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Network-based Identification of Conserved Gene Targets of Heavy Metal Toxicity through Zebrafish–Human Integrative Analysis

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Abstract

Heavy metal pollution from industrial, agricultural, and urban activities poses serious concerns for human and ecosystem health, particularly near industrial zones and agricultural lands. Toxic heavy metals profoundly alter biological functions, including embryonic development, oxidative stress, and abnormal cell behaviour, which may also be linked to developmental disorders and cancer. While recent innovations in computational toxicology have enabled detailed analysis of molecular responses to toxicants through comprehensive multi-omics approaches, incorporating this information into human remains remains challenging. Zebrafish, due to their genetic compatibility with humans, are gradually used in toxicogenomics. However, few studies integrate zebrafish data with relevant human bioinformatics to verify shared molecular pathways involved in metal toxicity, and no direct *in silico* analyses have been documented. This study addresses this gap by integrating computational tools with zebrafish to identify conserved gene targets and molecular pathways disrupted by heavy metal exposure, using platforms such as Cytoscape, STRING, CHEA3, and CTD. Analysis showed that heavy metals affect signalling pathways involved in stress responses, immunity, apoptosis, and oxidative damage in both zebrafish and humans. Shared genes were connected to diseases such as Alzheimer's, diabetes, cancer, and cardiovascular disorders.

Major Findings: The study indicates that heavy metals disrupt development, immunity, and organ function, and that conserved genes, transcription factors, and pathways shared by zebrafish and humans underscore the suitability of zebrafish for research on human disease and toxicology. Our integrative approach shows zebrafish with their conserved genes and pathways, providing a powerful model for investigating toxicity, disease mechanisms, and potential biomarkers for human health.

Keywords: CHEA3, CTD, Cytoscape, Heavy Metals, Humans, STRING, Toxicogenomics, Zebrafish

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1. Introduction

Heavy metal pollution from industrial activities, agriculture, waste disposal, urban runoff, and fossil fuel combustion is a significant global issue, especially near factories, cities, and farmlands, presenting major dangers to humans and the environment[1-5]. During embryonic development, metals such as arsenic, lead, mercury, cadmium, and chromium are particularly dangerous, as they can cause teratogenic effects, oxidative stress that disrupts gene expression, neurotoxicity, and developmental problems, including ADHD[6-8]. These metals are associated with cancer and long-term immune dysfunction[9,10]. Cadmium triggers the Nrf2 antioxidant pathway. Arsenic promotes oxidative stress and contributes to chronic diseases, lead interferes with neurotransmitter synthesis, affecting cognition, and mercury and chromium cause neurodegeneration and cancer primarily through oxidative stress[11-15].

Recent developments in computational tools have greatly enhanced our understanding of toxic effects on cellular and molecular processes[16]. These tools are crucial to toxicology because they enable the analysis of large biological datasets, the identification of molecular pathways of toxicity, and a more accurate evaluation of the risks to human and environmental

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health[17-21]. Computational methods uncover toxicant-induced alterations in gene expression, protein interactions, and signalling cascades by combining data from transcriptomics, proteomics, and genomes. This comprehensive method facilitates the identification of biomarkers and advances understanding of the harmful effects on genes and biological pathways[22-25]. Tools like Cytoscape, STRING, CHEA3, and ShinyGO support data integration and network analysis to identify critical molecular hubs and pathways affected by toxicant exposures[26-30]. Computational toxicogenomics plays an important role in determining heavy metal toxicity and minimising environmental and health risks through the Comparative Toxicogenomic Database (CTD) and associated computational tools, which enhance biomarker discovery, facilitate therapeutic target identification, improve predictive models, and map chemical-gene- disease interactions[31-38].

Zebrafish (*Danio rerio*) are commonly used in research on *in vivo* heavy metal toxicity because of their genetic similarity to humans, rapid development, and transparent embryos that enable real-time monitoring of developmental and toxicological responses. With approximately 70% of their genes like those in humans, zebrafish enable extensive toxicogenomic screening and provide important insights into mechanisms of heavy metal toxicity[39-45]. The use of zebrafish in toxicology research is increasing, but there hasn't been much use of toxicological methods to forecast the effects of heavy metals on human health. To confirm common molecular targets and pathways across species, we have combined computational methods between zebrafish and humans[46-48].

Zebrafish have significant latent potential in approaching predictive toxicology. Our study addresses this gap by integrating zebrafish and human data to explore the toxicogenomic effects of heavy metals. Using computational analyses, we pinpoint molecular targets that are commonly affected, focusing on those most relevant to human health effects without *in vivo* analysis. By combining these strategies, the accuracy of computational predictions is improved, and a quicker way to uncover the mechanisms of toxicity cost-effectively and their potential implications for human treatments is achieved.

