

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

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24 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.

40 Ethics and dissemination: The study has been approved by Medical ethics 41 committee of Guangxi Medical University. The results will be published by 42 peer-reviewed publications and presented at international conferences.

45 Keywords

46 Manganese; Occupational exposure; Manganese toxicity; Genetic Susceptibility

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2	47	Strengths and limitations of this study
2	48	■ In this study, we can collect an abundant database because of large samples in the
2	49	heavy metals cohort.
4	50	■ The Ferro-manganese refinery Factory is the largest metallurgical factory of
4	51	manganese processing in China so that it can provide an extremely rich dataset of
4	52	analysis.
4	53	• The GXMEWHC is the longitudinal study which can continuous follow up and
4	54	repeated investigate the participants. We can explore the relations between
4	55	occupational manganese exposure and the early health injure.
4	56	• The GWAS are implemented for seeking the susceptibility genes of chronic
4	57	low-level manganese exposure, and exploring the interactions between genetic
4	58	factors and environmental factors. Those provide an important opportunity to
4	59	identify the more susceptible individuals so that prevent the early health injure of
e	50	workers.
6	51	• Potential limitations are that loss of follow up may be a weakness with our study.
6	52	There are some temporary workers in the factory and they may leave the factory
(53	after a period of time working in factory. We can reduce the probability of the
(54	loss of follow up through strict controlled the inclusion criteria when established
(55	the cohort.
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70 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 speed⁵ 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.⁸⁹ 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

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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 Mn²⁺ 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.

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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,

iovascular disease) in the be
factors (such as Cu, Pb, C
sychiatric disease, languag
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every three years and col
examination, biological
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ng the early healthy effec
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posure on genetic field are
nd final objectives are ex
nan body by gene-environm
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hepatic disease, kidney disease and cardi eginning of work, the contact of various occupational risk Cr, Hg, et al) and unable to provide informed consent (pa ge barrier, mental deficiency). All participants were divide oups according to the type of work.

Follow-up

We will follow up the participants e lect the data of questionnaire interview, physical e specimens and environmental monitoring repeatedly. F study plan of the GXMEWHC. The retrospective surv perfecting the GXMEWHC by collecting baseline da mation, lifestyle, biological specimens as well as history pational exposure. t of occupational The short-term objectives are researching manganese exposure interact with enviro lition, preliminary exploring the effects of manganese exp also one of our studies. In the future, our long-term as ploring the early healthy injure on various systems in hum nental interactions for long-term and continuous low levels of

- **Building database**
- Questionnaire
- The trained interviewers used a specifi ire to collect the

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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 (FEV₁), the ratio of forced expiratory volume at one second (FEV₁%), 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

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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),
contained total bilirubin (T-BIL), direct bilirubin (D-BIL), indirect bilirubin (I-BIL), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and AST/ALT.
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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.

216 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 $-80\Box$ 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 $4\square$ 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 All the hair specimens were stored in a cool and dry area. cm. 30

Page 11 of 23

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231	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.

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248 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

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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

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275	are calculated by the efficient mixed-model association eXpedited (EMMAX)
276	program which can correct for sample structure within human GWASs by taking an
277	expedited mixed linear model approach ³¹ The Binary Trait are calculated through the
278	Logistic Score Test (LST) which can test with rare variants and relate the enriched
279	genetic information to disease phenotypes through Logistic regression models. ³²
280	When the Gene-wise or Group-wise Tests are conducted, the optimal sequence kernel
281	association tests (SKAT-O) are used ³³
282	
283	Preliminary result
284	Demographic description of the cohort
285	The 1991 individuals were recruited from the Ferro-manganese refinery Factory. After
286	completing the questionnaire, a total of 1888 participants entered into the
287	GXMEWHC who accord with standard and the effective rate is 94.8%.
288	In the cohort, 63.4% were male and 36.6% were female. The mean age was
289	40.31 years and the percent of the four-stages were similar. Thereinto, 34.5%, 31.0%,
290	and 34.5% of the participants had the seniority of <10, 10-20, and >20 years,
291	respectively. In the factory, 31.2%, 15.7%, 20.1% and 33.0% were smelter, raw
292	material processor, high exposed auxiliary and low exposed auxiliary, respectively.
293	The mean seniority was 15.34 years. The mean Body Mass Index (BMI) was normal
294	(22.47kg·m ⁻²). Among the participants, 48.5% was Han Chinese. A majority of the
295	participants (83.7%) were married. In the midst of the participants, 43.9% graduated
296	from middle school, 45.0% had finished high school and 11.1% achieved college or
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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**.

305 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/m³·year), the low exposed group ($1.0 \le Mn$ -CEI < 2.0 mg/m³·year), the medium exposed group $(2.0 \le \text{Mn-CEI} \le 5.0 \text{ mg/m}^3 \cdot \text{year})$ and the high exposed group (Mn-CEI \geq 5.0 mg/m³·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/m³·year and the range was 0.01 mg/m³·year~9.77 mg/m³·year. The details of Mn-CEI are show in **Table 3**.

319 Main results of occupational health examination of the cohort

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

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321	125.43 and 78.81 mmHg, respectively. The median of uric Mn was 2.63 μ g/L, the
322	male was 3.67 μ g/L, and the female was 2.84 μ g/L. The values of the Blood Routine,
323	Hepatic Function and Pulmonary Function are show in the Table 4.
324	
325	Detection of biological specimens and GWAS of the cohort
326	We pay attention to the potential gene-environment interaction. Therefore, we
327	performed the GWAS of QTL and BTL using the Illumina Infinium HumanExome
328	BeadChip for 500 exposed workers, such as uric Mn and various kinds of index of
329	pulmonary function, liver function and blood routine. The Illumina's GenomeStudio
330	Genotyping Module was used for genotyping and data analysis which is an integrated
331	platform for data visualization and analysis. About twenty -five thousand locus was
332	involved in the analysis after Quality Control (QC). And then the QTL, BTL and
333	Gene-wise or Group-wise Tests were conducted by EMMAX, LST and SKAT-O,
334	respectively. We will analyze the differential gene expression further. The results of
335	GWAS and other indexes will be reported in separate articles. We plan to conduct
336	GWAS in a larger number of manganese exposure workers for exploring the genic
337	risk factors and the gene-environment interaction.
338	
339	
340	ETHICS AND DISSEMINATION
341	The study has been approved via the Medical ethics committee of Guangxi Medical
342	University. All the original files and data are maintained and stored at the research

343 office, in the Department of Occupational Health and Environmental Health, School

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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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366 Patient consent Obtained.

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4 5	368	Data s	sharing statement No additional data are available.
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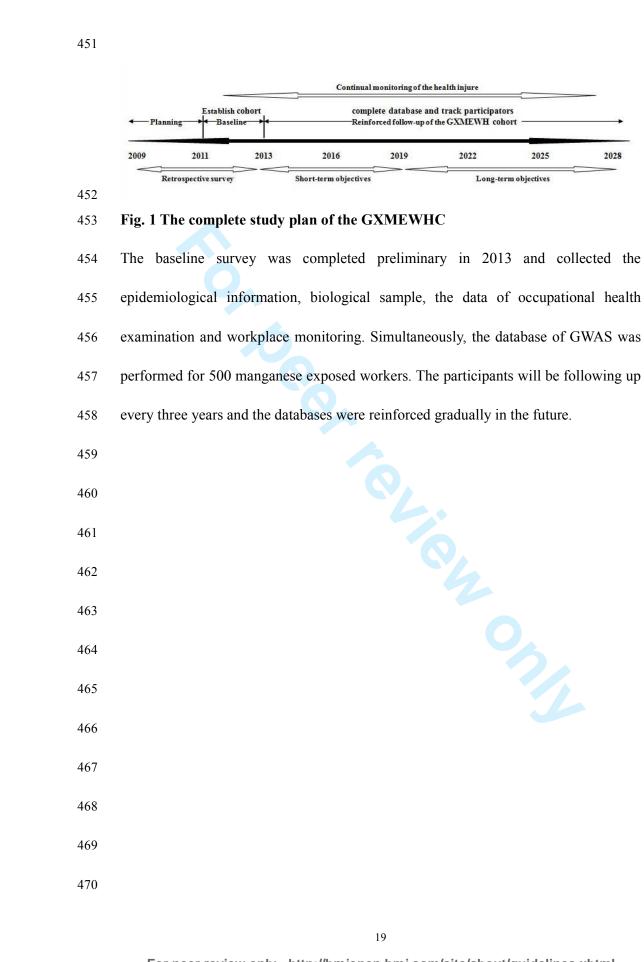
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Demographic characteristics of the GXMEWHC

