SUPPLEMENTAL MATERIAL
Circulating multiple metals and incident stroke in Chinese adults: the Dongfeng-Tongji cohort
Yang Xiao, Yu Yuan, Yiyi Liu, Yanqiu Yu, Ningning Jia, Lue Zhou, Hao Wang, Suli Huang, Wilson Wang, Yanwei Zhang, Handong Yang, Xiulou Li, Frank B. Hu, Liming Liang, An Pan, Xiaomin Zhang, Meian He, Jinquan Cheng, Tangchun Wu

Author Affiliations:
Department of Occupational and Environmental Health, Key Laboratory of Environment and Health, Ministry of Education and State Key Laboratory of Environmental Health (Incubating) (Y.X., Y.Y., Y.L., Y.Y., N.J., L.Z., H.W., X.Z., M.H., T.W.), and Department of Epidemiology and Biostatistics (A.P.), School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China. Key Laboratory of Molecular Biology (S.H., Y.Z.), and Department of Molecular Epidemiology (J.C.), Shenzhen Center for Disease Control and Prevention, Shenzhen 518055, China. Department of Cardiovascular Diseases, Dongfeng Central Hospital, Hubei University of Medicine, Shiyan 44208, China (H.Y., X.L.). Department of Nutrition and Department of Epidemiology (F.B.H.), and Department of Biostatistics and Department of Epidemiology (L.L.), Harvard T.H. Chan School of Public Health, Boston, Massachusetts 02115, United States
Supplemental Methods

Confirmation of incident stroke cases

Stroke was diagnosed following the WHO criteria as a constellation of neurologic deficits of sudden or rapid onset that lasted at least 24 hours or until death with no apparent cause other than that of vascular origin. The follow-up questionnaires recorded the information of history of chronic disease. The DMC health-care service system covered all the participants and provided complete morbidity and mortality records until December 31, 2016. Possible stroke cases were initially identified through follow-up questionnaires, medical insurance documents, death certificates and hospital records. An expert panel of physicians reviewed medical records and adjudicated definite stroke cases according to clinical symptoms and confirmatory imaging findings. Moreover, fatal stroke cases were identified by death certificates with International Classification of Diseases (ICD) codes (ICD-10 I60, I61, I63, I64 and ICD-10 I69.0, I69.1, I69.3, I69.4). The researchers croschecked all the dates of stroke events through medical records and death certificates. Incident stroke cases were identified as the first occurrence of definite stroke between the sampling date and December 31, 2016. All the definite stroke cases included in our analysis received CT or MRI examination. Among the 1,304 stroke cases, a total of 619 subjects received CT and 411 received MRI, and the other 274 participants underwent both CT and MRI.

Covariate data collection

Trained interviewers used a semi-structured questionnaire to collect the information of socio-demographics (e.g., age and sex), lifestyle habits (e.g., smoking status, drinking status and physical activity), personal and family history of chronic diseases (e.g., stroke, CHD, cancer, hypertension, hyperlipidemia and diabetes mellitus) and medication use. Smokers were classified as current smokers who smoked at least one cigarette per day over the last six months, ever smokers who quitted smoking for more than one month, and never smokers. Drinkers were classified as current drinkers who drunk more than once per week over the last six months, ever drinkers who quitted drinking for more than one month, and never drinkers. Regular exercise was defined as physical exercise for at least 30 min in no less than 5 days per week for more than half a year.

Physical examinations including measurements of weight, height, blood pressures (BPs), blood lipids and fasting glucose were taken by qualified physicians in Dongfeng General Hospital. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters and overweight was defined as BMI≥24 kg/m². Hyperlipidemia was defined as self-reported hyperlipidemia, or using anti-hyperlipidemia medications, or total cholesterol (TC)≥6.22 mmol/L, or triglycerides (TG)≥2.26 mmol/L, or high-density lipoprotein cholesterol (HDL-C)<1.04 mmol/L, or high-density lipoprotein cholesterol (LDL-C)≥4.14 mmol/L. Diabetes was defined as self-reported diabetes mellitus, or use of anti-diabetic medications, or fasting glucose≥7.0 mmol/L. Hypertension was defined as self-reported hypertension, using antihypertensive medication, or having systolic blood pressure (SBP)≥140 mmHg or diastolic blood pressure (DBP)≥90 mmHg.

Quality control methods for metal measurement

Matched case-control sets were assessed in the same analytical run but in random order with laboratory personnel blinded to the case-control status. The methods of accuracy evaluation and quality control have been described previously. We assayed a spiked pooled specimen of 100 randomly selected plasma samples for the metals without certified reference samples (rubidium, mercury, titanium and tungsten). The spike recovery values of those four metals ranged from 84.40% to 104.07%. Intra-assay and inter-assay coefficients of variations for all plasma metals were below 15% (Table I). The limits of detection (LOD) for plasma metals were presented in Table I. For participants below the LOD, the metal concentrations were substituted with the value half of the detection limit.
Table I. Limits of detection, percentages of samples below detection limits, intra-assay and inter-assay coefficients of variation (total n=2608)

