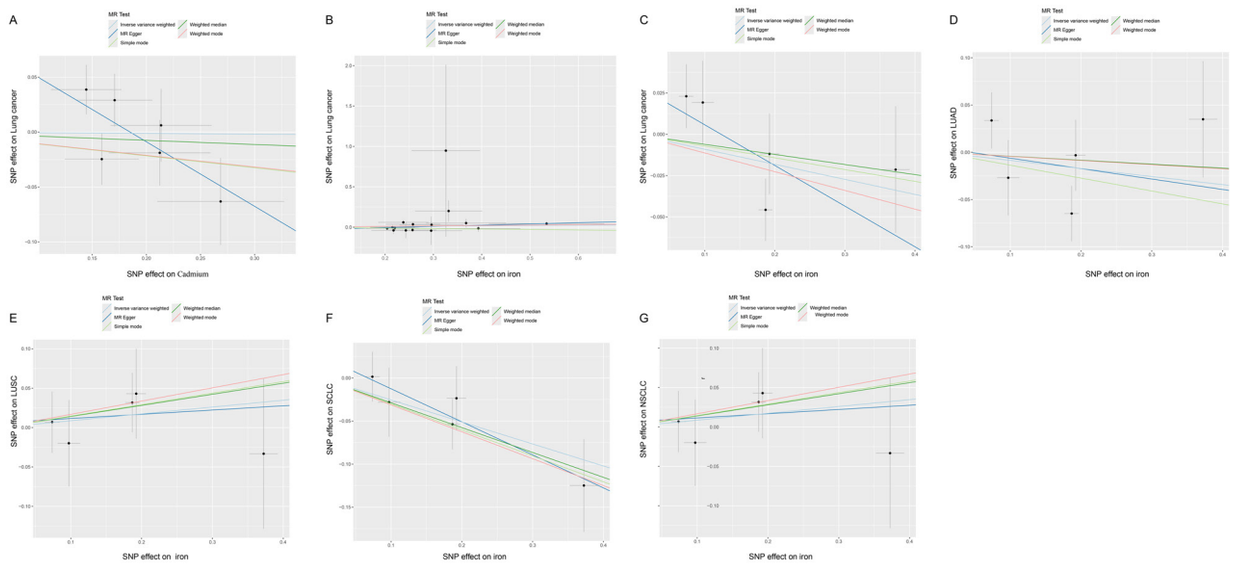

Figure S1 Funnel plot. (A) Causal relationship between cadmium and lung cancer in the Asian population. (B) Causal relationship between iron and lung cancer in the Asian population. (C) Causal relationship between iron and lung cancer in the European population. (D) Causal relationship between iron and LUAD in the European population. (E) Causal relationship between iron and LUSC in the European population. (F) Causal relationship between iron and SCLC in the European population. (G) Causal association between iron and NSCLC in the European population. SNP, single nucleotide polymorphism; MR, Mendelian randomization; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; SCLC, small cell lung cancer; NSCLC, non-small cell lung cancer.

