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COHORT PROFILE

Cohort Profile: The China Jintan Child Cohort Study

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How did the study come about?

Environmental toxicity, including lead exposure, is an important global health problem adversely affecting millions of children worldwide. In developing countries, the prevalence of lead exposure is significantly worse.[1–3] Children in China are exposed to diverse environmental pollutants.[4] Although leaded gasoline was phased out in China, the prevalence of childhood lead exposure still remains very high at 20%.[5] The toxic effects of lead on neurodevelopment are well known, impacting both cognitive and behavioural development.[6–10] In addition, malnutrition caused by micronutrient deficiencies is still prevalent in developing countries like China.[11–12] Nutrition deficiency has been linked to children's poor cognition and negative mental wellbeing.[13–15] Furthermore, youth aggression and juvenile delinquency are being increasingly viewed as a public health problem.[16–17] One recent large epidemiologic study reported considerable consistency across 24 countries in adolescents' self-reported problems.[18] Like many other developing counties, China has been undergoing major social, economic and cultural changes in the last two decades. These changes have had significant implications for children's social and psychological functioning.[19,20] It has been reported from large-scale epidemiological surveys that 10–30% of Chinese children and adolescents experience mental health problems,[21] including negative behavioural outcomes.

The Jintan Cohort Project was established in the summer of 2004 to help address these concerns on lead exposure and other environmental and health-related issues. This population-based pre-school cohort study was initiated in 2004 and reflects collaboration between American scientists from the University of Pennsylvania and members of Shanghai Jiaotong University, Southeastern University, Jintan Hospital and Jintan Maternal Health Center. The study will follow children into adolescence to investigate the influence of lead exposure and micronutrient deficiency on their behaviour. Other biological and psychosocial risk factors such as sleep patterns, family conflict and parenting affecting children's cognitive and behavioural outcomes are also examined.

The initial focus of this cohort study was on the impact of lead exposure and micronutrient deficiency on children's cognitive and behaviour outcomes. Child development is complex and likely reflects the individual and combined effects of neuro-toxicants, micronutrients and the social environments.[22] Given this complexity, we have extended our assessments to include broad aspects of environment health factors (both biological and psychosocial factors) in our assessments.

How was it funded?

Initially, the study on the impact of high levels of lead and low levels of micronutrients on children's behavioural outcome was supported by a grant from the Wacker Foundation to Dr Jianghong Liu in the USA. The Shanghai Lead Prevention Center (Shanghai Jiao Tong University) has assisted in lead analyses, whereas the Jintan study team helped in data collection. The study is currently supported by a NIEHS Career Development Award to the PI (Dr Jianghong Liu) together with Jintan city funds.

What does it cover?

The overarching goal of the Jintan cohort study is to examine the effects of lead exposure and micronutrients deficiency in relation to the development of children and adolescents neurocognitive and neurobehavioural outcomes. We are also interested in what other heath factors that directly, indirectly or

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interactively affect children and adolescents' physical and mental health and subsequent well being over the life course.

Where is Jintan located and why was Jintan selected for this study?

The city of Jintan is located in the south-eastern coastal region of China, 50 miles south of Nanjing and 120 miles north of Shanghai (Figure 1). Geographically, the city is a unique place for studying environmental factors such as lead on children's health because the the city centre is surrounded by busy construction sites, whereas the rural region has a mining area and a lake area. In addition, Jintan city is ideal for a longitudinal project because it is a relatively small county-level city of 976 square kilometres and a population of 540 000. Due to low mobility, it is ideal for following up and tracking subjects in a longitudinal study. Furthermore, Jintan is a fast-developing city with a range of socio-economic levels, which will help in assessing the role of the social environment. Finally, our established collaborative network makes Jintan an ideal place for studying the environmental effects on the developmental of child behaviour.

Who is in the sample?

