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Abstract

Our study examined the relationships and interactions among 30 genes related to the NLRP3 inflammasome. We identified 368 interconnections between these 30 genes, with NLRP3 involved in 38 interactions. ~~By utilizing protein-protein interaction networks and co-expression analysis, we assessed t~~The potential roles of these genes in Atherosclerosis (AS) were evaluated based on protein-protein interaction networks as well as co-expression analysis. We identified differential expression of 20 genes, five of which were significantly upregulated: P2RX7, CASP1, CD36, GBP5, and PYCARD. We also discovered a ~~strong~~ high positive ~~correlation~~ association ~~between~~ of P2RX7 ~~and~~ with PYCARD and a ~~strong~~ high negative ~~correlation~~ association ~~between~~ of RELA ~~and~~ with CD36. Further analysis indicated a clear ~~correlation~~ association between the expression of inflammasome-~~related~~ associated genes and immune cell infiltration in disease ~~samples~~specimens. A logistic regression model for diagnosing AS, based on six inflammasome-related genes, achieved an AUC of 0.996, indicating excellent diagnostic performance. ~~–~~Genomic enrichment analysis indicated that inflammasome-related genes were mainly ~~involved~~ implicated in various pathways, ~~including~~ like hypertrophic cardiomyopathy and ribosome. In the end, we substantiated the expression of risk genes in AS cells utilizing qRT-PCR and Western blot techniques. Moreover, we discovered that the knockdown of P2RX7 instigated a shift in THP-1 macrophages towards M2, thereby reinforcing our findings.

Keywords: NLRP3, Gene interaction, Differential gene expression, Immune cell infiltration, Atherosclerosis, Diagnostic model

Introduction

Cardiovascular and cerebrovascular diseases persist as prevalent and highly detrimental conditions among the elderly population, characterized by high morbidity, disability, and mortality rates[1]. With population aging and an increase in patients aged 80 and above, the prevalence of these diseases remains high even with standard secondary prevention medication due to the coexistence of other underlying conditions. Atherosclerosis (AS) is one of the critical pathological bases for the occurrence of cardiovascular and cerebrovascular events[2, 3]. Elderly patients often experience symptoms such as hypertension, leading to long-term high blood pressure, endothelial cell damage, and gradual changes in vascular tension. High blood pressure can also cause monocytes, lymphocytes, and macrophages to settle in the intima, which reduces the elasticity of arterial walls and thickens them. As a result, AS has become one of the most common diseases among the elderly[4-6].

Atherosclerosis (AS) underpins these conditions, marked by the build-up of low-density lipoprotein (LDL) particles in ~~large to~~ mid- to large-sized arteries, the translocation of monocytes and other immune entities through dysfunctional endothelial cells as well as~~, and~~ the creation of lipid plaques. This condition could trigger chronic inflammatory processes within the arterial wall, accompanied by the secretion of inflammatory mediators[7, 8]. Disruptions such as plaque rupture, clot

formation, and arterial narrowing can hinder the flow of blood, setting off a chain of serious negative cardiovascular occurrences (MACE). Statins, which work by decreasing LDL cholesterol concentrations, are often the go-to medication for addressing AS. Nonetheless, such therapies fall short in effectively mitigating MACE levels[9]. Consequently, a deeper understanding of AS's origin and progression could aid in enhancing clinical diagnostic procedures, therapeutic approaches, and improving patient prognoses.

The NLRP3 inflammasome is an essential immune response complex in our bodies and ~~belongs to~~ one member of the NLR (nucleotide-binding oligomerization domain-like receptors) family. The members of this family are mainly immune system proteins that can perceive pathogens[10]. The inflammasome is ~~a~~ one multi-protein complex ~~whose~~ with primary functions ~~is to~~of perceiving pathogens or harmful signals inside cells and triggering a series of immune responses[11]. When triggered, the NLRP3 inflammasome can direct the engagement of the apoptosis-associated protease caspase-1. This ~~leads to~~brings the subsequent maturation and discharge of ~~pro-inflammatory cytokines~~ IL-1β and IL-18, instigating an inflammatory reaction[12]. Despite the necessity of NLRP3 inflammasome activation in combating pathogenic infiltration, the association between genes ~~related to~~associated with the NLRP3 inflammasome and atherosclerosis is yet to be completely understood.

Therefore, our study aims to analyze the relationships and regulatory mechanisms between NLRP3 inflammasome-related genes and AS. Understanding these relationships is vital for comprehending the pathophysiological processes of these diseases and for developing new treatment strategies.

Methods and Materials

Sample Source

In our research, we procured datasets ~~related to~~ associated with AS gene expression from the Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo)[13]. Employing a keyword-oriented approach, we chose dataset GSE100927 (AS=69, control=35) for further scrutiny. ~~A~~ Through Gene Set Enrichment Analysis (GSEA) ~~was performed, yielding,~~ 30 genes associated with NLRP3 were yielded.

Spotlighting Divergent Gene Expression

To identify genes displaying significant expression shifts in the GSE100927 dataset, we deployed the Limma R package[14], referencing normalized count data. We employed criteria of |Log2fold change| >1 and adjusted P<0.05 for sifting differentially expressed genes (DEGs). ~~Differentially expressed genes~~DEGs were visually represented in heatmaps and volcano plots, created using the pheatmap and ggplot2 R packages.

