Environmental Research 236 (2023) 116835

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Occupational exposure to potentially toxic elements alters gene expression profiles in formal and informal Brazilian workers

Fernanda Junqueira Salles [a,h,*], Ilias S. Frydas [b,c], Nafsika Papaioannou [b,c], Dayna R. Schultz [b,c], Maciel Santos Luz [d], Marcelo Macedo Rogero [e], Dimosthenis A. Sarigiannis [b,c,f,g], Kelly Polido Kaneshiro Olympio [a,h,**]

[a] Department of Environmental Health, School of Public Health, University of Sao Paulo, Av. Dr. Arnaldo, 715, Cerqueira Cesar, CEP 01246-904, São Paulo, SP, Brazil
[b] Environmental Engineering Laboratory, Department of Chemical Engineering, Aristotle University of Thessaloniki, Thessaloniki, 54124, Greece
[c] HERACLES Research Center on the Exposome and Health, Center for Interdisciplinary Research and Innovation, Balkan Center, Bldg. B, 10th Km Thessaloniki-Thermi Road, 57001, Greece
[d] Laboratory of Metallurgical Process, Institute for Technological Research, Sao Paulo, SP, Brazil
[e] Nutritional Genomics and Inflammation Laboratory, Department of Nutrition, School of Public Health, University of Sao Paulo, 01246-904 São Paulo, Brazil
[f] National Hellenic Research Foundation, Athens, Greece
[g] Environmental Health Engineering, Science, Technology and Society Department, School for Advanced Study (IUSS), Pavia, Italy
[h] The Human Exposome Research Group/ Expossoma e Saúde do Trabalhador – eXsat, School of Public Health, University of Sao Paulo, Av. Dr. Arnaldo, 715, Cerqueira César, Sao Paulo, SP, 01246-000, Brazil

ARTICLE INFO

Handling Editor: Jose L Domingo

Keywords:
Occupational exposure
Toxic elements
Inflammation
Immune response

ABSTRACT

Chemical elements, such as toxic metals, have previously demonstrated their ability to alter gene expression in humans and other species. In this study, microarray analysis was used to compare the gene expression profiles of different occupational exposure populations: a) informal workers who perform soldering of jewelry inside their houses (n = 22) in São Paulo (SP) State; and b) formal workers from a steel company (n = 10) in Rio de Janeiro (RJ) state, Brazil. Control participants were recruited from the same neighborhoods without occupational chemical exposure (n = 19 in SP and n = 8 in RJ). A total of 68 blood samples were collected and RNA was extracted and hybridized using an Agilent microarray platform. Data pre-processing, statistical and pathway analysis were performed using GeneSpring software. Different expression was detected by fold-change analysis resulting in 16 up- and 33 down-regulated genes in informal workers compared to the control group. Pathway analysis revealed genes enriched in MAPK, Toll-like receptor, and NF-kappa B signaling pathways, involved in inflammatory and immune responses. In formal workers, 20 up- and 50 down-regulated genes were found related to antimicrobial peptides, defensins, neutrophil degranulation, Fc-gamma receptor-dependent phagocytosis, and pathways associated with atherosclerosis development, which is one of the main factors involved in the progression of cardiovascular diseases. The gene IFI27 was the only one commonly differentially expressed between informal and formal workers and is known to be associated with various types of cancer. In conclusion, differences in gene expression related to occupational exposure are mainly associated with inflammation and immune response. Previous research has identified a link between inflammation and immune responses and the development of chronic diseases, suggesting that prolonged occupational exposures to potentially toxic elements in Brazilian metal workers could lead to negative health outcomes. Further analysis should be carried out to investigate its direct effects and to validate causal associations.

* Corresponding author.
** Corresponding author.
E-mail addresses: fjsalles@usp.br (F.J. Salles), ilias.frydas@gmail.com (I.S. Frydas), nafsikapapaioannou@gmail.com (N. Papaioannou), dayna.schultz@usask.ca (D.R. Schultz), macielluz@ipt.br, maciel.luz@gmail.com (M.S. Luz), mmrogero@usp.br (M.M. Rogero), sarigiannis@auth.gr, d.a.sarigiannis@gmail.com (D.A. Sarigiannis), kellypko@usp.br (K.P.K. Olympio).

https://doi.org/10.1016/j.envres.2023.116835
Received 26 May 2023; Received in revised form 14 July 2023; Accepted 3 August 2023
Available online 4 August 2023

1. Introduction

The transcriptome is the set of RNA molecules synthesized during the transcription process of an organism, organ, tissue, or cell and therefore directly reflects the gene expression of an organism, organ, tissue, or cell (Passos, 2014; Pećina-Šlaus and Pećina, 2015; DeBord et al., 2016). Hence, transcriptomics is the identification of total RNAs expressed in each organism, the study of interactions between these genes and the environment, characterization of polymorphisms associated with transcribed genes, recognition of markers for molecular diagnosis of diseases, and may indicate changes in the proteome and/or metabolome (Van Breda et al., 2015; DeBord et al., 2016). A common technology utilized in transcriptomics and more specifically in gene expression and the analysis of mRNAs is microarray technology. The latter is capable of simultaneously measuring thousands of genes and is an effective method to identify gene expression patterns. Microarray has been widely utilized in the biomedical field to study complex biological processes, including disease progression and cellular signaling pathways. They have also proven extremely useful in the field of environmental toxicology to determine subtle differences in gene expression profiles associated with specific environmental exposure scenarios (Korashy et al., 2017; Espín-Pérez et al., 2018; Papaioannou et al., 2021).

Some chemical elements found in the environment have already demonstrated their ability to change the transcriptome, among them benzene, dioxins, phthalates, polycyclic aromatic hydrocarbons, diesel, metals, metallic fumes, and tobacco (Smith et al., 2005; van Leeuwen et al., 2006; Wild et al., 2013; Korashy et al., 2017; Papaioannou et al., 2021). According to Idaghdour et al. (2008), 30% of the transcriptome can be altered by different environmental conditions, which can be represented by a combination of biotic, abiotic, and cultural factors. Whereas toxic elements can effectively cause gene expression alterations, and thus, studying molecular responses on the blood transcriptome in exposed human populations might elucidate in more detail the biological processes associated with these chemicals (Wild et al., 2013; Espín-Pérez et al., 2018; Papaioannou et al., 2021).

Among the sources of exposure that humans are subjected to during life, a very important one is occupational exposure, especially considering that a high portion of adult life is spent in working hours. Whole blood transcriptomic analysis performed in adults exposed to metal fumes in a U.S. cohort study, identified differences in the expression of genes related to inflammatory responses, oxidative stress, cell cycle, and apoptosis (Wang et al., 2005). The working population can be occupationally exposed to hazardous chemicals that cause toxicity, and dysregulation in gene expression which leads to negative health outcomes (Pronk et al., 2022) and it is therefore pertinent to identify the hazards associated with certain occupations in order to reduce the country-wide burden of disease and enforce safer workplace practices. The Global Burden of Disease (GBD) estimates that environmental and occupational risks in Brazil are responsible for 1.39% of total deaths by chronic respiratory diseases and for 3.57% of deaths by cardiovascular diseases, being that the main causes of death in the country, with stroke and ischemic heart disease accounting for over 20% of deaths.

The city of Limeira in Brazil is known as the capital of plated jewelry. The production of jewelry and fashion jewelry in the municipality is characterized by a high level of informality and outsourcing in the production stages, and thus, a significant percentage of industrial work is being replaced by improvised work in homes (Ferreira et al., 2019; Barrozo et al., 2022). Metal parts are hand-crafted manipulating manually a welding torch or acids without adequate occupational hygiene and the use of protective equipment (Lacorte et al., 2013; Ferreira et al., 2019; Barrozo et al., 2022). The city of Volta Redonda is the home of one of the largest steel companies in Latin America, which produces approximately 5.8 million tons of steel annually (CSN, 2017). The main production units are the coke oven, sintering, steelworks, continuous casting, hot and cold rolling, zinc plating, chroming, and electrolytic tinning. These processes involve activities known to impact the environment and the local population health due to the use and disposal of chemical compounds such as polycyclic aromatic hydrocarbons, potentially toxic elements, and solvents (Chen et al., 2015).

The aim of this study is to compare the gene expression profiles of different occupational exposure populations: informal workers from Limeira city in São Paulo state (SP) and formal workers from Volta Redonda city in Rio de Janeiro State (RJ) state, Brazil. To do so, blood samples were collected from volunteers and subjected to microarray analysis and then analyzed to identify trends and differences. Human transcriptomic analysis methodologies are rarely used in Brazil and are important to deepen the knowledge about the effects caused by occupational exposure to potentially toxic elements.

2. Methodology

The present study is part of a larger research investigation entitled, "The 'omics' era applied to society: the impact of formal and informal labor on the exposome of workers with an emphasis on metabolomics, transcriptomics and lipidomics" by the Human Exposome Research Group of the School of Public Health, University of São Paulo. All individuals participating in this study were volunteers and reside in two Brazilian cities. Ethical approval was obtained from the Institutional Review Board of the School of Public Health of the University of Sao Paulo (Protocol N° 32580820.8.0000.5421).

2.1. Informal work population

The first population who participated in this study resides in Limeira, State of São Paulo. The participants were recruited and selected with the assistance of the Health Secretariat of Limeira city and Family Health Care Centers of the Brazilian Public Health System. The local community health workers indicated possible participants and helped in researcher communication. All selected informal workers sold jewelry at home using torches or acids.

2.2. Formal work population

The second population who participated in this study resides in Volta Redonda, State of Rio de Janeiro. The participants from this population were selected by snowball technique where an initial number of subjects from the target population were selected and these subjects then identified other potential participants. Steelworkers included in this study carry out activities in different sectors of the company, such as coke plant, galvanization, chrome plating, and maintenance (90% of them perform soldering, the only one who did not carry out this activity worked in the coke plant). The average number of years working in the steel industry was 10 (minimum: 2 years, maximum: 22 years).

2.3. Study selection

To control the genetic variability that exists in the transcriptome and ensure the reliability of results, certain criteria were adopted for study population selection. These criteria were defined based on the existing transcriptome literature, which selects healthy individuals, since the effects of age, diet, disease, and drug consumption may alter gene expression (Whitney et al., 2003; Bahr et al., 2013; McHale et al., 2013; Vrijheid et al., 2014). Exclusion criteria were: a) aged under 18 years and over 50 years; b) athletes; c) smokers; d) pregnant women; e) women with menopausal symptoms; f) diagnosed with cancer and/or autoimmune disease; g) use of anti-inflammatory drugs in the last 30 days before recruitment.

The exposed group was composed of formal and informal workers from each city while the control group was recruited from the same neighborhoods as the exposed participants (at least four houses away) and of which none worked in activities with known chemical exposures. Individuals who were omitted through the exclusion criteria and who

agreed to sign the consent form were included in this study.

In addition, a specific criterion was considered for each of the populations. For Limeira, the population was composed exclusively of females since most informal workers were women who carry out soldering activities at home while also undertaking domestic duties and childcare responsibilities. In the city of Volta Redonda, the population profile was the opposite, since most formal steelworkers were men; therefore, only male individuals were included in this sample.

Therefore, in Limeira, the participants (all female, n = 41) were divided into the exposed group (n = 22) composed of informal outsourced workers who perform soldering of jewelry and fashion jewelry inside their houses; and the control group (n = 19). In Volta Redonda, participants (all male, n = 18) were divided into an exposed group (n = 10) composed of formal steelworkers and a control group (n = 8).

2.4. Sample collection

Blood samples (2.5 mL) were collected in Paxgene Blood RNA tubes (PreAnalytix, Qiagen) for transcriptomics analysis. Additional 6 mL of whole blood was collected in heparinized tubes free of trace elements (Vacutainer®) for potentially toxic elements (PTEs) determination. In Limeira, sample collection was carried out between October and November 2019 (n = 41), whereas, in Volta Redonda, sample collection occurred between November and December 2021 (n = 18). All participants were instructed to refrain from any type of physical exercise 48 h before the blood collection and to avoid consumption of any type of alcoholic beverage for 1 week prior to blood collection. The research team provided the last meal of the day before the collection to standardize the dietary influence on the findings. Blood was drawn by qualified nurses and transported on ice to the Laboratory of Human Exposure to Environmental Contaminants (LEHCA) of the University of São Paulo, Brazil, where the samples were frozen and stored at −80 °C until downstream analysis.

