FABAD J. Pharm. Sci., 49, 2, 359-370, 2024
Doi: 10.55262/fabadeczacilik.1471982
RESEARCH ARTICLE

A Network Toxicology Analysis of the Molecular Pathways and Novel Targets in TCDD-Induced Cardiovascular Toxicity

Fuat KARAKUŞ*°

A Network Toxicology Analysis of the Molecular Pathways and Novel Targets in TCDD-Induced Cardiovascular Toxicity

SUMMARY

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), an environmental contaminant, disrupts multiple systems including endocrine, immun, nervous, reproductive, developmental, and cardiovascular. This study aimed to identify the molecular pathways and potential therapeutic targets for TCDD-induced cardiovascular toxicity using CTD, ShinyGO, STRING, GeneMANIA, ChEA3, MIENTURNET, and Cytoscape computational tools. The analysis identified the AGE-RAGE signaling pathway, blood circulation, and cytokine receptor binding as the top 3 among ten key molecular pathways, biological processes, and molecular functions associated with TCDD-induced cardiovascular toxicity. Additionally, ten hub proteins/genes were found to play a critical role, with NFKB1 being the most essential regulating transcription factor and hsa-miR-19a-3p and hsa-miR-125b-5p as the most crucial microRNAs. This study sheds light on the molecular mechanisms underlying TCDD-induced cardiovascular toxicity, revealing novel potential targets for therapeutic intervention.

Key Words: *Cardiovascular toxicity, hsa-miR-19a-3p, hsa-miR-125b-5p, NFKB1, TCDD.*

TCDD ile İndüklenen Kardiyovasküler Toksisitede Moleküler Yolakların ve Yeni Hedeflerin Ağ Toksikolojisi Analizi

ÖZ

2,3,7,8-Tetraklorodibenzo-p-dioksin (TCDD), çevresel bir kirleticidir ve endokrin, immün, sinir, üreme, gelişimsel ve kalp-damar sistemleri dahil olmak üzere birçok sistemi bozmaktadır. Bu çalışma, CTD, ShinyGO, STRING, GeneMANIA, ChEA3, MIENTURNET ve Cytoscape gibi hesaplamalı araçlar kullanarak TCDD kaynaklı kalp-damar toksisitesi için moleküler yolları ve potansiyel terapötik hedefleri belirlemeyi amaçlamaktadır. Analiz, TCDD kaynaklı kalp-damar toksisitesi ile ilişkili 10 önemli moleküler yol, biyolojik süreç ve moleküler fonksiyon arasında AGE-RAGE sinyal yolu, kan dolaşımı ve sitokin reseptör bağlanmasının en önemli 3'ü olduğunu belirlemiştir. Ek olarak, 10 hub protein/genin kritik bir rol oynadığı, NFKB1'in en önemli regüle edici transkripsiyon faktörü ve hsa-miR-19a-3p ile hsa-miR-125b-5p'nin en önemli mikroRNA'lar olduğu bulunmuştur. Bu çalışma, TCDD kaynaklı kalp-damar toksisitesinin altında yatan moleküler mekanizmaları aydınlatarak terapötik müdahale için yeni potansiyel hedefler ortaya koymaktadır.

Anahtar Kelimeler: *Kardiyovasküler toksisite, hsa-miR-19a-3p, hsa-miR-125b-5p, NFKB1, TCDD.*

Received: 22.04.2024
Revised: 18.07.2024
Accepted: 19.07.2024

* ORCID: 0000-0002-5260-3650, Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Van Yuzuncu Yil University, Van/Türkiye

° Corresponding Author; Fuat Karakuş
E-posta: fuatkarakus@yyu.edu.tr

INTRODUCTION

Dioxins are a class of persistent organic pollutants, wherein 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) stands out as a well-known representative (Figure 1A). TCDD is unintentionally produced during various processes, including the combustion of organic materials and as a side product in organic synthesis (Ogino et al., 2016). This highly stable compound exhibits a long half-life in humans (5–10 years) and persists in the environment at varying concentrations (0.01-1.31 ng/g) (Sorg et al., 2009; Colombo et al., 2011; Sforzini et al., 2014). While human exposure to TCDD primarily occurs through contaminated food or occupational environments, the industrial accident that occurred in Seveso, Italy, in 1976 also caused years of exposure. With the international legal measures taken in accordance with the Stockholm Convention, the level of TCDD in the environment, and therefore human exposure, has gradually decreased, especially in developed countries. TCDD is one of the most toxic chemicals. While short-term high-levels exposure can cause liver damage and chloracne, long-term exposure is linked to a wide range of health problems affecting the endocrine, immune, nervous, reproductive, and cardiovascular systems (Gogal and Holladay, 2008; Humblet et al., 2008; Sorg et al., 2009; Marinković et al., 2010; Pelcl et al., 2018; Gaspari et al., 2021).

TCDD-induced cardiovascular toxicity has been observed in various animals, such as fish, birds, and rodents. While animal studies have identified the aryl hydrocarbon receptor pathway as a critical contributor, this pathway alone does not fully explain the observed effects in humans (Kopf and Walker, 2009; Mohsenzadeh et al., 2018; Mi et al., 2023). This knowledge gap hinders the development of effective preventive and therapeutic strategies for TCDD-related cardiovascular diseases in humans. Therefore, this study aims to explore the molecular pathways, biomarkers, and potential therapeutic targets associated with TCDD-induced cardiovascular toxicity in humans using computational tools.

