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Rationale, design and baseline results of the Guangxi manganese-exposed workers healthy cohort (GXMEWHC) study

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ABSTRACT

Objective: To determine the relationship between biomarkers of exposure, disease and susceptibility, and early health effects and long-term diseases related to occupational manganese (Mn) exposure.

Design: Baseline survey of a longitudinal cohort study of workers in a ferromanganese refinery.

Participants: A total of 1888 individuals (1197 men, 691 women; average seniority 15.34 years) were enrolled in the Guangxi manganese-exposed workers healthy cohort (GXMEWHC) study. Participants were between 18 and 60 years of age (mean 40.31 years), had worked in the ferromanganese refinery for at least 1 year and lived in the local area.

Results: The GXMEWHC study included a baseline survey. Participants were divided into four groups according to manganese (Mn) cumulative exposure index (Mn-CEI) levels: an internal control group (Mn-CEI <1.0 mg/m^3 year), a low exposure group (1.0 mg/m^3 year≤Mn-CEI<2.0 mg/m^3 year), a medium exposure group (2.0 mg/m^3 year≤Mn-CEI<5.0 mg/m^3 year) and a high exposure group (Mn-CEI≥5.0 mg/m^3 year). Genome-wide association studies of quantitative trait loci and binary trait loci in 500 Mn-exposed workers were performed using Illumina Infinium HumanExome BeadChip arrays. Stored plasma, DNA, hair and urine are available for further study. Participants will be followed up every 3 years.

Conclusions: The GXMEWHC study provides abundant data for exploring the systemic health effects of occupational Mn exposure using biomarkers of exposure, disease and susceptibility.

Strengths and limitations of this study

- We can collect a large database due to the size of the heavy metals cohort.
- The ferromanganese refinery is the largest such operation in China and therefore can provide an extremely rich dataset for analysis.
- The Guangxi manganese-exposed workers healthy cohort (GXMEWHC) is a longitudinal study that allows exploration of the relationships between occupational Mn exposure and early health effects.
- Genome-wide association studies were used to determine the susceptibility genes related to chronic low-level Mn exposure and explore genetic–environmental interactions, allowing susceptible workers to be identified before they experience early health effects.
- A potential limitation is the loss to follow-up of temporary workers, but this can be minimised through strict inclusion criteria when recruiting participants.

INTRODUCTION

Manganese (Mn) is essential for life. It is obtained from food, and can also be absorbed through environmental and occupational exposure. Mn accumulates in some organs with adverse effects when the Mn concentration exceeds the capacity of human metabolism.[1]

Many studies have shown that Mn can cause neurological abnormalities when it accumulates in the human brain,[2–4] such as early impaired finger tapping speed,[5] cognitive deficits, terminal Parkinsonian-like symptoms[6] and manganism.[7] The level of Mn in the human body can be detected using biomarkers, neurobehavioral tests and functional neuroimaging.[8 9] Increased concentrations of Mn in the kidney have been found in Mn-exposed workers because the kidneys are involved in excreting Mn.[4] In addition, repeated respiratory exposure to Mn may cause impaired lung function. In one study, a dose–effect relationship was found between occupational Mn exposure and reduced lung function.[10] Compared with non-exposed workers, lung function in Mn-exposed workers evaluated by spirometry tests showed

a significant decrease in forced vital capacity (FVC), forced expiratory volume in 1 s (FEV_1) and the ratio of forced expiratory volume in 1 s and forced vital capacity ($FEV_1\%$).[11] Increased Mn levels in blood serum are due to the fact that the liver stores, biotransforms and detoxifies Mn.[12] Overexposure to Mn can cause liver toxicity and exacerbate liver dysfunction.[13 14] Chronic Mn exposure causes significant cardiovascular problems including an abnormal ECG and inhibition of myocardial contraction, which can affect blood pressure (BP).[15] Additionally, Mn cytotoxicity has been shown to induce cell apoptosis and DNA damage in avian immune cells.[16] Low Mn^{2+} levels can induce oxidative DNA damage via an apoptotic pathway, but this DNA damage can be reduced using antioxidants. A risk assessment of inhaled Mn utilising genetics and genomics identified genetic biomarkers of exposure, disease and susceptibility.[17]