All participants completed questionnaires about demographic characteristics (education level, ethnicity, and length of time residing in the region) and lifestyle habits (yes/no) including alcoholic consumption, physical activity level, and use of medications. Body mass index (BMI) values were calculated using height and weight measured on the day of sample collection. The BMI classification follows those laid out by the World Health Organization (WHO, 2000). Glucose, insulin, and lipid profile exams were performed on all participants. The HOMA-IR value, a method used to estimate insulin resistance, was also calculated based on the measured glucose and insulin values. HOMA-IR values above 2.71 indicated insulin resistance (Geloneze et al., 2006, 2009). Additionally, plasma samples collected by the research group were used to quantify high-sensitivity C-reactive protein (hs-CRP) by immunoturbidimetry.

2.5. Potentially toxic elements determination

Levels of PTEs in blood were determined by Triple Quadrupole Inductively Coupled Plasma Mass Spectrometry (iCAP TQ ICP-MS, Thermo Fisher ScientificTM, Bremen, Germany) at the Institute for Technological Research. Blood aliquots (200 μL) were diluted (1:50) with 1 mL of internal standard solution and then made up to 10 mL with a diluent of 0.01% (w/v) of Triton X-100 and 0.5% (v/v) of doubled distilled HNO_3 (Merck®). Quality control of the analytical results was performed with the use of calibration curves with mono-elemental standard solutions (Ga, Ir, Y, and Tb), blank, and the certified reference material (Seronorm® TE Whole Blood Level II - Stasjonsveien) for blood. All dilutions and blanks analyses were performed in triplicate (n = 3).

2.6. Microarray analysis

Blood samples were transported by a certified biomaterial transportation company (AmerisourceBergen Corporation, World Courier) on dry ice at −80 °C to Thessaloniki, Greece. Sample preparation, RNA isolation, and microarray analysis were performed at the HERACLES Research Center (Health and Exposome Research: Assessing Contributors to Lifetime Exposure and State of Health) at the Center for Interdisciplinary Research and Innovation (KEDEK) in Greece.

According to the manufacturer's instructions, RNA was extracted with a Paxgene Blood RNA kit (PreAnalytiX Company, 2015). Briefly, 2.5 mL of sample was incubated for 2 h at room temperature in PAXgene® Blood RNA tubes and centrifuged to obtain cell pellets that were incubated with a binding buffer and proteinase K. The resultant lysate was centrifuged in a PAXgene® Shredder spin column, and the supernatant was mixed with 100% ethanol. A DNase mix was added to remove any DNA fragments. The purified RNA was eluted with elution buffer and denatured by incubation at 65 °C for 5 min. RNA concentration and quality were analyzed by spectrophotometric measurement (A260/A280 ratios) using a Nanodrop ND-1000 instrument (Marshall Scientific, Hampton, NH, USA). All analyzed samples were of >1.8 A260/280 ratios. Samples with low RNA Integrity Number (RIN) values or low RNA concentration after extraction were appropriately excluded to ensure reliable data (n = 8 samples from RJ, n = 1 sample from SP). The final RNA samples were stored at −20 °C until microarray analysis.

The isolated RNA was reversed transcribed and labeled with Cy3 in a cDNA synthesis reaction following the "Low input quick amp labeling protocol G4140-90040 version 6.5" (Agilent, 2015). Agilent RNA Spike-In mix was added to 200 ng of total RNA used for the reactions to monitor both labeling reactions and microarray performance. After the first step of sample purification, dye incorporation, and cRNA yield were checked with Nanodrop ND-1000 (Marshall Scientific, Hampton, NH, USA). Then, samples were hybridized on the SurePrint G3 Human Gene Expression 8 × 60 K v2 Microarray ID:039494 (Agilent Technologies, Inc., CA). After hybridization and post-hybridization washes, microarrays were scanned for the quantification of signals in a SureScan Microarray Scanner (Agilent Technologies, Inc., CA). QC reports were used to evaluate microarray performance. Raw data were extracted using Feature Extraction software (version 10.7.1.1; Agilent Technologies) and feature extraction files were imported into Genespring GX 13.1.1 software (Agilent Technologies, Inc., CA) for further analysis. The microarray quality parameters are presented in Table S1 of the Supplementary Material.

2.7. Data processing and statistical analysis

Statistical analysis of PTEs results in blood was performed using STATA 13.1 software (STATA Corporation, TX, USA). Elements, where blood concentrations were below the limit of detection (LD), were assigned a value of $LD/\sqrt{2}$ (Croghan and Egeghy, 2003). Differences in blood concentrations between the Exposed and Control groups were tested by the Mann-Whitney test (non-parametric).

Transcriptomic data pre-processing included filtering of compromised and non-detected probes and quantile normalization. The resulting entity list was further filtered to remove control and alignment probes from the analysis as well as probes with less than a 50% coefficient of variation. Statistical evaluation included a fold change analysis (FC) to identify genes with different expression ratios in terms of up- or down-regulation between exposed and control conditions (FC > 2). Hierarchical clustering analysis was performed to visualize differences between samples based on their gene expression profiles. Principal component analysis (PCA) was employed for visual exploration providing information on the overall structure of the analyzed dataset, sample discrimination, and analytical performance.

Differentially expressed genes (DEGs) were mapped to available pathways from WikiPathways and KEGG databases. Gene ontology (GO) analysis was performed on PANTHER 14.1 (Mi et al., 2019). A network analysis was performed on NetworkAnalyst 3.0 online platform to investigate Gene-Disease Associations (Zhou et al., 2019).

3. Results

3.1. Participant characteristics

Participants from Limeira, Sao Paulo State (SP), Brazil (n = 41) were all females (mean age: 33.5 ± 9.2 years; mean BMI: 27.65) of these, less than 50% had completed high school, more than 36% considered themselves to be white and more than 50% lived in the region between 10 and 20 years. Participants from Volta Redonda, Rio de Janeiro State (RJ), Brazil (n = 18) were all males (mean age: 31.4 ± 8.9 years; mean BMI: 25.76) of these, more than 60% had completed high school, more than 50% considered themselves to be of mixed race and more than 70% lived in the region for more than 20 years. Insulin, glucose, lipid profile, and hs-CRP levels were not significantly different between exposed and control groups for both populations. Demographic variables, such as ethnicity and educational level, also showed no significant differences. A detailed volunteer profile is presented in the Supplementary material (Table S2 for SP and Table S3 for RJ).

The concentration of 11 elements in the blood was determined for arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), mercury (Hg), manganese (Mn), nickel (Ni), lead (Pb), strontium (Sb), tin (Sn), and zinc (Zn). The geometric mean (GM), the 95% confidence interval (CI), and the 95th percentile of each of the PTEs in each population are shown in Table 1. Blood Cd and Pb concentrations were significantly higher in the exposed group than in the control group from the city of Limeira. In the city of Volta Redonda, no statistically significant difference was observed between the groups. The contribution of each PTE in each study group is shown in the Supplementary material (Fig. S1).

3.2. Transcriptomics results of informal workers in limeira

Differential gene expression analysis revealed 16 up- and 33 down-regulated genes (FC > 2) in the informal workers compared to controls. Fig. 1 shows the expression of DEGs in each sample; the resulting list of pathways (p-value <0.05) and their functions are presented in Table 2.

The results indicate that many of the enriched pathways associated with differentially expressed genes are related to the inflammation process and immune response. Some of the significant pathways ($p < 0.05$) are involved in MAPK signaling and cascade or are mediated by MAP kinases, in addition, there are significant chemokine, Toll-like receptor, and NF-kappa B signaling pathways.

Functional classification was performed using gene ontology (GO) analysis on the DEGs to investigate results associated with molecular functions and biological processes in informal work-related occupational exposure (Fig. 2). The top GO terms identified for biological processes were “cellular processes”, “biological regulation”, and “metabolic processes”. Regarding molecular functions, in the top GO terms were “binding” and “catalytic activity”.

The gene-disease network showed one of the DEGs, the CCL4, is associated with the inflammation process, atherosclerosis, and myocardial ischemia, among other outcomes (Fig. 3).

3.3. Transcriptomics results of formal workers in Volta Redonda

Differential gene expression analysis revealed 20 up- and 50 down-regulated genes in the formal workers compared to controls (FC > 2). Fig. 4 depicts the status of gene expression of each of the detected DEGs across samples and the resulting list of enriched pathways (p-value <0.05) found in association with formal occupational exposure. The list of all genes and their functions is presented in Table 3.

The results indicate that many of the enriched pathways associated with the perturbed genes are involved in immune response and with the process of atherosclerosis development. Antimicrobial peptides, defensins, neutrophil degranulation, and phagocytosis dependent on the Fc gamma receptor pathways play a defensive role in the immune system. Whilst, scavenger receptor pathways, pathways of cell surface interactions in the vessel wall, and the pathway of smooth muscle cell proliferation and differentiation can be related to atherosclerosis.

Functional classification was performed using gene ontology (GO) analysis on the DEGs to investigate results associated with molecular functions and biological processes in formal work-related occupational exposure (Fig. 5). The top GO terms identified for biological processes were “cellular processes”, “metabolic processes”, and “response to a

Table 1
Geometric mean (GM), confidence interval (CI 95%), and 95th percentile of potentially toxic elements (PTEs; given in μg L^{-1}) detected in the blood, of participants in Exposed and Control groups from Limeira and Volta Redonda. The p-value is provided for comparisons between exposure groups from the respective cities.

PTEs	LIMEIRA					VOLTA REDONDA				
	Exposed		Control			Exposed		Control		
	95th percentile	GM (CI 95%)	95th percentile	GM (CI 95%)	p-value	95th percentile	GM (CI 95%)	95th percentile	GM (CI 95%)	p-value
As	1.11	0.45 (0.36–0.57)	7.45	0.37 (0.24–0.56)	0.17	1.47	0.74 (0.53–1.02)	2.45	0.92 (0.57–1.48)	0.37
Mn	17.7	7.57 (6.22–9.20)	13.4	7.88 (6.70–9.28)	0.52	14.63	9.72 (7.98–11.82)	14.77	9.88 (7.84–12.45)	0.91
Ni	6.48	0.81 (0.27–2.42)	5.75	1.29 (0.42–3.93)	0.35	–	–	–	–	–
Cd	6.27	0.36 (0.20–0.66)	0.53	0.04 (0.01–0.09)	<0.01	1.03	0.21 (0.05–0.90)	1.70	0.19 (0.03–1.11)	0.89
Cr	6.09	1.05 (0.75–1.47)	6.96	1.22 (0.90–1.65)	0.21	–	–	–	–	–
Sb	2.96	2.14 (1.98–2.32)	2.93	2.10 (1.94–2.28)	0.62	3.21	0.97 (0.68–1.39)	1.88	1.04 (0.75–1.45)	0.75
Sn	1.32	0.80 (0.65–0.98)	1.42	0.85 (0.66–1.08)	0.72	12.61	6.29 (4.85–8.15)	9.89	6.52 (5.17–8.21)	0.96
Cu	1818	1163 (1073–1261)	1744	1181 (1088–1283)	0.61	1013	811.17 (732.56–898.21)	1028	785.19 (677.06–910.59)	0.71
Zn	7076	4228 (3721–4804)	6774	4737 (4306–5211)	0.92	6778	5584 (4984–6255)	8029	6444 (5719–7260)	0.06
Pb[a]	5.01	1.37 (0.98–1.93)	2.03	0.86 (0.69–1.07)	0.02	3.60	2.17 (1.75–2.69)	4.03	1.97 (1.45–2.68)	0.68
Hg	2.69	1.11 (0.93–1.33)	6.06	1.02 (0.79–1.33)	0.29	2.42	0.38 (0.20–0.71)	5.30	0.55 (0.11–2.83)	0.16

[a] Pb value given in μg dL^{-1}.

