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Cohort Profile: The Guangxi manganese exposure workers healthy cohort (GXMEWHC)

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Abstract

Introduction: Manganese (Mn) is an essential element for growth and maintenance of human health. Inhaled manganese can be excrete by normal homeostatic mechanisms, but it also can be accumulate in the bodily organ when excess the ability of metabolism. Occupational exposure to Mn in air can cause the adverse health effects in the human bodies. Consequently, we established the Guangxi manganese exposure workers healthy cohort (GXMEWHC) to explore the early healthy effect and the long-term related disease of occupational manganese exposure by the biomarkers of exposure, effect and susceptibility.

Methods and analysis: The GXMEWHC is a prospective study. We recruited the workers in Ferro-manganese refinery Factory and presently conducted the baseline surveys including epidemiological investigation, neurological function test, occupational health examination and environmental monitoring. The genome-wide association study (GWAS) are also implemented further. We will follow up the participators every three years and ultimately the appropriate measures will be taken to prevent and control the early healthy injure and the related disease.

Ethics and dissemination: The study has been approved by Medical ethics committee of Guangxi Medical University. The results will be published by peer-reviewed publications and presented at international conferences.

Keywords

Manganese; Occupational exposure; Manganese toxicity; Genetic Susceptibility

Strengths and limitations of this study

- In this study, we can collect an abundant database because of large samples in the heavy metals cohort.
- The Ferro-manganese refinery Factory is the largest metallurgical factory of manganese processing in China so that it can provide an extremely rich dataset of analysis.
- The GXMEWHC is the longitudinal study which can continuous follow up and repeated investigate the participants. We can explore the relations between occupational manganese exposure and the early health injure.
- The GWAS are implemented for seeking the susceptibility genes of chronic low-level manganese exposure, and exploring the interactions between genetic factors and environmental factors. Those provide an important opportunity to identify the more susceptible individuals so that prevent the early health injure of workers.
- Potential limitations are that loss of follow up may be a weakness with our study. There are some temporary workers in the factory and they may leave the factory after a period of time working in factory. We can reduce the probability of the loss of follow up through strict controlled the inclusion criteria when established the cohort.

INTRODUCTION

Mn is an essential nutrient and it is necessary to inhaled manganese for maintain the daily life. In addition to food intake, environmental exposure to Mn was the way to absorption of Mn, especially occupational exposure. Mn was accumulated in some bodily organ and induced the adverse effects when the Mn concentration in *vivo* over the capacity of human metabolism.[1]

Many studies showed that Mn can caused neurological abnormalities when it accumulated in brain in human bodies,[2-4] such as early impaired Finger Tapping speed[5] or cognitive deficits, terminal Parkinsonian-like symptoms,[6] and Manganism.[7] The values of Mn in the human bodies were detected through some internal biomarkers, neurobehavioral tests and functional neuroimaging.[8 9] The concentrations of Mn in kidney were increased in the manganese exposure workers because the kidney is a way to excrete manganese.[4] In addition, the repeated respiratory exposed to Mn resulted in accumulation in the lung. One study showed that it was a dose-effect relationship between occupational manganese exposures and the reduction of pulmonary function.[10] Compared with the non-exposure workers, the pulmonary function in the manganese exposure workers were evaluated by the spirometry test and the values of them were a significant decrease in FEV_1, FVC and FEV_1% values.[11] Increased manganese levels in blood serum ascribed to that liver is a mainly organ to store, biotransformation and detoxify the poisonous substance.[12] Over exposure to manganese can cause liver toxicity as well as exacerbate liver dysfunction.[13 14] Chronic manganese exposures lead to a series of significant cardiovascular toxicities including the abnormal electrocardiogram (ECG) and

inhibition of myocardial contraction which can alters the blood pressure (BP).[15] Besides, the manganic cytotoxicity could induce cell apoptosis and the DNA damage of bird immune cells.[16] Low Mn^{2+} can induce oxidative DNA damage via an apoptotic pathway so that the DNA damage could be reduced using antioxidants. A research conducted a risk assessment of inhaled manganese through incorporating genetics and genomics to identify genetically based biomarkers of exposure, disease and susceptibility.[17]

From the above, manganic toxicity in humans played a significant role in several systems. Currently, most studies were explored separately the effect of manganese exposure for different system in the human body. To explore further the effect and the interaction of manganese exposure in various systems, we will establish a prospective cohort study which includes the situation of individual manganese exposure and regular occupational examination. Simultaneously, we will detect the biological exposure indicators by means of hair, urine and the blood samples. Blood and urine can reflect the extents of manganese exposure for a short term so that they can as the biomarker of manganese exposure. Previous research has shown that hair also can act as a biomarker of manganese exposure because that it may reflect the levels of manganese exposure for longer timeframes.[18] Moreover, a suitable Mn-biomonitoring including Mn-citrate can be used to determine the early onset of Mn concentrations in human bodies and therefore it can prevent the early onset of manganism or Mn-induced Parkinsonism as far as possible.[19] Accordingly, the risk of manganese exposure in sensitive effective biomarkers and the effect of health injure are also the main emphasis of this study.

METHODS AND ANALYSIS

Establishing a cohort

To explore the early healthy effect, the potential biomarkers of exposure, susceptibility and disease, as well as the related disease of occupational manganese exposure, we establish the GXMEWHC. The cohort consists of the workers in the Ferro-manganese refinery Factory. It is a long-term prospective cohort study of manganese exposure workers. The study investigates a variety of lifestyle, socio-economic status, environmental and occupational factors as well as genetic factors in relation to the early health injure for manganese exposure. This is an opportunity to explore the relations between various kinds of risk factors and the early health injure of manganese exposure, particularly the genetic and environmental factors and their interactions.

Sample source

The entire samples in this study were collected from a Ferro-manganese refinery Factory. The workers who participated in physical examination every year and accorded with the following conditions were recruited. The study was approved by the Ethics Committee. Inclusion criteria included the age of 18-60 years, living in the local, working in this company for a long time (at least one year) and being able to long term follow-up, the inexistence of obvious diseases for each system, outing of touch with other risk factors except manganese (such as Cu, Pb, Cr, Hg, et al) and voluntary participating after informed consent. Exclusion criteria contained the presence of obvious diseases for any system (such as a serious neurologic disease,

hepatic disease, kidney disease and cardiovascular disease) in the beginning of work, the contact of various occupational risk factors (such as Cu, Pb, Cr, Hg, et al) and unable to provide informed consent (psychiatric disease, language barrier, mental deficiency). All participants were divided into different exposed groups according to the type of work.

Follow-up

We will follow up the participants every three years and collect the data of questionnaire interview, physical examination, biological specimens and environmental monitoring repeatedly. **Fig. 1** shows the complete study plan of the GXMEWHC. The retrospective survey are establishing and perfecting the GXMEWHC by collecting baseline data on demographic information, lifestyle, biological specimens as well as history of environmental and occupational exposure. The short-term objectives are researching the early healthy effect of occupational manganese exposure interact with environmental influences. In addition, preliminary exploring the effects of manganese exposure on genetic field are also one of our studies. In the future, our long-term and final objectives are exploring the early healthy injure on various systems in human body by gene-environmental interactions for long-term and continuous low levels of manganese exposure.

Building database

Questionnaire

The trained interviewers used a specifically designed questionnaire to collect the

baseline data after obtaining written informed consent. In order to obtain real and accurate information, we take face-to-face interviews during the physical examination. The self-reported diseases are tested and verified through the diagnosis of specialists, which is based on recognized international standards. The questionnaires consist of demographic information, socio-economic status, smoking history and alcohol consumption and occupational history.

Occupational health examination

The occupational health examination was implemented at the same time. All participants took part in the general health examination and were checked by trained physicians, nurses and the medical technicians.

The physical measurements covered height, weight, blood pressure (BP) and pulmonary function. The pulmonary function was estimated using a spirometry test which comprised the test value of forced vital capacity (FVC) , forced expiratory volume at one second (FEV_1), the ratio of forced expiratory volume at one second (FEV_1%), maximal medexpiratory flow curve (MMEF), peak expiratory flow ratio (PEFR), maximal voluntary ventilation (MVV), the predicted value and the ratio percentage of all above.

The clinical examinations included high kilovar chest radiograph (HKV), Neurology inspection, ECG, Uncorrected visual acuity (UCVA), pure tone audiometry and physical examination of the heart, lungs, liver, spleen and abdomen.

The laboratory tests included blood routine tests, urine routine tests and liver function tests. The blood routine tests were measured in the laboratory covering white

blood cell count(WBC), lymphocyte ratio(LYR), neutrophile granulocyte ratio(GRANR), middle cell ratio (MIDR), lymphocyte count(LYC), neutrophile granulocyte count(NGC), middle cell count (MIDC), red blood cell count (RBC), hemoglobin (Hb), platelet count (PLT), hematokrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), thrombocytocrit (THR), erythrocyte hemoglobin distribution width (RDW), platelet volume distribution width (PDW) and mean platelet volume (MPV). The urine routines were detected by urobilinogen(URO), bilirubin,(BIL), ketobody(KET), blood(BLD), protein(PRO), nitrite(NIT), white blood cell(WBC), glucose(GLU), specific gravity(SG), power of hydrogen(PH) and vitamin C. Furthermore, we examined the content of manganese in urine. The liver function test contained total bilirubin (T-BIL), direct bilirubin (D-BIL), indirect bilirubin (I-BIL), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and AST/ALT.