Thus, Mn toxicity in humans significantly affects several systems. So far, most studies have explored the effect of Mn exposure on different parts of the human body separately. To further investigate the effects of Mn exposure on various systems, we have established a prospective cohort study which includes data on individual Mn exposure and the results of regular occupational examination. Simultaneously, we will determine biological exposure using hair, urine and blood samples. Blood and urine can be used as biomarkers to determine short-term Mn exposure. Previous research has shown that hair can also be used as a biomarker of longer term Mn exposure.[18] Moreover, Mn-citrate can be used to determine excess Mn concentrations in the human body before manganism or Mn-induced Parkinsonism develops.[19] Accordingly, the main aims of this study are to determine Mn exposure using sensitive biomarkers and identify associated health effects.

METHODS

Establishing the cohort

We established the Guangxi Mn-exposed workers healthy cohort (GXMEWHC) in order to explore early health effects, potential biomarkers of exposure, disease and susceptibility, as well as diseases related to occupational Mn exposure. The prospective cohort study started in 2011 and included workers aged 18 years or older working in a ferromanganese refinery. The study investigates lifestyle, socio-economic status, environmental and occupational factors as well as genetic factors in relation to the early health effects of Mn exposure. This is an opportunity to explore the relationships between various risk factors and Mn exposure, particularly genetic and environmental factors and their interactions.

All members of the cohort were recruited from a ferromanganese refinery. Workers who participated in annual physical examinations and met the requirements were enrolled. The study was approved by the local ethics committee. Inclusion criteria were age 18–60 years, residence in the local area, employment in the factory for at least 1 year, ability to complete long-term follow-up, absence of disease, absence of risk factors other than Mn exposure (such as Cu, Pb, Cr or Hg exposure) and voluntary participation after providing informed consent. Participants were divided into different exposure groups according to their type of work.

Follow-up

We will follow up participants every 3 years. The same information as gathered at baseline will be collected through questionnaires, physical examination, biological specimens and environmental monitoring. Figure 1 shows the GXMEWHC study plan. Baseline data on demographic information, lifestyle, biological measurements as well as history of environmental and occupational exposure were collected by retrospective survey. The short-term objectives are to explore the early health effects of occupational Mn exposure and interactions with environmental factors. In addition, a preliminary exploration of the genetic effects of Mn exposure will also be carried out. Our long-term objective is to explore the early health effects on various systems of the human body of genetic–environmental interactions following long-term and continuous low levels of Mn exposure.

Building the database

Questionnaire

After obtaining written informed consent, trained interviewers used a structured questionnaire to collect baseline data in face-to-face interviews at the time of physical examination. Self-reported diseases were verified by specialists according to recognised international standards. The questionnaire collected information on demographics, socio-economic status, smoking history, alcohol consumption and occupational history.

Occupational health examination

The occupational health examination was conducted at the same visit. All participants underwent a general physical examination and were checked by trained physicians, nurses and medical technicians.

Height, weight, BP and lung function were measured. Lung function was assessed using a spirometry test which determined the value, predicted value and percentage of FVC, FEV_1, $FEV_1\%$, maximal mid-expiratory flow, peak expiratory flow ratio, and maximal voluntary ventilation.

Clinical examination included a high kV chest radiograph, neurological examination, ECG, uncorrected visual acuity test, pure tone audiometry and physical examination of the heart, lungs, liver, spleen and abdomen.

