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Integrative Network Toxicology, Transcriptomics Profiling, and Molecular Docking Analysis of Potential Mechanisms Linking Tris(1,3-dichloro-2-propyl) Phosphate (TDCPP) Exposure to Atherosclerosis

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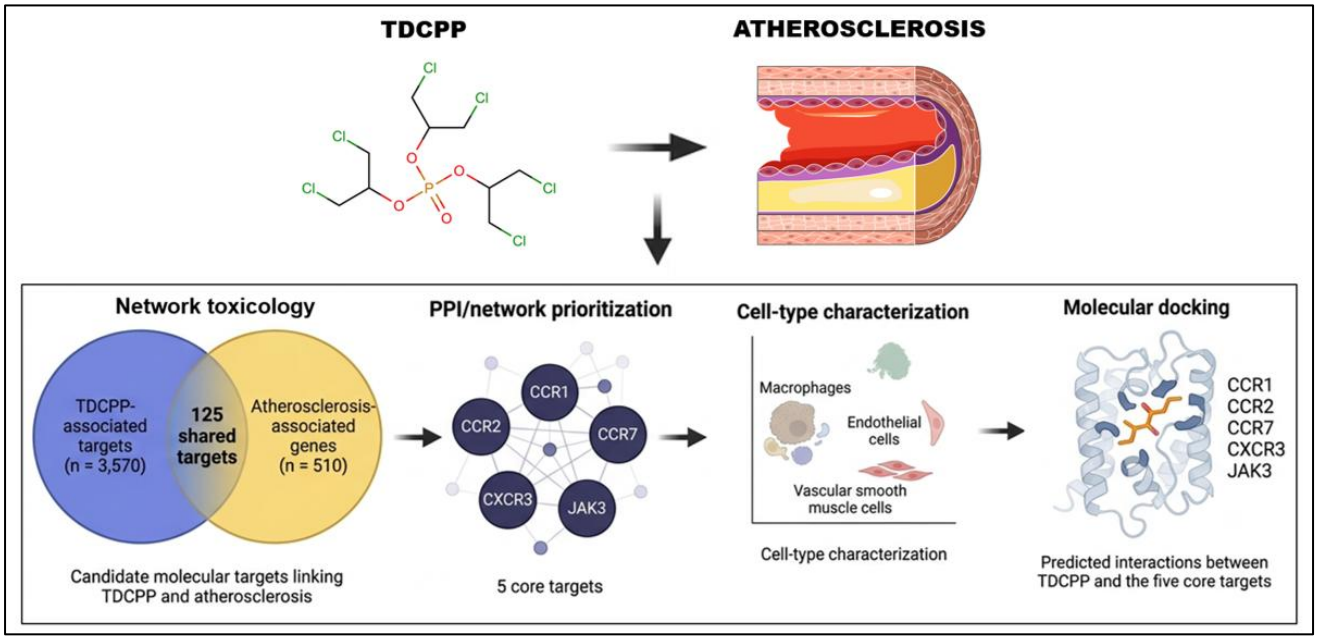
Integrative Network Toxicology, Transcriptomics Profiling, and Molecular Docking Analysis of Potential Mechanisms Linking Tris(1,3-dichloro-2-propyl) Phosphate (TDCPP) Exposure to Atherosclerosis

Abstract

Tris(1,3-dichloro-2-propyl) phosphate (TDCPP) is an organophosphate flame retardant widely detected in the environment, raising concerns regarding its long-term health effects. Epidemiological studies have associated TDCPP exposure with the risk of cardiovascular diseases. However, the mechanisms by which TDCPP exposure may contribute to cardiovascular diseases, particularly through atherosclerosis formation, remain unclear. Our study aimed to investigate the potential biomolecular mechanisms linking TDCPP to atherosclerosis through in silico analyses, including network toxicology, protein-protein interaction (PPI) network analysis, transcriptomic profiling, and molecular docking. Genes related to TDCPP and atherosclerosis were retrieved from open-access databases. We identified 125 intersecting genes between TDCPP- and atherosclerosis-related gene sets, which were associated with inflammatory signaling pathways, particularly cytokine- and chemokine-related processes and leukocyte migration. PPI network analysis identified CCR1, CCR2, CCR7, CXCR3, and JAK3 as potential core gene targets. Further analysis of potential cellular targets by mapping to a human atherosclerotic plaque single-cell transcriptomic atlas indicated that these five genes were mainly expressed in immune-cell populations, particularly myeloid and lymphoid cells. Transcriptomic analysis showed higher expression of all five genes in atherosclerotic tissues than in controls across arterial territories, while CCR1, CCR2, and JAK3 consistently showed higher expression in advanced atherosclerotic lesions. Molecular docking revealed that CCR7 and CCR2 had favorable docking scores with TDCPP (-5.801 and -5.627 kcal/mol, respectively). Overall, our findings indicate potential molecular mechanisms linking TDCPP to atherosclerosis through chemokine-mediated immune-cell trafficking and inflammatory signaling. Further studies are necessary to validate the relevance of these candidate targets and pathways.

Keywords: TDCPP; atherosclerosis; chemokine signaling; network toxicology; single-cell transcriptomics; molecular docking

Graphical Abstract



Introduction

Atherosclerosis is a major pathological basis of atherosclerotic cardiovascular disease (ASCVD), including ischemic heart disease, ischemic stroke, and peripheral arterial disease. ASCVD remains a leading cause of morbidity and mortality worldwide. It may be induced by chronic inflammation associated with known traditional risk factors, including metabolic and behavioral factors [1-3]. Recently, evidence regarding environmental contaminants as non-traditional risk factors for cardiovascular diseases has gained increasing attention [4]. Among them, organophosphate flame retardants (OPFRs) have become a potential concern because of their widespread use in consumer and industrial products [5].

Tris(1,3-dichloro-2-propyl) phosphate (TDCPP) is a chlorinated organophosphate triester widely used as an additive flame retardant in polyurethane foams, resins, plastics, textile coatings, and rubber [6]. TDCPP is used as an additive material and is not tightly bound to the polymer matrix, allowing it to migrate easily from the original products into the environment. TDCPP has been detected in environmental matrices, including indoor dust, air, water, and soil, indicating multiple potential routes of exposure for living organisms [6, 7]. Furthermore, biomonitoring studies detecting TDCPP-related metabolites support the widespread occurrence of human exposure and highlight concerns regarding the potential long-term health impacts of TDCPP, particularly in relation to cardiovascular disease [8-10].

Several epidemiological and experimental studies suggest that exposure to organophosphate esters may influence biological processes relevant to the development of cardiovascular disease. In a cross-sectional analysis of the National Health and Nutrition Examination Survey, combined exposure to organophosphate esters was associated with a higher prevalence of cardiovascular disease, although associations with individual metabolites were less consistent [10]. In addition, urinary bis(1,3-dichloro-2-propyl) phosphate (BDCPP), a major metabolite of TDCPP, has been inversely associated with high-density lipoprotein cholesterol concentrations, suggesting a potential relationship with lipid metabolism [5]. Experimental studies have also reported that TDCPP can disrupt vascular development through altered Nrf2-dependent VEGF signaling and induce intracellular lipid accumulation and mitochondrial dysfunction [11, 12]. Collectively, these findings provide biological plausibility for a potential contribution between TDCPP exposure and processes relevant to atherogenesis.