MATERIAL AND METHOD

Identification of common genes between TCDD and cardiovascular diseases

The identification of genes linked to TCDD and their associations with cardiovascular diseases (CVDs) was accomplished using the Comparative Toxicogenomics Database (CTD; https://ctdbase.org) and its tools (Davis et al., 2023). CTD curates and connects a wide range of data on chemical exposures and their biological effects across various species. This process involves manually curating and connecting data on chemicals, genes, phenotypes, anatomies, diseases, taxa, and exposures from published literature. CTD currently provides 45 million toxicogenomic relationships for over 16,300 chemicals, 51,300 genes, 5,500 phenotypes, 7,200 diseases, and 163,000 exposure events from 600 comparative species (Davis et al., 2023). However, the limitations of the CTD rely on the manual curation of scientific literature, which can lead to incomplete coverage. Not all relevant studies might be included, and newly published data may not be immediately available.

To analyze genes related to TCDD, the term "TCDD" was entered in the "Chemicals" section of CTD, and all resulting genes were downloaded. The genes associated with CVDs were acquired from the "Direct Evidence" section of CTD, where "M" indicates "marker/mechanism" and "T" represents "therapeutic." To identify common genes related to both TCDD and CVDs, the MyVenn CTD tool (Davis et al., 2023) was utilized. It can process up to three input lists and display the results as a Venn diagram. All findings in this study are based on data collected in March 2024.

GO bioprocess analysis and KEGG enrichment analysis

Gene Ontology (GO) terms were analyzed for annotated genes associated with TCDD and CVDs using ShinyGO 0.80 (Ge et al., 2020). ShinyGO is a gene list enrichment analysis tool accessible at (Ge et al., 2020). It is based on a vast annotation database

that includes 1,678 bacterial species, 59 plant species, 256 animal species, and 115 archaeal species from Ensembl and STRING-db. Among its attributes are the graphical representation of enrichment outcomes and gene attributes. Additionally, it offers program interface access to KEGG (Kanehisa et al., 2023) and STRING (Szklarczyk et al., 2015) for retrieving pathway diagrams and protein-protein interaction networks, respectively. However, ShinyGO has some limitations; for instance, it does not offer dynamic or real-time updates to reflect changes in GO annotations or underlying databases, and the enrichment analysis may be influenced by bias in GO annotations, such as overrepresentation of well-studied genes or biological processes.

For the analysis, the common gene list was input into ShinyGO, with *Homo sapiens* selected as the target species. For gene ontology analysis, the top 10 molecular pathways (MP), biological processes (BP), and molecular functions (MF) were determined. The significance of the results was established by applying a false discovery rate (FDR) correction and adhering to a recommended p-value cut-off of 0.05.

Protein-protein interaction and centrality analysis

For protein-protein interactions (PPIs) of the common genes between TCDD and CVDs, STRING v.12.0 (https://string-db.org/cgi) was used. The STRING database systematically gathers and consolidates information on protein-protein interactions, including direct physical associations and functional relationships. This data is sourced from various channels, including automated text mining of scientific literature, computational predictions based on coexpression and conserved genomic context, databases housing interaction experiments, and established complexes/pathways curated from reliable sources (Szklarczyk et al., 2015). However, it's important to note some limitations of STRING. Firstly, the accuracy of predicted interactions can vary, and false positives may be present due to the inherent complexity of protein interactions and limitations in computational algorithms. Secondly, STRING's reliance on existing annotations and curated data may not capture newly discovered or less well-studied protein interactions, leading to potential gaps in coverage.

For the analysis, the protein set was entered into the "Multiple Proteins by Names/Identifiers" section, with *Homo sapiens* designated as the chosen species. A minimum interaction score of 0.4 was established. The final PPI network was constructed using Cytoscape version 3.10.1 (http://www.cytoscape.org/). Cytoscape is an open-source software for interactive analysis, integration, and visualization of network data. It provides a versatile platform for researchers to explore biological networks, including protein-protein interactions, gene regulatory networks, and metabolic pathways. Additionally, Cytoscape supports the integration of additional plugins, expanding its functionality for specific analysis tasks such as functional enrichment, network clustering, and pathway analysis. However, Cytoscape also has some limitations. Firstly, while it offers extensive flexibility, the complexity of the software may present a steep learning curve for users unfamiliar with network analysis tools. Secondly, Cytoscape's performance may degrade when handling large-scale networks with thousands of nodes and edges, requiring substantial computational resources.

Additionally, centrality analysis was evaluated using the Network Analyzer Cytoscape plugin and the cytoHubba plugin (https://apps.cytoscape.org/apps/cytohubba) to identify core proteins TCDD-induced CVDs. This evaluation was based on six topological algorithms: degree, closeness, radiality, edge percolated component (EPC), maximum neighborhood component (MNC), and stress.

Gene network analysis

The common genes between TCDD and CVDs

were inputted into GeneMANIA (http://genemania.org) to study the network of gene-gene interactions (Warde-Farley et al., 2010). GeneMANIA identifies other genes associated with a group of input genes by utilizing an extensive array of functional connection data, encompassing protein and genetic interactions, pathways, coexpression, co-localization, and protein domain similarity. Currently, it maps 166,691 genes from nine organisms (Warde-Farley et al., 2010). However, GeneMANIA also has some limitations. Firstly, while it provides valuable insights into functional relationships among genes, the accuracy of predictions may vary depending on the quality and completeness of input data. Secondly, the reliance on pre-existing databases and computational algorithms means that GeneMANIA's results may not capture emerging or context-specific gene associations, potentially leading to incomplete or biased interpretations.

In this study, *Homo sapiens* was chosen as the target organism for analysis, and an automatically selected weighting method was employed.

