Identification of the regulatory network and potential markers for type 2 diabetes mellitus related to internal exposure to metals in Chinese adults

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Abstract

People intake metals from their environment. This study investigated type 2 diabetes mellitus (T2DM) related to internal exposure to metals and attempted to identify possible biomarkers. A total of 734 Chinese adults were enrolled and urinary levels of 10 metals were measured. Multinomial logistic regression model was used to assess the association between metals and impaired fasting glucose (IFG) and T2DM. Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), and protein–protein interaction (PPI) were used to explore the pathogenesis of T2DM related to metals. After adjustment, lead (Pb) was positively associated with IFG (odds ratio [OR] 1.31, 95% confidence interval [CI] 1.06–1.61) and T2DM (OR 1.41, 95% CI 1.01–1.98), but cobalt (Co) was negatively associated with IFG (OR 0.57, 95% CI 0.34–0.95). Transcriptome analysis showed 69 target genes involved in the Pb-target network of T2DM. GO enrichment indicated the target genes are enriched mainly in the biological process category. KEGG enrichment indicated Pb exposure leads to non-alcoholic fatty liver disease, lipid and atherosclerosis, and insulin resistance. Moreover, there is alteration of four key pathways, and six algorithms were used to identify 12 possible genes in T2DM related to Pb. *SOD2* and *ICAM1* show strong similarity in expression, suggesting a functional correlation between these key genes. This study reveals that *SOD2* and *ICAM1* may be potential targets of Pb exposure-induced T2DM and provides novel insight into the biological effects and underlying mechanism of T2DM related to internal exposure to metals in the Chinese population.

Introduction

In the past 50 years, the incidence of diabetes has been continued increasing globally, reaching the level of a pandemic (Aschner et al., 2021). In 2019, approximately 463 million adults (20–79 years) had diabetes mellitus, and 36 years later, this number will increase to 700 million (Saeedi et al., 2019). Type 2 diabetes mellitus (T2DM) is a metabolic disorder which caused by genetic and environmental factors, and accounts for more than 90% of diabetes mellitus cases (Hu et al., 2021). Genetic predisposition to a given disease is estimated to be 10% (mostly monogenic, Mendelian disorders); thus, environmental factors account for 80–90% of cases (i.e., complex, polygenic diseases) (Misra & Misra, 2020). Researchers have identified modifiable environmental factors, including specific aspects of diet, overweight, inactivity, and smoking, that account for over 90% of T2DM cases. (Willett, 2002).

Metals may occur as a result of industrial exposure, occupational hazards, air or water pollution, foods, medicines, improperly coated food containers, or the ingestion of lead (Pb)-based paints. Metals can be either toxic (arsenic [As], cadmium [Cd], Pb, and mercury) or essential (chromium, cobalt [Co], molybdenum, manganese [Mn], nickel [Ni], and selenium [Se]) to health, as they could induce the body to produce a large amount of reactive oxygen species (ROS), causing oxidative stress and cellular damage (Peana et al., 2021), or maintain metabolic homeostasis, protecting macromolecules and membrane lipids from oxidative damage (Lv et al., 2021). Thus, it is crucial to explore the association between metal levels in the body and T2DM in the population.

We recently inferred a strong positive association between urinary Cd and T2DM, and a benchmark dose and its low limit estimated for a 5% benchmark response was 0.297 (0.198) μg/g creatinine (Shi et al., 2021). The high-exposure group, based on urinary As, Pb and Cd concentrations, had significantly higher mortality

related to nephritis (Yao et al., 2021). The blood Pb level was significantly different between subjects with T2DM and those without T2DM; however, annual changes in fasting plasma glucose (FPG) according to the Pb concentration only at the beginning of exposure showed a positive correlation (Ji et al., 2021). At the same time, it would have been difficult to observe the true association between local environmental stressor exposure and chronic disease if study subjects with shorter residence times were included. Therefore, in the present study the associations between impaired fasting glucose (IFG) and T2DM with the urinary levels of 10 metals were examined in 734 Chinese adults from two residential communities (lived in the community for at least 20 years). The examined metals were As, Co, iron (Fe), Mn, Ni, Pb, Se, strontium (Sr), vanadium (V), and zinc (Zn).

Furthermore, in the current study molecular mechanisms and potential markers of T2DM related to metal co-exposure were explored based on analysis of the Comparative Toxicogenomics Database (CTD). Then, the target genes for potential effects of metal exposure on T2DM were corroborated. Subsequently, a protein–protein interaction (PPI) network was established, and hub and overlapped genes were identified. The relative expression levels of these hub genes and their relationship with patients with T2DM were verified by using multiple databases.

Materials And Methods

Study population

A cross-sectional survey was conducted between December 2019 and December 2020 in Northeast China. By random group sampling, 800 participants between the ages of 40 and 89 were included, consisting of community residents who had lived in the community for at least twenty years. All participants were examined after an overnight fast. Information about age, gender, the use of alcohol and tobacco, and medical history was obtained by trained reviewers using a standardized structured questionnaire. In addition, information on a participant's history of T2DM included questions about prior diagnoses of T2DM by a physician and current use of insulin and oral hypoglycemic drugs. Basic physical examination including height, weight, blood pressure and abdominal ultrasonographic examination was performed by qualified physicians. Blood and morning spot urine samples were collected from each participant and stored at -80℃ until laboratory analysis. All participants provided written informed consent, and the ethics committee of the China Medical University approved the study.

Exclusion criterion

For the current analyses, those who lacked the complete data of urinary metals or questionnaire (n = 16), and lost data on blood pressure or plasma glucose (n = 50) were excluded for further analysis. Finally, a total of 734 participants were enrolled in the study (Fig. 1).