2. Methodology

The overall toxicogenomic analysis aims to identify common heavy metal targets between zebrafish and humans, as shown in Figure 1.

2.1 Identification of the Top Five Toxic Heavy Metals

The top 5 toxic heavy metals were selected based on literature and according to ATSDR's Substance Priority list (https://www.atsdr.cdc.gov/spl/index.html), which are arsenic, lead, mercury, cadmium, and chromium (Table 1).

2.2 Dataset Analysis from Comparative Toxicogenomic Database (CTD)

In this study, the top 5 toxic heavy metals (Arsenic, Cadmium, Chromium, Mercury, Lead) were chosen for

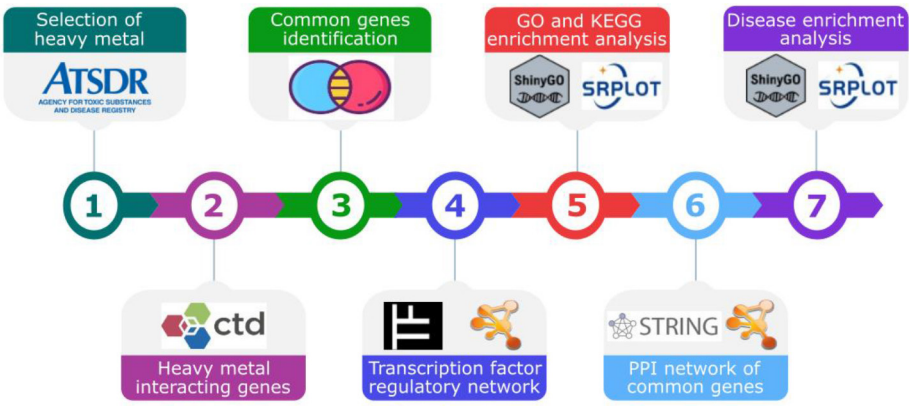


Figure 1. The toxicogenomic methodological workflow used to identify heavy-metal target genes shared between zebrafish and humans.

Table 1. Top 5 toxic heavy metals according to ATSDR's Substance Priority List

Serial no.	Heavy Metal name	Pubchem ID	ATSDR's Substance Priority List	Total Points	Molecular Weight (g/mol)
1	ARSENIC	5359596	1	1675	74.92160
2	LEAD	5352425	2	1531	207.2000
3	MERCURY	23931	3	1455	200.59
4	CADMIUM	23973	7	1317	112.411
5	CHROMIUM	23976	17	1151	51.9961

2022 rankings (https://www.atsdr.cdc.gov/spl/index.html).

analysis. Common genes altered by heavy metal exposure were identified using CTD (http://CTD.mdibl.org). CTD is publicly available, manually curated data for over 17,677 chemicals and their gene-protein interactions. CTD is commonly referred to as a "golden set database" because it uses a restricted vocabulary, ensuring internal consistency[49]. It is updated monthly with consistent data, which improves accuracy, integration, and cohesion. The database contains correlations between gene-disease, chemical-disease, and phenotypes following chemical exposure, which are used to investigate the biological impacts of diverse xenobiotic exposures. Furthermore, the routes and data provided enable us to use the database to generate hypotheses to better understand environmental diseases. From this database, the datasets for five heavy metals were downloaded in Excel format.

2.3 Identification of Common Genes

Common genes among the top 5 heavy metals (Arsenic, Cadmium, Chromium, Mercury, Lead) were identified using Interactivenn (https://www.interactivenn.net/)[50]. The obtained result was also plotted using the same web browser. The common genes were used independently for further analysis.

2.4 Transcription Factor Analysis

To identify key transcription factors (TFs) regulating the identified genes, the CHEA3 tool (https://maayanlab.cloud/chea3/) was utilised. CHEA3 predicts TFs using ENCODE datasets[29]. A Fisher's Exact Test was performed using a background of 20,000 genes to compare input genes and determine the most relevant TFs. The top 8 TFs most likely involved in regulating the common genes were selected, and a regulatory network was subsequently constructed using Cytoscape.

