BMJ Open

Cohort profile: Aichi regional adjunct sub-cohort of the Japan Environment and Children's Study (JECS-A)

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-028105
Article Type:	Cohort profile
Date Submitted by the Author:	24-Nov-2018

Complete List of Authors:	Ebara, Takeshi; Nagoya City University, Occupational and Environmental Health, Graduate school of Medical Sciences Yamada, Yasuyuki; Nagoya City University Graduate School of Medical Sciences and Medical School, Department of Occupational and Environmental Health; Juntendo University School of Health and Sports Science Graduate School of Health and Sports Science Shoji, Naoto; Nagoya City University Graduate School of Medical Sciences and Medical School, Department of Occupational and Environmental Health; Asahi University, School of Health Sciences Ito, Yuki; Nagoya City University Graduate School of Medical Sciences and Medical School, Department of Occupational and Environmental Health Nakagawa, Atsuko; Nagoya City University Faculty of Humanities and Social Sciences Graduate School of Humanities and Social Sciences Miyachi, Taishi; Nagoya City University Graduate School of Medical Sciences and Medical School, Pediatrics and Neonatology; Nagoya West District Care Center for Disabled Children Ozaki, Yasuhiko; Nagoya City University Graduate School of Medical Sciences and Medical School, Obstetrics and Gynecology Omori, Toyonori; Nagoya City University Graduate School of Medical Sciences and Medical School, Health Care Policy and Management Suzuki, Sadao; Nagoya City University Graduate School of Medical Sciences, Department of Public Health Kojima, Masayo ; Nagoya City University Graduate School of Medical Sciences and Medical School, Medical Education Ueyama, Jun; Nagoya University Graduate School of Medicine Faculty of Medicine, Department of Pathophysiological Laboratory Sciences, Field of Radiological and Medical Laboratory Sciences Tomizawa, Motohiro; Nagoya City University Graduate School of Medical Sciences and Medical School, Department of Occupational and Environmental Health; Tokyo University of Agriculture Kato, Sayaka; Nagoya City University Graduate School of Medical Sciences and Medical School, Department of Occupational and Environmental Health; Nagoya City University Graduate School of Medical Sciences and Medical School, Pediatrics and Neonatology Oguri, Tomoko; Nagoya City University Graduate School of Medical Sciences and Medical School, Department of Occupational and Environmental Health; National Institute of Advanced Industrial Science and Technology (AIST) Matsuki, Taro; Nagoya City University Graduate School of Medical Sciences and Medical School, Department of Occupational and

	Environmental Health Sato, Hirotaka; Nagoya City University Graduate School of Medical Sciences and Medical School, Department of Occupational and Environmental Health Oya, Naoko; Nagoya City University Graduate School of Medical Sciences and Medical School, Department of Occupational and Environmental Health Sugiura-Ogasawara , M; Nagoya City University Graduate School of Medical Sciences and Medical School, Obstetrics and Gynecology Saitoh, Shinji; Nagoya City University Graduate School of Medical Sciences and Medical School, Pediatrics and Neonatology Kamijima, Michihiro; Nagoya City University Graduate School of Medical Sciences, Department of Occupational and Environmental Health
Keywords:	JECS, birth cohort, EPIDEMIOLOGY

Cohort profile: Aichi regional adjunct sub-cohort of the Japan Environment and Children's Study (JECS-A)

Takeshi Ebara[1], Yasuyuki Yamada[1,9], Naoto Shoji[1,10], Yuki Ito[1], Atsuko Nakagawa[7], Taishi Miyachi[2,11], Yasuhiko Ozaki[3], Toyonori Omori[4], Sadao Suzuki[5], Masayo Kojima[6], Jun Ueyama[8], Motohiro Tomizawa[1†], Sayaka Kato[1,2], Tomoko Oguri[1††], Taro Matsuki[1], Hirotaka Sato[1], Naoko Oya[1], Mayumi Sugiura-Ogasawara[3], Shinji Saitoh[2], Michihiro Kamijima[1]

Department of [1]Occupational and Environmental Health, [2]Pediatrics and Neonatology, [3]Obstetrics and Gynecology, [4]Health Care Policy and Management, [5]Public Health, [6]Medical Education, Nagoya City University, Graduate School of Medical Sciences, Nagoya, Japan
[7]School of Humanities and Social Sciences, Nagoya City University, Nagoya, Japan
[8]Department of Pathophysiological Laboratory Sciences, Field of Radiological and Medical Laboratory Sciences, Nagoya University Graduate School of Medicine, Japan.
[9]Juntendo University Graduate School of Health and Sports Science, Chiba, Japan
[10] Asahi University, School of Health Sciences
[11]Nagoya West District Care Center for Disabled Children, Nagoya, Japan

Present affiliation: [†]Tokyo University of Agriculture, [††]National Institute of Advanced Industrial Science and Technology (AIST)

Corresponding Author:
Takeshi Ebara, PhD.
Department of Occupational and Environmental Health, Nagoya City University, Graduate School of Medical Sciences. Mizuho-ku, Nagoya, 4678601, Japan
TEL: 81-52-853-8171 FAX: 81-52-859-1228
e-mail: ebara@med.nagoya-cu.ac.jp

ABSTRACT

Purpose: Effects of fetal, perinatal and childhood environmental factors on the health of children at birth and during later life have recently become a topic of concern. The Aichi regional adjunct subcohort of the Japan Environment and Children's Study (JECS-A) is an ongoing birth cohort of pregnant women and their children that has been used to provide unique data on multifaceted potential factors affecting children's health.

Participants: The JECS-A is part of the Japan Environment and Children's Study (JECS) which follows a total of 100,000 pairs of children and their parents across 15 regions in Japan. Of the 8,134 pregnant women living in Ichinomiya City and Nagoya City, Japan, who were eligible for recruitment, a total of 5,721 pregnant women and their 5,555 children were included. Sociodemographic and psychological data as well as biological specimens were available for all the pregnant women in the cohort. Information on children included in the JECS-A was collected from their mothers and includes demographic, behavioral, childcare, psychological and psychiatric data. Biological specimens of urine extracted from disposable diapers and anthropometric data were also obtained.

Findings to date: Regarding the representativeness of the JECS-A, a similar distribution trend for age at delivery was confirmed between the pregnant women enrolled in the JECS-A and the national statistics of the relevant areas. However, differences in education level and household income were observed. A total of 5,502 children were enrolled in the cohort at 18 months after delivery. Compared with the national statistics, the basic demographics of the children in the cohort were representative of the population in the study area.

Future plans: The enrolled children in the JECS-A will be followed until the age of 13 years. The studies that comes from JECS-A will complement JECS and bring novel results with a high level of generalizability.

Strengths and limitations of this study

- The main strength of the JECS-A of children is its large sample size, reflecting a representative population.
- Another strength can be found in considering multifaceted potential factors affecting children's health in the prospective birth cohort over two generations.
- The main limitation of the cohort is that the population with low-income households is not included.

INTRODUCTION

The effects of fetal, perinatal and childhood environmental factors on the health of children at birth and during later life have recently become a topic of concern. A number of birth cohort studies are thus being conducted worldwide to address this issue, and the Japan Environment and Children's Study (JECS) is one of the largest. The JECS was launched in 2011 by the Ministry of the Environment, Japan, after 3 years of planning.[1] The JECS consists of nationwide regional sub-cohorts that can be used to conduct independent studies related to children's health. This article describes the cohort profile of a regional sub-cohort of the JECS, the Aichi regional adjunct sub-cohort of the JECS (JECS-A), containing 5,721 pregnant women and their 5,555 children. The following research themes are the possible main focuses of the JECS-A.

Special attention has been paid to neurodevelopmental disorders including intellectual disability, communication disorders, autism spectrum disorder (ASD), attention deficit hyperactivity disorder (ADHD), specific learning disorder, and motor disorders.[2] A review has indicated that the estimated prevalence of ASD in Asia before 1980 was around 1.9/10,000, but this figure has recently increased by ten times.[3] Though a previous study conducted in Denmark claimed that 33% of the increase in the prevalence of ASD in recent years can be accounted for by changes in the diagnostic criteria and reporting methods,[4] this increased prevalence remains a complex and highly controversial issue that needs to be addressed. Recent epidemiological studies have linked neurodevelopmental outcomes to prenatal exposure to environmental toxicants such as heavy metals,[5,6] prenatal tobacco exposure,[7,8] environmental tobacco exposure,[9,10] phthalates,[11] persistent organic pollutants,[12,13] and organophosphate pesticides (OPs).[14,15 16] Furthermore, interest is growing regarding the potential role of social stressors in modifying the relationship between neurodevelopmental outcomes and early

childhood exposure. A cohort study of seven-year-old children suggested that social adversities including a poor learning environment and parent-child interactions were significantly associated with a moderate decrease in the intelligence quotient (IQ) score and its subset scores.[17] Several recent studies have also discussed the effects of parent-child interactions, focusing on the effects of alexithymia in mothers[18] and of depressive symptoms on parenting stress.[19] These lines of evidence suggest that factors influencing children's neurodevelopment have multifaceted aspects extending from the prenatal period to early childhood. Accumulating knowledge gained from epidemiological studies suggests the need to investigate postnatal influences, such as the effects of interactions between mothers and their children, as well as prenatal exposures on the neurodevelopment of children.