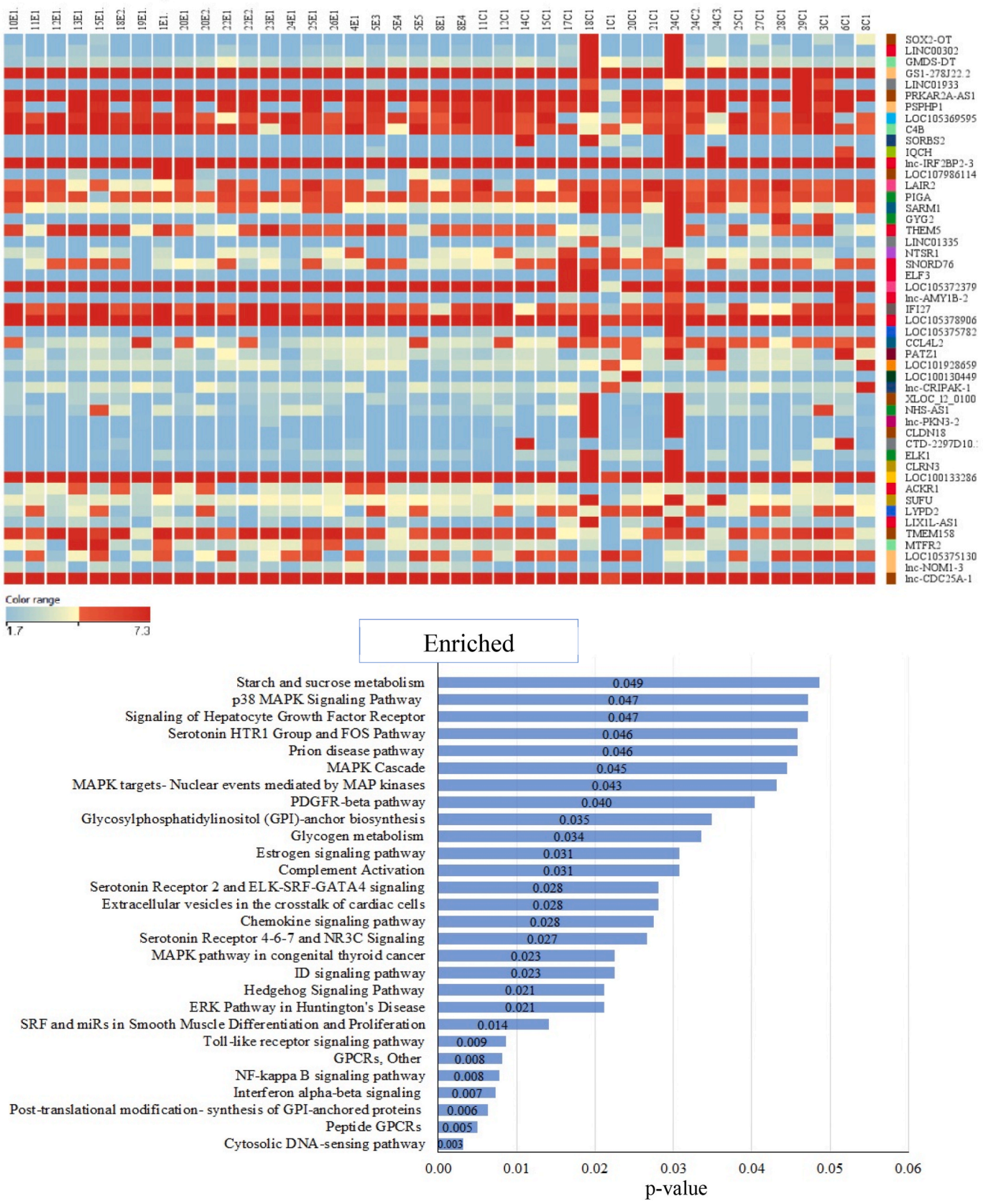

Fig. 1. Top: Heat map of the 49 significantly differentially expressed genes (DEGs) in informal workers compared to controls from Limeira where columns represent samples and rows represent genes and red depicts upregulation while blue depicts downregulation. Bottom: Enriched biological pathways and respective p-values associated with the perturbed DEGs.

Table 2
Gene Symbol, name, direction, and function of the 49 differentially expressed genes detected between Control and Exposed groups from Limeira.

Gene Symbol	Gene Name	Regulation	Function
CCL4	C–C motif chemokine ligand 4 like 2	down	Chemokine receptor binding, signaling receptor activity, cytokine activity.
ELK	ETS transcription factor ELK1	down	RNA polymerase II transcription regulatory region sequence-specific DNA binding. DNA-binding transcription factor activity.
NTSR1	Neurotensin receptor 1	down	Neuropeptide binding and receptor activity.
ELF3	E74 like ETS transcription factor 3	down	DNA-binding transcription activator activity, RNA polymerase II-specific and binding activity.
SUFU	SUFU negative regulator of hedgehog signaling	down	Transcription factor binding.
PIGA	Phosphatidylinositol glycan anchor biosynthesis class A	down	Acetylglucosaminyltransferase activity.
THEM5	Thioesterase superfamily member 5	up	Thioester hydrolase activity.
IFI27	Interferon alpha inducible protein 27	up	Interferon alpha-inducible protein 27.
PATZ1	POZ/BTB and AT hook containing zinc finger 1	down	Regulation of cytokine production, negative regulation of transcription by RNA polymerase II, regulation of immune system process.
MTRF2	Mitochondrial fission regulator 2	up	Unknown.
CLDN18	Claudin 18	down	Cell adhesion and the bicellular tight junction assembly.
C4B	Complement C4B	up	Carbohydrate, immune complex, and complement binding.
SORBS2	Sorbin and SH3 domain containing 2	down	Protein and RNA binding, cytoskeletal anchor activity.
IQCH	IQ motif containing H	down	Molecular function. May play a regulatory role in spermatogenesis.
LAIR2	Leukocyte-associated immunoglobulin-like receptor 2	down	Protein binding, encodes a protein member of the immunoglobulin superfamily.
SARM1	Sterile alpha and TIR motif containing 1	down	Protein binding, NAD + nucleosidase activity, cyclic ADP-ribose generating.
GYG2	Glycogenin 2	down	Glycosyltransferase activity.
CLRN3	Clarin 3	down	Protein binding.
ACKR1	Atypical chemokine receptor 1	up	Chemokine binding, signaling receptor activity.
LYPD2	LY6/PLAUR domain containing 2	down	Unknown.
TMEM158	Transmembrane protein 158	up	Receptor for brain injury-derived neurotrophic peptide (BINP), a synthetic 13-mer peptide.
SNORD76	Small nucleolar RNA, C/D box 76	down	Non-coding
NHS-AS1	NHS antisense RNA 1	down	Non-coding
LIX1L-AS1	LIX1L antisense RNA 1	down	Non-coding
LINC01335	Long intergenic non-protein coding	down	Non-coding
lnc-IRF2BP2-3	lnc-IRF2BP2-3:1	up	Non-coding
lnc-AMY1B-2	lnc-AMY1B-2:1	down	Non-coding
lnc-CRIPAK-1	lnc-CRIPAK-1:4	down	Non-coding
lnc-PKN3-2	lnc-PKN3-2:1	down	Non-coding
lnc-NOM1-3	lnc-NOM1-3:1	up	Non-coding
lnc-CDC25A-1	lnc-CDC25A-1:1	up	Non-coding
SOX2-OT	SOX2 overlapping transcript	down	Non-coding
LINC00302	Long intergenic non-protein coding	down	Non-coding
GMDS-DT	GMDS divergent transcript	down	Non-coding
LINC01933	Long intergenic non-protein coding	down	Non-coding
PRKAR2A-AS1	PRKAR2A antisense RNA 1	up	Non-coding

Differentially expressed genes classified as uncharacterized were not included in this table.

stimulus". Regarding molecular functions, in the top GO terms were similar to informal workers with "binding activities" and "catalytic activities".

The gene-disease network showed that the genes NKX2-5, NDUFAF4, and FHL1 forms a network and are linked to cardiac, EKG, and electrocardiogram pathology (Fig. 6).

4. Discussion

To the best of our knowledge, this is the first study investigating changes in the transcriptome profile of informal welders from a jewelry production chain and formal steelworkers. The results indicate changes in gene expression associated with inflammation and immune response related to occupational exposure to potentially toxic elements in both formal and informal work environments.

A total of 49 DEGs were observed in the informal work population, including CCL4L1, CCL4L2, ELK1, NTSR1, PIGA, IFI27, and SUFU (the complete list can be seen in Table 2). Pathway analysis revealed that these genes are mainly involved in inflammatory and immune responses; more specifically, the detected DEGs are enriched in MAPK, Toll-like receptor, and NF-kappa B chemokine signaling pathways. The Toll-like receptors activate innate immune responses, such as the induction of inflammatory cytokines, and protein recruitment triggering downstream signaling cascades and leading to the activation of transcription factor nuclear factor-kappaB (NF-κB) and MAP kinases (Kawai and Akira, 2007; Kawasaki and Kawai, 2014). NF-κB proteins control the induction of proinflammatory cytokines and chemokines; they also regulate the expression of genes involved in immunity, inflammation, stress responses, and cancer progression (Kawai and Akira, 2007; Mitchell and Carmody, 2018). MAP kinases activate protein kinases that regulate cell proliferation, differentiation, and death (Morrison, 2012). Other pathways that appear enriched in this study are also related to the activation of the MAP kinase cascade, such as the platelet-derived growth factor receptor (PDGFR) and G-protein-coupled receptors (GPCRs) (Jain et al., 2018). The results of the GO analysis also demonstrated that most of the differentially expressed genes in informal workers are involved with cellular process activities, and as observed in the pathway analysis, some of these genes are also associated with inflammatory processes, including CCL4L1, CCL4L2, and ELK1.

Uncontrolled or dysregulated inflammation signaling has been shown to manifest in a range of diseases including rheumatoid arthritis, autoimmune diseases, atherosclerosis, and cancer (Mitchell and Carmody, 2018). The pathways associated with these disease outcomes can be activated by external factors to which the population is exposed, such as exposure to toxic elements. Various epidemiological studies have demonstrated that exposure to PTEs, such as Pb, Cr, As, Hg, Ni, and Cd,