Definition of IFG and T2DM

FPG concentrations were measured using an Aero set automatic analyzer (by glucose oxidase method; Abbott Laboratories. Abbott Park, Illinois, USA). According to the World Health Organization guidelines

(Alberti & Zimmet, 1998), The definition of IFG was that the FPG 6.1 mmol/L to 7.0 mmol/L; the definition of T2DM was that the FPG 7.0 mmol/L or use of antidiabetic medications or have been diagnosed as T2DM.

Definition of other confounders

Smoking and drinking status were classified into yes and never smoker or drinker groups. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Hypertension was defined as either having a systolic blood pressure (SBP) that was greater than or equal to 140 mmHg or a diastolic blood pressure (DBP) that was greater than or equal to 90 mmHg, or having been diagnosed with hypertension by a physician. The definition of fatty liver was having been diagnosed with abdominal ultrasonographic examination performed by two experienced ultrasonographers.

Determination of urinary metals and creatinine concentrations

Added 0.25 mL of thawed urine into a 15 mL polypropylene centrifuge tube, and then diluted with 10 mL of 5% (vol/vol) HNO_3. Urinary concentrations of As, Co, Fe, Mn, Ni, Pb, Se, Sr, V, and Zn were determined using an inductively coupled mass spectrometer (ICP-MS; Agilent Technologies, Waldbronn, USA). Percent recovery was between 95% and 105%, and relative standard deviation was less than 10%. Added deionized water to a blank urine sampling tube as a control to identify possible contamination. The limits of detection (LOD) for ten metals ranged from 0.0004 to 0.49 mg/L, and values of below the LOD were replaced with a value of $\sqrt{\mathrm{LOD}/2}$. The coefficients (r^2) of the calibration standard solutions of the 10 metals were all greater than 0.999. Urinary creatinine was determined by the creatinine oxidase method on a urinary creatinine assay kit (Nanjing Jiancheng Institute of Biological Engineering, Nanjing, China).

Construct a network diagram of target protein interaction

All the chemicals and their corresponding target genes were downloaded from the CTD (http://ctdbase.org/) [August, 2022], with “T2DM” as the keyword in its “Chemicals” entry, then, the chemicals unrelated to this research were excluded manually (Davis et al., 2021). It was constructed corresponding data tables for the remaining chemicals, T2DM targets and related pathways to construct the relationship data the table was used as the input file of Cytoscape 3.8.0 to construct a network diagram showing the relationship between the chemicals and T2DM (Shannon et al., 2003).

The potential targets of T2DM related to co-exposure of heavy metals were imported into the String database (https://string-db.org/) [August, 2022], restricted the species to “Homo sapiens”, obtained the protein interaction relationship, and exported the results in the TSV format (Szklarczyk et al., 2019). Used Cytoscape 3.8.0 software to draw PPI network diagram to visualize the data; used the cytoHubba plugin to discover the key gene (Chin et al., 2014). The computational plug-in cytoHubba integrates 11 algorithms that can calculate network local feature parameters and global feature parameters to infer the importance of nodes in the network and thus identify the core elements of the network. We selected three local network feature parameter algorithms and three global network feature parameter algorithms based on a comprehensive evaluation of the prediction accuracy of each algorithm: Density of Maximum Neighborhood Component (DMNC), Maximal Clique Centrality (MCC), Degree, Edge Percolated Component (EPC), closeness and Betweenness algorithms to calculate PPI networks' network characteristic parameters and discover key genes

(Chin et al., 2014). At the same time, the calculation results were sorted, and the top 30 genes were selected, and various algorithms were analyzed through the online jvenn tool (Bardou et al., 2014). The selected genes were crossed, and if the same gene could appear in the six algorithms at the same time, it was considered as the key gene.

Gene enrichment and pathway analysis

Gene Ontology (GO) annotated genes through three dimensions: biological process (BP), cellular component (CC), molecular function (MF). Kyoto Encyclopedia of Genes and Genomes (KEGG) could visually detect the pathways involved in the target (Kanehisa & Goto, 2000). In order to reveal the enrichment of the interacting target proteins in the gene function, the potential target genes that associated with T2DM caused by the co-exposure of heavy metals were for GO analysis and KEGG analysis using package “Clusterprofiler” of the R software (Yu et al., 2012), and statistical significance level was set at $\alpha = 0.05$..

Statistical analysis

Descriptive statistics were calculated for all demographic and clinical characteristics of the study subjects. The demographic characteristics of different groups were present as mean ± standard deviation (SD) for continuous variables and n (%) for categorical variables.

Used Mann-Whitney U tests to compare the distribution characteristics of metal concentrations in different groups, and evaluate the correlation between metals by Spearman rank correlation analysis. The variance inflation factor (VIF) was used to determine whether there was collinearity between metals. If the VIF was greater than 10, the collinearity was stronger. It was performed log-transformation on urinary metals concentrations, because of the distribution of urinary metals was left-skewed. Multinomial logistic regression was used to compare the odds ratio (OR) and 95% confidence interval (95% CI) of IFG and T2DM risks. Age (continuous), gender, BMI (continuous), smoking status (yes/no), alcohol drinking status (yes/no), hypertension, fatty liver, and urinary creatinine concentrations (continuous) were the covariates adjusted in the multi-metal models.

Considering the influence of FPG outliers on the results, it was undertaken sensitivity analyse in the study. FPG was performed log-transformation to improve the left-skewed distribution, then FPG outliers were selected by standard deviation method. It was reanalyzed the association between urinary metals and IFG or T2DM by excluding participants who had FPG outliers.