Neurological function test

The neurological function test consists of neurocognitive function test, neurobehavioral function tests, and neuropsychological test. The Montreal Cognitive Assessment (MoCA) is a neurocognitive function test and it is an assessment method which rapid screen the Mild Cognitive Impairment (MCI) with high sensitivity and specificity.[20-22] We assessed the influence of manganese exposure on 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 an acceptable and valid

assessment means for non-motor symptoms in Parkinson's disease (PD).[24 25] SCOPA-AUT is a self-administered scale and can be used for the screening of autonomic symptoms in PD.[26 27] We used NMSS and SCOPA-AUT to evaluate the neurobehavioral function of workers who exposed to occupational manganese. The Profile of Mood States (POMS) is a neuropsychological test and is a scale to assess the emotions of manganese exposed workers.[28 29] We used POMS as an assessment instrument for assessing neuropsychological of workers.

Database of biological specimens

The biological specimens were composed blood samples, urine specimen and the hair samples. Three vacuum tubes (two ethylene diamine tetraacetic teraacetic (EDTA) anticoagulant tubes and a coagulation tube) filled with five milliliters of fasting blood respectively through intravenous access. The blood sample in the coagulation tube was used to detect the liver function and one of the EDTA anticoagulant tubes was measured the blood routine. The blood sample in another EDTA anticoagulant tube was separated into blood plasma and blood corpuscle which was extracted the DNA from it as soon as possible. All the blood specimens were stored in -80□ refrigerators. In addition, a minimum of 10 milliliter of the urine were collected in the urine bottles by the participants and then as the urine specimen stored in 4□ refrigerators. A tuft of hair of proximate 0.5 cm diameter was cut off with a stainless steel scissor and collected in the special sacks which close to the scalp in the occipital region about 2 cm. [30] All the hair specimens were stored in a cool and dry area.

Determining manganese exposure in the workplace

We will track the levels of manganese exposure workers who participated in the present cohort study by means of the workplace monitoring. We will record the basic information of the factory, the technological processes of production, and the distributions of occupational risk factors, the work mode and the situation of manganese in this company. The concentrations of manganese dust and fume in the workplace were detected through the air point sampler. At the same time, we monitor the individual levels of manganese by the individual sampler in their working time. Permissible concentration-time-weighted average (PC-TWA) is the average permissible exposure levels on the regulation eight hours working day weighting by time. The Permissible concentration-Short Term Exposure Limit (PC-STEL) is the permissible exposure levels on no more than 15 minutes any time weighting by time within a working day. The cumulative exposure index (CEI) is calculated through TWA, STEL and the seniority in working. The CEI as an external exposure index of manganese and was calculated for each job combining the airborne monitoring with the individual monitoring both at working time and break time.

Genetic determination

GWAS is implemented in this study which researched the effect of manganese exposure on genetic side. The GWAS of Quantative Trait Loci (QTL) and Binary Trait Loci (BTL) are also performed for the exposed workers using the Infinium HumanExome BeadChip from Illumina Company (Illumina Infinium HumanExome

v1.0 BeadChips, 12-sample HD). The Illumina's HumanExome BeadChips covered emphatically human exonic regions and the exonic content contains more than 240,000 variant markers. The markers represented a variety of common diseases and the different groups which contained the individuals of China, Europe, Africa and Spain. We will focus on the potential interaction of environmental manganese exposure and genetics which based on the significant effects of Mn on the targeted phenotypes. Furthermore, the potential gene-environment interaction is explored through the genomes of the Manganism patients and healthy individuals who exposed manganese in workplace.

Statistical analyses

After collecting the complete questionnaire, the data of physical examination and neurological function test, the trained investigators enter all the above data into the computer twice using the EpiData software. The GXMEWHC study database is established and it is gradually improved in later follow-up. Simultaneously, the experimental data is contained by the database. All the data is analyzed by the SPSS 16.0 software. The data of genetic determination is obtained and analyzed through the Illumina's GenomeStudio which is an integrated software platform for data visualization and analysis. The GenomeStudio Genotyping Module is an application for extracting genotyping data from the Illumina iScan systems. We use the Efficient and Parallelizable Association Container Toolbox (EPACTS) which can perform various statistical tests for identifying genome-wide association. The Quantative Trait

are calculated by the efficient mixed-model association eXpedited (EMMAX) program which can correct for sample structure within human GWASs by taking an expedited mixed linear model approach[31] The Binary Trait are calculated through the Logistic Score Test (LST) which can test with rare variants and relate the enriched genetic information to disease phenotypes through Logistic regression models.[32] When the Gene-wise or Group-wise Tests are conducted, the optimal sequence kernel association tests (SKAT-O) are used[33]

Preliminary result

Demographic description of the cohort

The 1991 individuals were recruited from the Ferro-manganese refinery Factory. After completing the questionnaire, a total of 1888 participants entered into the GXMEWHC who accord with standard and the effective rate is 94.8%.

In the cohort, 63.4% were male and 36.6% were female. The mean age was 40.31 years and the percent of the four-stages were similar. Thereinto, 34.5%, 31.0%, and 34.5% of the participants had the seniority of <10, 10-20, and >20 years, respectively. In the factory, 31.2%, 15.7%, 20.1% and 33.0% were smelter, raw material processor, high exposed auxiliary and low exposed auxiliary, respectively. The mean seniority was 15.34 years. The mean Body Mass Index (BMI) was normal ($22.47 kg \cdot m^{-2}$). Among the participants, 48.5% was Han Chinese. A majority of the participants (83.7%) were married. In the midst of the participants, 43.9% graduated from middle school, 45.0% had finished high school and 11.1% achieved college or higher education. In the cohort, 38.6% was current smoker, 7.0% was former smoker

and 54.4% was never smoker. Current passive smoking rates were 87.3%. The proportion of current drinker was 48.1%, the former drinker was 15.9% and the never drinker was 36.0%. Detailed information of the demographic characteristics of this cohort is provided in **Table 1**. Among the participators, 31.5% was smelters, 16.9% was human crushing workers and 6.8% was welder. The other types of work, the proportion of them, the mean age and seniority show in **Table 2**.

Determining manganese exposure in the workplace

All the participators were divided according to different the types of work in the factory. Then the extents of the manganese exposure were confirmed using the working positions combine with the results of workplace detection. The CEI is calculated through TWA or STEL. Finally, all workers were classified into four exposed groups on the basis of the Mn-CEI which are respectively the internal control group (Mn-CEI＜1.0 mg/m^3·year), the low exposed group (1.0≤Mn-CEI＜2.0 mg/m^3·year), the medium exposed group (2.0≤Mn-CEI＜5.0 mg/m^3·year)and the high exposed group (Mn-CEI≥5.0 mg/m^3·year). The percent of internal control group, low exposed group, medium exposed group and high exposed group were 34.5%, 17.6%, 37.6% and 10.3%, respectively. The median of total Mn-CEI was 1.85 mg/m^3·year and the range was 0.01 mg/m^3·year～9.77 mg/m^3·year. The details of Mn-CEI are show in **Table 3**.

Main results of occupational health examination of the cohort

The mean systolic blood pressure (SBP) and diastolic blood pressure (DBP) was

125.43 and 78.81 mmHg, respectively. The median of uric Mn was 2.63μg/L, the male was 3.67μg/L, and the female was 2.84μg/L. The values of the Blood Routine, Hepatic Function and Pulmonary Function are show in the **Table 4**.

Detection of biological specimens and GWAS of the cohort

We pay attention to the potential gene-environment interaction. Therefore, we performed the GWAS of QTL and BTL using the Illumina Infinium HumanExome BeadChip for 500 exposed workers, such as uric Mn and various kinds of index of pulmonary function, liver function and blood routine. The Illumina's GenomeStudio Genotyping Module was used for genotyping and data analysis which is an integrated platform for data visualization and analysis. About twenty –five thousand locus was involved in the analysis after Quality Control (QC). And then the QTL, BTL and Gene-wise or Group-wise Tests were conducted by EMMAX, LST and SKAT-O, respectively. We will analyze the differential gene expression further. The results of GWAS and other indexes will be reported in separate articles. We plan to conduct GWAS in a larger number of manganese exposure workers for exploring the genic risk factors and the gene-environment interaction.

ETHICS AND DISSEMINATION

The study has been approved via the Medical ethics committee of Guangxi Medical University. All the 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 accessible by the data manager. All the biological samples are marked in a sequential order and stored in secure freezer. The results will be disseminated to relevant scientific forums which included publishing in peer-reviewed journals and presenting at international conferences.

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Competing interests All authors declare that they have no conflict of interest.

Ethics approval Medical ethics committee of Guangxi Medical University.

Patient consent Obtained.

Provenance and peer review Not commissioned; externally peer reviewed.