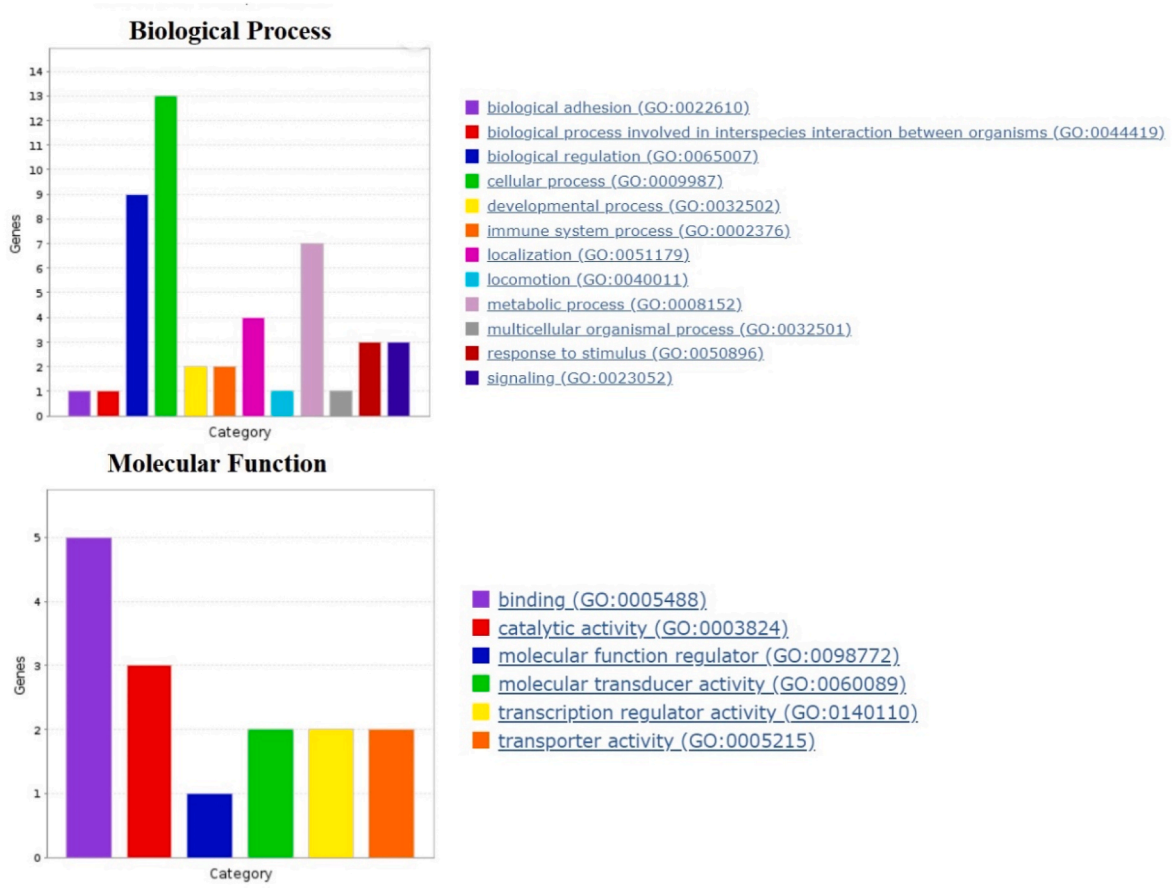

Fig. 2. Informal occupational exposure of jewelry welders affects mostly biological processes related to cellular and metabolic processes and biological regulation, and molecular functions related to binding and catalytic activity, according to the performed GO analysis data using PANTHER14.1.

leads to dysregulation of pro- and anti-inflammatory cytokines and is related to inflammatory processes involving the activity of TNF-α, and MAPK/ERK pathways (Brucker et al., 2015; Machoń-Grecka et al., 2017; López-Vanegas et al., 2020; Zhang et al., 2020; Renu et al., 2021). Brucker et al. (2015) report that concentrations of Hg, As, and Pb in the blood of Brazilian taxi drivers exposed to PTEs in vehicle emissions were positively associated with pro-inflammatory cytokines (such as IL-1β, IL-6, and TNF-α), increasing inflammatory biomarkers and contributing to the development of cardiovascular diseases. Zhang et al. (2020) observed that Pb, Cd, Hg, and As can cause dysregulation in the immune response and an increase in systemic inflammation (Zhang et al., 2020). Furthermore, battery recycling workers with high blood lead levels showed an increase of chronic inflammatory processes, dysfunction in leukocytes, inadequate immune response, and increased susceptibility to infections (López-Vanegas et al., 2020). Occupational exposure to Pb has been associated with the promotion of inflammatory response via the induction of cytokines and the modulation of angiogenesis which, once stimulated, may be related to cancer progression (Machoń-Grecka et al., 2017).

Other studies in welders indicate that exposure to the set of chemicals present in welding fumes is associated with DNA damage (Raffie et al., 2022), and can change the expression of genes involved in inflammation, defense response, oxidative stress, phosphate metabolism, cell proliferation, and programmed cell death (Wang et al., 2005; Jönsson et al., 2011). Other investigations to detect welding-related gene expression changes observed genes associated with inflammatory responses, toxic chemical metabolism, stress proteins, transcription factors, and signal transduction (Rim et al., 2007; Audureau et al., 2018).

As we observed in this study previous studies carried out in the city of Limeira have reported higher PTEs concentrations in the blood and breathing zone of informal workers (Ferreira et al., 2019) and in their urine (Salles et al., 2021) compared to controls. Correlations were also observed between the concentration of PTEs in the dust of houses where welding activities take place and the blood of children residing in those environments (Barrozo et al., 2022). Another study in the same jewelry production chain found that 30 proteins were differentially expressed in the saliva of welders and correlated with at least one blood PTE (Araujo et al., 2023). The MNDA protein was upregulated in welders and correlated with blood concentrations of tin (Araujo et al., 2023), an element that can be found in soldering wires used in jewelry production (Barrozo et al., 2022). This protein is also related to NF-kappa-B signaling pathways which corroborate with the results of the present study. Metabolomic results from the plasma of welders and multi-omics analysis revealed disturbance in amino acid pathways (Araujo et al., 2023).

A total of 70 DEGs were observed in the formal work population of Volta Redonda, including CAMP, DEFA3 E 4, LTF, BPI, OLFM4, NKX2-5, and others. Pathway analysis revealed that these genes are mainly involved in immune responses, some of which play a defensive role in the immune system or have antimicrobial functions (Table 3). Further analysis showed a predominance in pathways related to the immune system processes, such as antimicrobial peptides and defensins, neutrophil degranulation, and phagocytosis dependent on the Fc gamma receptor.

The Fc gamma receptor is an important mediator between humoral and cellular immune responses through the binding of the Fc domains of IgG. This binding stimulates a variety of biological responses, including processes directly related to antigen clearance, such as phagocytosis (Daëron, 1997). Defensins are antimicrobial peptides naturally

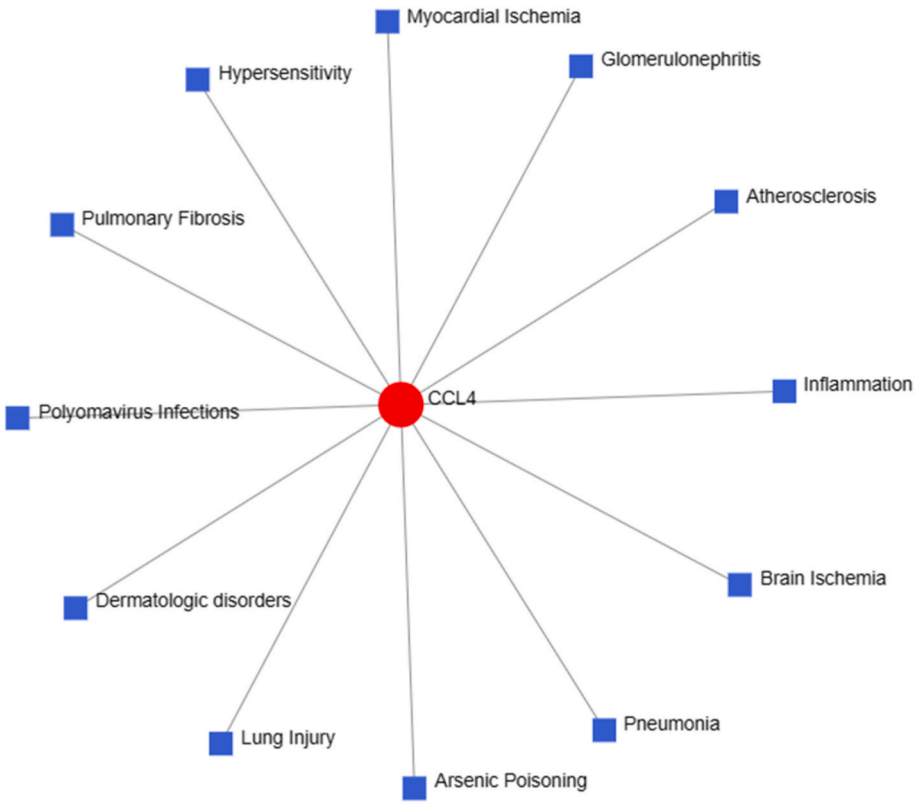

Fig. 3. Gene-disease network from Limeira population. Red circle nodes represent the CCL4 gene, and blue squares represent associated diseases.

synthesized by humans in neutrophils and gastrointestinal cells encoded by DEFA genes. Its antimicrobial activity is part of the innate immune defense against bacterial, viral, and fungal infections (Contreras et al., 2020; Xu and Lu, 2020). Neutrophil degranulation is one of the first cellular responses of the innate immune response (Effah et al., 2021; Klopf et al., 2021). Pro- and anti-inflammatory substances are the main components of neutrophil granules that are released to destroy invading pathogens, triggering a series of antimicrobial activities (Effah et al., 2021). Neutrophils have regulatory activities in the immune system and in the inflammatory response.

Pathway enrichment analysis of Volta Redonda also indicated important associations with the process of atherosclerosis development, one of the main factors involved in the progression of cardiovascular diseases. Scavenger receptor pathways, pathways of cell surface interactions in the vessel wall, and the pathway of smooth muscle cell proliferation and differentiation are all related to atherosclerosis. Scavenger receptors constitute a family of recognition proteins related to the uptake of oxidized LDL in macrophages, therefore related to lipid metabolism and atherogenesis. Through interaction with lipoproteins, these receptors play an important role in modulating vascular inflammation, in the accumulation of lipids, and, consequently, in the formation of plaques in vessels, which are all part of the pathophysiology of atherosclerosis progression (Mineo, 2020). Cell surface interactions in the vascular wall may include regulation of blood transport to underlying cells and tissues, permeability, vascular tone, cell adhesion, smooth muscle cell proliferation, angiogenesis, and vessel wall inflammation (Neubauer and Zieger, 2022). Dysfunctions in these processes precede the clinical complications of atherosclerosis (Mineo, 2020). Smooth muscle cell proliferation and differentiation pathways are also involved in more advanced stages of atherosclerosis (Frismantiene et al., 2018; Neubauer and Zieger, 2022). Smooth muscle cells are essential components of vascular development, maturation, repair, and regeneration. Over time, these cells move to the lining of the artery wall and multiply, together with fat cells forming a lumpy deposit called an atheroma or atherosclerotic plaque (Frismantiene et al., 2018). In addition, the network analysis shows the connection of three genes (NKX2-5, NDUFAF4, and FHL1) that are associated with different types of cardiac problems, including arrhythmias, EKG abnormalities, and heart conduction disorders (Zhang and Liu, 2015; Baban et al., 2022).

The mean blood concentrations of PTEs were not found to be significantly different between the groups from Volta Redonda in this study, which may indicate that other risk factors may be contributing to the alteration in gene expression. Other studies with steelworkers point to associations between risk factors such as obesity, insomnia, and shift work with atherosclerosis (Yu et al., 2022a, 2022b, Wang et al., 2022a, 2022b). Other pollutants such as polycyclic aromatic hydrocarbons (PAHs) and volatile organic compounds (VOCs) emitted from the iron and steelmaking operations should be investigated (Chen et al., 2015; Hou et al., 2023) and may be responsible for differences in gene expression. Furthermore, it is important to consider the levels of air pollution in Volta Redonda, which affects both the exposed and control groups and may have influenced the results. Scientific research has already been carried out in the region to evaluate the effects of pollution on workers and the surrounding population (Paiva, 2014; Nascimento et al., 2016; Reis et al., 2017). Higher levels of tin were determined in household dust in the eastern region of the city, and in atmospheric air samples in the southwest and southeast region (Azevedo et al., 2019), however, blood levels of tin (3.85 μg L^{-1}) in adults living in the municipality were lower compared to this study. Steel companies have great potential for the emission of air pollutants, mainly emissions of particulate matter, and in Volta Redonda, it was possible to verify that the total suspended particles, particulate material (PM_{10}), and ozone (O_3) exceeded the limits of Brazilian legislation (Rocha and Guimarães, 2017). Additionally, the mandatory use of personal protective equipment (PPE) seems to be an important protective factor for formal workers, while air pollution seems to be an important exposure factor for the general population in Volta Redonda, reflecting similar blood PTE concentrations.