On the other hand, the establishment of a methodology for exposure assessments during early childhood is also urgently required. Although the central nervous system develops rapidly during early childhood, the amount of exposure to environmental chemical substances taken into the bodies of infants has rarely been investigated because most infants wear diapers that fully absorb all urine. Noninvasive biomonitoring using urine samples is required for exposure assessments during early childhood, and a methodology for extracting urine from used disposable diapers has also been studied.[20 21]

Furthermore, public health services in local communities must be capable of providing early intervention support for children with neurodevelopmental disorders. Preventive screening for the detection of disorders during the critical period of brain development during early childhood can help to minimize later difficulties and to improve the trajectory of subjects with ADHD in later life. A validated screening checklist for autism at the age of 2 years, called M-CHAT-R/F,[22] is also available as part of early medical checkups, but this screening protocol requires a 2-step screening test that must be conducted by a physician. The development of

secondary tools allowing easy and objective screening would provide a reliable means of detecting disabilities at earlier stages, enabling the provision of early intervention support for children with disabilities. One solution for earlier screening might be the second to fourth digit (2D:4D) ratio, which is defined as the proportion between the lengths of the index and ring fingers. Recent studies[23 24] have investigated whether the 2D:4D ratio, a controversial but commonly used proxy marker of prenatal androgen concentrations based on the extreme male brain theory, is associated with ASD. If anthropometric differences in the 2D:4D ratio could be confirmed at an earlier stage of childhood, the 2D:4D ratio might be available as an alternative and indirect screening measure. Another recent trend for early intervention can be found in neonatal oral-motor assessments, such as sucking behavior. While contradictory findings have been obtained,[25] a recent longitudinal study also showed interesting evidence that some sucking behaviors in preterm infants are associated with later abnormal neurodevelopmental outcomes at the age of 2 years.[26] As the available early screening tools for the prediction of later neurodevelopmental outcomes are presently insufficient, further research on the development of screening tools for early intervention is needed.

The JECS-A was established in 2011 and has four main objectives: (1) to ascertain the effects of prenatal and postnatal chemical exposures on pregnant women and their children; (2) to investigate potential exposures for which the neurodevelopmental outcomes were modified by the mothers' interactions with their children during early childhood; (3) to develop a biomonitoring method using urine samples extracted from diapers for measuring internal exposure to chemical substances; and (4) to ascertain the availabilities of secondary tools such as the 2D:4D ratio or neonatal oral-motor assessments at earlier stages of childhood. This article outlines the JECS-A cohort and its baseline data to date.

COHORT DESCRIPTION

Setting

The JECS is an ongoing nationwide birth cohort study with a total of 100,000 children and their parents across 15 regions in Japan.[27 28 29] To identify harmful factors in the environment affecting children's growth and health, a longitudinal study was planned and will be conducted from the fetal stage until the age of 13 years. An interdisciplinary team composed of toxicologists, psychologists, epidemiologists, hygienists, pediatricians, obstetricians and gynecologists, biostatisticians, and ergonomists at the Aichi Regional Center of the JECS designed the JECS-A as part of the JECS cohort. The study areas covered by the Aichi Regional Center of the JECS consist of Ichinomiya City (population of 387,000 in 2012) and Kita-ward in Nagoya City (population of 165,000 in 2012). Nagoya City consists of 16 wards with a population of over 2 million and is Japan's third largest industrial metropolis, next to Tokyo and Osaka. Kita-ward is in the northern part of Nagoya City. Ichinomiya City neighbors Nagoya City and traditionally was known as an area involved in textile production but is now a regional commercial and residential area with a mixed economy of manufacturing and agriculture. Both areas are relatively urban and widely known in the automobile and ceramics industries.

Figure 1 shows a flowchart depicting each stage of study recruitment. A community-based recruitment strategy at 32 obstetric hospitals and clinics providing medical care for pregnant women in the study areas was adopted. Women in the early stage of pregnancy who visited an obstetrics facility were invited to participate in the JECS if they met the following criteria: (1) residence within the study areas, (2) an estimated delivery date after August 2011, and (3) an ability to read and write the Japanese language so as to complete the self-administered questionnaire. Of the 8,134 pregnant women who were invited as eligible recruits during the

recruitment period from January 2011 to March 2014, a total of 5,721 (70.3%) participants were enrolled as the baseline cohort of pregnant women.

After recruitment, the registered JECS participants were also invited to participate in the JECS-A adjunct studies if they provided any of the following three types of consent: agreement to complete the adjunct questionnaire survey only (Sub-cohort A1, n = 3,426), agreement to provide maternal biological specimens in addition to agreeing to participate in Sub-cohort A1 (Sub-cohort A2, n = 2,924), and agreement to undergo genetic analyses in addition to agreeing to participate in Sub-cohorts A1 and A2 (Sub-cohort A3, n = 1,753). Subsequently, of the 5,555 children, including 49 pairs of twins, born from the enrolled mothers, 57 children dropped out of the study because of infant death (n = 7), mother death (n = 1), withdrawal of consent (n = 48), or a change in residence to an area outside the surveyed area (n = 1). On the other hand, 4 children who moved into the surveyed area from another area were included. As of 18 months after delivery, a total of 5,502 children remained within the JECS-A cohort, accounting for approximately 40% of the children born in the study area.

A second recruitment for the JECS-A adjunct studies was also planned, focusing on children. We approached the guardians of targeted children at legal check-ups for 18-month-olds provided at regional public health centers and health consultation centers. The recruitment period was divided into two phases. Of the 5,502 children remaining in the JECS-A cohort at 18 months of age, a total of 2,576 children had reached the age of 18 months during the second year of the 3-year recruitment period. During this second-year period, we conducted a survey of the anthropometric measurements of the hands of 18-month-old children to confirm the applicability of the 2D:4D ratio during early childhood. By gradually increasing the number of recruitment facilities capable of obtaining the consent of the children's guardians during this period, a total of 1,357 children (coverage of target samples: 52.7%, consent rate: 99.0%)

were enrolled in Sub-cohort C. Subsequent to this recruitment, another recruitment was also set for 18-month-old children born from mothers registered during the last year of the pregnant women recruitment (Sub-cohort B). This cohort was specifically planned to collect biological samples from diapers, and written informed consent for the adjunct survey involving the provision of biological specimens from each child was obtained from 1,192 children (82.9%). All the enrolled children had reached the age of 1.5 years as of June 2016. The follow-up schedule for children beyond the age of 3 years has yet to be finalized, but these sub-cohorts will be followed until the children reach the age of 13 years. Note that the above figures were based on the data set jecs-ag-20160424, which was released in June, 2016, and on the provisional data set determined as of August 1, 2018.

Patient and Public Involvement

To develop the JECS-A cohort, we have established a system for public involvement in research, organizing an annual advisory committee consisting of the representatives from local government, medical association, nursing association, women's group, lawyers and mass media. The role of the committee offers advice and research questions from public concern, as members of an external project supporting group. Moreover, the committee has great contribution not only to sharing knowledge or engaging and creating a dialogue with the public, but also to playing a valuable role in advising on recruitment of participants and suggesting ideas for conducting the research. We also have held an open lecture for the participants every year since 2012, as an opportunity in which information and knowledge about research is provided and disseminated.

Data collection and measurements

The measurements collected for the adjunct studies of JECS in the JECS-A are summarized in **Table 1**. Data acquisition for some socio-demographics and neurodevelopmental outcomes was planned within the main body of the JECS.

The adjunct cohorts were designed for four specific main objectives. First, to challenge the multifaceted problems surrounding children's development, Sub-cohort A1 was formed with the intention of collecting data on longitudinal changes in prenatal and postnatal exposures considered to affect neurodevelopmental outcomes. One of our special concerns is the effect of alexithymia in mothers on early child development. The 20-item Toronto Alexithymia Scale (TAS-20)[30] will be used to measure this trait as a primary factor, and studies will investigate the pregnancy and perinatal outcomes and/or the developmental outcomes of postnatal infants. As there are many potential determinants extending from the prenatal to early childhood period, the risks will be estimated using the mother's and child's demographic variables or other related factors as confounding factors.

Sub-cohort A1 will also be used to examine the relationship between neonatal oral-motor assessments and subsequent abnormal neurodevelopmental outcomes. Using the short version of Infant Behavior Questionnaire (IBQ) and Early Childhood Behavior Questionnaire (ECBQ) as factors reflecting temperamental self-regulation, the utility of such assessments as early screening tools for predicting subsequent neurodevelopmental outcomes will be verified.

Sub-cohorts A2 and A3 will mainly focus on studies using biological specimens (urine, serum, and/or whole blood) obtained from pregnant women. These biological samples will be used for exposure assessments, single nucleotide polymorphism analyses, and activity determinations of chemical metabolism-related enzymes, and so on. Data on the levels of urinary exposure biomarkers including chemical metabolites will be combined with those in sub-cohort B and, eventually, the longitudinal exposure trajectory and the relationships

between exposure and health outcomes will also be analyzed. These cohorts will be used to derive human biomonitoring reference values (e.g., RV95) for chemicals in urine samples for the general population of Japanese pregnant women.