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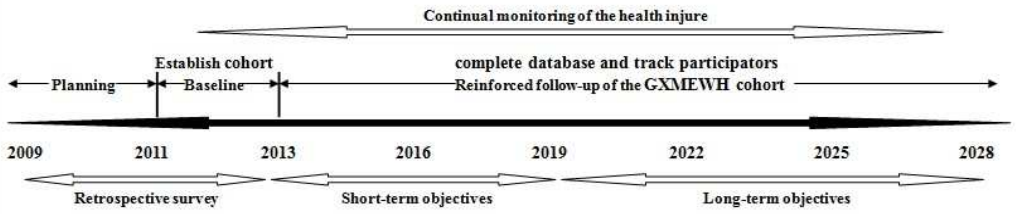

Fig. 1 The complete study plan of the GXMEWHC

The baseline survey was completed preliminary in 2013 and collected the epidemiological information, biological sample, the data of occupational health examination and workplace monitoring. Simultaneously, the database of GWAS was performed for 500 manganese exposed workers. The participants will be following up every three years and the databases were reinforced gradually in the future.

471 **Table 1**

472 Demographic characteristics of the GXMEWHC

Variables	Number (n=1888)	Percent (%)
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
Other ethnic groups	972	51.5
Marital status		
Single	233	12.3
Married	1580	83.7
Windowed or divorced	75	4
Education or lower		
Middle school	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

Table 2

Different types of work of the GXMEWHC

Types of work	Number（n）	Percent（%）	Age（years）	Seniority（years）
			Mean± SD	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	6.2	39.01 ± 9.96	15.09 ± 12.07
Total	1888	100	40.31 ± 7.85	15.23 ± 9.60

Table 3

The Mn-CEI of the GXMEWHC

Mn-CEI (mg/m^3·year)	Number (n)	Percent (%)	Median (Interquartile Range)	Range
Internal Control Group (Mn-CEI <1.0)	651	34.5	0.51 (0.55)	0.01～0.99
Low Exposed Group (1.0≤Mn-CEI<2.0)	333	17.6	1.49 (0.46)	1.01～1.99
Medium Exposed Group (2.0≤Mn-CEI<5.0)	710	37.6	3.04 (1.20)	2.00～4.98
High Exposed 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

Table 4

The results of occupational health examination of the GXMEWHC

Variables	Male(n=1197)	Female(n=691)	Total(n=1888)
	Mean ± SD	Mean ± SD	Mean ± SD
Systolic blood pressure, mmHg	127.68 ± 12.11	121.54 ± 11.53	125.43 ±1 2.26
Diastolic blood pressure, mmHg	79.93 ± 8.29	76.86 ± 7.93	78.81 ± 8.29
Blood routine			
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
Hepatic 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
Pulmonary function			
Test value of FVC, L	4.25 ± 0.86	3.18 ± 0.64	3.86 ± 0.94
Test value of FEV1, L	3.61 ± 0.72	2.71 ± 0.54	3.28 ± 0.79
Uric Mn, μg/L			
Median (Interquartile Range)	2.63 (2.37)	3.67 (4.12)	2.84 (2.79)

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

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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 explore the early health effect and long-term related diseases of occupational manganese (Mn) exposure according to biomarkers of exposure, effect and susceptibility.

Design: The baseline survey of a longitudinal cohort study from a Ferromanganese Refinery.

Participants: A total of 1888 individuals (1197 men, 691 women) involved in Guangxi manganese-exposed worker healthy cohort (GXMEWHC) study. Participants were aged between 18 and 60 years (average age 40.31 years) and worked in Ferromanganese Refinery at least one year and lived in the local area.

Results: The baseline survey was completed and the GXMEWHC study was established, which included 1888 workers (average seniority 15.34 years). All participants were divided into four groups according to the levels of Mn cumulative exposure index (Mn-CEI), which included 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). GWAS of quantitative trait loci (QTL) and binary trait loci (BTL) were performed using Illumina Infinium HumanExome BeadChip for 500 manganese-exposed workers. Stored plasma, DNA, hair and urine are available in further study. Participants will be followed up every three years.

Conclusions: The GXMEWHC study provides abundant data to explore the multi-organ health effects of occupational Mn exposure by biomarkers of exposure, response and susceptibility, respectively.

Strengths and limitations of this study

- In this study, we can collect an abundant database because of the large samples in the heavy metals cohort.
- The Ferromanganese Refinery is the largest metallurgical factory of Mn processing in China; therefore, it can provide an extremely rich dataset for analysis.
- The GXMEWHC is a longitudinal study that can continuously follow up and repeatedly investigate the participants. We can explore the relationships between occupational Mn exposure and the early health effects.
- GWAS was implemented to determine the susceptibility genes related to chronic low-level Mn exposure, and to explore the interactions between genetic factors and environmental factors. These data will provide an important opportunity to identify more susceptible individuals to prevent early health effect in workers.
- The potential limitations are that loss to follow-up may be a weakness with our study. There are some temporary workers in the factory and they may leave the factory after a period of time. We can reduce the probability of the loss to follow up through strictly controlled inclusion criteria when establishing the cohort.

INTRODUCTION

Manganese (Mn) is an essential nutrient and is necessary to inhale Mn for maintaining daily life. In addition to food intake, environmental exposure to Mn allows the absorption of Mn, including occupational exposure. Mn accumulated in some organs and induces adverse effects when the Mn concentration *in vivo* 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] or cognitive deficits, terminal Parkinsonian-like symptoms,[6] and manganism.[7] The level of Mn in the human body can be detected through some internal biomarkers, neurobehavioral tests and functional neuroimaging.[8 9] Increased concentrations of Mn in the kidney have been found in the manganese-exposed workers because the kidney is a pathway of Mn excretion.[4] In addition, repeated respiratory exposure to Mn may cause impaired lung function. In one study, there was a dose-effect relationship between occupational Mn exposures and a reduction in pulmonary function.[10] Compared with the non-exposed workers, the pulmonary function in the manganese-exposed workers evaluated by the spirometry tests showed a significant decrease in forced vital capacity (FVC), forced expiratory volume at one second (FEV1), and the ratio of forced expiratory volume at one second (FEV_1%) values.[11] Increased Mn levels in blood serum ascribed to that liver is the main organ to store, biotransformation and detoxify Mn.[12] Overexposure to Mn can cause liver toxicity and exacerbate liver dysfunction.[13 14] Chronic Mn exposure leads to a series of significant cardiovascular toxicities including an abnormal electrocardiogram (ECG)

and the inhibition of myocardial contraction which can alter the 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 incorporating genetics and genomics identified genetically based biomarkers of exposure, disease and susceptibility.[17]

Thus, Mn toxicity in humans plays a significant role in several systems. Currently, most studies have explored separately the effect of Mn exposure on different systems of the human body. To further explore the effects and the interaction of Mn exposure in various systems, we will establish a prospective cohort study which includes individual Mn exposure and regular occupational examinations. Simultaneously, we will determine biological exposure indicators by means of hair, urine and blood samples. Blood and urine can reflect the extent of Mn exposure in the short term so they can be used as biomarkers of Mn exposure. Previous research has shown that hair can also act as a biomarker of Mn exposure because it may reflect the levels of Mn exposure over longer timeframes.[18] Moreover, suitable Mn biomonitoring including Mn-citrate can be used to determine the early onset of excess Mn concentrations in the human body and can prevent the early onset of manganism or Mn-induced Parkinsonism as far as possible.[19] Accordingly, the risk of Mn exposure using sensitive effective biomarkers and the associated health effects are also the main emphasis of this study.

METHODS

Establishing a cohort

To explore the early healthy effects, potential biomarkers of exposure, susceptibility and disease, as well as the related disease of occupational Mn exposure, we established the GXMEWHC. The prospective cohort study started in 2011 and the targeted population was the workers aged 18 years or older working in the Ferromanganese Refinery. The study investigates a variety of 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 the early health effects of Mn exposure, particularly genetic and environmental factors and their interactions.

Sample source

The entire sample of this study was collected from a Ferromanganese Refinery. The workers who participated in physical examinations every year and met the following conditions were recruited. The study was approved by the local Ethics Committee. Inclusion criteria were age of 18-60 years, living in the local area, working in the factory for a long time (at least one year) and being able to complete long-term follow-up, the lack of obvious diseases in each system, absence if other risk factors except Mn exposure (such as Cu, Pb, Cr, or Hg) and voluntary participation after providing informed consent. People were excluded if they had an obvious diseases in any system (such as a serious neurological disease, hepatic disease, kidney disease or cardiovascular disease), contact with other occupational risk factors (such as Cu, Pb,

Cr, or Hg) and inability to provide informed consent (psychiatric disease, language barrier, or mental deficiency). All participants were divided into different exposure groups according to the type of work.

Follow-up

We will follow up the participants every three years. The information collected will be the same as the baseline data and will be collected by questionnaire interviews, physical examinations, biological specimens and environmental monitoring. **Fig. 1** shows the complete study plan of the GXMEWHC. The retrospective survey are establishing and perfecting the GXMEWHC by collecting baseline data on demographic information, lifestyle, biological specimens as well as history of environmental and occupational exposure. The short-term objectives are to explore the early health effects of occupational Mn exposure interact with environmental influences. In addition, preliminary exploring the effects of Mn exposure on genetics are also one of our studies. Our long-term and final objectives are to explore the early health effects on various systems of the human body by gene-environmental interactions for long-term and continuous low levels of Mn exposure.

