RESEARCH ARTICLE / ARAŞTIRMA MAKALESİ
Journal of Advanced Research in Health Sciences /
Sağlık Bilimlerinde İleri Araştırmalar Dergisi 2024
DOI: 10.26650/JARHS2024-1397075

LINKING METAL EXPOSURE TO ISCHAEMIC HEART DISEASE: A BIOINFORMATIC ANALYSIS

METAL MARUZİYETİNİN İSKEMİ KALP HASTALIĞIYLA BAĞLANTISI: BİYOİNFORMATİK ANALİZ

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Citation/Atıf: Karakuş F, Kuzu B. Linking metal exposure to ischaemic heart disease: A bioinformatic analysis. Journal of Advanced Research in Health Sciences 2024;7(3):201-208. https://doi.org/10.26650/JARHS2024-1397075

ABSTRACT

Objective: Cardiovascular disease causing the most deaths worldwide is ischaemic heart disease. In addition to modifiable and non-modifiable risk factors, there is a growing consideration that environmental factors, especially heavy metals, may also contribute to the risk of ischaemic heart disease. The study identified the potential molecular mechanisms associated with ischaemic heart disease induced by arsenic, cadmium, lead, and mercury.

Materials and Methods: In this study, we used toxicogenomic data and various bioinformatic databases and tools, including the Comparative Toxicogenomic Database, ToppGene Suite, GeneMANIA, String, Cytoscape, CHEA3, and MIENTURNET.

Results: We observed an overlap of the CRP, HMOX1, PON1, and PTGS2 genes among the metals and ischaemic heart disease. The most prevalent interactions among these genes were identified as physical interactions, constituting 77.64% of the total. Several pathways were determined as the principal molecular mechanisms that might be influenced by the examined metals involved in the aetiology of ischaemic heart disease (e.g., regulation of plasma lipoprotein particle levels, response to inorganic substances, blood circulation, circulatory system processes, and cellular response to metal ions). CRP and HMOX1 emerged as key genes, whereas CREB3L3 and ATF5 were identified as key transcription factors related to ischaemic heart disease caused by the combination of the examined metals. Furthermore, we identified two miRNAs (hsa-miR-128-3p and hsa-miR-1273g-3p) associated with ischaemic heart disease.

Conclusion: These observations make a substantial contribution to our understanding of the processes underlying ischaemic heart disease induced by arsenic, cadmium, lead, and mercury.

Keywords: Ischaemic heart disease, arsenic, cadmium, lead, mercury, big data

ÖZ

Amaç: Dünya çapında en çok ölüme neden olan kardiyovasküler hastalık iskemik kalp hastalığıdır. Değiştirilebilir ve değiştirilemez risk faktörlerinin yanı sıra, özellikle ağır metaller olmak üzere çevresel faktörlerin de iskemik kalp hastalığı riskine katkıda bulunabileceği düşünülmektedir. Çalışmamızın amacı, arsenik, kadmiyum, kurşun ve cıva tarafından indüklenen iskemik kalp hastalığı ile ilişkilendirilebilecek potansiyel moleküler mekanizmaları belirlemektir.

Gereç ve Yöntem: Bu çalışmada toksikogenomik verileri ve Comparative Toxicogenomic Database, ToppGene Suite, GeneMANIA, STRING, Cytoscape, ChEA3 ve MIENTURNET gibi biyoinformatik veritabanlarını ve araçları kullandık.

Bulgular: Çalışılan metaller ve iskemik kalp hastalığı ile ilişkili genlerden CRP, HMOX1, PON1 ve PTGS2'nin örtüştüğünü gözlemledik. Bu örtüşen genler arasında en yaygın etkileşim fiziksel etkileşimdi (%77,64). Çalışılan metallerin etkileyebilecekleri temel moleküler mekanizmalar olarak çeşitli yolaklar tanımlandı ve bunlar, iskemik kalp hastalığının etiyolojisinde rol oynayan yolakları içeriyordu (örneğin, plazma lipoprotein parçacık seviyelerinin düzenlenmesi, inorganik maddelere yanıt, kan dolaşımı, dolaşım sistemi süreçleri ve metal iyonlarına hücresel yanıt). Bu metallerin indüklediği iskemik kalp hastalığı ile ilişkili olarak temel genler CRP ve HMOX1 iken, ATF5 ve CREB3L3 de temel transkripsiyon faktörleri olarak belirlendi. Ayrıca, iskemik kalp hastalığıyla ilişkili iki miRNA da (hsa-miR-128-3p ve hsa-miR-1273g-3p) belirledik.

Sonuç: Bu gözlemler arsenik, kadmiyum, kurşun ve cıvanın neden olduğu iskemik kalp hastalığının altında yatan süreçleri anlamamıza önemli bir katkı sağlamaktadır.