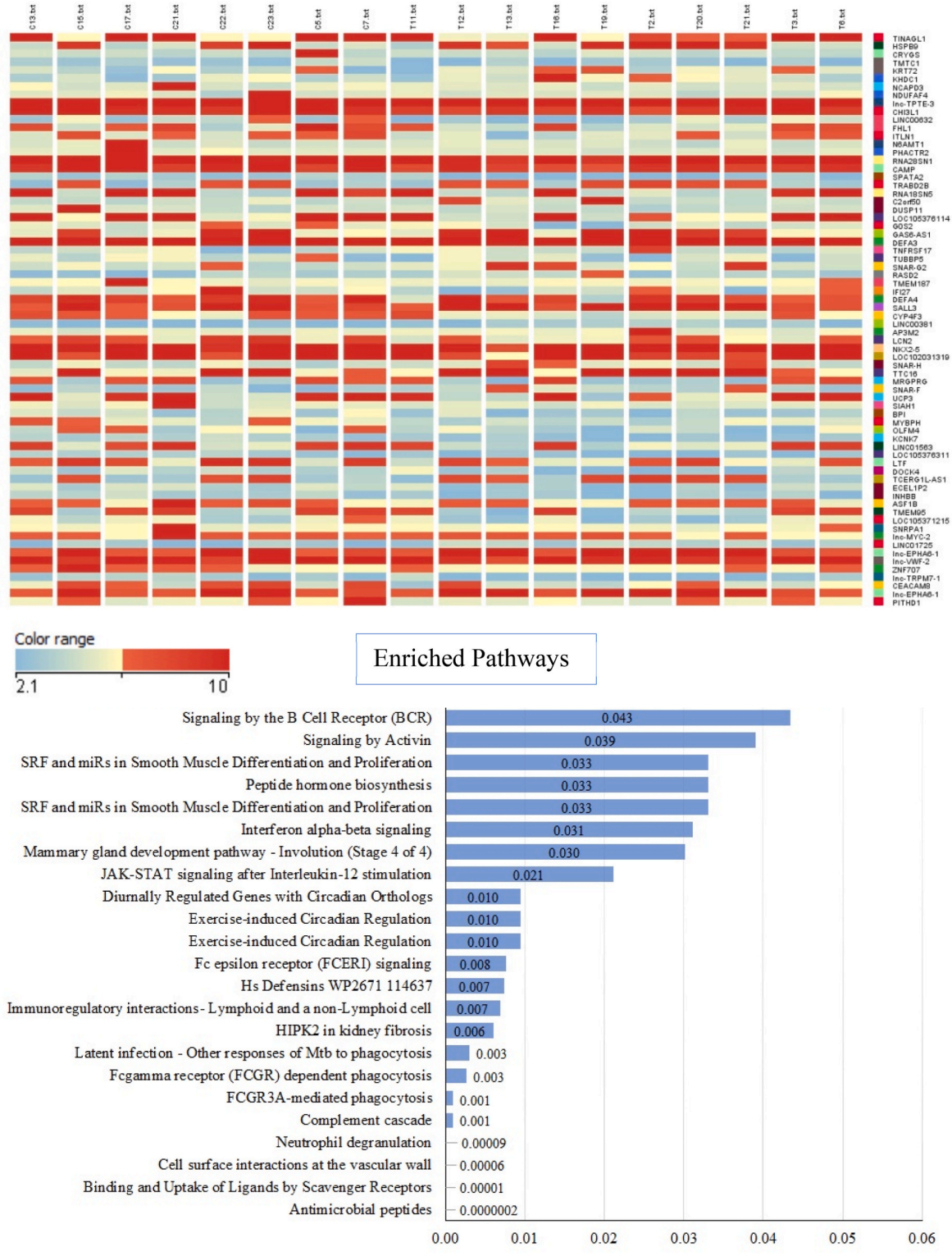

Fig. 4. Top: Heat map of the 70 significantly differentially expressed genes (DEGs) in formal workers compared to controls from Volta Redonda where columns represent samples and rows represent genes and red depicts upregulation while blue depicts downregulation. Bottom: Enriched biological pathways and respective p-values associated with the perturbed DEGs.

Great emphasis has been placed on the association between high-sensitivity C-reactive protein (hs-CRP) plasma concentration and cardiovascular risk and mortality. The American Heart Association established, in 2003, relative risk categories with cutoff points for hs-CRP, including low (<1 mg L^{-1}), medium (1–3 mg L^{-1}), and high risk (>3 mg L^{-1}) (Pearson et al., 2003). The plasma concentration of hs-CRP is positively correlated with the components of metabolic syndrome, blood glucose, waist circumference, concentrations of triacylglycerols and cholesterol associated with HDL and blood pressure, as well as fasting insulinemia, microalbuminuria, and reduced fibrinolysis (Pearson et al., 2003). In this study, higher geometric means of hs-CRP were observed in the Limeira population (2.61 mg L^{-1}), with a medium to high risk, while

Table 3
Gene Symbol, name, direction, and function of the 70 differentially expressed genes detected between Control and Exposed groups from Volta Redonda.

Gene Symbol	Gene Name	Regulation	Function
TINAGL1	Tubulointerstitial nephritis antigen like 1	down	Enables extracellular matrix structural constituent, laminin, and protein binding.
HSPB9	Heat shock protein family B (small) member 9	up	Protein binding.
CRYGS	Crystallin gamma S	down	Protein binding enables the structural constituents of the eye lens.
TMTC1	Transmembrane O-mannosyltransferase targeting cadherins 1	down	Mannosyltransferase activity.
KRT72	Keratin 72	up	Enables structural constituents of skin epidermis, protein binding, and molecular function.
KHDC1	KH domain containing 1	up	RNA binding. Predicted to be involved in the apoptotic process.
NCAPD3	Non-SMC condensin II complex subunit D3	down	Histone binding and protein binding.
NDUFAF4	NADH:ubiquinone oxidoreductase complex assembly factor 4	down	Protein binding. Involved in the mitochondrial respiratory chain.
CHI3L1	Chitinase 3 like 1	down	Chitin and carbohydrate binding. Chitinase activity.
FHL1	Four and a half LIM domains 1	down	Metal ion binding, protein binding, molecular function, transmembrane transporter binding.
ITLN1	Intelectin 1	down	Calcium ion binding, protein, and oligosaccharide binding.
N6AMT1	N-6 adenine-specific DNA methyltransferase 1	down	Nucleic acid and protein binding. Protein methyltransferase activity.
PHACTR2	Phosphatase and actin regulator 2	down	Actin binding. Protein phosphatase inhibitor activity.
CAMP	Cathelicidin antimicrobial peptide	down	Encodes a member of an antimicrobial peptide family. Lipopolysaccharide binding.
SPATA2	Spermatogenesis associated 2	down	Protein-coding complex binding. Signaling receptor complex adaptor activity. Ubiquitin-specific protease binding.
TRABD2B	TraB domain containing 2 B	up	Wnt-protein binding. Metal ion binding. Metalloendopeptidase activity.
C2orf50	Chromosome 2 open reading frame 50	up	Protein binding.
DUSP11	Dual specificity phosphatase 11	down	RNA binding. Phosphatase activity. Polynucleotide 5′-phosphatase activity. Protein tyrosine phosphatase activity.
G0S2	G0/G1 switch 2	down	Molecular function and protein binding. Involved in the extrinsic apoptotic signaling pathway.
DEFA3	Defensin alpha 3	down	Molecular function. Protein homodimerization activity. Involved in host defense.
DEFA4	Defensin alpha 4	down	Molecular function. Protein homodimerization activity. Involved in host defense.
TNFRSF17	TNF receptor superfamily member 17	up	Protein binding. Signaling receptor activity. Encode a protein member of the TNF-receptor superfamily.
RASD2	RASD family member 2	up	G-protein beta-subunit binding. GTP binding. GTPase activity.
TMEM187	Transmembrane protein 187	down	Molecular function and protein binding.
IFI27	Interferon alpha inducible protein 27	down	Interferon alpha-inducible protein 27
SALL3	Spalt like transcription factor 3	up	DNA-binding transcription factor activity. RNA polymerase II cis-regulatory region sequence-specific DNA binding. Metal ion binding.
CYP4F3	Cytochrome P450 family 4 subfamily F member 3	down	Aromatase activity. Docosanoate omega-hydroxylase activity. Monooxygenase activity. Heme binding. Iron ion binding
AP3M2	Adaptor related protein complex 3 subunit mu 2	up	Protein trafficking to lysosomes and specialized organelles.
LCN2	Lipocalin 2	down	Enterobactin binding. Iron ion binding. Protein and small molecule binding.
NKX2-5	NK2 homeobox 5	down	DNA-binding transcription factor activity. RNA polymerase II cis-regulatory region sequence-specific DNA binding. Chromatin binding. Protein homodimerization activity.
TTC16	Tetratricopeptide repeat domain 16	up	Unknown.
MRGPRG	MAS related GPR family member G	down	G protein-coupled receptor activity.
UCP3	Uncoupling protein 3	down	Oxidative phosphorylation uncoupler activity. Protein binding.
SIAH1	Siah E3 ubiquitin protein ligase 1	down	Protein binding. Ubiquitin-conjugating enzyme binding. Zinc ion binding. Ubiquitin-protein transferase activity. Ubiquitin protein ligase activity.
BPI	Bactericidal permeability increasing protein	down	Lipopolysaccharide binding. Encodes a protein associated with human neutrophil granules with antimicrobial activity.
MYBPH	Myosin binding protein H	down	Protein binding. Enables structural constituent of muscle.
OLFM4	Olfactomedin 4	down	Cadherin and protein binding. Enables structural molecule activity.
KCNK7	Potassium two pore domain channel subfamily K member 7	down	Potassium channel and potassium ion leak channel activity.
LTF	Lactotransferrin	down	DNA, protein, lipopolysaccharide, heparin, and iron ion binding. Cysteine-type

(*continued on next page*)

Table 3 (*continued*)

Gene Symbol	Gene Name	Regulation	Function
			endopeptidase inhibitor activity. Protein serine/threonine kinase activator activity. Serine-type endopeptidase activity.
DOCK4	Dedicator of cytokinesis 4	down	PDZ and SH3 domain binding. Receptor tyrosine kinase and small GTPase binding. Guanyl-nucleotide exchange factor activity. GTPase activator activity.
INHBB	Inhibin subunit beta B	down	Protein and host cell surface receptor binding. Cytokine activity. Growth factor and hormone activity. Protein homodimerization activity.
ASF1B	Anti-silencing function 1 B histone chaperone	down	Histone and protein binding. Histone chaperone activity.
TMEM95	Transmembrane protein 95	down	Protein binding.
SNRPA1	Small nuclear ribonucleoprotein polypeptide A′	down	RNA binding. U2 snRNA binding. Protein binding.
CEACAM8	CEA cell adhesion molecule 8	down	Protein binding. Protein heterodimerization activity
PITHD1	PITH domain containing 1	down	Involved in positive regulation of megakaryocyte differentiation and positive regulation of transcription, DNA-templated.
ZNF707	Zinc finger protein 707	down	Metal ion and protein binding. DNA-binding transcription factor activity. RNA polymerase II cis-regulatory region sequence-specific DNA binding.
RNA28SN1	RNA, 28 S ribosomal N1	down	rRNA.
RNA18SN5	RNA, 18 S ribosomal N5	down	rRNA.
GAS6-AS1	GAS6 antisense RNA 1	up	Non-coding.
SNAR-G2	Small NF90 (ILF3) associated RNA G2	up	Non-coding.
SNAR-F	Small NF90 (ILF3) associated RNA F	up	Non-coding.
SNAR-H	Small NF90 (ILF3) associated RNA H	up	Non-coding.
LINC01725	Long intergenic non-protein coding.	up	Non-coding.
LINC00381	Long intergenic non-protein coding.	up	Non-coding.
LINC00632	Long intergenic non-protein coding	down	Non-coding.
LINC01563	Long intergenic non-protein coding.	down	Non-coding.
TCERG1L-AS1	TCERG1L antisense RNA 1	up	Non-coding.
lnc-EPHA6-1	lnc-EPHA6-1	up	Non-coding.
lnc-VWF-2	lnc-VWF-2	down	Non-coding.
lnc-MYC-2	lnc-MYC-2	down	Non-coding.
lnc-TRPM7-1	lnc-TRPM7-1	down	Non-coding.
lnc-TPTE-3	lnc-TPTE-3	up	Non-coding.
lnc-EPHA6-1	lnc-EPHA6-1	up	Non-coding.