Analysis of transcription factors and microRNAs

The common genes between TCDD and CVDs were input into ChIP-X Enrichment Analysis Version 3 (ChEA3) (https://maayanlab.cloud/chea3) to identify the transcription factors (TFs) responsible for their regulation. ChEA3 is an online tool for TF enrichment analysis, which evaluates and prioritizes TFs linked to gene sets submitted by users (Keenan et al., 2019). The ChEA3 background repository encompasses various gene set libraries compiled from diverse origins, such as TF-gene coexpression data derived from RNA-seq investigations, TF-target associations identified through ChIP-seq experiments, and TF-gene cooccurrence patterns computed from gene lists submitted by users (Keenan et al., 2019). However, ChEA3 also has a limitation. The accuracy of TF enrichment analysis heavily depends on the quality and coverage of underlying datasets, which may vary across different experimental sources and conditions.

Next, the common genes were also subjected to the MIcroRNA Enrichment TURned NETwork (MIENTURNET) tool (http://userver.bio.uniroma1.it/apps/mienturnet/), and *Homo sapiens* was selected as the target to determine potential miRNA networks from miRTarBase that were experimentally confirmed. MIENTURNET uses computationally predicted or experimentally validated miRNA-target interactions from several organisms, including *Homo sapiens, Mus musculus, Rattus norvegicus, Caenorhabditis elegans, Drosophila melanogaster,* and *Danio rerio* (Licursi et al., 2019). MIENTURNET integrates multiple sources of miRNA-target interaction data, including databases curated from literature mining, computational predictions based on sequence complementarity, and experimental validation assays. Additionally, it offers advanced analysis features such as network visualization, pathway enrichment analysis, and prioritization of crucial regulatory hubs. The limitation of MIENTURNET is that the accuracy of predicted miRNA-target interactions may vary depending on the underlying computational algorithms and experimental validation methods. False positives and negatives in the predicted interactions could lead to misinterpretation of regulatory networks.

In the analysis, the threshold for the minimum number of miRNA-target interactions was set at 2, and the adjusted p-value (FDR) was 0.5.

Chemical-gene binary interaction analysis

To establish correlations between protein/genes linked to CVDs and genes associated with TCDD, a manual analysis was conducted using CTD (https://ctdbase.org). This involved scrutinizing the "gene interaction" card in the CTD chemical profile, specifically identifying interactions between proteins/genes and TCDD regarding protein activity, mRNA expression, and protein expression. The resulting table enumerates the interactions between TCDD and selected genes, excluding interactions involving a combination of two or more chemicals and their collective impact on the genes.

RESULTS AND DISCUSSION

TCDD, a persistent organic pollutant, causes various adverse effects, including cardiovascular toxicity. While animal studies suggest mechanisms involving AhR activation, the underlying causes of TCDD-induced CVD in humans remain unclear. This study aimed to elucidate potential molecular mechanisms and targets associated with this toxicity using toxicogenomic analysis and other bioinformatics tools.

Common genes associated with TCDD and CVDs

A search of the CTD database showed that TCDD targets 17,055 genes. Additionally, the database identified 3,782,920 genes associated with CVDs, of which 1,674 were marked as "markers/mechanisms" and/or "therapeutics" in the "Direct Evidence" section. Furthermore, 1,438 genes were common between TCDD and CVDs (Figure 1). All common genes are presented in Table S1.

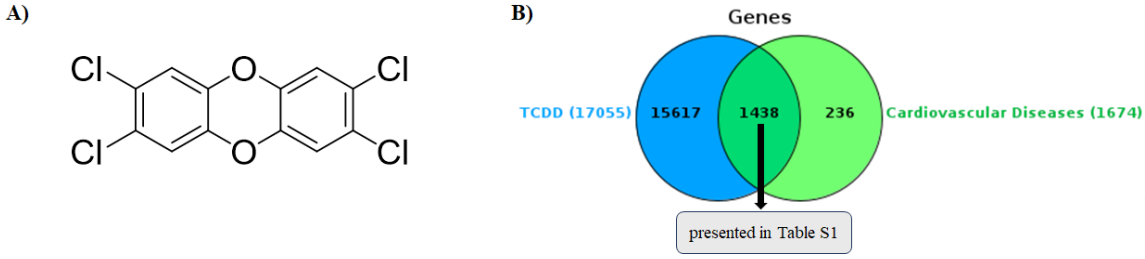

Figure 1. A) Molecular structure of TCDD.

B) Venn diagram illustrating the 1,438 genes common between TCDD and CVDs.

GO enrichment analyses of common genes

GO analysis identified the top 10 critical molecular pathways, biological processes, and molecular functions involved in the development of CVDs induced by TCDD (Figure 2). The results highlight the AGE-RAGE signaling pathway as a primary molecular pathway related to the 1,438 common genes (Figure 2A). Advanced Glycation End Products (AGEs) are harmful molecules formed naturally during metabolism and aging. These arise when sugars bond with proteins or fats in a non-enzymatic process. Significantly, AGEs bind to receptors for AGEs (RAGE), triggering a cascade of cellular events contributing to CVD development. Chronic inflammation, oxidative stress, and endothelial dysfunction are all hallmarks of CVD, and research shows the AGE-RAGE pathway plays a crucial role in their activation (Lee et al., 2019). Clinical studies support this connection. Sabbatinelli et al. (2022) found that higher levels of circulating AGEs and RAGEs were linked to increased mortality and major cardiovascular complications in patients with type 2 diabetes. Similarly, Singh et al. (2022) suggested that AGEs are not only associated with arterial stiffness and atherosclerosis but also with impaired cellular signaling in the endothelium, vascular smooth muscle cells, and platelets. These detrimental effects on blood vessel function and blood cell activity further solidify AGEs as crucial risk factors for CVD. Identifying of the AGE-RAGE signaling pathway as the most crucial molecular pathway associated with common genes suggests that TCDD may exert its cardiovascular effects, at least in part, through modulation of this pathway.