Anahtar Kelimeler: İskemik kalp hastalığı, arsenik, kadmiyum, kurşun, cıva, büyük veri

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Submitted/Başvuru: 28.11.2023 • **Revision Requested/Revizyon Talebi:** 03.03.2024 • **Last Revision Received/Son Revizyon:** 04.03.2024 • **Accepted/Kabul:** 19.04.2024 • **Published Online/Online Yayın:** 01.10.2024

INTRODUCTION

Ischaemic heart disease (IHD), alternatively referred to as coronary artery disease or coronary heart disease, refers to an insufficient blood supply to the myocardium due to blockage of the epicardial coronary arteries, typically caused by atherosclerosis. IHD accounts for 16% of global deaths, with a notable increase since 2000, reaching 8.9 million deaths in 2019 (1). Although factors such as age, gender, and family history are non-modifiable risks, there are also modifiable risks such as smoking, hypertension, increased cholesterol levels, physical inactivity, diabetes, obesity, an unhealthy diet, excessive alcohol consumption, and stress (2). Nevertheless, emerging evidence suggests that these factors alone may not entirely elucidate the complexities of IHD. Environmental factors, particularly heavy metal exposure, have been implicated as additional risk factors for IHD (3-5).

The widespread use of organic and inorganic chemicals in the past century has led to an escalation of environmental pollutants within the human body. Arsenic (As), cadmium (Cd), lead (Pb), and mercury (Hg) are extremely toxic metals linked to significant environmental contamination and health issues (6-9). Exposure to these heavy metals, either individually or in combination, is unavoidable because of their persistence in various environmental settings, such as industrial environments, food, water, air, and soil. The primary sources of human exposure include consuming contaminated water and foods, exposure to cigarette smoke, and inhaling polluted air in occupational or residential areas near industrial facilities. Recent studies have revealed that these metals may collectively induce IHD (10-14). Consequently, millions of people worldwide are exposed to elevated levels of these metals through various means.

The objectives of this study were to uncover the potential key genes, proteins, transcription factors, microRNAs, and molecular pathways that underlie heavy metal-linked IHD through the analysis of toxicogenomic data. These data were processed from databases that collect experimental and epidemiological data. Thus, inferences regarding the relationship between heavy metals and IHD were made using bioinformatics tools.

MATERIALS and METHODS

Identifying common genes for the metals and ischaemic heart disease

To investigate the potential relationship between the metals (As, Cd, Hg, and Pb) and IHD, we analysed data gathered from the Comparative Toxicogenomics Database (CTD; https://ctdbase.org) (15). The data were acquired from the "Direct Evidence" section of CTD, where "M" denotes "marker/mechanism" and "T" denotes "therapeutic." The data were downloaded on November 25, 2023, and the DiVenn tool was employed to identify common genes associated with both metals and IHD (16).

Determining the interplay between metals and overlapping genes in the context of IHD

To establish the correlation between genes linked to IHD and those connected to exposure to metals, we conducted a manual analysis using CTD (15). This involved scrutinising the "gene interaction" section in the CTD chemical profile and specifically identifying interactions between genes and metals from the array of interactions of protein activity, mRNA, and protein expression. The resulting table enumerates the interplay between the metals and the chosen genes, excluding interactions involving a combination of two or more chemicals and their collective impact on genes.

Enrichment analysis

Enrichment analysis for molecular functions and biological processes was conducted on annotated genes linked to IHD using the ToppGene Suite (17). This tool, available at https://toppgene.cchmc.org, serves as a platform for gene list enrichment analysis. ToppGene Suite offers ToppFun, accessible at https://toppgene.cchmc.org/enrichment.jsp, which facilitates the identification of functional enrichment within your gene list. It leverages diverse data sources, encompassing the transcriptome, proteome, regulate (TFBS and miRNA), ontologies (GO, Pathway), phenotype (human disease and mouse phenotype), pharmacome (Drug-Gene associations), literature co-citation, and additional features. The significance of the results can be determined by applying a false discovery rate (FDR) correction and adhering to a recommended p-value cut-off of 0.05, as suggested by the ToppGene Suite (17).

Exploring gene-gene and protein-protein interactions

GeneMANIA (http://genemania.org) was used to study the network of gene-gene interactions. The analysis focussed on *Homo sapiens* as the target organism (18).

The protein-protein interactions (PPI) associated with IHD induced by metals were investigated using String v.12 Database (https://string-db.org/cgi) by selecting a medium confidence threshold of 0.4 as the minimum required interaction score (19). Cytoscape version 3.10.1 (http://www.cytoscape.org/) was also utilised for the analysis (20). Additionally, to identify the core proteins contributing to the development of IHD caused by metals, the Network Analyser Cytoscape plugin was utilised to assess betweenness (BC), closeness (CC), and degree centralities (DC).

Analysis of transcription factors and microRNAs

We submitted overlapping genes linked to both the metals and IHD to ChIP-X Enrichment Analysis Version 3 (CHEA3) (https://maayanlab.cloud/chea3) to identify the transcription factors responsible for their regulation (21). Next, the genes were also subjected to the MIENTURNET tool (http://userver.bio.uniroma1.it/apps/mienturnet/) to determine potential miRNA networks from miRTarBase that were experimentally confirmed (22).