All statistical analyses were performed using IBM SPSS version 25 for Windows (SPSS Inc., Chicago, IL, USA) and R software (version 4.0.3, R Core Team). Two-side test $P < 0.05$ was considered statistically significant.

Results

Basic characteristics of study participants

As shown in Table 1, the participants in this study consisted of 354 men (48.2% of the participants) and 380 women (51.8% of the participants), with a mean age of 63.5 years. The prevalence of IFG and T2DM was

7.5% and 10.6% respectively. In the Normoglycemia group, the median FPG was 4.80 mmol/L, whereas in the IFG and T2DM groups, the median FPG was 6.40 mmol/L and 8.65 mmol/L, respectively (Supplementary Fig. 1). Nearly overweight was observed in normoglycemia and IFG with BMI of 24.6 kg/m^2, and obesity was observed in T2DM with BMI of 25.3 kg/m^2. With regard to lifestyle, the majority of the participants were non-drinker, while the rate of smoking was 25.1% in normoglycemia, 16.4% in IFG, and 20.5% in T2DM. Less than half of normoglycemia had hypertension, but the prevalence of hypertension was dramatically increased with 58.2% in IFG and 64.1% in T2DM. The prevalence of fatty liver was 25.0%, 34.5% and 37.2% in the three divided groups of normoglycemia, IFG and T2DM.

Table 1
Participants' characteristics among the normoglycemia, IFG and T2DM.

	Total (*N*= 734)	Normoglycemia (*N*= 601)	IFG (*N*= 55)	T2DM (*N*= 78)
Age (years)	63.5 ± 11.5	63.3 ± 11.5	65.5 ± 11.3	63.5 ± 11.6
BMI (kg/m^2)	24.7 ± 3.5	24.6 ± 3.5	24.6 ± 3.1	25.3 ± 3.8
Urinary creatinine (mmol/L)	9.3 ± 5.0	9.3 ± 5.0	9.5 ± 4.7	8.6 ± 4.6
Male, n(%)	354 (48.2)	286 (47.6)	26 (47.3)	42 (53.8)
Smocking, n(%)	176 (24.0)	151 (25.1)	9 (16.4)	16 (20.5)
Alcohol drinking, n(%)	57 (7.8)	44 (7.3)	6 (10.9)	7 (9.0)
Hypertension, n(%)	353 (48.1)	271 (45.1)	32 (58.2)	50 (64.1)
Fatty liver, n(%)	198 (27.0)	150 (25.0)	19 (34.5)	29 (37.2)
Abbreviations: IFG, impaired fasting glucose; T2DM, type 2 diabetes mellitus; BMI, body mass index.				
Continuous variables were present as *mean ± SD*.				
Categorical variables were present as *n (%)*.				

Distribution of urinary metals

The geometric means of 10 urinary metals in normoglycemia, IFG and T2DM were presented in Table 2. Detection rates (N% >LOD) of all the metals were > 77%. The distribution of 10 urinary metals (unadjusted and adjusted for urinary creatinine) was given in Supplementary Table 1. Compared with the normoglycemia, the levels of Pb (2.0 μg/L vs 1.9 μg/L) and Zn (628.6 μg/L vs 428.1 μg/L) in urine were higher in IFG and T2DM ($P < 0.05$). As shown in Supplementary Fig. 2, no significant correlations were observed among most of the metals, and significant co-exposures were only identified between Fe and Mn (r = 0.58), As and Se (r = 0.46), and Se and Sr (r = 0.45).

Table 2
Concentrations of urine metals of the participants. (g/L)

μ				
	Total (*N* = 734)	Normoglycemia (*N* = 601)	IFG (*N* = 55)	T2DM (*N* = 78)
As	43.1 (23.4, 85.3)	43.3 (24.3, 85.4)	36.5 (21.2, 101.7)	44.7 (19.8, 74.4)
Co	0.3 (0.2, 0.5)	0.3 (0.2, 0.5)	0.3 (0.2, 0.4)	0.3 (0.2, 0.6)
Fe	63.8 (6.2, 258.2)	63.7 (7.2, 245.7)	50.6 (0.1, 394.2)	70.9 (14.8, 286.5)
Mn	3.0 (0.4, 9.8)	2.8 (0.2, 9.3)	4.3 (0.8, 13.8)	3.8 (1.1, 9.9)
Ni	3.4 (1.0, 6.7)	3.4 (0.9, 7.0)	2.9 (0.5, 5.7)	4.1 (1.9, 7.3)
Pb	1.9 (1.0, 4.0)	1.8 (0.9, 3.8)	2.0 (1.2, 6.9)*	2.1 (1.1, 7.2)
Se	18.8 (10.9, 27.8)	18.8 (11.1, 27.6)	20.9 (11.3, 29.1)	18.2 (10.2, 29.9)
Sr	182.5 (116.7, 275.5)	185.8 (117.7, 276.5)	154.7 (96.2, 258.8)	183.4 (122.7, 280.2)
V	1.5 (0.9, 2.2)	1.5 (1.0, 2.2)	1.3 (0.8, 1.9)	1.3 (0.9, 2.2)
Zn	482.1 (275.0, 812.0)	470.5 (268.6, 784.5)	555.7 (320.8, 896.7)	628.6 (297.0, 993.4)*

Abbreviations: IFG, impaired fasting glucose, T2DM, type 2 diabetes mellitus.

[a] Data were presented as *median* and *quartile interval*.

[b] *p* values were derived from *Mann-Whitney U tests.* * Compared with the normoglycemia, $p < 0.05$.