Laboratory tests included routine blood tests, routine urine tests and liver function tests. The routine blood tests included white blood cell (WBC) count, lymphocyte ratio, neutrophil granulocyte ratio, middle cell ratio, lymphocyte count, neutrophil granulocyte count,

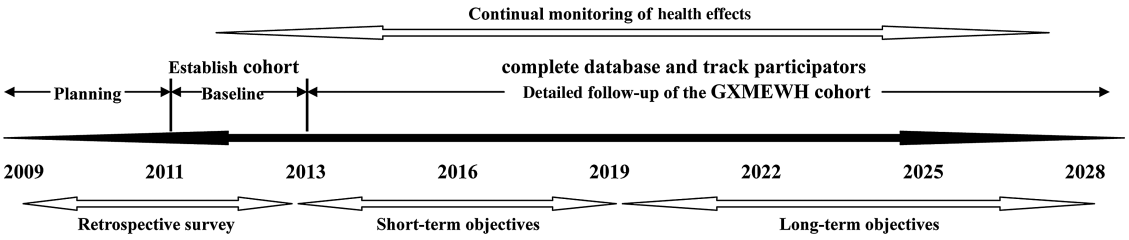

Figure 1 Study plan for the Guangxi manganese-exposed workers healthy cohort (GXMEWHC). The preliminary baseline survey was completed in 2013 and collected epidemiological information, biological samples, and data from the occupational health examination and workplace monitoring. Simultaneously, the GWAS database was established for 500 manganese-exposed workers. Participants will be followed up every 3 years and databases updated. GWAS, genome-wide association study

middle cell count, red blood cell count, haemoglobin (Hb), platelet count, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin (MCH), MCH concentration, thrombocytocrit (THR), erythrocyte haemoglobin distribution width, platelet volume distribution width, and mean platelet volume. The routine urine tests included urobilinogen, bilirubin (BIL), ketobodies, blood, protein, nitrite, WBCs, glucose, specific gravity, pH and vitamin C. We also measured urine Mn levels. The liver function tests consisted of total BIL (T-BIL), direct BIL (D-BIL), indirect BIL (I-BIL), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and AST/ALT ratio.

Neurological function test

The neurological function test consisted of a neurocognitive function test, neurobehavioral function test and neuropsychological test. The Montreal Cognitive Assessment (MoCA) is a neurocognitive function test which rapidly screens for mild cognitive impairment with high sensitivity and specificity.[20–22] We assessed the affect of Mn exposure on the nervous system using the MoCA as a cognitive screening tool.[23] The Non-Motor Symptoms Scale (NMSS) and the Scales for Outcomes in Parkinson's Disease-Autonomic (SCOPA-AUT) are neurobehavioral function tests. NMSS is a validated test for non-motor symptoms in Parkinson's disease (PD).[24 25] SCOPA-AUT is a self-administered scale and can be used for screening for autonomic symptoms in PD.[26 27] We used NMSS and SCOPA-AUT to evaluate the neurobehavioral function of workers who were exposed to occupational Mn. The Profile of Mood States (POMS) was used to assess the neuropsychological condition of Mn-exposed workers.[28 29]

Database of biological specimens

The biological specimens consisted of blood samples, urine specimens and hair samples. Three vacuum tubes (two EDTA anticoagulant tubes and a coagulation tube) were filled with 5 mL of fasting blood obtained intravenously. The blood sample in the coagulation tube was used to assess liver function and that in one of the EDTA anticoagulant tubes was used for routine blood tests. The blood sample in the other EDTA anticoagulant tube was separated into blood plasma and blood cells from which DNA was extracted as soon as possible. All blood specimens were stored at −80°C. In addition, a minimum of 10 mL of urine were collected in a urine bottle and stored at 4°C. A tuft of hair close to the scalp in the occipital region, about 2 cm in length and approximately 0.5 cm in diameter, was cut off with stainless steel scissors and collected in a special bag.[30] All hair specimens were stored in a cool, dry place.