Among biological processes, blood circulation (Figure 2B) was identified as the most significant contributor to TCDD-induced CVDs, while cytokine receptor binding was found to be the most critical molecular function (Figure 2C). Efficient blood circulation is crucial for maintaining cardiovascular health. Disruptions in blood flow, often due to atherosclerotic plaque formation, lead to ischemia and infarction. The

association of blood circulation as the critical biological process with the common genes implies that these genes may play a crucial role in mediating the effects of TCDD on vascular health. TCDD exposure has been shown to disrupt normal vascular function and impair blood circulation, contributing to the development of CVDs such as atherosclerosis and hypertension (Kopf et al., 2010; Walsh-Wilcox et al., 2019; Bock, 2019).

The identification of cytokine receptor binding as the primary molecular function associated with the common genes suggests that TCDD may influence cardiovascular health by modulating cytokine signaling pathways and cytokines play a central role in mediating inflammatory responses. Dysregulated cytokine receptor binding could lead to aberrant immune responses and chronic inflammation, contributing to the pathogenesis of TCDD-induced CVDs (Bock, 2019).

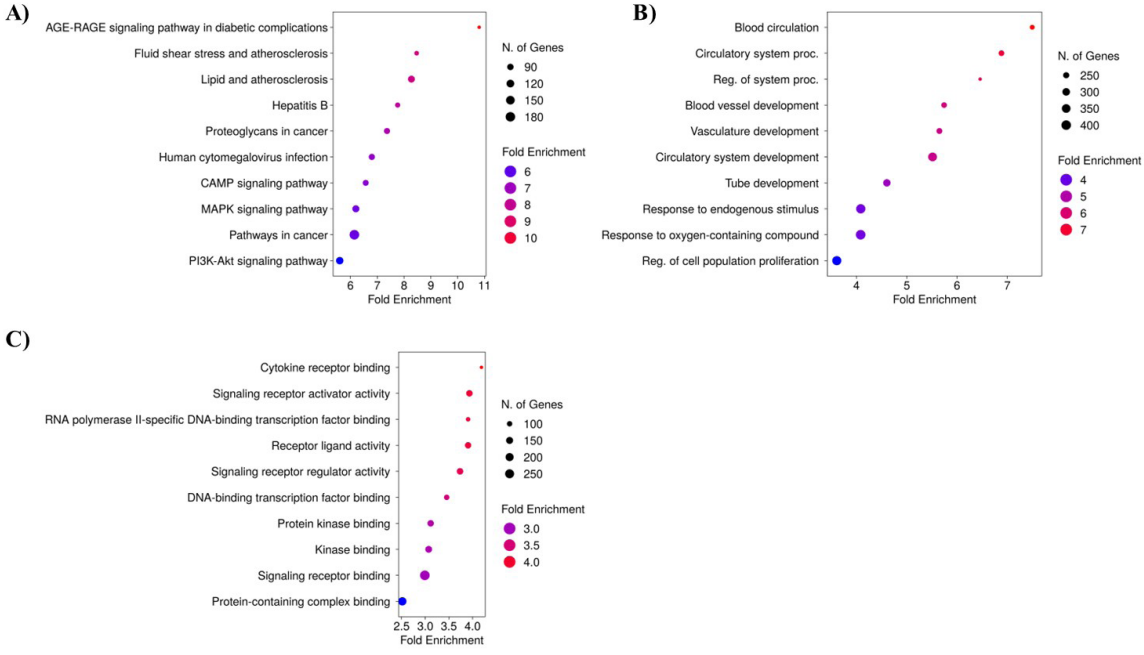

Figure 2. GO enrichment analysis of 1,438 common genes: **A)** Top 10 KEGG pathways of the common genes. **B)** Top 10 biological processes associated with the common genes. **C)** Top 10 molecular functions associated with the common genes.

PPI interactions, hub proteins/genes involved in TCDD-induced CVDs, and gene-gene interactions

The PPI network revealed 1,371 nodes and 43,318 edges, as illustrated in Figure 3A, with a PPI enrichment p-value of $<1.0x10^{-16}$. Centrality analysis was also performed to determine the core proteins. As a result of the analysis, top 10 hub proteins/genes of 1,438 common proteins/genes in the list was selected based on their six topological algorithms (Figure 3B, Table 1). This hub proteins/genes alphabetically: Actin, cytoplasmic 1 (ACTB), RAC-alpha serine/threonine-protein kinase (AKT1), Albumin (ALB), Epidermal growth factor receptor (EGFR), Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), Interleukin-1 beta (IL1B), Interleukin-6 (IL6), Insulin (INS), Tumor necrosis factor (TNF), and Cellular tumor antigen p53 (TP53).

