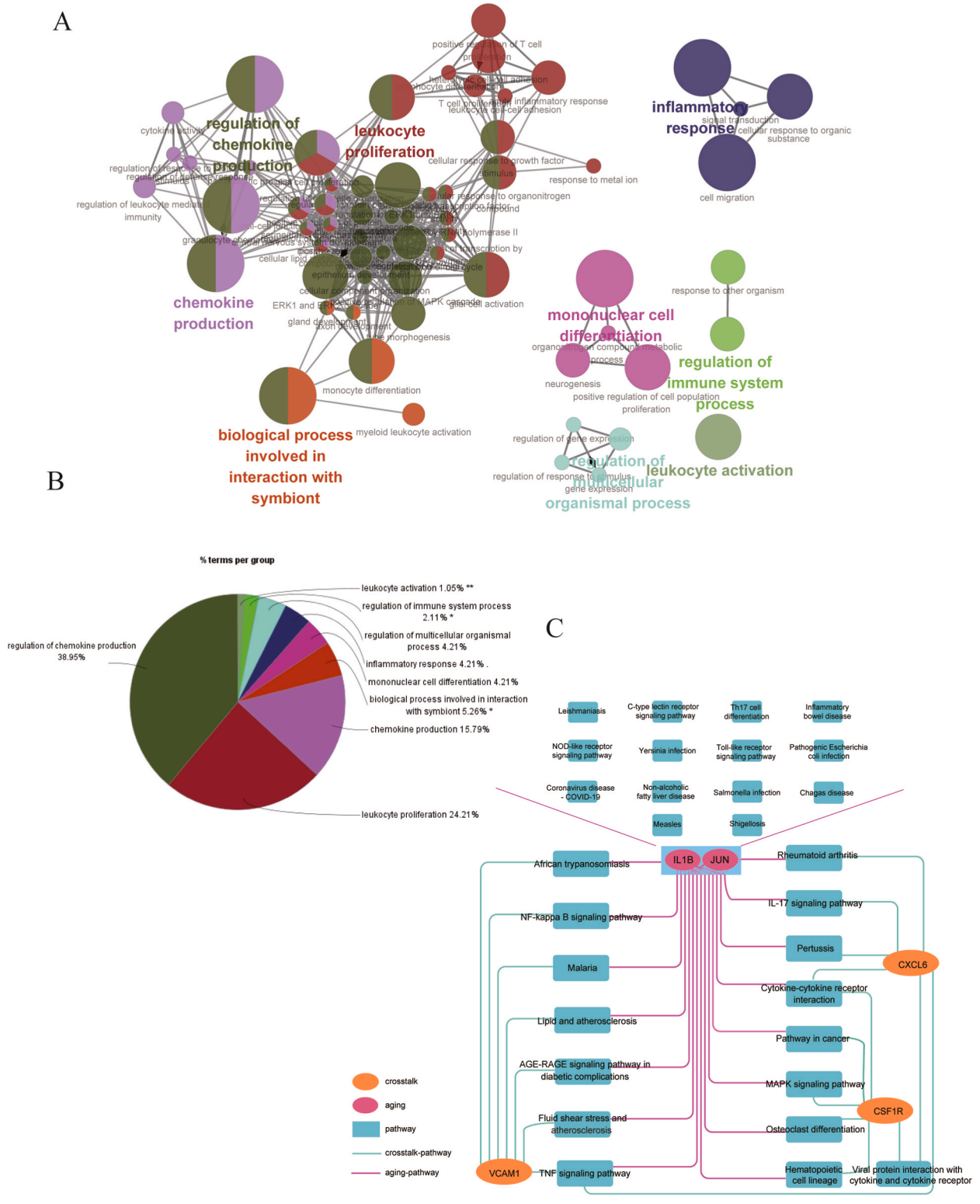

Fig. 12. Enrichment analysis of key genes. (A) ClueGO fusion results for biological processes implicated in key genes. (B) Pie chart for each GO term group scale. (C) Pathway enrichment networks for key genes.