| Plasma metals | LOD (μg/L) | Total No. (%) | Ischemic stroke | Hemorrhagic stroke | Intra-assay | Inter-assay |
|---------------|------------|---------------|-----------------|-------------------|-------------|-------------|
|               | <LOD<sup>*</sup> | No. (%) <LOD<sup>*</sup> | No. (%) <LOD<sup>*</sup> | CV% | CV% |
| Aluminum      | 0.4267     | 56 (2.15)     | 48 (2.32)       | 8 (1.49)          | 6.00        | 6.86        |
| Antimony      | 0.0019     | 606 (23.24)   | 472 (22.80)     | 134 (24.91)       | 7.07        | 4.60        |
| Arsenic       | 0.0141     | 6 (0.23)      | 5 (0.24)        | 1 (0.19)          | 3.60        | 5.37        |
| Barium        | 0.0347     | 0 (0.00)      | 0 (0.00)        | 0 (0.00)          | 4.09        | 4.44        |
| Cadmium       | 0.0015     | 15 (0.58)     | 14 (0.68)       | 1 (0.19)          | 5.39        | 6.33        |
| Chromium      | 0.0680     | 2 (0.08)      | 2 (0.10)        | 0 (0.00)          | 5.74        | 5.90        |
| Cobalt        | 0.0033     | 24 (0.92)     | 21 (1.01)       | 3 (0.56)          | 5.02        | 4.55        |
| Copper        | 0.0128     | 0 (0.00)      | 0 (0.00)        | 0 (0.00)          | 4.29        | 4.98        |
| Iron          | 0.5879     | 0 (0.00)      | 0 (0.00)        | 0 (0.00)          | 5.26        | 6.23        |
| Lead          | 0.0114     | 0 (0.00)      | 0 (0.00)        | 0 (0.00)          | 9.92        | 4.75        |
| Manganese     | 0.0169     | 9 (0.35)      | 8 (0.39)        | 1 (0.19)          | 6.49        | 4.66        |
| Mercury       | 0.0050     | 42 (1.61)     | 34 (1.64)       | 8 (1.49)          | 4.38        | 8.32        |
| Molybdenum    | 0.0042     | 0 (0.00)      | 0 (0.00)        | 0 (0.00)          | 7.67        | 5.51        |
| Nickel        | 0.0382     | 61 (2.34)     | 41 (1.98)       | 20 (3.72)         | 6.27        | 5.70        |
| Rubidium      | 0.0075     | 0 (0.00)      | 0 (0.00)        | 0 (0.00)          | 3.69        | 5.33        |
| Selenium      | 0.0323     | 0 (0.00)      | 0 (0.00)        | 0 (0.00)          | 5.05        | 8.53        |
| Strontium     | 0.0228     | 0 (0.00)      | 0 (0.00)        | 0 (0.00)          | 4.34        | 5.61        |
| Thallium      | 0.0003     | 0 (0.00)      | 0 (0.00)        | 0 (0.00)          | 4.83        | 4.25        |
| Tin           | 0.0100     | 1368 (52.45)  | 1048 (50.63)    | 320 (59.48)       | 9.38        | 5.93        |
| Titanium      | 0.1527     | 0 (0.00)      | 0 (0.00)        | 0 (0.00)          | 5.11        | 9.53        |
| Tungsten      | 0.0010     | 222 (8.51)    | 177 (8.55)      | 45 (8.36)         | 12.43       | 6.78        |
| Uranium       | 0.0004     | 567 (21.74)   | 434 (20.97)     | 133 (24.72)       | 13.14       | 11.72       |
| Vanadium      | 0.0043     | 0 (0.00)      | 0 (0.00)        | 0 (0.00)          | 4.57        | 3.46        |
| Zinc          | 0.7324     | 0 (0.00)      | 0 (0.00)        | 0 (0.00)          | 3.67        | 5.45        |

Abbreviations: LOD, limit of detection; CV, coefficient of variation.
<sup>*</sup>Number and percentage of samples below LOD.
| Variables                       | Ischemic stroke | Hemorrhagic stroke |
|--------------------------------|-----------------|-------------------|
|                                | Controls (n=1035) | Cases (n=1035) | P value* | Controls (n=269) | Cases (n=269) | P value* |
| Age (year)                     | 66.8±7.5        | 66.8±7.6         | 0.79     | 65.62±7.7        | 65.62±7.7        | 1.00     |
| Male, No. (%)                  | 653 (63.1)      | 653 (63.1)       | 1.00     | 157 (58.4)       | 157 (58.4)       | 1.00     |
| Body mass index (kg/m²)        | 24.4±3.0        | 24.7±3.2         | 0.019    | 24.2±3.1        | 24.4±3.5         | 0.44     |
| Smoking status, No. (%)        |                 |                  |          |                 |                  |          |
| Current smoker                 | 251 (24.2)      | 296 (28.6)       | 0.08     | 63 (23.4)        | 65 (24.2)        | 0.63     |
| Former smoker                  | 152 (14.7)      | 142 (13.7)       |          | 34 (12.7)        | 41 (15.2)        |          |
| Never smoker                   | 632 (61.1)      | 597 (57.7)       |          | 172 (63.9)       | 163 (60.6)       |          |
| Drinking status, No. (%)       |                 | 0.21              |          |                 | 0.48              |          |
| Current drinker                | 277 (26.8)      | 310 (30.0)       |          | 62 (23.1)        | 72 (26.8)         |          |
| Former drinker                 | 72 (6.9)        | 61 (5.9)         |          | 14 (5.2)         | 17 (6.3)          |          |
| Never drinker                  | 686 (66.3)      | 664 (64.1)       |          | 193 (71.7)       | 180 (66.9)        |          |
| Regular exercise, No. (%)†     | 761 (73.5)      | 735 (71.0)       | 0.20     | 202 (75.1)       | 196 (72.9)        | 0.56     |
| Family history of stroke, No. (%) | 46 (4.4) | 29 (2.8)         | 0.046    | 14 (5.2)         | 7 (2.6)           | 0.12     |
| Hyperlipidemia, No. (%)‡       | 443 (42.8)      | 485 (46.9)       | 0.06     | 129 (48.0)       | 134 (49.8)        | 0.67     |
| Diabetes, No. (%)§             | 186 (18.0)      | 274 (26.5)       | <0.001   | 51 (19.0)        | 63 (23.4)         | 0.21     |
| Hypertension, No. (%)¶         | 568 (54.9)      | 725 (70.1)       | <0.001   | 158 (58.7)       | 200 (74.4)        | <0.001   |
| Plasma metals (μg/L)           |                 |                  |          |                 |                  |          |
| Aluminum                       | 56.78 (36.96-103.64) | 57.13 (37.51-114.25) | 0.50     | 60.23 (38.98-131.83) | 56.84 (36.65-97.21) | 0.17     |
| Arsenic                        | 1.86 (1.09-3.77) | 1.95 (1.06-4.07)  | 0.34     | 1.91 (1.04-4.38)  | 1.83 (1.03-4.07)  | 0.20     |
| Barium                         | 38.33 (23.04-67.81) | 40.57 (23.62-70.73) | 0.20     | 41.37 (23.12-69.01) | 37.16 (22.05-72.71) | 1.00     |
| Cobalt                         | 0.17 (0.14-0.21) | 0.17 (0.13-0.21)  | 0.99     | 0.16 (0.13-0.21)  | 0.17 (0.13-0.22)  | 0.91     |
| Copper                         | 953.14 (850.30-1071.60) | 979.48 (875.80-1112.71) | <0.001   | 999.38 (884.52-1120.40) | 1003.70 (883.30-1115.17) | 0.68     |
| Lead                           | 13.30 (8.75-23.75) | 14.34 (9.22-25.67) | 0.043    | 13.18 (8.79-23.70) | 14.37 (9.02-26.16) | 0.91     |
| Manganese                      | 2.79 (2.12-3.90) | 2.87 (2.10-3.97)  | 0.97     | 2.97 (2.06-4.05)  | 2.76 (2.03-3.85)  | 0.28     |
| Element     | Mean (Range) | Median (25th–75th Percentiles) | SD (95% CI) |
|------------|--------------|--------------------------------|-------------|
| Mercury    | 0.55 (0.36-0.84) | 0.53 (0.35-0.78) | 0.47 | 0.55 (0.33-0.86) | 0.51 (0.32-0.77) | 0.046 |
| Molybdenum | 1.27 (1.00-1.66) | 1.29 (1.02-1.74) | 0.003 | 1.34 (1.02-1.71) | 1.34 (1.03-1.83) | 0.04 |
| Nickel     | 2.44 (1.83-3.55) | 2.49 (1.84-3.64) | 0.11 | 2.36 (1.77-3.36) | 2.29 (1.64-3.08) | 0.24 |
| Rubidium   | 348.50 (309.66-394.25) | 345.22 (308.26-390.88) | 0.56 | 357.90 (318.82-396.32) | 348.31 (298.94-384.20) | 0.009 |
| Selenium   | 66.36 (57.50-78.59) | 66.02 (55.91-77.15) | 0.027 | 68.86 (57.14-78.66) | 63.85 (55.37-76.20) | 0.03 |
| Strontium  | 33.92 (28.56-41.63) | 34.86 (29.23-41.96) | 0.42 | 34.59 (28.30-40.55) | 36.08 (29.64-43.44) | 0.038 |
| Thallium   | 0.10 (0.08-0.13) | 0.10 (0.08-0.14) | 0.32 | 0.10 (0.08-0.14) | 0.10 (0.08-0.13) | 0.12 |
| Titanium   | 49.09 (41.49-56.95) | 50.23 (42.41-58.13) | 0.07 | 44.13 (35.50-55.49) | 43.35 (34.24-55.25) | 0.82 |
| Tungsten   | 0.05 (0.03-0.07) | 0.05 (0.04-0.07) | 0.55 | 0.05 (0.03-0.07) | 0.05 (0.03-0.07) | 0.73 |
| Vanadium   | 1.41 (1.13-1.95) | 1.43 (1.14-1.98) | 0.81 | 1.42 (1.14-2.01) | 1.41 (1.12-1.93) | 0.76 |
| Zinc       | 1383.21 (1046.25-2775.78) | 1454.18 (1045.67-3399.51) | 0.31 | 1461.55 (1072.69-2830.71) | 1432.18 (1027.95-2742.95) | 0.77 |