The Jintan Child Study consists of a pre-school cohort of 1656 children accounting for 24.3% all children in this age range in Jintan city. The cohort includes 55.5% boys, 44.5% girls and 99.8% Han ethnicity. Census data for the city as a whole shows 53.5% boys and 46.5% girls in this age range, and 99.8% Han ethnicity, indicating that the study largely achieved its goal of selecting a sample representative of the city in terms of sex and Han ethnicity distribution. Between autumn 2004 and spring 2005, all children (age 3–5 years old) attending the following four pre-schools in Jintan were invited to participate in our study: Jianshe, Huacheng, Xuebu and Huashan. The response rate to the initial recruitment was 97%. Among the respondents, 98% agreed to participate. There were no clear differences between initial responders and non-responders who, agreed or refused to participate in relation to the children's profiles. Since only 3% of children were in the non-responder group, the numbers were too small to compare statistically. The main reason we received a high response and agreement rate is due to the effort of our collaborator—the Jintan Maternal Child Health Center. In China, pre-schools (where they are called kindergarten) are administrated by the City Maternal-Child Health Center, which is a division of the City Health Department. As part of the public health work for the children in the city, the Jintan Maternal-Child Health Center periodically goes to the pre-school for health education and health check-ups for children. Consequently, recruitment was not a challenge at the beginning of this study.

The four pre-schools were chosen to be representative of the geographic, social and economic profile of the whole city. The profile of these four pre-schools also fitted rationally with our study interest. Jianshe is situated in the city centre (39.4%), and Huacheng is located in the peripheral region (40%), a newly-developed zone with more middle-class families. The other two pre-schools are from separate rural areas (20.6%), with one having several cement factories nearby. Such locations allow us to assess for any localization of the effects of lead exposure and micronutrients. It was hypothesized that each area may yield different levels of lead and micronutrients.

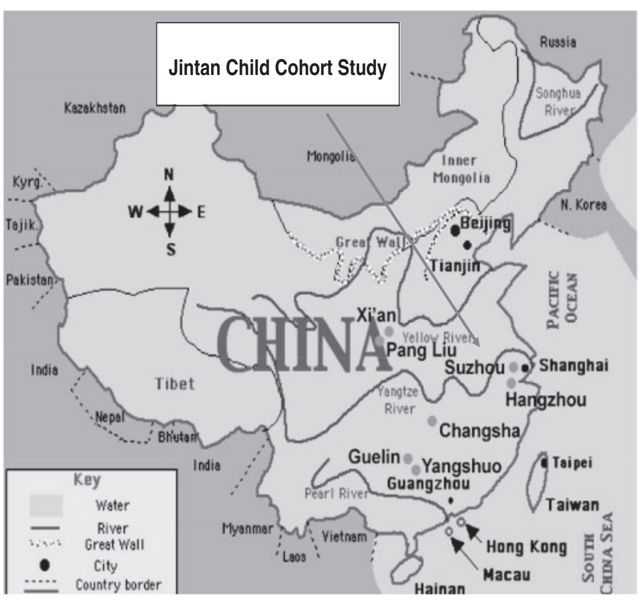

Figure 1 Location of Jintan

How often have they been followed up?

Chinese pre-schools are divided into junior (3–4 years old), middle (4–5 years old) and senior levels (5–6 years old). Baseline data on blood lead and micronutrients were assessed during 2004–2005 on 1656 children. The first follow-up in 2005–2007 included IQ tests, behavioural outcomes and psychosocial, health and demographic information, which were assessed when the children were in their senior level. The children are now all enrolled in several Jintan elementary schools and the second follow-up is imminent/to be carried out shortly.

What has been measured?

The list of measures is outlined in Table 1. The baseline recruitment included blood specimen

Table 1 Outline of the status/locations of the measures in the Jintan Cohort Study