Intricate Interplay within Protein Network

~~We harnessed t~~The Search Tool was adopted for the Retrieval of Interacting

Genes/Proteins (STRING) database (https://string-db.org)[15] ~~to~~ for construction of a PPI network associated with NLRP3 inflammasome-related genes. Interactions scoring >0.4 were deemed ~~statistically~~ significant in statistical sense, and solitary target protein nodes were made invisible.

Building a Diagnostic Model of NLRP3-related Genes through LASSO Regression

In the context of GSE100927 data, we initially employed univariate logistic regression to probe differences ~~in the expression of NLRP3 inflammasome-related genes~~ between disease ~~samples~~specimens and healthy tissues in the expression of NLRP3 inflammasome-associated genes. Using the glmnet package, LASSO regression was implemented ~~with~~ based on the ~~following~~ risk equation: Risk scores= Σ(X: coefficient of ~~each~~ every gene, Y: expression of ~~every~~each gene). Superfluous genes were removed to create a streamlined model. GraphPad Prism 9 was adopted for calculation of t~~T~~he Receiver Operating Characteristic (ROC) curves and Area Under Curve (AUC) ~~were calculated utilizing GraphPad Prism 9~~.

GSEA for Biological Functions

~~Based on~~In the light of the established risk score threshold, ~~samples~~specimens were split into groups with high and low gene expression. GSEA (http://software.broadinstitute.org/gsea/index.jsp) was ~~performed~~ conducted to elucidate biological functions, focusing on the ~~Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway,~~ Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway, ~~and~~ as well as Hallmarkgene feature gene sets, with annotations undertaken by the "clusterProfiler" package[16].

Evaluating the Immune Landscape

~~Utilizing the GSVA package[17], s~~Single-sample Gene Set Enrichment Analysis (ssGSEA) was ~~performed~~ conducted through GSVA package ~~to~~ for inspecting differences in the immune landscape[17], particularly the infiltration variance of 22 ~~types~~ kinds of immune cells between groups.

Creating Inflammatory Models with RAW264.7 Cells Exposed to LPS

We procured mouse macrophages (RAW264.7) from the ATCC cell bank and cultured them in RPMI1640 base medium, fortified with 10% fetal bovine serum and 1% antibiotics until growth was observed. Subsequently, the RAW264.7 cells were ~~transferred to~~placed into 6-well plates, and each well was supplemented with 1 g/ml LPS (Sigma-Aldrich, Inc.). Following a 24-hour period, we confirmed the successful establishment of the RAW264.7 inflammation model. RAW264.7 cells not exposed to LPS were designated as the control group, and the cells showing successful modeling post-LPS intervention were classified as the AS group.

Protein Quantification via Western Blot Analysis

Cellular lysis was achieved with ice-cold radioimmunoprecipitation assay buffer

to extract total protein. Proteins were differentiated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, followed by ~~and subsequently transfer~~transferring~~red~~ to polyvinylidene fluoride membranes. After ~~blocking~~ 1-h immersion ~~with~~ in 5% nonfat milk~~ for an hour~~, the membranes were ~~then incubated~~subjected to overnight incubation (4°C) with primary antibodies against ATAT1, CPTP, NLRC3, CD36, NFKB1, NFKB2, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH)~~ at 4°C overnight~~. After ~~washing~~ cleaning away the primary antibodies, the membranes were ~~incubated with~~treated by 1-h incubation (37°C) through ~~—~~horseradish peroxidase-labeled goat anti-rabbit secondary antibodies (1:1000)~~ at 37°C for 1 hour~~. The membranes were rinsed ~~with~~ through PBS thrice, for 5 minutes~~/ each~~ time. Protein bands were visually captured, scanned, and grayscale values were assessed in Quantity One software, where the relative protein expression ~~level~~= grayscale value of the target protein band / ~~grayscale value~~ that of the GAPDH protein band.

Quantitative Real-Time PCR (qRT-PCR)

~~For qRT-PCR analysis, e~~Cells were gathered for qRT-PCR analysis. ~~and t~~Total RNA was ~~extracted~~ acquired through~~using~~ the GenElute Total RNA Purification Kit, and its ~~. The~~ concentration and quality ~~of total RNA~~ were examined using NanoDrop 2000. RNA with an OD260/OD280 ratio ~~close to~~about 2.0 was selected for ~~subsequent~~ later reverse transcription. With the SuperScrip® Vilo cDNA Synthesis Kit, ~~A total of~~ 2.0μg of RNA was reverse transcribed~~ using the SuperScrip® Vilo cDNA Synthesis Kit~~. The relative expression levels of P2X7R, NLRP3, iNOs, TNF-α, CXCL10, Arg-1, CD163, ATAT1, CPTP, NLRC3, CD36, NFKB1, NFKB2 were determined by qPCR, employing the SYBR green I Master Mix kit (Invitrogen; Thermo Fisher Scientific, Inc.) on a 7500 real-time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.), ~~as per~~under the manufacturer's ~~instructions~~guidelines. GAPDH ~~was used~~ served as an internal control. The qPCR thermal cycle conditions were as follows: initial denaturation at 95°C for 10 minutes; followed by 40 cycles of 95°C for 20 seconds, 60°C for 15 seconds, and 72°C for 20 seconds. The 2 -ΔΔCq method was adopted for calculation of the final expression values~~ were calculated using the 2 -ΔΔCq method~~.

Statistical ~~Analysis~~Analyses

Data in ~~this~~ the present article are ~~expressed as~~described by mean± standard error and were analyzed ~~using~~ through SPSS 26.0. Comparisons between two or more groups were ~~made~~ conducted through ~~using~~ the independent samples t-test or one-way analysis of variance, followed by Tukey's post hoc test. P<0.05 ~~was considered statistically significant~~suggests a notable difference.