Normally distributed variables were presented as mean±SD or median (25th–75th percentiles). Categorical variables were presented as numbers (percentage). Plasma antimony, tin and uranium with low detection rates were excluded from further analyses because of many samples below the limits of detection (23.24%, 52.45% and 21.74%, respectively). Plasma cadmium, chromium, and iron were excluded from further analyses because of concerns about the use of plasma concentrations as biomarkers.

*P values were derived from Student’s t tests or Mann-Whitney U tests for continuous variables according to the data distribution, as well as Chi-square tests for the category variables.

†Regular exercise was defined as physical exercise for at least 30 min in no less than 5 days per week for more than half a year.

‡Hyperlipidemia was defined as self-reported hyperlipidemia, or using anti-hyperlipidemia medications, or total cholesterol (TC)≥6.22 mmol/L, or triglycerides (TG)≥2.26 mmol/L, or high-density lipoprotein cholesterol (HDL-C)<1.04 mmol/L, or high-density lipoprotein cholesterol (LDL-C)≥4.14 mmol/L.

§Diabetes was defined as self-reported diabetes mellitus, or use of anti-diabetic medications, or fasting glucose≥7.0 mmol/L.

∥Hypertension was defined as self-reported hypertension, using antihypertensive medication, or having systolic blood pressure (SBP) ≥ 140 mmHg or diastolic blood pressure (DBP)≥90 mmHg.
| Plasma metals  | Tertiles of plasma metals (μg/L) | P-trend | FDR* | Linear model† |
|----------------|----------------------------------|---------|------|---------------|
|                | T1                               | T2      | T3   |               |
| Ischemic stroke|                                  |         |      |               |
| Aluminum       | <43.15                           | 43.15-80.49 | >80.49 |               |
| N (cases/controls) | 354/347                        | 324/344            | 356/344      |               |
| Model 1‡       | 1.00                             | 0.91 (0.72-1.14)  | 1.02 (0.81-1.28) | 0.72  | 0.81  | 1.09 (0.98-1.20) |
| Model 2§       | 1.00                             | 0.90 (0.71-1.14)  | 0.99 (0.78-1.26) | 0.94  | 0.94  | 1.08 (0.97-1.20) |
| Arsenic        | <1.30                            | 1.30-3.03     | >3.03 |               |
| N (cases/controls) | 339/345                        | 320/344            | 376/346      |               |
| Model 1‡       | 1.00                             | 0.97 (0.77-1.22)  | 1.15 (0.90-1.48) | 0.21  | 0.34  | 1.02 (0.88-1.18) |
| Model 2§       | 1.00                             | 0.98 (0.77-1.25)  | 1.14 (0.88-1.47) | 0.29  | 0.47  | 1.01 (0.87-1.18) |
| Barium         | <27.40                           | 27.40-56.68     | >56.68 |               |
| N (cases/controls) | 316/345                        | 366/344            | 353/346      |               |
| Model 1‡       | 1.00                             | 1.20 (0.96-1.50)  | 1.16 (0.91-1.48) | 0.27  | 0.41  | 1.06 (0.93-1.20) |
| Model 2§       | 1.00                             | 1.20 (0.95-1.53)  | 1.09 (0.84-1.41) | 0.57  | 0.73  | 1.03 (0.90-1.18) |
| Cobalt         | <0.15                            | 0.15-0.19       | >0.19 |               |
| N (cases/controls) | 356/347                        | 326/345            | 353/343      |               |
| Model 1‡       | 1.00                             | 0.91 (0.71-1.16)  | 0.99 (0.77-1.27) | 0.98  | 0.94  | 1.01 (0.91-1.11) |
| Model 2§       | 1.00                             | 0.87 (0.67-1.13)  | 0.95 (0.73-1.24) | 0.77  | 0.87  | 0.98 (0.89-1.09) |
| Copper         | <892.63                          | 892.63-1025.91   | >1025.91     |               |
| N (cases/controls) | 299/345                        | 304/345              | 430/345      |               |
| Model 1‡       | 1.00                             | 1.05 (0.83-1.33)  | 1.56 (1.24-1.97) | <0.001 | <0.001 | 1.30 (1.15-1.46) |
| Model 2§       | 1.00                             | 1.03 (0.81-1.32)  | 1.53 (1.20-1.96) | <0.001 | <0.001 | 1.29 (1.13-1.46) |
Table III. Adjusted odds ratios (95% CI) for incident stroke according to plasma metals (continued)