Urinary metals and T2DM and IFG risk

After adjustment for conventional confounders such as age, BMI, urinary creatinine concentration, gender, smoking status, alcohol drinking status, hypertension, and fatty liver, Co (OR 0.57, 95% CI:0.34–0.95, $P = 0.032$) was significantly negatively associated with IFG, but Se (OR 1.85, 95% CI:1.10–3.13, $P = 0.021$) was significantly associated with IFG (Table 3). Pb was significantly associated with both IFG (OR 1.31, 95% CI: 1.06–1.61, $P = 0.012$) and T2DM (OR 1.41, 95% CI: 1.01–1.98, $P = 0.048$). In the sensitivity analysis, we also obtained similar results (Supplementary Table 2)

Genes associated with T2DM related to Pb exposure

To identify the potential molecular mechanism of T2DM related to Pb exposure, the CTD database was searched. Pb and four Pb containing chemicals including lead acetate, lead chloride, lead chromate and lead nitrate were chosen, and 80 related genes associated with T2DM were found for further analyses in Supplementary Table 3. No-homo sapiens genes (*INS1*, *MIR204*, *MIR222*, *MIR375*, and *MIR4516*) and no-interaction genes (*AUTS2*, *BHMT*, *CMIP*, *JADE2*, *RNF6*, and *SLC22A3*) were excluded. From Pb-target network of T2DM, the top 10 hub target genes with higher degrees were filtered, including Serine / threonine protein kinase (*AKT1*), Interleukin-6 (*IL6*), Tumor necrosis factor (*TNF*), Caspase-3 (*CASP3*), Peroxisome proliferator-activated receptor gamma (*PPARG*), catalase (*CAT*), epidermal growth factor receptor (*EGFR*), superoxide

dismutase 2 (*SOD2*), leptin (*LEP*) and sirtuin (*SIRT1*), and included 69 nodes and 569 edges. (Fig. 2A). Detailed information of these 69 nodes was shown in Supplementary Table 4.

Functional analysis by GO and KEGG enrichment

In order to better understand the effects of Pb exposure on biological function in T2DM, GO enrichment analysis mainly including three categories: biological process, cellular components and molecular function were performed (Fig. 2B). In the biological process category, the target genes were enriched mainly in response to oxidative stress, response to nutrient levels, neuron death, regulation of lipid metabolic process, and response to peptide hormone. In cellular components category, membrane raft, membrane microdomain and membrane region contained the most target genes. In molecular function category, DNA-binding transcription factor binding, RNA polymerase II-specific DNA-binding transcription factor binding, ubiquitin protein ligase binding and ubiquitin-like protein ligase binding were the more abundant functions. The detailed information of GO enrichment analysis was listed in Supplementary Table 5–7.

To further elucidate the function of target genes in signaling pathways, the differently expressed unigenes were located in the KEGG database to analyze the significantly abundant pathways. KEGG pathway enrichment analysis results showed a total of 136 pathways ($P < 0.05$) in Supplementary Table 8. The top 20 KEGG pathways results indicated that enriched pathways played important role in liver and kidney diseases, such as non-alcoholic fatty liver disease, lipid and atherosclerosis, insulin resistance, AGE-RAGE signaling pathway in diabetic complications (Fig. 2C). Meanwhile, four main signal transduction pathways including adipocytokine signaling pathway, longevity regulating pathway, hypoxia-inducible factor 1 (HIF-1) signaling pathway and TNF signaling pathway were also found in the KEGG enrichment pathways (Fig. 2C).

PPI network of intersection target genes and identified key genes

In order to explore the target genes in depth, six algorithms, DMNC, MCC, EPC, Degree, Closeness and Betweenness were used to analyze the network topology of the PPI network (Supplementary Table 9). 12 possible genes related to T2DM were shared in these six datasets (Fig. 2D, Supplementary Table 10). Therefore, the 12 possible genes were mainly focused in the subsequent analyses. Based on the STRING database, a co-occurrence network of total 12 nodes and 56 edges was constructed (Fig. 2E). The local clustering coefficient was 0.861, and the PPI enrichment P value was less than 1 × 10 − 16. It could be concluded that genes such as TNF, endothelial nitric oxide synthase (*NOS3*), adiponectin (*ADIPOQ*), *SIRT1*, mitochondrial uncoupling protein 2 (*UCP2*), *SOD2*, insulin receptor substrate 1 (*IRS1*), *LEP*, endothelin-1 (*EDN1*), intercellular adhesion molecule 1 (*ICAM1*), peroxisome proliferator-activated receptor gamma coactivator 1-alpha (*PPARGC1A*), and resistin (*RETN*) had a strong correlation with T2DM caused by Pb exposure. It was found that there was a strong similarity in expression between *SOD2* and *ICAM1*, suggesting functional correlation between the two genes (Fig. 2F).