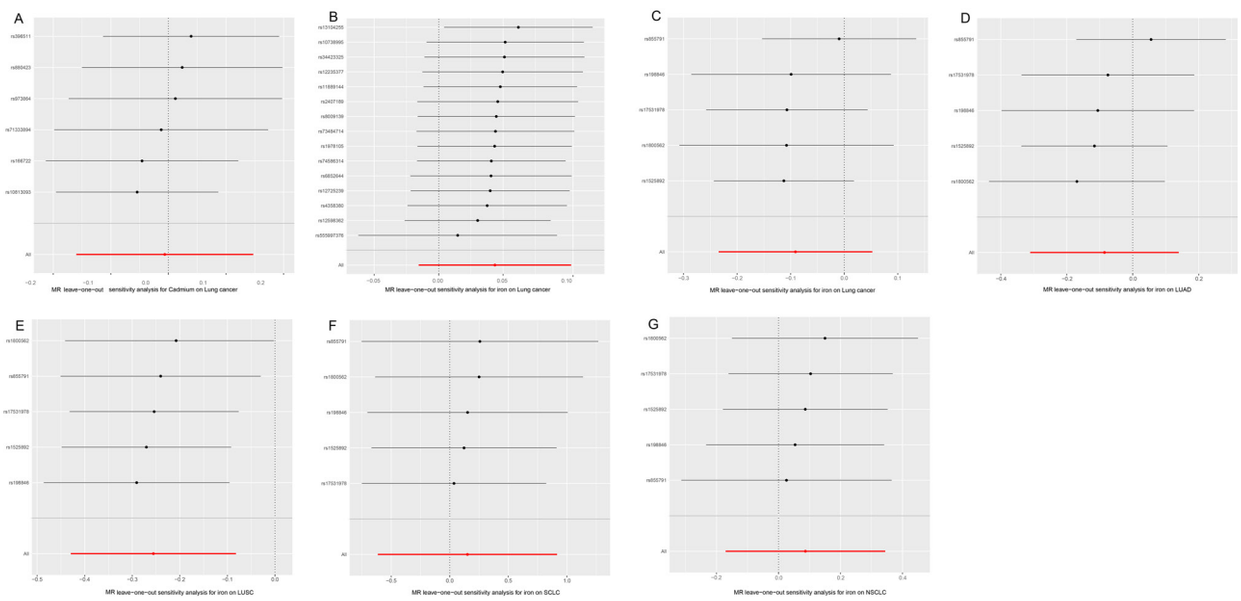

Figure S2 Leave-one-out sensitivity analysis. (A) Causal relationship between cadmium and lung cancer in the Asian population. (B) Causal relationship between iron and lung cancer in the Asian population. (C) Causal relationship between iron and lung cancer in the European population. (D) Causal relationship between iron and LUAD in the European population. (E) Causal relationship between iron and LUSC in the European population. (F) Causal relationship between iron and SCLC in the European population. (G) Causal association between iron and NSCLC in the European population. MR, Mendelian randomization; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; SCLC, small cell lung cancer; NSCLC, non-small cell lung cancer.

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Table S1 Key elements of the study design

Phase	Element	Details
NHANES Observational Study	Setting	Cross-sectional study conducted by the CDC, utilizing NHANES data from 1999–2018. Participants were recruited across the U.S. using a multistage probability sampling method. Blood samples were collected at Mobile Examination Centers (MECs) and processed at CDC labs
	Participants	Eligibility criteria: adults aged 18+ with blood tests for metallic elements (cadmium, lead, mercury, selenium, manganese, cobalt, copper, zinc, iron). Exclusion: participants <18 years, those without blood tests, unspecified lung cancer, missing covariate data, pregnant women. Final sample size: 48,132 participants. No prior power or sample size calculation was reported
	Measurement, Quality Control, and Genetic Variant Selection	Blood metal levels were measured using ICP-MS and HPLC. Quality control: Blood samples were cryopreserved and analyzed under standardized conditions. Genetic variants were selected based on genome-wide significance ($P<5\times10^{-8}$), with adjustments for linkage disequilibrium ($r^2<0.01$).
	Assessment Methods	Exposure: blood metal levels were the primary exposures, assessed through validated laboratory methods. Outcome: Lung cancer status was determined based on participant self-report, corroborated by responses to specific NHANES questions about cancer diagnoses. Covariates included demographic and socioeconomic factors (age, gender, race/ethnicity, education, marital status, income, smoking status, and BMI). Diagnostic criteria: Self-reported lung cancer was cross-referenced with age at diagnosis to ensure consistency
	Ethics and Consent	NHANES was approved by the National Center for Health Statistics (NCHS) Research Ethics Review Board, and written informed consent was obtained from all participants. The study used de-identified, publicly available data, exempting it from further ethical review
Mendelian randomization (MR) analysis	Setting	Bidirectional two-sample MR using GWAS data from various sources: ILCCO, FinnGen, BioBank Japan. These datasets include genetic information on European and Asian populations related to lung cancer and blood metal levels, and focus on lung cancer and its subtypes, as well as blood metal levels
	Participants	GWAS data were sourced from large international cohorts. The ILCCO and FinnGen datasets provided data on European populations, while BBJ provided data on Asian populations. The selection criteria for participants in these GWAS included diagnosis of lung cancer or related subtypes, as well as genetic information on blood metal levels. The GWAS data included tens of thousands of participants, ensuring a robust sample size for MR analysis. No additional participant recruitment or power calculations were performed for this MR analysis as it relied on existing GWAS summary statistics
	Measurement, Quality Control, and Genetic Variant Selection	Genetic variants (SNPs) were selected based on strict significance criteria ($P<5\times10^{-8}$), with linkage disequilibrium parameters set to $r^2<0.01$, ensuring robust instrumental variables for MR analysis. The F-statistic for selected SNPs was calculated to ensure strong instruments, with an F>10 considered adequate. Quality control procedures were applied by the original GWAS studies, including checks for population stratification and genotyping errors
	Assessment Methods	The MR analysis examined the causal relationship between blood metal levels and lung cancer outcomes. The primary method used was inverse variance weighting (IVW), with additional sensitivity analyses conducted using MR-Egger, weighted median, and simple mode approaches to validate the findings. Cochrane's Q test was employed to assess heterogeneity, and the MR-Egger intercept was used to detect horizontal pleiotropy. MR-PRESSO was also used to identify and correct for pleiotropy. Leave-one-out analyses were performed to evaluate the influence of individual SNPs on the overall results
	Ethics and Consent	Each GWAS used was reviewed and approved by local ethics committees, with participants providing informed consent. The MR analysis did not involve direct interaction with participants but utilized publicly available GWAS summary statistics