Sub-cohort B will be used to clarify the chemical exposure levels in infants and toddlers. Biological monitoring data for chemical exposure during early childhood are limited because the collection of urine from non-toilet-trained children is difficult. To estimate the amount of exposure to environmental chemical substances in the bodies of infants, the sub-cohort will be used to study a noninvasive biomonitoring approach using urine samples extracted from used disposable diapers. This cohort will also be used to determine the reference values for chemicals in urine samples for the general population of Japanese young children.

Sub-cohort C will be used to study anthropometric measurements of the lengths of digits on both hands in children. A previous study suggested that autistic children aged 2 to 14 years have a smaller 2nd digit to 4th digit (2D:4D) ratio than normal children of the same generation.[31] However, little is known about whether the 2D:4D ratio in Japanese infants and children under the age of 2 years can be used for the early diagnosis of autism, and the results of studies are contradictory. Thus, Sub-cohort C was designed to investigate the longitudinal changes in 2D:4D ratios in 18-month-old and 36-month-old infants. As for the anthropometric measurements of the palms and digits of the children's hands, we have established a specialized protocol involving photographic records and digit measurements that has a high reliability (Intra/Inter-Class Correlations: ICC_1 = 0.97 [95% CI, 0.87-0.99], ICC_3 = 0.93 [0.83-0.98]). In short, our easy-to-use photocopying method, which involves placing the child's palm on a box composed of transparent acrylic thin plates and photographing it from a digital camera fixed inside the box, was devised for application in mandatory health checkup settings. All the 2D:4D ratio data will be obtained using this protocol.

The JECS-A sub-cohorts do not include any information regarding medical diagnoses affecting neurodevelopmental outcomes at this time, but such information will be available in the future through the main data of the JECS, which is being conducted under the same protocol across 15 regions in Japan.

FINDINGS TO DATE

Table 2 shows the main socio-demographic characteristics of the pregnant women. To ascertain whether the JECS-A baseline profiles are representative of the pregnant women in the study areas in general, the national statistics for Aichi prefecture including Nagoya City and Ichinomiya City (since data for smaller areas were not available) were substituted and included in the table. Similar distribution trends for variables such as "Age at delivery" were confirmed when the JECS-A baseline cohort of pregnant women and the national statistics were compared; however, differences in "Education level" and "Household income" were observed. Regarding education level, the percentage of JECS participants who had graduated from high school was relatively low, compared with the national statistics (25.1% vs. 39.7%), while the percentage of college/junior college/technology college students was higher among the JECS participants, compared with the national statistics (35.3% vs. 20.8%). Moreover, the number of JECS-A participants with a household income of 2 million yen or less was about 1/8 of the national statistics. Likewise, unemployed participants (mainly housewives) accounted for 45% of the JECS-A cohort. These results suggest a possible selection bias. We recruited pregnant women who had visited the obstetric facilities during the daytime; this might have resulted in a lower study participation rate among pregnant employees.

As for the baseline cohort of children, a total of 5,502 (99.0%) of the 5,555 children remained in the JECS-A at 18 months after delivery (provisional figures as of August 1, 2018). **Table 3**

compares the baseline data of the newborns with the national statistics. Basic demographics including “Sex”, “Birth weight” and “Birth height” had similar distributions, indicating the representativeness of the sample.

Similarly, **Table 4** shows the fundamental characteristics of the pregnant women and their children in each sub-cohort of the JECS-A. No notable differences in the descriptive statistics were observed among the sub-cohorts. Furthermore, the drop-out rates for each sub-cohort, to date, have been maintained at less than 10% relative to the baseline. However, 10% of the sub-cohort values for household income are missing because many participants did not provide information on the income. Therefore, caution is needed when using this variable as a confounding factor in multiple regression analyses.

STRENGTHS AND LIMITATIONS

The main strength of the JECS-A of children is its large sample size, reflecting a representative population. Approximately 40% of the children born in the study area have been included in the study to date. The participating children will be followed until they reach the age of 13 years. The study protocol[32] of the JECS decided on a target retention rate of 80% or higher at the age of 13 years. The linkage between the JECS-A sub-cohort and the main data of the JECS study will further allow novel and challenging studies with a high generalizability.

The main weakness of the JECS-A cohort concerns the pregnant women participants. A relatively large proportion of single-income, middle-class households were included in the JECS-A; many low-income households refused to participate in the surveys, and some participants did not provide information concerning their household income. Regarding the latter, the impact of the missing values can be adjusted to some extent using the multiple imputation method. However, researchers must take into account the effect of response biases

when conducting statistical analyses using paired data for pregnant women and their children and when interpreting these results.

COLLABORATION

The original data and specimens will be made available to investigators and stakeholders working within the JECS-A project. The study must adhere to the JECS policy on the availability of research results, publications, intellectual property rights, and data sharing. At the moment, the Ethical Guidelines for Medical and Health Research Involving Human Subjects enforced by the Japan Ministry of Education, Culture, Sports, Science and Technology and the Ministry of Health, Labour and Welfare restrict the open sharing of epidemiologic data. This means that researchers interested in using the data must collaborate with and participate in the JECS-A project. This project was recently started and is still ongoing. We are open to new proposals that fall within the nature of the JECS-A cohort.

Acknowledgements

We would like to express our gratitude to all the JECS-A cohort participants. We also express our sincere appreciation to the collaborating hospitals, clinics, local healthcare centers, local government agencies in Nagoya City and Ichinomiya City, and the JECS-A staff members for their continuous assistance in promoting this study. The authors also specially thank Takaaki Hiraiwa, Hiroki Yoshizoe, Maiko Miyata, Hirohisa Kano and Takahiro Kurihara for the data management and their assistance in the data analysis.

Contributors

TE designed the JECS-A cohort architecture, developed the protocol, analyzed the data, and

wrote the first draft of the manuscript. YY and MT organized the study team, obtained approvals and contributed to the development of the protocol, design, and data collection tools. TOm was in charge of coordination with relevant organizations and organized the JECS-A members. NS, SK, TMa and TOg performed the analysis and interpreted the data. YI, HS, NO and JU designed and developed the protocol for the JECS-A sub-cohorts A2, A3 and B and edited and analyzed the data. AN, MK, YO, TMi, SSu, MSO and SSa designed each adjunct study, supervised the data collection, and drafted the manuscript. MK was a member of the JECS Steering Committee, was responsible for the study design and protocol, supervised the data collection, and edited and drafted the manuscript. All the authors interpreted the data, contributed to the writing of the manuscript, revised it critically for important intellectual content, and agreed with the final version and the findings.

Funding The Japan Environment and Children's Study was funded by the Ministry of the Environment, Japan. This work was also supported by grants from the Environment Research and Technology Development Fund (ERTDF, No 5-1551 and 5-1851), Japan Society for the Promotion of Science (JSPS) KAKENHI Grant Number 16H03733, 25293151, 25285185 and Grants-in-aid for research of Nagoya City University (2011-6, 2012-4, 2013-2, 2013-10, 2014-4, 2015-10, 2016-4).

Competing Interests None declared

Ethical approval This study was approved by the Review Board of the Ministry of the Environment, by the Institutional Review Board of Nagoya City University Graduate School of Medical Sciences (No. 70-00-0134; No. 00000544-6, 00000574-2) and by the Ethics

Committees of all participating institutions.

Data sharing statement The original data and specimens will be made available to investigators and stakeholders working within the JECS-A project.

Researchers interested in collaborations or further information should contact the JECS-A secretariat by e-mail at eisei@med.nagoya-cu.ac.jp.

Disclaimer The findings and conclusions of this article are solely the responsibility of the authors and do not represent the official views of the Japanese government.

REFERENCES

1. Ishitsuka K, Nakayama SF, Kishi R, et al. Japan Environment and Children's Study: backgrounds, activities, and future directions in global perspectives. *Environ Health Prev Med* 2017;22(1):61. doi: 10.1186/s12199-017-0667-y
2. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders (DSM–5): American Psychiatric Association Publishing 2013:xiii-xv.
3. Xiang S, Carrie A. A review of the prevalence of Autism Spectrum Disorder in Asia, Research in Autism Spectrum Disorders. *Autism Spectrum Disorders* 2010;4(2):156-67. doi: http://dx.doi.org/10.1016/j.rasd.2009.10.003.
4. Hansen SN, Schendel DE, Parner ET. Explaining the increase in the prevalence of autism spectrum disorders: the proportion attributable to changes in reporting practices. *JAMA Pediatr* 2015;169(1):56-62. doi: 10.1001/jamapediatrics.2014.1893
5. Rodriguez-Barranco M, Lacasana M, Aguilar-Garduno C, et al. Association of arsenic,

cadmium and manganese exposure with neurodevelopment and behavioural disorders in children: a systematic review and meta-analysis. *Sci Total Environ* 2013;454-455:562-77. doi: 10.1016/j.scitotenv.2013.03.047

6. Rodrigues EG, Bellinger DC, Valeri L, et al. Neurodevelopmental outcomes among 2- to 3-year-old children in Bangladesh with elevated blood lead and exposure to arsenic and manganese in drinking water. *Environ Health* 2016;15:44. doi: 10.1186/s12940-016-0127-y

7. Melchior M, Hersi R, van der Waerden J, et al. Maternal tobacco smoking in pregnancy and children's socio-emotional development at age 5: The EDEN mother-child birth cohort study. *Eur Psychiatry* 2015;30(5):562-8. doi: 10.1016/j.eurpsy.2015.03.005