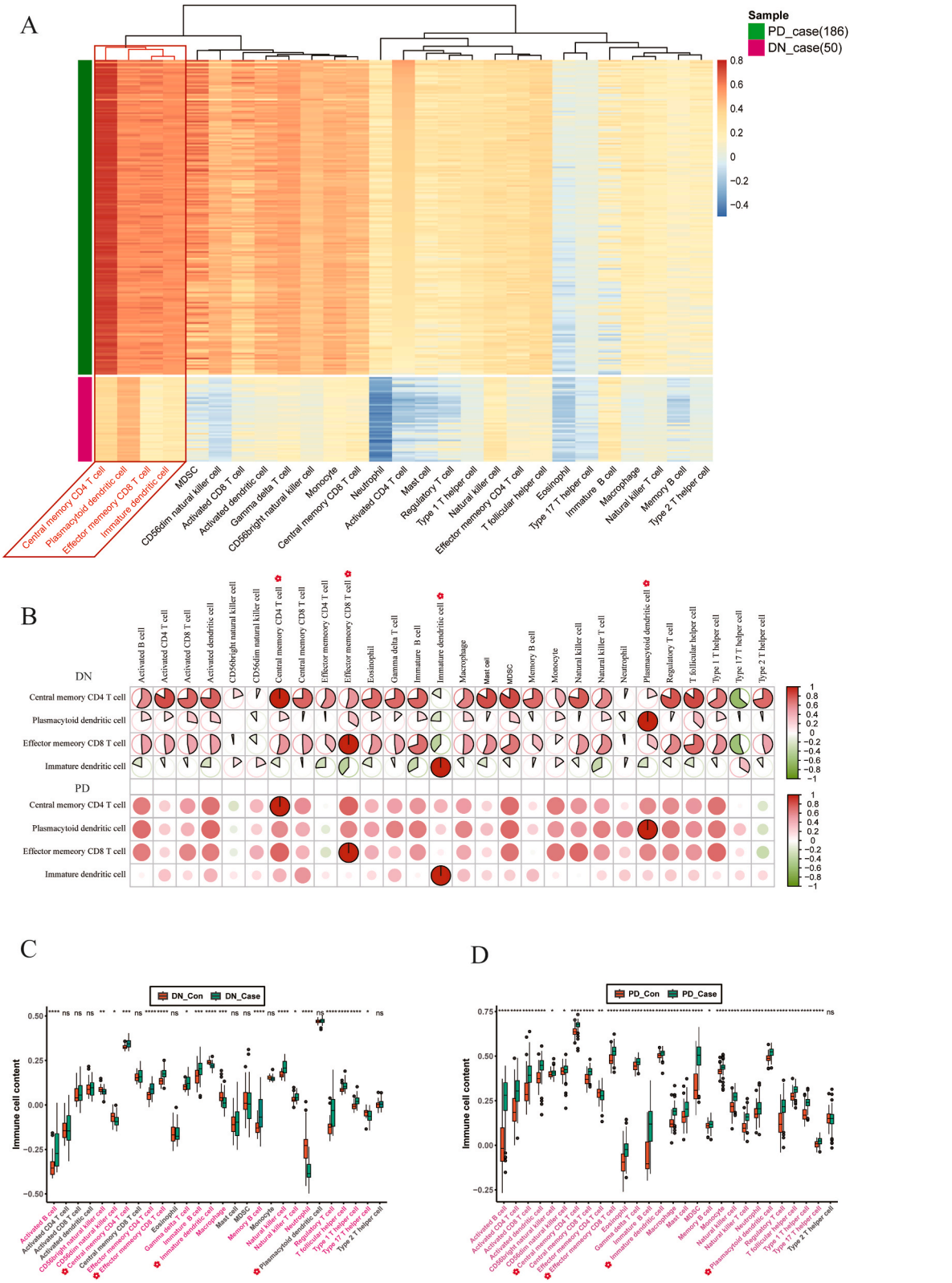

Fig. 13. Analysis of immune characteristics of DN and PD. (A) Heatmap displaying the immune abundance of DN and PD. (B) Correlation analysis between high-level immune cells and various immune cells. Pie charts in DN and circle charts in PD represent correlations. (C and D) Comparison of the levels of distinct groups of immune cells in DN (C) and PD (D).

PD, and a diagnostic model consisting of these markers has good predictive power for both diseases. CSF1R is an important transmembrane receptor tyrosine kinase that mediates the biological effects of colony stimulating factor-1 (CSF-1) [53]. Regulation of CSF-1R stimulation affects the innate immune system and plays a central role in many diseases including cancer, chronic inflammatory diseases and fibrosis [54,55]. CXCL6 is a class of small molecular peptides with chemotactic and inducible properties that were first identified in osteosarcoma cell lines and play a key role in various types of tumor diseases [56]. As an important inflammatory cytokine, CXCL6 can recruit inflammatory cells to sites of inflammation. Combined with crosstalk gene pathway analysis, we can boldly speculate that inflammatory chemotaxis is an important factor in the common pathogenesis of DN and PD. Initially identified as a membrane protein promoting immune cell recruitment, VCAM1 plays a vital role in endothelial adhesion [57]. Aging is a powerful independent predictor of soluble VCAM1 levels [58]. In diabetic nephropathy, VCAM1 emerges a crucial immune-related gene that worsens interstitial inflammation by promoting the recruitment of immune cells such as macrophages and T cells into diabetic glomeruli [59,60]. Furthermore, VCAM1 gene polymorphisms were associated with the severity of periodontal disease [61]. c-Jun (encoded by JUN) is a transcription factor that is activated by various extracellular signals, such as growth factors, pathogenic infections, cytokines, and chemokines, and plays an important role in cell proliferation, differentiation, apoptosis, survival, and immune cell immune response [62]. IL-1β is one of the most important pro-inflammatory cytokines that plays a key role in human innate and adaptive immunity and acts as a mediator of inflammatory and aging processes [63,64]. Aberrant IL-1β-related signaling pathways have been shown to be involved in nephron hyperfiltration, loss of podocytes, and progressive decline in glomerular filtration rate (GFR) in DN [65]. IL-1β gene polymorphisms have been found to be significantly associated with DN risk in some regional populations [66]. IL-1β is not only a promising option for improving renal prognosis in DN patients [67], but also a potential target for PD [68].