Discussion

In the present study, we integrated a crowd survey with metallomics to identify potential metabolic signatures (metabolites, metabolic pathways, and potential markers) associated with metals and T2DM development. In

our overall analysis, urinary Se concentrations were positively associated with IFG risk, whereas urinary Co concentrations showed a negative association with IFG risk. Urinary Pb concentrations were positively associated with the risk of IFG and T2DM. Pb is a toxic heavy metal with complex biological functions, and the mechanism of action of Pb-mediated T2DM is unclear. Therefore, metallomics was used to identify the metabolic pathways and gene networks of Pb to understand the potential markers of Pb-mediated T2DM. On a molecular scale, there were five chemical components and 80 potential targets of Pb that are related T2DM. Both GO and KEGG enrichment results indicate that Pb exposure leading to T2DM is a multi-target, multi-functional, multi-pathway process. Genes such as *TNF*, *NOS3*, *AADIPOQ*, *SIRT1*, *UCP2*, *SOD2*, *IRS1*, *LEP*, *EDN1*, *ICAM1*, *PPARGC1A*, and *RETN* have a strong correlation with T2DM caused by Pb exposure. There is strong similarity in the expression of *SOD2* and *ICAM1.*

The results of the current study showed for the first time that high urinary Se status in the Chinese population correlates with an increased risk of onset of IFG. Several previous studies have reported that high blood Se status is associated with the occurrence of IFG (Akbaraly et al., 2010; Li et al., 2018; Zhou et al., 2019). In humans, Se is an essential metal: it is part of 25 selenoproteins that have selenocysteine at their active center (Kryukov et al., 2003). Selenoprotein P (SeP) functions as a Se-supply protein; it is a secretory protein primarily produced by the liver (Burk & Hill, 2005; Saito & Takahashi, 2002). Treatment of primary hepatocytes with purified SeP could induce a reduction in insulin-stimulated phosphorylation of insulin receptor and AKT, leading to impaired insulin signal transduction and dysregulated cellular glucose metabolism (Misu et al., 2010). This mechanism might provide a comprehensible explanation for the effect of Se on glucose homeostasis.

As the active center of vitamin B12, Co is an essential trace element that is of great

Table 3
Associations of 10 urinary metals concentrations with IFG and T2DM risk.

	VIF	Model 1 [a]				Model 2 [b]			
		IFG		T2DM		IFG		T2DM	
		OR (95%CI)	*P*	OR (95%CI)	*P*	OR (95%CI)	*P*	OR (95%CI)	*P*
As	1.733	1.03 (0.74, 1.45)	0.857	0.96 (0.71, 1.30)	0.793	1.08 (0.76, 1.53)	0.657	0.98 (0.72, 1.32)	0.869
Co	1.641	**0.60 (0.36, 0.98)**	**0.043**	1.15 (0.92, 1.45)	0.226	**0.57 (0.34, 0.95)**	**0.032**	1.00 (0.64, 1.54)	0.984
Fe	1.619	0.93 (0.85, 1.02)	0.137	0.98 (0.90, 1.06)	0.621	0.94 (0.86, 1.04)	0.228	0.99 (0.91, 1.07)	0.783
Mn	1.955	1.13 (0.98, 1.31)	0.090	1.06 (0.93, 1.19)	0.390	1.11 (0.96, 1.29)	0.149	1.04 (0.92, 1.18)	0.491
Ni	1.349	0.92 (0.79, 1.06)	0.252	0.99 (0.86, 1.13)	0.870	0.90 (0.77, 1.05)	0.179	0.98 (0.85, 1.13)	0.789
Pb	1.523	**1.31 (1.07, 1.62)**	**0.010**	**1.43 (1.02, 1.99)**	**0.036**	**1.31 (1.06, 1.61)**	**0.012**	**1.41 (1.01, 1.98)**	**0.048**
Se	1.981	**1.81 (1.09, 3.00)**	**0.022**	0.83 (0.58, 1.19)	0.321	**1.85 (1.10, 3.13)**	**0.021**	0.90 (0.61, 1.33)	0.601
Sr	1.496	0.88 (0.70, 1.10)	0.252	0.99 (0.65, 1.52)	0.980	0.87 (0.69, 1.09)	0.222	1.17 (0.92, 1.47)	0.201
V	1.275	0.87 (0.71, 1.06)	0.167	0.98 (0.81, 1.19)	0.833	0.88 (0.72, 1.08)	0.228	0.95 (0.78, 1.17)	0.647
Zn	1.386	0.87 (0.66, 1.14)	0.303	0.96 (0.83, 1.12)	0.605	0.86 (0.65, 1.15)	0.313	0.95 (0.82, 1.10)	0.490

Abbreviations: IFG, impaired fasting glucose, T2DM, type 2 diabetes mellitus, VIF, variance inflation factor, OR, odds ratio, CI, confidence interval.

[a] *No adjustment*.

[b] *Adjusted* for age, body mass index, urinary creatinine concentration, gender, smoking status, alcohol drinking status, hypertension, fatty liver.

significance to human cell metabolism (Rizzo et al., 2016). Hypoxia, decreased immune function, sexual dysfunction and anemia are the adverse symptoms of the body when Co deficiency occurs, and death may occur in severe Co deficiency (González-Montaña et al., 2020). Previous studies have elucidated that vitamin B12 deficiency may be attributed to increased adiposity, insulin resistance, and diabetes (Krishnaveni et al., 2009; Yajnik et al., 2008). Previous studies have elucidated that vitamin B12 deficiency may be attributed to increased adiposity, insulin resistance, and diabetes (Nomura et al., 2005; Saker et al., 1998; Ybarra et al., 1997). Studies had reported that Co has good hypoglycemic properties and diabetes could be prevented by Co supplementation. There have also been some epidemiological studies reporting the link between Co and glucose metabolism. Chen et al. found in average adult women in the United States that insulin resistance gradually weakened as the blood Co concentrations increased (Chen et al., 2021). Cao et al. demonstrated a U-shaped association between Co and T2DM (Cao et al., 2021). In addition, our findings inferred that Co may be beneficial to glucose metabolism, although this eventuality needed to be verified in a larger sample.