RESULTS

Overlapping genes between metals and IHD

The outcomes derived from the initial CTD data exploration showed that 38, 36, 10, 18, and 87 genes are related to As, Cd,

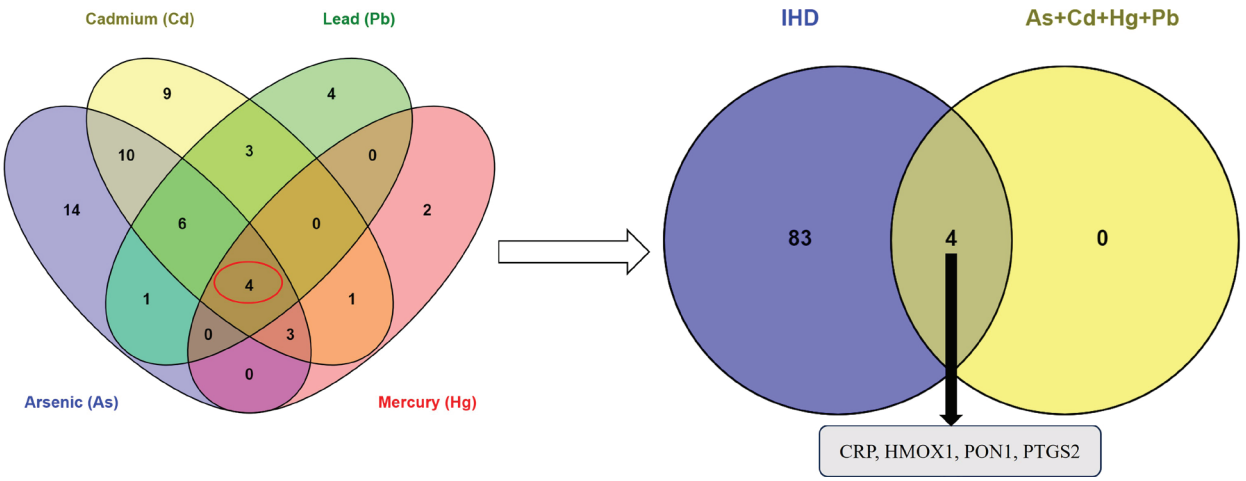

Figure 1: The Venn diagrams show the common genes between IHD and metals.

Hg, Pb, and IHD, respectively. Four genes (CRP, HMOX1, PON1, and PTGS2) were found to be common between the chemicals and IHD (Figure 1, Suppl. Table S1).

Overlapping gene alterations induced by metals

The results of the manually conducted gene interaction analysis are presented in Table 1, which provides detailed insights into the interactions between IHD and the studied chemicals. This includes information on the activity and expression of proteins and mRNA. Arsenic induced CRP and HMOX1 protein expression and increased HMOX1 mRNA expression. In contrast, it either increased or decreased PTGS2 mRNA expression. Cadmium increased both the protein and mRNA expression of HMOX1 and the protein activity of HMOX1. It decreased CRP mRNA expression. However, Cd increased PTGS2 protein expression while affecting mRNA expression in both an increase and a decrease. While lead and mercury decreased PON1 protein activity, they increased HMOX1 mRNA expression. Lead also increased CRP protein expression and PTGS2 mRNA and protein expression.

Molecular processes related to common genes between metals and IHD

The terms related to molecular function delineate actions occurring at the molecular level, typically aligning with activities achievable by individual gene products, such as proteins or RNA molecules. Gene ontology enrichment analysis showed that "lipoprotein particle binding", "protein-lipid complex binding", "protein homodimerization activity", "haem binding", and "tetrapyrrole binding" were among the molecular functions linked to genes induced by the metals and IHD (Table 2). The biological processes, or 'biological programmes' accomplished by multiple molecular activities. The five most significant biological processes involved in the aetiology of IHD induced by metals were "regulation of plasma lipoprotein particle levels", "response to inorganic substance", "blood circulation", "circulatory system process", and "cellular response to metal ion" (Table 3).

Analysis of core genes and proteins

The overlapping genes were input into the GeneMania online plug-in, generating a linked network. The analysis revealed that physical interactions (77.64%) were the predominant factors among the overlapping genes (Figure 2). In addition, the PPIs of four common genes (CRP, HMOX1, PON1, and PTGS2) showed four nodes and five edges, and the PPI enrichment p-value was $9.9e^{-05}$. The pivotal genes associated with both metals and IHD were also identified. CRP and HMOX1 were notably detected

Table 1: Overlapping gene alterations linked to the metals and ischaemic heart disease