Variables	Number (n=1888)	Percent (%)
Sex		
Male	1197	63.4
Female	691	36.6
Age, years (mean \pm 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 \pm 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	209	11.1
higher		
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

Different types of work of the GXMEWHC

Types of work	Number (n)	Percent (%)	Age (years)	Seniority (years
Types of work		Percent (%)	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

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Table 3

The Mn-CEI of the GXMEWHC

	Mn-CEI (mg/m ³ ·year)	Number (n)	Percent (%)	Median (Interquartile Range)	Range
	Internal Control Group	(51	24.5	0.51 (0.55)	0.01 0.00
	(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
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Table 4

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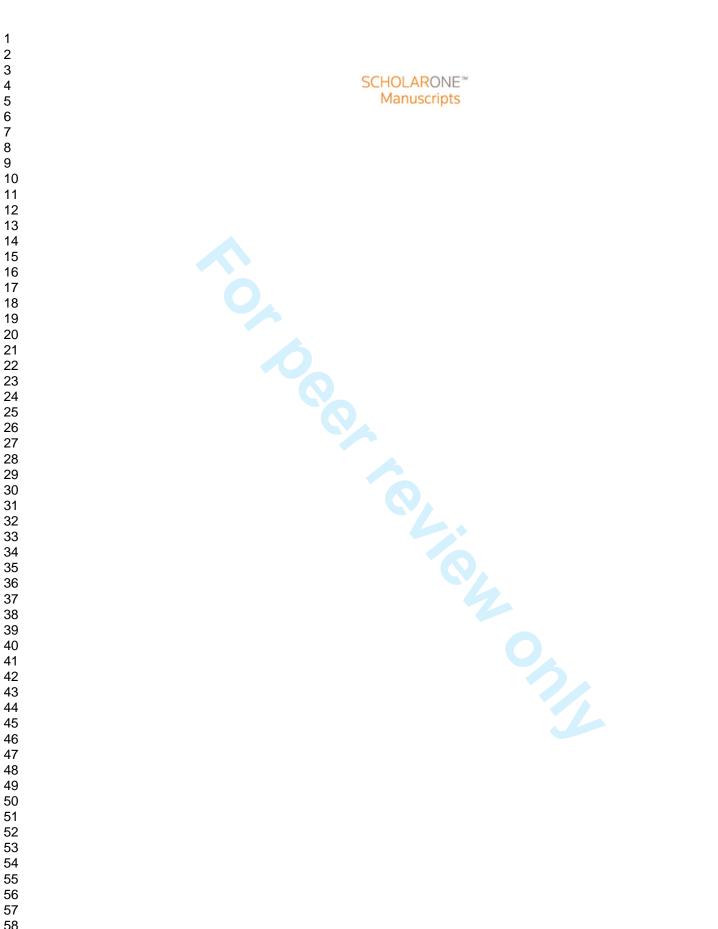
	Male(n=1197)	Female(n=691)	Total(n=
Variables	Mean ± SD	Mean ± SD	Mean
Systolic blood pressure, mmHg	127.68 ± 12.11	121.54 ± 11.53	125.43 ±
Diastolic blood pressure, mmHg	79.93 ± 8.29	76.86 ± 7.93	78.81 ±
Blood routine			
WBC, 10 ⁹ /L	6.91 ± 1.51	6.32 ± 1.52	6.69 ±
RBC, 10 ¹² /L	5.13 ± 0.52	4.61 ± 0.44	4.94 ±
Haemoglobin, g/L	148.69 ± 12.73	128.8 ± 14.37	141.38 ±
Platelet count, 10 ⁹ /L	241.76 ± 54.13	256.29 ± 62.86	247.1 ±
Hepatic function			
Total bilirubin, µmol/l	12.48 ± 5.3	11.94 ± 4.49	12.28 ±
Direct bilirubin, µmol/l	3.98 ± 2.16	3.66 ± 2.19	3.86±
Indirect bilirubin, µmol/l	8.5 ± 3.44	8.24 ± 2.51	8.4 ± 3
ALT, U/L	25.35 ± 17.62	17.23 ± 14.74	22.38 ±
AST, U/L	27.06 ± 15.7	23.32 ± 21.75	25.69 ±
Pulmonary function			
Test value of FVC, L	4.25 ± 0.86	3.18 ± 0.64	3.86±
Test value of FEV1, L	3.61 ± 0.72	2.71 ± 0.54	3.28±
Uric Mn, µg/L			
Median (Interquartile Range)	2.63 (2.37)	3.67 (4.12)	2.84 (2

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BMJ Open

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

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1	Rationale, design and baseline results of the Guangxi
2	manganese-exposed workers healthy cohort (GXMEWHC)
3	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/m³·year), low exposure group (1.0 mg/m³·year \leq Mn-CEI \leq 2.0 mg/m³·year), medium exposure group (2.0 mg/m³·year \leq Mn-CEI<5.0 mg/m³·year), and high exposure group (Mn-CEI \geq 5.0 mg/m³·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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70	Strengths and limitations of this study
71	■ In this study, we can collect an abundant database because of the large samples in
72	the heavy metals cohort.
73	■ The Ferromanganese Refinery is the largest metallurgical factory of Mn
74	processing in China; therefore, it can provide an extremely rich dataset for
75	analysis.
76	• The GXMEWHC is a longitudinal study that can continuously follow up and
77	repeatedly investigate the participants. We can explore the relationships between
78	occupational Mn exposure and the early health effects.
79	GWAS was implemented to determine the susceptibility genes related to chronic
80	low-level Mn exposure, and to explore the interactions between genetic factors
81	and environmental factors. These data will provide an important opportunity to
82	identify more susceptible individuals to prevent early health effect in workers.
83	• The potential limitations are that loss to follow-up may be a weakness with our
84	study. There are some temporary workers in the factory and they may leave the
85	factory after a period of time. We can reduce the probability of the loss to follow
86	up through strictly controlled inclusion criteria when establishing the cohort.
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93 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 speed⁵ 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.⁸⁹ 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 (FEV₁%) 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)

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116	and the inhibition of myocardial contraction which can alter the blood pressure
117	(BP). ¹⁵ Additionally, Mn cytotoxicity has been shown to induce cell apoptosis and
118	DNA damage in avian immune cells. ¹⁶ Low Mn ²⁺ levels can induce oxidative DNA
119	damage via an apoptotic pathway, but this DNA damage can be reduced using
120	antioxidants. A risk assessment of inhaled Mn incorporating genetics and genomics
121	identified genetically based biomarkers of exposure, disease and susceptibility. ¹⁷
122	Thus, Mn toxicity in humans plays a significant role in several systems.
123	Currently, most studies have explored separately the effect of Mn exposure on
124	different systems of the human body. To further explore the effects and the interaction
125	of Mn exposure in various systems, we will establish a prospective cohort study
126	which includes individual Mn exposure and regular occupational examinations.
127	Simultaneously, we will determine biological exposure indicators by means of hair,
128	urine and blood samples. Blood and urine can reflect the extent of Mn exposure in the
129	short term so they can be used as biomarkers of Mn exposure. Previous research has
130	shown that hair can also act as a biomarker of Mn exposure because it may reflect the
131	levels of Mn exposure over longer timeframes. ¹⁸ Moreover, suitable Mn
132	biomonitoring including Mn-citrate can be used to determine the early onset of excess
133	Mn concentrations in the human body and can prevent the early onset of manganism
134	or Mn-induced Parkinsonism as far as possible. ¹⁹ Accordingly, the risk of Mn
135	exposure using sensitive effective biomarkers and the associated health effects are
136	also the main emphasis of this study.
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METHODS

140 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.