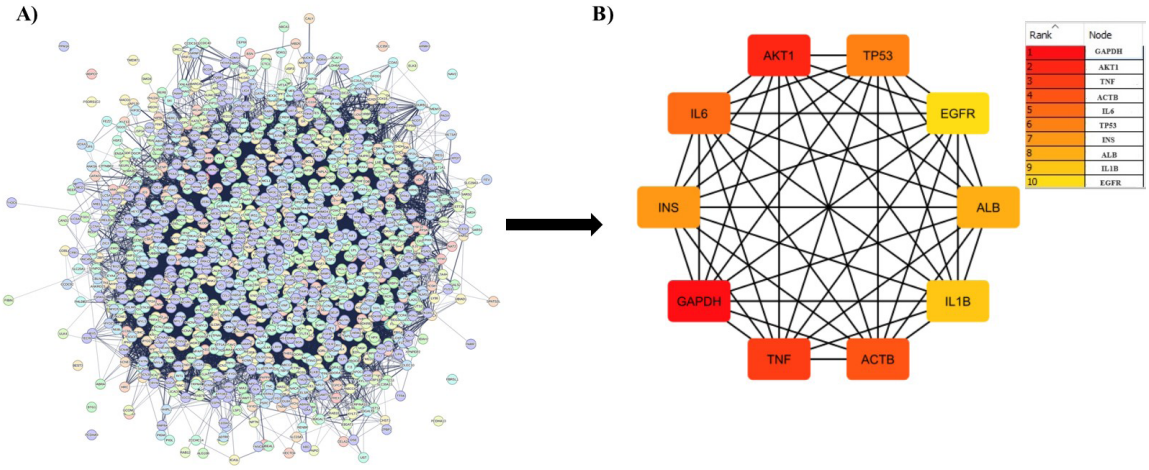

Figure 3. A) PPI network from STRING analysis of 1,438 proteins/genes common between TCDD exposure and CVDs. **B)** Centrality analysis of the common proteins/genes by Cytoscape. The darker-colored nodes score higher in most of the six topology algorithms (Table 1).

Table 1. Centrality analysis of 1438 common proteins revealed the ten most essential proteins.

Hub proteins	Degree score	Closeness Score	Radiality Score	EPC Score	MNC Score	Stress Score
GAPDH	1	1	1	2	1	1
AKT1	2	2	2	3	2	2
TNF	3	3	3	1	3	5
ACTB	4	4	4	8	4	4
IL6	5	5	5	4	5	9
TP53	6	6	6	9	6	3
INS	7	7	7	7	7	6
ALB	8	8	8	6	8	7
IL1B	9	9	10	5	9	10
EGFR	10	10	9	10	10	8

The GeneMANIA online plug-in created a linked network among hub genes. The results showed that most genes associated with CVDs were involved in coexpression (54.37%) and physical interactions (25.82%). Other types of interactions were less pronounced: genetic interactions (9.94%), predicted interactions (7.06%), colocalization (2.48%), and pathways (0.33%) (Figure 4). Coexpression, the dominant interaction among hub genes, suggests similar expression levels across conditions in a gene expression study. Namely, when one gene is upregulated or downregulated, the others coexpressed with it also show a similar expression pattern. Co-expressed genes may be involved in the same biological processes, pathways, or cellular functions.

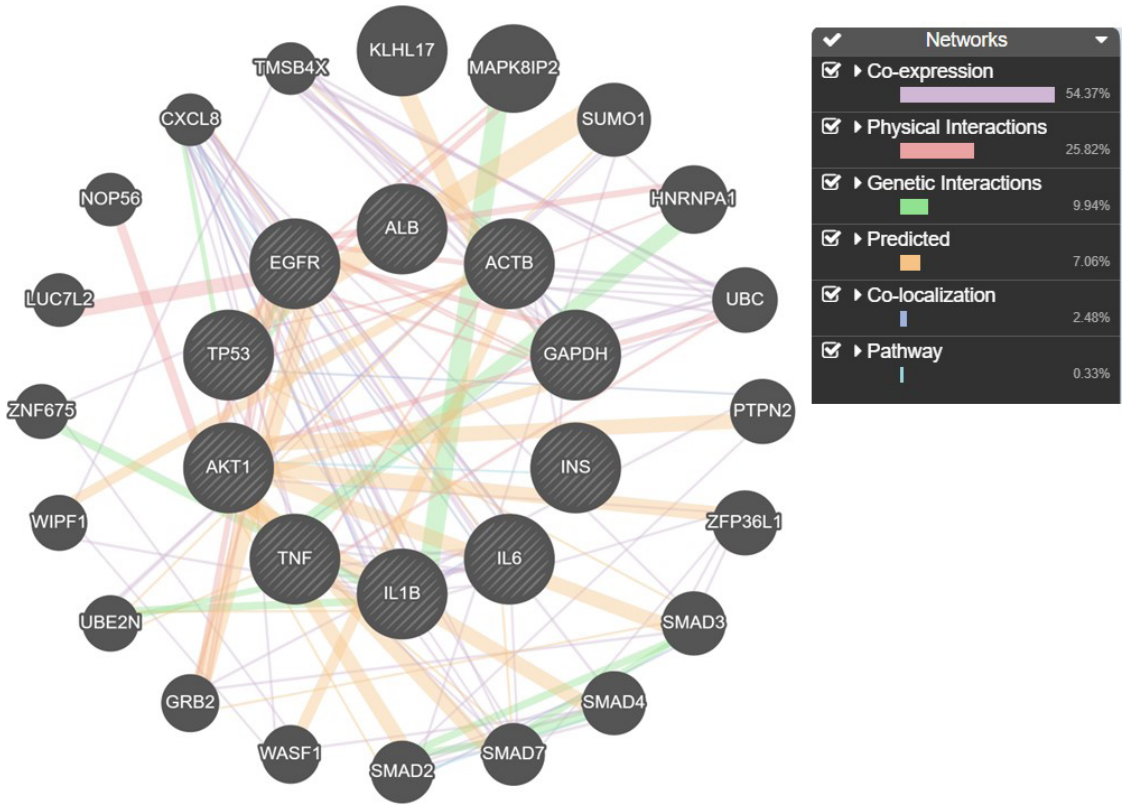

Figure 4. Gene network from GeneMANIA analysis of 10 hub genes involved in TCDD-induced CVDs.