However, the mechanistic basis linking TDCPP exposure to atherosclerosis remains poorly characterized. In particular, the molecular targets, signaling pathways, and disease-relevant cell populations that may contribute to this relationship have not been systematically defined. Therefore, this study aimed to explore potential pathways

linking TDCPP exposure to atherosclerosis through an integrative analysis of candidate molecular targets, signaling pathways, and disease-relevant cell populations.

Material and methods

Study design

This study performed an integrative in silico framework combining network toxicology, protein-protein interaction (PPI) network analysis, single-cell transcriptomic analysis, and molecular docking. All analyses were based on publicly available secondary data obtained from open-access databases; no human or animal specimens were newly collected for this study. Database searches and data retrieval were completed on June 2, 2026. Details of the databases and resources used in this study are provided in Supplementary Table S1.

Toxicity assessment and identification of TDCPP-associated targets

The toxicity profile of TDCPP was initially evaluated using ADMETlab 3.0 and ProTox-3.0. Potential TDCPP-associated molecular targets were subsequently identified using SwissTargetPrediction and STITCH. For these analyses, the Simplified Molecular-Input Line-Entry System (SMILES) representation of TDCPP was retrieved from PubChem and used as the chemical input. Additional TDCPP-associated genes were retrieved from the Comparative Toxicogenomics Database (CTD) using the chemical name "Tris(1,3-dichloro-2-propyl) phosphate" as the search term.

Identification of atherosclerosis-related genes

Transcriptomic data from the GSE100927 dataset were obtained from the Gene Expression Omnibus (GEO) database. Data processing and differential expression analysis were performed in R using the *GEOquery* and *limma* packages. Expression values were log2-transformed, followed by between-array normalization using the *normalizeBetweenArrays* function in *limma*. Probes without corresponding gene symbols were excluded. For genes represented by multiple probes, the probe with the lowest p-value was retained for subsequent analysis. Principal component analysis (PCA) was performed to assess the overall distribution of samples and explore clustering patterns between the atherosclerosis and control groups.

Differential expression analysis was then performed to identify genes whose expression differed between the atherosclerosis and control groups. p-values were adjusted for multiple comparisons using the Benjamini–Hochberg method. Genes with an adjusted p-value <0.05 and an absolute log2 fold change ($|\log2FC| > 1$ were considered differentially expressed genes (DEGs). Volcano plots and heatmaps were generated to visualize the overall distribution and expression patterns of the identified DEGs.

Additional atherosclerosis-associated genes were retrieved from the Online Mendelian Inheritance in Man (OMIM) and GeneCards databases using the search term “atherosclerosis”. For GeneCards, only genes with relevance scores above the median value of all retrieved entries were retained for further analysis. For OMIM, entries without an associated phenotype annotation were excluded.

Identification of overlapping targets

A Venn diagram was used to identify genes shared between the TDCPP-associated and atherosclerosis-associated gene sets obtained from the respective databases and transcriptomic analysis. The intersecting genes were considered potential TDCPP-related atherosclerosis targets and were subsequently subjected to PPI network analysis.

Pathway enrichment analysis

Pathway enrichment analysis was performed in R to characterize the biological functions and pathways associated with the candidate genes. Gene symbols were first converted to Entrez Gene IDs using the *org.Hs.eg.db* annotation package.

Gene Ontology (GO) enrichment analysis was conducted using the *enrichGO()* function in the *clusterProfiler* package and included the Biological Process (BP), Molecular Function (MF), and Cellular Component (CC) domains. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis was subsequently performed using the *enrichKEGG()* function. p-values were adjusted for multiple testing using the Benjamini-Hochberg method, and enrichment terms with an adjusted p-value <0.05 were considered statistically significant. Dot plots were generated to visualize the top enriched GO terms and KEGG pathways ranked by adjusted p-value. Gene-concept network plots were additionally generated to illustrate the relationships between enriched biological terms or pathways and their associated genes.

PPI network analysis and core target identification

The overlapping genes between the TDCPP-associated and atherosclerosis-associated gene sets were submitted to the STRING database to construct a PPI network. The analysis was restricted to *Homo sapiens*, and a high-confidence interaction score (>0.7) was applied.

The resulting PPI network was imported into Cytoscape (v3.10.4) for topological analysis using the cytoNCA plugin. Six centrality measures were evaluated: degree centrality (DC), betweenness centrality (BC), closeness centrality (CC), eigenvector centrality (EC), local average connectivity (LAC), and network centrality (NC). Nodes with values equal to or greater than the median for all six centrality measures were retained as topologically important candidates.

The Molecular Complex Detection (MCODE) plugin was subsequently used to identify densely interconnected network modules. Genes that met the cytoNCA centrality criteria and were grouped within the same interconnected MCODE module were identified as core candidate targets.

Transcriptomic analysis of core target genes

Single-cell transcriptomic mapping

The core candidate genes were mapped to a human atherosclerotic plaque single-cell RNA-sequencing atlas reported by Traeuble et al [13]. Data were analyzed in R using an HDF5-based workflow. Gene annotations, cell metadata, the expression matrix, and UMAP coordinates were extracted from the original *.h5ad* file.

The core candidate genes were first matched against the gene annotation data to determine their presence in the single-cell dataset. For genes detected in the atlas, expression values were extracted directly from the sparse expression matrix using a chunk-based approach. Gene expression patterns were subsequently visualized using UMAP feature plots and dot plots according to the cell-type annotations provided in the original atlas.

Differential expression analysis in atherosclerosis tissue

Publicly available transcriptomic datasets using human atherosclerotic tissues, GSE28829, GSE43292, and GSE100927 were accessed from GEO and analyzed. The data were retrieved using the *GEOquery* package in R. Differential expression was analyzed using the *limma* package with empirical Bayes variance moderation. Results were expressed as log2 fold changes (Log2FC) relative to the comparator, with 95% confidence intervals (CIs). Statistical significance was assessed using adjusted p-values calculated with the Benjamini-Hochberg correction. An adjusted p-value <0.05 was considered statistically significant.

Molecular docking

The three-dimensional structure of TDCPP was obtained from PubChem and subjected to geometry optimization using Avogadro version 2.0.0. Experimentally determined protein structures of the target proteins were retrieved from the RCSB Protein Data Bank (PDB). Protein structures were prepared using AutoDockTools version 1.5.7 by removing water molecules, ions, and other non-receptor components, followed by the addition of hydrogens and Gasteiger charges.

