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Sex-specific associations of a ferroalloy metal mixture with motor function in Italian adolescents

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Background: Motor function is critical for children's health, yet remains an understudied neurodevelopmental domain. Exposure to metals has been linked with motor function, but no study has examined the joint effects of metal mixtures.

Methods: We evaluated cross-sectional associations between a metal mixture and motor function among 569 adolescents (10–14 years old) living near the ferroalloy industry. Concentrations of blood lead, hair manganese, hair copper, and hair chromium were quantified using inductively coupled plasma mass spectrometry. Neuropsychologists administered multiple fine motor function assessments: pursuit aiming, finger tapping, visual reaction time (VRT), and subtests from the Luria Nebraska battery. We estimated associations between motor function and the metal mixture using quantile-based g-computation and multivariable linear regression, adjusting for child age, sex, and socioeconomic status. We explored sex-specific associations in stratified models.

Results: Associations between the metal mixture and motor function were mostly null but were modified by sex. We observed a beneficial association among females: a quartile increase in all metals in the mixture was associated with a 2.6% faster average response time on the VRT (95% confidence interval [CI] = −4.7%, −0.5%), driven by Cu and Cr. In contrast, this association was adverse among males (β = 1.5% slower response time [95% CI = −0.7%, 3.9%]), driven by Cu and Mn.

Conclusions: Results suggest that males may be more susceptible to the adverse effects of metal exposure on motor function during adolescence than females. Future studies, particularly prospective study designs, are warranted to further understand the associations of metal mixtures with motor function.

Keywords: Metals; Neurodevelopment; Mixtures; Adolescents; Motor function

Introduction

Metals are ubiquitous in the environment and many are neurotoxic.[1,2] Ferroalloy production (also commonly referred to as ferromanganese or steel production) results in anthropogenic releases of multiple metals to the environment, notably manganese (Mn), lead (Pb), copper (Cu), and iron (Fe).[3,4] Metals concentrations, particularly Mn, in environmental media and biospecimens have been shown to be higher among populations residing near ferroalloy sites.[5–9] Further, environmental metals, including key ferroalloy components, have been associated both individually and as a mixture with neurobehavioral effects.[7,9–20] Given that the ferroalloy industry is predicted to expand globally in overall production due to increasing demand for construction, especially in lower- to middle-income countries,[21,22] understanding the neurodevelopmental consequences of ferroalloy exposure is important for protecting the health of communities living near ferroalloy sites.

Epidemiological data suggest that adults exposed to ferroalloy emissions, such as from living near or working in ferroalloy sites, experience neurological sequelae, including motor function disturbances.[23–26] These effects on motor function are consistent with the accumulation of metals in the basal ganglia and frontal cortex, brain regions that regulate motor control, and with the known interference of metals such as manganese with the dopaminergic system.[24,27] Motor function, however, is generally an understudied domain of neurodevelopment in younger populations, although it is essential for healthy development.

What this study adds:
This study is among the first to examine an industrially relevant metal mixture of lead, manganese, copper, and chromium with motor function outcomes in an adolescent population. We observed that the metal mixture was associated with adolescent motor function in a sex-specific manner. Our findings support the hypothesis that biological sex and exposure timing are important for elucidating the relationship between metal mixtures and neurodevelopment.

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Adequate motor function, defined as control of voluntary movements, is critical to a child's physical, academic, and overall well-being.[28,29] Strong (i.e., well-developed) motor skills have been positively associated with improved neurologic function, such as higher executive function, better language development, and increased social engagement through participation in activities and sports.[30–33] Subclinical decrements in motor ability (e.g., difficulty using writing utensils) can lead to long-term declines in academic performance and social development. As motor function is an essential part of neurodevelopment, but understudied in children's environmental health, research is needed to elucidate the relationship between environmental metal exposure and motor function in pediatric populations.

Adolescence is a period of physiological, hormonal, and psychosocial growth that includes further structural and functional brain development.[34–37] For example, during adolescence there is rapid growth and maturation of multiple brain regions. In particular, the prefrontal cortex, the basal ganglia, and the catecholaminergic neurons of the brain undergo dendritic pruning and increased myelination.[35,38] These changes are unique to the adolescent period and suggest that this age group may be susceptible to specific types of neuropathological effects following exposure to neurotoxicants such as metals, which have been shown to disrupt the dopaminergic system.[39–41]

Some metals, including Mn and Cu, are essential nutrients at physiologic levels but neurotoxicants at higher exposure levels, meaning that they are required for multiple biological processes but have the potential to adversely impact neurodevelopment when exposure occurs in excess.[1,6,42,43] The magnitude and direction of effect for a given metal may depend not only on dose but also on levels of co-occurring metals,[17] which has been demonstrated in previous epidemiologic studies for cognitive and other behavioral outcomes.[15–17,44–51] Individual metals, such as Mn,[27,52–55] Pb,[56–58] and Cu,[59] have been associated with decreased motor function,[20,45,46,55] and toxicological data support metal mixture effects on motor function.[39,60] However, no prior epidemiologic study has examined associations of a metal mixture with adolescent motor function.

The objective of this study was to investigate associations between a metal mixture (Pb, Mn, Cu, and Cr) and motor function in a cohort of Italian adolescents residing near ferroalloy production. Given that exposure to individual metals has been associated with adverse motor function in prior epidemiological studies,[27,52–59] we hypothesized that the metal mixture would be associated with poorer adolescent motor function. Additionally, given previous findings of sex-specific associations between metals and neurodevelopment,[1,15,53,61–64] we explored whether associations differed by biological sex.