Building database

Questionnaire

Trained interviewers used a structured questionnaire to collect the baseline data after obtaining written informed consent. In order to obtain real and accurate information, we conducted face-to-face interviews during the physical examination. Self-reported

diseases were tested and verified through the diagnosis of specialists, based on recognized international standards. The questionnaire consisted of demographic information, socio-economic status, smoking history, alcohol consumption and occupational history.

Occupational health examination

The occupational health examination was conducted at the same time. All participants took part in the general health examination and were checked by trained physicians, nurses, and medical technicians.

The physical measurements covered height, weight, BP, and pulmonary function. The pulmonary function was estimated using a spirometry test which included FVC , FEV_1, FEV_1%, maximal mid-expiratory flow (MMEF), the peak expiratory flow ratio (PEFR), maximal voluntary ventilation (MVV), the predicted value of them and the ratio percentage of all above.

The clinical examinations included a high kilovar chest radiograph (HKV), neurology inspection, ECG, uncorrected visual acuity (UCVA) test, pure tone audiometry and physical examination of the heart, lungs, liver, spleen and abdomen.

The laboratory tests included routine blood tests, routine urine tests and liver function tests. The routine blood tests were measured in the laboratory and included the white blood cell count (WBC), lymphocyte ratio (LYR), neutrophil granulocyte ratio (GRANR), middle cell ratio (MIDR), lymphocyte count (LYC), neutrophil granulocyte count (NGC), middle cell count (MIDC), red blood cell count (RBC), hemoglobin (Hb), platelet count (PLT), hematocrit (HCT), mean corpuscular volume

(MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), thrombocytocrit (THR), erythrocyte hemoglobin distribution width (RDW), platelet volume distribution width (PDW), and mean platelet volume (MPV). The routines urine tests included urobilinogen (URO), bilirubin (BIL), ketobodies (KET), blood (BLD), protein (PRO), nitrite (NIT), white blood cells (WBC), glucose (GLU), specific gravity (SG), pH and vitamin C. Furthermore, we examined the content of Mn in urine. The liver function tests contained total bilirubin (T-BIL), direct bilirubin (D-BIL), indirect bilirubin (I-BIL), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and the 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 and is an assessment method which rapidly screens for Mild Cognitive Impairment (MCI) with high sensitivity and specificity.[20-22] We assessed the influence 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 an acceptable and valid assessment means for non-motor symptoms in Parkinson's disease (PD).[24 25] SCOPA-AUT is a self-administered scale and can be used for the screening of 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) is a neuropsychological test and is a scale to assess the emotions of manganese-exposed workers.[28 29] We used POMS as an assessment instrument for assessing neuropsychological of workers.

Database of biological specimens

The biological specimens were composed blood samples, urine specimen and the hair samples. Three vacuum tubes (two ethylene diamine tetraacetic teraacetic (EDTA) anticoagulant tubes and a coagulation tube) were filled with 5 mL of fasting blood obtained through intravenous access. The blood sample in the coagulation tube was used to assess liver function and 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 cell from which was DNA extracted as soon as possible. All the blood specimens were stored at -80ºC. In addition, a minimum of 10 mL of the urine were collected in a urine bottle and stored at 4ºC. 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 and dry area.

Determining Mn exposure in the workplace

We will track the levels of manganese-exposed workers who participated in the present cohort study by means of workplace monitoring. We will record the basic information of the factory, the technological processes of production, the distributions of occupational risk factors, the work mode, and the level of Mn in this factory. The

concentrations of Mn dust and fumes in the workplace are detected through an air point sampler. At the same time, we will monitor the individual levels of Mn using individual samplers during working hours. The permissible concentration-time-weighted average (PC-TWA) is the average permissible exposure levels during the regulation eight-hour working day, weighted by time. The permissible concentration-short term exposure limit (PC-STEL) is the permissible exposure levels in no more than 15 minutes at any time, weighted by time within a working day. The cumulative exposure index (CEI) is calculated through TWA, STEL, and workplace seniority in working. The CEI as an external exposure index of ma Mn nganese and was calculated for each job, combining the airborne monitoring with the individual monitoring both during work time and break time.

Database of biomarkers

The database of biomarkers included biomarkers of exposure, effect and susceptibility. The biomarkers of exposure will be detected through atomic absorption spectrometry (AAS) which are the levels of Mn and Fe in plasma, urine and hair. The levels of plasma brain-derived neurotrophic factor (BDNF), dopamine (DA) were determined by Sandwich ELISA kits which are biomarkers of effect. The biomarkers of susceptibility are also assessed by GWAS which are shown in the following Genetic assessments part in detail.

Genetic assessments

A sub-cohort of GWAS is implemented in this study to assess the effect of Mn exposure on genetics. The GWAS of quantitative trait loci (QTL) and binary trait loci (BTL) will also be performed for exposed workers using the Infinium HumanExome BeadChip from Illumina Company (Illumina Infinium HumanExome v1.0 BeadChips, 12-sample HD). Illumina's HumanExome BeadChips are covered with human exonic regions, the exonic content contains more than 240,000 variant markers. The markers represent a variety of common diseases. The different groups include individuals from China, Europe, and Africa. We will focus on the potential interactions of environmental Mn exposure and genetics based on the significant effects of Mn on the targeted phenotypes. Furthermore, potential gene-environment interactions will be explored through the genomes of manganism patients and healthy individuals exposed to Mn in the workplace.

Statistical analyses

After collecting the complete questionnaires, the physical examination results and neurological function test data, trained investigators will enter the above data into the computer twice using EpiData software. The GXMEWHC study database is established and will be gradually improved in subsequent follow-up. Simultaneously, the experimental data will be contained in the database. All the data will be analyzed by SPSS 16.0 software. The genetic determination data will be obtained and analyzed through Illumina's GenomeStudio, which is an integrated software platform for data

visualization and analysis. The GenomeStudio Genotyping Module is an application for extracting genotyping data from the Illumina iScan systems. We will use the Efficient and Parallelizable Association Container Toolbox (EPACTS), which can perform various statistical tests for identifying genome-wide associations. The quantitative traits will be calculated by the efficient mixed-model association eXpedited (EMMAX) program, which can correct 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 test rare variants and relate the enriched genetic information to disease phenotypes through Logistic regression models.[32] When the gene-wise or group-wise tests are conducted, optimal sequence kernel association tests (SKAT-O) will be used[33]

PRELIMINARY RESULTS

Demographic description of the cohort

In total, 1,991 individuals were recruited to participate in the study from the Ferromanganese Refinery. After completing the questionnaire, a total of 1,888 participants who met the standards were entered into the GXMEWHC study,with an effective rate of 94.8%.

Table 1 summarizes the baseline characteristics of the cohort. Of the cohort, 63.4% were male and 36.6% were female. The mean age was 40.31 years and the percent of the age distribution was similar. 34.5%, 31.0%, and 34.5% of the participants had seniority of <10, 10-20, and >20 years, respectively. In the factory, 31.2%, 15.7%, 20.1% and 33.0% were smelters, raw material processors, high

exposure auxiliary, and low exposure auxiliary, respectively. The mean seniority was 15.34 years. The mean body mass index (BMI) was normal (22.47kg·m^{-2}). Among the participants, 48.5% were Han Chinese. A majority of the participants (83.7%) were married. Of these participants, 43.9% graduated from middle school, 45.0% finished high school and 11.1% completed college or higher education. In 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%, former drinkers was 15.9% and never drinkers was 36.0%. Among the participants, 31.5% were smelters, 16.9% were human crushing workers and 6.8% were welder. The other types of work, the proportion of them, the mean age, and seniority are shown in **Table 2**.

Determining Mn exposure in the workplace

All the participants were divided according to different types of work in the factory. Then, the extent of the Mn exposure was confirmed using the working positions combined with the results of workplace detection. The CEI was calculated through TWA or STEL. Finally, all workers were classified into four exposure groups on the basis of the Mn-CEI results, including the internal control group (Mn-CEI＜1.0 mg/m^3·year), the low exposure group (1.0 mg/m^3·year≤Mn-CEI＜2.0 mg/m^3·year), the medium exposure group (2.0 mg/m^3·year≤Mn-CEI＜5.0 mg/m^3·year) and the high exposure group (Mn-CEI≥5.0 mg/m^3·year). The percentages of the internal control group, low exposure group, medium exposure group and high exposure group were 34.5%, 17.6%, 37.6% and 10.3%, respectively. The median of total Mn-CEI was

1.85 mg/m^3·year and the range was 0.01 mg/m^3·year-9.77 mg/m^3·year. The details of Mn-CEI are shown in **Table 3**.

Main results of the occupational health examination of the cohort

The mean systolic blood pressure (SBP) and diastolic blood pressure (DBP) were 125.43 and 78.81 mmHg, respectively. The median urine Mn level was 2.63μg/L, 3.67μg/L in males, and 2.84μg/L in females. The results of the routine blood tests, hepatic function tests and pulmonary function tests are shown in the **Table 4**.