In the key gene pathway analysis, the network of pathways operating from the identified five key genes is a major key to our mechanistic studies, including AGE-receptor for AGE (RAGE) pathway and immune inflammatory pathways such as NF-kappa B, TNF, MAPK, IL-17 signaling pathways and cytokine-cytokine receptor interaction. The receptor for advanced glycation end-products (RAGE) is a multiligand transmembrane receptor that can be linked to the pathophysiology of multiple cellular environments such as diabetes, inflammation, oxidative stress and aging [69]. The kidney produces abundant AGEs and upregulates RAGE expression under diabetic or aging conditions, and activated RAGE promotes reactive oxygen species (ROS) production and amplifies inflammation, thereby enhancing renal tissue damage [69,70]. Additionally, RAGE stimulation is able to regulate different intracellular signaling pathways in the kidney, such as NF-κB, a key pro-inflammatory pathway that enhances the expression and production of various inflammatory factors, chemokines and adhesion molecules in DN [71], and the MAPK signaling pathway [72]. Similarly, increased sugar-fermenting bacteria and hyperglycemia increase AGEs in periodontal tissue, induce the release of pro-inflammatory factors by binding to immune cell surface RAGE, thereby promoting periodontal destruction [73], impede bone regeneration and remodeling by disrupting the RANKL/osteoprotegerin (OPG) axis [74]. Besides the appeal pathway, the crosstalk gene-enriched complement and coagulation cascade pathways also appear to be an important link between PD and DN. Inhibition of complement cascade components such as C3 reduces the dysbiosis of oral periodontal microbiota and the inflammatory process of bone destruction [75]. Also, abundant studies point to the involvement of the complement system in the pathogenesis of DN and, likewise, as a promising target for the treatment of DN [76].

Changes in immune cells appear to play a large role in the relationship between DN and PD. Several immune cells such as central memory CD4 T cells, plasmacytoid dendritic cells, effector memeory CD8 T cells, immature dendritic cells are highly expressed in DN and PD. Dendritic cells (DCs) draw our attention. As the most potent bone marrow-derived antigen-presenting cells, DCs can serve as a nexus for coordinating innate and adaptive immunity by inducing T-cell responses, and are critical for immune protection against overwhelming pathogens. Porphyromonas gingivalis, the main pathogen of PD, not only enhances the differentiation of monocytes to immature DCs, but also induces anti-apoptotic proteins that disrupt immune homeostasis in human dendritic cells [77,78]. Highly migratory hematopoietic DCs play a role in carrying periodontal tissue bacteria that spread to distant systems via the body circulation [77]. DCs are not only considered as potential contributors to the development of systemic disease associated with periodontitis, but are also closely associated with higher inflammatory infiltration [79]. Aging decreases DCs' recruitment in response to bacterial attack, and the mechanism may involve the effect of high glucose or late glycosylation end products on DCs migration [80]. DCs recruitment also activates effector T cells, which release toxic mediators that mediate various types of kidney injury. A subpopulation of CD103 DCs was found to reduce the activation and proliferation of renal CD8 T cells in DN, which in turn reduced pro-inflammatory cytokine release and renal infiltration to protect against renal injury and fibrosis [81]. We also explored immune cell correlations that could lead to a better understanding of pathogenesis in the immune context of disease.

Overall, different hypotheses for the interaction between PD and DN can be drawn from the results: first, potential periodontal causative agents and their associated cytokines and chemokines recur in the context of chronic inflammation, triggering periodontitis and renal inflammation. Second, common risk factors such as high glucose, aging, obesity, and smoking, involving changes in immune responses and immune cells like T cells, dendritic cells, as well as their contents, seem to emphasize the possibility of interconnection. Third, aging may affect key genes, involving AGE-RAGE pathway signaling and multiple immune-inflammatory pathways to impact PD and DN. Thus, these could be key points linking these two single entities to the pathophysiology of age-related diseases.

Strengths and limitations: we applied comprehensive bioinformatics analysis methods to explore for the first time the link between DN and PD of aging-related genes. The results have potential clinical relevance and reveal new concepts for future research in this field. Nevertheless, our study has certain limitations. Firstly, due to the limited availability of RNA-seq data, we merged the transcriptomic data of PD and DN using microarray datasets. To strengthen our study, we aspire to acquire additional RNA-seq data for validation in the future. Secondly, the study included patients with DN and PD without considering individual patient characteristics, such as age, gender, smoking habits, medications, and other health conditions. This made the patient group quite diverse. In a word, it's important to note that finding clear and common mechanisms in a clinical setting is challenging because each patient is unique and influenced by

factors like genetics, epigenetics, environment, and lifestyle. Lastly, our study was obtained based on computational predictions from published PD and DN-related datasets, and despite integrating scattered literature support, relevant experiments are needed to validate the biological functions of the studied markers and the associated signaling pathways. For example, the use of CRISPR knockout or overexpression research techniques has helped to validate the role of acquired genes in disease. The science of cell behavior has explored the role of these genes in inflammation and aging. Flow cytometry has provided a deeper understanding of the role of immunity in disease. Clinically, a more comprehensive treatment and prevention strategy may be required, including consensus between the two diseases in dental and nephrology practices, reduction of common risk factors, and an interdisciplinary approach to care for these diseases.