Differentially expressed genes classified as uncharacterized were not included in this table.

in Volta Redonda the mean insinuates low risk (0.75 mg L^{-1}). However, it was not possible to observe a relationship between informal or formal work and an increase in this risk, which may indicate the influence of other factors, such as habits, lifestyle, and diet, and points to the complex nature of cohort studies.

Both work populations impacted similar GO ontologies but do not present many common DEGs. The only in common in both populations was the IFI27 (Interferon alpha-inducible protein 27). IFI27 was found to be upregulated in informal workers and downregulated in formal workers. This gene is part of the family of interferon-inducible proteins-that regulate resistance to viral infections, enhance innate and immune responses, and modulate normal and tumor cell survival and death. Upregulation of IFI27 is proven to be related to different types of cancer, including breast, ovarian, and pancreatic (Li et al., 2015; Huang et al., 2021). The downregulation can also be involved in tumor development by interfering with apoptosis through the TNF-related ligand and favoring cancer cell survival (Liu et al., 2014).

The main difference between the two populations of this study is the work activities. Informal workers perform their job in a domestic environment, replacing industrial steps with little or no training, guidance, or supervision. They also have irregular working hours, improvised and precarious workplaces, fewer opportunities to file complaints, and complete duties without the use of personal protective equipment (PPE). This scenario can promote an increased likelihood of occupational accidents and hazardous exposures, culminating in diseases and/or incapacities (Schulte et al., 2022). On the contrary, formal workers have a working agreement with basic rights assured, including health monitoring. Usually, the employer follows safety standards to mitigate risks and prevent hazardous exposures, such as the mandatory use of PPE and overall workplace safety procedures. Thus, formal work can expose an individual to a higher load of pollutants inside the industry than an informal job, however, they are normally more protected by work safety measures. It is also possible to consider differences between control conditions of each city, the air pollution and contamination of soil and water by industrial activities in the region of Volta Redonda should be considered and can explain PTE levels. The fact that the two populations are of different sexes also makes it difficult to compare the expressed genes. There are important population-related differences and so each case study should be handled separately, and extrapolation should be done carefully in future cohort studies and risk assessments.

The sample size included in this study is relatively small compared to other epidemiological studies. Unfortunately, workers commonly express concerns about the potential repercussions of their work, including being reported to authorities and facing retaliatory actions from competing factories, each of which may endanger their livelihood. As a result, there are frequently hesitancies, lack of engagement, and skepticism from these workers when it comes to engaging in scientific studies such as this and are likely the cause for the low recruitment numbers. Still, this exposure scenario is unique and crucial for understanding the health impacts of neglected informal activities commonly found in emerging and developing countries from across Africa, Asia, and Latin America (ILO, 2018).

There are a lot of factors from the human biology compartment that can affect results and complicate the interpretation of human population samples. However, these populations should not be avoided for scientific investigation. In this study, exploratory results based on microarray analysis (without further quantification of target genes and comprehension of the potential regulatory mechanisms) generate a starting point to set a basis for analysis in sensitive, understudied populations, especially from developing countries. The lack of validation of detected differentially expressed genes is a limitation. The observed patterns of occupational exposure effects on workers, as well as the use of specific genes as potential biomarkers, need to be further explored and validated using qPCR or other quantitative methods. Nevertheless, the knowledge gap and need for preliminary analyses far outweigh the limitations of the study. Future studies will select genes to perform target analyses, related

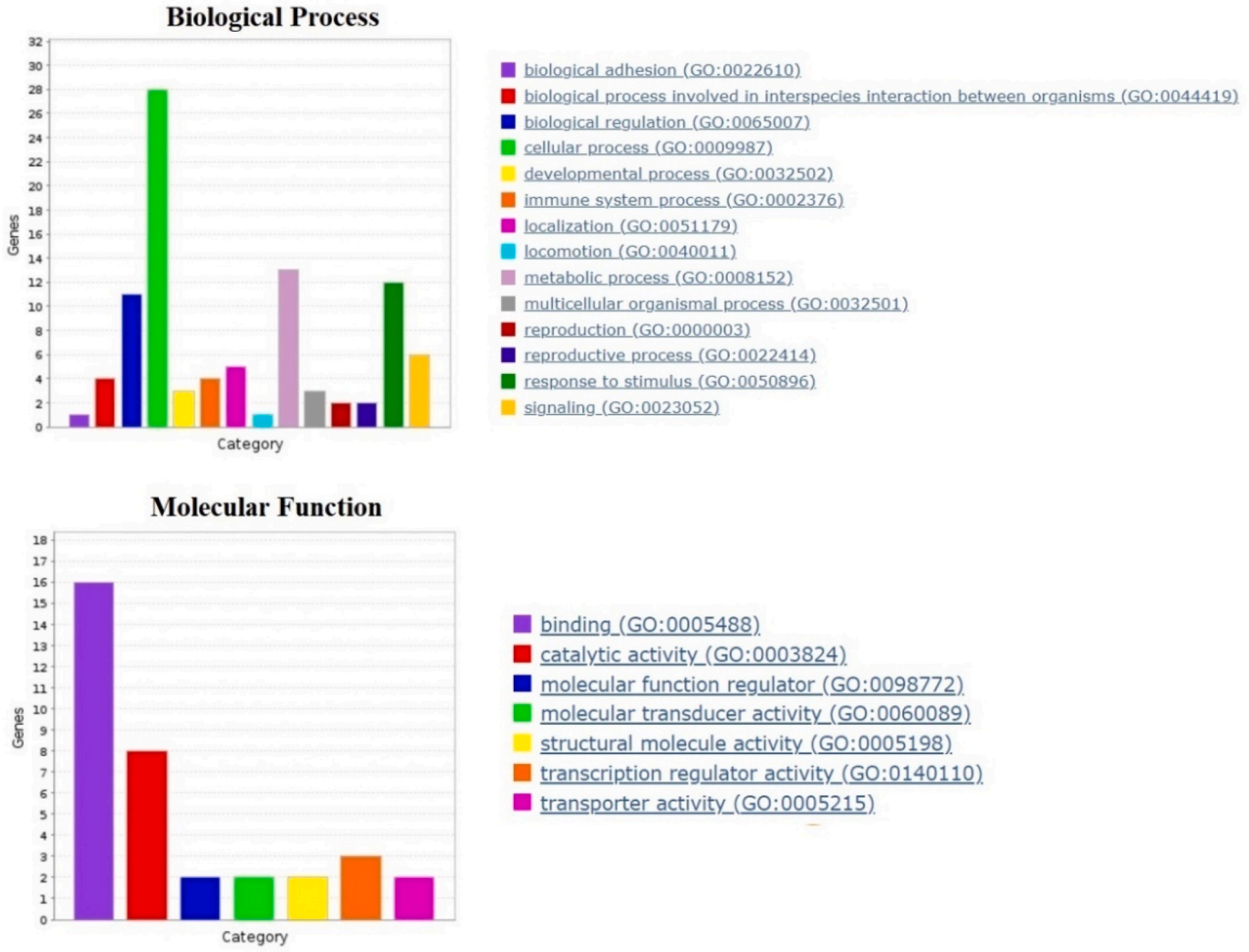

Fig. 5. Formal occupational exposure of steelworkers affects mostly biological processes related to cellular and metabolic processes and molecular functions related to binding and catalytic activity, according to the performed GO analysis data using PANTHER14.1.

to paths of interest and different exposures.

This study provides evidence that occupational exposures, whether formal or informal, have the potential to alter gene expression profiles in humans and highlights the effect of occupational activity on the exposome of laborers. By investigating how occupational exposure may influence systemic inflammatory processes and immune responses, and by association its contribution to the development of cardiovascular and metabolic diseases, we contribute to the growing body of literature that suggests such activities may contribute to public health problems responsible for high mortality and morbidity rates. Moreover, identifying specific patterns of gene expression may provide clues about how occupational exposures can alter the human transcriptome and lead to changes in metabolic patterns (van Breda et al., 2015). Correlations between the differentially expressed genes of this study, the diseases reported by the population, and the blood levels of PTEs are currently being investigated and will be discussed in future studies.

5. Conclusions

The results of the microarray whole blood analysis found 49 DEGs between informal jewelry welders and the control group in Limeira and 70 DEGs between the formal steelworkers and the control group in Volta Redonda. By comparing the outcomes from each of these populations, we identified that differences in gene expression related to occupational exposure are mainly associated with inflammation and immune responses. Moreover, despite the fact that PTE levels in participant blood were not elevated between exposed and control groups, it was possible to observe DEGs in the two occupationally-exposed populations compared to the controls. These results may indicate that, over time, the impact of occupational exposures contribute to biological responses with potential outcomes in chronic diseases.

Credit author statement

Fernanda Junqueira Salles: Investigation, Methodology, Formal analysis, Writing – original draft. Ilias S. Frydas: Methodology, Writing – review & editing. Nafsika Papaioannou: Formal analysis, Writing – review & editing. Dayna R. Schultz: Formal analysis, Writing – review & editing. Maciel Santos Luz: Resources, Writing – review & editing. Marcelo Macedo Rogero: Conceptualization, Writing – review & editing. Dimosthenis A. Sarigiannis: Resources, Supervision, Writing – review & editing. Kelly Polido Kaneshiro Olympio: Conceptualization, Supervision, Project administration, Funding acquisition, Writing – review & editing.

Funding source

This research was funded by “Fundação de Amparo à Pesquisa do

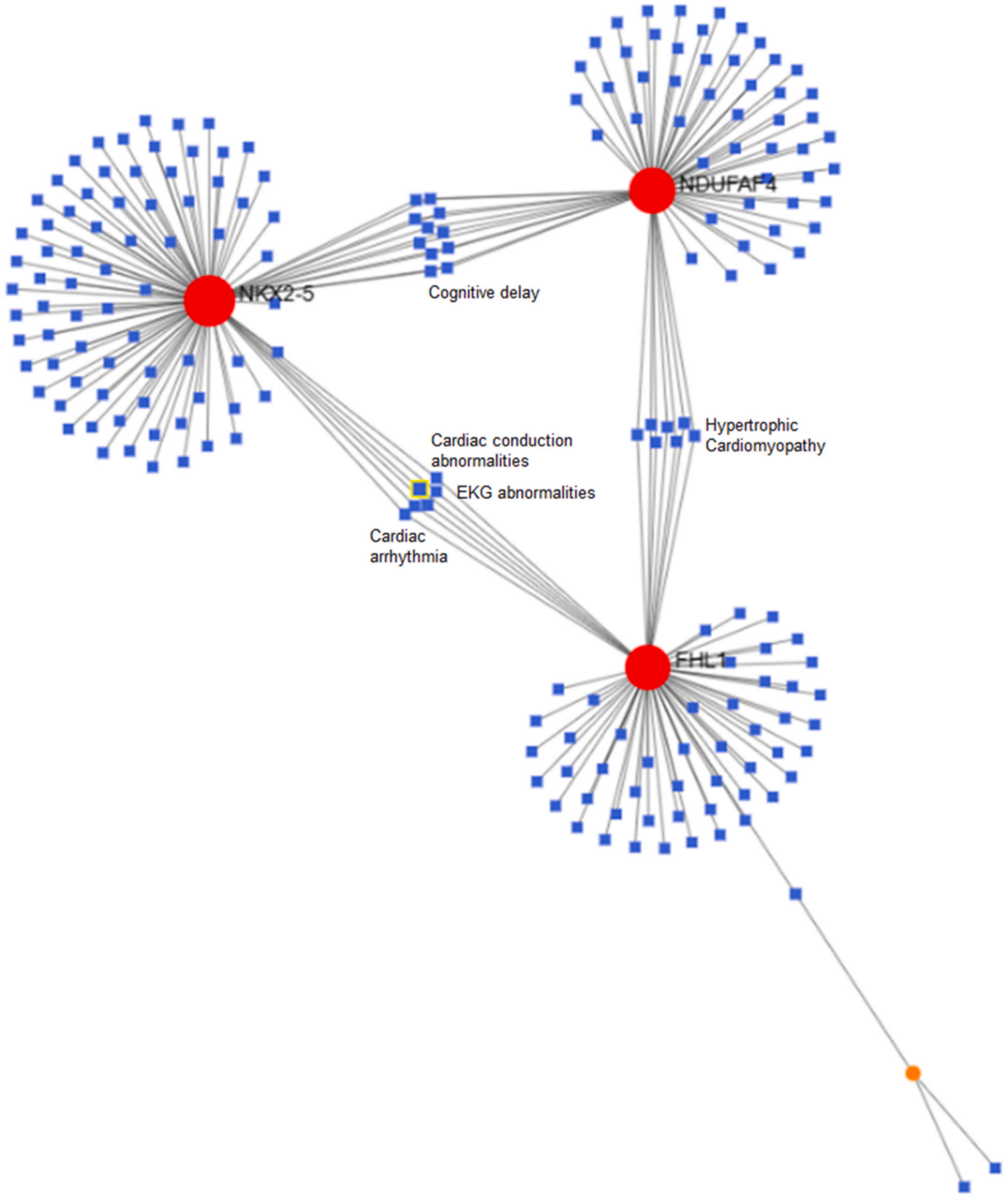

Fig. 6. Gene-disease network from Volta Redonda population. Red circle nodes represent the NKX2-5, NDUFAF4, and FHL1 genes, and blue squares represent associated diseases.