Assessment of biomarkers

The liver function was analyzed between different manganese-exposed groups of the cohort in 2013. Our conclusions in this study were that occupational Mn exposure can cause a dose-dependent increase of liver enzyme levels and interact with alcohol drinking to potentially aggravate the liver damage.[14] The plasma BDNF levels and cognitive function of different manganese-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 greatly paid attention to potential gene-environment interactions. Therefore, we performed GWAS of QTL and BTL using the Illumina Infinium HumanExome BeadChip for 500 exposed workers, including urine Mn and various indices of pulmonary function, liver function and blood detection. Illumina's GenomeStudio

Genotyping Module was used for genotyping and data analysis, using an integrated platform for data visualization and analysis. About 25,000 locu were involved in the analysis after quality control (QC). The QTL, BTL, gene-wise and group-wise tests were conducted by EMMAX, LST, and SKAT-O, respectively. We will further analyze differential gene expression. The results of GWAS and other indices will be reported in ongoing articles. We plan to conduct GWAS in a larger number of manganese-exposed workers to explore the genic risk factors and gene-environment interactions.

ETHICS AND DISSEMINATION

The study was approved via the medical ethics committee of Guangxi Medical University. All the 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 the biological samples are marked in sequential order and stored in secure freezers. The results will be disseminated to relevant scientific forums which include publishing in peer-reviewed journals and presentation at international conferences.

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Contributors Xiaobo Yang and Yunfeng Zou contributed in conception and design; Jing Liu, Kangcheng Chen, Yingnan Lv, Damin Huang, Yuefei Shen, Yaoqiu Zhong, Zhihao Liu, Bei Jiang, Qin Li, Li Qing,Wei Zhang, Lang Chen, Fenfen Wang, Bing Xia and Li Yang contributed in acquisition of the data; Yingnan Lv analysed the data and drafted the manuscript; all authors contributed to review and revision of the manuscript and approved the final version.

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Competing interests All authors declare that they have no conflict of interest.

Ethics approval Medical ethics committee of Guangxi Medical University.

Participant consent Obtained.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement No additional data are available.

Fig. 1 The complete study plan of the GXMEWHC

The preliminary baseline survey was completed in 2013 and collected epidemiological information, biological samples, data from the occupational health examination and workplace monitoring. Simultaneously, the GWAS database was established for 500 manganese-exposed workers. The participants will be followed up every three years and the databases will be amended in the future.

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Table 1

Demographic characteristics of the GXMEWHC

Variables	Number (n=1888)	Percent (%)
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 or lower		
Middle school	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

Table 2

Different types of work of the GXMEWHC

Types of work	Number (n)	Percent (%)	Age (years)	Seniority (years)
			Mean± SD	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	6.2	39.01 ± 9.96	15.09 ± 12.07
Total	1888	100	40.31 ± 7.85	15.23 ± 9.60

Table 3

The Mn-CEI of the GXMEWHC

Mn-CEI (mg/m^3·year)	Number (n)	Percent (%)	Median (Interquartile Range)	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

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Table 4

546 The results of occupational health examination of the GXMEWHC

Variables	Male(n=1197)	Female(n=691)	Total(n=1888)
	Mean ± SD	Mean ± SD	Mean ± SD
Systolic blood pressure, mmHg	127.68 ± 12.11	121.54 ± 11.53	125.43 ±1 2.26
Diastolic blood pressure, mmHg	79.93 ± 8.29	76.86 ± 7.93	78.81 ± 8.29
Blood routine			
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
Hemoglobin, 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
Hepatic 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
Pulmonary function			
Test value of FVC, L	4.25 ± 0.86	3.18 ± 0.64	3.86 ± 0.94
Test value of FEV1, L	3.61 ± 0.72	2.71 ± 0.54	3.28 ± 0.79
Uric Mn, μg/L	2.63 (2.37)	3.67 (4.12)	2.84 (2.79)

Median (interquartile range)

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Rationale, design and baseline results of t~~Cohort Profile:~~ ~~T~~he Guangxi ~~manganese~~ manganese-~~exposure~~ exposed workers healthy cohort (GXMEWHC) study

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Abstract

Objective: To explore the early health effect and long-term related diseases of occupational manganese (Mn) exposure according to biomarkers of exposure, effect and susceptibility.

Design: The baseline survey of a longitudinal cohort study from a Ferromanganese Refinery.

Participants: A total of 1888 individuals (1197 men, 691 women) involved in Guangxi manganese-exposed worker healthy cohort (GXMEWHC) study. Participants were aged between 18 and 60 years (average age 40.31 years) and worked in Ferromanganese Refinery at least one year and lived in the local area.

Results: The baseline survey was completed and the GXMEWHC study was established, which included 1888 workers (average seniority 15.34 years). All participants were divided into four groups according to the levels of Mn cumulative exposure index (Mn-CEI), which included 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). GWAS of quantitative trait loci (QTL) and binary trait loci (BTL) were performed using Illumina Infinium HumanExome BeadChip for 500 manganese-exposed workers. Stored plasma, DNA, hair and urine are available in further study. Participants will be followed up every three years.

Conclusions: The GXMEWHC study provides abundant data to explore the multi-organ health effects of occupational Mn exposure by biomarkers of exposure, response and susceptibility, respectively.

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Introduction: Manganese (Mn) is an essential element for growth and maintenance of human health. Inhaled manganese can be excrete by normal homeostatic mechanisms, but it also can be accumulate in the bodily organ when excess the ability of metabolism. Occupational exposure to Mn in air can cause the adverse health effects in the human bodies. Consequently, we established the Guangxi manganese exposure workers healthy cohort (GXMEWHC) to explore the early healthy effect and the long term related disease of occupational manganese exposure by the biomarkers of exposure, effect and susceptibility.

Methods and analysis: The GXMEWHC is a prospective study. We recruited the workers in Ferro manganese refinery Factory and presently conducted the baseline surveys including epidemiological investigation, neurological function test, occupational health examination and environmental monitoring. The genome wide association study (GWAS) are also implemented further. We will follow up the participators every three years and ultimately the appropriate measures will be taken to prevent and control the early healthy injure and the related disease.

Ethics and dissemination: The study has been approved by Medical ethics committee of Guangxi Medical University. The results will be published by peer-reviewed publications and presented at international conferences.

Keywords

Manganese; Occupational exposure; Manganese toxicity; Genetic Susceptibility

Strengths and limitations of this study

- In this study, we can collect an abundant database because of the large samples in the heavy metals cohort.
- The Ferro-manganese ~~refinery~~ Refinery ~~Factory~~ is the largest metallurgical factory of ~~manganese~~ Mn processing in China ~~so that~~; therefore, it can provide an extremely rich dataset for~~of~~ analysis.
- The GXMEWHC is ~~the~~ a longitudinal study ~~which~~ that can continuously follow up and repeatedly investigate the participants. We can explore the relationships between occupational ~~manganese~~ Mn exposure and the early health ~~injure~~effects.
- ~~The~~ GWAS ~~are~~ was implemented ~~for seeking~~to determine the susceptibility genes ~~of~~ related to chronic low-level ~~manganese~~ Mn exposure, and to ~~exploring~~ explore the interactions between genetic factors and environmental factors. ~~Those~~ These data will provide an important opportunity to identify ~~the~~ more susceptible individuals ~~so that~~to prevent ~~the~~ early health ~~injure~~ effect ~~of~~ in workers.
- The p~~P~~otential limitations are that loss ~~of~~ to ~~follow~~ follow-up may be a weakness with our study. There are some temporary workers in the factory and they may leave the factory after a period of time ~~working in factory~~. We can reduce the probability of the loss ~~of~~ to follow up through strictly controlled ~~the~~ inclusion criteria when ~~established~~ establishing the cohort.

INTRODUCTION

Manganese (Mn) is an essential nutrient and ~~it~~ is necessary to inhale~~d~~ ~~manganese~~ Mn for maintaining ~~the~~ daily life. In addition to food intake, environmental exposure to Mn ~~was~~ allows the ~~way to~~ absorption of Mn, ~~especially~~ including occupational exposure. Mn ~~was~~ accumulated in some ~~bodily~~ organs and ~~induced~~ induces ~~the~~ adverse effects when the Mn concentration *in vivo* ~~over~~ exceeds the capacity of human metabolism.[1]

Many studies have ~~showed~~ shown that Mn can cause~~d~~ neurological abnormalities when it ~~accumulated~~ accumulates in the human brain ~~in human bodies~~,[2-4] such as early impaired ~~Finger~~ finger ~~Tapping~~ tapping speed[5] or cognitive deficits, terminal Parkinsonian-like symptoms,[6] and ~~Manganism~~manganism.[7] The ~~values~~ level of Mn in the human ~~bodies~~ body ~~were~~ can be detected through some internal biomarkers, neurobehavioral tests and functional neuroimaging.[8 9] ~~The~~ Increased concentrations of Mn in the kidney ~~were increased~~have been found in the ~~manganese~~ manganese-~~exposure~~ exposed workers because the kidney is a ~~way to~~pathway of ~~excrete~~ Mn~~manganese~~ excretion.[4] In addition, ~~the~~ repeated respiratory exposure~~d~~ to Mn may cause impaired~~resulted in accumulation in the~~ lung function. In o~~O~~ne study, ~~showed that it~~there was a dose-effect relationship between occupational ~~manganese~~ Mn exposures and ~~the~~ a reduction ~~of~~ in pulmonary function.[10] Compared with the non-~~exposure~~ exposed workers, the pulmonary function in the ~~manganese~~ manganese-~~exposure~~ exposed workers ~~were~~ evaluated by the spirometry tests ~~and the~~