5. Conclusion

There is little evidence to support a causal relationship between PD and DN. Five key genes, CSF1R, CXCL6, VCAM1, JUN and IL1B, were employed to reveal shared mechanisms between PD and DN in the context of aging, supporting a close interconnection between PD and DN. It is conceivable that PD and DN are associated with RAGE pathway signaling, the complement system, and multiple immune inflammatory pathways, and these findings could act as the foundation for future research and should be assessed in experimental and/or clinical studies.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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CRediT authorship contribution statement

Peng Yan: Writing – review & editing, Writing – original draft, Methodology, Conceptualization. **Ben Ke:** Formal analysis, Data curation. **Xiangdong Fang:** Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e24872.

References

[1] J.L. Gross, M.J. De Azevedo, S.P. Silveiro, L.H. Canani, M.L. Caramori, T. Zelmanovitz, Diabetic nephropathy: diagnosis, prevention, and treatment, Diabetes Care 28 (1) (2005) 164–176, https://doi.org/10.2337/diacare.28.1.164.
[2] F. Chiarelli, D. Trotta, A. Verrotti, A. Mohn, Kidney involvement and disease in patients with diabetes, Panminerva medica 45 (1) (2003) 23–41.
[3] S. Jepsen, J. Suvan, J. Deschner, The association of periodontal diseases with metabolic syndrome and obesity, Periodontology 200083 (1) (2020) 125–153, https://doi.org/10.1111/prd.12326.
[4] E. Lalla, P.N. Papapanou, Diabetes mellitus and periodontitis: a tale of two common interrelated diseases, Nat Rev Endocrinol 7 (12) (2011) 738–748, https://doi.org/10.1038/nrendo.2011.106.
[5] X. Zhang, M. Wang, X. Wang, H. Qu, R. Zhang, J. Gu, Y. Wu, T. Ni, W. Tang, Q. Li, Relationship between periodontitis and microangiopathy in type 2 diabetes mellitus: a meta-analysis, Journal of periodontal research 56 (6) (2021) 1019–1027, https://doi.org/10.1111/jre.12916.
[6] D. Zhang, W. Liu, R. Miao, L. Wang, X. Zhou, H. Wang, Y. Li, Z. Wang, Chronic periodontitis is a risk factor of renal dysfunction in patients with type 2 diabetes, Exp Clin Endocrinol Diabetes 129 (6) (2021) 407–412, https://doi.org/10.1055/a-0895-5275.
[7] K. Parsegian, D. Randall, M. Curtis, E. Ioannidou, Association between periodontitis and chronic kidney disease, Periodontology 200089 (1) (2022) 114–124, https://doi.org/10.1111/prd.12431.
[8] W.A. Shultis, E.J. Weil, H.C. Looker, J.M. Curtis, M. Shlossman, R.J. Genco, W.C. Knowler, R.G. Nelson, Effect of periodontitis on overt nephropathy and end-stage renal disease in type 2 diabetes, Diabetes care 30 (2) (2007) 306–311, https://doi.org/10.2337/dc06-1184.
[9] A. Saremi, R.G. Nelson, M. Tulloch-Reid, R.L. Hanson, M.L. Sievers, G.W. Taylor, M. Shlossman, P.H. Bennett, R. Genco, W.C. Knowler, Periodontal disease and mortality in type 2 diabetes, Diabetes care 28 (1) (2005) 27–32, https://doi.org/10.2337/diacare.28.1.27.

[10] J.F. Navarro-González, C. Mora-Fernández, The role of inflammatory cytokines in diabetic nephropathy, J Am Soc Nephrol 19 (3) (2008) 433–442, https://doi.org/10.1681/asn.2007091048.

[11] P.M. Preshaw, J.J. Taylor, How has research into cytokine interactions and their role in driving immune responses impacted our understanding of periodontitis? Journal of clinical periodontology 38 (Suppl 11) (2011) 60–84, https://doi.org/10.1111/j.1600-051X.2010.01671.x.

[12] B.G. Loos, Systemic markers of inflammation in periodontitis, Journal of periodontology 76 (11 Suppl) (2005) 2106–2115, https://doi.org/10.1902/jop.2005.76.11-S.2106.

[13] L.T.R. Tavares, M. Saavedra-Silva, J.F. López-Marcos, N.J. Veiga, R.M. Castilho, G.V.O. Fernandes, Blood and Salivary inflammatory biomarkers profile in patients with chronic kidney disease and periodontal disease: a systematic review, Diseases 10 (1) (2022), https://doi.org/10.3390/diseases10010012.

[14] J.R. Tran, H. Chen, X. Zheng, Y. Zheng, Lamin in inflammation and aging, Current opinion in cell biology 40 (2016) 124–130, https://doi.org/10.1016/j.ceb.2016.03.004.

[15] S. Borgoni, K.S. Kudryashova, K. Burka, J.P. De Magalhães, Targeting immune dysfunction in aging, Ageing Research Reviews 70 (2021) 101410, https://doi.org/10.1016/j.arr.2021.101410.

[16] F. Barbé-Tuana, G. Funchal, C.R.R. Schmitz, R.M. Maurmann, M.E. Bauer, The interplay between immunosenescence and age-related diseases, Seminars in immunopathology 42 (5) (2020) 545–557, https://doi.org/10.1007/s00281-020-00806-z.