| Plasma metals | Tertiles of plasma metals (µg/L) | P-trend | FDR* | Linear model† |
|---------------|---------------------------------|---------|------|---------------|
|               | T1                              | T2      | T3   |                |
| Lead         | <10.16                          | 10.16-18.62 | >18.62 |                |
| N (cases/controls) | 322/344                         | 338/346  | 375/344 |                |
| Model 1‡     | 1.00                            | 1.08 (0.87-1.34) | 1.22 (0.97-1.54) | 0.09 | 0.23 | 1.12 (1.01-1.24) |
| Model 2§     | 1.00                            | 1.08 (0.68-1.70) | 1.50 (0.92-2.44) | 0.09 | 0.51 | 1.14 (0.91-1.42) |
| Manganese    | <2.35                           | 2.35-3.43 | >3.43 |                |
| N (cases/controls) | 336/344                         | 334/346  | 365/345 |                |
| Model 1‡     | 1.00                            | 0.98 (0.79-1.23) | 1.10 (0.87-1.39) | 0.41 | 0.53 | 1.05 (0.94-1.18) |
| Model 2§     | 1.00                            | 1.02 (0.80-1.29) | 1.06 (0.83-1.36) | 0.62 | 0.75 | 1.06 (0.94-1.19) |
| Mercury      | <0.42                           | 0.42-0.71 | >0.71 |                |
| N (cases/controls) | 369/345                         | 351/345  | 314/345 |                |
| Model 1‡     | 1.00                            | 0.93 (0.75-1.16) | 0.83 (0.65-1.05) | 0.13 | 0.23 | 0.97 (0.87-1.09) |
| Model 2§     | 1.00                            | 0.92 (0.73-1.16) | 0.83 (0.64-1.07) | 0.16 | 0.44 | 0.97 (0.86-1.09) |
| Molybdenum   | <1.09                           | 1.09-1.48 | >1.48 |                |
| N (cases/controls) | 337/345                         | 308/345  | 389/345 |                |
| Model 1‡     | 1.00                            | 0.93 (0.75-1.16) | 1.19 (0.95-1.50) | 0.10 | 0.23 | 1.15 (1.02-1.30) |
| Model 2§     | 1.00                            | 1.03 (0.82-1.30) | 1.24 (0.97-1.57) | 0.07 | 0.43 | 1.19 (1.05-1.35) |
| Nickel       | <2.04                           | 2.04-3.03 | >3.03 |                |
| N (cases/controls) | 349/347                         | 337/345  | 349/343 |                |
| Model 1‡     | 1.00                            | 0.96 (0.75-1.22) | 1.00 (0.78-1.29) | 0.93 | 0.94 | 1.03 (0.95-1.12) |
| Model 2§     | 1.00                            | 0.92 (0.72-1.18) | 0.98 (0.75-1.28) | 0.97 | 0.94 | 1.04 (0.95-1.13) |
| Rubidium     | <321.85                         | 321.85-375.09 | >375.09 |                |
| N (cases/controls) | 349/345                         | 351/344  | 335/346 |                |
| Model 1‡     | 1.00                            | 1.00 (0.80-1.24) | 0.95 (0.75-1.20) | 0.65 | 0.78 | 1.04 (0.94-1.15) |
| Model 2§     | 1.00                            | 0.97 (0.77-1.22) | 0.89 (0.70-1.14) | 0.35 | 0.50 | 1.02 (0.93-1.13) |
Table III. Adjusted odds ratios (95% CI) for incident stroke according to plasma metals (continued)

| Plasma metals | Tertiles of plasma metals (μg/L) | P-trend | FDR* | Linear model† |
|---------------|----------------------------------|---------|------|---------------|
|               | T1 | T2 | T3 |               |       |               |
| Selenium      |      |      |    |               |       |               |
| N (cases/controls) | <60.75 | 60.75-74.36 | >74.36 |       |       |               |
| Model 1‡ | 1.00 | 0.81 (0.65-1.01) | 0.81 (0.64-1.02) | 0.07   | 0.23   | 0.89 (0.79-1.01) |
| Model 2§ | 1.00 | 0.85 (0.67-1.07) | 0.87 (0.68-1.11) | 0.25   | 0.47   | 0.92 (0.82-1.05) |
| Strontium     |      |      |    |               |       |               |
| N (cases/controls) | <30.25 | 30.25-38.80 | >38.80 |       |       |               |
| Model 1‡ | 1.00 | 1.17 (0.94-1.45) | 1.19 (0.96-1.48) | 0.13   | 0.23   | 1.09 (0.97-1.23) |
| Model 2§ | 1.00 | 1.16 (0.93-1.46) | 1.12 (0.89-1.41) | 0.36   | 0.50   | 1.05 (0.93-1.19) |
| Thallium      |      |      |    |               |       |               |
| N (cases/controls) | <0.08 | 0.08-0.12 | >0.12 |       |       |               |
| Model 1‡ | 1.00 | 1.04 (0.83-1.31) | 1.22 (0.96-1.54) | 0.09   | 0.23   | 1.10 (0.97-1.24) |
| Model 2§ | 1.00 | 1.03 (0.81-1.31) | 1.17 (0.92-1.49) | 0.20   | 0.44   | 1.07 (0.95-1.22) |
| Titanium      |      |      |    |               |       |               |
| N (cases/controls) | <44.48 | 44.48-53.71 | >53.71 |       |       |               |
| Model 1‡ | 1.00 | 1.23 (0.93-1.64) | 1.63 (1.18-2.26) | 0.003  | 0.027  | 1.34 (1.11-1.62) |
| Model 2§ | 1.00 | 1.25 (0.93-1.68) | 1.60 (1.14-2.25) | 0.006  | 0.05   | 1.30 (1.07-1.59) |
| Tungsten      |      |      |    |               |       |               |
| N (cases/controls) | <0.04 | 0.04-0.06 | >0.06 |       |       |               |
| Model 1‡ | 1.00 | 1.40 (1.08-1.80) | 1.18 (0.88-1.59) | 0.36   | 0.50   | 1.02 (0.90-1.17) |
| Model 2§ | 1.00 | 1.46 (1.12-1.90) | 1.24 (0.90-1.69) | 0.26   | 0.47   | 1.02 (0.89-1.17) |
| Vanadium      |      |      |    |               |       |               |
| N (cases/controls) | <1.21 | 1.21-1.69 | >1.69 |       |       |               |
| Model 1‡ | 1.00 | 1.24 (0.94-1.65) | 1.37 (1.00-1.88) | 0.07   | 0.23   | 1.08 (0.93-1.25) |
| Model 2§ | 1.00 | 1.31 (0.97-1.77) | 1.35 (0.97-1.88) | 0.14   | 0.44   | 1.06 (0.91-1.24) |
Table III. Adjusted odds ratios (95% CI) for incident stroke according to plasma metals (continued)