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values of them wereshowed a significant decrease in forced vital capacity (FVC), forced expiratory volume at one second (FEV1), and the ratio of forced expiratory volume at one second (FEV$_1$, FVC and FEV$_1$%) values.[11] Increased manganese Mn levels in blood serum ascribed to that liver is a the mainly organ to store, biotransformation and detoxify the poisonous substanceMn.[12] Over–exposure to manganese Mn can cause liver toxicity as well asand exacerbate liver dysfunction.[13 14] Chronic manganese Mn exposures leads to a series of significant cardiovascular toxicities including the an abnormal electrocardiogram (ECG) and the inhibition of myocardial contraction which can alters the blood pressure (BP).[15] AdditionallyBesides, the manganicMn cytotoxicity could has been shown to induce cell apoptosis and the DNA damage of birdin avian immune cells.[16] Low Mn^{2+} levels can induce oxidative DNA damage via an apoptotic pathway, so that thebut this DNA damage could can be reduced using antioxidants. A research conducted a risk assessment of inhaled manganese Mn through incorporating genetics and genomics to identifyied genetically based biomarkers of exposure, disease and susceptibility.[17]

From the aboveThus, manganic Mn toxicity in humans played plays a significant role in several systems. Currently, most studies were have explored separately the effect of Mnmanganese exposure for on different systems in of the human body. To further explore further the effects and the interaction of manganese Mn exposure in various systems, we will establish a prospective cohort study which includes the situation of individual manganese Mn exposure and regular occupational examinations. Simultaneously, we will determinet the biological exposure indicators by means of hair, urine and the blood samples. Blood and urine can reflect the extents

of ~~manganese~~ Mn exposure ~~for~~ in ~~a~~ the short term so ~~that~~ they can be used as ~~the~~ biomarkers of ~~manganese~~ Mn exposure. Previous research has shown that hair ~~also~~ can also act as a biomarker of Mn~~manganese~~ exposure because ~~that~~ it may reflect the levels of Mn~~manganese~~ exposure ~~for~~ over longer timeframes.[18] Moreover, ~~a~~ suitable Mn- biomonitoring including Mn-citrate can be used to determine the early onset of excess Mn concentrations in the human ~~bodies~~ body and ~~therefore it~~ can prevent the early onset of manganism or Mn-induced Parkinsonism as far as possible.[19] Accordingly, the risk of ~~manganese~~ Mn exposure ~~in~~ using sensitive effective biomarkers and the associated health effects ~~of health injure~~ are also the main emphasis of this study.

METHODS ~~AND ANALYSIS~~

Establishing a cohort

To explore the early healthy effects, ~~the~~ potential biomarkers of exposure, susceptibility and disease, as well as the related disease of occupational Mn~~manganese~~ exposure, we established the GXMEWHC. The prospective cohort study started in 2011 and the targeted population was ~~consists of~~ the workers aged 18 years or older working in the Ferro-manganese ~~refinery~~ Refinery~~Factory~~. ~~It is a long-term prospective cohort study of manganese exposure workers.~~ The study investigates a variety of lifestyle, socio-economic status, environmental and occupational factors as well as genetic factors in relation to the early health ~~injure~~ effects ~~for~~ of ~~manganese~~ Mn exposure. This is an opportunity to explore the

relationshhips between various ~~kinds of~~ risk factors and the early health ~~injure~~ effects of ~~manganese~~ Mn exposure, particularly ~~the~~ genetic and environmental factors and their interactions.

Sample source

The entire sample~~s~~ ~~in~~ of this study ~~were~~ was collected from a Ferro-manganese ~~refinery~~ Refinery~~Factory~~. The workers who participated in physical examinations every year and ~~accorded with~~met the following conditions were recruited. The study was approved by the local Ethics Committee. Inclusion criteria ~~included~~ were ~~the~~ age of 18-60 years, living in the local area, working in ~~this~~ the ~~company~~ factory for a long time (at least one year) and being able to complete ~~long~~ long-term follow-up, the ~~inexistence~~ lack of obvious diseases ~~for~~ in each system, absence if~~outing of touch with~~ other risk factors except ~~manganese~~ Mn exposure (such as Cu, Pb, Cr~~,~~ ~~—~~ or Hg~~, et al~~) and voluntary ~~participating~~ participation after providing informed consent. People were excluded if they had ~~Exclusion criteria contained the presence of~~an obvious diseases ~~for~~ in any system (such as a serious neurological disease, hepatic disease, kidney disease ~~and~~ or cardiovascular disease) ~~in the beginning of work~~, ~~the~~ contact ~~of various~~with other occupational risk factors (such as Cu, Pb, Cr, or Hg~~, et al~~) and ~~unable~~ inability to provide informed consent (psychiatric disease, language barrier, or mental deficiency). All participants were divided into different ~~exposed~~ exposure groups according to the type of work.

Follow-up

We will follow up the participants every three years. The information collected will be the same as the baseline data and will be collected ~~the data of~~ questionnaire interviews, physical examinations, biological specimens and environmental monitoring ~~repeatedly~~. **Fig. 1** shows the complete study plan of the GXMEWHC. The retrospective survey are establishing and perfecting the GXMEWHC by collecting baseline data on demographic information, lifestyle, biological specimens as well as history of environmental and occupational exposure. The short-term objectives are to ~~researching~~ explore the early health~~y~~ effects of occupational Mn~~manganese~~ exposure interact with environmental influences. In addition, preliminary exploring the effects of Mn~~manganese~~ exposure on genetic ~~fields~~ are also one of our studies. ~~In the future,~~ ~~o~~Our long-term and final objectives are to ~~exploring~~ explore the early health~~y~~ ~~injure~~ effects on various systems ~~in~~ of the human body by gene-environmental interactions for long-term and continuous low levels of ~~manganese~~ Mn exposure.

Building database

Questionnaire

~~The t~~Trained interviewers used a structured~~specifically designed~~ questionnaire to collect the baseline data after obtaining written informed consent. In order to obtain real and accurate information, we ~~take~~ conducted face-to-face interviews during the physical examination. ~~The s~~Self-reported diseases ~~are~~ were tested and verified through the diagnosis of specialists, ~~which is~~ based on recognized international standards. The questionnaire~~s~~ consisted of demographic information, socio-economic

status, smoking history ~~and~~, alcohol consumption and occupational history.

Occupational health examination

The occupational health examination was ~~implemented~~ conducted at the same time. All participants took part in the general health examination and were checked by trained physicians, nurses, and ~~the~~ medical technicians.

The physical measurements covered height, weight, ~~blood pressure (~~BP~~)~~ , and pulmonary function. The pulmonary function was estimated using a spirometry test which ~~comprised the test value of forced vital capacity~~included ~~(~~FVC~~)~~ , ~~forced expiratory volume at one second (~~FEV_1~~)~~, ~~the ratio of forced expiratory volume at one second (~~FEV_1%~~)~~, maximal ~~medexpiratory~~ mid-expiratory flow ~~curve~~ (MMEF), the peak expiratory flow ratio (PEFR), maximal voluntary ventilation (MVV), the predicted value of them and the ratio percentage of all above.

The clinical examinations included a high kilovar chest radiograph (HKV), ~~Neurology~~ neurology inspection, ECG, ~~Uncorrected~~ uncorrected visual acuity (UCVA) test, pure tone audiometry and physical examination of the heart, lungs, liver, spleen and abdomen.

The laboratory tests included ~~blood~~ routine blood tests, ~~urine~~ routine urine tests and liver function tests. The ~~blood~~ routine blood tests were measured in the laboratory ~~covering~~ and included the white blood cell count (WBC), lymphocyte ratio (LYR), neutrophil~~e~~ granulocyte ratio (GRANR), middle cell ratio (MIDR), lymphocyte count (LYC), neutrophil~~e~~ granulocyte count (NGC), middle cell count (MIDC), red blood cell count (RBC), hemoglobin (Hb), platelet count (PLT), ~~hematokrit~~ hematocrit

(HCT), mean corpuscular volume (MCV), ~~–~~mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), thrombocytocrit (THR), erythrocyte hemoglobin distribution width (RDW), platelet volume distribution width (PDW), and mean platelet volume (MPV). The ~~urine~~ routines urine tests ~~were detected by~~included urobilinogen (URO), bilirubin~~,(~~ (BIL), ~~ketobody~~ketobodies (KET), blood (BLD), protein (PRO), nitrite (NIT), white blood cells (WBC), glucose (GLU), specific gravity (SG), ~~power of hydrogen(P~~pH~~)~~ and vitamin C. Furthermore, we examined the content of Mn~~manganese~~ in urine. The liver function tests contained total bilirubin (T-BIL), direct bilirubin (D-BIL), indirect bilirubin (I-BIL), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and the AST/ALT ratio.

Neurological function test

The neurological function test consisted~~s~~ of a neurocognitive function test, neurobehavioral function test~~s~~, and neuropsychological test. The Montreal Cognitive Assessment (MoCA) is a neurocognitive function test and ~~it~~ is an assessment method which rapidly screens for ~~the~~ Mild Cognitive Impairment (MCI) with high sensitivity and specificity.[20-22] We assessed the influence of ~~manganese~~ 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 an acceptable and valid assessment means for non-motor symptoms in Parkinson~~'~~s disease (PD).[24 25] SCOPA-AUT is a self-administered scale and can be used for the screening of autonomic symptoms in PD.[26 27] We used NMSS and SCOPA-AUT to

evaluate the neurobehavioral function of workers who were exposed to occupational Mnmanganese. The Profile of Mood States (POMS) is a neuropsychological test and is a scale to assess the emotions of manganese manganese-exposed workers.[28 29] We used POMS as an assessment instrument for assessing neuropsychological of workers.