The docking grid was centered on the binding site defined by the co-crystallized ligand. Docking simulations were performed using AutoDock Vina version 1.2.7 with a uniform grid box of 22.5×22.5×22.5 Å, an exhaustiveness value of 16, a maximum of 10 output binding modes, and an energy range of 3 kcal/mol. The top-ranked TDCPP pose was selected based on the lowest predicted binding energy.

Three-dimensional protein-ligand binding poses were visualized using the open-source version of PyMOL. Two-dimensional interaction diagrams were generated using BIOVIA Discovery Studio Visualizer to characterize the predicted interactions between TDCPP and the surrounding amino acid residues.

Results

Predicted toxicological profile of TDCPP and identification of associated genes

In silico toxicity assessment using ADMETlab 3.0 and ProTox-3.0 predicted potential carcinogenic, endocrine-disrupting, neurotoxic, immunotoxic, and environmental toxicity-related properties of TDCPP (Supplementary Fig. S1). Collectively, these predictions suggest that TDCPP may affect multiple biological systems and processes, supporting further investigation of its potential long-term health effects.

Database searches identified 3,610 TDCPP-associated genes in the CTD, 103 predicted targets in SwissTargetPrediction, and 10 targets in STITCH. After combining the retrieved gene sets and removing duplicate entries, 3,695 unique TDCPP-associated gene targets were retained for subsequent analyses (Supplementary File 1).

Identification of atherosclerosis-associated genes

Atherosclerosis-associated genes were first identified using transcriptomic data from human atherosclerotic plaque samples obtained from the carotid, femoral, and infrapopliteal arteries in the GSE100927 dataset (69 atherosclerotic plaque vs 35 control samples). PCA indicated differences in sample distribution between the atherosclerosis and control groups, with PC1 and PC2 accounting for 14.6% and 5.8% of the total variance, respectively (Fig. 1A). Differential expression analysis identified 548 DEGs, comprising 396 upregulated and 152 downregulated genes (Fig. 1B-C). GO and KEGG enrichment analyses further characterized the biological functions and pathways associated with these DEGs (Fig. 1D-E). The 548 DEGs were retained as transcriptomics-derived atherosclerosis-associated genes for subsequent analysis.

To broaden the identification of atherosclerosis-associated genes, additional candidates were retrieved from GeneCards (n=3,323) and OMIM (n=112). Intersection of these gene sets yielded 103 shared genes, which were retained for subsequent analysis (Supplementary File 1).

Identification and functional enrichment of intersecting TDCPP-atherosclerosis genes

Intersection of the TDCPP-associated and atherosclerosis-associated gene sets identified 125 shared genes, which were considered candidate TDCPP-atherosclerosis targets (Fig. 2). The complete list of intersecting genes is provided in Supplementary File 1.

Functional enrichment analysis was subsequently performed to characterize the biological processes and pathways associated with these 125 genes. GO enrichment analysis identified terms related to cytokine production, leukocyte migration, chemotaxis, and immune defense responses. Molecular function analysis was predominantly enriched for G protein-coupled chemoattractant receptor activity and chemokine receptor activity (Fig. 3A-B). Consistent with the GO findings, KEGG pathway analysis showed significant enrichment in the lipid and atherosclerosis pathway, cytokine-cytokine receptor interaction, NF-κB signalling pathway, and C-type lectin receptor signalling pathway (Fig. 3C-D). Collectively, these enrichment patterns indicate that the intersecting TDCPP-atherosclerosis genes are predominantly associated with immune-cell recruitment, cytokine and chemokine signalling, and inflammatory pathways relevant to atherosclerosis.

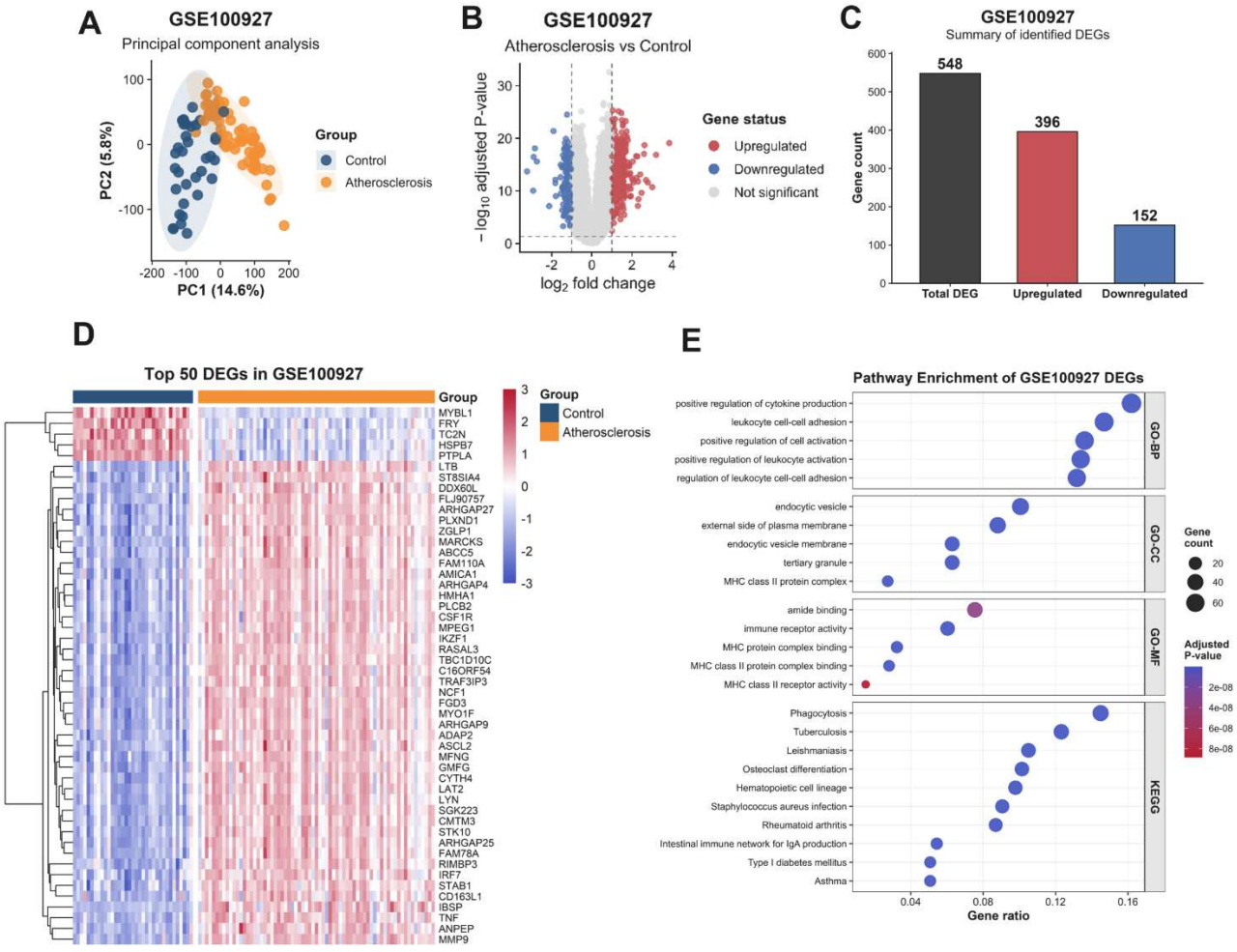


Fig. 1 Transcriptomic analysis of atherosclerosis-associated genes using the GSE100927 dataset. (A) Principal component analysis (PCA) of the study samples; (B) Heatmap of differentially expressed genes (DEGs); (C) Bar plot showing the numbers of total, upregulated, and downregulated DEGs; (D) Top 50 DEGs; and (E) Pathway enrichment analysis