Results

Interactions Among NLRP3 Inflammasome-Related Genes

We conducted PPI co-expression analysis on 30 genes, assessing their

interrelationships. We found a total of 368 interactions among these 30 genes, with NLRP3 involved in 38 interactions (~~Figure~~Fig. 1A-B).

Expression of NLRP3 Inflammasome-~~Related~~ Associated Genes in AS

~~Our~~ The present study involved 30 NLRP3 inflammasome-related genes. However, three NLRP3 inflammasome-~~related~~ associated genes lacked certain expression data. ~~As a result~~Finally, we selected 27 NLRP3 genes with available expression data for further analysis. By comparing, we identified 20 ~~differentially expressed genes~~DEGs in inflammatory ~~samples~~specimens, namely APP, ATAT1, CARD8, NLRC3, NLRP3, P2RX7, RELA, TLR4, CASP1, CD36, GBP5, GSDMD, HSP90AB1, NFKB1, NFKB2, PSTPIP1, PYCARD, EIF2AK2, DHX33 and SUGT1 (~~Figure~~Fig. 2A). After that, using the limma package, we screened the 20 genes and identified 5 ~~differentially expressed genes~~DEGs: P2RX7, CASP1, CD36, GBP5, and PYCARD, which were all upregulated in AS (~~Figure~~Fig. 2B,C).

Examining the Interplay of NLRP3 Inflammasome-Associated Genes

Further probing was conducted to comprehend the interrelationships among the 27 NLRP3 inflammasome-~~related~~ associated genes in both ~~diseased and~~ healthy and diseased specimens (~~Figure~~Fig. 3A). Upon integration of ~~diseased and~~ normal and diseased ~~samples~~specimens, a striking positive association was uncovered between P2RX7 and PYCARD expression ($r = 0.933$, $P < 0.001$) (~~Figure~~Fig. 3B), juxtaposed with a pronounced negative link noted between RELA and CD36 expression ($r = -0.540$, $P < 0.001$) (~~Figure~~Fig. 3C).

Assessing the Association ~~between~~ of NLRP3 Inflammasome-Associated Genes ~~and~~ with Immune Penetration

In an attempt to probe deeper into the disparities in immune infiltration and the association ~~between~~ of NLRP3 inflammasome-associated genes ~~and~~ with immune cell penetration, we executed an ssGSEA evaluation. A significant enrichment of virtually all immune cell types was noticed within the diseased sample microenvironment (~~Figure~~Fig. 4A). An increase in the expression of ~~the majority of~~ most NLRP3 inflammasome-associated genes was notably linked with ~~enhanced~~ intensified immune cell infiltration (~~Figure~~Fig. 4B). The most profound positive correlation was evident between P2RX7 and M0 Macrophages infiltration ($r=0.777$, $P < 0.001$; ~~Figure~~Fig. 4C). Conversely, the strongest negative association was between P2RX7 and the infiltration of resting CD4 memory T cells ($r = -0.745$, $P < 2.2e-16$; ~~Figure~~Fig. 4D).

Downregulation of P2RX7 Promotes the Polarization of THP-1 Macrophages Towards M2

To study the immune mechanisms of P2RX7 in AS, we induced THP-1 cells to M0 state using PMA. Both pHBLV-P2X7R and P2X7R si-RNA were transfected into the induced THP-1 cells. According to qRT-PCR and Western Blot analysis, ~~revealed that~~ after transfection with pHBLV-P2X7R, the expression of P2X7R,

NLRP3, iNOS, TNF-α, and CXCL10 were significantly increased compared to pHBLV-NC, whereas Arg-1 and CD163 levels were significantly reduced (~~Figure~~Fig. 5A, B). However, we found in THP-1 cells transfected with P2X7R si-RNA, that the downregulation of P2X7R, NLRP3, iNOS, TNF-α, and CXCL10 expression were significantly decreased compared to si-NC, while Arg-1 and CD163 levels were significantly increased (~~Figure~~Fig. 5C, D). This suggests that P2RX7 may mediate the polarization of THP-1 cells from M0 to M2 via regulation of NLRP3, which could be associated with the stability of atherosclerotic plaques.

Construction of AS Diagnostic Model Related to NLRP3 Inflammasome-Related Genes

To construct an AS diagnostic model related to NLRP3 inflammasome-related genes, we first screened 20 NLRP3 inflammasome-related genes related to AS through univariate logistic regression (~~Figure~~Fig. 6A). To further screen predictive genes, LASSO regression analysis was performed, and 6 out of 20 genes (ATAT1, NFKB1, NFKB2, NLRC3, RELA, and CD36) were included in the predictive model (~~Figure~~Fig. 6B-D). According to the risk formula:=(ATAT1-0.261598503)+(NLRC3-1.486750281)+(RELA+4.722730188)+(CD36-1.297310476)+(NFKB1-2.396361885)+(NFKB2-1.291709974). We calculated the scores for each sample. According to t~~T~~he results, ~~showed that~~ the scores calculated by the prediction model were significantly lower for diseased ~~samples~~specimens (~~Figure~~Fig. 7A). ~~The ROC curve showed that t~~The AUC ~~of~~ the prediction model in the ROC curve ~~–~~was 0.996 (~~Figure~~Fig. 7B).