| Plasma metals | Tertiles of plasma metals (μg/L) |  |  |  |  |  |
|---------------|----------------------------------|---|---|---|---|---|
|               | T1                               | T2                        | T3                        | P-trend | FDR* | Linear model† |
| Zinc          | <1120.59                         | 1120.59-2030.87           | >2030.87                  | 0.13    | 0.23 | 1.09 (0.97-1.22) |
| N (cases/controls) | 338/344                         | 320/344                   | 377/345                   |         |      |               |
| Model 1‡      | 1.00                             | 0.94 (0.74-1.21)          | 1.14 (0.90-1.44)          | 0.19    | 0.44 | 1.06 (0.94-1.20) |
| Model 2§      | 1.00                             | 0.89 (0.69-1.15)          | 1.10 (0.86-1.41)          | 0.13    | 0.23 | 1.09 (0.97-1.22) |
| Hemorrhagic stroke |  |  |  |  |  |  |
| Aluminum      | <44.88                           | 44.88-90.31               | >90.31                    | 0.17    | 0.42 | 0.83 (0.67-1.02) |
| N (cases/controls) | 92/89                           | 104/91                    | 72/89                     |         |      |               |
| Model 1‡      | 1.00                             | 1.08 (0.70-1.68)          | 0.77 (0.49-1.22)          | 0.16    | 0.51 | 0.82 (0.65-1.02) |
| Model 2§      | 1.00                             | 1.21 (0.75-1.96)          | 0.76 (0.47-1.24)          | 0.16    | 0.51 | 0.82 (0.65-1.02) |
| Arsenic       | <1.24                            | 1.24-3.23                 | >3.23                     | 0.96    | 0.94 | 0.90 (0.66-1.22) |
| N (cases/controls) | 89/90                           | 92/90                     | 88/89                     |         |      |               |
| Model 1‡      | 1.00                             | 1.03 (0.66-1.61)          | 1.02 (0.63-1.65)          | 0.80    | 0.85 | 0.86 (0.63-1.20) |
| Model 2§      | 1.00                             | 0.97 (0.60-1.57)          | 0.93 (0.56-1.56)          | 0.80    | 0.85 | 0.86 (0.63-1.20) |
| Barium        | <28.42                           | 28.42-54.12               | >54.12                    | 0.50    | 0.60 | 0.98 (0.77-1.26) |
| N (cases/controls) | 104/90                          | 73/89                     | 92/90                     |         |      |               |
| Model 1‡      | 1.00                             | 0.71 (0.46-1.10)          | 0.86 (0.54-1.39)          | 0.28    | 0.51 | 0.98 (0.75-1.28) |
| Model 2§      | 1.00                             | 0.74 (0.46-1.17)          | 0.77 (0.46-1.28)          | 0.28    | 0.51 | 0.98 (0.75-1.28) |
| Cobalt        | <0.14                            | 0.14-0.19                 | >0.19                     | 0.16    | 0.42 | 1.11 (0.91-1.37) |
| N (cases/controls) | 80/89                           | 86/90                     | 103/90                    |         |      |               |
| Model 1‡      | 1.00                             | 1.13 (0.74-1.75)          | 1.41 (0.87-2.28)          | 0.25    | 0.51 | 1.09 (0.88-1.36) |
| Model 2§      | 1.00                             | 1.09 (0.68-1.73)          | 1.36 (0.80-2.32)          | 0.25    | 0.51 | 1.09 (0.88-1.36) |
### Table III. Adjusted odds ratios (95% CI) for incident stroke according to plasma metals (continued)