[17] B.J. North, D.A. Sinclair, The intersection between aging and cardiovascular disease, Circ Res 110 (8) (2012) 1097–1108, https://doi.org/10.1161/circresaha.111.246876.

[18] C.W. Hung, Y.C. Chen, W.L. Hsieh, S.H. Chiou, C.L. Kao, Ageing and neurodegenerative diseases, Ageing research reviews 9 (Suppl 1) (2010) S36–S46, https://doi.org/10.1016/j.arr.2010.08.006.

[19] S. Khosla, J.N. Farr, T. Tchkonia, J.L. Kirkland, The role of cellular senescence in ageing and endocrine disease, Nat Rev Endocrinol 16 (5) (2020) 263–275, https://doi.org/10.1038/s41574-020-0335-y.

[20] J.L. Ebersole, C.L. Graves, O.A. Gonzalez, D. Dawson 3rd, L.A. Morford, P.E. Huja, J.K. Hartsfield Jr., S.S. Huja, S. Pandruvada, S.M. Wallet, Aging, inflammation, immunity and periodontal disease, Periodontology 200072 (1) (2016) 54–75, https://doi.org/10.1111/prd.12135.

[21] R. López, P.C. Smith, G. Göstemeyer, F. Schwendicke, Ageing, dental caries and periodontal diseases, Journal of clinical periodontology44 Suppl 18 (2017), https://doi.org/10.1111/jcpe.12683. S145-s52.

[22] J. Dybiec, M. Szlagor, E. Młynarska, J. Rysz, B. Franczyk, Structural and functional changes in aging kidneys, Int J Mol Sci 23 (23) (2022), https://doi.org/10.3390/ijms232315435.

[23] S. Shen, C. Ji, K. Wei, Cellular senescence and regulated cell death of tubular epithelial cells in diabetic kidney disease, Front. Endocrinol. 13 (2022) 924299, https://doi.org/10.3389/fendo.2022.924299.

[24] Burgess Stephen, Butterworth Adam, G. Thompson Simon, Mendelian randomization analysis with multiple genetic variants using summarized data, Genetic epidemiology 37 (7) (2013) 658–665, https://doi.org/10.1002/gepi.21758.

[25] D. Shungin, S. Haworth, K. Divaris, C.S. Agler, Y. Kamatani, M. Keun Lee, K. Grinde, G. Hindy, V. Alaraudanjoki, P. Pesonen, A. Teumer, B. Holtfreter, S. Sakaue, J. Hirata, Y.H. Yu, P.M. Ridker, F. Giulianini, D.I. Chasman, P.K.E. Magnusson, T. Sudo, Y. Okada, U. Völker, T. Kocher, V. Anttonen, M.L. Laitala, M. Orho-Melander, T. Sofer, J.R. Shaffer, A. Vieira, M.L. Marazita, M. Kubo, Y. Furuichi, K.E. North, S. Offenbacher, E. Ingelsson, P.W. Franks, N.J. Timpson, I. Johansson, Genome-wide analysis of dental caries and periodontitis combining clinical and self-reported data, Nature communications 10 (1) (2019) 2773, https://doi.org/10.1038/s41467-019-10630-1.

[26] N.M. Davies, M.V. Holmes, G. Davey Smith, Reading Mendelian randomisation studies: a guide, glossary, and checklist for clinicians, BMJ (Clinical research ed) 362 (2018) k601, https://doi.org/10.1136/bmj.k601.

[27] S. Burgess, A. Butterworth, S.G. Thompson, Mendelian randomization analysis with multiple genetic variants using summarized data, Genetic epidemiology 37 (7) (2013) 658–665, https://doi.org/10.1002/gepi.21758.

[28] J. Bowden, G. Davey Smith, P.C. Haycock, S. Burgess, Consistent estimation in mendelian randomization with some invalid instruments using a weighted median estimator, Genetic epidemiology 40 (4) (2016) 304–314, https://doi.org/10.1002/gepi.21965.

[29] J. Bowden, G. Davey Smith, S. Burgess, Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression, International journal of epidemiology 44 (2) (2015) 512–525, https://doi.org/10.1093/ije/dyv080.

[30] M. Verbanck, C.Y. Chen, B. Neale, R. Do, Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases, Nat Genet 50 (5) (2018) 693–698, https://doi.org/10.1038/s41588-018-0099-7.

[31] D. Saul, R.L. Kosinsky, E.J. Atkinson, M.L. Doolittle, X. Zhang, N.K. Lebrasseur, R.J. Pignolo, P.D. Robbins, L.J. Niedernhofer, Y. Ikeno, D. Jurk, J.F. Passos, L. J. Hickson, A. Xue, D.G. Monroe, T. Tchkonia, J.L. Kirkland, J.N. Farr, S. Khosla, A new gene set identifies senescent cells and predicts senescence-associated pathways across tissues, Nature communications 13 (1) (2022) 4827, https://doi.org/10.1038/s41467-022-32552-1.

[32] N.R. Smith, P.N. Zivich, L.M. Frerichs, J. Moody, A.E. Aiello, A guide for choosing community detection algorithms in social network studies: the question alignment approach, American journal of preventive medicine 59 (4) (2020) 597–605, https://doi.org/10.1016/j.amepre.2020.04.015.