2.5 Enrichment Analysis of the Identified Common Genes

The ShinyGO (version 0.80) web server (http://bioinformatics.sdstate.edu/go/) is a publicly available graphical gene-set enrichment tool for animals and plants[30]. GO (Gene Ontology) and KEGG (Kyoto Encyclopaedia of Genes and Genomes) pathway enrichment analysis of common genes harvested from the top 5 heavy metals (Arsenic, Cadmium, Chromium, Mercury, Lead). Each enrichment analysis was used for data visualisation to examine their functional roles in zebrafish and humans, including Biological Processes (BP) and Molecular Functions (MF), as well as other cell signalling pathways related to them. The enrichment bubble and enrichment bar plots were generated for GO terms and KEGG pathways, respectively, using the SRplot web server (https://www.bioinformatics.com.cn/en), a graphical gene-enrichment tool for visualising overlapping genes among enriched gene sets. It also identifies statistically significant differences in gene type, length, and gene count content between query genes[51]. Enrichment analysis of charts was performed with a False Discovery Rate (FDR) cut-off of 0.05, fold enrichment on the X-axis, and pathways on the Y-axis, ordered by the number of genes in each pathway.

2.6 Protein-Protein Interaction (PPI) Network

To understand the interactions among the identified proteins, a PPI network was constructed using the STRING database version 12.0 (https://string-db.org/). The STRING database integrates experimentally verified and computationally predicted PPIs, including physical and functional interactions, using a minimum interaction score of 0.900[27,52]. The whole PPI network was constructed using multiple

protein search queries and default settings, with *D. rerio* and *H. sapiens* as the reference organisms. The network was represented using Cytoscape software 3.10.3. (http://www.cytoscape.org/), An open-source bioinformatics tool for the visualisation of complex biological networks[53]. Common genes between interconnected regions in PPI networks were identified in both organisms using Cytoscape.

2.7 Disease Enrichment Analysis

The top 10 diseases linked to the common genes were identified using the ShinyGO (version 0.80) web server (http://bioinformatics.sdstate.edu/go/) with a FDR cutoff of <0.05. The enriched diseases linked to the common genes were visualised using the SRplot web server (https://www.bioinformatics.com.cn/en).

3. Result

Toxicogenomic data mining from CTD was performed to identify common genes, the CGPD-tetramer (a 4-unit block of information linking an initiating chemical, an interacting gene, a phenotype, and a disease outcome), and interactions with heavy metals.

3.1 Common Genes Identification

In total, 78 common genes (Table 2) were identified for heavy metal interactions (Figure 2). These genes were analysed individually.

3.2 Transcription Factor Regulatory Network

Transcription Factors (TF) gene regulatory relationships were identified for 78 common genes associated with the top 5 toxic heavy metals using ChEA3 and visualised in Cytoscape. A total of 269 regulatory interactions (edges) involving 71 intersecting genes were detected. The top 8 TFs, including GATA1, JUND, ETS1, FOS, MYOG, MAFK, TFAP2A, and TFAP2C, regulated between 18 and 24 genes in common (Figure 3).

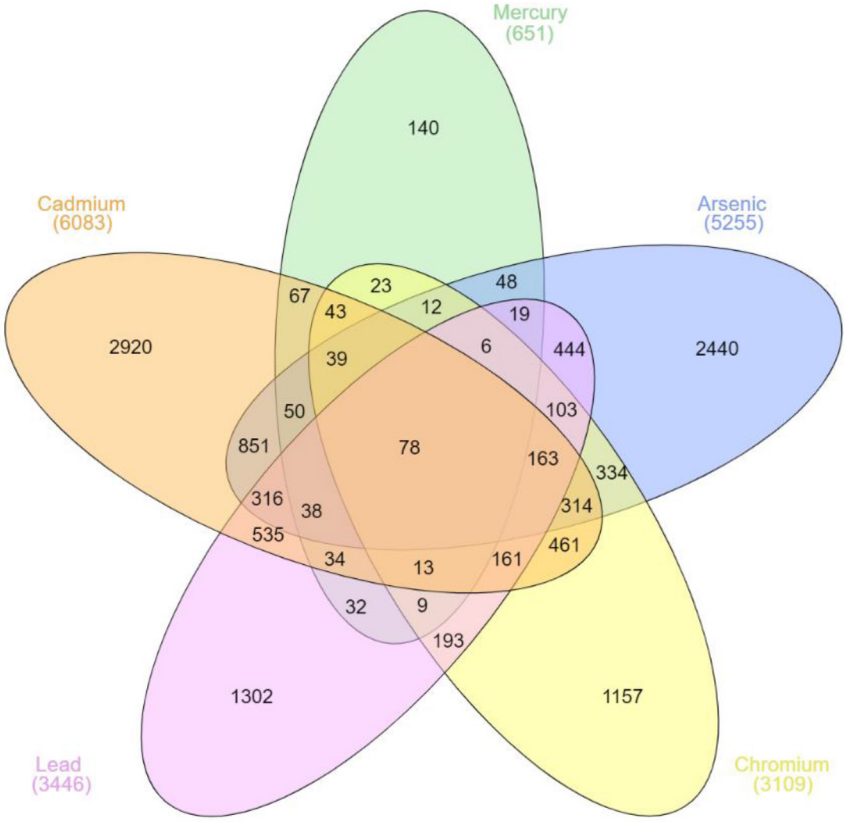


Figure 2. Common genes identified from Cadmium, Mercury, Arsenic, Chromium, and Lead.