Core transcription factors and miRNAs involved in TCDD-induced CVDs

After analyzing the hub genes for TFs in ChEA3, the top 10 TFs were identified. Subsequently, nodes and edges representing the relationships between TFs and hub genes were manually prepared in Excel and schematized in Cytoscape 3.10.1, as shown in Figure 5A. The results are sorted by mean rank (Table S2). MeanRank refers to the mean rank of each TF across all libraries containing that TF, serving as the score by which a composite list of TFs is reranked.

In Figure 4A, green nodes represent TFs, whereas light brown nodes represent hub genes. ChEA3 revealed that the most significant TF regulating these hub genes, with eight connections and a mean rank of 13.0, was Nuclear Factor Kappa B Subunit 1 (NFKB1) (Table S2). These results are supported by previous studies. For instance, Luo et al. (2022) identified the NFKB1 polymorphism as associated with susceptibility to coronary heart disease in populations of different genetic backgrounds. In a meta-analysis, authors suggested that a polymorphism in the NFKB1 promoter region was associated with susceptibility to coronary artery disease in both Asian and Caucasian populations (Chen et al., 2016).

As a result of miRNA-target analysis, the top ten miRNAs are shown in Figure 4B, while the corresponding p-values, FDR, odds ratios, and target genes are presented in Table S3. Among these 10 miRNAs, hsa-miR-19a-3p and hsa-miR-125b-5p emerged as the most critical miRNAs regulating these hub genes in TCDD-induced CVDs, which is consistent with the findings of prior studies. Bulent Vatan et al. (2016) reported that hsa-miR-19a-3p is downregulated in patients with mitral valve rupture. Jia et al. (2016) suggested that hsa-miR-125b-5p has diagnostic value for early diagnosis of acute myocardial infarction. Overall, these TFs and miRNAs may be used as predictors of TCDD-induced CVDs.

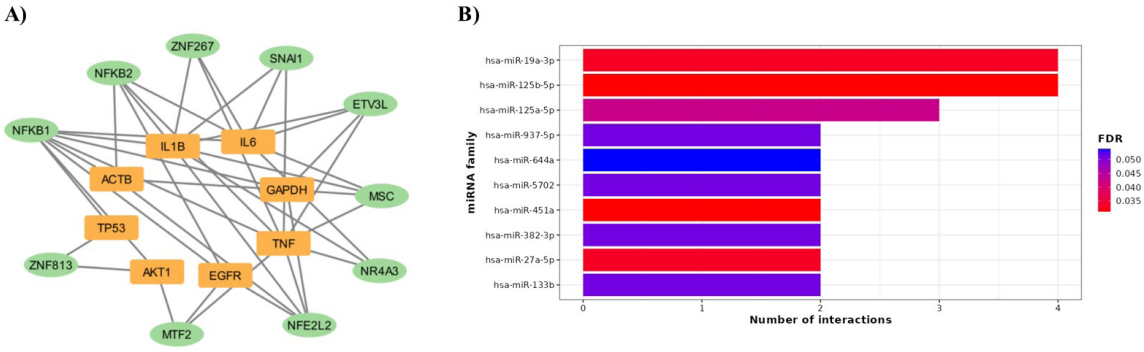

Figure 5. A) The top 10 transcription factors related to hub genes are highlighted, with green nodes indicating TFs and light brown color indicating the hub genes. **B)** miRNA-target analysis showed that the top 10 miRNAs related to the hub genes were predicted by MIENTURNET.

TCDD-hub protein/gene binary analysis results

After identifying ten hub proteins/genes involved in TCDD-induced CVDs (Figure 3B, Table 1), a manual search of the CTD database revealed the impact of TCDD on the mRNA and protein expression as well as the protein activity of these proteins/genes, except for INS. Additionally, only studies where TCDD was applied alone were considered and focused on human plasma/serum or human cells.

TCDD was found to increase the protein activities of AKT1 and EGFR; mRNA expression of TNF, IL1B, and EGFR; and protein expression of ACTB, ALB, and EGFR. Interestingly, TCDD had a bidirectional effect on the mRNA expression of IL6 (Table 2). However, the studies summarized in Table 2 are not direct cardiovascular system studies, so they offer a prediction that TCDD could similarly affect these hub proteins/genes in the cardiovascular system.

Table 2. TCDD – hub proteins/genes interactions.

Hub genes	**TCDD** (2,3,7,8-Tetrachlorodibenzo-*p*-dioxin)		
	Protein activity	mRNA expression	Protein expression
GAPDH		↓	
AKT1	↑		
TNF		↑	
ACTB			↑
IL6		↑↓	
TP53		↓	↓
INS			
ALB		↓	↑
IL1B		↑	↑
EGFR	↑	↑	↑

↑—increase; ↓—decrease; ↑↓—both increase and decrease

CONCLUSION

This study elucidates how TCDD may contribute to the development of CVDs by modulating ten hub proteins/genes, as presented in Table 2. Additionally, TFs (especially NFKB1) and miRNAs (especially hsa-miR-19a-3p and hsa-miR-125b-5p) were predicted as potential mediators of TCDD-induced CVDs. Furthermore, these proteins/genes, TFs, and miRNAs may be potential biomarkers or therapeutic targets for TCDD-induced CVDs. Importantly, these molecules hold promise as not only biomarkers for disease diagnosis and prognosis but also as therapeutic targets for mitigating TCDD-induced cardiovascular toxicity. By targeting these key molecules, it may be possible to modulate the pathological processes underlying TCDD-induced CVDs and alleviate their adverse effects. Future research should focus on validating the therapeutic potential of these targets and developing innovative intervention strategies to combat TCDD-induced cardiovascular toxicity effectively.