Methods

Study population

We used cross-sectional data from the Public Health Impact of Metals Exposure (PHIME) study, which was designed to examine associations between ferroalloy industry metal exposure and neurodevelopment. A detailed description of the PHIME study design has been previously published.[52] Briefly, adolescents aged 10–14 years old were recruited between 2007 and 2014 from three different areas within the Brescia region of Italy that have varied ferroalloy industry activity: (1) Bagnolo Mella, which had active ferroalloy industry since 1974; (2) Valcamonica, which had historical ferroalloy production that ceased in 2001; and (3) Garda Lake, which had no history of ferroalloy activity.

To be eligible for the PHIME study, adolescents must have (1) been born to families living in the designated study area since the 1970s; (2) lived in the study area since birth, and (3) been 10–14 years old at the time of enrollment. Exclusion criteria included (1) having had any diagnosed metabolic, neurological, hepatic, or endocrine diseases, or clinically diagnosed hand/finger motor deficits that would affect neuropsychological assessments; (2) currently taking any prescription psychoactive drugs; (3) having had any visual deficits not adequately corrected; or (4) having ever received total parenteral nutrition. A total of 721 participants were enrolled in the study. The present analysis comprises PHIME participants who had no missing data on exposures (i.e., metal concentrations) or outcomes (i.e., motor function outcomes) (N = 612).

Informed consent was obtained from eligible participants before participation. The Institutional Review Boards at the Ethical Committee of Brescia, the Icahn School of Medicine at Mount Sinai, and the University of California, Santa Cruz approved all PHIME study protocols.

Measurement of metals

Blood and hair samples were collected and analyzed for Pb, Mn, Cu, and Cr at the time of enrollment. Whole blood samples (4 ml) were collected using a 19-gauge butterfly catheter into trace metal-free vacutainers.[65] Analysis of blood metal concentrations was performed at the University of California, Santa Cruz, using previously described methods.[65] A 2–3 cm section of hair (~100 to 150 strands) from the occipital lobe region proximal to the scalp was collected from participants using stainless steel scissors. Details about sample preparation for hair metal measurement are also available elsewhere.[66,67] Briefly, hair samples were sonicated sequentially in Triton and weak nitric acid to remove exogenous metal contamination. Then, hair samples were dried overnight and digested in ultrapure concentrated nitric acid. Metal concentrations (Pb, Mn, Cr, and Cu) in all biospecimens were measured using magnetic sector, inductively coupled plasma mass spectrometry (ICP-MS).[52] The analytical limits of detection (LOD) were determined based on repeated measurements of procedural blanks over four separate days as described in Butler et al (2019) and listed in Supplemental Table 1; http://links.lww.com/EE/A290. Nearly all metal measurements in hair and blood were above their respective LODs, except for two values (hair Mn, n = 1; hair Cr, n = 1); samples below the LOD were assigned a value of LOD/2. Based on histograms and boxplots, we visually observed several extreme values for metal concentrations; thus, we excluded participants with concentrations of any metal that were ±3 standard deviations (SD) from the mean (blood Pb, n = 8; hair Mn, n = 19; hair Cu, n = 8; hair Cr, n = 9). The final analytic sample size was 569 participants.

Motor function outcomes

Neurobehavioral assessments were administered by trained psychologists to PHIME participants concurrent with biomarker collection. We examined fine motor function using multiple assessments: (1) pursuit aiming (PA) test; (2) finger tapping (FT) and visual reaction time (VRT) tests from the Swedish Performance Evaluation System;[68] and (3) select subtests from the Luria Nebraska (LN) battery.[69] The outcomes of the PA test measure hand-eye dexterity, motor skills, and perceptual speed: the subject uses a pencil to place one dot inside a circle following a given pattern, performed as quickly as possible within two trials (60 seconds each). Over the two trials, the number of dots placed correctly inside the circle (i.e., total correct dots), the number of dots placed outside the circle (i.e., total error dots), and the sum of correct and error dots (i.e., total dots) are recorded and reported as raw scores. The FT test assesses manual motor speed. Participants are asked to finger tap a computer key with their forearm in a fixed position, as quickly as possible over eight 10-second trials (with 15-second breaks between trials), alternating between the dominant and nondominant hand. The average number of finger taps across the trials is recorded for each hand. During the VRT test, which

Table 1.

Description and range of scores for motor function assessments among 569 participants included in analysis

Outcome	Description	Mean (SD)	Range	Direction of beneficial effect
Pursuit aiming test				
Total correct dots	Number of dots placed correctly in a circle across two 60-second trials	126 (27.7)	20–214	Higher score
Total dots	Total number of dots placed on testing sheet across two 60-second trials	166 (38.7)	85–317	
Swedish Performance Evaluation System				
Finger tapping	Average number of finger taps over eight 10-second trials in the dominant hand	59.8 (5.6)	39–77	Higher score
	Average number of finger taps over eight 10-second trials in the nondominant hand	51.2 (5.8)	23–69	
Visual reaction time	Mean time (ms) between visual stimulus appearing on screen and subject pressing a button in response	308 (46)	219–598	Lower score
	Variability in time (ms) between visual stimulus appearing on screen and subject pressing a button in response (i.e., standard deviation of responses)	70 (26)	23–184	
Luria Nebraska battery		Mean across five tests	Sum across five tests	
Subtest 1	Number of hand openings and closings in the dominant hand for one 10-second trial	Mean (SD): 12.8 (2.7)	Mean (SD): 63.8 (13.5)	Higher score
Subtest 2	Number of hand openings and closings in the nondominant hand for one 10-second trial	Range: 6.2–21.4	Range: 31.0–107.0	
Subtest 3	Number of openings and closings of both hands (alternating between both hands) for one 10-second trial			
Subtest 4	Number of thumb touches with each finger in the dominant hand for one 10-second trial			
Subtest 5	Number of thumb touches with each finger in the nondominant hand for one 10-second trial			