Database of biological specimens

The biological specimens were composed blood samples, urine specimen and the hair samples. Three vacuum tubes (two ethylene diamine tetraacetic teraacetic (EDTA) anticoagulant tubes and a coagulation tube) were filled with five 5 milliliters mL of fasting blood respectively obtained through intravenous access. The blood sample in the coagulation tube was used to detect assess the liver function and one of the EDTA anticoagulant tubes was measured used for the blood routine blood tests. The blood sample in the another EDTA anticoagulant tube was separated into blood plasma and blood corpuscle cell from which was DNA extracted the DNA from it as soon as possible. All the blood specimens were stored in at -80ºC refrigerators. In addition, a minimum of 10 milliliter mL of the urine were collected in the a urine bottles by the participants and then as the urine specimen stored in at 4ºC refrigerators. A tuft of hair close to the scalp in the occipital region, about 2 cm in length and of approximately 0.5 cm in diameter was cut off with a stainless steel scissors and collected in the a special sacks bagwhich close to the scalp in the occipital region about 2 cm.[30] All the hair specimens were stored in a cool and dry area.

Determining Mn~~manganese~~ exposure in the workplace

We will track the levels of ~~manganese~~ manganese ~~exposure~~ exposed workers who participated in the present cohort study by means of ~~the~~ workplace monitoring. We will record the basic information of the factory, the technological processes of production, ~~and~~ the distributions of occupational risk factors, the work mode, and the ~~situation~~ level of ~~manganese~~ Mn in this ~~company~~factory. The concentrations of ~~manganese~~ Mn dust and fumes in the workplace ~~were~~ are detected through ~~the~~ an air point sampler. At the same time, we will monitor the individual levels of ~~manganese~~ ~~by~~Mn using ~~the~~ individual samplers ~~in their~~during working ~~time~~hours. The ~~Permissible~~ permissible concentration-time-weighted average (PC-TWA) is the average permissible exposure levels ~~on~~ during the regulation ~~eight~~ eight-hour~~s~~ working day, weighted~~ing~~ by time. The ~~Permissible~~ permissible concentration-~~Short~~ short ~~Term~~ term ~~Exposure~~ exposure ~~Limit~~ limit (PC-STEL) is the permissible exposure levels ~~on~~ in no more than 15 minutes at any time, ~~weighting~~ weighted by time within a working day. The cumulative exposure index (CEI) is calculated through TWA, STEL, and ~~the~~ workplace seniority in working. The CEI as an external exposure index of ma Mn nganese and was calculated for each job, combining the airborne monitoring with the individual monitoring both ~~at~~ during work~~ing~~ time and break time.

Database of biomarkers

The database of biomarkers included biomarkers of exposure, effect and susceptibility.

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The biomarkers of exposure will be detected through atomic absorption spectrometry (AAS) which are the levels of Mn and Fe in plasma, urine and hair. The levels of plasma brain-derived neurotrophic factor (BDNF), dopamine (DA) were determined by Sandwich ELISA kits which are biomarkers of effect. The biomarkers of susceptibility are also assessed by GWAS which are shown in the following Genetic assessments part in detail.

Genetic ~~determination~~assessments

A sub-cohort of GWAS is implemented in this study ~~which researched~~to assess the effect of ~~manganese~~ Mn exposure on genetic ~~sides~~. The GWAS of ~~Quantative~~ quantitative ~~Trait~~ trait ~~Loci~~ loci (QTL) and ~~Binary~~ binary ~~Trait~~ trait ~~Loci~~ loci (BTL) will also ~~are~~ be ~~also~~ performed for ~~the~~ exposed workers using the Infinium HumanExome BeadChip from Illumina Company (Illumina Infinium HumanExome v1.0 BeadChips, 12-sample HD). ~~The~~ Illumina's HumanExome BeadChips are covered with ~~emphatically~~ human exonic regions ~~and~~, the exonic content contains more than 240,000 variant markers. The markers represent~~ed~~ a variety of common diseases. ~~and t~~The different groups ~~which contained the~~include individuals ~~of~~ from China, Europe, and Africa ~~and Spain~~. We will focus on the potential interactions of environmental ~~manganese~~ Mn exposure and genetics ~~which~~ based on the significant effects of Mn on the targeted phenotypes. Furthermore, ~~the~~ potential gene-environment interactions ~~is~~ will be explored through the genomes of ~~the~~ ~~M~~manganism patients and healthy individuals ~~who~~ exposed to ~~manganese~~ Mn in the

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workplace.

Statistical analyses

After collecting the complete questionnaires, the ~~data of~~ physical examination results and neurological function test data, ~~the~~ trained investigators will enter ~~all~~ the above data into the computer twice using ~~the~~ EpiData software. The GXMEWHC study database is established and ~~it is~~ will be gradually improved in ~~later~~ subsequent follow-up. Simultaneously, the experimental data ~~is~~ will be contained ~~by~~ in the database. All the data ~~is~~ will be analyzed by ~~the~~ SPSS 16.0 software. The ~~data of~~ genetic determination data ~~is~~ will be obtained and analyzed through ~~the~~ Illumina's GenomeStudio, which is an integrated software platform for data visualization and analysis. The GenomeStudio Genotyping Module is an application for extracting genotyping data from the Illumina iScan systems. We will use the Efficient and Parallelizable Association Container Toolbox (EPACTS), which can perform various statistical tests for identifying genome-wide associations. The ~~Quantative~~ quantitative ~~Trait~~ traits ~~are~~ will be calculated by the efficient mixed-model association eXpedited (EMMAX) program, which can correct for sample structure within human GWAS~~s~~ by taking an expedited mixed linear model approach.[31] The ~~Binary~~ binary ~~Trait~~ traits ~~are~~ will be calculated through the Logistic Score Test (LST) which can test ~~with~~ rare variants and relate the enriched genetic information to disease phenotypes through Logistic regression models.[32] When the ~~Gene~~gene-wise or ~~Group~~group-wise ~~Tests~~ tests are conducted, ~~the~~ optimal sequence kernel association tests (SKAT-O) ~~are~~ will

be used[33]

~~Preliminary result~~PRELIMINARY RESULTS

Demographic description of the cohort

~~The~~ In total, 1,991 individuals were recruited to participate in the study from the ~~Ferro~~Ferro-manganese ~~refinery~~ Refinery~~Factory~~. After completing the questionnaire, a total of 1,888 participants who met the standards were entered into the GXMEWHC study,~~who accord with standard and the~~with an effective rate ~~is~~ of 94.8%.

Table 1 summarizes the baseline characteristics of the cohort. ~~In~~ Of the cohort, 63.4% were male and 36.6% were female. The mean age was 40.31 years and the percent of the ~~four stages~~age distribution was ~~were~~ similar. ~~Thereinto,~~ 34.5%, 31.0%, and 34.5% of the participants had ~~the~~ seniority of <10, 10-20, and >20 years, respectively. In the factory, 31.2%, 15.7%, 20.1% and 33.0% were smelters, raw material processors, high ~~exposed~~ exposure auxiliary, and low ~~exposed~~ exposure auxiliary, respectively. The mean seniority was 15.34 years. The mean ~~Body~~ body ~~Mass~~ mass ~~Index~~ index (BMI) was normal (22.47kg·m^{-2}). Among the participants, 48.5% ~~was~~ were Han Chinese. A majority of the participants (83.7%) were married. ~~In the midst of the~~Of these participants, 43.9% graduated from middle school, 45.0% ~~had~~ finished high school and 11.1% ~~achieved~~ completed college or higher education. In the cohort, 38.6% ~~was~~ were current smokers, 7.0% ~~was~~ were former smokers and 54.4% ~~was~~ were never smokers. Current passive smoking rates were 87.3%. The proportion of current drinkers was 48.1%, ~~the~~ former drinkers was 15.9% and ~~the~~ never drinkers was 36.0%. ~~Detailed information of the demographic characteristics of~~

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~~this cohort is provided in **Table 1**.~~ Among the ~~participators~~participants, 31.5% ~~was~~ were smelters, 16.9% ~~was~~ were human crushing workers and 6.8% ~~was~~ were welder. The other types of work, the proportion of them, the mean age, and seniority are shown in **Table 2**.

Determining Mn~~manganese~~ exposure in the workplace

All the ~~participators~~ participants were divided according to different ~~the~~ types of work in the factory. Then, the extent~~s~~ of the ~~manganese~~ Mn exposure ~~were~~ was confirmed using the working positions combined with the results of workplace detection. The CEI ~~is~~ was calculated through TWA or STEL. Finally, all workers were classified into four ~~exposed~~ exposure groups on the basis of the Mn-CEI results, including~~which are respectively~~ the internal control group (Mn-CEI＜1.0 mg/m^3·year), the low ~~exposed~~ exposure group (1.0 mg/m^3·year≤Mn-CEI＜2.0 mg/m^3·year), the medium ~~exposed~~ exposure group (2.0 mg/m^3·year≤Mn-CEI＜5.0 mg/m^3·year) and the high ~~exposed~~ exposure group (Mn-CEI≥5.0 mg/m^3·year). The percentages of the internal control group, low ~~exposed~~ exposure group, medium exposure~~exposed~~ group and high exposure~~exposed~~ group were 34.5%, 17.6%, 37.6% and 10.3%, respectively. The median of total Mn-CEI was 1.85 mg/m^3·year and the range was 0.01 mg/m^3·year~~~~-9.77 mg/m^3·year. The details of Mn-CEI are shown in **Table 3**.