Functional Analysis of High and Low Risk Groups via GSEA

Subsequently, we ~~performed~~ carried out a ~~gene set enrichment analysis (~~GSEA~~)~~ to study the ~~potential~~ possible biological functions of the~~our~~ high-risk and low-risk groups. The results suggested that within the KEGG pathways, NLRP3 inflammasome-related genes predominantly participated in biological processes such as HYPERTROPHIC CARDIOMYOPATHY HCM, RIBOSOME, VASCULAR SMOOTH MUSCLE CONTRACTION, ARRHYTHMOGENIC RIGHT VENTRICULAR CARDIOMYOPATHY ARVC, DILATED CARDIOMYOPATHY, TGF BETA SIGNALING PATHWAY, and CARDIAC MUSCLE CONTRACTION (~~Figure~~Fig. 8A). Meanwhile, in the GO analysis, we identified 1232 biological processes (BP), 175 molecular functions (MF), and 140 cellular components (CC) ~~related to~~ associated with our genes of interest (~~Figure~~Fig. 8B-D).

In Vitro Validation

At the end of the study, we used qRT-PCR and WB experiments ~~to~~ for detecting the expression of risk genes in AS cells. According to t~~T~~he results, ~~showed that~~ ATAT1, CPTP, NLRC3, CD36, NFKB1, and NFKB2mRNA and proteins were highly expressed in the AS group, while RELA was expressed at lower levels in the AS group ($P<0.05$, ~~Figure~~Fig. 9A,B).

Discussion

In our research, we delved into and assessed the significance of NLRP3 inflammasome-associated genes ~~in~~ during the development of AS. Their expression ~~of NLRP3 inflammasome-related genes~~ was found to be generally upregulated in AS ~~samples~~specimens, suggesting that the genetic profile could assist in diagnosing AS. Additionally, there was an increase in immune cell infiltration in the diseased ~~samples~~specimens, which might be modulated by NLRP3 inflammasome-~~related~~ associated genes. ~~Furthermore~~Moreover, our in vitro experiments identified 7 NLRP3 inflammasome-~~related~~ associated genes ~~associated with~~linked to the development and progression of AS.

Considering the complex nature of AS, which includes genetic, environmental, and immune factors, its root causes remain unclear1. It's generally accepted that AS is associated with abnormal inflammation and immune reactions. Various inflammasomes, especially the NLRP3 inflammasome - the most well-documented signaling platform, have been extensively associated with different ~~inflammatory and~~ immune and inflammatory disorders[19]. The exact ~~role~~ effect of the NLRP3 inflammasome ~~in~~ on AS is still a matter of debate since its activation may either safeguard endothelial cells through the preservation of homeostasis, or intensify vascular injury by promoting inflammation[20]. Our study highlights the importance of NLRP3 inflammasome-associated genes in AS, providing insights into the disease's initiation and advancement.

However, the primary diagnostic methods for AS currently include cardiac stress tests and coronary angiography. While coronary angiography is considered one of the most accurate diagnostic methods, its high cost imposes a significant financial burden on many patients, resulting in challenges in diagnosing AS[21]. Additionally, in certain regions, a lack of disease awareness, unavailability of diagnostic services, diseases mimicking AS, and the widespread use of drug treatments pose significant challenges to the diagnosis of AS. Zhao et al. previously constructed an AS diagnostic model based on 28 aging genes with an ~~area under the curve (~~AUC~~)~~ of 0.898[22]. Another study by Tang et al. built an AS diagnostic model based on 4 genes, achieving an AUC of 0.967[23]. Our model demonstrated excellent performance in distinguishing between AS and normal ~~samples~~specimens with an AUC of 0.996, highlighting its immense clinical value in AS diagnosis.

~~The majority of~~Most NLRP3 inflammasome-~~related~~ associated genes are ~~associated with~~bound up with ~~enhanced~~ intensified immune cell infiltration and activation as well as immune signaling. In arterial sclerosis, both T and B cells ~~play~~ take pivotal ~~roles~~ parts in the pathological process[24]. For instance, CD4+ T cells have been proven to ~~play~~ take a crucial ~~role~~ part in AS inflammatory responses, while B cells are significant in antibody-mediated reactions[25]. Macrophages and neutrophils also have a vital role in AS, where macrophages influence lesion formation and progression through inflammatory responses and cytokine secretion, whereas neutrophils are believed to potentially cause endothelial damage in AS[26, 27]. ~~Although~~ Despite the disputable ~~the~~ role of the NLRP3 inflammasome in AS

~~remains disputed~~, our findings indicate that the heightened expression of NLRP3 inflammasome-~~related~~ associated genes correlates with increased immune cell infiltration, activated immune pathways, ~~and~~ as well as enhanced immune signaling in AS.