measures psychomotor response and attention, the subject is asked to press a button as soon as a stimulus appears on the computer screen. The reaction time, or the time required for the participant to respond to the presence of a visual stimulus, is recorded in milliseconds. Scores are reported as mean reaction time and standard deviation (i.e., between-trial variability of motor responses) of reaction time for each subject. In addition, a total of five subtests were selected from the LN Battery (administered for 10 seconds each) that measure overall motor coordination. These include dominant hand clench (i.e., opening and closing), nondominant hand clench, alternating hand clenches, finger-thumb touching with the dominant hand, and finger-thumb touching with nondominant hand. For each subtest of the LN, the number of clenches (subtests 1–3) or touches (subtests 4 and 5) was recorded; the outcome scores include the mean and sum of all LN subtest scores. Outcome descriptions with mean (SD) and range of raw scores are provided in Table 1.

Covariates

Sociodemographic information was collected at enrollment using a standardized questionnaire administered either in person or by phone by trained researchers. Information included the child's biological sex (male or female), age (years, continuous), birth order (first, second, third, or higher), and area of residence (Bagnolo Mella, Valcamonica, and Garda Lake). Socioeconomic status (SES; low, medium, high) was determined using a methodology developed in Italy that combines parent education and occupation.[53,65] In addition to metal concentrations, ferritin (ng/ml) and hemoglobin (g/dl), two markers of iron (Fe) status, were quantified in serum and whole blood. Ten questions from the abbreviated version of the Home Observation Measurement of the Environment (HOME) Short Form[70] were used to estimate cognitive and emotional stimulation in the home (e.g., access to books and newspapers; range of scores: 0–9).

Statistical analysis

Univariate distributions were examined, and bivariate analyses were conducted for each exposure and outcome variable. Pb concentrations in whole blood were used in this analysis as blood is widely accepted as a Pb biomarker.[71,72] For Mn, Cu, and Cr, there is no gold standard exposure biomarker. We used hair as a biomarker of exposure for these metals as hair has been previously validated and used as the preferred biomarker in multiple previous epidemiological studies.[1,20,66,73–75] Metal concentrations were z-standardized in all subsequent models to account for varying units of the biomarkers and to facilitate interpretation. The total number of correct dots and total error dots from the PA test both capture the same information (i.e., both outcomes reflect a measure of accuracy), and the total error dots outcome was nonnormally distributed before and after natural log (ln)-transformation; therefore, we excluded total error dots from further analysis to avoid model misspecification. Mean reaction time and standard deviation (i.e., variability) of reaction time from the VRT test were not normally distributed; we therefore ln-transformed these outcomes to meet modeling assumptions of normality given that these scores were normally distributed after ln-transformation. Correlations between metal concentrations and motor function assessment scores were examined using Spearman's correlation coefficients.

Potential confounders were selected using a priori knowledge of the literature[52,53,76,77] and the construction of a directed acyclic graph (Supplemental Figure 1; http://links.lww.com/EE/A290).[78] Final confounders included child age (continuous), child biological sex (binary: male vs. female), and family SES (categorical: low, medium, vs. high). A small number of participants (n = 10, 1.8%) were missing data on SES; these participants were assigned the middle value for SES (i.e., medium SES).

Selection of statistical model

We considered three statistical approaches for evaluating the individual and joint associations of the metal mixture with

motor function: Bayesian kernel machine regression (BKMR), quantile g-computation (Qgcomp), and multipollutant multivariable linear regression. There is evidence to support pairwise or higher-order interactions among the metals in relation to neurodevelopmental outcomes[1,15,79] as well as nonlinearity between some metals and neurodevelopment;[15,59,80] therefore, we first conducted analyses with BKMR because it is a highly flexible model that allows for nonlinearity and visualization of higher-order interactions (described in more detail in Supplemental Material; http://links.lww.com/EE/A290).[81,82] We visually inspected BKMR exposure–response plots for nonlinearity and evidence of interaction. In the absence of nonlinearity or interactions (Supplemental Figures 2–5; http://links.lww.com/EE/A290), we prioritized Qgcomp (described below) to estimate the effects of the mixture on motor outcomes. To ground the mixture analysis in a more traditional regression framework and to facilitate the comparison of findings with other studies of individual metal associations, we also fit multivariable linear regression models (described below).

Quantile g-computation

Qgcomp is a parametric, generalized linear modeling approach, where the estimated effect of the mixture represents the change in motor function score per quantile increase in all mixture components. Using Qgcomp, we categorized metals into quartiles, which were then modeled in relation to each motor function outcome assuming additivity and linearity between exposures and outcomes, while controlling for confounders. Within the Qgcomp model, a positive or negative weight is estimated for each metal based on its relationship with the outcome, which can be interpreted as each metal's relative contribution to the estimated association.[83] If all mixture components act in the same direction on the outcome, the weight of a given metal is interpreted as the proportion of effect due to that metal, and weights sum to 1.0 across all mixture components. If metals act in opposing directions on the outcome, then the weight of a given metal is interpreted as the proportion of the positive (or negative) partial effect, and the positive and negative weights together sum to 2.0.[84–86] The Qgcomp model simultaneously estimates the joint association of the mixture by summing the beta coefficients for a quartile increase in each individual metal, allowing the metals to act in either the positive or negative direction in relation to motor function score. Thus, the beta coefficients (with 95% confidence intervals [CIs]) from Qgcomp models are interpreted as the cumulative association of the mixture on the outcome per quartile increase in all metals (i.e., the sum of beta coefficients of individual metals).[86,87] For ln-transformed outcomes (i.e., VRT mean and standard deviation), beta estimates (with 95% CIs) reflect percent change in motor function per quartile increase in all metals in the mixture and was calculated using the following equation: ([exp(β) – 1] × 100). We explored effect measure modification by stratifying models by sex.