151 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,

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162 Cr, or Hg) and inability to provide informed consent (psychiatric disease, language 163 barrier, or mental deficiency). All participants were divided into different exposure 164 groups according to the type of work.

166 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.

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Building database

Questionnaire

182 Trained interviewers used a structured questionnaire to collect the baseline data after 183 obtaining written informed consent. In order to obtain real and accurate information, 184 we conducted face-to-face interviews during the physical examination. Self-reported

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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.

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190 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 ,
FEV₁, FEV₁%, 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.

199 The clinical examinations included a high kilovar chest radiograph (HKV), 200 neurology inspection, ECG, uncorrected visual acuity (UCVA) test, pure tone 201 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

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208	(MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin
209	concentration (MCHC), thrombocytocrit (THR), erythrocyte hemoglobin distribution
210	width (RDW), platelet volume distribution width (PDW), and mean platelet volume
211	(MPV). The routines urine tests included urobilinogen (URO), bilirubin (BIL),
212	ketobodies (KET), blood (BLD), protein (PRO), nitrite (NIT), white blood cells
213	(WBC), glucose (GLU), specific gravity (SG), pH and vitamin C. Furthermore, we
214	examined the content of Mn in urine. The liver function tests contained total bilirubin
215	(T-BIL), direct bilirubin (D-BIL), indirect bilirubin (I-BIL), alanine aminotransferase
216	(ALT), aspartate aminotransferase (AST), and the AST/ALT ratio.
217	

218 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

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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.

234

235 Database of biological specimens

236 The biological specimens were composed blood samples, urine specimen and the hair 237 samples. Three vacuum tubes (two ethylene diamine tetraacetic teraacetic (EDTA) 238 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 239 240 used to assess liver function and one of the EDTA anticoagulant tubes was used for 241 routine blood tests. The blood sample in the other EDTA anticoagulant tube was 242 separated into blood plasma and blood cell from which was DNA extracted as soon as 243 possible. All the blood specimens were stored at -80°C. In addition, a minimum of 10 244 mL of the urine were collected in a urine bottle and stored at 4°C. A tuft of hair close to the scalp in the occipital region, about 2 cm in length and approximately 0.5 cm in 245 diameter was cut off with stainless steel scissors and collected in a special bag.³⁰ 246 All hair specimens were stored in a cool and dry area. 247

248

249

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

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

266 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. BMJ Open: first published as 10.1136/bmjopen-2014-005070 on 3 July 2014. Downloaded from http://bmjopen.bmj.com/ on June 12, 2025 at Agence Bibliographique de l Enseignement Superieur (ABES)

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276 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.

290 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

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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 used³³

310 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%. BMJ Open: first published as 10.1136/bmjopen-2014-005070 on 3 July 2014. Downloaded from http://bmjopen.bmj.com/ on June 12, 2025 at Agence Bibliographique de l Enseignement Superieur (ABES) .

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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

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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.

333 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/m³·year), the low exposure group (1.0 mg/m³·year \leq Mn-CEI \leq 2.0 mg/m³·year), the medium exposure group (2.0 mg/m³·year ≤ Mn-CEI < 5.0 mg/m³·year) and the high exposure group (Mn-CEI \geq 5.0 mg/m³ 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

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344	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
345	Mn-CEI are shown in Table 3 .
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347	Main results of the occupational health examination of the cohort
348	The mean systolic blood pressure (SBP) and diastolic blood pressure (DBP) were
349	125.43 and 78.81 mmHg, respectively. The median urine Mn level was 2.63µg/L,
350	$3.67\mu g/L$ in males, and $2.84\mu g/L$ in females. The results of the routine blood tests,
351	hepatic function tests and pulmonary function tests are shown in the Table 4 .
352	
353	Assessment of biomarkers
354	The liver function was analyzed between different manganese-exposed groups of the
355	cohort in 2013. Our conclusions in this study were that occupational Mn exposure can
356	cause a dose-dependent increase of liver enzyme levels and interact with alcohol
357	drinking to potentially aggravate the liver damage. ¹⁴ The plasma BDNF levels and
358	cognitive function of different manganese-exposed groups were also measured. Our
359	results showed that occupational Mn exposure may be related to decreased plasma
360	BDNF levels and cognition impairment. ²³
361	
362	Assessment of GWAS in the cohort
363	We greatly paid attention to potential gene-environment interactions. Therefore, we
364	performed GWAS of QTL and BTL using the Illumina Infinium HumanExome
365	BeadChip for 500 exposed workers, including urine Mn and various indices of
366	pulmonary function, liver function and blood detection. Illumina's GenomeStudio

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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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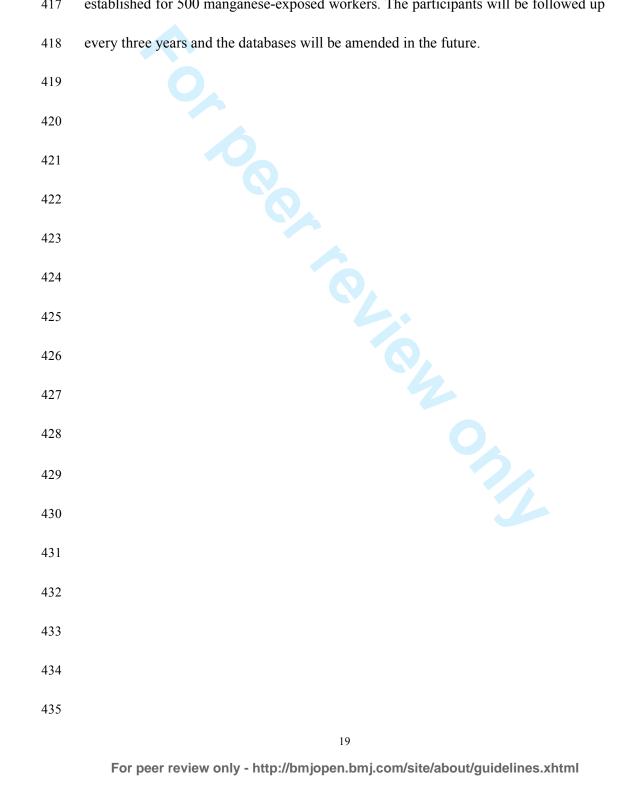
390 Acknowledgements We thank all participants who volunteered to take part in this study, all 391 members of the GXMEWHC research team, the nurses and administrators in the Ferro-manganese 392 refinery Factory. 393 Contributors Xiaobo Yang and Yunfeng Zou contributed in conception and design; Jing Liu, 394 Kangcheng Chen, Yingnan Lv, Damin Huang, Yuefei Shen, Yaoqiu Zhong, Zhihao Liu, Bei Jiang, 395 Qin Li, Li Qing, Wei Zhang, Lang Chen, Fenfen Wang, Bing Xia and Li Yang contributed in 396 acquisition of the data; Yingnan Lv analysed the data and drafted the manuscript; all authors 397 contributed to review and revision of the manuscript and approved the final version. 398 **Funding** This study was supported by National Natural Science Foundation of China (81060234, 399 21167004, and 81160339); Guangxi Science Fund for Distinguished Young Scholars 400 (2012jjFA40011); Guangxi Natural Science Foundation (2011jjA40294); Guangxi science and 401 technology development project (1355007-1); and Program for New Century Excellent Talents in 402 University (NCET-12-0653). 403 **Competing interests** All authors declare that they have no conflict of interest. 404 Ethics approval Medical ethics committee of Guangxi Medical University. 405 Participant consent Obtained. Provenance and peer review Not commissioned; externally peer reviewed. 406 407 Data sharing statement No additional data are available. 408 409 410 411 412