| Plasma metals | Tertiles of plasma metals (μg/L) | P-trend | FDR* | Linear model† |
|---------------|----------------------------------|---------|------|---------------|
|               | T1                               | T2      | T3   |                |
| Copper        | <936.36                          | 936.36-1073.46 | >1073.46 |        |
| N (cases/controls) | 89/90                           | 89/89  | 91/90 |                |
| Model 1†      | 1.00                             | 1.00 (0.65-1.54) | 1.04 (0.66-1.64) | 0.87 | 0.92 | 0.96 (0.76-1.20) |
| Model 2‡      | 1.00                             | 1.13 (0.70-1.81) | 1.06 (0.64-1.74) | 0.81 | 0.94 | 0.96 (0.75-1.23) |
| Lead          | <9.96                            | 9.96-18.24 | >18.24 |        |
| N (cases/controls) | 84/89                           | 80/90  | 105/90 |                |
| Model 1†      | 1.00                             | 0.94 (0.61-1.45) | 1.32 (0.84-2.08) | 0.20 | 0.42 | 1.09 (0.89-1.34) |
| Model 2‡      | 1.00                             | 1.08 (0.68-1.70) | 1.50 (0.92-2.44) | 0.09 | 0.51 | 1.14 (0.91-1.42) |
| Manganese     | <2.36                            | 2.36-3.60 | >3.60  |        |
| N (cases/controls) | 99/90                           | 94/89  | 76/90  |                |
| Model 1†      | 1.00                             | 0.92 (0.59-1.43) | 0.73 (0.46-1.16) | 0.18 | 0.42 | 0.85 (0.67-1.07) |
| Model 2‡      | 1.00                             | 0.96 (0.60-1.54) | 0.72 (0.44-1.18) | 0.18 | 0.51 | 0.85 (0.66-1.09) |
| Mercury       | <0.38                            | 0.38-0.74 | >0.74  |        |
| N (cases/controls) | 92/90                           | 104/90 | 73/89  |                |
| Model 1†      | 1.00                             | 1.05 (0.67-1.63) | 0.71 (0.42-1.21) | 0.21 | 0.42 | 0.77 (0.60-1.01) |
| Model 2‡      | 1.00                             | 1.21 (0.75-1.94) | 0.74 (0.42-1.30) | 0.29 | 0.51 | 0.82 (0.62-1.07) |
| Molybdenum    | <1.11                            | 1.11-1.54 | >1.54  |        |
| N (cases/controls) | 83/89                           | 85/90  | 100/90 |                |
| Model 1†      | 1.00                             | 1.04 (0.67-1.61) | 1.20 (0.78-1.85) | 0.41 | 0.52 | 1.14 (0.91-1.41) |
| Model 2‡      | 1.00                             | 1.00 (0.62-1.62) | 1.17 (0.74-1.87) | 0.50 | 0.60 | 1.14 (0.90-1.44) |
| Nickel        | <2.01                            | 2.01-2.96 | >2.96  |        |
| N (cases/controls) | 102/89                          | 92/90  | 75/90  |                |
| Model 1†      | 1.00                             | 0.84 (0.52-1.36) | 0.62 (0.37-1.04) | 0.07 | 0.42 | 0.88 (0.74-1.04) |
| Model 2‡      | 1.00                             | 0.72 (0.43-1.20) | 0.72 (0.41-1.27) | 0.26 | 0.51 | 0.88 (0.74-1.06) |
| Plasma metals | Tertiles of plasma metals (μg/L) | P-trend | FDR* | Linear model† |
|---------------|---------------------------------|---------|------|---------------|
|               | T1 | T2 | T3 |                |             |             |
| Rubidium      | <331.22 | 331.22-381.65 | >381.65 |                |             |             |
| N (cases/controls) | 118/90 | 82/89 | 69/90 | 0.008 | 0.07 | 0.68 (0.52-0.88) |
| Model 1‡       | 1.00 | 0.66 (0.43-1.01) | 0.54 (0.34-0.86) |             |             |             |
| Model 2§       | 1.00 | 0.69 (0.44-1.09) | 0.51 (0.31-0.85) | 0.009 | 0.14 | 0.66 (0.50-0.87) |
| Selenium       | <62.31 | 62.31-75.03 | >75.03 |                |             |             |
| N (cases/controls) | 120/89 | 74/91 | 74/91 | 0.006 | 0.07 | 0.70 (0.53-0.91) |
| Model 1‡       | 1.00 | 0.57 (0.37-0.88) | 0.54 (0.34-0.86) |             |             |             |
| Model 2§       | 1.00 | 0.51 (0.32-0.81) | 0.57 (0.35-0.95) | 0.015 | 0.14 | 0.68 (0.51-0.91) |
| Strontium      | <29.94 | 29.94-38.31 | >38.31 |                |             |             |
| N (cases/controls) | 72/89 | 93/90 | 104/90 | 0.10 | 0.42 | 1.28 (1.01-1.61) |
| Model 1‡       | 1.00 | 1.30 (0.85-2.00) | 1.45 (0.94-2.24) |             |             |             |
| Model 2§       | 1.00 | 1.15 (0.72-1.81) | 1.28 (0.80-2.04) | 0.31 | 0.51 | 1.19 (0.92-1.53) |
| Thallium       | <0.09 | 0.09-0.12 | >0.12 |                |             |             |
| N (cases/controls) | 105/90 | 79/90 | 85/90 | 0.28 | 0.47 | 0.79 (0.62-1.00) |
| Model 1‡       | 1.00 | 0.73 (0.47-1.13) | 0.78 (0.49-1.24) |             |             |             |
| Model 2§       | 1.00 | 0.72 (0.45-1.16) | 0.84 (0.51-1.39) | 0.46 | 0.60 | 0.82 (0.63-1.07) |
| Titanium       | <38.04 | 38.04-51.50 | >51.50 |                |             |             |
| N (cases/controls) | 98/90 | 85/89 | 86/90 | 0.32 | 0.48 | 0.91 (0.54-1.54) |
| Model 1‡       | 1.00 | 0.71 (0.38-1.33) | 0.65 (0.30-1.44) |             |             |             |
| Model 2§       | 1.00 | 0.70 (0.35-1.40) | 0.69 (0.28-1.70) | 0.50 | 0.60 | 0.91 (0.50-1.64) |
| Tungsten       | <0.04 | 0.04-0.06 | >0.06 |                |             |             |
| N (cases/controls) | 79/89 | 94/91 | 96/89 | 0.29 | 0.47 | 1.03 (0.80-1.31) |
| Model 1‡       | 1.00 | 1.22 (0.74-2.01) | 1.34 (0.79-2.29) |             |             |             |
| Model 2§       | 1.00 | 1.33 (0.78-2.26) | 1.39 (0.78-2.47) | 0.28 | 0.51 | 1.05 (0.81-1.37) |
Table III. Adjusted odds ratios (95% CI) for incident stroke according to plasma metals (continued)

| Plasma metals | Tertiles of plasma metals (μg/L) | P-trend | FDR* | Linear model† |
|---------------|----------------------------------|---------|------|---------------|
|               | T1 | T2-1.78 | T3 |       |               |
| Vanadium      | <1.20 | 1.20-1.78 | >1.78 |       |               |
| N (cases/controls) | 87/90 | 97/90 | 85/89 |       |               |
| Model 1‡      | 1.00 | 1.21 (0.72-2.02) | 0.93 (0.53-1.65) | 0.75 | 0.84 | 0.92 (0.70-1.22) |
| Model 2§      | 1.00 | 1.10 (0.64-1.92) | 0.94 (0.51-1.71) | 0.80 | 0.85 | 0.90 (0.67-1.22) |
| Zinc          | <1152.07 | 1152.07-2135.07 | >2135.07 |       |               |
| N (cases/controls) | 107/90 | 79/90 | 83/89 |       |               |
| Model 1‡      | 1.00 | 0.65 (0.40-1.06) | 0.75 (0.47-1.19) | 0.39 | 0.52 | 0.95 (0.78-1.15) |
| Model 2§      | 1.00 | 0.77 (0.45-1.29) | 0.78 (0.48-1.27) | 0.43 | 0.60 | 0.94 (0.76-1.16) |