Multipollutant linear regression

We fit multivariable linear regression models to quantify associations of each individual metal (Pb, Mn, Cu, and Cr) with motor function, adjusted for confounders and other metals. Given the low pairwise correlations between metals (range: –0.02 to 0.36), we were able to include all metals in a single linear regression model for each outcome (Supplemental Figure 6; http://links.lww.com/EE/A290). Consistent with Qgcomp models, we assumed linearity in these models because we did not observe evidence of nonlinear associations or interactions between metals in BKMR models (Supplemental Figures 2–5; http://links.lww.com/EE/A290). Beta estimates (with 95% CIs) in final models represent the covariate-adjusted change in motor function score per 1-SD increase in metal concentration. For ln-transformed outcomes (i.e., VRT mean and standard deviation), beta estimates (with 95% CIs) reflect percent change in motor function per 1-SD increase in metal concentration, calculated using the following equation: ([exp(β) – 1] × 100). Effect measure modification by sex was examined in multivariable regression models by stratifying models by sex.

Sensitivity analyses

We first evaluated the robustness of findings by changing the specifications of Qgcomp models; specifically, we changed the seed and, instead of using the nonbootstrapping setting in the *qgcomp* package in R (the *qgcomp.noboot()* function), we ran a secondary analysis where we used 300 nonparametric bootstrap iterations (using the *qgcomp.boot()* function).[88] Additionally, we examined the impact of excluding extreme values in the associations between metals and motor function by including previously excluded outliers. For all sensitivity analyses, we present metal-motor function associations from both Qgcomp and multipollutant linear regression models. All statistical analyses were conducted in R version 4.1.0 (The R Foundation for Statistical Computing, www.r-project.org).

Results

Participant characteristics, exposure, and outcome distributions

A total of 569 participants were included in the current analyses after excluding participants with outlying concentrations of metals (n = 43). Participants included in this analysis were, on average, 12 years old, and about half (53%) were from families classified as medium SES (Table 2). Median concentrations of blood Pb, hair Mn, hair Cu, and hair Cr were 1.30 µg/dl, 0.08 µg/g, 9.30 µg/g, and 0.05 µg/g, respectively. Compared to females, males had higher median blood Pb (1.50 µg/dl vs. 1.20 µg/dl) and lower median hair Cu levels (8.24 µg/g vs. 10.70 µg/g), while concentrations of hair Mn and hair Cr were similar between females and males (Table 2). Pairwise correlations for blood Pb, hair Mn, hair Cu, and hair Cr were weak to moderate (Spearman rho = –0.02 to 0.36), where the strongest correlation was estimated between hair Mn and hair Cr (Supplemental Figure 6; http://links.lww.com/EE/A290). Scores across different motor function assessments tended to be weakly correlated. Within the same motor function assessment, however, scores between outcomes were highly correlated (e.g., Spearman rho = 0.75 within the VRT; rho = 1.0 within the LN summary metrics) (Supplemental Figure 7; http://links.lww.com/EE/A290). Participants who were excluded from this analysis due to missing exposure or outcome data were more likely to be male and from a lower SES household compared to those included in the analysis (Supplemental Table 2; http://links.lww.com/EE/A290).

Associations between the metal mixture with motor function scores

There was little evidence of nonlinearity or interaction in the overall or the sex-stratified analyses based on BKMR models (Supplemental Figures 2–4; http://links.lww.com/EE/A290); therefore, we prioritized Qgcomp to estimate the effects of the mixture on motor outcomes. Based on Qgcomp models, associations between the metal mixture and motor function scores were mostly null and imprecise (Figure 1). In sex-stratified Qgcomp models, there was suggestive evidence of sexual dimorphism, where associations tended to be beneficial among females but not in males. For example, a quartile increase in all metals in the mixture was associated with a 2.6% decrease in average (mean) time on the VRT test (95% CI = –4.7%, –0.5%) in females, indicative of a faster (i.e., beneficial) reaction time for psychomotor

Table 2.

Sociodemographic characteristics of PHIME participants included in this analysis, for all participants and stratified by sex

Characteristic	All participants (N = 569)	Males (n = 279)	Females (n = 290)
		n (%) or median (IQR)	
Age (years)	12 (1)	12 (1)	12 (1)
Child SES status			
Low	120 (21.1%)	46 (16.5%)	74 (25.5%)
Medium	311 (54.5%)	160 (57.3%)	151 (52.0%)
High	138 (24.3%)	73 (26.2%)	65 (22.4%)
Study area			
Bagnolo Mella	177 (31.1%)	92 (33.0%)	85 (29.3%)
Garda Lake	187 (32.9%)	93 (33.3%)	94 (32.4%)
Valcamonica	205 (36.0%)	94 (33.7%)	111 (38.3%)
Biomarkers			
Blood Pb (μg/dL)	1.30 (0.99–1.88)	1.50 (1.07–2.02)	1.20 (0.92–1.62)
Hair Mn (μg/g)	0.08 (0.05–0.14)	0.08 (0.05–0.14)	0.07 (0.05–0.13)
Hair Cu (μg/g)	9.30 (6.9–14.72)	8.24 (6.29–12.26)	10.70 (7.83–17.33)
Hair Cr (μg/g)	0.05 (0.03–0.08)	0.05 (0.03–0.07)	0.06 (0.04–0.08)

IQR indicates interquartile range.