[33] X. Lin, C. Li, Y. Zhang, B. Su, M. Fan, H. Wei, Selecting feature subsets based on SVM-RFE and the overlapping ratio with applications in bioinformatics, Molecules 23 (1) (2017), https://doi.org/10.3390/molecules23010052.

[34] M.W. Beck, NeuralNetTools: visualization and analysis tools for neural networks, Journal of statistical software 85 (11) (2018) 1–20, https://doi.org/10.18637/jss.v085.i11.

[35] Bindea Gabriela, Mlecnik Bernhard, Tosolini Marie, Kirilovsky Amos, Waldner Maximilian, C. Obenauf Anna, Angell Helen, Fredriksen Tessa, Lafontaine Lucie, Berger Anne, Bruneval Patrick, Fridman Wolf Herman, Becker Christoph, Pagès Franck, R. Speicher Michael, Trajanoski Zlatko, Galon Jérôme, Spatiotemporal dynamics of intratumoral immune cells reveal the immune landscape in human cancer, Immunity (4) (2013) 782–795, https://doi.org/10.1016/j.immuni.2013.10.003.

[36] V. Villalobos, M. Garrido, A. Reyes, C. Fernández, C. Diaz, V.A. Torres, P.A. González, M. Cáceres, Aging envisage imbalance of the periodontium: a keystone in oral disease and systemic health, Front Immunol 13 (2022) 1044334, https://doi.org/10.3389/fimmu.2022.1044334.

[37] C.H. Chin, S.H. Chen, H.H. Wu, C.W. Ho, M.T. Ko, C.Y. Lin, cytoHubba: identifying hub objects and sub-networks from complex interactome, BMC systems biology8 Suppl 4 (Suppl 4) (2014) S11, https://doi.org/10.1186/1752-0509-8-s4-s11.

[38] H. Löe, Periodontal disease. The sixth complication of diabetes mellitus, Diabetes care 16 (1) (1993) 329–334.

[39] D.T. Graves, R.A. Kayal, Diabetic complications and dysregulated innate immunity, Frontiers in bioscience : a journal and virtual library 13 (2008) 1227–1239, https://doi.org/10.2741/2757.

[40] G.L. King, The role of inflammatory cytokines in diabetes and its complications, Journal of periodontology 79 (8 Suppl) (2008) 1527–1534, https://doi.org/10.1902/jop.2008.080246.

[41] H. Terheyden, B. Stadlinger, M. Sanz, A.I. Garbe, J. Meyle, Inflammatory reaction - communication of cells, Clinical oral implants research 25 (4) (2014) 399–407, https://doi.org/10.1111/clr.12176.

[42] J. Wada, H. Makino, Innate immunity in diabetes and diabetic nephropathy, Nat Rev Nephrol 12 (1) (2016) 13–26, https://doi.org/10.1038/nrneph.2015.175.

[43] T. Tchkonia, Y. Zhu, J. Van Deursen, J. Campisi, J.L. Kirkland, Cellular senescence and the senescent secretory phenotype: therapeutic opportunities, J Clin Invest 123 (3) (2013) 966–972, https://doi.org/10.1172/jci64098.

[44] C.D. Wiley, Role of senescent renal cells in pathophysiology of diabetic kidney disease, Current diabetes reports20 (8) (2020) 33, https://doi.org/10.1007/s11892-020-01314-y.

[45] E.A. Huttner, D.C. Machado, R.B. De Oliveira, A.G. Antunes, E. Hebling, Effects of human aging on periodontal tissues, Special care in dentistry : official publication of the American Association of Hospital Dentists, the Academy of Dentistry for the Handicapped, and the American Society for Geriatric Dentistry 29 (4) (2009) 149–155, https://doi.org/10.1111/j.1754-4505.2009.00082.x.

[46] G. Hajishengallis, Aging and its impact on innate immunity and inflammation: implications for periodontitis, Journal of oral biosciences 56 (1) (2014) 30–37, https://doi.org/10.1016/j.job.2013.09.001.