8. Joelsson P, Chudal R, Talati A, et al. Prenatal smoking exposure and neuropsychiatric comorbidity of ADHD: a finnish nationwide population-based cohort study. *BMC Psychiatry* 2016;16:306. doi: 10.1186/s12888-016-1007-2

9. Han JY, Kwon HJ, Ha M, et al. The effects of prenatal exposure to alcohol and environmental tobacco smoke on risk for ADHD: a large population-based study. *Psychiatry Res* 2015;225(1-2):164-8. doi: 10.1016/j.psychres.2014.11.009

10. Padron A, Galan I, Garcia-Esquinas E, et al. Exposure to secondhand smoke in the home and mental health in children: a population-based study. *Tob Control* 2016;25(3):307-12. doi: 10.1136/tobaccocontrol-2014-052077

11. Polanska K, Ligocka D, Sobala W, et al. Phthalate exposure and child development: the Polish Mother and Child Cohort Study. *Early Hum Dev* 2014;90(9):477-85. doi: 10.1016/j.earlhumdev.2014.06.006

12. Kyriklaki A, Vafeiadi M, Kampouri M, et al. Prenatal exposure to persistent organic pollutants in association with offspring neuropsychological development at 4years of

age: The Rhea mother-child cohort, Crete, Greece. *Environ Int* 2016;97:204-11. doi: 10.1016/j.envint.2016.09.012

13. Goudarzi H, Nakajima S, Ikeno T, et al. Prenatal exposure to perfluorinated chemicals and neurodevelopment in early infancy: The Hokkaido Study. *Sci Total Environ* 2016;541:1002-10. doi: 10.1016/j.scitotenv.2015.10.017
14. Yolton K, Cornelius M, Ornoy A, et al. Exposure to neurotoxicants and the development of attention deficit hyperactivity disorder and its related behaviors in childhood. *Neurotoxicol Teratol* 2014;44:30-45. doi: 10.1016/j.ntt.2014.05.003
15. Engel SM, Wetmur J, Chen J, et al. Prenatal exposure to organophosphates, paraoxonase 1, and cognitive development in childhood. *Environ Health Perspect* 2011;119(8):1182-8. doi: 10.1289/ehp.1003183
16. Bouchard MF, Chevrier J, Harley KG, et al. Prenatal exposure to organophosphate pesticides and IQ in 7-year-old children. *Environ Health Perspect* 2011;119(8):1189-95. doi: 10.1289/ehp.1003185
17. Stein LJ, Gunier RB, Harley K, et al. Early childhood adversity potentiates the adverse association between prenatal organophosphate pesticide exposure and child IQ: The CHAMACOS cohort. *Neurotoxicology* 2016;56:180-87. doi: 10.1016/j.neuro.2016.07.010
18. Yurumez E, Akca OF, Ugur C, et al. Mothers' alexithymia, depression and anxiety levels and their association with the quality of mother-infant relationship: a preliminary study. *Int J Psychiatry Clin Pract* 2014;18(3):190-6. doi: 10.3109/13651501.2014.940055
19. Li Y, Jiang WQ, Du YS, et al. Relationships between behavioral symptoms of non-medicated Chinese children with attention deficit hyperactivity disorder and parenting stress: Comparison of different subtypes and comorbidities. *Asia Pac Psychiatry*

2016;8(2):127-35. doi: 10.1111/appy.12213

20. Oya N, Ito Y, Hioki K, et al. Quantitative analysis of organophosphate insecticide metabolites in urine extracted from disposable diapers of toddlers in Japan. *Int J Hyg Environ Health* 2017;220(2 Pt A):209-16. doi: 10.1016/j.ijheh.2016.10.009

21. Saito S, Ueyama J, Kondo T, et al. A non-invasive biomonitoring method for assessing levels of urinary pyrethroid metabolites in diapered children by gas chromatography-mass spectrometry. *J Expo Sci Environ Epidemiol* 2014;24(2):200-7. doi: 10.1038/jes.2013.31

22. Robins DL, Casagrande K, Barton M, et al. Validation of the modified checklist for Autism in toddlers, revised with follow-up (M-CHAT-R/F). *Pediatrics* 2014;133(1):37-45. doi: 10.1542/peds.2013-1813

23. Teatero ML, Netley C. A critical review of the research on the extreme male brain theory and digit ratio (2D:4D). *J Autism Dev Disord* 2013;43(11):2664-76. doi: 10.1007/s10803-013-1819-6

24. Guyatt AL, Heron J, Knight Ble C, et al. Digit ratio and autism spectrum disorders in the Avon Longitudinal Study of Parents and Children: a birth cohort study. *BMJ Open* 2015;5(8):e007433. doi: 10.1136/bmjopen-2014-007433

25. Slattery J, Morgan A, Douglas J. Early sucking and swallowing problems as predictors of neurodevelopmental outcome in children with neonatal brain injury: a systematic review. *Dev Med Child Neurol* 2012;54(9):796-806. doi: 10.1111/j.1469-8749.2012.04318.x

26. Wolthuis-Stigter MI, Luinge MR, da Costa SP, et al. The association between sucking behavior in preterm infants and neurodevelopmental outcomes at 2 years of age. *J Pediatr* 2015;166(1):26-30. doi: 10.1016/j.jpeds.2014.09.007

27. Kawamoto T, Nitta H, Murata K, et al. Rationale and study design of the Japan environment and children's study (JECS). *BMC Public Health* 2014;14:25. doi: 10.1186/1471-2458-14-25

28. Michikawa T, Nitta H, Nakayama SF, et al. The Japan Environment and Children's Study (JECS): A Preliminary Report on Selected Characteristics of Approximately 10 000 Pregnant Women Recruited During the First Year of the Study. *J Epidemiol* 2015;25(6):452-8. doi: 10.2188/jea.JE20140186

29. Michikawa T, Nitta H, Nakayama SF, et al. Baseline Profile of Participants in the Japan Environment and Children's Study (JECS). *J Epidemiol* 2018;28(2):99-104. doi: 10.2188/jea.JE20170018

30. Moriguchi Y, Maeda M, Igarashi T, et al. Age and gender effect on alexithymia in large, Japanese community and clinical samples: a cross-validation study of the Toronto Alexithymia Scale (TAS-20). *Biopsychosoc Med* 2007;1:7. doi: 10.1186/1751-0759-1-7

31. Manning JT, Baron-Cohen S, Wheelwright S, et al. The 2nd to 4th digit ratio and autism. *Dev Med Child Neurol* 2001;43(3):160-4.

32. National Centre for Japan Environment and Children's study. Japan Environment and Children's study (JECS), Study Protocol (ver. 1.4) [Available from: https://www.env.go.jp/en/chemi/hs/jecs/data/about/jecs-study_protocol_14_en.pdf.

Table 1 Summary of measurements collected for the adjunct JECS-A study

Main items		Sub-cohort covered					Timing of measurement						
							prenatal (weeks)		postnatal (months after birth)				
		A1	A2	A3	B	C	<22	≥22	1 m	6 m	18 m	24 m	36 m
Pregnant women													
Risk and confounding factors													
Socio-demographic *	Age, Maternal age, Marital status, Household income, Education level	●	●	●	●	●	●						
Lifestyle *	Smoking, Second-hand smoking, Drinking habits, Dietary status, Sleeping habits	●	●	●	●		●	●	●	●	●	●	●
Psychological and psychiatric	TAS–20	●					●	●					
	POMS-SF	●					●						
	Readiness of Parenthood Scale	●					●	●	●	●			
Obstetric *	History of pregnancy, infertility treatment, prenatal diagnosis and gestational duration	●											
Biomonitoring data	Urine, serum, umbilical cord blood		●	●	●		●	●					
Children													
Risk and confounding factors													
Demographic*	Height, Weight, BMI, Sex	●	●	●	●	●							
Behavioral	Neonatal oral-motor assessment	●							●				
	DCDQ	●								●		●	
Childcare*	Breast feeding and weaning food status	●								●			
Psychological and psychiatric	Mary Rothbart's Temperament Questionnaires	●											
	・IBQ-R-SF									●			
	・ECBQ-SF											●	
Biomonitoring data	Urine extracted from disposable diapers				●						●		●
Anthropometric data	2D:4D					●					●		●
Outcome examples possibly to be analyzed													
LBW *			●	●					●				
ASD *		●	●	●	●	●							●
ADHD *		●	●	●	●								●

*Refer to the Japan Environment and Children's Study (JECS)

BMI, body mass index; **TAS-20**, the 20–item Toronto Alexithymia Scale; **POMS-SF**, Short form of the Profile of Mood States; **IBQ-R-SF**, The Infant Behavior Questionnaire Revised Short Form; **ECBQ-SF**, Early Childhood Behavior Questionnaire Short Form; **DCDQ**, Developmental Coordination Disorder Questionnaire, **2D:4D**, the second to fourth digit ratio; **PARS**, Pervasive Developmental Disorders Autism Society Japan Rating Scale; **ASD**, autism spectrum disorder; **ADHD**, attention deficit hyperactivity disorder; LBW, low birth weight; **<22 weeks**, Early pregnancy defined in the JECS is a gestational age of less than 22 weeks; **>=22 weeks**, Late pregnancy defined in the JECS is a gestational age of more than 22 weeks.