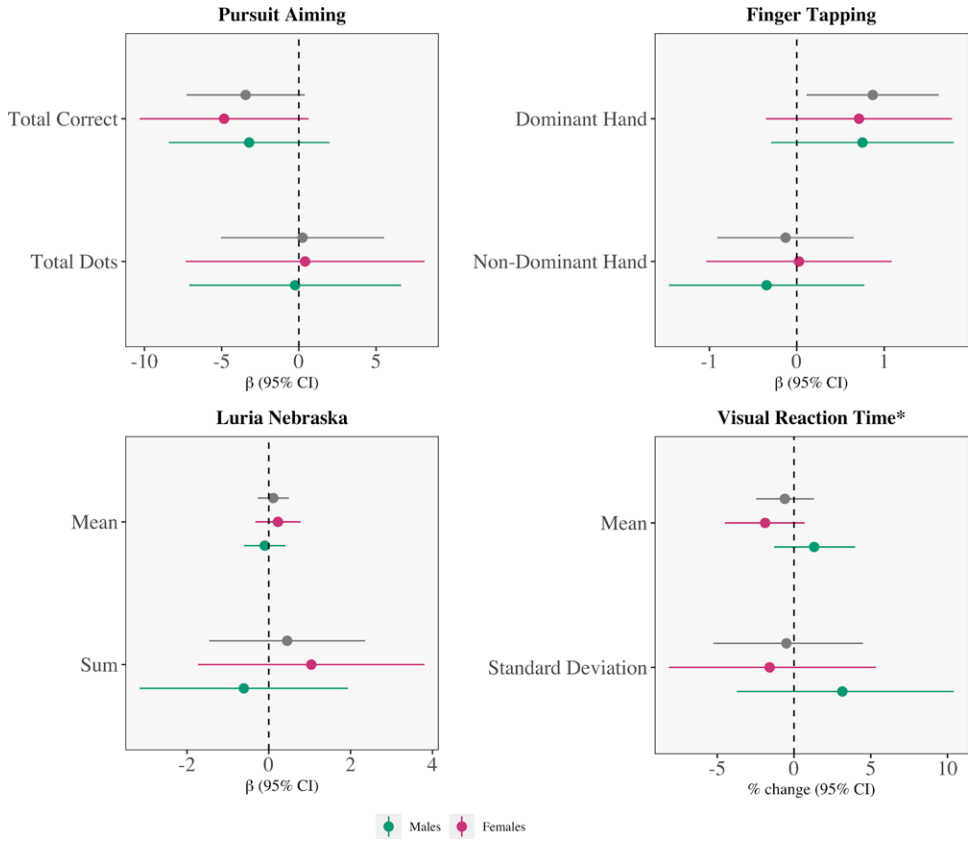

Figure 1. Adjusted associations between the metal mixture with adolescent motor function based on quantile g-computation models. Estimates reflect a change in motor function score per quartile increase in the mixture. Asterisk (*) indicates models where outcomes were natural log (ln)-transformed; effect estimates thus reflect percent change in motor function score per quartile increase in the metal mixture (calculated using the equation: ([exp(β) – 1] × 100). Models including all participants are shown in black and were adjusted for child sex, child age, and family SES. Sex-stratified models were adjusted for child age and family SES.

response and attention (Figure 1; Supplemental Table 3; http://links.lww.com/EE/A290). This beneficial association between the mixture and mean time on the VRT test among females was driven by Cu (weight = 0.38) and Cr (weight = 0.39), which had the largest weights for mixture components acting in the negative (beneficial) direction (Supplemental Figure 8; http://links.lww.com/EE/A290). In contrast, among males, a quartile increase in all metals in the mixture was associated with a 1.5% increase in average time on the VRT test, indicative of a slower reaction time (95% CI = –0.7%, 3.9%) (Figure 1; Supplemental Table 3; http://links.lww.com/EE/A290). The adverse, albeit imprecise, association in males was driven by Cu, which had the largest weight (0.42) of the mixture components acting in the positive (adverse) direction, followed by Mn (weight = 0.29) and Cr (weight = 0.29) (Supplemental Figure 8; http://links.lww.com/EE/A290). A similar pattern was observed for the standard deviation outcome on the VRT test, which is a measure of consistency between responses (Figure 1; Supplemental Table 3; http://links.lww.com/EE/A290). We observed similar sexually dimorphic trends for other motor function assessments

where associations of the mixture tended to be beneficial among females and adverse among males, including total dots on the PA test, FT in the nondominant hand on the FT test, and summary scores of the LN (Figure 1; Supplemental Table 3; http://links.lww.com/EE/A290).