[47] G.N. Belibasakis, Microbiological changes of the ageing oral cavity, Archives of oral biology 96 (2018) 230–232, https://doi.org/10.1016/j.archoralbio.2018.10.001.
[48] X. Wang, J.V. Bonventre, A.R. Parrish, The aging kidney: increased susceptibility to nephrotoxicity, Int J Mol Sci 15 (9) (2014) 15358–15376, https://doi.org/10.3390/ijms150915358.
[49] B. Hernández, A. Parnell, S.R. Pennington, Why have so few proteomic biomarkers "survived" validation? (Sample size and independent validation considerations), Proteomics 14 (13–14) (2014) 1587–1592, https://doi.org/10.1002/pmic.201300377.
[50] C.A. Tsai, Y.J. Chang, Efficient selection of Gaussian kernel SVM parameters for imbalanced data, Genes 14 (3) (2023), https://doi.org/10.3390/genes14030583.
[51] S. Huang, N. Cai, P.P. Pacheco, S. Narrandes, Y. Wang, W. Xu, Applications of support vector machine (SVM) learning in cancer genomics, Cancer genomics & proteomics 15 (1) (2018) 41–51, https://doi.org/10.21873/cgp.20063.
[52] T. Srebotnjak, A.H. Mokdad, C.J. Murray, A novel framework for validating and applying standardized small area measurement strategies, Population health metrics 8 (2010) 26, https://doi.org/10.1186/1478-7954-8-26.
[53] E.R. Stanley, V. Chitu, CSF-1 receptor signaling in myeloid cells, Cold Spring Harbor perspectives in biology 6 (6) (2014), https://doi.org/10.1101/cshperspect.a021857.
[54] M.B. Buechler, W. Fu, S.J. Turley, Fibroblast-macrophage reciprocal interactions in health, fibrosis, and cancer, Immunity54 (5) (2021) 903–915, https://doi.org/10.1016/j.immuni.2021.04.021.
[55] A. Kumari, O. Silakari, R.K. Singh, Recent advances in colony stimulating factor-1 receptor/c-FMS as an emerging target for various therapeutic implications, Biomed Pharmacother 103 (2018) 662–679, https://doi.org/10.1016/j.biopha.2018.04.046.
[56] Q. Chen, D. Liu, Z. Hu, C. Luo, S.L. Zheng, miRNA-101-5p Inhibits the Growth and Aggressiveness of NSCLC Cells through Targeting CXCL6, OncoTargets and Therapy 12 (2019) 835–848, https://doi.org/10.2147/ott.S184235.
[57] D.H. Kong, Y.K. Kim, M.R. Kim, J.H. Jang, S. Lee, Emerging roles of vascular cell adhesion molecule-1 (VCAM-1) in immunological disorders and cancer, Int J Mol Sci 19 (4) (2018), https://doi.org/10.3390/ijms19041057.
[58] N. Morisaki, I. Saito, K. Tamura, J. Tashiro, M. Masuda, T. Kanzaki, S. Watanabe, Y. Masuda, Y. Saito, New indices of ischemic heart disease and aging: studies on the serum levels of soluble intercellular adhesion molecule-1 (ICAM-1) and soluble vascular cell adhesion molecule-1 (VCAM-1) in patients with hypercholesterolemia and ischemic heart disease, Atherosclerosis 131 (1) (1997) 43–48, https://doi.org/10.1016/s0021-9150(97)06083-8.
[59] K. Ina, H. Kitamura, T. Okeda, K. Nagai, Z.Y. Liu, M. Matsuda, Y. Fujikura, Vascular cell adhesion molecule-1 expression in the renal interstitium of diabetic KKAy mice, Diabetes Res Clin Pract 44 (1) (1999) 1–8, https://doi.org/10.1016/s0168-8227(99)00011-x.
[60] Y. Jia, H. Xu, Q. Yu, L. Tan, Z. Xiong, Identification and verification of vascular cell adhesion protein 1 as an immune-related hub gene associated with the tubulointerstitial injury in diabetic kidney disease, Bioengineered 12 (1) (2021) 6655–6673, https://doi.org/10.1080/21655979.2021.1976540.
[61] L. Wang, X.H. Li, W.C. Ning, Evaluation of ICAM-1 and VCAM-1 gene polymorphisms in patients with periodontal disease, medical science monitor, international medical journal of experimental and clinical research 22 (2016) 2386–2391, https://doi.org/10.12659/msm.896979.
[62] A.G. Papavassiliou, A.M. Musti, The multifaceted output of c-jun biological activity: focus at the junction of CD8 T cell activation and exhaustion, Cells 9 (11) (2020), https://doi.org/10.3390/cells9112470.
[63] A. Weber, P. Wasiliew, M. Kracht, Interleukin-1beta (IL-1beta) processing pathway, Science signaling 3 (105) (2010) cm2, https://doi.org/10.1126/scisignal.3105cm2.
[64] C.A. Dinarello, Interleukin 1 and interleukin 18 as mediators of inflammation and the aging process, The American journal of clinical nutrition 83 (2) (2006), https://doi.org/10.1093/ajcn/83.2.447S, 447s-55s.
[65] Y. Lei, S.K. Devarapu, M. Motrapu, C.D. Cohen, M.T. Lindenmeyer, S. Moll, S.V. Kumar, H.J. Anders, Interleukin-1β inhibition for chronic kidney disease in obese mice with type 2 diabetes, Front Immunol 10 (2019) 1223, https://doi.org/10.3389/fimmu.2019.01223.