In the present study, we found that urinary lead levels were significantly associated with increased risk of IFG and T2DM in a long-term resident population. A positive association of lead with increased risk of IFG was also found in the short-term exposed population(Feng et al., 2015). The present study adds new evidence for lead-induced disturbances in glucose homeostasis. Other studies have reported that blood Pb concentrations among people with diabetes are higher than among control individuals (Afridi et al., 2008; Serdar et al., 2009). *In vivo* studies have revealed that Pb exposure is pro-diabetic, causing fasting hyperglycemia and glucose intolerance in rats, and chronic exposure to Pb could disrupt glucose homeostasis by affecting the pancreas and liver mainly through induction of insulin resistance (Mostafalou et al., 2015; Tyrrell et al., 2017). These findings illustrate that Pb might adversely affect insulin function and eventually lead to T2DM development. We identified the potential metabolites and metabolic pathways that are associated with Pb and T2DM development. In our analysis, we identified 80 targets of Pb and four Pb-containing chemicals resulting in T2DM, and these targets are part of different metabolic pathways. The KEGG pathway enrichment analysis results showed that Pb affects 136 biological pathways; the top four are the adipocytokine signaling pathway, the longevity regulating pathway, the HIF-1 signaling pathway, and the TNF signaling pathway. Meanwhile, 12 possible target genes related to T2DM were explored: *TNF*, *NOS3*, *AADIPOQ*, *SIRT1*, *UCP2*, *SOD2*, *IRS1*, *LEP*, *EDN1*, *ICAM1*, *PPARGC1A*, and *RETN*.

In yellow catfish, Pb exposure induces transcriptional upregulation of superoxide dismutase (*SOD*), *CAT*, interleukin-10 (*IL10*), transforming growth factor-β (*TGFB*), and *TNF* in the head kidney and spleen, suggesting that Pb impairs immune function and tissue integrity through oxidative stress, inflammation, and apoptosis (Guo et al., 2021). The role of *SIRT1* in the neurotoxicity of Pb has received increasing attention. Researchers have reported that *SIRT1* overexpression or *in vivo* administration can ameliorate the effect of Pb on the nervous system (Bai et al., 2021). Transgenic mice overexpressing *SIRT1* can inhibit liver glycogen production and enhance adiponectin levels to improve glucose tolerance and prevent the occurrence of diabetes (Pardo & Boriek, 2020). Based on the current research results, chronic Pb exposure might lead to the development of T2DM by inhibiting the expression of *SIRT1*. ADIPOQ, which is an adipocytokine rich in blood, can activate AMPK to promote glucose utilization and induce fatty acid oxidation to regulate glucose metabolism and insulin sensitivity (Choe et al., 2016). A prospective study found that LEP was positively correlated with insulin sensitivity, and LEP levels were higher in people with impaired IFG and T2DM (D'Elia et

al., 2019). Pb could increase fasting glucose and serum LEP levels (Beier et al., 2015). Impaired Insulin-stimulated glucose uptake occurs in mice or adipocytes treated with RETN or recombinant RETN, indicating that RETN could promote insulin resistance and affect normal glucose metabolism (Kershaw & Flier, 2004). Meanwhile, we have found that the adipocytokine signaling pathway is one of main signal transduction pathways by which Pb exerts its effects. These findings indicate that fat metabolism is closely associated with the molecular mechanism of Pb-mediated T2DM. Our findings provide possible clues for studying the molecular mechanism of T2DM caused by Pb exposure.

We also carried out a functional investigation by examining the co-expression of potential targets. We found strong similarity in the expression of *SOD2* and *ICAM1*, suggesting a functional correlation between the two genes. SOD are important antioxidant enzymes that decrease ROS levels, meanwhile. SOD2 is the predominant antioxidant enzyme in mitochondria to dismutate superoxide to generate hydrogen peroxide (H_2O_2) (Muscoli et al., 2003). Overexpressing *SOD2* greatly improves the ROS-scavenging capacity of the mitochondria, decreasing ROS levels, normalizing blood glucose levels, and reducing insulin levels (Zhai et al., 2011). ICAM1 belongs to the immunoglobulin superfamily. It is expressed most strongly in vascular endothelial cells and plays a key role in the adhesion of monocytes, lymphocytes, and endothelial cells (Cockerill et al., 1995). The *ICAM1* gene is located on chromosome 19p13, an area that is associated with diabetes (Vora et al., 1994). Previous studies have confirmed that increased *ICAM1* expression is positively correlated with insulin resistance (Hsu et al., 2009; Kent et al., 2004). In a study of the cognitive function of mice, the ROS and MDA contents in the Pb group increased while the GSH and SOD levels decreased compared with those in the control group (Zhang et al., 2021). Therefore, Pb exposure might stimulate the body to generate oxidative stress and an immune response, and thus affect the development of T2DM by regulating the expression of *SOD2* and *ICAM1*.

We designed this study to explore the association between exposure to multiple metals and the T2DM risk of residents in a certain area in Northeast China. Furthermore, we focused on the relationship between metal exposure and IFG, providing important epidemiological evidence for the effective prevention and control of the occurrence and development of T2DM. Finally, we applied metalomics to study the effects of metal metabolites on metabolic pathways. In the current study, we have explored the metal-related metabolomics pathways and possible potential markers with the CTD database and have provided important preliminary evidence for the subsequent identification of the molecular mechanism of T2DM caused by exposure to metals.