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413 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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5		T 11 4	
6 522 7		Table 1	
8 523	Demographic chara	acteristics of the GXME	WHC
9 10	Variables	Number (n=1888)	Percent (%)
11	Sex		
12 13	Male	1197	63.4
14	Female	691	36.6
15	Age, years (mean \pm SD)	40.31 ± 7.85	
16 17	<35	482	25.5
18	35~40	402	21.3
19 20	40~45	440	23.3
20	≥45	564	29.9
22	Seniority, years (mean \pm SD)	15.34 ± 9.63	
23 24	<10	652	34.5
25	10~20	585	31
26	>20	651	34.5
27 28	BMI, kg/m ² (mean \pm SD)	22.47 ± 2.8	
29	<18.5	95	5
30 31	18.5~24	1289	68.3
32	24~28	422	22.4
33	≥28	74	3.9
34 35	Missing	8	0.4
36	Race/ethnicity		
37	Han Chinese	916	48.5
38 39	Zhuang Minority	885	46.9
40	Other ethnic groups	80	4.2
41 42	Marital status		
43	Single	233	12.3
44	Married	1580	83.7
45 46	Widowed or divorced	75	4
47	Education or lower		
48	Middle school	829	43.9
49 50	High school	850	45
51	University or college or higher	209	11.1
52 53	Smoking status		
54	Current smoker	729	38.6
55	Former smoker	132	7
56 57	Never smoker	1027	54.4
58	Drinking status		
59 60		22	
60			

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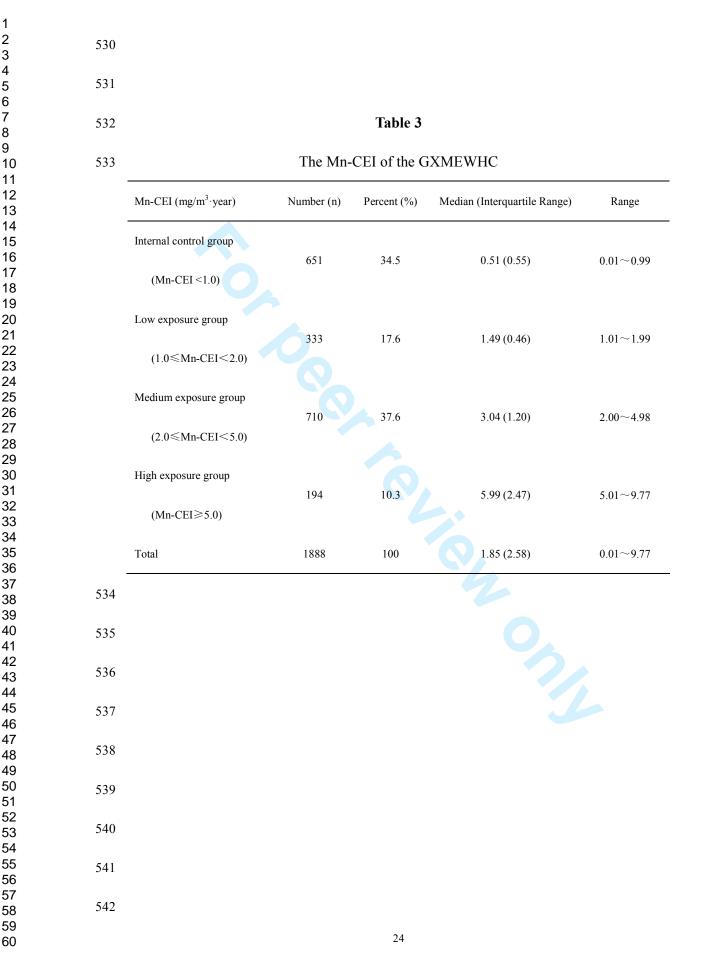
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Cu	rent drinker	907	48.1
			15.9
Nev	ver drinker	680	36

Table 2

Different types of work of the GXMEWHC

T			Age (years)	Seniority (years
Types of work	Number (n)	Percent (%)	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



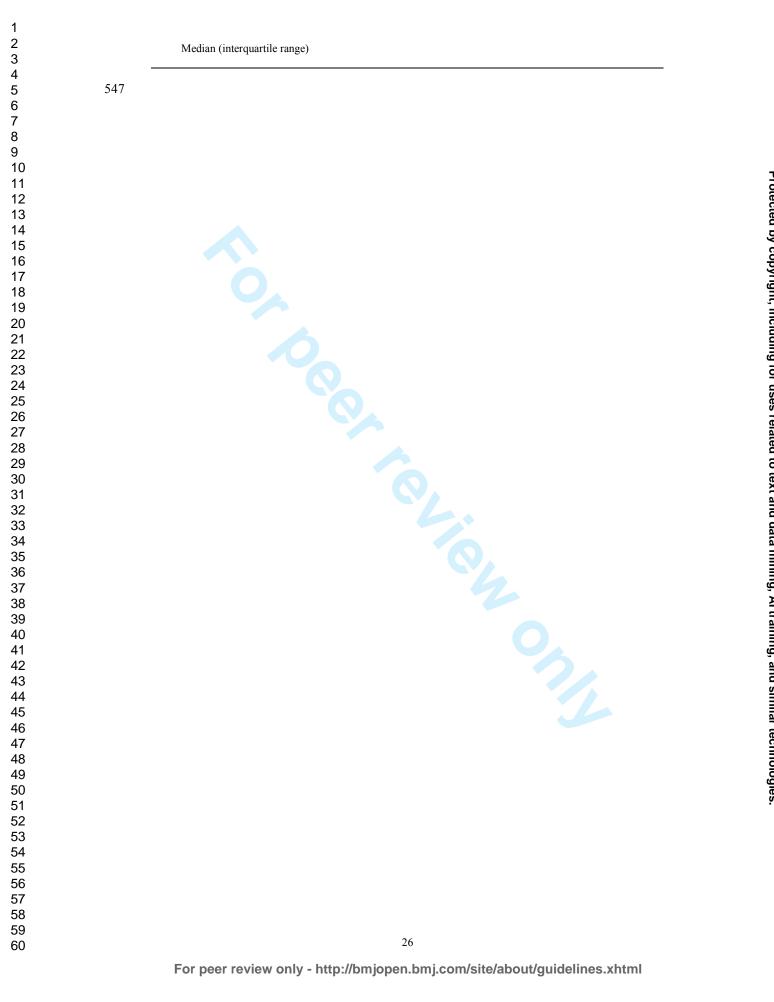
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543	
544	
545	Table 4

The results of occupational health examination of the GXMEWHC

	Male(n=1197)	Female(n=691)	Total(n=1888)
Variables	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)