Determining Mn exposure in the workplace

We will track the levels of Mn exposure in workers in the present study through workplace monitoring. We will record data on factory conditions, production processes, occupational risk factors, type of work, and levels of Mn. Concentrations of Mn dust and fumes in the workplace are detected with an air point sampler. We will also monitor individual levels of Mn using individual samplers during working hours. The permissible concentration-time weighted average (PC-TWA) is the average permissible exposure level during the 8 h working day, weighted by time. The permissible concentration-short term exposure limit (PC-STEL) is the permissible exposure level in any 15 min period, weighted by time within a working day. The cumulative exposure index (CEI) is calculated using TWA, STEL and workplace seniority. The CEI is an external exposure index of Mn and was calculated for each job by combining the results of airborne monitoring and of individual monitoring during both work time and break time.

Database of biomarkers

The database of biomarkers includes biomarkers of exposure, disease and susceptibility. Levels of plasma brain-derived neurotrophic factor (BDNF) and dopamine, which are biomarkers of effect, will be determined using sandwich ELISA kits. Levels of Mn and Fe in plasma, urine and hair will be determined using atomic absorption spectrometry. Biomarkers of susceptibility will be assessed using genome-wide association studies

(GWAS), which are described in the 'Genetic assessments' section below.

Genetic assessments

GWAS will be used to assess the genetic effects of Mn exposure. GWAS of quantitative trait loci (QTL) and binary trait loci (BTL) will be performed for exposed workers using the Infinium HumanExome BeadChip array (V.1.0, 12-sample HD; Illumina). The exonic content of these BeadChip arrays consists of over 240 000 variant markers representing a variety of common diseases and diverse populations from China, Europe and Africa. We will focus on potential interactions between environmental Mn exposure and genetics based on significant effects of Mn on the targeted phenotypes. Furthermore, potential gene–environment interactions will be explored through the genomes of patients with manganism and healthy individuals exposed to Mn in the workplace.

Statistical analyses

Trained investigators will enter the results of the questionnaires, physical examinations and neurological function tests twice into a computer using EpiData software. The GXMEWHC study database has already been set up and will be gradually improved; experimental data will also be entered. Data will be analysed by SPSS V.16.0 software. Genetic data will be analysed using Illumina's GenomeStudio, which is an integrated software platform for data visualisation and analysis. The GenomeStudio Genotyping Module is an application for extracting genotyping data from the Illumina iScan systems. We will use the Efficient and Parallelisable Association Container Toolbox (EPACTS), which can perform various statistical tests for identifying genome-wide associations. Quantitative traits will be calculated by the efficient mixed-model association eXpedited (EMMAX) programme, which corrects for sample structure within human GWAS by taking an expedited mixed linear model approach.[31] The binary traits will be calculated through the Logistic Score Test (LST) which can for test rare variants and relate the enriched genetic information to disease phenotypes through logistic regression models.[32] Optimal sequence kernel association tests (SKAT-O) will be used for the gene-wise and group-wise tests.[33]

PRELIMINARY RESULTS

Demographic characteristics of the cohort

A total of 1991 individuals from a ferromanganese refinery were considered. After completing the questionnaire, 1888 (94.8%) participants who met the criteria were enrolled in the GXMEWHC study.

Table 1 summarises the baseline characteristics of the cohort, 63.4% of whom were male and 36.6% female. Their mean age was 40.31 years and age distributions were similar. Overall, 34.5%, 31.0% and 34.5% of the participants had worked in the refinery for <10, 10–20 and >20 years, respectively. Mean length of service was 15.34 years. Mean body mass index was normal (22.47 kg/m^2). Among the participants, 48.5% were Han Chinese. A majority of participants (83.7%) were married. Some 43.9% had graduated from middle school or lower, 45.0% had finished high school and 11.1% had completed college or higher education. Of the cohort, 38.6% were current smokers, 7.0% were former smokers and 54.4% were never smokers. Current passive smoking rates were 87.3%. The proportion of current drinkers was 48.1%, while 15.9% were former drinkers and 36.0% never drinkers. Regarding type of work, 31.5%, 15.7%, 20.1% and 33.0% were smelters, raw material processors, high exposure auxiliary and low

Table 1 Demographic characteristics of the Guangxi manganese-exposed workers healthy cohort (GXMEWHC)