Gene	Protein name	Arsenic (As)			Cadmium (Cd)			Lead (Pb)			Mercury (Hg)		
		Prot. act.	mRNA exp.	Prot. exp.	Prot. act.	mRNA exp.	Prot. exp.	Prot. act.	mRNA exp.	Prot. exp.	Prot. act.	mRNA exp.	Prot. exp.
CRP	C-reactive protein	-	-	↑	-	↓	-	-	-	↑	-	-	-
HMOX1	Haem oxygenase 1	-	↑	↑	↑	↑	↑	-	↑	-	-	↑	-
PON1	Paraoxonase/ arylesterase 1	-	-	-	-	-	-	↓	-	-	↓	-	-
PTGS2 (COX2)	Prostaglandin G/H synthase 2	-	↑↓	-	-	↑↓	↑	-	↑	↑	-	-	-

↑: Increase, ↓: Decrease, ↑↓: Either increase or decrease, Prot. act.: Protein activity, mRNA exp.: mRNA expression, Prot. exp.: Protein expression

Table 2: Top 5 molecular functions associated with the metals and ischaemic heart disease **(https://**toppgene.cchmc.org/output.jsp).

	ID	Name	pValue	Genes from the input	Genes used in annotation
1	GO:0071813	Lipoprotein particle binding	$1.797E^{-5}$	CRP, PON1	35
2	GO:0071814	Protein-lipid complex binding	$1.797E^{-5}$	CRP, PON1	35
3	GO:0042803	Protein homodimerization activity	$2.737E^{-4}$	HMOX1, PON1, and PTGS2	824
4	GO:0020037	Haem binding	$3.349E^{-4}$	HMOX1, PTGS2	150
5	GO:0046906	Tetrapyrrole binding	$3.809E^{-4}$	HMOX1, PTGS2	160

Table 3: Top 5 biological processes associated with the metals and ischaemic heart disease (https://toppgene.cchmc.org/output.jsp).

	ID	Name	pValue	Genes from the Input	Genes used in Annotation
1	GO:0097006	Regulation of plasma lipoprotein particle levels	$4.393E^{-7}$	CRP, HMOX1, and PON1	100
2	GO:0010035	Response to an inorganic substance	$1.699E^{-6}$	CRP, HMOX1, PON1, and PTGS2	747
3	GO:0008015	Blood circulation	$1.783E^{-6}$	CRP, HMOX1, PON1, and PTGS2	756
4	GO:0003013	circulatory system process	$2.746E^{-6}$	CRP, HMOX1, PON1, and PTGS2	842
5	GO:0071248	Cellular response to metal ions	$6.543E^{-6}$	CRP, HMOX1, and PTGS2	245

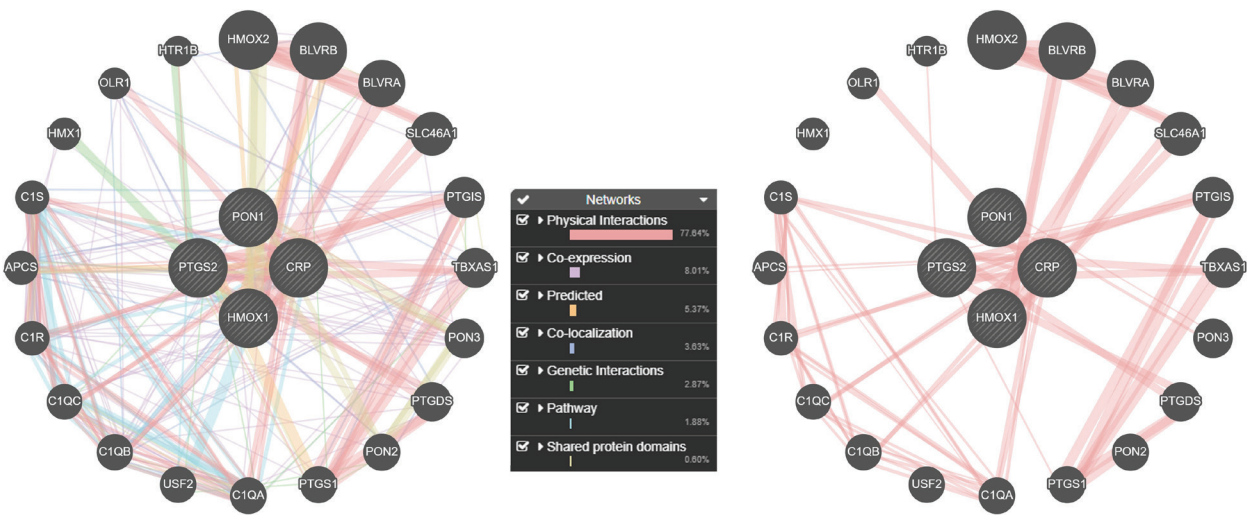

Figure 2: GeneMANIA-predicted gene-gene and physical interactions of the four common genes

in three centrality parameters: BC, CC, and DC (Figure 3a and Table 4).

Key transcription factors and miRNAs related to metals and IHD

To investigate the regulatory connexions between genes and transcription factors, the CHEA3 tool was employed. A total of 22 connexions were recognised between 10 transcription factors and 4 genes. As illustrated in Figure 3b, CREB3L3 and ATF5 regulated four genes, NR1H4 and NR1I3 regulated three genes, and ZNF267, NFE2L2, and NR4A3 regulated two genes each, with the others regulating one gene each. The primary transcription factors in this group were CREB3L3 and ATF5 (Suppl. Table S2).