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1	Rationale, design and baseline results of t Cohort Profile:	Formatted: Highlight
2	The Guangxi manganese manganese-exposure exposed	Formatted: Highlight Formatted: Highlight
3	workers healthy cohort (GXMEWHC) <u>study</u>	i of matteria (fighting).
4		
5	Yingnan Lv ¹ , Yunfeng Zou ² , Jing Liu ¹ , Kangcheng Chen ¹ , Damin Huang ¹ , Yuefei	
6	Shen ³ , Yaoqiu Zhong ¹ , Zhihao Liu ⁴ , Bei Jiang ⁴ , Qin Li ² , Li Qing ⁵ , Wei Zhang ³ ,	
7	Lang Chen ³ , Fenfen Wang ¹ , Bing Xia ¹ , Li Yang ¹ , Xiaobo Yang ^{1,6,*}	
8		
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17	Guangxi Medical University, Nanning, Guangxi, China	
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19	Nanning, Guangxi, China	
20	Yingnan Lv and Yunfeng Zou are contributed equally.	
21		
22	* Corresponding Author: Dr. Xiaobo Yang, Department of Occupational Health and	
23	Environmental Health, School of Public Health, Guangxi Medical University,	



47 Abstract

48	Objective: To explore the early health effect and long-term related diseases of
49	occupational manganese (Mn) exposure according to biomarkers of exposure, effect
50	and susceptibility.
51	Design: The baseline survey of a longitudinal cohort study from a Ferromanganese
52	Refinery,
53	Participants: A total of 1888 individuals (1197 men, 691 women) involved in
54	Guangxi manganese-exposed worker healthy cohort (GXMEWHC) study. Participants
55	were aged between 18 and 60 years (average age 40.31 years) and worked in
56	Ferromanganese Refinery at least one year and lived in the local area.
57	Results: The baseline survey was completed and the GXMEWHC study was
58	established, which included 1888 workers (average seniority 15.34 years). All
59	participants were divided into four groups according to the levels of Mn cumulative
60	exposure index (Mn-CEI), which included internal control group (Mn-CEI <1.0
61	mg/m ³ •year), low exposure group (1.0 mg/m ³ •year≤Mn-CEI<2.0 mg/m ³ •year),
62	medium exposure group (2.0 mg/m ³ •year≤Mn-CEI<5.0 mg/m ³ •year), and high
63	exposure group (Mn-CEI≥5.0 mg/m ³ •year). GWAS of quantitative trait loci (QTL)
64	and binary trait loci (BTL) were performed using Illumina Infinium HumanExome
65	BeadChip for 500 manganese-exposed workers. Stored plasma, DNA, hair and urine
66	are available in further study. Participants will be followed up every three years.
67	Conclusions: The GXMEWHC study provides abundant data to explore the
68	multi-organ health effects of occupational Mn exposure by biomarkers of exposure,
69	response and susceptibility, respectively.
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4 5	70	Introduction: Manganese (Mn) is an essential element for growth and maintenance
6 7 8	71	of human health. Inhaled manganese can be excrete by normal homeostatic
9 10	72	mechanisms, but it also can be accumulate in the bodily organ when excess the ability
11 12	73	of metabolism. Occupational exposure to Mn in air can cause the adverse health
13 14 15	74	effects in the human bodies. Consequently, we established the Guangxi manganese
16 17	75	exposure workers healthy cohort (GXMEWHC) to explore the early healthy effect
18 19	76	and the long term related disease of occupational manganese exposure by the
20 21	77	biomarkers of exposure, effect and susceptibility.
22 23 24	78	Methods and analysis: The GXMEWHC is a prospective study. We recruited the
25 26	79	workers in Ferro manganese refinery Factory and presently conducted the baseline
27 28	80	surveys including epidemiological investigation, neurological function test,
29 30 31	81	occupational health examination and environmental monitoring. The genome wide
32 33	82	association study (GWAS) are also implemented further. We will follow up the
34 35	83	participators every three years and ultimately the appropriate measures will be taken
36 37 38	84	to prevent and control the early healthy injure and the related disease.
39 40	85	Ethics and dissemination: The study has been approved by Medical ethics
41 42	86	committee of Guangxi Medical University. The results will be published by
43 44 45	87	peer-reviewed publications and presented at international conferences.
45 46 47	88	
48 49	89	
50 51 52	90	
52 53 54	91	Keywords
55 56	92	Manganese; Occupational exposure; Manganese toxicity; Genetic Susceptibility 4
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59 60		

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93	Strengths and limitations of this study	
94	■ In this study, we can collect an abundant database because of <u>the</u> large samples in	
95	the heavy metals cohort.	
96	■ The Ferro-manganese refinery <u>Refinery</u> Factory is the largest metallurgical	
97	factory of manganese Mn processing in China-so that: therefore, it can provide an	
98	extremely rich dataset <u>for</u> of analysis.	
99	■ The GXMEWHC is the a longitudinal study which that can continuous ly follow	
100	up and repeatedly investigate the participants. We can explore the relationships	
101	between occupational manganese Mn exposure and the early health injure effects.	
102	■ The GWAS are was implemented for seekingto determine the susceptibility genes	
103	of related to chronic low-level manganese Mn exposure, and to exploring explore	
104	the interactions between genetic factors and environmental factors. Those These	
105	data will provide an important opportunity to identify the more susceptible	
106	individuals so that<u>to</u> prevent the c arly health injure <u>effect of in</u> workers.	
107	• <u>The p</u> Potential limitations are that loss <u>of to follow-follow-</u> up may be a weakness	
108	with our study. There are some temporary workers in the factory and they may	
109	leave the factory after a period of time-working in factory. We can reduce the	
110	probability of the loss of to follow up through strictly controlled the inclusion	
111	criteria when established establishing the cohort.	
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118	INTRODUCTION	
119	Manganese (Mn) is an essential nutrient and it is necessary to inhaled manganese Mn	Formatted: Highlight
120	for maintaining the daily life. In addition to food intake, environmental exposure to	
121	Mn was allows the way to absorption of Mn, especially including occupational	
122	exposure. Mn was accumulated in some bodily organs and induced induces the	
123	adverse effects when the Mn concentration in vivo over exceeds the capacity of	Formatted: Font: Italic
124	human metabolism. ¹	
125	Many studies have showed shown that Mn can caused neurological abnormalities	Formatted: Highlight
126	when it accumulated accumulates in the human brain in human bodies, ²⁻⁴ such as	
127	early impaired Finger Tapping tapping speed ⁵ or cognitive deficits, terminal	
128	Parkinsonian-like symptoms, ⁶ and Manganismmanganism. ⁷ The values level of Mn in	
129	the human bodies-body were-can be detected through some internal biomarkers,	
130	neurobehavioral tests and functional neuroimaging. ⁸⁹ The Increased concentrations of	
131	Mn in the kidney were increased have been found in the manganese	
132	manganese-exposure exposed workers because the kidney is a way topathway of	
133	exercte <u>Mnmanganese</u> excretion. ⁴ In addition, the repeated respiratory exposured to	
134	Mn may cause impairedresulted in accumulation in the lung function. In oOne study	
135	showed that itthere was a dose-effect relationship between occupational manganese	
136	<u>Mn</u> exposures and the <u>a</u> reduction of <u>in</u> pulmonary function. ¹⁰ Compared with the	
137	non-exposure exposed workers, the pulmonary function in the manganese	
138	manganese-exposure exposed workers were evaluated by the spirometry tests and the	
	6	