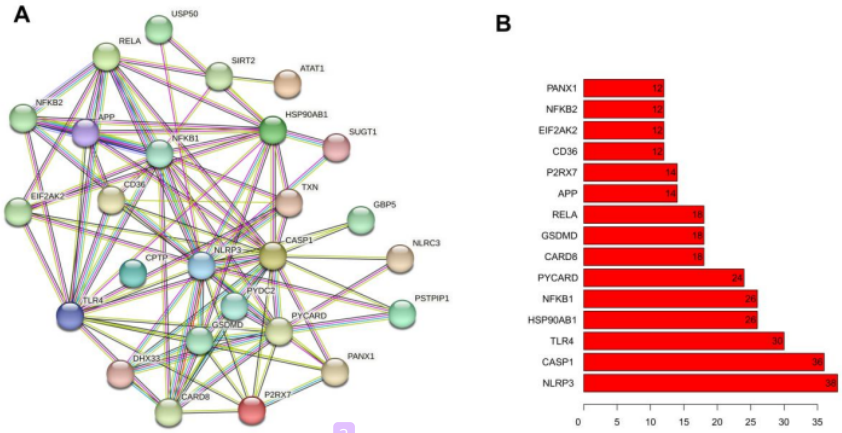
In our study, we discovered a close relationship between P2RX7 and immune infiltration in AS ~~samples~~specimens. Whether positively or negatively correlated, the differences between P2RX7 and immune infiltrating cells were most pronounced. P2RX7, also known as the Purinergic receptor P2X 7, is a type of acid receptor ~~belonging to~~ that belongs to the purinergic P2X receptor family. P2RX7 is widely distributed in many ~~various~~ kinds~~types~~ of immune cells, ~~including~~ like macrophages, dendritic cells, T cells, ~~and~~ as well as B cells, and ~~plays~~ takes an essential part~~role~~ in regulating cell activation, inflammation, and cell death[28]. Activation of P2RX7 is mainly driven by ATP released during cell injury or death, during which ATP can be recognized as a danger-associated molecular pattern (DAMP) by the immune system[29]. Reportedly, ~~Studies have reported that~~ P2RX7 ~~plays~~ takes a crucial ~~role~~ part in the pathophysiology of AS[30]. P2RX7 can induce the production of pro-inflammatory cytokines and chemokines, further amplifying the inflammatory response and impacting endothelial function[31]. Additionally, P2RX7 can influence macrophage behavior, promoting foam cell formation, a critical step in plaque formation in AS[32]. Furthermore, P2RX7 is believed to affect cell apoptosis and necrosis, potentially leading to increased cell death and inflammation, further aggravating the pathological process of AS. To verify our speculation, we induced THP-1 cells by PMA. Further regulating the expression of P 2 RX 7 in THP-1 cells, found that P2X7R, NLRP 3 in P 2 RX 7, TNOs, TNF- α, and CXCL10 compared to si-NC, and the levels of Arg-1 and CD163 suggested that P 2 RX 7 may induce THP-1 cells by regulating NLRP 3 from M0 to M2 associated with stable atherosclerotic plaques.

In the end, ~~based on~~ in the light of the risk score, patients were ~~divided into~~assigned to the high ~~and~~ or low-risk group~~s~~. Through GSEA enrichment analysis, we found the ~~that~~ involvement of NLRP3 inflammasome-related genes ~~are~~ mainly ~~involved~~ in a series of biological pathways related to heart disease. Hypertrophic cardiomyopathy (HCM) is a ~~common~~ frequently-seen hereditary heart disease ~~characterized by~~featured with hypertrophy of the left and/or right ventricular walls, which is not caused by other diseases[33]. HCM differs from AS mainly because the pathophysiology of HCM results from anomalies in cardiac muscle cells, leading to hypertrophy, whereas AS is primarily due to a series of inflammatory responses and lipid accumulation following endothelial damage. In AS, changes in the function and behavior of vascular smooth muscle cells (VSMCs) are critical. Under normal circumstances, VSMCs participate in maintaining vascular homeostasis2. However, in AS, they might proliferate, migrate to plaques, and produce extracellular matrix, leading to arteriosclerosis and narrowing. These enriched pathways further affirm the significance of these genes in the pathogenesis of AS.

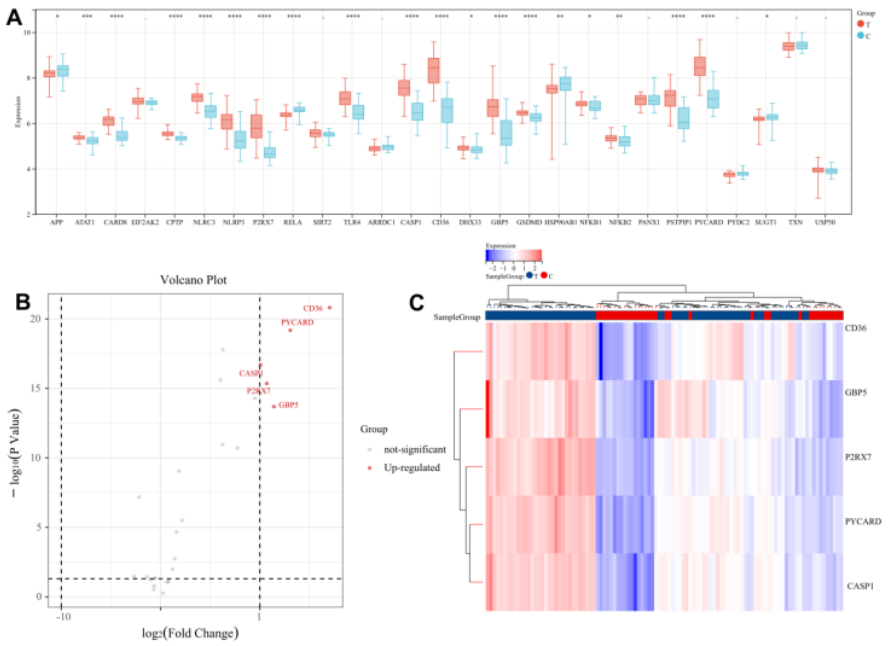
Nonetheless, our study has certain limitations. Firstly, there were no in vivo tests to corroborate our findings. Secondly, due to the absence of our clinical data on AS,

even though our results bear credibility, they still require verification with our clinical data. Hence, we anticipate conducting various experiments in future studies to further elucidate the relationship between these genes and AS.