Table 2. Common genes identified for the top 5 toxic heavy metals

Cadmium, Mercury, Arsenic, Chromium, and Lead	ABCC1,ACHE,AHR,ALB,ANXA2,AZGP1,BAX,BCL2,CASP3,CASP9,CAT,CFB,CRP,CRYAA,CXCL8,CYP1A1,CYP7A1,FABP5,FAS,FN1,FOS,FSHB,G6PD,GCLC,GCLM,GGT1,GPT,GPX1,GPX3,GSR,GSTM1,GSTP1,HAVCR1,HMOX1,HSPA5,HSPD1,IFNG,IGF1,IL10,IL1B,IL2,IL4,IL6,INS1,JUN,LDHA,MAFF,MAPK1,MAPK3,MMP2,MT1,MT1A,MT2A,NFE2L2,NFKBIA,NLRP3,NOS2,NOX4,NQO1,OCLN,OGG1,PARP1,PIK3R1,PRKCA,PTGS2,RELA,SERPING1,SLC11A2,SLC13A1,SLC2A4,SOD1,SQSTM1,TGFB1,TNF,TXN,TXNRD1,VDR,VIM

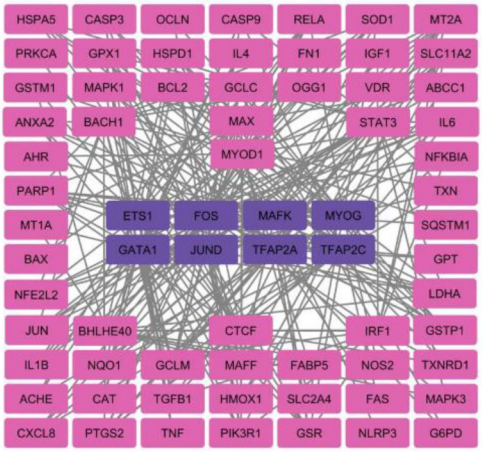


Figure 3. The top 8 transcription factors associated with intersecting gene interactions were identified and analysed using CHEA3 and Cytoscape, based on 78 common genes across the top 5 toxic heavy metals. A purple-colored node denotes TF, and a pink node denotes an intersecting gene.

3.3 Enrichment Analysis of the Identified Common Genes

The GO and KEGG pathway enrichment analyses of the identified common genes for heavy metals were performed in *D. rerio* and *H. sapiens*. The top 15 Gene Ontology (GO) terms were selected for further visualisation in the BP and MF categories. In contrast, no terms were chosen for the Cellular Component (CC) category due to limited data availability (Figures 4 and 5). In zebrafish, the BP category was enriched for genes involved in cellular response to stress, chemical stimuli, external stimuli, organic substances, the immune system, multicellular organismal processes, glutathione metabolic and biosynthetic processes, cell and leukocyte activation, non-ribosomal peptide biosynthetic processes, and anatomical structure development. The MF category was enriched with genes involved in various activities like signalling receptor regulation and activation, receptor ligand, sulfur compound transmembrane transportation, protein-disulfide reduction, disulfide oxidoreduction, cytokine, oxidoreductase, growth factor, molecular function regulation, MAP kinase; binding of growth factor receptor, signalling receptor, cytokine receptor; ATP-dependent protein folding chaperone. Furthermore, KEGG pathway enrichment analysis showed interaction with signalling pathways like C-type lectin receptor, Toll-like receptor, MAPK, VEGF, ErbB, GnRH, mTOR, p53, NOD-like receptor, and FoxO; and infections like Salmonella and Herpes simplex virus 1; Glutathione, Cysteine, and methionine metabolism; Autophagy and Mitophagy-animal; Apoptosis, Cellular senescence, Cytokine-cytokine receptor interaction, Progesterone-mediated oocyte maturation, Intestinal immune network for IgA production, Focal adhesion, Cytosolic DNA-sensing pathway, Necroptosis, and Metabolic pathways.