139	values of them wereshowed a significant decrease in forced vital capacity (FVC).
140	forced expiratory volume at one second (FEV1), and the ratio of forced expiratory
141	volume at one second (FEV ₁ , FVC and FEV ₁ %) values. ¹¹ Increased manganese Mn
142	levels in blood serum ascribed to that liver is a <u>the</u> main ly organ to store,
143	biotransformation and detoxify the poisonous substanceMn. ¹² Over-exposure to
144	manganese Mn cause liver toxicity as well as and exacerbate liver dysfunction. ^{13 14}
145	Chronic manganese-Mn exposures leads to a series of significant cardiovascular
146	toxicities including the-an_abnormal electrocardiogram (ECG) and the_inhibition of
147	myocardial contraction which can alters the blood pressure (BP). ¹⁵
148	AdditionallyBesides, the manganicMn cytotoxicity could has been shown to induce
149	cell apoptosis and the -DNA damage of birdin avian immune cells. ¹⁶ Low Mn ²⁺ levels
150	can induce oxidative DNA damage via an apoptotic pathway, so that the but this DNA
151	damage eould can be reduced using antioxidants. A research conducted a risk
152	assessment of inhaled manganese Mn through incorporating genetics and genomics to
153	identifyied genetically based biomarkers of exposure, disease and susceptibility. ¹⁷
154	From the above <u>Thus</u> , manganic- <u>Mn</u> toxicity in humans played plays a significant
155	role in several systems. Currently, most studies were have explored separately the
156	effect of <u>Mnmanganese</u> exposure for <u>on</u> different system <u>s</u> in <u>of</u> the human body. To
157	further explore further the effects and the interaction of manganese Mn exposure in
158	various systems, we will establish a prospective cohort study which includes the
159	situation of individual manganese Mn exposure and regular occupational
160	examinations. Simultaneously, we will determinet the biological exposure indicators
161	by means of hair, urine and the blood samples. Blood and urine can reflect the extents
	7

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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 Mnmanganese exposure because that it may reflect the levels of Mnmanganese 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.

174 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 Mnmanganese 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-RefineryFactory. 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 manganese Mn exposure. This is an opportunity to explore the

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relations<u>hips</u> between various <u>kinds of risk</u> factors and the early health <u>injure effects</u>
of <u>manganese Mn</u> exposure, particularly <u>the genetic</u> and environmental factors and
their interactions.

189 Sample source

The entire samples 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 withmet 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 ifouting of touch with other risk factors except manganese Mn exposure (such as Cu, Pb, Cr_a, 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 <u>unable inability</u> 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

209	We will follow up the participants every three years. The information collected will be		
210	the same as the baseline data and will be collected the data obyf questionnaire		
211	interviews, physical examinations, biological specimens and environmental		
212	monitoring-repeatedly. Fig. 1 shows the complete study plan of the GXMEWHC. The		
213	retrospective survey are establishing and perfecting the GXMEWHC by collecting		
214	baseline data on demographic information, lifestyle, biological specimens as well as		
215	history of environmental and occupational exposure. The short-term objectives are to		
216	researching-explore the early healthy effects of occupational Mnmanganese exposure		
217	interact with environmental influences. In addition, preliminary exploring the effects		
218	of <u>Mnmanganese</u> exposure on genetic fields are also one of our studies. In the future,		
219	•Our long-term and final objectives are to exploring explore the early healthy injure		
220	effects on various systems in of the human body by gene-environmental interactions		
221	for long-term and continuous low levels of manganese <u>Mn</u> exposure.		
222			
223	Building database		
224	Questionnaire		

Building database

Questionnaire

The tTrained 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 sSelf-reported diseases are were tested and verified through the diagnosis of specialists, which is based on recognized international standards. The questionnaires consisted of demographic information, socio-economic

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231	status, smoking history-and, alcohol consumption and occupational history.
232	
233	Occupational health examination
234	The occupational health examination was implemented conducted at the same time.
235	All participants took part in the general health examination and were checked by
236	trained physicians, nurses, and the medical technicians.
237	The physical measurements covered height, weight, blood pressure (BP)., and
238	pulmonary function. The pulmonary function was estimated using a spirometry test
239	which comprised the test value of forced vital capacityincluded (FVC), forced
240	expiratory volume at one second (FEV1), the ratio of forced expiratory volume at one
241	second (FEV1%), maximal medexpiratory mid-expiratory flow eurve (MMEF), the
242	peak expiratory flow ratio (PEFR), maximal voluntary ventilation (MVV), the
243	predicted value <u>of them</u> and the ratio percentage of all above.
244	The clinical examinations included <u>a</u> high kilovar chest radiograph (HKV),
245	Neurology neurology inspection, ECG, Uncorrected uncorrected visual acuity (UCVA)
246	test, pure tone audiometry and physical examination of the heart, lungs, liver, spleen
247	and abdomen.
248	The laboratory tests included blood routine blood tests, urine routine urine tests
249	and liver function tests. The blood routine blood tests were measured in the laboratory
250	covering-and included the white blood cell count_(WBC), lymphocyte ratio_(LYR),
251	neutrophile granulocyte ratio_(GRANR), middle cell ratio (MIDR), lymphocyte count
252	(LYC), neutrophile granulocyte count_(NGC), middle cell count (MIDC), red blood
253	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), ketobodyketobodies (KET), blood (BLD), protein (PRO), nitrite (NIT), white blood cells (WBC), glucose (GLU), specific gravity (SG), power of hydrogen(PpH) and vitamin C. Furthermore, we examined the content of Mnmanganese 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.

265 Neurological function test

The neurological function test consisteds of a 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 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

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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 -80°C - refrigerators. In addition, a minimum of 10 milliliter mL of the urine were collected in the <u>a</u>urine bottles by the participants and then as the urine specimen stored in at 4°C - 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.

1 2 3 4		
5 6	300	Determining <u>Mnmanganese</u> exposure in the workplace
7 8	301	We will track the levels of manganese-manganese-exposure exposed workers who
9 10 11	302	participated in the present cohort study by means of the workplace monitoring. We
12 13	303	will record the basic information of the factory, the technological processes of
14 15	304	production,-and the distributions of occupational risk factors, the work mode, and the
16 17	305	situation level of manganese Mn in this company factory. The concentrations of
18 19 20	306	manganese-Mn_dust and fumes in the workplace were-are detected through the-an_air
20 21 22	307	point sampler. At the same time, we will monitor the individual levels of manganese
23 24	308	byMn using the individual samplers in theirduring working timehours. The
25 26	309	Permissible permissible concentration-time-weighted average (PC-TWA) is the
27 28 29	310	average permissible exposure levels on <u>during</u> the regulation eight <u>eight</u> hours
29 30 31	311	working day, weighteding by time. The Permissible permissible concentration-Short
32 33	312	short Term term Exposure exposure Limit limit (PC-STEL) is the permissible
34 35	313	exposure levels on in no more than 15 minutes at any time, weighting weighted by
36 37 28	314	time within a working day. The cumulative exposure index (CEI) is calculated
38 39 40	315	through TWA, STEL, and the workplace seniority in working. The CEI as an external
41 42	316	exposure index of ma <u>Mn</u> nganese and was calculated for each job, combining the
43 44	317	airborne monitoring with the individual monitoring both at during working time and
45 46	318	break time.
47 48 49	319	
50 51 52	320	Database of biomarkers
53 54 55	321	The database of biomarkers included biomarkers of exposure, effect and susceptibility.
56 57 58 59 60		14

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322	The biomarkers of exposure will be detected through atomic absorption spectrometry	11	Formatted: Highlight
323	(AAS) which are the levels of Mn and Fe in plasma, urine and hair. The levels of		Formatted: Highlight
525	(AAS) which are the levels of will and i e in plasma, drine and han, The levels of		Formatted: Highlight
324	plasma brain-derived neurotrophic factor (BDNF), dopamine (DA) were determined		Formatted: Highlight
325	by Sandwich ELISA kits which are biomarkers of effect. The biomarkers of		Formatted: Highlight
326	susceptibility are also assessed by GWAS which are shown in the following Genetic		Formatted: Highlight
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327	assessments part in detail		Formatted: Highlight
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330	Genetic determinationassessments		
331	<u>A sub-cohort of GWAS</u> is implemented in this study which researched <u>to assess</u> the		
332	effect of manganese-Mn_exposure on genetic-sides. The GWAS of Quantative		
333	quantitative Trait-trait Loci-loci (QTL) and Binary binary Trait-trait Loci-loci (BTL)		
334	will also are be also performed for the exposed workers using the Infinium		
335	HumanExome BeadChip from Illumina Company (Illumina Infinium HumanExome		
336	v1.0 BeadChips, 12-sample HD). The-Illumina's HumanExome BeadChips are		
337	covered with emphatically human exonic regions and, the exonic content contains		
338	more than 240,000 variant markers. The markers represented a variety of common		
339	diseases. and tThe different groups which contained theinclude individuals of from		
340	China, Europe, and Africa-and Spain. We will focus on the potential interactions of		
341	environmental manganese Mn exposure and genetics which based on the significant		
342	effects of Mn on the targeted phenotypes. Furthermore, the potential		
343	gene-environment interactions is will be explored through the genomes of the		
344	Mmanganism patients and healthy individuals who-exposed to manganese Mn in the 15		