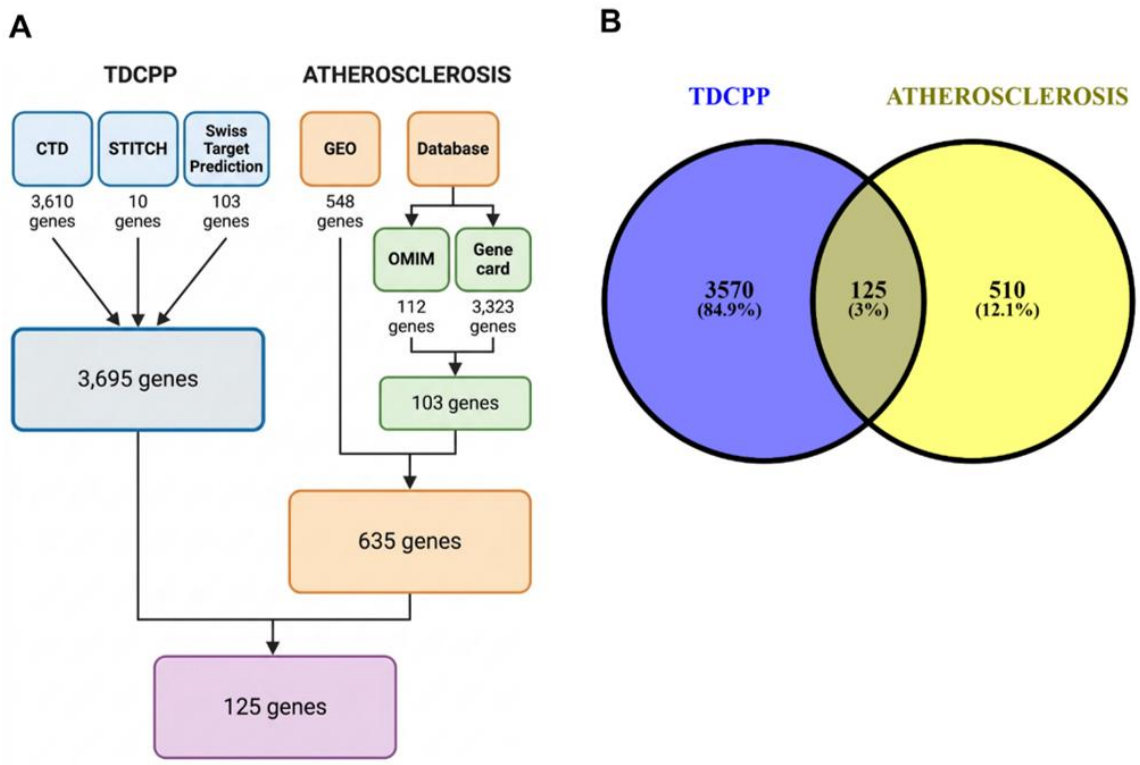


Fig. 2 Identification of intersecting TDCPP-atherosclerosis genes. (A) Flowchart illustrating the gene retrieval and screening process; (B) Venn diagram showing the genes shared between TDCPP-associated and atherosclerosis-associated dataset