Variables	Number (n=1888)	Per cent
Sex		
Male	1197	63.4
Female	691	36.6
Age, years (mean±SD)	40.31±7.85	
<35	482	25.5
35–40	402	21.3
40–45	440	23.3
≥45	564	29.9
Seniority, years (mean±SD)	15.34±9.63	
<10	652	34.5
10–20	585	31
>20	651	34.5
BMI, kg/m^2 (mean±SD)	22.47±2.8	
<18.5	95	5
18.5–24	1289	68.3
24–28	422	22.4
≥28	74	3.9
Missing	8	0.4
Race/ethnicity		
Han Chinese	916	48.5
Zhuang minority	885	46.9
Other ethnic groups	80	4.2
Marital status		
Single	233	12.3
Married	1580	83.7
Widowed or divorced	75	4
Education		
Middle school or lower	829	43.9
High school	850	45
University or college or higher	209	11.1
Smoking status		
Current smoker	729	38.6
Former smoker	132	7
Never smoker	1027	54.4
Drinking status		
Current drinker	907	48.1
Former drinker	301	15.9
Never drinker	680	36

BMI, body mass index.

Table 2 Job titles of members of the Guangxi manganese-exposed workers healthy cohort (GXMEWHC)

Job title	Number (n)	Per cent	Age (years),mean±SD	Seniority (years),mean±SD
Smelter	594	31.5	38.95±8.20	15.82±9.02
Human crushing worker	320	16.9	41.08±5.30	9.04±6.00
Craneman	74	3.9	37.15±8.76	16.24±8.88
Finishing machining worker	99	5.2	40.36±6.10	10.20±8.79
Scaleman	105	5.6	42.30±4.92	17.53±6.88
Sampleman	21	1.1	45.75±7.02	23.07±6.57
Welder	128	6.8	40.75±10.13	18.29±10.76
Chemical analyst	54	2.9	45.52±7.02	24.29±8.37
Repairman	151	8.0	41.63±9.10	19.19±10.64
Electrician	91	4.8	40.28±7.31	19.45±8.00
Alkali recovery worker	133	7.0	40.89±6.33	13.74±8.74
Car driver	118	6.3	39.01±9.96	15.09±12.07
Total	1888	100	40.31±7.85	15.23±9.60

exposure auxiliary, respectively. In addition, 16.9% were human crushing workers and 6.8% were welders. Other types of work, proportions of workers, mean age, and seniority are shown in table 2.

Determining Mn exposure in the workplace

The participants were divided into groups according to type of work. Mn exposure was then determined according to working position and the results of workplace measurements. The CEI was calculated using TWA or STEL. Finally, all workers were classified into four exposure groups on the basis of the Mn-CEI results: internal control group (Mn-CEI <1.0 mg/m^3 year), low exposure group (1.0 mg/m^3 year≤Mn-CEI<2.0 mg/m^3 year), medium exposure group (2.0 mg/m^3 year≤Mn-CEI<5.0 mg/m^3 year) and high exposure group (Mn-CEI≥5.0 mg/m^3 year). The percentages of workers in the internal control, low exposure, medium exposure and high exposure groups were 34.5%, 17.6%, 37.6% and 10.3%, respectively. The Mn-CEI median was 1.85 mg/m^3 year and the range was 0.01–9.77 mg/m^3 year. The details of the Mn-CEI results are shown in table 3.

Results of the occupational health examination of the cohort

Mean systolic and diastolic BP were 125.43 and 78.81 mm Hg, respectively. The median urine Mn level was 2.84 µg/L (2.63 µg/L in males and 3.67 µg/L in females). The results of routine blood tests, liver function tests and lung function tests are shown in table 4.