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 GWASs 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

will be calculated through the Logistic Score Test (LST) which call test with late
 variants and relate the enriched genetic information to disease phenotypes through
 Logistic regression models.³² When the Genegene-wise or Groupgroup-wise Tests
 tests are conducted, the optimal sequence kernel association tests (SKAT-O) are will

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367	be_used ³³	
368		
369	Preliminary result PRELIMINARY RESULTS	
370	Demographic description of the cohort	
371	The-In total, 1,991 individuals were recruited to participate in the study from the	
372	FerroFerro-manganese refinery-RefineryFactory. After completing the questionnaire,	
373	a total of 1 ₂ 888 participants who met the standards were entered into the GXMEWHC	
374	study. who accord with standard and the with an effective rate is of 94.8%.	
375	Table 1 summarizes the baseline characteristics of the cohort. In Of the cohort,	For
376	63.4% were male and 36.6% were female. The mean age was 40.31 years and the	
377	percent of the four-stagesage distribution was-were similar. Thereinto, 34.5%, 31.0%,	
378	and 34.5% of the participants had the-seniority of <10, 10-20, and >20 years,	
379	respectively. In the factory, 31.2%, 15.7%, 20.1% and 33.0% were smelters, raw	
380	material processors, high exposed exposure auxiliary, and low exposed exposure	
381	auxiliary, respectively. The mean seniority was 15.34 years. The mean Body-body	
382	Mass-mass Index-index (BMI) was normal (22.47kg·m ⁻²). Among the participants,	
383	48.5% was-were Han Chinese. A majority of the participants (83.7%) were married. In	
384	the midst of the <u>Of these</u> participants, 43.9% graduated from middle school, 45.0%	
385	had-finished high school and 11.1% achieved completed college or higher education.	
386	In the cohort, 38.6% was were current smokers, 7.0% was were former smokers and	
387	54.4% was-were never smokers. Current passive smoking rates were 87.3%. The	
388	proportion of current drinkers was 48.1%, the former drinkers was 15.9% and the	
389	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 participatorsparticipants, 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.

395 Determining <u>Mnmanganese</u> exposure in the workplace

All the participators participants were divided according to different the types of work in the factory. Then, the extents 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/m³ year), the low exposed exposure group (1.0 mg/m³·year ≤ Mn-CEI < 2.0 mg/m³·year), the medium exposed <u>exposure</u> group (2.0 mg/m³·year \leq Mn-CEI \leq 5.0 mg/m³·year) and the high exposed exposure group (Mn-CEI \geq 5.0 mg/m³ year). The percentages of the internal control group, low exposed exposure group, medium exposure exposed group and high exposureexposed group were 34.5%, 17.6%, 37.6% and 10.3%, respectively. The median of total Mn-CEI was 1.85 mg/m³ year and the range was 0.01 mg/m³ year <u>-9.77 mg/m³ year. The details of Mn-CEI are shown in Table 3.</u>

410 Main results of <u>the</u> 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 uriene Mn level was 2.63µg/L,

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413	the male was $3.67 \mu g/L$ in males, and the female was $2.84 \mu g/L$ in females. The values	
414	results of the Blood Rroutine blood tests, Hepatic hepatic Function function tests and	
415	Pulmonary pulmonary Function function tests are shown in the Table 4.	
416		
417	Assessment of biomarkers	Formatted: Highlight
418	The liver function was analyzed between different manganese-exposed groups of the	Formatted: Highlight
419	cohort in 2013. Our conclusions in this study were that occupational Mn exposure can	Formatted: Highlight
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420	cause a dose-dependent increase of liver enzyme levels and interact with alcohol	
421	drinking to potentially aggravate the liver damage. ¹⁴ The plasma BDNF levels and	Formatted: Highlight
422	cognitive function of different manganese-exposed groups were also measured. Our	Formatted: Highlight
722	cognitive function of unificient mangalese exposed groups were also measured. Our	Formatted: Highlight
423	results showed that occupational Mn exposure may be related to decreased plasma	Formatted: Highlight
424	BDNF levels and cognition impairment. ²³	
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426	Detection <u>Assessment</u> of biological specimens and GWAS of <u>in</u> the cohort–	
427	We <u>greatly pay paid</u> attention to the potential gene-environment interactions.	
428	Therefore, we performed the GWAS of QTL and BTL using the Illumina Infinium	
429	HumanExome BeadChip for 500 exposed workers, such as including urie-urine_Mn	
430	and various kinds of indexindices of pulmonary function, liver function and blood	
431	routine <u>detection</u> . The-Illumina2's GenomeStudio Genotyping Module was used for	
432	genotyping and data analysis, which isusing an integrated platform for data	
433	visualization and analysis. About twenty five thousand 25,000 locus was were	
434		
	involved in the analysis after Quality quality <u>Control control</u> (QC). And then tThe	
435	involved in the analysis after <u>Quality_quality_Control_control_(QC)</u> . <u>And then tThe</u> QTL, BTL-and, <u>Genegene</u> -wise <u>or and Groupgroup</u> -wise <u>Tests-tests</u> were conducted	
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by EMMAX, LST, and SKAT-O, respectively. We will <u>further analyze the differential</u>
gene expression<u>further</u>. The results of GWAS and other <u>indexes_indices</u> will be
reported in <u>separate_ongoing</u> articles. We plan to conduct GWAS in a larger number of
manganese<u>manganese_exposure_exposed</u> workers <u>for_to_exploring_explore</u> the genic
risk factors and <u>the gene-environment interactions</u>.

443 ETHICS AND DISSEMINATION

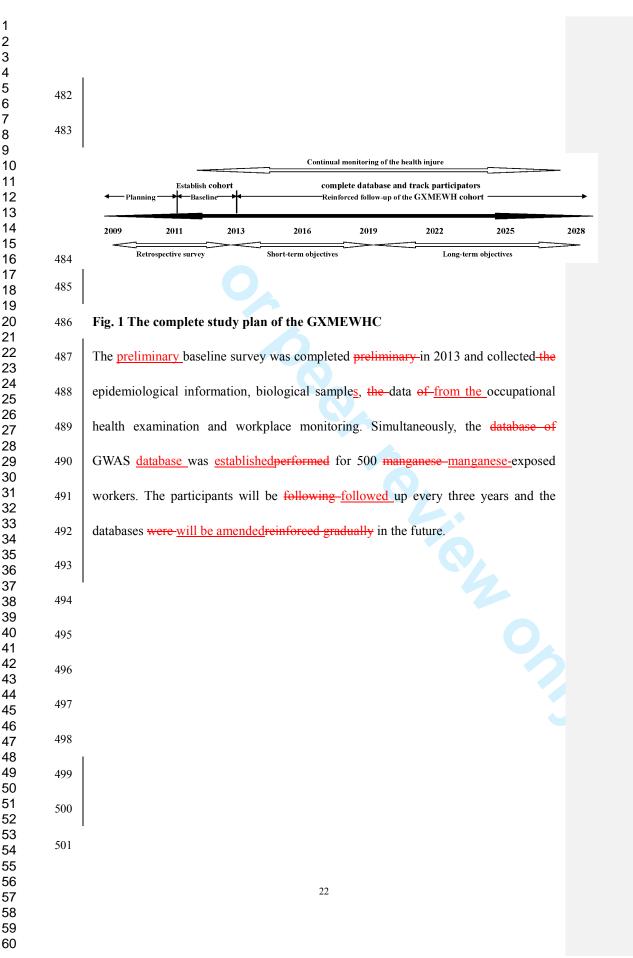
The study has been was approved via the Medical medical ethics committee of Guangxi Medical University. All the original files and data are maintained and stored at the research office, in the Department of Occupational Health and Environmental Health, School of Public Health, Guangxi Medical University, Nanning, China. Electronic materials are stored in a safe system file and <u>are accessible only</u> 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 included publishing in peer-reviewed journals and presenting presentation at international conferences.