Measures/instruments[b]	Status	Locations	Status	Child age (years)
Enrolments (informed consent)[a]	Baseline	Pre-school	General consent completed	3–5
Blood sample collection	Baseline	Pre-school	Completed in 2004–2005	3–5
Blood assay: lead (graphite furnace atomic absorption spectrophotometry)	Baseline	Shanghai Lead Prevention Center	Completed in 2005	4–5
Blood assay: micronutrients	Baseline	Pre-school	Completed in 2005	4–5
IQ testing: age 5 years (WPPSI–R)	First follow-up	Pre-school	Completed in 2005–2007	5–6
Growth data: height/weight	First follow-up	Pre-school	Completed in 2005–2007	4–6
Health data	First follow-up	Pre-school	Completed in 2005–2007	5–6
Retrospective birth complication				
Infant developmental data				
Breastfeeding history				
Secondhand smoking exposure				
Nutrition survey				
Sleep behaviour				
Demographic/psychosocial data	First follow-up	Pre-school	Completed in 2005–2007	5–6
Parental education, occupation				
Marital status				
Size of house				
Neighbourhood environment				
Child-rearing disagreement				
Parental report				
Child behaviour checklist (CBCL, TRF)	First follow-up	Pre-school	Completed in 2005–2007	5–6
Academic records	Second follow-up	Elementary school	On going 2008	7–10 Grades 1–4
IQ testing: age 10–12 (WISC)	Second follow-up	Elementary school	Planned	10–12 Grades 4–6
Health data Nutrition survey	Second follow-up	Elementary school	Planned	10–12 Grades 4–6
Social competency and peer relationship	Second follow-up	Elementary school	Planned	10–12 Grades 4–6
Child behaviour checklist: (CBCL/TRF/YSR)	Second follow-up	Elementary school	Planned	10–12 Grades 4–6

[a]General consent has been obtained from the subjects during the first phase of blood drawing.
[b]RAs are Pediatric Nurses (R.N., B.S.) who received training in IQ testing and data collection.

collection and analysis of lead and micronutrients (iron, copper, zinc, calcium, magnesium) on 1656 children. Venous blood specimens were taken from children at their pre-school in late 2004 by trained pediatric nurses using a strict research protocol to avoid lead contamination.[23] Blood was collected in lead-free EDTA tubes. Blood specimens were frozen and shipped to the Research Center for Environmental Medicine of Children in Shanghai at Shanghai Jaotong University for lead analyses and shipped to Nanjing Medial University for micronutrient analyses. Blood lead levels were measured by graphite furnace atomic absorption spectrophotometer (instrument AA100—Perkin-Elmer Company). The reliability and validity of the analysis and the detailed analytic procedures have been described previously in Shen *et al.*[24] and Yan *et al.*[23] Analysis of each specimen was conducted using a replication procedure, and the mean of the repeated measurements was taken as the final measure. Blood lead reference materials for quality controls were provided by Kaulson Laboratories, NJ, USA. This laboratory has participated successfully in a CDC-administered quality-control program (Blood Lead Proficiency

Testing Program) for the measurement of lead in whole blood.

The first follow-up at age 6 included measures of cognitive ability, behavioural outcomes, health and psychosocial demographic information. Cognitive ability was assessed using the Chinese version and norms of the Wechsler Preschool and Primary Scale of Intelligence–Revised (WPPSI–R).[25,26] The WPPSI–R is an internationally accepted measure of cognitive functioning in children from 3 to 7 years of age and consists of five subtests of the verbal IQ scale (Information, Comprehension, Arithmetic, Vocabulary, Similarities) and five subtests of performance IQ (Object Assembly, Geometric Design, Block Design, Mazes, Picture Completion, Scale). Verbal, performance and full-scale IQ indices have been computed. The test provides three IQ measures: full-scale IQ (a total of the 10 subtests), verbal IQ and performance IQ, each with a mean of 100 (SD 15). The Chinese versions of the WPPSI and WPPSI–R have established reliability and validity in Chinese children.[26–28] IQ tests were administered by two trained research assistants (RAs), and overseen by a psychologist who specialized in cognitive and behaviour assessment from Nanjing Brain Hospital. We have obtained data on test–retest reliability ($r = 0.87$, $P < 0.001$); and inter-rater reliability ($r = 0.91$, $P < 0.001$). Behavioural outcomes were assessed by the newly revised Chinese translation of Achenbach's Child Behaviour Checklist (CBCL) and the Caregiver-Teacher Report Form/1 1/2-5 (CTRF/1 1/2-5)[29] completed by parents and pre-school teachers. Parents filled out questionnaires on psychosocial, health and demographic information during their meeting at the pre-schools at the end of the school year. RAs were on site to assist parents' filling out the forms.