Association between individual metals with motor function scores

Multipollutant (i.e., adjusted for all metals) linear regression models were used to supplement findings from mixture models and to ground mixture findings in a more traditional statistical framework. In general, results from multipollutant models were consistent with findings from Qgcomp models: associations between metals and motor function scores tended to be beneficial among females, and associations were strongest for hair Cr and Cu (Figure 2; Supplemental Table 4; http://links.lww.com/EE/A290). For example, a 1-SD increase in hair Cr was associated with a 2.0% decrease in mean time on the VRT test for females (95% CI = −3.9%, 0.2%), whereas this association was null for males (β = 0% [95% CI = −1.0%, 1.6%]) (Figure 2; Supplemental Table 4; http://links.lww.com/EE/A290). Similarly, a 1-SD increase in hair Cu was associated with 1.0% decrease in mean time on the VRT test for females (95% CI = −2.0%, 0.9%), whereas this association was null for males (β = 0% increase [95% CI = −1.0%, 2.4%]). Consistent with Qgcomp findings, a sex-specific pattern was also observed between individual metals and standard deviation on the VRT. For example, in multipollutant regression models, among males, a 1-SD increase in hair Mn was associated with a 5.1% increase (95% CI = 0.0%, 9.5%) in SD on the VRT test, suggesting greater variability between responses and thus worse performance. In contrast, the association between hair Mn and this outcome in females was beneficial (β = −3.0% [95% CI = −6.8%, 2.0%]), though modest (Supplemental Table 4; http://links.lww.com/EE/A290).

Sensitivity analysis

After varying specifications of Qgcomp (i.e., using 300 bootstraps and varying seed), results between main models and sensitivity analyses were nearly identical (Supplemental Table 5; http://links.lww.com/EE/A290). We also performed a sensitivity analysis in which we included participants with outlying metals concentrations who were excluded from main analyses, for a total sample size of N = 612 (vs. n = 569 for main analyses). Most findings were similar, though some associations were stronger in sensitivity analyses (Supplemental Table 6; http://links.lww.com/EE/A290). For example, when models included outlier values, there was a sex-specific effect of the metal mixture on the number of total correct dots on the PA test: a quartile increase in the metal mixture was associated with 4.6 fewer total correct dots among females (95% CI = −8.9, −0.3) and 1.9 fewer correct dots among males (95% CI = −6.1, 2.3) This sex-specific association with correct dots was not observed in main analyses (females: β = −2.7 [95% CI = −7.2, 1.8]; males: β = −2.5 [−7.1, 2.0]) (Supplemental Tables 3 and 6; http://links.lww.com/EE/A290). Similar to the main findings, multipollutant linear regression models including outliers in sensitivity analyses supported Qgcomp mixture findings (Supplemental Tables 6 and 7; http://links.lww.com/EE/A290).

Discussion

To date, little is known about the effects of metal exposure on motor function in younger populations, particularly adolescents. In a cohort of Italian adolescents living near ferroalloy production, we evaluated associations between a metal mixture of Pb, Mn, Cu, and Cr with multiple assessments of motor function. Among all participants, associations of the metal mixture with motor function were imprecise and the direction of effects differed by motor function assessment. In sex-stratified models, however, among females, there was a beneficial association between the metal mixture and psychomotor response and attention, as assessed by the VRT test. This association was driven by Cu and Cr. In contrast, this association was adverse among males and driven by Cu and Mn. These results suggest that associations between exposure to this mixture of ferroalloy metals and motor function as assessed by the VRT test differ by sex.

To our knowledge, there are no prior epidemiologic studies that have examined the effects of multiple metals as a mixture on motor function in adolescence. However, prior epidemiological evidence supports that metal mixtures are associated with changes in other neurodevelopmental domains (e.g., other aspects of cognition, behavior, and mental health), with variability in direction and magnitude of associations depending on sex, exposure timing, and the neurobehavioral domain that was examined.[1,50,63,89,90] In addition, toxicological evidence suggests that metals may act jointly on motor function.[39,60] In an experimental study, adult rats were dosed with either Pb, Mn, arsenic (As), or a mixture of all 3 metals, and motor function was evaluated using an open-field apparatus. Compared with animals exposed to each metal individually, those who received the triple metal mixture had the greatest decline in motor activity.[39] Although this study is not directly comparable to human epidemiological studies and examined a different mixture of metals than in the present study, it nonetheless generally supports our findings of joint metal effects on motor function.

We observed sex-specific associations of the metal mixture on scores of motor function, in particular for psychomotor response and attention. In sex-stratified models, the metal mixture was associated with improved performance on the VRT test, among females only. While this beneficial association was small in magnitude, it is consistent with the essentiality of several of the metals in the mixture we examined.[6,42,91–93] The VRT test requires the subject to respond to a visual stimulus by pressing a key on a computer, therefore evaluating fine motor function while simultaneously requiring input from other cognitive domains. Among females, the metal mixture may benefit the functional connectivity of the brain and/or the areas of the brain responsible for integrating visual input with motor output. In contrast, the metal mixture was associated with slower and less consistent response time on VRT among males. Our results therefore suggest that females and males are not equally susceptible to the effects of metal exposure during adolescence.

Sexually dimorphic effects of individual metals and their mixtures on neurodevelopmental outcomes have been previously observed in the PHIME cohort in relation to visuospatial learning[63,94] and motor function,[53] as well as in other cohorts.[59,95–99] For example, a prior analysis in the PHIME cohort examined the association between the same metal mixture (Pb, Mn, Cu, and Cr) with visuospatial learning in adolescence and similarly observed sex-specific effects.[63] However, in contrast to the present analysis, authors reported that the metal mixture was associated with improved visuospatial learning among males (driven by Cr) and decreased visuospatial learning among females (driven by Mn and Cu). The discrepancy between sexes in the direction of effects and in the metals driving associations may be related in part to differences in exposure levels, sample size (Rechtman et al, 2020: N = 188 vs. present study, N = 569), and statistical approach (e.g., Rechtman et al, 2020 used multiple biomarkers while we relied on a single biomarker for each metal exposure), but could also relate to differences in the neurodevelopmental outcomes that were assessed. Further, sex-specific findings are mixed among studies even for a given metal. For example, some studies have found more pronounced adverse effects of Mn exposure among males[98] whereas other studies have found more pronounced adverse associations