Main results of the occupational health examination of the cohort~~–~~

The mean systolic blood pressure (SBP) and diastolic blood pressure (DBP) ~~was~~ were 125.43 and 78.81 mmHg, respectively. The median ~~of~~ urie~~n~~e Mn level was 2.63μg/L,

the male was 3.67μg/L in males, and the female was 2.84μg/L in females. The values results of the Blood Rroutine blood tests, Hepatic hepatic Function function tests and Pulmonary pulmonary Function function tests are shown in the **Table 4**.

Assessment of biomarkers

The liver function was analyzed between different manganese-exposed groups of the cohort in 2013. Our conclusions in this study were that occupational Mn exposure can cause a dose-dependent increase of liver enzyme levels and interact with alcohol drinking to potentially aggravate the liver damage.[14] The plasma BDNF levels and cognitive function of different manganese-exposed groups were also measured. Our results showed that occupational Mn exposure may be related to decreased plasma BDNF levels and cognition impairment.[23]

Detection Assessment of biological specimens and GWAS of in the cohort

We greatly pay paid attention to the potential gene-environment interactions. Therefore, we performed the GWAS of QTL and BTL using the Illumina Infinium HumanExome BeadChip for 500 exposed workers, such asincluding urie urine Mn and various kinds of indexindices of pulmonary function, liver function and blood routinedetection. The Illumina''s GenomeStudio Genotyping Module was used for genotyping and data analysis, which isusing an integrated platform for data visualization and analysis. About twenty five thousand25,000 locus was were involved in the analysis after Quality quality Control control (QC). And then tThe QTL, BTL and, Genegene-wise or and Groupgroup-wise Tests tests were conducted

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by EMMAX, LST, and SKAT-O, respectively. We will further analyze ~~the~~ differential gene expression ~~further~~. The results of GWAS and other ~~indexes~~ indices will be reported in ~~separate~~ ongoing articles. We plan to conduct GWAS in a larger number of ~~manganese~~ manganese-~~exposure~~ exposed workers ~~for~~ to ~~exploring~~ explore the genic risk factors and ~~the~~ gene-environment interactions.

ETHICS AND DISSEMINATION

The study ~~has been~~was approved via the ~~Medical~~ medical ethics committee of Guangxi Medical University. All the 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 the biological samples are marked in ~~a~~ sequential order and stored in secure freezers. The results will be disseminated to relevant scientific forums which include~~d~~ publishing in peer-reviewed journals and ~~presenting~~ presentation at international conferences.

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Contributors Xiaobo Yang and Yunfeng Zou contributed in conception and design; Jing Liu,

Kangcheng Chen, Yingnan Lv, Damin Huang, Yuefei Shen, Yaoqiu Zhong, Zhihao Liu, Bei Jiang, Qin Li, Li Qing,Wei Zhang, Lang Chen, Fenfen Wang, Bing Xia and Li Yang contributed in acquisition of the data; Yingnan Lv analysed the data and drafted the manuscript; all authors contributed to review and revision of the manuscript and approved the final version.

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Competing interests All authors declare that they have no conflict of interest.

Ethics approval Medical ethics committee of Guangxi Medical University.

~~Patient~~ Participant consent Obtained.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement No additional data are available.

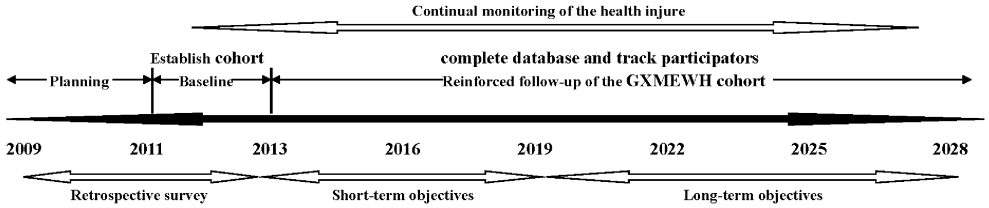

Fig. 1 The complete study plan of the GXMEWHC

The preliminary baseline survey was completed ~~preliminary~~ in 2013 and collected ~~the~~ epidemiological information, biological samples, ~~the~~ data ~~of~~ from the occupational health examination and workplace monitoring. Simultaneously, the ~~database of~~ GWAS database was established~~performed~~ for 500 ~~manganese~~ manganese-exposed workers. The participants will be ~~following~~ followed up every three years and the databases ~~were~~ will be amended~~reinforced gradually~~ in the future.

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Table 1

Demographic characteristics of the GXMEWHC

Variables	Number (n=1888)	Percent (%)
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	80972	4.251.5
Marital status		
Single	233	12.3
Married	1580	83.7
Windowed or divorced	75	4
Education or lower		
Middle school	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

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Table 2

Different types of work of the GXMEWHC

Types of work	Number (n)	Percent (%)	Age (years)	Seniority (years)
			Mean± SD	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	6.2	39.01 ± 9.96	15.09 ± 12.07
Total	1888	100	40.31 ± 7.85	15.23 ± 9.60

Table 3

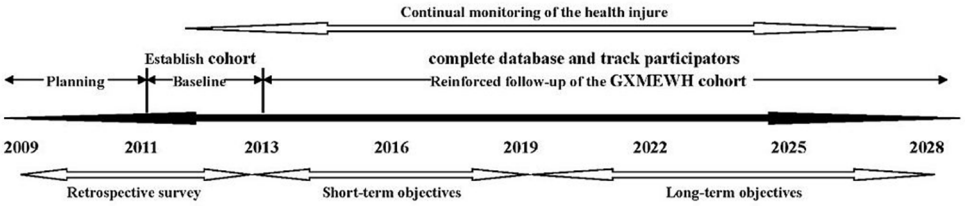
The Mn-CEI of the GXMEWHC

Mn-CEI (mg/m^3·year)	Number (n)	Percent (%)	Median (Interquartile Range)	Range
Internal cControl gGroup (Mn-CEI <1.0)	651	34.5	0.51 (0.55)	0.01～0.99
Low cExposureed gGroup (1.0≤Mn-CEI<2.0)	333	17.6	1.49 (0.46)	1.01～1.99
Medium cExposureed gGroup (2.0≤Mn-CEI<5.0)	710	37.6	3.04 (1.20)	2.00～4.98
High cExposureed gGroup (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

Table 4

The results of occupational health examination of the GXMEWHC

Variables	Male(n=1197)	Female(n=691)	Total(n=1888)
	Mean ± SD	Mean ± SD	Mean ± SD
Systolic blood pressure, mmHg	127.68 ± 12.11	121.54 ± 11.53	125.43 ±1 2.26
Diastolic blood pressure, mmHg	79.93 ± 8.29	76.86 ± 7.93	78.81 ± 8.29
Blood routine			
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
Hepatic function			
Total bilirubin, μmol/lL	12.48 ± 5.3	11.94 ± 4.49	12.28 ± 5.02
Direct bilirubin, μmol/Ll	3.98 ± 2.16	3.66 ± 2.19	3.86 ± 2.17
Indirect bilirubin, μmol/Ll	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
Pulmonary function			
Test value of FVC, L	4.25 ± 0.86	3.18 ± 0.64	3.86 ± 0.94
Test value of FEV1, L	3.61 ± 0.72	2.71 ± 0.54	3.28 ± 0.79
Uric Mn, μg/L			
Median (iInterquartile rRange)	2.63 (2.37)	3.67 (4.12)	2.84 (2.79)



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STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5, 6
Objectives	3	State specific objectives, including any prespecified hypotheses	6
Methods			
Study design	4	Present key elements of study design early in the paper	7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7,8
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	7,8
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	Non
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	8-13
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*7*
Bias	9	Describe any efforts to address potential sources of bias	4
Study size	10	Explain how the study size was arrived at	Non
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8-13
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	13, 14
		(*b*) Describe any methods used to examine subgroups and interactions	Non
		(*c*) Explain how missing data were addressed	Non
		(*d*) If applicable, explain how loss to follow-up was addressed	Non
		(*e*) Describe any sensitivity analyses	Non
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	14
		(b) Give reasons for non-participation at each stage	Non
		(c) Consider use of a flow diagram	Non
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	14-16
		(b) Indicate number of participants with missing data for each variable of interest	Non
		(c) Summarise follow-up time (eg, average and total amount)	Non
Outcome data	15*	Report numbers of outcome events or summary measures over time	14-17
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Non
		(*b*) Report category boundaries when continuous variables were categorized	Non
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Non
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Non
Discussion			
Key results	18	Summarise key results with reference to study objectives	Non
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	4
Generalisability	21	Discuss the generalisability (external validity) of the study results	Non
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	18

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.