[66] X. Liao, Y. Xiao, U. Elbelt, K.H. Weylandt, K. Li, J. Deng, N. Zeng, C. Xue, Association of interleukin-1 beta and interleukin-1 receptor antagonist gene polymorphisms and plasma levels with diabetic nephropathy, Biomed Res Int 2022 (2022) 9661823, https://doi.org/10.1155/2022/9661823.
[67] J.A. Moreno, C. Gomez-Guerrero, S. Mas, A.B. Sanz, O. Lorenzo, M. Ruiz-Ortega, L. Opazo, S. Mezzano, J. Egido, Targeting inflammation in diabetic nephropathy: a tale of hope, Expert opinion on investigational drugs 27 (11) (2018) 917–930, https://doi.org/10.1080/13543784.2018.1538352.
[68] R. Cheng, Z. Wu, M. Li, M. Shao, T. Hu, Interleukin-1β is a potential therapeutic target for periodontitis: a narrative review, International journal of oral science 12 (1) (2020) 2, https://doi.org/10.1038/s41368-019-0068-8.
[69] R. Ramasamy, A. Shekhtman, A.M. Schmidt, The multiple faces of RAGE–opportunities for therapeutic intervention in aging and chronic disease, Expert opinion on therapeutic targets 20 (4) (2016) 431–446, https://doi.org/10.1517/14728222.2016.1111873.
[70] R. Ramasamy, S.J. Vannucci, S.S. Yan, K. Herold, S.F. Yan, A.M. Schmidt, Advanced glycation end products and RAGE: a common thread in aging, diabetes, neurodegeneration, and inflammation, Glycobiology 15 (7) (2005) 16r–28r, https://doi.org/10.1093/glycob/cwi053.
[71] D. Sanajou, A. Ghorbani Haghjo, H. Argani, S. Aslani, AGE-RAGE axis blockade in diabetic nephropathy: current status and future directions, Eur J Pharmacol 833 (2018) 158–164, https://doi.org/10.1016/j.ejphar.2018.06.001.
[72] P.C. Chang, T.H. Chen, C.J. Chang, C.C. Hou, P. Chan, H.M. Lee, Advanced glycosylation end products induce inducible nitric oxide synthase (iNOS) expression via a p38 MAPK-dependent pathway, Kidney Int 65 (5) (2004) 1664–1675, https://doi.org/10.1111/j.1523-1755.2004.00602.x.
[73] V. Blasco-Baque, L. Garidou, C. Pomié, Q. Escoula, P. Loubieres, S. Le Gall-David, M. Lemaitre, S. Nicolas, P. Klopp, A. Waget, V. Azalbert, A. Colom, M. Bonnaure-Mallet, P. Kemoun, M. Serino, R. Burcelin, Periodontitis induced by Porphyromonas gingivalis drives periodontal microbiota dysbiosis and insulin resistance via an impaired adaptive immune response, Gut 66 (5) (2017) 872–885, https://doi.org/10.1136/gutjnl-2015-309897.
[74] M. Alikhani, Z. Alikhani, C. Boyd, C.M. Maclellan, M. Raptis, R. Liu, N. Pischon, P.C. Trackman, L. Gerstenfeld, D.T. Graves, Advanced glycation end products stimulate osteoblast apoptosis via the MAP kinase and cytosolic apoptotic pathways, Bone 40 (2) (2007) 345–353, https://doi.org/10.1016/j.bone.2006.09.011.
[75] G. Hajishengallis, T. Abe, T. Maekawa, E. Hajishengallis, J.D. Lambris, Role of complement in host-microbe homeostasis of the periodontium, Seminars in immunology 25 (1) (2013) 65–72, https://doi.org/10.1016/j.smim.2013.04.004.
[76] A. Flyvbjerg, The role of the complement system in diabetic nephropathy, Nat Rev Nephrol 13 (5) (2017) 311–318, https://doi.org/10.1038/nrneph.2017.31.
[77] J. Carrion, E. Scisci, B. Miles, G.J. Sabino, A.E. Zeituni, Y. Gu, A. Bear, C.A. Genco, D.L. Brown, C.W. Cutler, Microbial carriage state of peripheral blood dendritic cells (DCs) in chronic periodontitis influences DC differentiation, atherogenic potential, Journal of immunology (Baltimore, Md : 1950) 189 (6) (2012) 3178–3187, https://doi.org/10.4049/jimmunol.1201053.
[78] M.M. Meghil, O.K. Tawfik, M. Elashiry, M. Rajendran, R.M. Arce, D.J. Fulton, P.V. Schoenlein, C.W. Cutler, Disruption of immune homeostasis in human dendritic cells via regulation of autophagy and apoptosis by porphyromonas gingivalis, Front Immunol 10 (2019) 2286, https://doi.org/10.3389/fimmu.2019.02286.
[79] G.R. Souto, C.M. Queiroz-Junior, M.H. De Abreu, F.O. Costa, R.A. Mesquita, Pro-inflammatory, Th1, Th2, Th17 cytokines and dendritic cells: a cross-sectional study in chronic periodontitis, PLoS One 9 (3) (2014) e91636, https://doi.org/10.1371/journal.pone.0091636.
[80] Y. Wu, G. Dong, W. Xiao, E. Xiao, F. Miao, A. Syverson, N. Missaghian, R. Vafa, A.A. Cabrera-Ortega, C. Rossa Jr., D.T. Graves, Effect of aging on periodontal inflammation, microbial colonization, and disease susceptibility, Journal of dental research 95 (4) (2016) 460–466, https://doi.org/10.1177/0022034515625962.
[81] F. Zhang, C. Wang, X. Wen, Y. Chen, R. Mao, D. Cui, L. Li, J. Liu, Y. Chen, J. Cheng, Y. Lu, Mesenchymal stem cells alleviate rat diabetic nephropathy by suppressing CD103(+) DCs-mediated CD8(+) T cell responses, J Cell Mol Med 24 (10) (2020) 5817–5831, https://doi.org/10.1111/jcmm.15250.