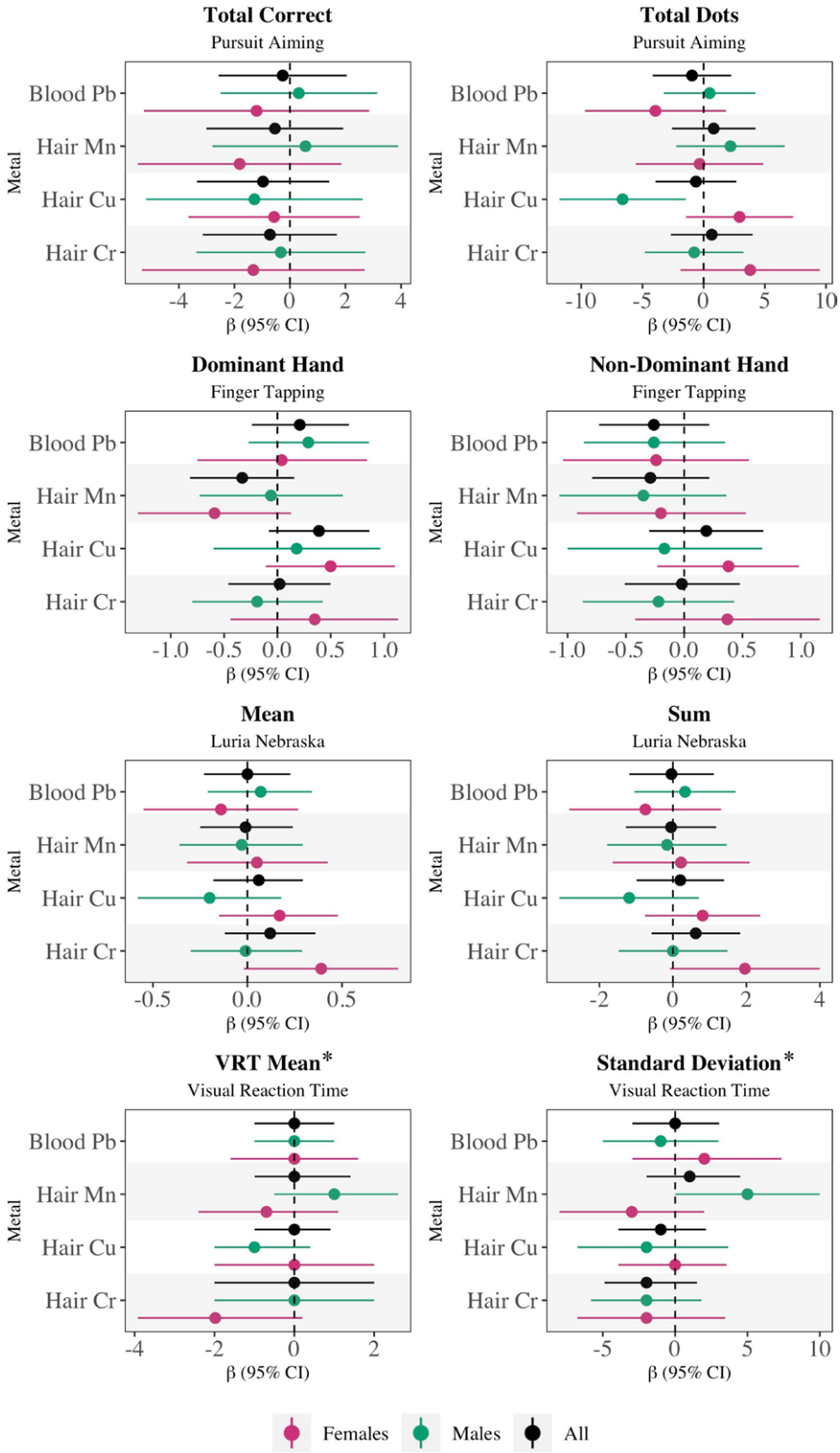

Figure 2. Sex-stratified associations between metals mixture and adolescent motor function based on multivariable linear models. Estimates reflect a one-unit change in motor function score per 1-SD increase in metal concentration. Asterisk (*) identifies models where outcomes were natural log (ln)-transformed; these estimates reflect percent change in motor function score per 1-SD increase in metal concentration (calculated using the equation: [(exp(β) − 1) × 100]). Models with all participants were adjusted for child sex, child age, family SES, and other metals. Sex-stratified models were adjusted for child age, family SES, and other metals.

among females.[94–96,99] The discrepancies in sex-specific findings between studies may be similarly related to differences in exposure timing, the neurodevelopmental outcomes that were assessed, or exposure levels across studies. There remains a paucity of epidemiological studies that examined metal mixtures in relation to neurodevelopment in adolescence,[15,18,63] and even fewer studies have formally examined effect measure modification by sex.[63] Therefore, future studies should be

designed to explicitly investigate the potential for biological sex to act as a modifier of associations between metal mixtures with neurodevelopment.