Abbreviations: FDR-False Discovery Rate.
*False Discovery Rate (FDR) adjusted p-values were calculated using software published by Pike (2011).
†Linear model: Odds ratios (95% CI) for incident stroke corresponding to an interquartile range increase in plasma metal levels were shown.
‡Model 1: Metals were included in the conditional logistic regression models separately without adjustment.
§Model 2: Metals were included in the conditional logistic regression models separately and adjusted for body mass index (kg/m²), smoking status (current, former, never), drinking status (current, former, never), regular exercise, family history of stroke, hyperlipidemia, diabetes and hypertension.
Table IV. Adjusted odds ratios (95% CI) for incident stroke according to plasma metals in sensitivity analysis

| Plasma metals | Tertiles of plasma metals (μg/L) | P-trend | Linear model* |
|---------------|---------------------------------|---------|---------------|
|               | T1 | T2 | T3 |                   |
| Ischemic stroke |                |       |       |                   |
| Copper        | <892.21 | 892.21-1025.81 | >1025.81 |         |
| N (cases/controls) | 261/298 | 263/297 | 366/297 |         |
| Model 1†      | 1.00 | 1.03 (0.80-1.33) | 1.52 (1.18-1.95) | 0.001 | 1.29 (1.13-1.46) |
| Model 2‡      | 1.00 | 1.00 (0.77-1.30) | 1.47 (1.13-1.91) | 0.002 | 1.26 (1.10-1.44) |
| Molybdenum    | <1.08 | 1.08-1.48 | >1.48 |         |
| N (cases/controls) | 287/298 | 272/297 | 332/297 |         |
| Model 1†      | 1.00 | 0.96 (0.76-1.22) | 1.20 (0.94-1.53) | 0.13 | 1.17 (1.03-1.33) |
| Model 2‡      | 1.00 | 1.05 (0.81-1.35) | 1.23 (0.95-1.59) | 0.11 | 1.18 (1.03-1.35) |
| Selenium      | <60.46 | 60.46-74.00 | >74.00 |         |
| N (cases/controls) | 332/297 | 272/297 | 288/298 |         |
| Model 1†      | 1.00 | 0.80 (0.63-1.01) | 0.83 (0.65-1.07) | 0.15 | 0.91 (0.80-1.04) |
| Model 2‡      | 1.00 | 0.86 (0.67-1.11) | 0.89 (0.68-1.15) | 0.37 | 0.94 (0.83-1.08) |
| Titanium      | <44.32 | 44.32-53.63 | >53.63 |         |
| N (cases/controls) | 272/298 | 284/296 | 336/298 |         |
| Model 1†      | 1.00 | 1.28 (0.94-1.75) | 1.66 (1.17-2.37) | 0.004 | 1.34 (1.09-1.64) |
| Model 2‡      | 1.00 | 1.30 (0.94-1.79) | 1.63 (1.12-2.37) | 0.010 | 1.28 (1.04-1.59) |
| Hemorrhagic stroke |                |       |       |                   |
| Rubidium      | <334.13 | 334.13-383.40 | >383.40 |         |
| N (cases/controls) | 103/77 | 66/77 | 61/76 |         |
| Model 1†      | 1.00 | 0.61 (0.39-0.97) | 0.56 (0.35-0.92) | 0.017 | 0.64 (0.48-0.85) |
| Model 2‡      | 1.00 | 0.67 (0.41-1.09) | 0.55 (0.32-0.95) | 0.027 | 0.62 (0.46-0.85) |
| Selenium      | <62.60 | 62.60-75.03 | >75.03 |         |
| N (cases/controls) | 108/76 | 60/78 | 61/76 |         |
| Model 1†      | 1.00 | 0.50 (0.31-0.81) | 0.48 (0.29-0.80) | 0.003 | 0.68 (0.51-0.91) |
| Model 2‡      | 1.00 | 0.47 (0.28-0.79) | 0.54 (0.31-0.93) | 0.013 | 0.69 (0.50-0.94) |

Metals which were significantly associated with incident stroke (P-trend <0.05) or selected by elastic net model were included in the table.

*Linear model: Odds ratios (95% CI) for incident stroke corresponding to an interquartile range increase in plasma metal levels were shown.

†Model 1: Metals were included in the conditional logistic regression models separately without adjustment.

‡Model 2: Metals were included in the conditional logistic regression models separately and adjusted for body mass index (kg/m²), smoking status (current, former, never), drinking status (current, former, never), regular exercise, family history of stroke, hyperlipidemia, diabetes and hypertension.
## Table V. Adjusted odds ratios (95% CI) for subtypes of stroke according to plasma metals