In humans, the BP category was enriched with genes involved in cellular response to chemical stimulus, oxygen-containing compound, stress, chemical, organic-inorganic substance, oxidative stress; regulation of programmed cell death; homeostatic and apoptotic process; regulation of biological quality. The MF category was enriched for genes involved in activities such as antioxidant, protein-disulfide reductase (NAD(P)), molecular function regulator, oxidoreductase, peroxidase, and protein homodimerization; and in binding of identical protein, enzyme, cytokine receptor, protein-containing complex, heme, tetrapyrrole, chaperone, and DNA-binding transcription factor. KEGG pathway enrichment analysis showed interaction with Pathways in cancer, IL-17 signaling, neurodegeneration-multiple diseases, C-type lectin receptor signaling, TNF signaling, T cell receptor signaling, and AGE-RAGE signaling in diabetic complications; infections such as Yersinia, Kaposi sarcoma-associated herpesvirus, Pathogenic E.coli, Salmonella, Human cytomegalovirus; Lipid and atherosclerosis, Chagas disease, Hepatitis B, Pertussis, Leishmaniasis, Influenza A, Chemical carcinogenesis-ROS, Fluid shear stress and atherosclerosis, Tuberculosis, Th17 cell differentiation, Apoptosis, Amoebiasis and Measles.

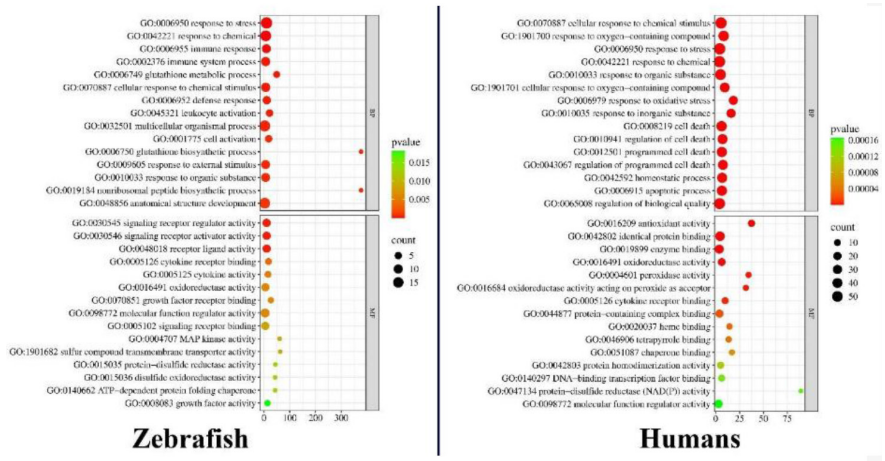


Figure 4. GO enrichment analysis using SRplot. This includes the biological processes and molecular functions of 78 common genes identified from the top 5 toxic heavy metals in zebrafish (*D. rerio*) and humans (*H. sapiens*).

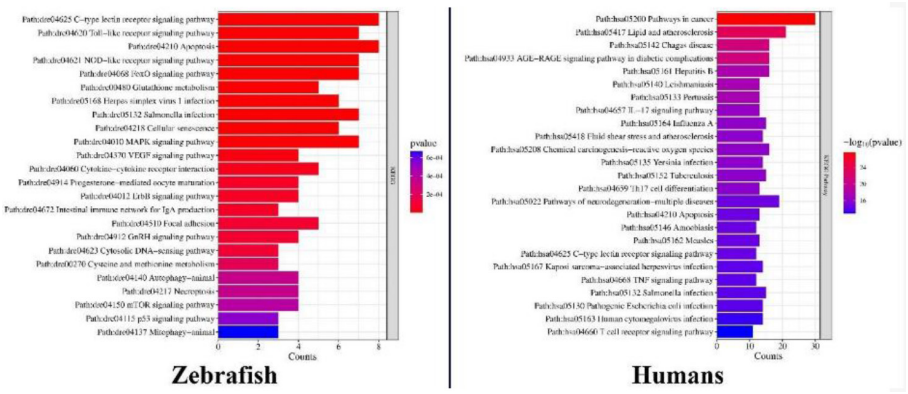


Figure 5. KEGG enrichment analysis using SRplot. Kyoto Encyclopedia of Genes and Genomes network pathway enrichment analysis of the 78 common genes identified from the top 5 toxic heavy metals in zebrafish (*D. rerio*) and humans (*H. sapiens*).