AUTHOR CONTRIBUTION STATEMENT

Concept, Design, Data Collection and/or Processing, Analysis and/or Interpretation, Writing manuscript (FK)

CONFLICT OF INTEREST

The author declares no conflicts of interest.

REFERENCES

Bock, K. W. (2019). Human AHR functions in vascular tissue: Pro- and anti-inflammatory responses of AHR agonists in atherosclerosis. *Biochemical pharmacology, 159,* 116–120. https://doi.org/10.1016/j.bcp.2018.11.021

Bulent Vatan, M., Kalaycı Yigin, A., Akdemir, R., Tarik Agac, M., Akif Cakar, M., Aksoy, M., Tatli, E., Kilic, H., Gunduz, H., Guzel, D., & Karacan, K. (2016). Altered Plasma MicroRNA Expression in Patients with Mitral Chordae Tendineae Rupture. *The Journal of heart valve disease, 25*(5), 580–588.

Chen, Q. J., Lai, H. M., Zhao, L., Ma, Y. T., Li, X. M., Zhai, H., Zhou, Y., He, C. H., Chen, B. D., Liu, F., & Yang, Y. N. (2016). Association Between the NFKB1-94ins/del ATTG Polymorphism (rs28362491) and Coronary Artery Disease: A Systematic Review and Meta-Analysis. *Genetic testing and molecular biomarkers, 20*(3), 105–111. https://doi.org/10.1089/gtmb.2015.0242

Colombo, A., Benfenati, E., Bugatti, S. G., Celeste, G., Lodi, M., Rotella, G., Senese, V., & Fanelli, R. (2011). Concentrations of PCDD/PCDF in soil close to a secondary aluminum smelter. *Chemosphere, 85*(11), 1719–1724. https://doi.org/10.1016/j.chemosphere.2011.09.018

Davis, A. P., Wiegers, T. C., Johnson, R. J., Sciaky, D., Wiegers, J., & Mattingly, C. J. (2023). Comparative Toxicogenomics Database (CTD): update 2023. *Nucleic acids research, 51*(D1), D1257–D1262. https://doi.org/10.1093/nar/gkac833

Gaspari, L., Paris, F., Kalfa, N., Soyer-Gobillard, M. O., Sultan, C., & Hamamah, S. (2021). Experimental Evidence of 2,3,7,8-Tetrachlordibenzo-p-Dioxin (TCDD) Transgenerational Effects on Reproductive Health. *International journal of molecular sciences, 22*(16), 9091. https://doi.org/10.3390/ijms22169091

Ge, S. X., Jung, D., & Yao, R. (2020). ShinyGO: a graphical gene-set enrichment tool for animals and plants. *Bioinformatics* (Oxford, England), *36*(8), 2628–2629. https://doi.org/10.1093/bioinformatics/btz931

Gogal, R. M., Jr, & Holladay, S. D. (2008). Perinatal TCDD exposure and the adult onset of autoimmune disease. *Journal of immunotoxicology, 5*(4), 413–418. https://doi.org/10.1080/10408360802483201

Humblet, O., Birnbaum, L., Rimm, E., Mittleman, M. A., & Hauser, R. (2008). Dioxins and cardiovascular disease mortality. *Environmental health perspectives, 116*(11), 1443–1448. https://doi.org/10.1289/ehp.11579

Jia, K., Shi, P., Han, X., Chen, T., Tang, H., & Wang, J. (2016). Diagnostic value of miR-30d-5p and miR-125b-5p in acute myocardial infarction. *Molecular medicine reports, 14*(1), 184–194. https://doi.org/10.3892/mmr.2016.5246

Kanehisa, M., Furumichi, M., Sato, Y., Kawashima, M., & Ishiguro-Watanabe, M. (2023). KEGG for taxonomy-based analysis of pathways and genomes. *Nucleic acids research, 51*(D1), D587–D592. https://doi.org/10.1093/nar/gkac963

Keenan, A. B., Torre, D., Lachmann, A., Leong, A. K., Wojciechowicz, M. L., Utti, V., Jagodnik, K. M., Kropiwnicki, E., Wang, Z., & Ma'ayan, A. (2019). ChEA3: transcription factor enrichment analysis by orthogonal omics integration. *Nucleic acids research, 47*(W1), W212–W224. https://doi.org/10.1093/nar/gkz446

Kopf, P. G., & Walker, M. K. (2009). Overview of developmental heart defects by dioxins, PCBs, and pesticides. Journal of environmental science and health. Part C, *Environmental carcinogenesis & ecotoxicology reviews, 27*(4), 276–285. https://doi.org/10.1080/10590500903310195

Kopf, P. G., Scott, J. A., Agbor, L. N., Boberg, J. R., Elased, K. M., Huwe, J. K., & Walker, M. K. (2010). Cytochrome P4501A1 is required for vascular dysfunction and hypertension induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Toxicological sciences*: *an official journal of the Society of Toxicology, 117*(2), 537–546. https://doi.org/10.1093/toxsci/kfq218

Lee, T. W., Kao, Y. H., Chen, Y. J., Chao, T. F., & Lee, T. I. (2019). Therapeutic potential of vitamin D in AGE/RAGE-related cardiovascular diseases. *Cellular and molecular life sciences: CMLS, 76*(20), 4103–4115. https://doi.org/10.1007/s00018-019-03204-3