Estado de São Paulo," FAPESP, grant number 2017/25424-9; 2020/00919-8; 2018/18391-0 and 2017/20752-8; 2014/50887-4". KPKO is the recipient of a scholarship from "Conselho Nacional de Desenvolvimento Científico e Tecnológico" (CNPq #314637/2021-4).

Ethical approval

Obtained from the Institutional Review Board of the School of Public Health of the University of Sao Paulo (Protocol N° 32580820.8.0000.5421).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgment

The authors would like to thank the volunteers who agreed to participate in this investigation. The authors acknowledge the support of the Health Secretariat of Limeira city and the collaboration of all community health agents and coordinators of the basic health units from Limeira. The authors acknowledge the support of the Center for Worker Health and Human Ecology (CESTEH) from the Sergio Arouca National School of Public Health, Oswaldo Cruz Foundation for the sample collection in the city of Volta Redonda. Also, the members of the eXsat (The Human Exposome Research Group) for the aid in sample collection.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2023.116835.

References

Agilent, 2015. One-color microarray-based gene expression analysis: low input quick amp labeling. Protocol. Version 6.9.1. Revision B6., 98p.

Araujo, A.N.M., Leroux, I.N., Furtado, D.Z.S., Ferreira, A.P.S.S., Batista, B.L., Silva, H.D. T., Handakas, E., Assunção, N.A., Olympio, K.P.K., 2023. Integration of proteomic and metabolomic analyses: new insights for mapping informal workers exposed to potentially toxic elements. Front. Public Health 10, 899638. https://doi.org/10.3389/fpubh.2022.899638.

Audureau, É., Simon-Deckers, A., Franco-Montoya, M.L., Annangi, B., Kermanizadeh, A., Boczkowski, J., Lanone, S., 2018. Substantial modification of the gene expression profile following exposure of macrophages to welding-related nanoparticles. Sci. Rep. 8 (1), 8554. https://doi.org/10.1038/s41598-018-26988-z.

Azevedo, S.V., Sobral, A., Moreira, M.F.R., 2019. Spatial pattern of the environmental exposure to tin in the vicinity of an alloy industry in Volta Redonda, Rio de Janeiro State, Brazil. Cad. Saúde Pública 35 (10), e00079819. https://doi.org/10.1590/0102-311X00079818, 14.

Baban, A., Lodato, V., Parlapiano, G., Drago, F., 2022. Genetics in congenital heart diseases: unraveling the link between cardiac morphogenesis, heart muscle disease, and electrical disorders. Heart Fail. Clin. 18 (1), 139–153. https://doi.org/10.1016/j.hfc.2021.07.016.

Bahr, D., Zambare, V., Azevedo, V. (Eds.), 2013. Omics: Application in Biomedical, Agricultural, and Environmental Sciences. CRC Press, Taylor & Francis Group, Florida, USA.

Barrozo, F., Almeida, G.A., Luz, M.S., Olympio, K.P.K., 2022. A low-cost method shows potentially toxic element levels in dust correlated with elevated blood levels of these chemicals in children exposed to an informal home-based production environment. Int. J. Environ. Res. Publ. Health 19 (23), 16236. https://doi.org/10.3390/ijerph192316236.

Brucker, N., Moro, A., Charão, M., Bubols, G., Nascimento, S., Goethel, G., Barth, A., Prohmann, A.C., Rocha, R., Moresco, R., Sangoi, M., Hausen, B.S., Saint'Pierre, T., Gioda, A., Duarte, M., Castro, I., Saldiva, P.H., Garcia, S.C., 2015. Relationship between blood metals and inflammation in taxi drivers. Clin. Chim. Acta 444, 176–181. https://doi.org/10.1016/j.cca.2015.02.032.

Chen, J., Aries, E., Collins, P., Anderson, D.R., Hodges, J.S., 2015. Characterization of priority substances in effluents from an integrated steelworks in the United Kingdom. Water Environ. Res. 87 (2), 132–144. https://doi.org/10.2175/106143014X14062131179311.

Contreras, G., Shirdel, I., Braun, M.S., Wink, M., 2020. Defensins: transcriptional regulation and function beyond antimicrobial activity. Dev. Comp. Immunol. 104, 103556 https://doi.org/10.1016/j.dci.2019.103556.

Croghan, C., Egeghy, P.P., 2003. Methods of dealing with values below the limit of detection using SAS. In: Presented at Southeastern SAS User Group, pp. 22–24. In: http://www8.sas.com/scholars/05/PREVIOUS/2001_200.4/2004_MOR/Proceed/_2003/Statistics/SD08-Croghan.pdf. (Accessed 30 April 2019).

CSN, 2017. Companhia Siderúrgica Nacional [internet]. Unidades Fabris: Usina Presidente Vargas [acesso em 19 out 2017]. http://www.csn.com.br/conteudo_pti.asp?idioma=0&conta=45&tipo=60851&prSv=1.

Daëron, M., 1997. Structural bases of FcγR functions. Int. Rev. Immunol. 16, 1–27. https://doi.org/10.3109/08830189709045701.

DeBord, D.G., Carreón, T., Lentz, T.J., Middendorf, P.J., Hoover, M.D., Schulte, P.A., 2016. Use of the "exposome" in the practice of epidemiology: a primer on –omic technologies. Am. J. Epidemiol. 184 (4), 302–314. https://doi.org/10.1093/aje/kwv325.

Effah, C.Y., Drokow, E.K., Agboyibor, C., Ding, L., He, S., Liu, S., Akorli, S.Y., Nuamah, E., Sun, T., Zhou, X., Liu, H., Xu, Z., Feng, F., Wu, Y., Zhang, X., 2021. Neutrophil-dependent immunity during pulmonary infections and inflammations. Front. Immunol. 12, 689866 https://doi.org/10.3389/fimmu.2021.689866.

Espín-Pérez, A., Font-Ribera, L., van Veldhoven, K., Krauskopf, J., Portengen, L., Chadeau-Hyam, M., Vermeulen, R., Grimalt, J.O., Villanueva, C.M., Vineis, P., Kogevinas, M., Kleinjans, J.C., de Kok, T.M., 2018. Blood transcriptional and microRNA responses to short-term exposure to disinfection by-products in a swimming pool. Environ. Int. 110, 42–50. https://doi.org/10.1016/j.envint.2017.10.003.

Ferreira, A.P.S.S., Pereira, E.C., Salles, F.J., da Silva, F.F., Batista, B.L., Handakas, E., Olympio, K.P.K., 2019. Home-based and informal work exposes the families to high levels of potentially toxic elements. Chemosphere 218, 319–327.

Frismantiene, A., Philippova, M., Erne, P., Resink, T.J., 2018. Smooth muscle cell-driven vascular diseases and molecular mechanisms of VSMC plasticity. Cell. Signal. 52, 48–64. https://doi.org/10.1016/j.cellsig.2018.08.019.

GBD, 2019. Global Burden of Disease. Institute for Health Metrics and Evaluation. GBD Compare. https://vizhub.healthdata.org/gbd-compare/. (Accessed 6 April 2023).

Geloneze, B., Repetto, E.M., Geloneze, S.R., Tambascia, M.A., Ermetice, M.N., 2006. The threshold value for insulin resistance (HOMA-IR) in an admixtured population IR in the Brazilian Metabolic Syndrome Study. Diabetes Res. Clin. Pract. 72 (2), 219–220.

Geloneze, B., Vasques, A.C., Stabe, C.F., Pareja, J.C., Rosado, L.E., Queiroz, E.C., et al., 2009. HOMA1-IR and HOMA2-IR indexes in identifying insulin resistance and metabolic syndrome: Brazilian Metabolic Syndrome Study (BRAMS). Arq. Bras. Endocrinol. Metabol. 53 (2), 281–287.

Hou, Y., Li, Y., Tao, H., Cao, H., Liao, X., Liu, X., 2023. Three-dimensional distribution characteristics of multiple pollutants in the soil at a steelworks mega-site based on multi-source information. J. Hazard Mater. 448, 130934 https://doi.org/10.1016/j.jhazmat.2023.130934.

Huang, S., Zhao, J., Song, J., Li, Y., Zuo, R., Sa, Y., Ma, Z., OuYang, H., 2021. Interferon alpha-inducible protein 27 (IFI27) is a prognostic marker for pancreatic cancer based on comprehensive bioinformatics analysis. Bioengineered 12 (1), 8515–8528. https://doi.org/10.1080/21655979.2021.1985858.

Idaghdour, Y., Storey, J.D., Jadallah, S.J., Gibson, G., 2008. A genome-wide gene expression signature of environmental geography in leukocytes of Moroccan amazighs. PLoS Genet. 4 (4), 1–10. https://doi.org/10.1371/journal.pgen.1000052.

International Labour Organization (ILO), 2018. Women and Men in the Informal Economy: A Statistical Picture, third ed. International Labour Organization, Geneva, Switzerland.

Jain, R., Watson, U., Vasudevan, L., Saini, D.K., 2018. ERK activation pathways downstream of GPCRs. Int Rev Cell Mol Biol 338, 79–109. https://doi.org/10.1016/bs.ircmb.2018.02.003.

Jönsson, L.S., Nielsen, J., Broberg, K., 2011. Gene expression analysis in induced sputum from welders with and without airway-related symptoms. Int. Arch. Occup. Environ. Health 84 (1), 105–113. https://doi.org/10.1007/s00420-010-0579-2.

Kawai, T., Akira, S., 2007. Signaling to NF-kappaB by toll-like receptors. Trends Mol. Med. 13 (11), 460–469. https://doi.org/10.1016/j.molmed.2007.09.002.

Kawasaki, T., Kawai, T., 2014. Toll-like receptor signaling pathways. Front. Immunol. 5, 461. https://doi.org/10.3389/fimmu.2014.00461.

Klopf, J., Brostjan, C., Eilenberg, W., Neumayer, C., 2021. Neutrophil extracellular traps and their implications in cardiovascular and inflammatory disease. Int. J. Mol. Sci. 22 (2), 559. https://doi.org/10.3390/ijms22020559.

Korashy, HM, Attafi, IM, Famulski, KS, Bakheet, SA, Hafez, MM, Alsaad, AMS, 2017. Al-Ghadeer ARM. Gene expression profiling to identify the toxicities and potentially relevant human disease outcomes associated with environmental heavy metal exposure. Environ Pollut 221, 64–74. https://doi.org/10.1016/j.envpol.2016.10.058.