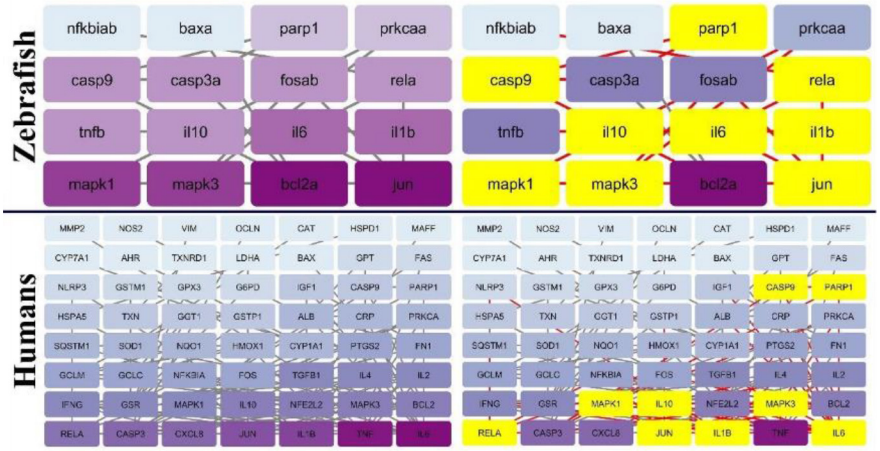


Figure 6. The protein-protein interaction network (PPIN) of heavy metals was constructed using the STRING for zebrafish (*D. rerio*) and humans (*H. sapiens*). Nodes are illustrated with a gradient colour scale ranging from blue to purple (ColorBrewer palette) to represent varying levels of interaction. Common genes shared between zebrafish and humans are highlighted in yellow to enhance visualisation. Network visualisation and analysis were conducted using Cytoscape.

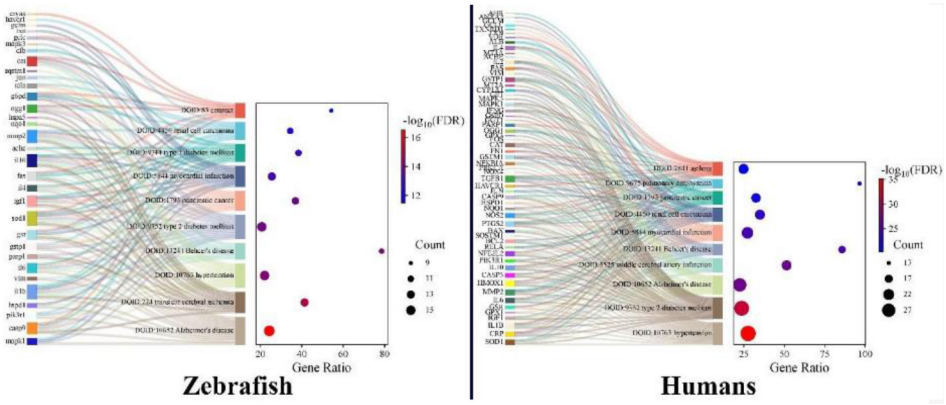


Figure 7. The top 10 disease categories associated with the gene interactions most common in heavy metals were identified using ShinyGo for zebrafish (*D. rerio*) (a) and human (*H. sapiens*), with an FDR of less than 0.05, and visualised using SRplot.

3.4 Protein-Protein Interaction (PPI) Network

The PPI network (PPIN) was constructed in Cytoscape using the STRING plugin to analyse connections among the identified common genes. The zebrafish Protein-Protein Interaction Network (PPIN) comprises 16 nodes and 27 edges after removing nodes without internal connections. In contrast, the human PPIN consists of 56 nodes and 135 edges. Common 9 nodes were identified in both PPINs, exhibiting 25 and 67 interactions (edges) in zebrafish and human, respectively. These nodes are conserved across the PPINs of both organisms, including IL6, IL1B, JUN, RELA, IL10, MAPK3, MAPK1, CASP9, and PARP1 (Figure 6).

3.5 Disease Enrichment Analysis

Diseases like Alzheimer's disease, type 2 diabetes mellitus, Behcet's disease, renal cell carcinoma, hypertension, myocardial infarction, and pancreatic cancer were commonly present in both organisms. In zebrafish, transient cerebral ischemia, type 1 diabetes mellitus, and cataract were highly enriched. In humans, middle cerebral artery infarction, pulmonary emphysema, and asthma were highly enriched (Figure 7).

4. Discussion

Heavy metals such as arsenic, cadmium, chromium, mercury, and lead are major environmental pollutants that pose serious health problems to both humans and wildlife. Arsenic, a common groundwater contaminant, is associated with skin lesions, cancers, and cardiovascular problems, and studies in zebrafish have confirmed its role in developmental disruptions and oxidative stress[54,55]. Cadmium affects kidneys and bones, as evidenced by kidney dysfunction and skeletal damage in zebrafish[56,57]. Chromium (Cr VI) is linked to lung cancer and DNA damage, and zebrafish experiments reveal related inflammation and developmental delays[58,59]. Methylmercury is a neurotoxin that harms fetal brain development and cognitive function, as shown by zebrafish linking it to neural damage and oxidative stress[60,61]. Lead exposure can result in cognitive impairment, behavioural changes, and kidney damage, and zebrafish studies have revealed disruptions in neural pathways[62,63]. Combined exposure of heavy metals such as mercury, cadmium, lead, and arsenic synergistically disrupts redox homeostasis, damages proteins and DNA, impairs mitochondrial function, and disturbs metal regulation through oxidative stress, altered signalling, competitive biomolecular binding, and compromised antioxidant and detoxification systems, often resulting in toxicity greater than additive individual effects. Although the individual effects of each heavy metal are well documented, the combined toxicity of multiple metals remains poorly understood, raising serious concerns about long-term human health.