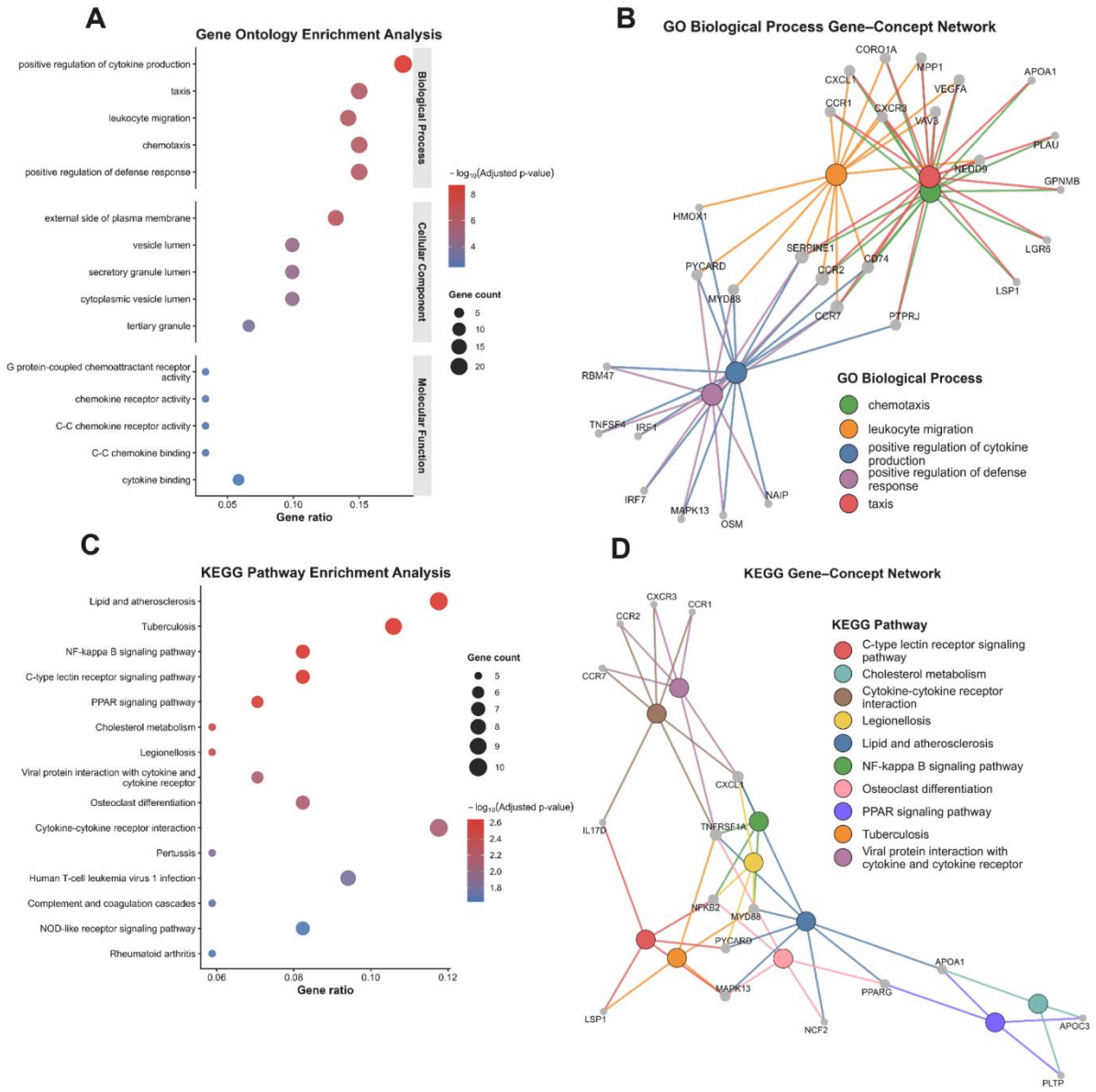


Fig. 3 Functional enrichment analysis of the 125 shared TDCPP-atherosclerosis genes. (A) Gene Ontology (GO) enrichment analysis; (B) gene-concept network plot of enriched GO biological processes; (C) Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis; and (D) gene-concept network plot of enriched KEGG pathways

Core candidate target identification by PPI network analysis

A PPI network analysis was constructed for the 125 intersecting TDCPP-atherosclerosis genes using STRING with a high-confidence interaction score (>0.7). The resulting network comprised 124 nodes and 72 edges, with an average node degree of 1.16. Topological analysis using cytoNCA in Cytoscape identified 11 candidate genes that met the predefined centrality criteria. Further analysis using MCODE identified densely interconnected

network modules among these candidates. Six genes, including CCR1, CCR2, CCR7, CXCR3, JAK3, and SERPINE1 were retained following the combined cytoNCA and MCODE analyses (Supplementary Table S2). SERPINE1 was located outside the interconnected JAK3-chemokine receptor module and showed no direct connection with the other genes within the selected subnetwork. Subsequent analyses therefore focused on CCR1, CCR2, CCR7, CXCR3, and JAK3 as the core candidate targets within this functionally coherent network module (Fig. 4).

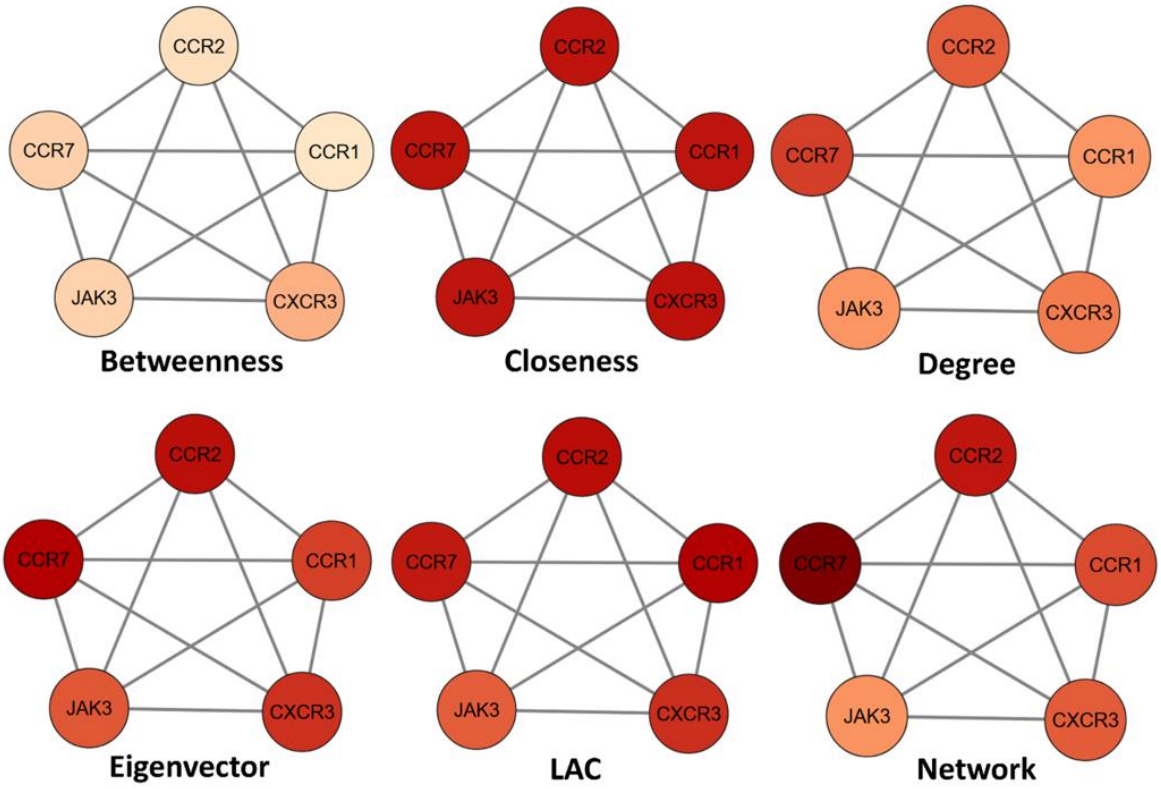


Fig. 4 Identification of five core candidate targets through protein-protein interaction (PPI) network analysis. Darker nodes indicate higher values of the corresponding metrics

Mapping of core candidate targets to single-cell RNA-sequencing and atherosclerotic tissue transcriptomic data

To characterize the cellular distribution of these core candidate genes within human atherosclerotic plaques, their expression was subsequently mapped to the single-cell RNA-sequencing atlas reported by Traeuble et al [13]. The atlas integrates single-cell transcriptomic data from human atherosclerotic lesions of the carotid, coronary, and femoral arteries and comprises 259,116 cells. Cell-type assignments were based on the annotations provided in the original atlas, with the corresponding marker profiles shown in Supplementary Fig. S2.

Single-cell expression profiling showed that the core candidate genes were predominantly expressed in immune-cell populations, including myeloid and lymphoid cells, rather than in structural vascular cell populations. CCR1 and CCR2 were primarily expressed in myeloid populations, including monocytes, macrophages, dendritic cells, and neutrophils. CCR7 expression was most prominent in B cells and T cells. CXCR3 was expressed across several immune-cell populations, particularly T cells, dendritic cells, and natural killer cells. In contrast, JAK3 showed a broader expression pattern across multiple cell populations (Fig. 5A-B).

Further analysis was performed to evaluate the expression of the five core target genes in human atherosclerotic tissues. As GSE100927 had been used for the initial identification of atherosclerosis-related targets, we further investigated whether the differential expression of these genes was consistent across distinct arterial territories. We identified that all five genes exhibited significantly higher expression in atherosclerotic tissues than in controls across the carotid, femoral, and infrapopliteal arteries (Supplementary Fig. S3). We subsequently examined their expression in relation to atherosclerotic lesion severity using two independent datasets, including GSE28829 (advanced and early carotid lesions) and GSE43292 (advanced carotid plaques with paired macroscopically intact arterial tissues). Notably, CCR1, CCR2, and JAK3 consistently exhibited higher expression in advanced lesions across both datasets. In contrast, CCR7 and CXCR3 showed no significant differential expression between advanced and early lesions in GSE28829 (Fig. 6A-B).