One limitation of this study is that we could not establish a causal relationship between exposure to metals and T2DM. We will use a prospective, longitudinal study method in future research. Another limitation is that the most representative biomarkers of the internal levels of certain metals may not be urinary metal concentrations due to recent exposure. In the future, the impact of other markers of internal exposure to metals on T2DM should be assessed to explore the relationship between different exposure states of metals and T2DM. The potential biomarkers obtained in our study were based on the analysis of data downloaded from public databases, which are accurate and reliable, but do not cover all information. Finally, the mechanisms behind T2DM are complex, involving activation of multiple signaling pathways and proteins, as well as cross-activation between various signaling pathways. We only focused on *SOD2* and *ICAM1* and did

not observe the effects of other signaling pathways and proteins. Therefore, further studies are needed to assess the potential effects of heavy metals on other signaling pathways and proteins.

In summary, urinary Pb is positively associated with the risk of T2DM and IFG. Urinary Se has a positive association with IFG risk among participants. On the contrary, urinary Co is negatively associated with IFG risk. Moreover, we examined the top 12 genes with a strong correlation with Pb exposure causing T2DM, including *TNF*, *NOS3*, *AADIPOQ*, *SIRT1*, *UCP2*, *SOD2*, *IRS1*, *LEP*, *EDN1*, *ICAM1*, *PPARGC1A*, and *RETN*. In addition, we identified some potential markers with a strong similarity in expression, namely *SOD2* and *ICAM1*. The findings of this study will help identify novel biomarkers, pathways of disease, and potential signatures of environmental metal exposure, to assist in developing and designing promising diagnostic biomarkers and novel therapeutic drugs for metal exposure.

Abbreviations

95% CI	95% confidence interval
BMI	Body mass index
CTD	Comparative Toxicogenomics Database
DMNC	Density of Maximum Neighborhood Component
EPC	Edge Percolated Component
FPG	Fasting plasma glucose
GO	Gene Ontology
IFG	Impaired fasting glucose
KEGG	Kyoto Encyclopedia of Genes and Genomes
LOD	Limits of detection
MCC	Maximal Clique Centrality
OR	Odds ratio
PPI	Protein–protein interaction
SD	Standard deviation
T2DM	Type 2 diabetes mellitus
VIF	Variance inflation factor

Declarations

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Author contributions YW and PS analyzed the data, and wrote the manuscript. CKZ, JGS, ZPQ, XSH, XW, NS, and ZJG collected the data. JHZ and MH edited, revised the manuscript, and approved the final version of the manuscript. All authors contributed to the article and approved the submitted version.

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Data availability and material Anonymised study data are available via the corresponding author. The CTD dabates that support the fndings of this study are openly available (see the Materials and methods section for the details)(Davis et al., 2021)

Conflict of interest The authors have no conflicts of interest to declare that are relevant to the content of this article, neither do they have any relevant financial or non-financial interests to disclose.

Consent to participate Participants signed an informed consent form prior to participation in the study.

Consent for publication Participants signed consent for publication of the study.

Ethical approval The studies involving human participants were reviewed and approved by the ethics committee of the China Medical University. The participants provided their written informed consent to participate in this study.