In this study, we have used *in silico* toxicogenomic data mining to identify genes, their interactions, and the molecular pathways they regulate. Our objective was to identify genes shared between datasets and investigate the impact of heavy metals on these common genes in both zebrafish and humans, noting variation in the number of shared genes. We identified eight key transcription factors, such as GATA1, JUND, ETS1, FOS, MYOG, MAFK, TFAP2A, and TFAP2C, that act as central

regulators of gene expression networks, controlling cellular identity and responses to environmental and developmental signals. GATA1 is important for blood cell development[64,65], while JUND and FOS are essential in cell proliferation and stress responses[66]. ETS1 contributes to immune system development and tissue repair[67], MYOG handles muscle differentiation[68], MAFK helps manage oxidative stress[69], and TFAP2A/C are essential in embryogenesis and cancer[70,71]. Network analyses revealed that these transcription factors interact synergistically, fine-tuning gene expression by regulating chromatin remodelling[72,73]. Disruption of these factors has been associated with numerous diseases, underscoring their potential as therapeutic targets. Further experimental validation using techniques such as ChIP-seq and CRISPR is recommended to confirm these findings.

Comparing Gene Ontology (GO) terms between zebrafish and humans reveals that many biological processes and molecular functions related to chemical and environmental stress responses are highly conserved. Both species show significant enrichment in BP related to stress responses, chemical reactions, and cellular adaptation to chemical stimuli, indicating that they rely on comparable mechanisms to detect and respond to environmental challenges, offering valuable insights for toxicology studies, especially those focused on heavy metal exposure[48,74,75]. Zebrafish are validated as a model organism, exhibiting conserved activation of stress-responsive transcription factors and detoxification enzymes, such as cytochrome P450[76,77]. In terms of MF, both organisms share activities critical for redox balance and signalling, including oxidoreductase activity, cytokine receptor binding, and protein-disulfide reductase activity, which support conserved antioxidant defences, such as glutathione metabolism[78]. Processes such as glutathione biosynthesis in zebrafish and oxidative stress responses in humans further underscore this conservation[79]. Shared MAP Kinase (MAPK) signalling also shows common regulatory pathways in environmental and inflammatory responses[80,81]. These overlaps reinforce the translational relevance of zebrafish for modelling human molecular functions and stress-related diseases in toxicogenomics analysis[82]. Analysis of common KEGG pathways between the two species shows conserved biological processes, including apoptosis and C-type lectin receptor signalling, which are central to programmed cell death and innate immunity[83-85]. Infection-related pathways, such as Salmonella infection, further demonstrate conserved host-pathogen interactions, supporting the use of zebrafish in infectious disease analysis[86]. Beyond exact matches, zebrafish pathways in MAPK, mTOR, p53 signalling, autophagy, and necrosis closely mirror human pathways involved in cancer, metabolism, and neurodegeneration[87,88]. Experimental studies in zebrafish cardiac disease models show that inhibiting MAPK or mTOR can rescue cardiomyopathy phenotypes, reflecting functional conservation[89,90]. Similarly, mTOR inhibition in zebrafish modulates senescence and angiogenesis in ways comparable to mammals[91]. Key molecular regulators, such as Dusp6, influence MAPK signalling and tissue repair in both zebrafish and mammals[92,93], while IGF signalling activates MAPK and PI3K pathways in zebrafish as it does in humans[94]. Cross-species transcriptomic analyses confirm a high degree of pathway correspondence, further supporting zebrafish as a valuable model for studying human disease, toxicology, and signalling biology[95,96]. Overall, these conserved KEGG pathways provide a strong mechanistic basis for using zebrafish to model human diseases.