NHANES, National Health and Nutrition Examination Survey; CDC, centers for disease control and prevention; ICP-MS, inductive coupled plasma mass spectrometry; HPLC, high-performance liquid chromatography; BMI, body mass index; GWAS, genome-wide association studies; ILCCO, international lung cancer consortium; BBJ, biobank japan; SNP, single nucleotide polymorphism.

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Table S2 Abbreviation for blood metal elements included in the study

Blood metal elements	Abbreviations	Years
Cadmium (μg/L)	Cd	1999–2018
Lead (μg/dL)	Pd	1999–2018
Mercury (μg/L)	Hg	1999–2018
Selenium (μg/L)	Se	2011–2018
Manganese (μg/L)	Mn	2011–2018
Cobalt (μg/L)	Co	2015–2018
Copper (μg/dL)	Cu	2011–2016
Iron (μg/dL)	Fe	1999–2018
Zinc (μg/dL)	Zn	2011–2016

Table S3 Genome wide association study (GWAS) source

Trait(s)	PMID	Year	Sample size	Number of SNPs	Population ancestry	Gender	The type of database
Serum iron measurement	25352340	2014	23,986	2,096,457	European	Males and females	GIS
Serum iron measurement	–	2020	1,469	9,797,409	South Asian	Males and females	UKB
Blood cadmium measurement	35501403	2022	1,775	6,148,846	East Asian	Males and females	EBI
Lung cancer	24880342	2014	27,209	8,945,893	European	Males and females	ILCCO
Lung cancer	34594039	2021	178,726	12,454,705	East Asian	Males and females	BBJ
LUAD	24880342	2014	18,336	8,881,354	European	Males and females	ILCCO
LUSC	24880342	2014	18,313	8,893,750	European	Males and females	ILCCO
SCLC	–	2021	174,185	16,380,303	European	Males and females	FinnGen biobank
NSCLC	–	2021	175,633	16,380,305	European	Males and females	FinnGen biobank

SNP, single nucleotide polymorphism; SCLC, small cell lung cancer; NSCLC, non-small cell lung cancer; LUSC, lung squamous cell carcinoma; LUAD, lung adenocarcinoma. GIS, Genetics of Iron Status; UKB, UK Biobank; EBI, European Bioinformatics Institute; ILCCO, international lung cancer consortium; BBJ, Biobank Japan.

Table S4 Distributions of blood metal elements in the study population.

Blood metal elements	Abbreviations	Years	Miss rate	Detection rate	Mean ± SD	Median (Q1, Q3)
Cadmium (μg/L)	Cd	1999–2018	10.91%	85.09%	0.54±0.59	0.37 (0.21, 0.61)
Lead (μg/dL)	Pd	1999–2018	10.91%	99.78%	1.82±1.86	1.37 (0.86, 2.20)
Mercury (μg/L)	Hg	1999–2018	23.42%	89.30%	1.59±2.49	0.86 (0.45, 1.73)
Selenium (μg/L)	Se	2011–2018	25.57%	100.00%	184.30±33.34	186.16 (165.77, 203.62)
Manganese (μg/L)	Mn	2011–2018	25.57%	100.00%	9.96±3.88	9.27 (7.41, 11.68)
Cobalt (μg/L)	Co	2015–2018	31.39%	99.58%	0.21±0.52	0.15 (0.12, 0.19)
Copper (μg/dL)	Cu	2011–2016	66.97%	100.00%	119.38±29.56	114.60 (99.40, 133.90)
Iron (μg/dL)	Fe	1999–2018	1.71%	100.00%	84.95±35.57	81.00 (61.00, 104.00)
Zinc (μg/dL)	Zn	2011–2016	66.98%	100.00%	81.49±15.18	80.40 (71.10, 90.22)

SD, standard deviation.