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Figures

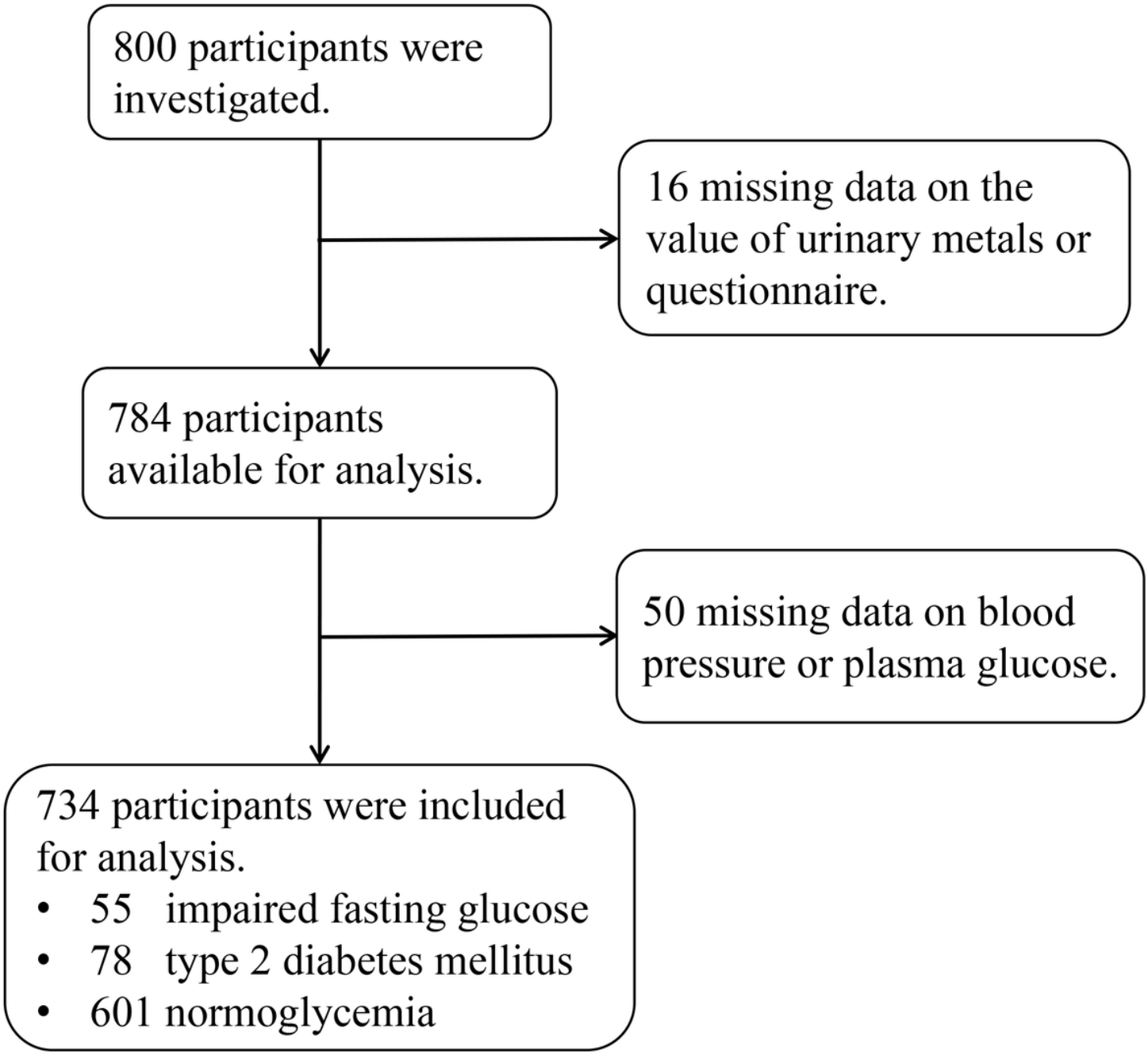

Figure 1

Flow chart of the inclusion of participants.

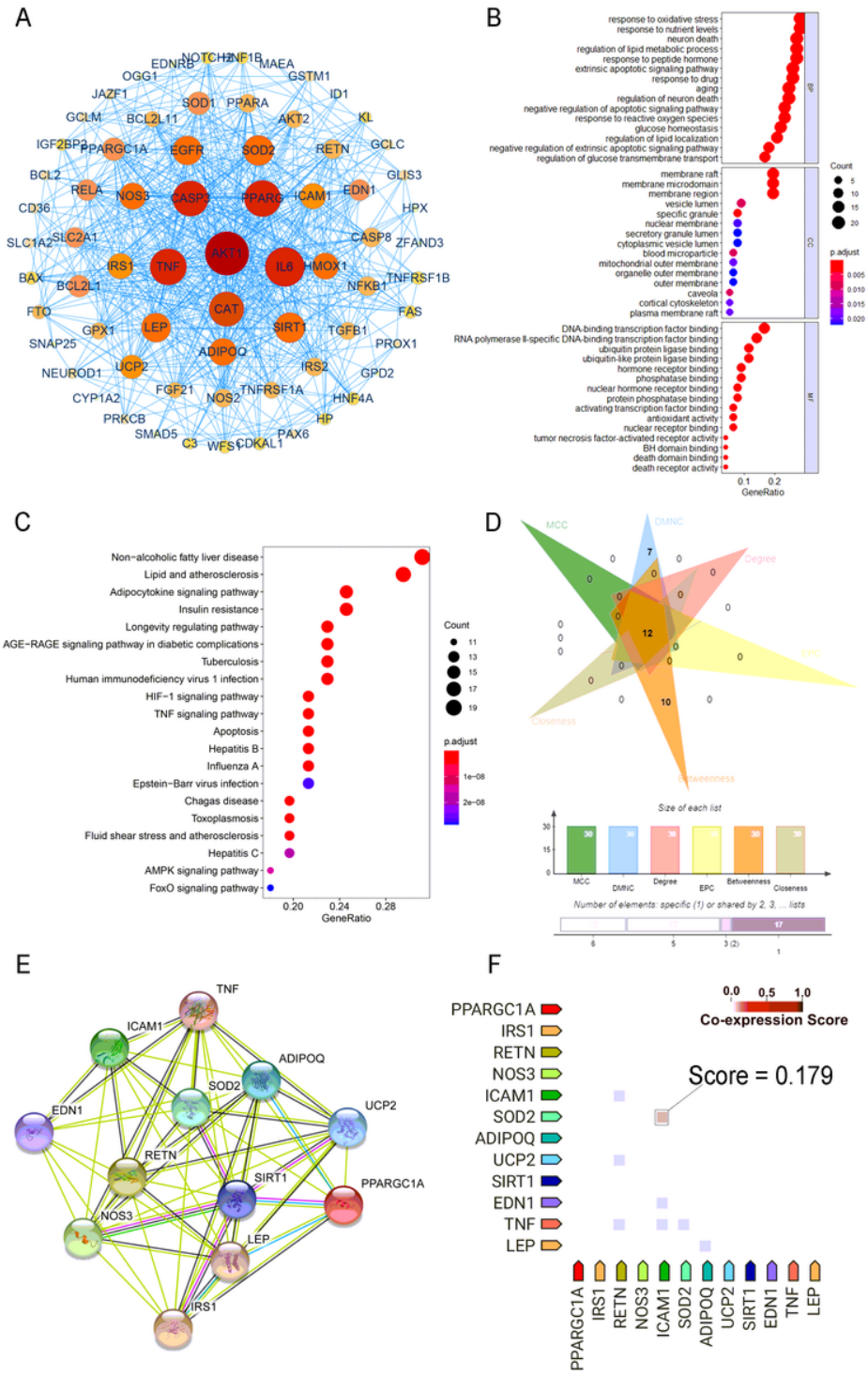

Figure 2

(A) PPI network diagram of target genes. **(B)**GO enrichment analyses of putative target genes. **(C)** KEGG pathway analysis of putative target genes. **(D)** Venn diagram of intersecting common genes identified by differential genes from six algorithms. (**E**) PPI network diagram of screened genes shared in the six algorithms datasets.**(F)** Combined genetic co-expression detected by the STRING database. Correlated gene were displayed with co-expression score. Color intensity reflect the reliability of co-expression. Co-expression of *SOD2* and *ICAM1*was found (score=0.179).

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