The second follow-up at ages of 7–12 in elementary schools includes measures of cognitive ability using the Chinese Wechsler Intelligence Scale for Children (WISC),[30] academic records, school performance, health data (nutrition and height/weight) and behavioural outcomes. Furthermore, recognizing the pre-adolescent period is a critical stage for the development of social functioning and peer relationship, so we also plan to measure children's social competence and peer relationship.[31–32]

What is the attrition rate likely to be?

Among 1656 children recruited at baseline, 1385 participated in the first follow-up data collection including measurement of IQ, behaviour, health and demographic and psychosocial data. Therefore, the attrition rate is ~16%. The follow-up group ($n = 1385$) was compared with those without follow-up ($n = 271$) on key variables that were available on all participants. Groups did not differ regarding blood lead level ($t = -1.556$; $P = 0.120$), zinc ($t = 0.494$; $P = 0.621$), calcium ($t = 0.510$; $P = .610$), iron ($t = 0.192$; $P = 0.848$), magnesium ($t = -0.927$; $P = 0.354$) and copper ($t = -0.777$; $P = 0.437$). The lost samples (271 children) is due to two reasons: (i) 43 (2.60% of the original sample) children whose blood samples were not validated due to sample collection and handling error were not involved in the follow-up; and (ii) among the remaining 228 children (13.77% of the original sample) children either moved to other pre-schools, did not respond or refused to participate in the follow-up. All our subjects currently have entered in grades 1–3 in five of Jinan's elementary schools. We anticipate a lower attrition rate for the next follow-up based on our recent pilot follow-up survey.

What has it found? Key findings and publications

Data collection and analyses are on going and papers are currently in preparation. We are currently working on factor analyses on the behavioural scales assessed by CBCL. The baseline data on lead exposure, micronutrients and cognitive ability are listed in Tables 2 and 3. A summary of the initial findings is as follows.

Analyses of micronutrients has indicated that there are high deficiency rates in zinc (38.14%), calcium (34.29%) and iron (24.32%), but only 0.67% and 0.61 have copper and magnesium deficiency, respectively. The criteria of deficiency are based on the recommendation of the Chinese Academy of Pediatrics. These data suggest that pre-school children in Jintan City have inadequate mineral status, which should be improved. Further analyses on identifying risk factors and health outcomes associated with minerals will be explored.

An assessment of lead exposure has shown that our cohort has mean blood lead level (BLL) of ~6.35 μg/dl, with 7.2% ⩾10 μg/dl, which is three times higher in the study sample than in the USA as a whole, which has a respective proportion of 2.2%.[33] Furthermore, preliminary analyses in a subset of the Jintan cohort study indicates that higher lead exposure correlates with both decreased IQ and increased behaviour problems.

Regarding cognitive ability, overall IQ was normally distributed, and performance IQ was significantly higher than verbal IQ. As expected, both are positively correlated with parents' education level ($P < 0.01$). From a subset of this cohort we have found that sleep behaviour is significantly related to pre-school children's cognitive ability, independent of parental education, with co-sleep negatively correlated with cognitive ability ($P < 0.05$). We have also found that consistently having breakfast is associated with higher verbal IQ in this cohort ($P < 0.001$).

Table 2 Key baseline data, China Jintan Cohort Study

		Area[a]			
		City centre	Suburb	Rural	**Total**
Demographic Information					
	n	578	651	346	1575
Age		4.67 ± 0.86	4.65 ± 0.90	4.92 ± 0.82	4.65 ± 0.87
Sex		322 (55.71%)	363 (55.76%)	189 (54.62%)	874 (55.49%)
Male					
Female		256 (44.29%)	288 (44.24%)	157 (45.38%)	701 (44.51%)
Blood assay					
Lead	n	601	582	346	1576
	$\bar{x} \pm s$	6.25 ± 2.45	6.12 ± 2.90	6.91 ± 2.45	6.35 ± 2.66
	G ± SD	5.80 ± 0.15	5.57 ± 0.16	6.54 ± 0.14	5.86 ± 0.15
Calcium	n	611	657	346	1614
	$\bar{x} \pm s$	1.64 ± 0.19	1.64 ± 0.16	1.67 ± 0.39	1.64 ± 0.24
Iron	n	612	657	345	1614
	$\bar{x} \pm s$	8.06 ± 0.84	8.23 ± 0.80	8.05 ± 0.80	8.13 ± 0.82
Zinc	n	612	657	346	1615
	$\bar{x} \pm s$	81.01 ± 14.71	85.21 ± 11.17	80.24 ± 13.00	82.55 ± 13.18
Magnesium	n	612	657	346	1615
	$\bar{x} \pm s$	1.47 ± 0.17	1.46 ± 0.16	1.46 ± 0.15	1.47 ± 0.16
Copper	n	612	657	346	1615
	$\bar{x} \pm s$	27.20 ± 6.83	27.03 ± 6.59	26.79 ± 6.61	27.04 ± 6.69