Regarding miRNAs, we established connexions between miRNAs and target genes using the tool provided by MIENTURNET. We observed that the miRNAs displaying the most elevated

Table 4: Centrality analysis of overlapping genes related to the metals and ischaemic heart disease

Overlapping genes	Degree	Betweenness centrality	Closeness centrality
CRP	3	0.166	1.00
HMOX1	3	0.1667	1.00
PON1	2	0	0.75
PTGS2	2	0	0.75

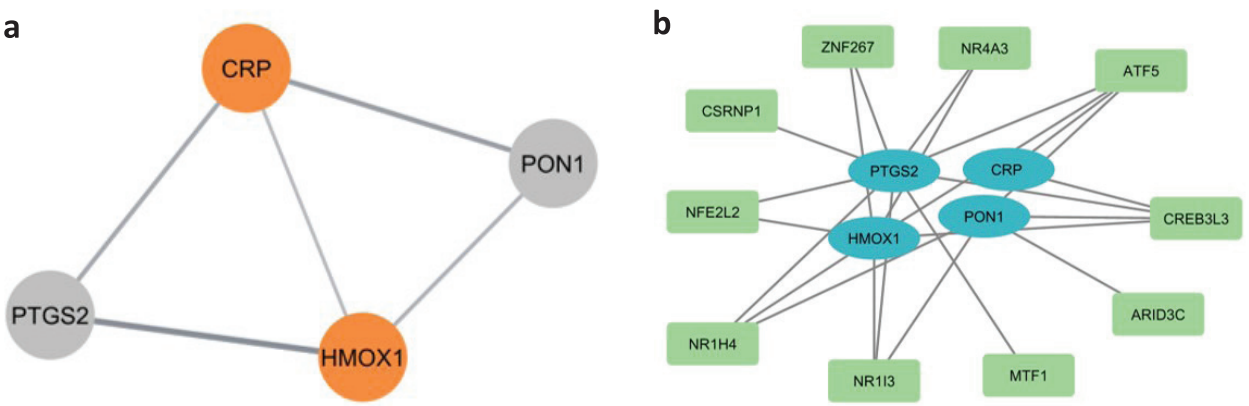

Figure 3: a) The centrality analysis of common genes reveals the core proteins/genes, shown as light brown nodes. b) The top 10 transcription factors related to the four common genes are highlighted, with green nodes indicating transcription factors. c) miRNA-target analysis shows the top 10 miRNAs related to the four common genes.

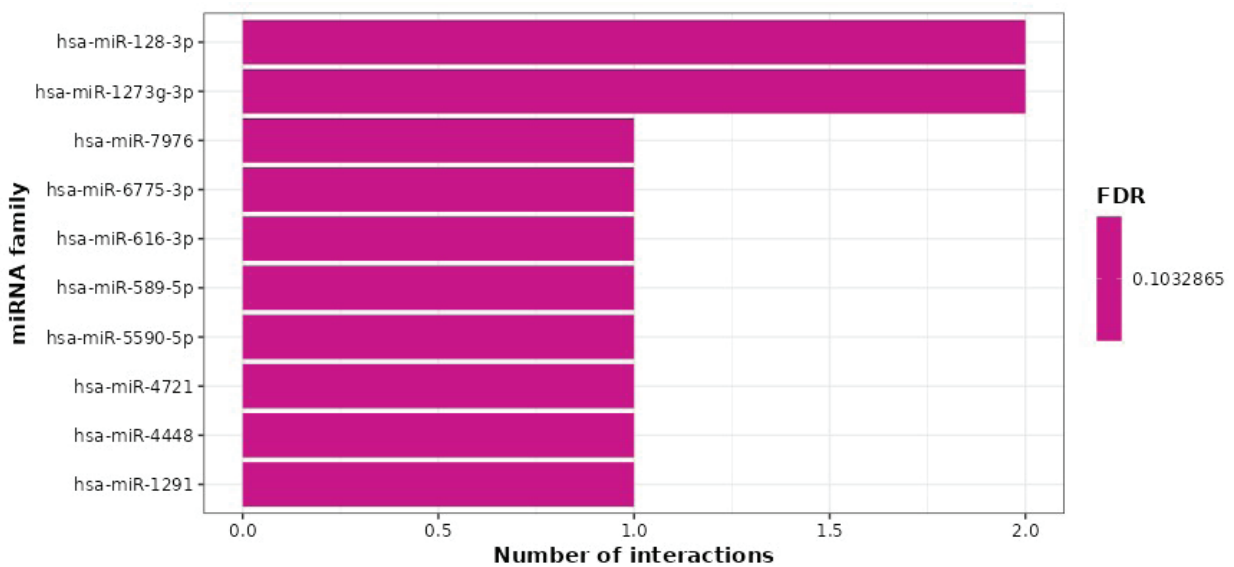

Figure 3: c) miRNA-target analysis shows the top 10 miRNAs related to the four common genes.

expression and interactions were hsa-miR-128-3p and hsa-miR-1273g-3p, which are related to four genes involved in the aetiology of IHD induced by metals (Figure 3c, Suppl. Table S3).