| Plasma metals | Tertiles of plasma metals (μg/L) | P for trend | Linear model* |
|---------------|----------------------------------|-------------|---------------|
|               | T1                                | T2          | T3            |
| **Large artery atherosclerosis infarction (n=382)** | | | |
| Copper        | 1 [Reference]                     | 1.16 (0.77, 1.75) | 2.01 (1.32, 3.06) | 0.001 | 1.36 (1.09, 1.70) |
| Molybdenum    | 1 [Reference]                     | 1.21 (0.81, 1.80) | 1.11 (0.74, 1.66) | 0.66 | 1.12 (0.91, 1.38) |
| Selenium      | 1 [Reference]                     | 1.04 (0.70, 1.55) | 1.15 (0.77, 1.74) | 0.49 | 1.01 (0.80, 1.29) |
| Titanium      | 1 [Reference]                     | 1.11 (0.68, 1.82) | 1.28 (0.73, 2.24) | 0.39 | 1.24 (0.87, 1.76) |
| **Cardioembolic infarction (n=206)** | | | |
| Copper        | 1 [Reference]                     | 0.86 (0.47, 1.59) | 1.08 (0.57, 2.05) | 0.75 | 1.17 (0.87, 1.58) |
| Molybdenum    | 1 [Reference]                     | 1.29 (0.72, 2.30) | 1.52 (0.84, 2.75) | 0.18 | 1.28 (0.95, 1.73) |
| Selenium      | 1 [Reference]                     | 0.97 (0.56, 1.65) | 0.67 (0.37, 1.23) | 0.21 | 0.87 (0.62, 1.23) |
| Titanium      | 1 [Reference]                     | 0.94 (0.45, 1.95) | 1.50 (0.65, 3.45) | 0.30 | 1.29 (0.77, 2.16) |
| **Lacunar infarction (n=228)** | | | |
| Copper        | 1 [Reference]                     | 0.63 (0.37, 1.06) | 0.61 (0.35, 1.05) | 0.07 | 1.33 (1.00, 1.77) |
| Molybdenum    | 1 [Reference]                     | 0.61 (0.37, 1.03) | 1.06 (0.64, 1.75) | 0.72 | 1.15 (0.85, 1.56) |
| Selenium      | 1 [Reference]                     | 0.78 (0.48, 1.28) | 0.88 (0.51, 1.50) | 0.60 | 1.00 (0.83, 1.20) |
| Titanium      | 1 [Reference]                     | 2.91 (1.21, 6.97) | 4.97 (1.83, 13.53) | 0.002 | 1.42 (0.94, 2.12) |
| **Undetermined cause of infarction (n=219)** | | | |
| Copper        | 1 [Reference]                     | 0.97 (0.56, 1.66) | 1.36 (0.81, 2.31) | 0.22 | 1.33 (1.00, 1.77) |
| Molybdenum    | 1 [Reference]                     | 1.29 (0.74, 2.24) | 2.00 (1.09, 3.65) | 0.024 | 1.38 (1.03, 1.84) |
| Selenium      | 1 [Reference]                     | 0.71 (0.40, 1.24) | 0.78 (0.45, 1.36) | 0.40 | 0.78 (0.58, 1.06) |
| Titanium      | 1 [Reference]                     | 0.69 (0.33, 1.43) | 1.20 (0.52, 2.76) | 0.63 | 1.32 (0.83, 2.11) |
| **Intracerebral hemorrhage (n=233)** | | | |
| Rubidium      | 1 [Reference]                     | 0.61 (0.37, 1.01) | 0.55 (0.33, 0.93) | 0.020 | 0.69 (0.51, 0.92) |
| Selenium      | 1 [Reference]                     | 0.57 (0.35, 0.92) | 0.51 (0.30, 0.88) | 0.010 | 0.68 (0.50, 0.92) |
| **Subarachnoid hemorrhage (n=36)** | | | |
| Rubidium      | 1 [Reference]                     | 1.87 (0.32, 10.81) | 0.61 (0.05, 7.58) | 0.98 | 0.44 (0.10, 1.90) |
| Selenium      | 1 [Reference]                     | 1.02 (0.26, 4.03) | 0.03 (0.00, 1.57) | 0.09 | 0.47 (0.12, 1.84) |

Metals associated with incident stroke (P-trend<0.05) or selected by elastic net model were included. Metals were included in the conditional logistic regression models separately and adjusted for body mass index (kg/m²), smoking status (current, former, never), drinking status (current, former, never), physical activity, family history of stroke, hyperlipidemia, diabetes and hypertension.

*Linear model: Odds ratios (95% CI) for incident stroke corresponding to an interquartile range increase in plasma metal levels were shown.
Figure I. The restricted cubic spline for the associations of plasma metals with incident ischemic stroke in sensitivity analysis
The restricted cubic spline for the associations of plasma copper (A) molybdenum (B) selenium (C) titanium (D) with ischemic stroke. The bars represent histograms of plasma metal distribution among the total population. The lines represent adjusted odds ratios for the log transformed levels of plasma metals in the conditional regression model. Knots were placed at the 20th 40th 60th and 80th percentiles of the plasma metal distribution and the reference value was set at the percentile of 10th. Models were adjusted for body mass index (kg/m²), smoking status (current, former, never), drinking status (current, former, never), regular exercise, family history of stroke, hyperlipidemia, diabetes and hypertension.
Figure II. The restricted cubic spline for the associations of plasma metals with incident hemorrhagic stroke in sensitivity analysis

The restricted cubic spline for the associations of plasma rubidium (A), selenium (B) with hemorrhagic stroke. The bars represent histograms of plasma metal distribution among the total population. The lines represent adjusted odds ratios for the log transformed levels of plasma metals in the conditional regression model. Knots were placed at the 20th 40th 60th and 80th percentiles of the plasma metal distribution and the reference value was set at the percentile of 10th. Models were adjusted for body mass index (kg/m²), smoking status (current, former, never), drinking status (current, former, never), regular exercise, family history of stroke, hyperlipidemia, diabetes and hypertension.
Natural log-transformed values of metal concentrations were included in the unconditional logistic regression models separately and adjusted for age, sex, smoking status (current, former, never), drinking status (current, former, never), regular exercise. Body mass index (kg/m²) and family history of hypertension were additionally adjusted for hypertension. Body mass index (kg/m²) and family history of hyperlipidemia were additionally adjusted for hyperlipidemia.
Figure IV. Adjusted odds ratios (95% CI) for incident stroke according to predictive plasma metal scores in subgroups

Adjusted odds ratio for incident ischemic stroke (A) and hemorrhagic stroke (B) according to predictive plasma metal scores were analyzed in subgroup stratified by age, sex, body mass index, ever-smokers (included current and former smokers), hypertension and hyperlipidemia. Unconditional logistic regression models were used and all stratified models were adjusted for age, sex, body mass index (kg/m²), smoking status (current, former, never), drinking status (current, former, never), regular exercise, family history of stroke, hyperlipidemia, diabetes and hypertension.
References

1. Walker AE, Robins M, Weinfeld FD. The national survey of stroke. Clinical findings. *Stroke*. 1981;12:113-44.

2. Yuan Y, Xiao Y, Feng W, Liu Y, Yu Y, Zhou L, et al. Plasma metal concentrations and incident coronary heart disease in Chinese adults: the Dongfeng-Tongji cohort. *Environ Health Perspect*. 2017;125:107007.