[a]Four pre-schools are categorized into: (1) City center: Jianshe; (2) Suburb: Huacheng; and (3); Rural: Xuebu and Huashan.

Table 3 Key variables of first follow-up (age 6 years), China Jintan Cohort Study

		Area[a]			
		City centre $n = 545$	Suburb $n = 554$	Rural $n = 286$	**Total** $n = 1385$
Cognitive ability (IQ)					
Performance IQ		109.07 ± 14.59	103.95 ± 13.65	94.16 ± 13.56	104.02 ± 15.04
Verbal IQ		107.96 ± 14.39	103.60 ± 15.13	96.72 ± 12.08	103.95 ± 14.84
Full-scale IQ		109.25 ± 13.64	103.88 ± 13.71	94.71 ± 12.09	104.17 ± 14.39
Behaviour					
CBCL total	Mother	33.40 ± 19.93	33.39 ± 21.38	34.41 ± 22.08	33.60 ± 20.95
TRF	Teacher	18.73 ± 16.79	17.36 ± 15.68	26.52 ± 18.37	19.76 ± 17.04

[a]Four pre-schools are categorized into: (1) City centre: Jianshe; (2) Suburb: Huacheng; and (3); Rural: Xuebu and Huashan.

What are the main strengths and weaknesses?

The key features of the Jintan cohort study include: (i) blood lead levels from 1656 children in a pre-school cohort were assessed by venous blood specimens using a strict research protocol. Consequently, we believe the assessment of BLLs has high reliability and validity; (ii) we employed a prospective longitudinal design at the beginning of this study aimed at following up children into adolescence; this cohort will yield behavioural outcomes at several time points, including the critical developmental stage of puberty; (iii) each child in this cohort has a health profile including height/weight taken twice a year since the age of 3; (iv) a biosocial approach to the study design including several biological and psychosocial measures; (v) a multidisciplinary collaboration involving epidemiology, nursing, environmental medicine, public health, psychology and psychiatry. In addition, the small size of the city with its low mobility helps subject-tracking for follow-up testing to assess whether early lead levels will particularly characterize a subgroup of children with sustained levels of neuro-cognitive and neuro-behavioural problems throughout childhood. Programmatically, the results of this study could provide the basis for planning future intervention studies to reduce lead exposure[34,35] and/or to assess the effect of nutritional interventions on the lead–behaviour relationship using a multidisciplinary approach for reducing behavioural problems in children.

A possible limitation is related to potential bias in sample selction in the context of recuriment of partcipaans. For example, it is possible that children already poisoned by lead never even got to go to pre-school. On the other hand, there is no report from Jintan Maternal Child Health Center, whose work includes monitoring children's lead level from age 1–6 years in Jintan City, to suport this possibility. It is also possible that children with high BBL did not continue to attend the four pre-schools to complete the follow-up data. Our analysis, however, indicates that those lost to the follow-up did not differ in BLL ($t = 1.64$; $P = 0.10$). The fact that the study is set in a developing Asian county may limit the generalizability of any findings to developed Western countries such as the USA.

Can I get hold of the data? Where can I find out more?

To learn more about this cohort and explore potential collaborations, please contact the Principle Investigator of the Jintan Child Cohort Study: Dr Jianghong Liu (jhliu@nursing.upenn.edu).

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