Lacorte, L.E.C., Vilela, R.A.G., Silva, R.C., Chiesa, A.M., Tulio, E.S., Franco, R.R., Bravo, E.S., 2013. The knots of the child labor eradication network within the jewelry and costume jewelry production in Limeira, São Paulo, Brazil. Rev. Bras. Saúde Ocup. 38 (128), 199–215. https://doi.org/10.1590/S0303-76572013000200009 [in Portuguese].

Li, S., Xie, Y., Zhang, W., Gao, J., Wang, M., Zheng, G., Yin, X., Xia, H., Tao, X., 2015. Interferon alpha-inducible protein 27 promotes epithelial-mesenchymal transition and induces ovarian tumorigenicity and stemness. J. Surg. Res. 193 (1), 255–264. https://doi.org/10.1016/j.jss.2014.06.055.

Liu, N., Zuo, C., Wang, X., Chen, T., Yang, D., Wang, J., Zhu, H., 2014. miR-942 decreases TRAIL-induced apoptosis through ISG12a downregulation and is regulated by AKT. Oncotarget 5 (13), 4959–4971. https://doi.org/10.18632/oncotarget.2067.

López-Vanegas, N.C., Hernández, G., Maldonado-Vega, M., Calderón-Salinas, J.V., 2020. Leukocyte apoptosis, TNF-α concentration and oxidative damage in lead-exposed workers. Toxicol. Appl. Pharmacol. 391, 114901 https://doi.org/10.1016/j.taap.2020.114901.

Machoń-Grecka, A., Dobrakowski, M., Boroń, M., Lisowska, G., Kasperczyk, A., Kasperczyk, S., 2017. The influence of occupational chronic lead exposure on the levels of selected pro-inflammatory cytokines and angiogenic factors. Hum. Exp. Toxicol. 36 (5), 467–473. https://doi.org/10.1177/0960327117703688.

McHale, C.M., Zhang, L., Thomas, R., Smith, M.T., 2013. Analysis of the transcriptome in molecular epidemiology studies. Environ. Mol. Mutagen. 54 (7), 500–517 doi: 10,1002/em.21798.

Mi, H., Muruganujan, A., Ebert, D., Huang, X., Thomas, P.D., 2019. PANTHER version 14: more genomes, a new PANTHER GO-slim and improvements in enrichment analysis tools. Nucleic Acids Res. 47, D419–d426.

Mineo, C., 2020. Lipoprotein receptor signalling in atherosclerosis. Cardiovasc. Res. 116 (7), 1254–1274. https://doi.org/10.1093/cvr/cvz338.

Mitchell, J.P., Carmody, R.J., 2018. NF-κB and the transcriptional control of inflammation. Int Rev Cell Mol Biol 335, 41–84. https://doi.org/10.1016/bs.ircmb.2017.07.007.

Morrison, D.K., 2012. MAP kinase pathways. Cold Spring Harbor Perspect. Biol. 4 (11), a011254. https://doi.org/10.1101/cshperspect.a011254.

Nascimento, L.F.C., Vieira, L.C.P.F., Mantovani, K.C.C., Moreira, D.S., 2016. Air pollution and respiratory diseases: ecological time series. Sao Paulo Med. J. 134 (4), 315–321. https://doi.org/10.1590/1516-3180.2015.0237250216.

Neubauer, K., Zieger, B., 2022. Endothelial cells and coagulation. Cell Tissue Res. 387 (3), 391–398. https://doi.org/10.1007/s00441-021-03471-2.

Paiva, R.F.P.S., 2014. Hospital morbidity due to diseases associated with air pollution in the city of Volta Redonda, Rio de Janeiro: cases and economic cost. Cad. Saúde Colet., Rio de Janeiro. 22 (2), 127–132. https://doi.org/10.1590/1414-462X201400020004.

Papaioannou, N., Distel, E., de Oliveira, E., Gabriel, C., Frydas, I.S., Anesti, O., Attignon, E.A., Odena, A., Díaz, R., Aggerbeck, M., Horvat, M., Barouki, R., Karakitsios, S., Sarigiannis, D.A., 2021. Multi-omics analysis reveals that co-exposure to phthalates and metals disturbs urea cycle and choline metabolism. Environ. Res. 192, 110041 https://doi.org/10.1016/j.envres.2020.110041.

Passos, G.A. (Ed.), 2014. Transcriptomics in Health and Disease. Springer. https://doi.org/10.1007/978-3-319-11985-4.

Pearson, T.A., Mensah, G.A., Alexander, R.W., et al., 2003. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: a statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation 107, 499–511.

Pećina-Šlaus, N., Pećina, M., 2015. Only one health, and so many omics. Cancer Cell Int. 15, 64. https://doi.org/10.1186/s12935-015-0212-2.

PreAnalytiX Company, 2015. Blood RNA Kit Handbook. PAXgene, Switzerland, Version 2.

Pronk, A., Loh, M., Kuijpers, E., Albin, M., Selander, J., Godderis, L., et al., 2022. Applying the exposome concept to working life health: the EU EPHOR project. Environ Epidem 6 (2), e185. https://doi.org/10.1097/EE9.0000000000000185.

Rafiee, A., Ospina, M.B., Pitt, T.M., Quémerais, B., 2022. Oxidative stress and DNA damage resulting from welding fumes exposure among professional welders: a systematic review and meta-analysis. Environ. Res. 214 (Pt 4), 114152 https://doi.org/10.1016/j.envres.2022.114152.

Reis, M.M., Guimarães, M.T., Braga, A.L.F., Martins, L.C., Pereira, L.A.A., 2017. Air pollution and low birth weight in an industrialized city in Southeastern Brazil, 2003-2006. Rev. Bras. Epidemiol 20 (2), 189–199. https://doi.org/10.1590/1980-5497201700020001.

Renu, K., Chakraborty, R., Myakala, H., Koti, R., Famurewa, A.C., Madhyastha, H., Vellingiri, B., George, A., Valsala Gopalakrishnan, A., 2021. Molecular mechanism of heavy metals (lead, chromium, arsenic, mercury, nickel and cadmium) - induced hepatotoxicity - a review. Chemosphere 271, 129735. https://doi.org/10.1016/j.chemosphere.2021.129735.

Rim, K.T., Park, K.K., Kim, Y.H., Lee, Y.H., Han, J.H., Chung, Y.H., Yu, I.J., 2007. Gene-expression profiling of human mononuclear cells from welders using cDNA microarray. J. Toxicol. Environ. Health 70 (15–16), 1264–1277. https://doi.org/10.1080/15287390701428986.

Rocha, N.L.T., Guimarães, C.S., 2017. Estudo da qualidade do ar e a atividade siderúrgica na cidade de Volta Redonda. Cadernos UniFOA, Volta Redonda 33, 25–36 [in portuguese].

Salles, F.J., Tavares, D.J.B., Freire, B.M., Ferreira, A.P.S.S., Handakas, E., Batista, B.L., Olympio, K.P.K., 2021. Home-based informal jewelry production increases exposure of working families to cadmium. Sci. Total Environ. 785, 147297 https://doi.org/10.1016/j.scitotenv.2021.147297.

Schulte, P.A., Iavicoli, I., Fontana, L., Leka, S., Dollard, M.F., Salmen-Navarro, A., Salles, F.J., Olympio, K.P.K., et al., 2022. Occupational safety and health staging framework for decent work. Int. J. Environ. Res. Publ. Health 19, 10842. https://doi.org/10.3390/ijerph191710842.

Smith, M.T., Vermeulen, R., Li, G., Zhang, L., Lan, Q., Hubbard, A.E., Forrest, M.S., McHale, C., Zhao, X., Gunn, L., Shen, M., Rappaport, S.M., Yin, S., Chanock, S., Rothman, N., 2005. Use of 'Omic' technologies to study humans exposed to benzene. Chem. Biol. Interact. 153–154, 123–127. https://doi.org/10.1016/j.cbi.2005.03.017.

Van Breda, S.G.J., Wilms, L.C., Gaj, S., Jennen, D.G.J., Briedé, J.J., Kleinjans, J.C.S., de Kok, T.M.C.M., 2015. The exposome concept in a human nutrigenomics study: evaluating the impact of exposure to a complex mixture of phytochemicals using transcriptomics signatures. Mutagenesis 30, 723–731. https://doi.org/10.1093/mutage/gev008.

van Leeuwen, D.M., van Herwijnen, M.H.M., Pedersen, M., Knudsen, L.E., Kirsch-Volders, M., Sram, R.J., Staal, Y.C.M., et al., 2006. Genome-wide differential gene expression in children exposed to air pollution in the Czech Republic. Mutat. Res. 600, 12–22. https://doi.org/10.1016/j.mrfmmm.2006.05.032.

Vrijheid, M., Slama, R., Robins, O., Chatzi, L., Coen, M., van den Hazel, P., et al., 2014. The human early-life exposome (HELIX): project rationale and design. Environ. Health Perspect. 122 (6), 535–544. https://doi.org/10.1289/ehp.1307204.

Wang, Z., Neuburg, D., Li, C., Su, L., Kim, J.Y., Chen, J.C., Christiani, D.C., 2005. Global gene expression profiling in whole-blood samples from individuals exposed to metal fumes. Environ. Health Perspect. 113 (2), 233–241. https://doi.org/10.1289/txg.7273.

Wang, L., Zhang, S., Yu, M., Yuan, J., 2022a. Association between insomnia and subclinical atherosclerosis among Chinese steelworkers: a cross-sectional survey. Arch. Publ. Health 80 (1), 80. https://doi.org/10.1186/s13690-022-00834-1.

Wang, L., Zhang, S., Yu, M., Yuan, J., 2022b. Association between rotating night shift work and carotid atherosclerosis among Chinese steelworkers: a cross-sectional survey. Hypertens. Res. 45 (4), 686–697. https://doi.org/10.1038/s41440-021-00821-z.

Whitney, A.R., Diehn, M., Popper, S.J., Alizadeh, A.A., Boldrick, J.C., Relman, D.A., Brown, P.O., 2003. Individuality and variation in gene expression patterns in human blood. Proc. Natl. Acad. Sci. USA 100 (4). https://doi.org/10.1073/pnas.252784499, 1896-90.

WHO, 2000. Obesity: preventing and managing the global epidemic. Report of a WHO consultation. World Health Organ Tech Rep Ser 894 (i-xii), 1–253.

Wild, C.P., Scalbert, A., Herceg, Z., 2013. Measuring the exposome: a powerful basis for evaluating environmental exposures and cancer risk. Environ. Mol. Mutagen. 54, 480–499. https://doi.org/10.1002/em.21777.

Xu, D., Lu, W., 2020. Defensins: a double-edged sword in host immunity. Front. Immunol. 11, 764. https://doi.org/10.3389/fimmu.2020.00764-z.

Yu, M., Wang, L., Zhang, S., Feng, H., Wu, J., Li, X., Yuan, J., 2022a. Association between neck circumference and subclinical atherosclerosis among Chinese steelworkers: a cross-sectional survey. Int. J. Environ. Res. Publ. Health 19 (11), 6740. https://doi.org/10.3390/ijerph19116740.

Yu, M., Zhang, S., Wang, L., Wu, J., Li, X., Yuan, J., 2022b. Metabolically healthy obesity and carotid plaque among steelworkers in north China: the role of inflammation. Nutrients 14 (23), 5123. https://doi.org/10.3390/nu14235123.

Zhang, Q.J., Liu, Z.P., 2015. Histone methylations in heart development, congenital and adult heart diseases. Epigenomics 7 (2), 321–330. https://doi.org/10.2217/epi.14.60.

Zhang, Y., Huo, X., Lu, X., Zeng, Z., Faas, M.M., Xu, X., 2020. Exposure to multiple heavy metals associate with aberrant immune homeostasis and inflammatory activation in preschool children. Chemosphere 257, 127257. https://doi.org/10.1016/j.chemosphere.2020.127257.

Zhou, G., Soufan, O., Ewald, J., Hancock, R.E.W., Basu, N., Xia, J., 2019. NetworkAnalyst 3.0: a visual analytics platform for comprehensive gene expression profiling and meta-analysis. Nucleic Acids Res. 47 (W1), W234–W241. https://doi.org/10.1093/nar/gkz240.