Molecular docking analysis

Molecular docking was performed between TDCPP and the five core candidate proteins. CCR7 and CCR2 showed the most favorable binding energies (<-5 kcal/mol), with docking scores of -5.801 and -5.627 kcal/mol, respectively (Supplementary Table S3).

Interaction analysis showed that the CCR7-TDCPP and CCR2-TDCPP complexes were mainly characterized by hydrophobic and aromatic interactions, including alkyl, π-alkyl, and π-sigma contacts, together with van der Waals interactions (Fig. 7A-B and Supplementary Fig. S4).

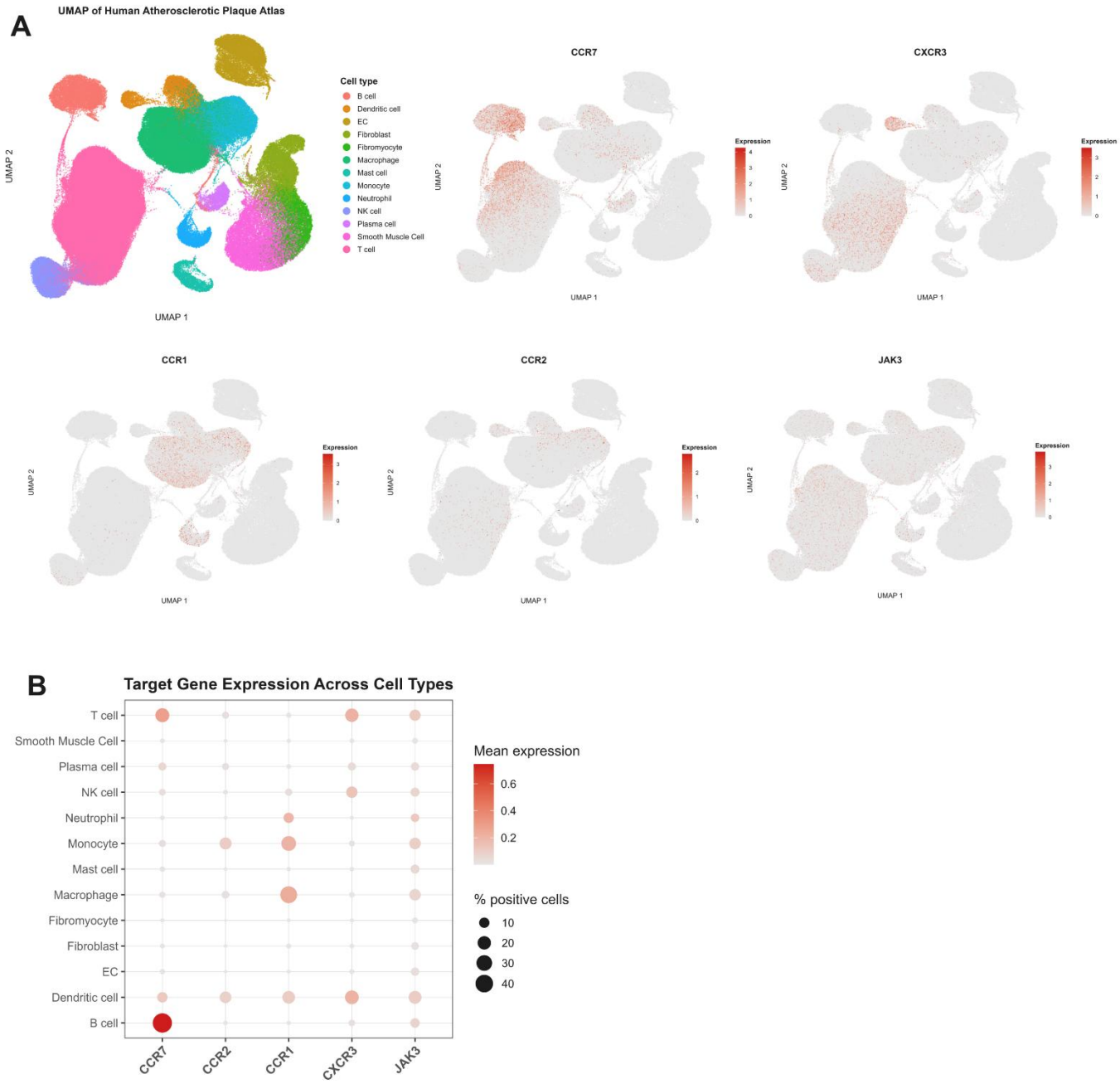


Fig. 5 Mapping of core candidate targets in a human atherosclerotic plaque single-cell RNA-sequencing atlas. (A) UMAP feature plots; (B) Dot plot showing expression across annotated cell populations

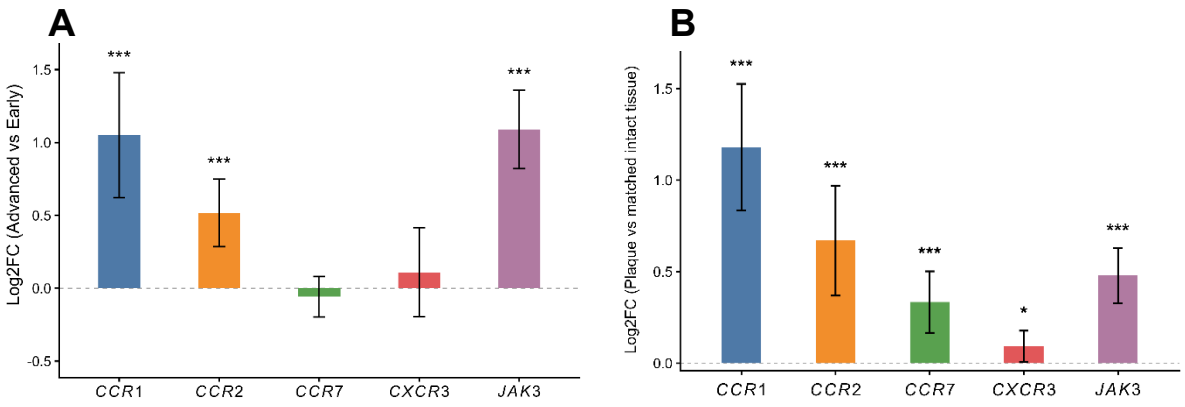


Fig. 6. Differential expression of core target genes in carotid atherosclerotic tissues. (A) GSE28829: advanced vs. early lesions; (B) GSE43292: advanced plaques vs. paired macroscopically intact tissues. Data are presented as log2 fold change (log2FC) with 95% confidence intervals. Benjamini-Hochberg-adjusted p-values: *p<0.05; ***p<0.001