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Table S5 Logistic regression analysis of the relationship between blood metal elements and lung cancer

Blood metal elements	Continues [OR (95% CI)]	Q1(Ref)	Q2 [OR (95% CI)]	Q3 [OR (95% CI)]	Q4 [OR (95% CI)]	P
Cadmium						
Cases/numbers	100/42,787	12/10,765	15/10,720	32/10,921	41/10,371	
Model 1	1.33 (1.09–1.62)	Ref	1.99 (0.74–5.39)	6.11 (2.57–14.51)	8.21 (3.50–19.25)	<0.001
Model 2	1.38 (1.09–1.75)	Ref	1.54 (0.51–4.61)	4.24 (1.63–10.99)	6.39 (2.48–16.51)	<0.001
Model 3	1.45 (1.09–1.93)	Ref	1.39 (0.46–4.18)	3.44 (1.32–8.98)	5.94 (2.23–15.85)	<0.001
Lead						
Cases/numbers	100/42,787	12/10765	15/10,720	32/10,931	41/10,371	
Model 1	1.08 (1.04–1.13)	Ref	1.26 (0.59–2.68)	2.63 (1.35–5.11)	3.56 (1.87–6.77)	<0.001
Model 2	1.05 (0.96–1.14)	Ref	0.71 (0.32–1.59)	1.04 (0.50–2.16)	0.97 (0.46–2.05)	0.67
Model 3	1.04 (0.94–1.14)	Ref	0.66 (0.29–1.47)	0.89 (0.42–1.86)	0.81 (0.38–1.73)	0.96
Mercury						
Cases/numbers	65/32,905	17/8,333	20/8,483	12/7,872	16/8,217	
Model 1	0.98 (0.88–1.09)	Ref	1.16 (0.61–2.21)	0.75 (0.36–1.57)	0.96 (0.48–1.89)	0.62
Model 2	0.90 (0.76–1.07)	Ref	1.33 (0.68–2.62)	0.60 (0.26–1.38)	0.74 (0.34–1.62)	0.19
Model 3	0.90 (0.76–1.07)	Ref	1.29 (0.66–2.54)	0.58 (0.25–1.35)	0.71 (0.33–1.56)	0.16
Selenium						
Cases/numbers	38/15,217	10/3,805	7/3,806	9/3,804	12/3,802	
Model 1	1.00 (0.99–1.01)	Ref	0.70 (0.27–1.84)	0.90 (0.37–2.22)	1.20 (0.52–2.78)	0.56
Model 2	1.00 (0.99–1.01)	Ref	0.88 (0.30–2.56)	1.43 (0.54–3.75)	1.47 (0.57–3.81)	0.30
Model 3	1.00 (0.99–1.01)	Ref	0.95 (0.32–2.77)	1.47 (0.56–3.88)	1.53 (0.59–4.00)	0.28
Manganese						
Cases/numbers	38/15,228	10/3,816	8/3,804	15/3,809	5/3,799	
Model 1	0.96 (0.87–1.05)	Ref	0.80 (0.32–2.03)	1.51 (0.68–3.36)	0.50 (0.17–1.47)	0.57
Model 2	1.02 (0.93–1.11)	Ref	1.48 (0.53–4.17)	2.62 (1.01–6.76)	1.11 (0.33–3.68)	0.42
Model 3	1.02 (0.93–1.11)	Ref	1.57 (0.56–4.43	2.69 (1.04–6.99)	1.15 (0.35–3.80)	0.40
Cobalt						
Cases/numbers	24/6,918	3/1,909	6/2,032	8/1,423	7/1,554	
Model 1	1.10 (0.72–1.68)	Ref	1.88 (0.47–7.53)	3.59 (0.95–13.56)	2.87 (0.74–11.14)	0.07
Model 2	1.10 (0.69–1.75)	Ref	1.14 (0.25–5.15)	2.74 (0.69–10.95)	1.89 (0.45–7.97)	0.23
Model 3	1.03 (0.65–1.64)	Ref	1.18 (0.26–5.36)	2.65 (0.66–10.62)	1.76(0.42–7.48)	0.29
Copper						
Cases/numbers	13/5,113	2/1,279	4/1,277	1/1,278	6/1,277	
Model 1	1.01 (1.00–1.03)	Ref	2.01 (0.37–10.97)	0.50 (0.05–5.52)	3.02 (0.61–14.99)	0.27
Model 2	1.01 (0.98–1.03)	Ref	1.97 (0.34–11.25)	0.54 (0.04–6.42)	2.98 (0.44–20.05)	0.44
Model 3	1.00 (0.98–1.03)	Ref	1.90 (0.32–11.22)	0.50 (0.04–6.02)	2.38 (0.33–17.24)	0.62
Iron						
Cases/numbers	112/47,309	42/12,234	35/11,840	22/11,410	13/11,825	
Model 1	0.99 (0.98–0.99)	Ref	0.86 (0.55–1.35)	0.56 (0.33–0.94)	0.32 (0.17–0.60)	<0.001
Model 2	0.99 (0.98–1.00)	Ref	0.71 (0.43–1.17)	0.53 (0.30–0.92)	0.35 (0.18–0.69)	<0.001
Model 3	0.99 (0.98–0.99)	Ref	0.69 (0.42–1.14)	0.50 (0.29–0.88)	0.34 (0.17–0.67)	<0.001
Zinc						
Cases/numbers	13/5,108	2/1,283	3/1,283	4/1,265	4/1,277	
Model 1	1.01 (0.98–1.05)	Ref	1.50 (0.25–9.00)	2.03 (0.37–11.11)	2.01 (0.37–11.01)	0.38
Model 2	1.00 (0.97–1.04)	Ref	1.58 (0.26–9.69)	2.50 (0.45–13.96)	1.10 (0.15–8.03)	0.76
Model 3	1.00 (0.97–1.04)	Ref	1.34 (0.21–8.37)	2.33 (0.41–13.16)	1.04 (0.14–7.68)	0.78