Sub cohort A1, Agreement on the adjunct questionnaire survey only; **Sub cohort A2**, Agreement on the adjunct questionnaire survey and provision of mother's biological specimen (urine); **Sub cohort A3**, Agreement on the adjunct questionnaire survey and provision of mother's biological specimen (urine) with gene analysis; **Sub cohort B**, Obtaining informed consent for provision of child's biological specimen from guardian (legally acceptable representatives); **Sub cohort C**, Obtaining informed consent for anthropometry measurement on child hands from guardian (legally acceptable representatives)

Table 2 Comparison with national statistics in Aichi prefecture and JECS-A baseline data for pregnant women: verification of representativeness of the sample

	JECS-A baseline cohort of pregnant women (n = 5,721)	National statistics (Aichi pref.)
Pregnant women [a] (n)	5721	65218
Age at Delivery [a]		
15-19 (%)	0.7	1.3
20-24	6.7	8.2
25-29	25.3	28.3
30-34	36.4	36.6
35-39	23.2	21.4
40-44	4.9	4.2
45-49	0.1	0.1
50-	0.0	0.0
Missing (n)	157	
Education level [b] (n)		2397000
Junior high school/High school (%)	29.9	47.2
College/Junior college/Technology college	35.3	20.8
University	27.6	28.4
Graduate school	1.6	3.6
Missing (n)	315	
Household income [b c d] (n)		3018900
<2,000 (%)	2.4	18.1
2,000-<4,000	24.4	26.7
4,000-<6,000	33.1	20.2
6,000-<8,000	17.2	13.4
8,000-<10,000	7.4	8.3
≥10,000	4.3	5.0
Missing (n)	643	

[a] Data of Aichi prefecture as reference, provided by national vital statistics, Ministry of Health, Labour and Welfare in 2014.

[b] Data of Aichi prefecture provided by Ministry of Internal Affaires and Communications in 2012.

[c] Population of Aichi Prefecture including single-person households and families

[d] Household income shows thousand Japanese Yen(JPY), 110 JPY = 1US$ as of 2017

-: no available data provided; Household income shows thousand Japanese Yen(JPY), 110 JPY = 1US$ as of 2017.

Table 3 Comparison with national statistics in Aichi prefecture and JECS-A baseline data for children: verification of representativeness of the sample

	JECS-A baseline cohort of children (n = 5457)			National statistics (Aichi pref.) [a]		
	Total	Male	Female	Total	Male	Female
Children (n) [b]	5457	2793	2659	65218	33649	31569
Sex (Singleton births)						
(%)	99.9	51.2	48.7		51.6	48.4
Missing (n)	5					
Birth weight, g (Singleton births)						
Mean	3034.3	3076.1	2990.7	3000.0	3040.0	2950.0
SD	427.0	439.8	408.6	-	-	-
Missing (n)	43	23	16			
Low birth weight (Singleton births)						
<2500 g (%)	7.8	6.6	9.0	9.8	8.5	11.1
Missing (n)	43	23	16			
Birth height, cm (Singleton births)						
Mean	49.7	49.9	49.5	49.3	49.6	49.1
SD	2.3	2.4	2.1	-	-	-
Missing (n)	51	28	19			

[a] Data of Aichi prefecture as reference, provided by national vital statistics, Ministry of Health, Labour and Welfare in 2014.

[b] Multiple births (49 pairs of twins) were excluded.

-: no available data provided

Table 4 Baseline characteristics of paired data of pregnant women and their children in the JECS-A cohort

	Sub-cohort				
	A1	A2	A3	B	C
Pregnant women (n)	3426	2924	1753	1187	1352
Age at Delivery					
Mean	31.6	31.7	31.8	32.0	31.8
SD	4.9	4.9	5.0	4.7	4.8
Missing (n)	44	14	7	0	0
Smoking habits					
Never smoked (%)	62.8	63.7	63.7	64.6	64.2
Ex-smokers who quit before pregnancy	21.6	22.0	23.1	23.1	21.8
Smokers during early pregnancy	9.0	9.0	8.3	7.7	8.4
Smokers	2.5	2.5	2.1	2.2	2.8
Missing (n)	144	82	51	29	38
Second-hand smoking					
Rarely (%)	67.5	69.1	69.6	71.9	68.6
A few days a week	18.6	18.6	18.0	17.1	19.1
Daily	10.4	10.1	10.3	9.6	10.1
Missing (n)	124	66	36	17	30
Household income (JPY)					
<2,000 (%)	2.3	2.1	2.5	2.1	1.8
2,000-<4,000	25.8	26.0	25.6	24.3	24.4
4,000-<6,000	33.4	33.7	34.2	35.9	35.1
6,000-<8,000	17.5	18.1	17.5	18.6	18.6
8,000-<10,000	7.3	7.5	7.8	8.3	7.5
≥10,000	4.5	4.7	4.5	4.5	3.9
Missing (n)	314	233	141	76	116

Children (n)[a]	3336	2884	1726	1182	1346
Sex (Singleton births)					
Male (%)	50.5	50.4	50.1	50.4	49.9
Female	49.5	49.6	49.8	49.6	50.1
Missing (n)	1	1	1	0	0
Birth weight, g (Singleton births)					
Mean	3041.6	3052.3	3054.1	3062.3	3039.1
SD	432.8	419.9	430.4	416.7	410.0
Missing (n)	22	15	9	7	4
Birth height, cm (Singleton births)					
Mean	49.6	49.7	49.7	49.7	49.7
SD	2.3	2.2	2.2	2.1	2.1
Missing (n)	28	18	11	8	6

[a] Multiple births (twin pairs) were excluded; A1: 20 (n = 40), A2: 16 (n = 32), A3: 12 (n = 24), B: 5 (n = 10), and C: 6 (n = 12).

Sub cohort A1, Agreement on the adjunct questionnaire survey only; **Sub cohort A2**, Agreement on the adjunct questionnaire survey and provision of mother's biological specimen (blood and urine); **Sub cohort A3**, Agreement on the adjunct questionnaire survey and provision of mother's biological specimen (blood and urine) with genetic analysis; **Sub cohort B**, Obtaining informed consent for provision of child's biological specimen from guardian (legally acceptable representatives); **Sub cohort C**, Obtaining informed consent for anthropometry measurement on child hands from guardians (legally acceptable representatives). Household income shows thousand Japanese Yen (JPY), 110 JPY = 1 US$ as of 2017

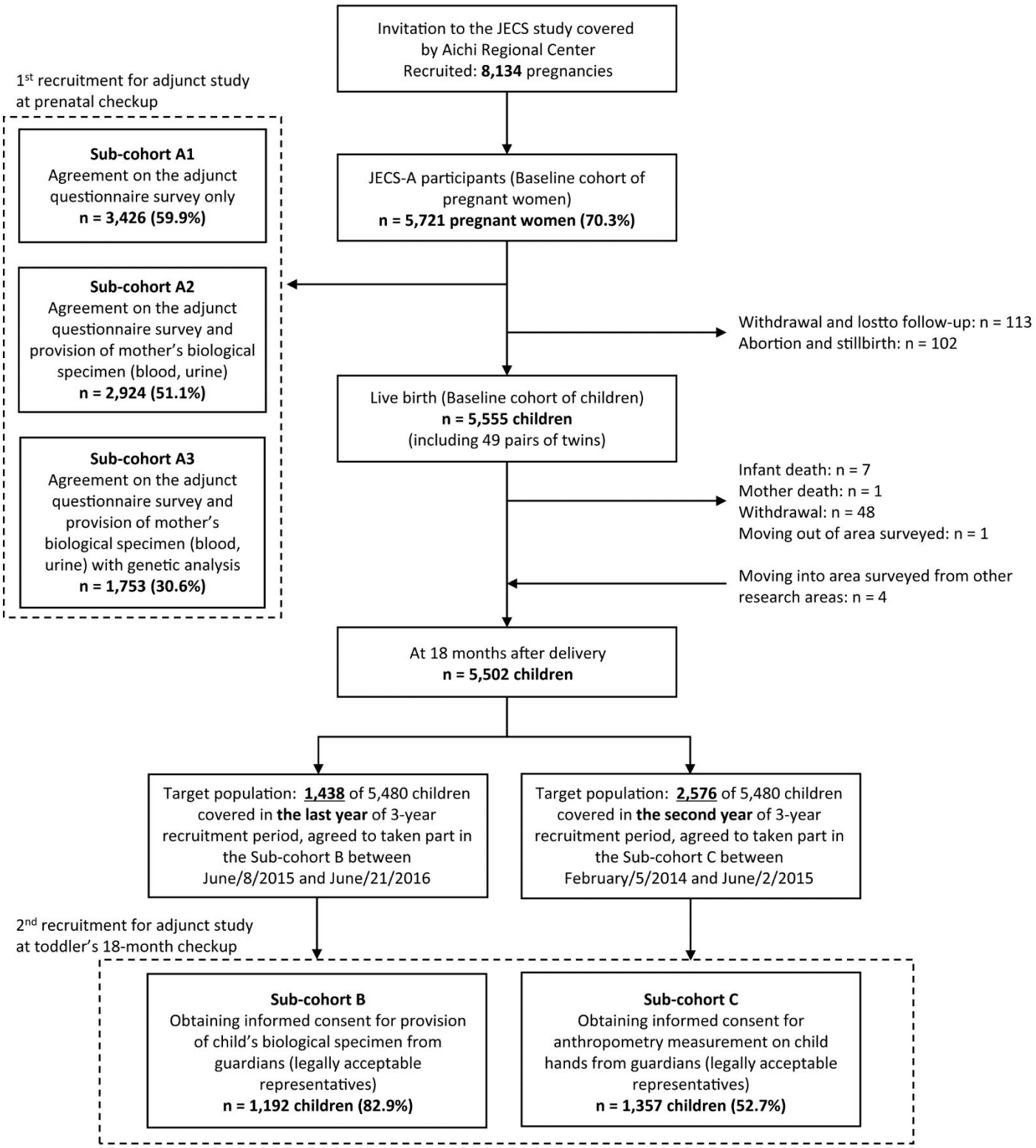

Figure 1. Flowchart showing each stage of study recruitment

BMJ Open

Cohort profile: Aichi regional sub-cohort of the Japan Environment and Children's Study (JECS-A)