In conclusion, we have identified the importance of NLRP3 inflammasome-~~related~~ associated genes. Their expression is ~~generally~~ upregulated in AS ~~samples~~specimens generally, suggesting they might play vital roles in the onset and progression of the disease. Our research also revealed enhanced immune cell infiltration, potentially regulated by NLRP3 inflammasome-~~related~~ associated genes.



~~Figure~~Fig. 1: Interactions Among NLRP3 Inflammasome-~~Related~~ Associated Genes
A. The network of protein-protein interactions among genes associated with NLRP3 inflammasome.
B. The top 15 gene interactions linked with NLRP3 inflammasome.



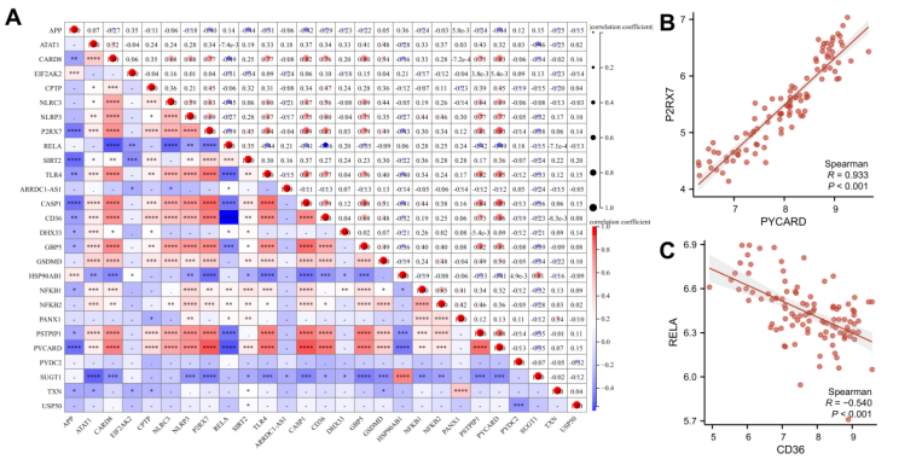
~~Figure~~Fig. 2: Analysis of NLRP3 Inflammasome-~~Related~~ Associated Genes Variance

A. Box plot showcasing the gene expression overview in the GSE100927 dataset.

B. Volcano plot ~~illustrating~~ displaying the divergent expression of genes in the GSE100927 dataset.

C. Heatmap depicting gene expression discrepancies in the GSE100927 dataset.

Note: C: Healthy ~~samples~~specimens; T: Diseased ~~samples~~specimens. Red: Upregulated; Gray: No significant change. Blue: From healthy control ~~samples~~specimens; Red: From ~~patients with~~ Arteriosclerosis (AS) patients.



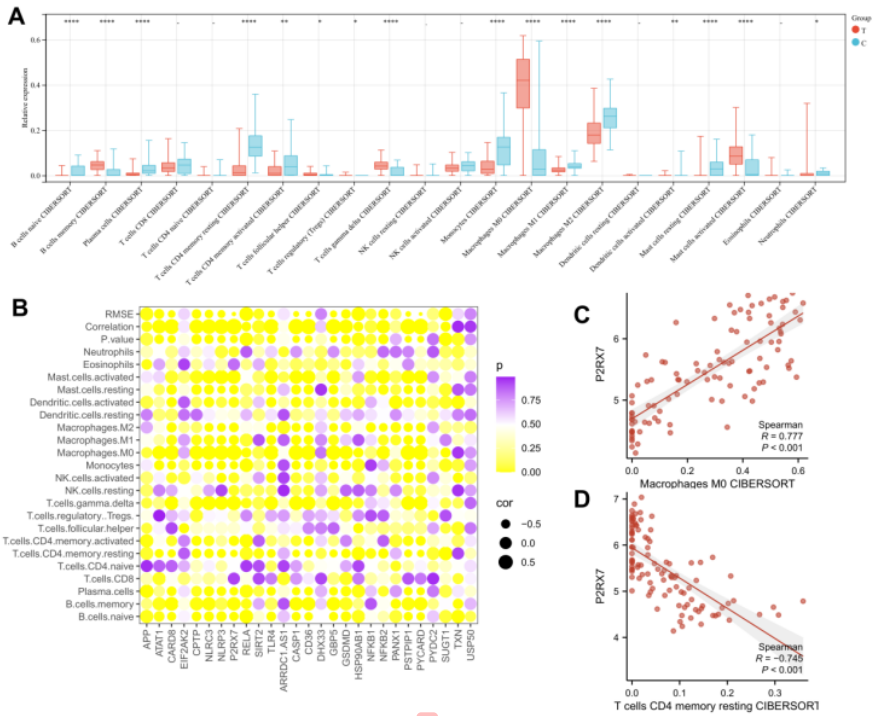
~~Figure~~Fig. 3: Relationships Among Expressions of the 27 NLRP3 Inflammasome-Linked Genes

A. ~~Correlation~~ Association plot exhibiting ~~significant~~ strong interrelations among genes.

B. Interaction between CARD8 and GBP5 expressions in the collective ~~samples~~specimens.

C. Interaction between TLR4 and PANX1 expressions in diseased ~~samples~~specimens.

Note: Red: Positive ~~correlation~~association; Blue: Negative association~~correlation~~. Darker shades ~~indicate~~ suggest~~stronger~~ closer correlation.