DISCUSSION

In this study, big data and toxicogenomic data mining were employed to illustrate a positive relationship between metals (As, Cd, Hg, and Pb) and ischaemic heart disease (IHD). *In silico* analysis of these metals revealed alterations in four genes (CRP, HMOX1, PON1, and PTGS2) involved in the aetiology of IHD. Lipoprotein particle binding emerged as the leading molecular function, whereas the regulation of plasma lipoprotein particle levels was identified as the foremost biological process related to IHD induced by these metals.

Centrality analysis revealed that CRP and HMOX1 are key genes in IHD induced by these metals. C-reactive protein (CRP) is encoded by the CRP gene and synthesised by the liver. CRP levels increase during inflammation in the body, which has been identified as a major risk factor for IHD (23-25). Manual CTD analysis indicated that As and Pb increased CRP protein expression (Table 1). Another core gene is haem oxygenase 1 (HMOX1), which degrades haem, generating CO and biliverdin, while simultaneously releasing iron. HMOX1 is critical for heart repair and survival. All studied metals upregulate HMOX1, although it is also frequently up-regulated in tumour tissues (26, 27) (Table 1).

ATF5 (Cyclic AMP-dependent transcription factor ATF5) and CREB3L3 (Cyclic AMP-responsive element-binding protein 3-like protein 3) were identified as the main transcription factors. Our findings align with those of prior research; for instance, ATF5 and CREB3L3 were identified as crucial transcription factors in cardiovascular diseases induced by cadmium, lead, and mercury. It has also been mentioned that the mitochondrial unfolded protein response, a cytoprotective signalling system, depends on ATF5 activation (28). Another core transcription factor is CREB3L3, which oversees various metabolic processes, including lipid metabolism, cholesterol absorption, and glucose. CREB3L3 performs a versatile protective function against atherosclerosis (29).

Regarding miRNAs, we observed that hsa-miR-128-3p and hsa-miR-1273g-3p were the predominant miRNAs triggered by the combination of metals associated with the aetiology of IHD. A study that reported the inhibition of hsa-miR-128-3p provides protection to human cardiomyocytes against ischaemia/reperfusion corroborates our results (30). However, there is no study in the literature regarding hsa-miR-1273g-3p.

In conclusion, our study highlights the crucial involvement of genes CRP and HMOX1, transcription factors ATF5 and CREB3L3, and miRNAs hsa-miR-128-3p and hsa-miR-1273g-3p in the development of IHD. ATF5 and CREB3L3, along with hsa-miR-128-3p and hsa-miR-1273g-3p, have the potential to open avenues for novel therapeutic targets in future IHD treatments.

Ethics Committee Approval: Since this study consists entirely of bioinformatics and in silico analyses, ethics committee approval is not required.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- F.K.; Data Acquisition- F.K., B.K. Data Analysis/Interpretation- F.K., B.K. Drafting Manuscript- F.K Critical Revision of Manuscript- F.K., B.K. Final Approval and Accountability- F.K., B.K. Material and Technical Support- F.K., B.K. Supervision- F.K.

Conflict of Interest: The authors declare that there is no conflict of interest.

Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES

1. Duggan JP, Peters AS, Trachiotis GD, Antevil JL. Epidemiology of coronary artery disease. Surg Clin North Am 2022;102(3):499-516.
2. Malakar AK, Choudhury D, Halder B, Paul P, Uddin A, Chakraborty S. A review on coronary artery disease, its risk factors, and therapeutics. J Cell Physiol 2019;234(10):16812-23.
3. Chen Z, Huo X, Chen G, Luo X, Xu X. Lead (Pb) exposure and heart failure risk. Environ Sci Pollut Res Int 2021;28(23):28833-47.
4. Wang Z, Xue K, Wang Z, Zhu X, Guo C, Qian Y, et al. Effects of e-waste exposure on biomarkers of coronary heart disease (CHD) and their associations with level of heavy metals in blood. Environ Sci Pollut Res Int 2022;29(33):49850-7.
5. Gump BB, Heffernan K, Brann LS, Hill DT, Labrie-Cleary C, Jandev V et al. Exposure to arsenic and subclinical cardiovascular disease in 9- to 11-year-old children, syracuse, new york. JAMA Netw Open 2023;6(6):e2321379.
6. Das S, Sultana KW, Ndhlala AR, Mondal M, Chandra I. Heavy metal pollution in the environment and its impact on health: exploring green technology for remediation. Environ Health Insights 2023;17:11786302231201259.
7. Xu L, Mondal D, Polya DA. Positive association of cardiovascular disease with chronic exposure to drinking water arsenic at concentrations below the who provisional guideline value: a systematic review and meta-Analysis. Int J Environ Res Public Health 2020;17(7):2536.
8. Glicklich D, Shin CT, Frishman WH. Heavy metal toxicity in chronic renal failure and cardiovascular disease: possible role for chelation therapy. Cardiol Rev 2020;28(6):312-8.
9. Hu XF, Lowe M, Chan HM. Mercury exposure, cardiovascular disease, and mortality: a systematic review and dose-response meta-analysis. Environ Res 2021;193:110538.
10. Tsuji JS, Perez V, Garry MR, Alexander DD. Association of low-level arsenic exposure in drinking water with cardiovascular disease: a systematic review and risk assessment. Toxicology 2014;323:78-94.
11. Ujueta F, Navas-Acien A, Mann KK, Prashad R, Lamas GA. Low-level metal contamination and chelation in cardiovascular disease-a ripe area for toxicology research. Toxicol Sci 2021;181(2):135-47.
12. Nigra AE, Moon KA, Jones MR, Sanchez TR, Navas-Acien A. Urinary arsenic and heart disease mortality in nhanes 2003-2014. Environ Res 2021;200:111387.
13. Liu J, Song W, Li Y, Wang Y, Cui Y, Huang J, et al. Burden of coronary heart disease and cancer from dietary exposure to inorganic arsenic in adults in china, 2016. Ann Glob Health 2022;88(1):28.
14. Liu J, Li Y, Li D, Wang Y, Wei S. The burden of coronary heart disease and stroke attributable to dietary cadmium exposure in chinese adults, 2017. Sci Total Environ 2022;825:153997.
15. Davis AP, Wiegers TC, Johnson RJ, Sciaky D, Wiegers J, Mattingly CJ. Comparative toxicogenomics database (ctd): update 2023. Nucleic Acids Res 2023;51(D1):D1257-62.
16. Sun L, Dong S, Ge Y, Fonseca JP, Robinson ZT, Mysore KS, Mehta P. Divenn: an interactive and integrated web-based visualization tool for comparing gene lists. Front Genet 2019;10:421.
17. Chen J, Bardes EE, Aronow BJ, Jegga AG. Toppgene suite for gene list enrichment analysis and candidate gene prioritization. Nucleic

Acids Res 2009;37:W305-11.
18. Franz M, Rodriguez H, Lopes C, Zuberi K, Montojo J, Bader GD, et al. Genemania update 2018. Nucleic Acids Res 2018;46(W1):W60-W64.
19. Szklarczyk D, Kirsch R, Koutrouli M, Nastou K, Mehryary F, Hachilif R, et al. The string database in 2023: protein-protein association networks and functional enrichment analyses for any sequenced genome of interest. Nucleic Acids Res 2023;51(D1):D638-46.
20. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 2003;13(11):2498-504.
21. Keenan AB, Torre D, Lachmann A, Leong AK, Wojciechowicz ML, Utti V, et al. Chea3: transcription factor enrichment analysis by orthogonal omics integration. Nucleic Acids Res 2019;47(W1):W212-24.
22. Licursi V, Conte F, Fiscon G, Paci P. Mienturnet: an interactive web tool for microrna-target enrichment and network-based analysis. BMC Bioinform 2019;20(1):545.
23. Kaptoge S, Di Angelantonio E, Lowe G, Pepys MB, Thompson SG, Collins R, et al. C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis. Lancet 2010;375(9709):132-40.
24. Ridker PM. From c-reactive protein to interleukin-6 to interleukin-1: moving upstream to identify novel targets for atheroprotection. Circ Res 2016;118(1):145-56.
25. Amezcua-Castillo E, González-Pacheco H, Sáenz-San Martín A, Méndez-Ocampo P, Gutierrez-Moctezuma I, Massó F, et al. C-Reactive Protein: The quintessential marker of systemic inflammation in coronary artery disease-advancing toward precision medicine. Biomedicines 2023;11(9):2444.
26. Jozkowicz A, Was H, Dulak J. Heme oxygenase-1 in tumors: is it a false friend? Antioxid Redox Signal 2007;9(12):2099-117.
27. Otterbein LE, Foresti R, Motterlini R. Heme oxygenase-1 and carbon monoxide in the heart: the balancing act between danger signaling and pro-survival. Circ Res 2016;118(12):1940-59.
28. Nguyen HD, Kim MS. Effects of heavy metals on cardiovascular diseases in pre and post-menopausal women: from big data to molecular mechanism involved. Environ Sci Pollut Res Int 2022;29(51):77635-55.
29. Nakagawa Y, Wang Y, Han SI, Okuda K, Oishi A, Yagishita Y, et al. Enterohepatic transcription factor creb3L3 protects atherosclerosis via srebp competitive inhibition. Cell Mol Gastroenterol Hepatol 2021;11(4):949-71.
30. Chen GH, Xu CS, Zhang J, Li Q, Cui HH, Li XD, et al. Inhibition of mir-128-3p by tongxinluo protects human cardiomyocytes from ischemia/reperfusion injury via upregulation of p70s6k1/p-p70s6k1. Front Pharmacol 2017;8:775.