Previous studies have documented U-shaped dose–response curves between Cu and neurodevelopment,[15,59] supporting the biphasic roles that Cu plays as both nutrient and toxicant. In a previous analysis conducted in PHIME, Bauer et al[15] (2020) reported that a metal mixture of Pb, Mn, and Cr was associated with lower verbal IQ scores, especially at low levels of Cu. Cu was associated with measured intelligence in a nonlinear (i.e., U-shaped) manner, whereby low and high levels were associated with worse verbal IQ, adjusted for all other metals (Pb, Mn, and Cr) as well as confounders. Compared to the present analysis, the sample size in Bauer et al (2020) was larger and the range of hair Cu levels was wider (Bauer et al, 2020: n = 709; range of hair Cu [1.72–151.00 μg/g] vs. in our study: n = 569; range of hair Cu [1.72–52.6 μg/g]), which may partially explain the lack of a nonlinear dose–response curve for Cu in our analysis. Furthermore, in the present study, females had slightly higher levels of hair Cu than males (mean hair Cu level: 14.2 μg/g vs. 10.4 μg/g). Given the differences in Cu concentrations between males and females in our analysis, the opposing direction of Cu associations between sexes may, in part, reflect different ranges of the Cu dose–response curve. More research focusing on elucidating the role of Cu in motor function and the levels at which Cu may be toxic, particularly for children and adolescents, is warranted.

There are other plausible biological mechanisms that may explain the sexually dimorphic effects of metals. For example, there is evidence that sex chromosomes act directly on the brain, resulting in sex-specific differences in the structure and function of multiple brain regions.[100] Further, adolescence is a dynamic time of brain development[34,35,101,102] and hormonal changes from puberty during this developmental period may further contribute to sex-related differences in neurodevelopment.[101,103] For example, studies have reported that reductions in gray matter and increases in white matter volume in the brain occur during adolescence. However, the age at which the reduction of gray matter begins is different between sexes, likely related to the onset of puberty.[36,104,105] Similarly, the change in white matter volume is different: males tend to have a sharper increase in white matter compared to females during the same developmental period.[36,102,104] There is also evidence that there are sex-specific differences in the striatal response to dopamine, a neurotransmitter important for adequate motor function.[106–108] Further, some brain imaging studies have found that females had greater levels of dopamine availability in the brain compared to males.[106,109] In one brain imaging study, this increase in dopamine transporter availability among females was correlated with better performance on tests of executive and motor function.[109] Thus, sex-specific effects of metal exposure are plausible and may be related to the influence of sex hormones on brain structure and function, differential impacts on dopamine neurotransmission, or differences in the underlying neuroanatomy between sexes.

The observed beneficial and adverse associations of Mn, Cu, and Cr are consistent with these metals being essential metals as well as neurotoxicants. Mn is required for healthy brain development and the proper functioning of multiple Mn-dependent enzymes, such as transferases, hydrolases, and ligases.[110–112] However, in excess, Mn can disrupt levels and signaling of neurotransmitters and dopaminergic neurons.[2,113–116] Cu is required for the myelination of neurons, cellular respiration, catecholamine synthesis, and neurotransmission.[117,118] However, impaired homeostasis of Cu is associated with cellular toxicity and oxidative damage.[43,91] Similarly, while trivalent Cr (Cr III) is required for energy metabolism,[42] disrupted homeostasis releases free radicals that can result in oxidative stress. While the essentiality of these metals in the body supports that these metals may be associated with improved neurodevelopment, it is also possible these metals would be adversely associated with aspects of motor function given their potential for neurotoxic effects, as we observed in our study.

There are both strengths and limitations to our study. We examined the association between an industrial metal mixture and an understudied domain of neurodevelopment in a sample of Italian adolescents. To our knowledge, this is the first study to examine a metal mixture and its associations with motor function in this adolescent age group. In addition, the metal mixture in this study (Pb, Mn, Cu, and Cr) represents several of the more common metals emitted from the ferroalloy industry, which is predicted to double in production by 2050.[21,22,119] However, given the cross-sectional nature of these data, we are unable to make inferences about causality. Further, in sex-stratified analyses, the sample size was reduced. We recommend that these findings be confirmed in larger, ideally prospective studies. In addition, there may be some coexposure amplification bias in models due to unmeasured confounding in the setting of correlated exposures,[120] although we are less concerned here because metals concentrations were not highly correlated (Spearman rho range: −0.02 to 0.36).

Our findings may also be subject to residual or unmeasured confounding by unmeasured covariates and/or other important coexposures. For example, we were unable to account for coexposure to other toxic metals (e.g., arsenic, mercury, cadmium), which have been shown to be associated with motor function in toxicological and epidemiological studies.[1,39,60,121] We are also unable to examine and adjust for metal exposures in early life, which have been shown to be associated with brain structure and neurodevelopment in childhood and adolescence and could similarly result in residual confounding.[89,90,122] Additionally, we were unable to account for the pubertal stage as we do not have data on hormone levels or other pubertal stage assessments like Tanner stages. There may be concerns about selection bias if study participation was related to both metal exposure and motor function. However, this is unlikely given that the sample was restricted to participants without diagnosed neurological and developmental outcomes and participants were unaware of their exposure status. Finally, we used hair to estimate exposure to Mn, Cu, and Cr, and there may be concerns that hair is prone to exogenous contamination.[123] However, we used a validated method that extensively cleans the hair shaft and has been shown to decrease exogenous contamination,[66] thereby decreasing concerns about exposure misclassification.

Conclusion

We evaluated associations of a metal mixture with multiple assessments of adolescent motor function. These data suggest that exposure to a ferroalloy metal mixture may have impacts on aspects of motor function that vary by biological sex: associations between the metal mixture and motor function tended to be beneficial among females (driven by Cu and Cr) but harmful among males (driven by Cu and Mn). This study contributes to the limited literature on associations between metal exposures and motor function in adolescence. Our findings are important for protecting the health of communities that reside near historic, current, or future ferroalloy industrial activity, which is projected to expand. Future studies are needed to further understand the effects of exposure to metal mixtures on motor function in childhood and adolescence, ideally in prospective studies.

Conflicts of interest statement

The authors declare that they have no conflicts of interest with regard to the content of this report.

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