Several common genes, including IL6, IL1B, JUN, RELA, IL10, MAPK3, MAPK1, CASP9, and PARP1, act as conserved molecular hubs that regulate inflammation, immune responses, stress signalling, apoptosis, and DNA repair in both zebrafish and humans. This underscores the value of zebrafish as a model for studying human disease[97-99]. Pro-inflammatory cytokines IL6 and IL1B drive immune activation and are implicated in autoimmune diseases and cancer, with IL6 signalling through both JAK/STAT3 and MAPK pathways[100-103]. The transcription factors JUN, which form the AP-1 complex with c-Fos, and RELA, a key NF-κB subunit, coordinate cellular stress and inflammatory responses across species[104-106]. In contrast, IL10 functions as an anti-inflammatory cytokine, inhibiting NF-κB and MAPK signalling through STAT3, a mechanism conserved in zebrafish and humans[107,108]. Key kinases MAPK1 (ERK2) and MAPK3 (ERK1) regulate proliferation, differentiation, and apoptosis by modulating transcription factors such as AP-1 and NF-κB[109,110]. The DNA repair enzyme PARP1 facilitates poly-ADP-ribosylation, though excessive activation can deplete cellular energy and trigger cell death[111,112]. CASP9 initiates intrinsic apoptosis and supports central nervous system development and immune function; its dysregulation is linked to disease[113,114]. Together, these genes form a conserved network that balances survival signalling through IL6/STAT3, NF-κB/RELA, and PARP1

and apoptosis via CASP9. This balance is essential for regulating inflammation, stress resilience, and potential therapeutic targets across species.

The shared links to diseases in both zebrafish and humans, such as Alzheimer's disease, type 2 diabetes (T2DM), hypertension, heart attacks, and various cancers, underscore how deeply conserved many essential molecular pathways are across vertebrate species. Zebrafish carry orthologs of many human disease genes, including those linked to Alzheimer's (e.g., APP, presenilin 1/2, ApoE), and exhibit similar neuropathological features when experimentally manipulated, such as neurodegeneration and impaired neurogenesis following Amyloid-β42 injections[115,116]. In diabetes research, overfed zebrafish develop elevated blood glucose levels, insulin resistance, and transcriptomic profiles that closely resemble those of human T2DM[117,118]. Zebrafish models of diabetic nephropathy reproduce key hallmarks of human kidney disease and involve shared signalling pathways, such as PI3K/Akt[118,119]. Cardiovascular studies show that Angiotensin II–induced cardiac hypertrophy and fibrosis in zebrafish mirror human hypertension and cardiac remodelling[120,121]. Moreover, zebrafish heart regeneration after cryoinjury provides valuable insights into the limited repair mechanisms in the human heart[122]. These comparisons demonstrate that zebrafish are powerful models for understanding disease mechanisms and for guiding drug discovery with strong translational potential.

Zebrafish are an important vertebrate model for studying human disease, and when combined with toxicogenomic databases and multi-omics approaches, they provide powerful tools for toxicity screening and risk assessment[123-126]. Their advantages for biomedical research are notable: zebrafish have remarkable regenerative capacities in organs such as the brain, heart, pancreas, and kidney, and their transparent embryos allow live imaging, high-throughput screening, and precise genetic manipulation. These features make zebrafish particularly valuable for investigating disease mechanisms and gene functions that are difficult to study directly in humans. However, there are inherent species differences, including lifespan, immune system complexity, organ structure, and certain pathological features, so findings in zebrafish often need validation in mammalian models or human tissues. Additional limitations include potential biases in the data, lack of direct causality, tissue-specific effects, and incomplete dose-response information, all of which can limit the interpretation of the results[127].

5. Conclusion

Zebrafish share extensive molecular, genetic, and pathway-level conservation with humans in their responses to heavy metal toxicity and disease processes. Key transcription factors, conserved genes, and signalling pathways, including MAPK, mTOR, and p53, make zebrafish a powerful translational model for toxicogenomic and biomedical research. The MAPK pathway regulates cell growth and stress responses, mTOR controls cell growth and metabolism in response to nutrient availability, and p53 maintains genomic stability by halting the division of damaged cells or triggering their programmed death. Together, these pathways determine whether a cell grows, divides, repairs itself, or undergoes apoptosis, and their dysregulation is closely linked to cancer and other diseases. The substantial overlap in molecular functions and disease associations, such as Alzheimer's disease, diabetes, heart disease, and cancer, further underscores the translational relevance of zebrafish. By integrating *in silico* analyses with experimental validation, zebrafish serve as a bridge between environmental toxicology and human health risk assessment. Nonetheless, complementary studies in mammalian models are essential to account for species-specific effects and improve the accuracy of translational applications. Using multi-omics approaches across species and employing gene-editing techniques in zebrafish, alongside human cell assays, can validate conserved heavy-metal response pathways, advancing biomarker discovery and therapeutic strategies for metal-induced diseases. This reinforces zebrafish as a valuable model for studying human toxicology and disease mechanisms.

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Authors' contributions

Both authors contributed to the design, execution, analysis, and reporting of this work.

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