Table S1.

Chemical/Disease name	Inference network from Comparative Toxicogenomics Database
Arsenic (As)	*ABCG5 | APCS | ATP2B1 | CCL2 | CD40LG | CDKN2B |* ***CRP*** *| ESR1 | ESR2 | FES | FURIN | GDF15 | GFOD1 |* ***HMOX1*** *| IRS1 | JCAD | KCNE2 | LDLR | LTA | MAT2A | MIA3 | MRAS | NOS3 | PDGFD | PECAM1 | PLPP3 |* ***PON1*** *| PRDM16 | PROCR |* ***PTGS2*** *| TBX2 | TERT | TLR4 | TRAF6 | TRIB1 | TWIST1 | VAMP5 | VEGFA |*
Cadmium (Cd)	*ACE | ANGPTL4 | APCS | APOA1 | ATP2B1 | CCL2 | CDKN2B |* ***CRP*** *| CXCL12 | EPO | ESR1 | ESR2 | FURIN | GDF15 | GGCX |* ***HMOX1*** *| IRAK1 | IRS1 | KL | LDLR | LTA | MIR146A | MMP3 | NOS3 | PECAM1 | PHACTR1| PLPP3 |* ***PON1*** *| PSRC1 |* ***PTGS2*** *| SLC5A3 | TLR4 | TRAF6 | TRIB1 | VAMP5 | VEGFA |*
Mercury (Hg)	***CRP*** *| ESR2 | FURIN | GGCX |* ***HMOX1*** *| IRS1 | LIPA |* ***PON1*** *|* ***PTGS2*** *| WDR12 |*
Lead (Pb)	*ACE | APCS | APOA1 | CCL2 |* ***CRP*** *| HECTD4 |* ***HMOX1*** *| ICA1L | LDLR | LTA | MAT2A | MIR146A | NOS3 |* ***PON1*** *|* ***PTGS2*** *| SORT1 | TBC1D7 | TLR4 | VEGFA |*
Ischemic heart disease (IHD)	*ABCG5 | ABCG8 | ABO | ACE | ADAMTS7 | ADTRP | ANGPTL4 | ANKS1A | APCS | APOA1 | APOA5 | APOC3 | ATP2B1 | CARF | CCL2 | CD40LG | CDKN2B | CELSR2 |* ***CRP*** *| CXCL12 | DDAH2 | EPO | ESR1 | ESR2 | FES | FURIN | GDF15 | GFOD1 | GGCX | GNB3 | GUCY1A1 | HECTD4 | HHIPL1 |* ***HMOX1*** *| ICA1L | IRAK1 | IRS1 | JCAD | KCNE2 | KIAA1462 | KL | LDLR | LIPA | LMOD1 | LPA | LRP6 | LTA | MAT2A | MEF2A | MIA3 | MIR146A | MIR146B | MMP3 | MRAS | MRPS6 | NAT2 | NBEAL1 | NOS3 | NPPB | PAPPA | PCSK9 | PDGFD | PECAM1 | PHACTR1 | PLPP3 |* ***PON1*** *| PRDM16 | PROCR | PSRC1 |* ***PTGS2*** *| SARS1 | SH2B3 | SLC5A3 | SORT1 | TBC1D7 | TBX2 | TCF21 | TERT| TLR4 | TRAF6 | TRIB1 | TWIST1 | VAMP5 |VAMP8 | VEGFA | WDR12 | ZC3HC1*
As+Cd+Pb+Hg+ IHD	*CRP | HMOX1 | PON1 | PTGS2*

Table S2. The top 10 transcription factors (TF) related to the metals and ischemic heart disease (https://maayanlab.cloud/chea3/)

Transcription factors	Genes from input
CREB3L3	*CRP, PON1, HMOX1, PTGS2*
ATF5	*CRP, PON1, HMOX1, PTGS2*
NR1I3	*PON1, HMOX1, PTGS2*
NR1H4	*PON1, HMOX1, PTGS2*
ZNF267	*HMOX1, PTGS2*
NFE2L2	*HMOX1, PTGS2*
NR4A3	*HMOX1, PTGS2*
CSRNP1	*PTGS2*
ARID3C	*PON1*
MTF1	*PTGS2*

Table S3. The top 10 miRNAs related to the metals and ischemic heart disease (https://maayanlab.cloud/Enrichr/)

miRNAs	p-value	Genes from input
hsa-miR-1273g-3p	0.003705	*CRP, PON1*
mmu-miR-26b-5p	0.003994	*PTGS2*
hsa-miR-128-3p	0.004014	*HMOX1, PTGS2*
mmu-miR-101a-3p	0.005389	*PTGS2*
hsa-miR-6775-3p	0.009963	*HMOX1*
hsa-miR-616-3p	0.010559	*PON1*
mmu-miR-5098	0.010757	*CRP*
hsa-miR-1291	0.010955	*HMOX1*
hsa-miR-7976	0.011947	*HMOX1*
hsa-miR-5590-5p	0.014125	*HMOX1*