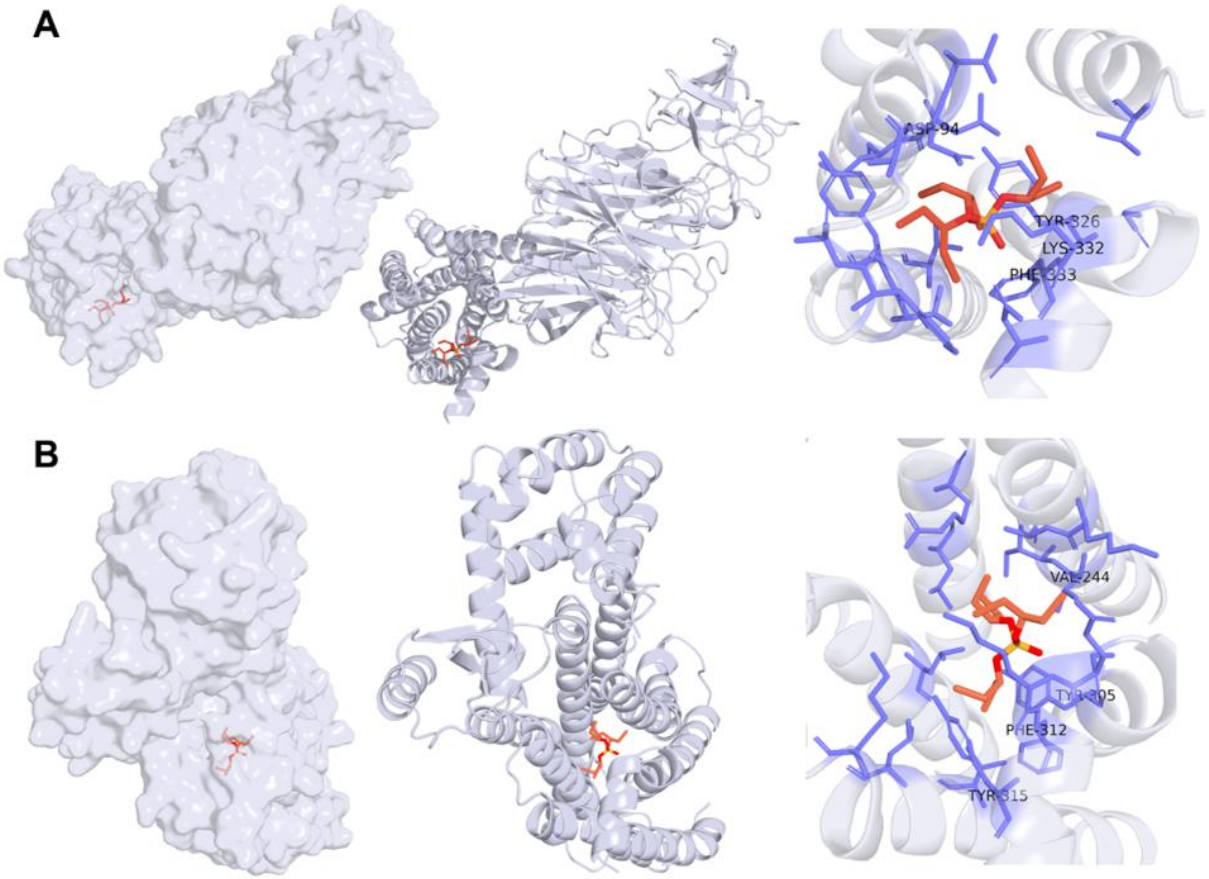


Fig. 7 Molecular docking visualization of TDCPP with selected core targets. (A) TDCPP-CCR7 complex; (B) TDCPP-CCR2 complex

Discussion

Atherosclerosis is a chronic inflammatory vascular disease characterized by lipid accumulation, endothelial activation, and progressive plaque formation, in which sustained recruitment and activation of innate and adaptive immune cells contribute to lesion development and progression [14, 15]. Environmental contaminants have increasingly been considered potential modifiers of these vascular and inflammatory processes, raising concern regarding the cardiovascular effects of TDCPP exposure [5]. In the present study, integrative network toxicology identified 125 genes shared between TDCPP-associated and atherosclerosis-associated datasets. Functional enrichment predominantly implicated cytokine production, leukocyte migration, chemotaxis, chemokine receptor activity, and inflammatory signaling pathways. Subsequent PPI network analysis prioritized five core candidate targets, including CCR1, CCR2, CCR7, CXCR3, and JAK3, which showed distinct immune-cell expression patterns within the single-cell plaque atlas.

Our findings indicate that CCR1 and CCR2 are mainly expressed in myeloid cells within human atherosclerotic plaques, pointing to their potential involvement in the TDCPP-atherosclerosis network. CCR1 and CCR2 are chemokine receptors that belong to the seven-transmembrane G-protein-coupled receptor family and are mainly involved in myeloid-cell recruitment and inflammatory responses. CCR1 binds to several CC chemokines, particularly CCL3 and CCL5, and is expressed on macrophages and T cells, two major immune-cell populations involved in the pathogenesis of atherosclerosis [16]. In atherosclerosis, this receptor contributes to leukocyte recruitment and infiltration across the vascular wall, processes that are critical for plaque initiation and progression. Through chemokine-mediated signaling, CCR1 may therefore influence the accumulation of inflammatory immune cells and the local vascular inflammatory microenvironment [17, 18]. CCR2 is mainly expressed on inflammatory monocytes/macrophages and lymphocytes and binds several chemokines, particularly CCL2 and CCL7, with the highest affinity for CCL2 [19, 20]. Activation of the CCL2-CCR2 axis promotes the mobilization of monocytes from the bone marrow into the circulation and their subsequent recruitment to inflamed vascular tissues, where they can differentiate into macrophages and contribute to their accumulation within atherosclerotic lesions. These macrophages further accumulate modified lipoproteins and develop into foam cells, implying that CCR2-mediated immune-cell recruitment is linked to a central pathological process in the development stage of atherogenesis [21-23].