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-028105.R1
Article Type:	Cohort profile
Date Submitted by the Author:	29-May-2019
Complete List of Authors:	Ebara, Takeshi; Nagoya City University, Occupational and Environmental Health, Graduate school of Medical Sciences Yamada, Yasuyuki; Nagoya City University Graduate School of Medical Sciences and Medical School, Department of Occupational and Environmental Health; Juntendo University School of Health and Sports Science Graduate School of Health and Sports Science Shoji, Naoto; Nagoya City University Graduate School of Medical Sciences and Medical School, Department of Occupational and Environmental Health; Asahi University, School of Health Sciences Ito, Yuki; Nagoya City University Graduate School of Medical Sciences and Medical School, Department of Occupational and Environmental Health Nakagawa, Atsuko; Nagoya City University Faculty of Humanities and Social Sciences Graduate School of Humanities and Social Sciences Miyachi, Taishi; Nagoya City University Graduate School of Medical Sciences and Medical School, Pediatrics and Neonatology; Nagoya West District Care Center for Disabled Children Ozaki, Yasuhiko; Nagoya City University Graduate School of Medical Sciences and Medical School, Obstetrics and Gynecology Omori, Toyonori; Nagoya City University Graduate School of Medical Sciences and Medical School, Health Care Policy and Management Suzuki, Sadao; Nagoya City University Graduate School of Medical Sciences, Department of Public Health Kojima, Masayo ; Nagoya City University Graduate School of Medical Sciences and Medical School, Medical Education Ueyama, Jun; Nagoya University Graduate School of Medicine Faculty of Medicine, Department of Pathophysiological Laboratory Sciences, Field of Radiological and Medical Laboratory Sciences Tomizawa, Motohiro; Nagoya City University Graduate School of Medical Sciences and Medical School, Department of Occupational and Environmental Health; Tokyo University of Agriculture Kato, Sayaka; Nagoya City University Graduate School of Medical Sciences and Medical School, Department of Occupational and Environmental Health; Nagoya City University Graduate School of Medical Sciences and Medical School, Pediatrics and Neonatology Oguri, Tomoko; Nagoya City University Graduate School of Medical Sciences and Medical School, Department of Occupational and Environmental Health; National Institute of Advanced Industrial Science and Technology (AIST) Matsuki, Taro; Nagoya City University Graduate School of Medical Sciences and Medical School, Department of Occupational and

	Environmental Health Sato, Hirotaka; Nagoya City University Graduate School of Medical Sciences and Medical School, Department of Occupational and Environmental Health Oya, Naoko; Nagoya City University Graduate School of Medical Sciences and Medical School, Department of Occupational and Environmental Health Sugiura-Ogasawara , M; Nagoya City University Graduate School of Medical Sciences and Medical School, Obstetrics and Gynecology Saitoh, Shinji; Nagoya City University Graduate School of Medical Sciences and Medical School, Pediatrics and Neonatology Kamijima, Michihiro; Nagoya City University Graduate School of Medical Sciences, Department of Occupational and Environmental Health
Primary Subject Heading:	Epidemiology
Secondary Subject Heading:	Epidemiology, Paediatrics
Keywords:	JECS, birth cohort, EPIDEMIOLOGY, JECS-A

Cohort profile: Aichi regional sub-cohort of the Japan Environment and Children's Study (JECS-A)

Takeshi Ebara[1], Yasuyuki Yamada[1,9], Naoto Shoji[1,10], Yuki Ito[1], Atsuko Nakagawa[7], Taishi Miyachi[2,11], Yasuhiko Ozaki[3], Toyonori Omori[4], Sadao Suzuki[5], Masayo Kojima[6], Jun Ueyama[8], Motohiro Tomizawa[1†], Sayaka Kato[1,2], Tomoko Oguri[1††], Taro Matsuki[1], Hirotaka Sato[1], Naoko Oya[1], Mayumi Sugiura-Ogasawara[3], Shinji Saitoh[2], Michihiro Kamijima[1]

Department of [1]Occupational and Environmental Health, [2]Pediatrics and Neonatology, [3]Obstetrics and Gynecology, [4]Health Care Policy and Management, [5]Public Health, [6]Medical Education, Nagoya City University, Graduate School of Medical Sciences, Nagoya, Japan
[7]School of Humanities and Social Sciences, Nagoya City University, Nagoya, Japan
[8]Department of Pathophysiological Laboratory Sciences, Field of Radiological and Medical Laboratory Sciences, Nagoya University Graduate School of Medicine, Japan.
[9]Juntendo University Graduate School of Health and Sports Science, Chiba, Japan
[10] Asahi University, School of Health Sciences
[11]Nagoya West District Care Center for Disabled Children, Nagoya, Japan

Present affiliation: [†]Tokyo University of Agriculture, [††]National Institute of Advanced Industrial Science and Technology (AIST)

Corresponding Author:
Takeshi Ebara, PhD.
Department of Occupational and Environmental Health, Nagoya City University, Graduate School of Medical Sciences. Mizuho-ku, Nagoya, 4678601, Japan
TEL: 81-52-853-8171 FAX: 81-52-859-1228
e-mail: ebara@med.nagoya-cu.ac.jp

ABSTRACT

Purpose: Effects of fetal, perinatal and childhood environment on the health of children at birth and during later life have become a topic of concern. The Aichi regional sub-cohort of the Japan Environment and Children's Study (JECS-A) is an ongoing birth cohort of pregnant women and their children that has been used to provide unique data, as adjunct studies of JECS, on multifaceted potential factors affecting children's health.

Participants: The JECS-A is part of the Japan Environment and Children's Study (JECS) which follows a total of 100,000 pairs of children and their mothers (fathers' participation is optional) across 15 regions in Japan. In JECS-A, of the 8,134 pregnant women living in Ichinomiya City and Nagoya City, Japan, a total of 5,721 pregnant women and their 5,555 children were included. Sociodemographic and psychological data as well as biological specimens were collected from the pregnant women and their spouses (if available) in the cohort during their pregnancy. Information on children included in the JECS-A was collected from their mothers and includes demographic, behavioral, childcare, psychological and psychiatric data. Urine extracted from disposable diapers and anthropometric data were also obtained from the children.

Findings to date: A similar distribution trend for age at delivery was confirmed between the pregnant women enrolled in the JECS-A and the national statistics of the relevant areas. However, differences in education level and household income were observed. A total of 5,502 children remained in the cohort at 18 months after delivery. Compared with the national statistics, the basic demographics of the children in the cohort represented the population in the study areas.

Future plans: The enrolled children in the JECS-A will be followed until the age of 13 years. The studies that come from JECS-A will complement JECS and bring novel results with a high level of generalizability.

(300 words)

Strengths and limitations of this study

- The main strength of the JECS-A of children is its large sample size, reflecting a representative population.
- Another strength can be found in considering multifaceted potential factors affecting children's health in the prospective birth cohort over two generations.
- The main limitation of the cohort is that the population with low-income households is not included.

INTRODUCTION

The effects of fetal, perinatal and childhood environmental factors on the health of children at birth and during later life are a topic of concern. A number of birth cohort studies have thus been conducted worldwide to address this issue, and the Japan Environment and Children's Study (JECS), which focuses on the effects of environmental chemical pollutants, is one of the largest. The JECS was launched in 2011 by the Ministry of the Environment, Japan, after 3 years of planning.[1] The JECS consists of nationwide regional sub-cohorts that can be used to conduct studies, so called as adjunct studies of JECS, which are independent of the main study of the JECS. This article describes the cohort profile of a regional sub-cohort of the JECS, the Aichi regional sub-cohort of the JECS (JECS-A), containing 5,721 pregnant women and their 5,555 children. The following research themes are the main focuses of the adjunct studies conducted in JECS-A.