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

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459 Kangcheng Chen, Yingnan Lv, Damin Huang, Yuefei Shen, Yaoqiu Zhong, Zhihao Liu, Bei Jiang, 460 Qin Li, Li Qing, Wei Zhang, Lang Chen, Fenfen Wang, Bing Xia and Li Yang contributed in 461 acquisition of the data; Yingnan Lv analysed the data and drafted the manuscript; all authors 462 contributed to review and revision of the manuscript and approved the final version. Funding This study was supported by National Natural Science Foundation of China (81060234, 463 464 21167004, and 81160339); Guangxi Science Fund for Distinguished Young Scholars 465 (2012jjFA40011); Guangxi Natural Science Foundation (2011jjA40294); Guangxi science and 466 technology development project (1355007-1); and Program for New Century Excellent Talents in 467 University (NCET-12-0653). 468 Competing interests All authors declare that they have no conflict of interest. 469 Ethics approval Medical ethics committee of Guangxi Medical University. 470 Patient Participant consent Obtained. 471 Provenance and peer review Not commissioned; externally peer reviewed. 472 Data sharing statement No additional data are available. 473 474 475 476 477 478 479 480 481 21



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	Table 1		
Demographic charac	cteristics of the GXME	WHC	
Variables	Number (n=1888)	Percent (%)	Formatted Table
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Male	1197	63.4	
Female	691	36.6	
ge <u>,</u> -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	
eniority, years (mean± SD)	15.34 ± 9.63		
<10	652	34.5	
10~20	585	31	
>20	651	34.5	
$MI_{-,}kg/m^2$ (mean \pm 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	
ace/ethnicity			
Han Chinese	916	48.5	
Zhuang Minority	<u>885</u>	<u>46.9</u>	Formatted: Highlight
Other ethnic groups	<u>80</u> 972	<u>4.251.5</u>	Formatted: Indent: First line: 2 ch
farital status	222		
Single	233	12.3	
Married	1580	83.7	Eaurattad. Highlight
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ducation or lower	820	12.0	
Middle school	829	43.9	
High school University or college or higher	850 209	45 11.1	
	209	11.1	
moking status Current smoker	729	20 6	
Former smoker	132	38.6 7	
Never smoker	132	54.4	
rinking status	1027	54.4	
Current drinker	907	48.1	
Former drinker	301	48.1	
Never drinker	680	36	

			Age (years)	Seniority
Types of work	Number((n)-)	Percent <u>-(%)</u>		-(_(years)-
			Mean± SD	Mean± SD
Smelter	594	31.5	38.95 ± 8.20	15.82 ± 9.02
Human <mark>c€</mark> rushing <u>w</u> ₩orker	320	16.9	41.08 ± 5.30	9.04 ± 6.00
Craneman	74	3.9	37.15 ± 8.76	16.24 ± 8.88
Finishing <u>m</u> Machining	99	5.2	40.36 ± 6.10	10.20 ± 8.79
<u>w</u> ₩orker			10.50 - 0.10	10.20 = 0.77
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 ± 10.7
Chemical <mark>aA</mark> nalyst	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 <u>r</u> Recovery <u>w</u> ₩orker	133	7.0	40.89 ± 6.33	13.74 ± 8.74
Car <u>d</u> D river	118	6.2	39.01 ± 9.96	15.09 ± 12.0
Total	1888	100	40.31 ± 7.85	15.23 ± 9.60

Table 2

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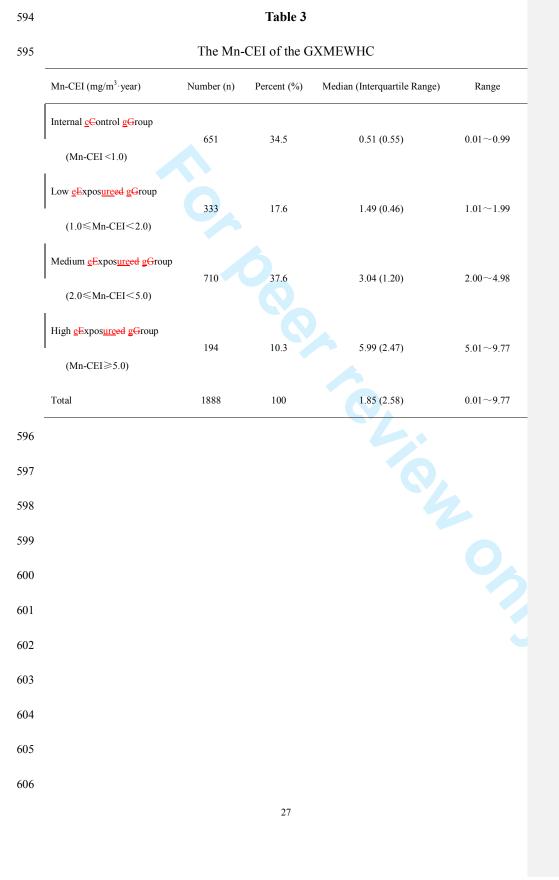


Table 4

The results of occupational health ex-	kamination of the GXMEWHC
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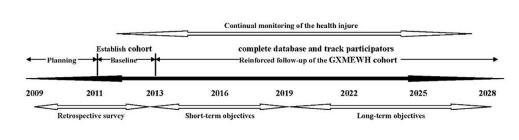
X	Male(n=1197)	Female(n=691)	Total(n=1888
Variables -	Mean ± SD	Mean ± SD	Mean ± SD
Systolic blood pressure, mmHg	127.68 ± 12.11	121.54 ± 11.53	125.43 ±1 2.2
Diastolic blood pressure, mmHg	79.93 ± 8.29	76.86 ± 7.93	78.81 ± 8.29
Blood routine			
WBC, 10 ⁹ /L	6.91 ± 1.51	6.32 ± 1.52	6.69 ± 1.54
RBC, 10 ¹² /L	5.13 ± 0.52	4.61 ± 0.44	4.94 ± 0.55
H ac moglobin, g/L	148.69 ± 12.73	128.8 ± 14.37	141.38 ± 16.4
Platelet count, 10 ⁹ /L	241.76 ± 54.13	256.29 ± 62.86	247.1 ± 57.9
Hepatic function			
Total bilirubin, μmol/4 <u>L</u>	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 (<u>i</u> Interquartile <u>r</u> Range)	2.63 (2.37)	3.67 (4.12)	2.84 (2.79)

Page 56 of 57

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90x38mm (300 x 300 DPI)

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STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of <i>cohort studies</i>

Section/Topic	ltem #	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			

Page	58	of	57
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Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed	14
		eligible, included in the study, completing follow-up, and analysed	
		(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	Non
		interval). Make clear which confounders were adjusted for and why they were included	
		(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	
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	18
		which the present article is based	

*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.