Further results depicted that CCR7 and CXCR3 were mainly expressed in lymphoid cells, with CCR7 expression most prominent in B cells and T cells, while CXCR3 showed a broader distribution across T cells, dendritic cells, and natural killer cells. They also belong to the seven-transmembrane G-protein-coupled chemokine receptor

family. The principal ligands of CCR7 are CCL19 and CCL21. CCR7 plays an important role in regulating immune-cell migration and trafficking, T-cell development, and lymphoid tissue organization through the CCR7–CCL19/CCL21 axis [24]. Dysregulation of this axis has been implicated in chronic inflammation, tumorigenesis, metastasis, and autoimmune diseases [25]. In atherosclerosis, dendritic cells process lipid-derived antigens and migrate to draining lymph nodes to activate naïve T cells, while CCR7 also facilitates T-cell recirculation between lymphoid tissues and the inflamed vascular wall [26, 27]. Another experimental study indicated that CCR7 also contributes to macrophage emigration during plaque regression, implying that the role of CCR7 may vary depending on the stage of atherosclerosis [28]. CXCR3 plays an important role in immune-cell migration and the regulation of inflammatory and adaptive immune responses [29, 30]. CXCR3 ligands, including CXCL9, CXCL10, and CXCL11, appear to influence atherosclerotic progression through distinct but complementary mechanisms involving immune-cell trafficking and lipid metabolism [17, 29]. However, these three ligands may exert distinct effects: CXCL9 and CXCL10 generally promote pro-inflammatory T-cell responses, which may contribute to sustained immune activation within established atherosclerotic lesions, whereas CXCL11 may favor regulatory T-cell responses and help limit inflammation [31]. These findings suggest that CCR7- and CXCR3-associated pathways may influence the adaptive immune microenvironment of atherosclerotic plaques, although their effects are likely dependent on specific ligands and immune-cell populations [17, 29, 31].

JAK3 represents a distinct but potentially complementary component of the identified network. Unlike the four chemokine receptors, JAK3 is an intracellular non-receptor tyrosine kinase that mediates signaling downstream of cytokine receptors containing the common γ-chain, including receptors for IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21 [32, 33]. JAK3 therefore links extracellular cytokine signals to intracellular pathways controlling immune-cell development, proliferation, survival, and activation. Experimental evidence also suggests that JAK3 may contribute to vascular remodeling by promoting vascular smooth muscle cell proliferation, neointima formation, and endothelial activation, although its contribution to lipid-driven atherosclerotic plaque progression remains unclear [34, 35]. In our single-cell analysis, JAK3 showed broad expression across nearly all cell populations within the atherosclerotic plaque. This broader distribution suggests that the TDCPP-atherosclerosis network may involve cytokine-responsive downstream signaling in addition to chemokine-directed immune-cell trafficking.

Atherosclerosis exhibits considerable heterogeneity across different vascular territories, reflecting variations in lesion characteristics, inflammatory pathways, and cellular composition [36]. Therefore, examining the expression of the identified core targets across distinct arterial territories may provide insights into whether their expression patterns reflect broader atherosclerosis-associated processes or vascular bed-specific alterations. Our bulk

transcriptomic analysis of GSE100927 revealed significantly higher expression of all five core genes in atherosclerotic tissues across the carotid, femoral, and infrapopliteal arteries compared with controls. These consistent expression patterns may reflect inflammatory processes shared across different arterial territories, despite the heterogeneity of immune cell populations identified in human atherosclerotic plaques [13]. Further analyses using the independent carotid datasets GSE28829 and GSE43292 demonstrated higher expression of CCR1, CCR2, and JAK3 in advanced atherosclerotic lesions compared with reference tissues. These findings are biologically plausible given the involvement of CCR2 in monocyte recruitment and accumulation within atherosclerotic plaques [23], the context-dependent immunoregulatory role of CCR1 [16], and the association of JAK3 with vascular smooth muscle cell proliferation and remodeling [34]. In contrast, CCR7 and CXCR3 showed no significant expression differences between early and advanced lesions in GSE28829, suggesting that their expression may not consistently correlate with lesion severity, despite their roles in immune cell trafficking and plaque-associated inflammatory responses [26, 28, 31]. Nevertheless, differences in patient characteristics, lesion classification, and reference tissues across datasets warrant cautious interpretation of these findings. Moreover, bulk transcriptomic analyses cannot distinguish changes in cellular composition from cell-specific transcriptional regulation. Collectively, these findings provide additional evidence supporting the involvement of the identified core target genes in atherosclerosis-associated biological processes and provide a rationale for prioritizing them for experimental validation to clarify their involvement in TDCPP-associated vascular effects.

Overall, our integrative in silico approach revealed an immune-centered framework involving immune-cell trafficking through chemokine-related mechanisms and inflammatory activation as potential molecular processes linking TDCPP to atherosclerosis. These findings provide a biologically coherent basis for prioritizing molecular targets and pathways potentially involved in TDCPP-associated atherosclerotic processes. However, there are several limitations regarding this study. First, it remains unclear whether the predicted molecular interactions occur at environmentally relevant TDCPP exposure levels or at concentrations commonly detected in human circulation. Second, these findings do not establish a causal relationship between TDCPP exposure and the development of atherosclerosis. Therefore, further in vitro studies using cell models relevant to vascular biology, together with in vivo studies using environmentally relevant TDCPP concentrations, are needed to validate the significance of these molecular targets and clarify their role to atherosclerotic development following TDCPP exposure.

Conclusion

In conclusion, this integrative computational study identified 125 genes shared between TDCPP-associated and atherosclerosis-associated datasets and prioritized CCR1, CCR2, CCR7, CXCR3, and JAK3 as candidate molecular targets involved in inflammatory signaling and chemokine-mediated immune-cell trafficking. Further analysis of potential cellular targets using single-cell transcriptomics indicated that these targets were mainly expressed in immune-cell populations, particularly myeloid and lymphoid cells. Finally, this potential biomolecular framework provides a basis for future experimental studies to validate the functional relevance of these candidate targets and pathways in the development of atherosclerosis related to TDCPP exposure.

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Author contributions

Conceptualization & methodology: I.G.G.S., M.Z.S. Data curation & analysis: I.G.G.S., N.K.V.I. Writing-original draft: I.G.G.S., N.K.S.D.G. Writing-review & editing: N.N.A.D., L.O.S.S., A.A.A.G.

Abbreviations

ADMET	: absorption, distribution, metabolism, excretion, and toxicity
ASCVD	: atherosclerotic cardiovascular disease
BC	: betweenness centrality
BDCPP	: bis(1,3-dichloro-2-propyl) phosphate
BP	: biological process
CC	: cellular component
CTD	: Comparative Toxicogenomics Database
DC	: degree centrality
DEG	: differentially expressed gene
EC	: eigenvector centrality
GEO	: Gene Expression Omnibus
GO	: Gene Ontology
HDF5	: Hierarchical Data Format version 5
KEGG	: Kyoto Encyclopedia of Genes and Genomes
LAC	: local average connectivity

MCODE : Molecular Complex Detection

MF : molecular function

NC : network centrality

NF-κB : nuclear factor kappa B

Nrf2 : nuclear factor erythroid 2-related factor 2

OMIM : Online Mendelian Inheritance in Man

OPFR : organophosphate flame retardant

PCA : principal component analysis

PDB : Protein Data Bank

PPI : protein-protein interaction

RCSB : Research Collaboratory for Structural Bioinformatics

SMILES : Simplified Molecular Input Line Entry System

TDCPP : tris(1,3-dichloro-2-propyl) phosphate

UMAP : Uniform Manifold Approximation and Projection

VEGF : vascular endothelial growth factor

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Declarations

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