The first one is neurodevelopmental disorders[2] including intellectual disability, communication disorders, autism spectrum disorder (ASD), attention deficit hyperactivity disorder (ADHD), specific learning disorder, and motor disorders. A review has indicated that the estimated prevalence of ASD in Asia before 1980 was around 1.9/10,000, but this figure has recently increased by ten times.[3] Though a previous study conducted in Denmark claimed that 33% of the increase in the prevalence of ASD in recent years can be accounted for by changes in the diagnostic criteria and reporting methods,[4] this increased prevalence remains a complex and highly controversial issue that needs to be addressed. Recent epidemiological studies have linked neurodevelopmental outcomes to prenatal exposure to environmental toxicants such as heavy metals,[5,6] prenatal tobacco exposure,[7,8] environmental tobacco exposure,[9,10] phthalates,[11] persistent organic pollutants,[12,13] and organophosphate pesticides (OPs).[14,15 16] Furthermore, interest is growing regarding the potential role of social

stressors in modifying the relationship between the above early childhood exposure and the neurodevelopmental outcomes. A cohort study of seven-year-old predominantly Mexican American children in California's Salinas Valley suggested that social adversities including a poor learning environment and parent-child interactions were significantly associated with a moderate decrease in the intelligence quotient (IQ) score and its subset scores.[17] Several recent studies have also discussed the effects of parent-child interactions, focusing on the effects of alexithymia in mothers[18] and of depressive symptoms on parenting stress.[19] Thus, there is the need to investigate postnatal influences, such as the effects of interactions between mothers and their children, as well as prenatal and postnatal toxicant exposures on the neurodevelopment of children. The above research questions will be addressed to complement those of the main study of the JECS.

The second theme of the adjunct studies conducted in JECS-A is the exposure assessments using urine collected during early childhood when the central nervous system develops rapidly. So far, the amount of exposure to environmental chemical substances taken into the bodies of infants has rarely been investigated because most infants wear diapers that fully absorb all urine. Noninvasive biomonitoring using urine samples is thus required especially for exposure assessments of chemicals with short biological half lives, and a methodology for extracting urine from used disposable diapers has been investigated in our previous studies.[20 21] Since the urine during early childhood was not collected in the main study of the JECS, the adjunct studies using such urine in JECS-A will address research questions regarding environmental exposure in that period.

The third theme of the adjunct studies is the investigation of objective screening tools to detect neurodevelopmental disorders at earlier stages. Public health services in local communities must be capable of providing early intervention support for children with such

disorders. Preventive screening during early childhood can help to minimize later difficulties and to improve the trajectory of subjects with neurodevelopmental disorders in later life. For example, a validated screening checklist for autism at the age of 2 years, called M-CHAT-R/F,[22] is available as part of early medical checkups, but this screening protocol requires a 2-step screening test that must be conducted by a physician. As a secondary tool allowing easy and objective screening the second to fourth digit (2D:4D) ratio, which is defined as the proportion between the lengths of the index and ring fingers, might be useful. A previous study suggested that autistic children aged 2 to 14 years have a smaller 2D:4D ratio than normal children of the same generation.[23] Recent studies[24 25] have also investigated whether the 2D:4D ratio, a controversial but commonly used proxy marker of prenatal androgen concentrations based on the extreme male brain theory, is associated with ASD. However, this hypothetical screening method needs further scientific evidence in different epidemiological settings in different part of the globe. Another recent trial aiming to early intervention for neurodevelopmental problems can be found in neonatal oral-motor assessments, such as sucking behavior. While contradictory findings have been obtained,[26] a recent longitudinal study also showed interesting evidence that some sucking behaviors in preterm infants are associated with later abnormal neurodevelopmental outcomes at the age of 2 years.[27] As such, further research on the development of screening tools for early intervention is needed.

The JECS-A was established in 2011 as a sub-cohort of the JECS and has three main objectives: (1) to clarify the effects of prenatal and postnatal chemical exposures and social stressors on pregnant women and/or their children, especially neurodevelopmental outcomes of the children; (2) to develop a biomonitoring method using urine samples extracted from diapers for measuring internal exposure to chemical substances; and (3) to develop secondary

tools such as the 2D:4D ratio or neonatal oral-motor assessments to screen future neurodevelopmental problems at earlier stages of childhood. This article outlines the JECS-A and its baseline data to date.

COHORT DESCRIPTION

Setting

The JECS is an ongoing nationwide birth cohort study with a total of 100,000 children and their parents (father's participation is optional, but suggested) across 15 regions in Japan.[28 29 30] To identify risk factors in the environment affecting children's growth and health, the participating children are being followed from their fetal stage until the age of 13 years. The JECS has 15 regional sub-cohorts. At Aichi Regional Center of the JECS, an interdisciplinary team composed of toxicologists, psychologists, epidemiologists, public health specialists, pediatricians, obstetricians and gynecologists, biostatisticians, and ergonomists designed the JECS-A as part of the JECS cohort. The study areas (**Figure 1**) covered by the Aichi Regional Center of the JECS consist of Ichinomiya City (population of 387,000 in 2012) and Kita-ward in Nagoya City (population of 165,000 in 2012). Nagoya City consists of 16 wards with a population of over 2 million and is Japan's third largest industrial metropolis, next to Tokyo and Osaka. Kita-ward is in the northern part of Nagoya City. Ichinomiya City neighbors Nagoya City and traditionally was known as an area involved in textile production but is now a regional commercial and residential area with a mixed economy of manufacturing and agriculture. Both areas are relatively urban and widely known in the automobile and ceramics industries.

Enrollment strategy

Figure 2 shows a flowchart depicting each stage of study recruitment. A

community-based recruitment strategy was adopted at designated 32 obstetric facilities including hospitals, clinics or midwifery units providing medical care for pregnant women in the study areas. The participants living in the areas can reach any of the 32 facilities within 30 minutes by car or public transportation such as subway or train. Women in the early stage of pregnancy who visited an obstetrics facility for prenatal health care were invited to participate in the JECS if they met the following criteria: (1) residence within the study areas, (2) an estimated delivery date after August 2011, and (3) an ability to read and write the Japanese language so as to complete the self-administered questionnaire. In addition to this recruitment, we asked public health centers of the local governments to help us approach hard-to-reach groups such as low socio-economic status (SES) or reluctant pregnant women. All pregnant women receive the Maternal and Child Health Handbook at the public health centers to get complimentary municipal maternal care for pregnancy, delivery, and childcare under the Maternal and Child Health Law in Japan. Taking advantages of the occasions enabling face-to-face communication with all pregnant women in the study area, our staffs carefully explained the importance of the JECS to them and requested their cooperation for the study at the obstetric facilities.

Of the 8,134 pregnant women who were invited as eligible recruits during the recruitment period from January 2011 to March 2014, a total of 5,721 (70.3%) participants from cooperating 32 obstetrics facilities were enrolled as the baseline regional cohort of pregnant women for main study of the JECS. The study area has 4,400 pregnant women annually on average, suggesting that 5,721 participants accounted for about 40% of the target population in the area.

After the recruitment to the main study of the JECS, the registered 5,721 JECS participants were also invited to participate in adjunct studies conducted solely in the JECS-A and asked

if they could provide any of the following three types of consent: agreement to complete the adjunct questionnaire survey only (Sub-cohort A1, n = 3,426), agreement to provide maternal biological specimens in addition to agreeing to participate in Sub-cohort A1 (Sub-cohort A2, n = 2,924), and agreement to undergo genetic analyses in addition to agreeing to participate in Sub-cohorts A1 and A2 (Sub-cohort A3, n = 1,753). Sub-cohorts A1, A2 and A3 were designed as a hierarchy, that is, the participants in sub-cohort A3 agreed to participate in studies conducted in sub-cohorts A1 and A2. Each participant in the adjunct studies gave written informed consent before any surveys began.

Adjunct questionnaires for Sub-cohort A1 were distributed three times at the obstetric facilities during 1st and 2nd/3rd trimesters, in principle, and one month after the delivery, and mailed thereafter. Selective attrition caused by low SES may result in the estimates of findings being biased [31], so that enrollment and retention strategies play an important role in longitudinal cohort studies. We sent 1,000 Japanese yen (JPY, 110 JPY = 1US$ as of 2019) worth of a prepaid card for every adjunct mail survey as monetary incentive to respondents. The prepaid card called 'Quo card' is familiar and can be used at all kinds of restaurants, convenience stores, gas stations, bookstores etc. in Japan. In addition to the monetary incentives to participate, we also conducted reminder calls or letters to retain participants who were likely to drop out of the study, focusing on non-respondents of the postal surveys.

In Sub-cohorts A2 and A3, the parental blood (up to 1.5 ml) and urine (up to 50 ml, mothers only) during 1st and 2nd/3rd trimesters in principle and the cord blood (up to 1.5 ml) were collected and stored at around -80 ˚C. Participants in the JECS-A were then followed regardless of whether or not they took part in the above adjunct studies. Subsequently, of the 5,555 children, including 49 pairs of twins, born from the enrolled mothers, 57 children dropped out of the JECS-A because of infant death (n = 7), mother death (n = 1), withdrawal

of consent (n = 48), or a change in residence to an area outside the surveyed area (n = 1). On the other hand, 4 children who moved into the surveyed area from another one were included. As of 18 months after delivery, a total of 5,502 children remained within the JECS-A, accounting for approximately 40% of the children born in the study area.