Assessment of biomarkers

Liver function was compared in the different Mn-exposed groups in 2013. We found that occupational Mn exposure can cause a dose-dependent increase in liver enzyme levels and can interact with alcohol consumption to aggravate liver damage.[14] Plasma BDNF levels and the cognitive function of the different Mn-exposed groups were also measured. Our results showed that occupational Mn exposure may be related to decreased plasma BDNF levels and cognition impairment.[23]

Assessment of GWAS in the cohort

We carefully studied potential gene–environment interactions. We performed GWAS of QTL and BTL for 500 exposed workers using the Illumina Infinium HumanExome BeadChip array, and the targeted phenotypes included urine Mn and various indices of lung function, liver function and blood composition. Illumina's GenomeStudio Genotyping Module was used for genotyping and data analysis, using an integrated platform for data visualisation and analysis. About 25 000 loci were used in the analysis after quality control. The QTL, BTL, gene- and group-wise tests were conducted using EMMAX, LST and SKAT-O, respectively. We will further analyse differential gene expression. The results of GWAS and other indices will be reported in later

Table 3 The Mn-CEI of the Guangxi manganese-exposed workers healthy cohort (GXMEWHC)

Mn-CEI (mg/m^3 year)	Number (n)	Per cent	Median (IQR)	Range
Internal control group (Mn-CEI<1.0)	651	34.5	0.51 (0.55)	0.01–0.99
Low exposure group (1.0≤Mn-CEI < 2.0)	333	17.6	1.49 (0.46)	1.01–1.99
Medium exposure group (2.0≤Mn-CEI < 5.0)	710	37.6	3.04 (1.20)	2.00–4.98
High exposure group (Mn-CEI≥5.0)	194	10.3	5.99 (2.47)	5.01–9.77
Total	1888	100	1.85 (2.58)	0.01–9.77

Mn-CEI, manganese cumulative exposure index.

Table 4 Health examination results of the Guangxi manganese-exposed workers healthy cohort (GXMEWHC)

Variable	Males (n=1197), mean±SD	Females (n=691), mean±SD	Total (n=1888), mean±SD
Systolic blood pressure, mm Hg	127.68±12.11	121.54±11.53	125.43±1 2.26
Diastolic blood pressure, mm Hg	79.93±8.29	76.86±7.93	78.81±8.29
Blood tests			
WBC, 10^9/L	6.91±1.51	6.32±1.52	6.69±1.54
RBC, 10^{12}/L	5.13±0.52	4.61±0.44	4.94±0.55
Haemoglobin, g/L	148.69±12.73	128.8±14.37	141.38±16.44
Platelet count, 10^9/L	241.76±54.13	256.29±62.86	247.1±57.9
Liver function			
Total bilirubin, μmol/L	12.48±5.3	11.94±4.49	12.28±5.02
Direct bilirubin, μmol/L	3.98±2.16	3.66±2.19	3.86±2.17
Indirect bilirubin, μmol/L	8.5±3.44	8.24±2.51	8.4±3.13
ALT, U/L	25.35±17.62	17.23±14.74	22.38±17.07
AST, U/L	27.06±15.7	23.32±21.75	25.69±18.24
Lung function			
FVC, L	4.25±0.86	3.18±0.64	3.86±0.94
FEV_1, L	3.61±0.72	2.71±0.54	3.28±0.79
Uric Mn, μg/L, median (IQR)	2.63 (2.37)	3.67 (4.12)	2.84 (2.79)

ALT, alanine aminotransferase; AST, aspartate aminotransferase; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; Mn, manganese; RBC, red blood cells; WBC, white blood cells.

articles. We plan to conduct GWAS in a larger number of Mn-exposed workers to explore genetic risk factors and gene–environment interactions.

ETHICS AND DISSEMINATION

The study was approved by the medical ethics committee of Guangxi Medical University. All original files and data are maintained and stored at the research office in the Department of Occupational Health and Environmental Health, School of Public Health, Guangxi Medical University, Nanning, China. Electronic materials are stored in a safe system file and are accessible only by the data manager. All biological samples are marked in sequential order and stored in secure freezers. The results will be disseminated to relevant scientific forums including peer-reviewed journals and international conferences.

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Competing interests None.

Patient consent Obtained.

Ethics approval The medical ethics committee of Guangxi Medical University approved this study.

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