Licursi, V., Conte, F., Fiscon, G., & Paci, P. (2019). MIENTURNET: an interactive web tool for microRNA-target enrichment and network-based analysis. *BMC bioinformatics, 20*(1), 545. https://doi.org/10.1186/s12859-019-3105-x

Luo, J. Y., Liu, F., Zhang, T., Tian, T., Luo, F., Li, X. M., & Yang, Y. N. (2022). Association of NFKB1 gene rs28362491 mutation with the occurrence of major adverse cardiovascular events. *BMC cardiovascular disorders, 22*(1), 313. https://doi.org/10.1186/s12872-022-02755-x

Marinković, N., Pašalić, D., Ferenčak, G., Gršković, B., & Stavljenić Rukavina, A. (2010). Dioxins and human toxicity. *Arhiv za higijenu rada i toksikologiju, 61*(4), 445–453. https://doi.org/10.2478/10004-1254-61-2010-2024

Mi, P., Li, N., Ai, K., Li, L., & Yuan, D. (2023). AhR-mediated lipid peroxidation contributes to TCDD-induced cardiac defects in zebrafish. *Chemosphere, 317,* 137942. https://doi.org/10.1016/j.chemosphere.2023.137942

Mohsenzadeh, M. S., Zanjani, B. R., & Karimi, G. (2018). Mechanisms of 2,3,7,8-tetrachlorodibenzo-p-dioxin- induced cardiovascular toxicity: An overview. *Chemico-biological interactions, 282,* 1–6. https://doi.org/10.1016/j.cbi.2018.01.002

Ogino, Y., Miyagawa, S., & Iguchi, T. (2016). Subchapter 101G - 2,3,7,8-Tetrachlorodibenzo-p-dioxin/Polychlorinated Biphenyls. In Y. Takei, H. Ando, & K. Tsutsui (Eds.), *Handbook of Hormones. Comparative Endocrinology for Basic and Clinical Research*. (pp. 583-584). San Diego, CA: Academic Press.

Pelcl, T., Skrha, J., Jr, Prazny, M., Vlckova, S., Fenclova, Z., Navratil, T., Malik, J., Diblik, P., Zikan, V., & Pelclova, D. (2018). Diabetes, Cardiovascular Disorders and 2,3,7,8-Tetrachlorodibenzo-p-Dioxin Body Burden in Czech Patients 50 Years After the Intoxication. *Basic & clinical pharmacology & toxicology, 123*(3), 356–359. https://doi.org/10.1111/bcpt.13013

Sabbatinelli, J., Castiglione, S., Macrì, F., Giuliani, A., Ramini, D., Vinci, M. C., Tortato, E., Bonfigli, A. R., Olivieri, F., & Raucci, A. (2022). Circulating levels of AGEs and soluble RAGE isoforms are associated with all-cause mortality and development of cardiovascular complications in type 2 diabetes: a retrospective cohort study. *Cardiovascular diabetology, 21*(1), 95. https://doi.org/10.1186/s12933-022-01535-3

Sforzini, S., Moore, M. N., Boeri, M., Benfenati, E., Colombo, A., & Viarengo, A. (2014). Immunofluorescence detection and localization of B[a]P and TCDD in earthworm tissues. *Chemosphere, 107,* 282–289. https://doi.org/10.1016/j.chemosphere.2013.12.062

Shannon, P., Markiel, A., Ozier, O., Baliga, N. S., Wang, J. T., Ramage, D., Amin, N., Schwikowski, B., & Ideker, T. (2003). Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome research, 13*(11), 2498–2504. https://doi.org/10.1101/gr.1239303

Singh, S., Siva, B. V., & Ravichandiran, V. (2022). Advanced Glycation End Products: key player of the pathogenesis of atherosclerosis. *Glycoconjugate journal, 39*(4), 547–563. https://doi.org/10.1007/s10719-022-10063-x

Sorg, O., Zennegg, M., Schmid, P., Fedosyuk, R., Valikhnovskyi, R., Gaide, O., Kniazevych, V., & Saurat, J. H. (2009). 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) poisoning in Victor Yushchenko: identification and measurement of TCDD metabolites. *Lancet (London, England), 374*(9696), 1179–1185. https://doi.org/10.1016/S0140-6736(09)60912-0

Szklarczyk, D., Franceschini, A., Wyder, S., Forslund, K., Heller, D., Huerta-Cepas, J., Simonovic, M., Roth, A., Santos, A., Tsafou, K. P., Kuhn, M., Bork, P., Jensen, L. J., & von Mering, C. (2015). STRING v10: protein-protein interaction networks, integrated over the tree of life. *Nucleic acids research,43*(Database issue), D447–D452. https://doi.org/10.1093/nar/gku1003

Walsh-Wilcox, M. T., Kaye, J., Rubinstein, E., & Walker, M. K. (2019). 2,3,7,8-Tetrachlorodibenzo-p-dioxin Induces Vascular Dysfunction That is Dependent on Perivascular Adipose and Cytochrome P4501A1 Expression. *Cardiovascular toxicology, 19*(6), 565–574. https://doi.org/10.1007/s12012-019-09529-6

Warde-Farley, D., Donaldson, S. L., Comes, O., Zuberi, K., Badrawi, R., Chao, P., Franz, M., Grouios, C., Kazi, F., Lopes, C. T., Maitland, A., Mostafavi, S., Montojo, J., Shao, Q., Wright, G., Bader, G. D., & Morris, Q. (2010). The GeneMANIA prediction server: biological network integration for gene prioritization and predicting gene function. *Nucleic acids research*, *38*(Web Server issue), W214–W220. https://doi.org/10.1093/nar/gkq537