Model 1 was not adjusted for any covariate factors; Model 2 was further adjusted for age, gender, race/ethnicity, education, marital status, PIR and AHI; Model 3 was additionally adjusted for BMI and smoking based on model 2. OR, odds ratio; CI, confidence interval; PIR, poverty income ratio; AHI, annual household income; BMI, body mass index; Ref, reference.

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Table S6 The sensitivity between blood metal elements (cadmium, iron) and lung cancer in the Asian population

Blood metal elements	nSNP	Heterogeneity		Pleiotropy P	MR-PRESSO P
		Type	P		
Cadmium	6	MR Egger	0.31	0.15	0.94
		Inverse variance weighted	0.13	–	–
Iron	15	MR Egger	0.36	0.19	0.17
		Inverse variance weighted	0.30	–	–

nSNP, the number of single nucleotide polymorphism; MR, Mendelian randomization; MR-PRESSO, MR-pleiotropy residual sum and outlier.

Table S7 The sensitivity between blood metal elements (iron) and lung cancer (lung cancer, subtypes of lung cancer) on the Asian population

Method	nSNP	Heterogeneity		Pleiotropy P	MR-PRESSO P
		Type	P		
Lung cancer	5	MR Egger	0.24	0.34	0.28
		Inverse variance weighted	0.19	–	–
LUAD	5	MR Egger	0.11	0.93	0.50
		Inverse variance weighted	0.19	–	–
LUSC	5	MR Egger	0.87	0.495	0.07
		Inverse variance weighted	0.86	–	–
SCLC	5	MR Egger	0.83	0.43	0.59
		Inverse variance weighted	0.79	–	–
NSCLC	5	MR Egger	0.78	0.91	0.28
		Inverse variance weighted	0.89	–	–

nSNP, the number of single nucleotide polymorphism; MR, Mendelian randomization; MR-PRESSO, MR-pleiotropy residual sum and outlier; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; SCLC, small cell lung cancer; NSCLC, non-small cell lung cancer.

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