~~Figure~~Fig. 4: Interaction ~~Between~~ of NLRP3 Inflammasome-~~Related~~ Associated Genes ~~and~~ with Immune Cell Infiltration in the Immune Landscape

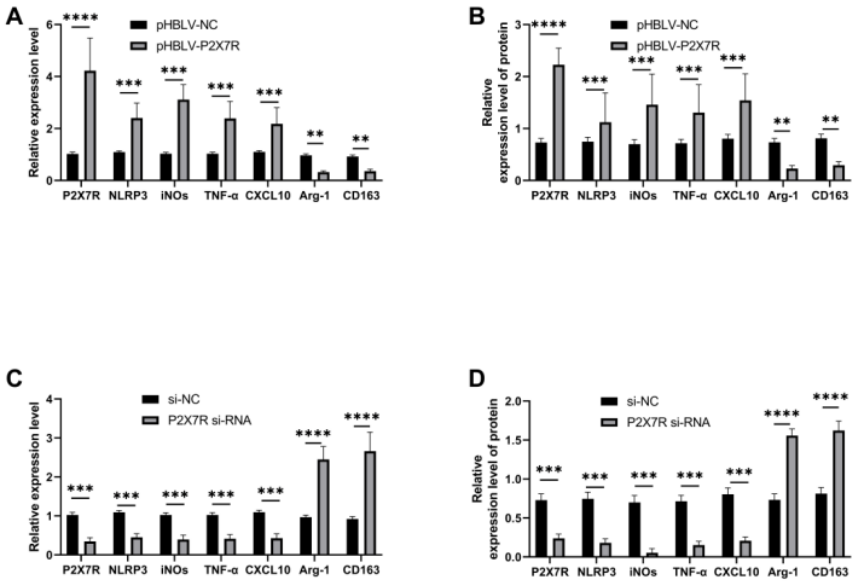
A. Comparison of immune cell infiltration degrees between AS and normal ~~samples~~specimens.

B. Significant interrelations between the 27 NLRP3 inflammasome-linked genes and immune cell infiltration.

C. ~~Correlation~~ Association assessment between P2RX7 and Macrophages M0 cell infiltration.

D. Association~~Correlation~~ assessment between P2RX7 and T cells CD4 memory resting cell infiltration.

Note: Purple: Positive correlation; Yellow: Negative correlation. Darker circles ~~indicate~~ imply ~~stronger~~ closer correlation.



~~Figure~~Fig. 5 Effect of P2RX7 Modulation on THP-1 Macrophage Polarization

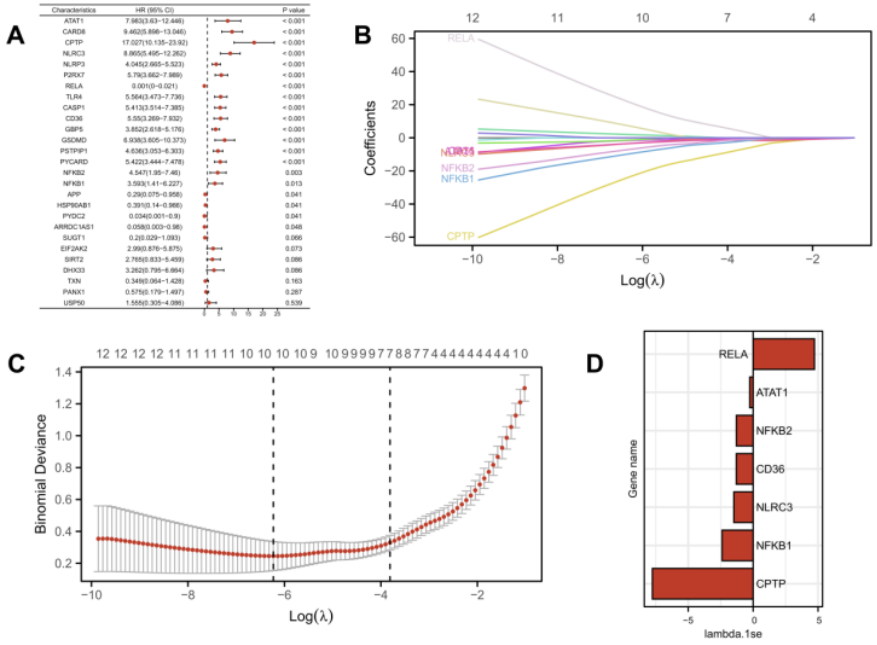
A. Variations in mRNA of M1 and M2 polarization-~~associated~~ linked genes in THP-1 cells after transfection with pHBLV-P2X7R

B. Variations in protein of M1 and M2 polarization- linked~~associated~~ genes in THP-1 cells after transfection with pHBLV-P2X7R

C. Variations in mRNA of M1 and M2 polarization- linked~~associated~~ genes in THP-1 cells after transfection with P2X7R si-RNA

D. Variations in protein of M1 and M2 polarization- linked~~associated~~ genes in THP-1 cells after transfection with P2X7R si-RNA

Note: ***P＜0.001, ****P＜0.00001



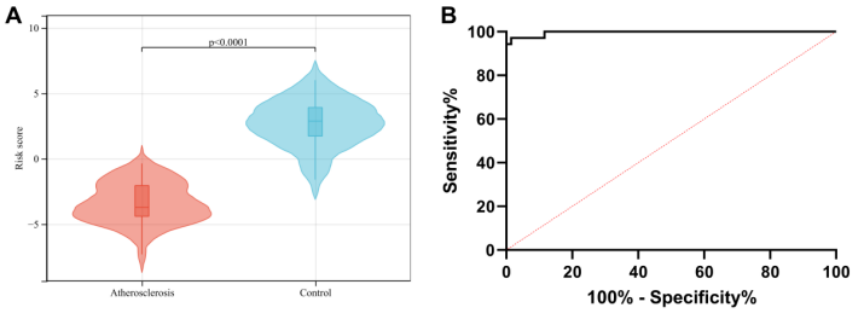
~~Figure~~Fig. 6: Construction of a LASSO Regression Model

A. Univariate logistic regression analysis of the 27 NLRP3 inflammasome-linked genes.

B. ~~Selection~~ Choosing of genes with non-zero coefficients for model construction.

C. ~~—~~Log lambda values of genes corresponding to the point with minimal cross-validation error.

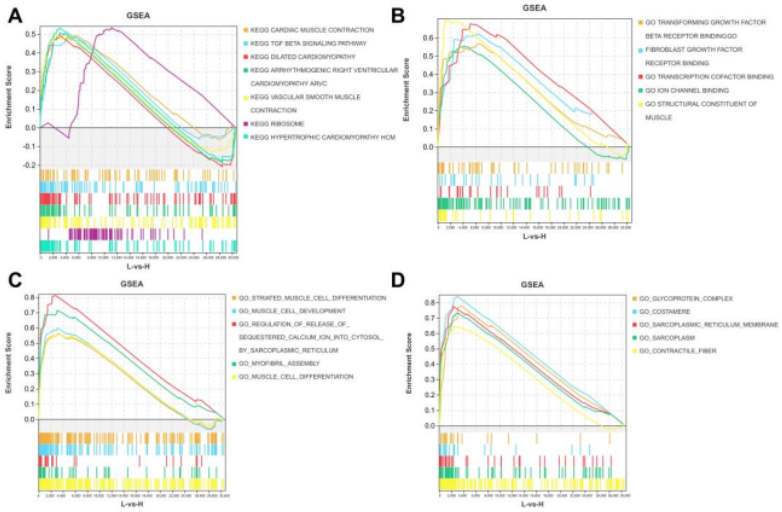
D. Coefficients of each gene in the predictive model.



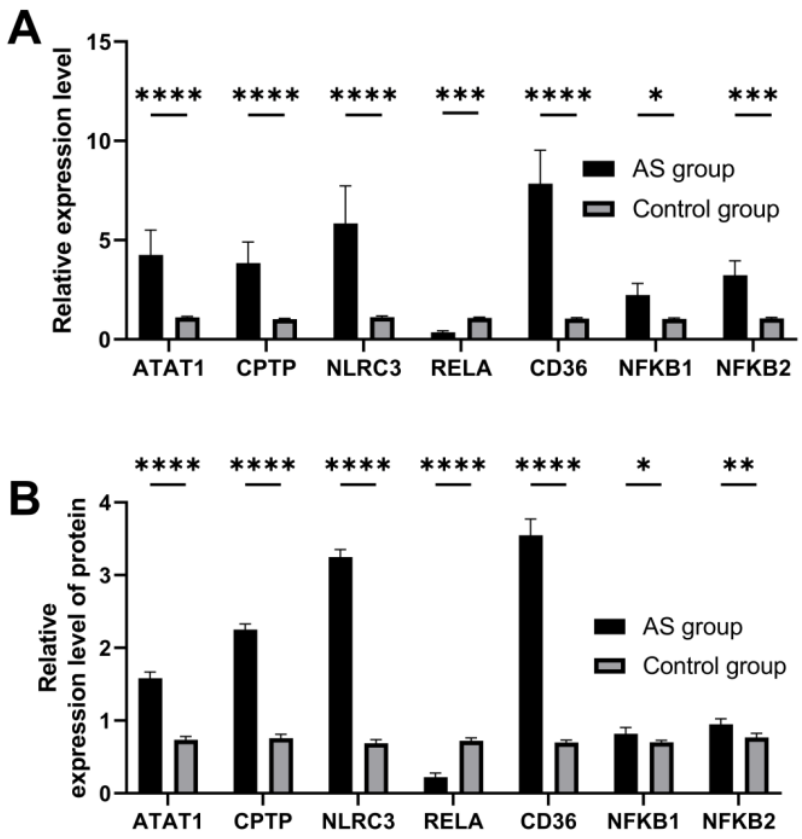
~~Figure~~Fig. 7: ROC Curve for Risk Score in Diagnosing Arteriosclerosis (AS) Patients

A. Risk score levels in AS ~~samples~~specimens in comparison to normal ~~samples~~specimens.

B. Risk score diagnostic curve in AS.



~~Figure~~Fig. 8: Enriched Gene Sets Analysis for Biological Functions and Pathways
A. KEGG, a reference resource from the Kyoto Encyclopedia of Genes and Genomes.
B-C. GO, denoting Gene Ontology.



~~Figure~~Fig. 9: Risk Genes Verification in AS Cells through qRT-PCR and WB Tests
A. Risk genes verification in AS cells utilizing qRT-PCR.
B. Risk genes verification in AS cells utilizing WB (Western Blot).
Note: *P＜0.05, **P＜0.01, ****P＜0.0001.

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