A second recruitment for the adjunct studies in the JECS-A was also conducted during the follow-up of the study participants of the main study of the JECS, focusing on children. We approached the guardians of targeted children at legal check-ups for 18-month-olds provided at regional public health centers and health consultation centers. The recruitment period was divided into two phases. Of the 5,502 children remaining in the JECS-A at 18 months of age, a total of 2,576 children had reached the age until June 2015. We conducted a survey of the anthropometric measurements of the 2D:4D ratio of the children on both hands (Sub-cohort B). A total of 1,357 children (coverage of target samples: 52.7%, consent rate: 99.0%) were enrolled in Sub-cohort B. Subsequent to this recruitment, another recruitment was also set for 18-month-old children born from mothers registered during the last year of the pregnant women recruitment (Sub-cohort C). This cohort was specifically planned to collect biological samples (mainly overnight urine) from diapers, and written informed consent for the adjunct survey was obtained from 1,192 children (82.9%). All the enrolled children had reached the age of 1.5 years as of June 2016. The follow-up schedule for children beyond the age of 3 years has yet to be finalized, but these sub-cohorts within the JECS-A will be followed until the children reach the age of 13 years. Informed parental consent from the legally authorized representative was obtained for all the enrollment of children in the studies. Note that the above figures were based on the data set jecs-ag-20160424, which was released in June 2016, and on the provisional data set determined as of August 1, 2018.

Patient and Public Involvement

To develop the JECS-A cohort, we have established a system for public involvement in research, organizing an annual advisory committee consisting of the representatives from local government, medical association, nursing association, women's group, lawyers and mass media. The role of the committee offers advice and research questions from public concern, as members of an external project supporting group. Moreover, the committee has great contribution not only to sharing knowledge or engaging and creating a dialogue with the public, but also to playing a valuable role in advising on recruitment of participants and suggesting ideas for conducting the research. We also have held an open lecture for the participants every year since 2012, as an opportunity in which information and knowledge about the research is provided and disseminated.

Data collection and measurements for adjunct studies of the JECS-A

The data collected for the adjunct studies in the JECS-A are summarized in **Table 1**. Data acquisition for some socio-demographics and neurodevelopmental outcomes was planned within the main study of the JECS.

Each of sub-cohorts in JECS-A was designed for the three main objectives as stated in the Introduction. First, to challenge the multifaceted problems surrounding children's development, Sub-cohort A1 was formed with the intention of collecting data on longitudinal changes in prenatal and postnatal exposures considered to affect neurodevelopmental outcomes. One of our special concerns is the effect of alexithymia in mothers on early child development. The 20-item Toronto Alexithymia Scale (TAS-20)[32] will be used to measure this trait as a primary factor, and studies will investigate the pregnancy and perinatal outcomes and/or the developmental outcomes of postnatal infants. As there are many

potential determinants extending from the prenatal to early childhood period, the risks will be estimated using the mother's and child's demographic variables or other related factors as confounding factors. Sub-cohorts A2 and A3 will mainly focus on studies using biological specimens (urine and/or blood) obtained from pregnant women and their spouses when available. These biological samples will be used for exposure assessments, genetic and epigenetic analyses, and activity determinations of chemical metabolism-related enzymes, and so on. Data on the levels of urinary exposure biomarkers including chemical metabolites will be combined with those in sub-cohort C and, eventually, the longitudinal exposure trajectory and the relationships between exposure and health outcomes will also be analyzed. Second, Sub-cohort C was designed to clarify the chemical exposure levels in toddlers. To estimate their amount of exposure to environmental chemicals, a noninvasive biomonitoring approach was adopted in which a paper diaper worn overnight was collected under refrigerated conditions at the age of 18 and 36 months. This cohort will also be used to derive human biomonitoring reference values (e.g., RV95) of urinary chemical concentrations in the Japanese children.

Third, sub-cohort B was designed to develop secondary tools such as the 2D:4D ratio or neonatal oral-motor assessments to screen future neurodevelopmental problems at earlier stages of childhood. One of our major interest is the lengths of digits on both hands in children especially the longitudinal changes in 2D:4D ratios between 18-month-old and 36-month-old infants. As for the anthropometric measurements of the palms and digits of the children's hands, we have established a specialized protocol involving photographic records and digit measurements that has a high reliability (Intra/Inter-Class Correlations: ICC_1 = 0.97 [95% CI, 0.87-0.99], ICC_3 = 0.93 [0.83-0.98]). In short, our easy-to-use photocopying method, which involves placing the child's palm on a box composed of transparent acrylic

thin plates and photographing it with a digital camera fixed inside the box, was devised for application in health checkup settings enforced by law. All the 2D:4D ratio data will be obtained using this protocol. Sub-cohort A1 will also be used to examine the relationship between neonatal oral-motor assessments and subsequent abnormal neurodevelopmental outcomes. Using the short version of Infant Behavior Questionnaire (IBQ) and Early Childhood Behavior Questionnaire (ECBQ) as factors reflecting temperamental self-regulation, the utility of such assessments as early screening tools for predicting subsequent neurodevelopmental outcomes will be verified.

The JECS-A do not include any information regarding medical diagnoses affecting neurodevelopmental outcomes at this time, but such information will be available in the future through the main study of the JECS, which is being conducted under the same protocol across 15 regions in Japan.

FINDINGS TO DATE

Participant characteristics of pregnant women

Table 2 shows the main socio-demographic characteristics of the pregnant women. To ascertain whether the JECS-A baseline profiles are representative of the pregnant women in the study areas in general, the national statistics for Aichi prefecture including Nagoya City and Ichinomiya City (since data for each city were not available) were included in the table. Similar distribution trends for variables such as “Age at delivery” were confirmed when the JECS-A baseline cohort of pregnant women and the national statistics were compared; however, differences in “Education level” and “Household income” were observed. Regarding education level, the percentage of JECS participants who had graduated from high school was relatively low, compared with the national statistics (25.1% vs. 39.7%), while the

percentage of college/junior college/technology college students was higher among the JECS participants, compared with the national statistics (35.3% vs. 20.8%). Moreover, the number of JECS-A participants with a household income of 2 million yen or less was about 1/8 of the national statistics. Likewise, unemployed participants (mainly housewives) accounted for 45% of the JECS-A cohort. These results suggest a possible selection bias. We recruited pregnant women who had visited the obstetric facilities during the daytime; this might have resulted in a lower study participation rate among pregnant employees.

Participant characteristics of children

As for the baseline cohort of children, a total of 5,502 (99.0%) of the 5,555 children remained in the JECS-A at 18 months after delivery (provisional figures as of August 1, 2018). **Table 3** compares the baseline data of the newborns with the national statistics. Basic demographics including "Sex", "Birth weight" and "Birth height" had similar distributions, indicating the representativeness of the sample.

Similarly, **Table 4** shows the fundamental characteristics of the pregnant women and their children in each sub-cohort of the JECS-A. No notable differences in the descriptive statistics were observed among the sub-cohorts. Furthermore, the drop-out rates for each sub-cohort, to date, have been maintained at less than 10% relative to the baseline. However, 10% of the sub-cohort values for household income are missing because many participants did not provide information on the income. Therefore, caution is needed when using this variable as a confounding factor in multiple regression analyses.

STRENGTHS AND LIMITATIONS

The main strength of the JECS-A of children is its large sample size, reflecting a representative population. Approximately 40% of the children born in the study area have been included in the study to date. The participating children will be followed until they reach the age of 13 years. The study protocol[33] of the JECS decided on a target retention rate of 80% or higher at the age of 13 years. The linkage between the JECS-A sub-cohort and the main data of the JECS study will further allow novel and challenging studies with a high generalizability.

The main weakness of the JECS-A cohort concerns the pregnant women participants related to a selective attrition. A relatively large proportion of single-income, middle-class households were included in the JECS-A; many low-income households refused to participate in the surveys, and some participants did not provide information concerning their household income. As enrollment strategies, though we set about recruiting from the first trimester of pregnancy (<12-week gestation), it was hard to get their cooperation in earlier weeks of a pregnancy, when the risk of miscarriage is higher. Most of pregnant women registered were actually after 10th week of pregnancy. Furthermore, we had no choice but to call on pregnant women for participation who had visited the obstetric facilities mainly during the daytime, owing to limited number of staffs during the evening shift at hospitals. Employed participants including all non-regular employees (part-time, temporary or contract employees) only accounted for 33% in the group of annual household income with 2 million Japanese yen or less. This means a selective attrition that working pregnant women of low SES might have been excluded in the cohorts. Such family socioeconomic status will have possibilities affecting several outcomes related to child development such as breastfeeding duration [34], obesity in children [35] and child maltreatment [36]. A British study [37], however, suggests that cognitive and behavioural development has a weak or absent direct effect of

income inequality after controlling potential confounders. Thus, possible biases as a result of selective attrition and direct SES effects for child should be carefully examined in future studies when using the JECS-A cohort data. The impact of the missing values can be adjusted to some extent by comparing estimates resulting from the multiple imputation and from complete case analysis, using the data of main study of the JECS as reference. However, researchers must take into account the effect of response biases when conducting statistical analyses using paired data for pregnant women and their children and when interpreting these results.

COLLABORATION

The original data and specimens will be made available to investigators and stakeholders working within the JECS-A project. The study must adhere to the JECS policy on the availability of research results, publications, intellectual property rights, and data sharing. At the moment, the Ethical Guidelines for Medical and Health Research Involving Human Subjects enforced by the Japan Ministry of Education, Culture, Sports, Science and Technology and the Ministry of Health, Labour and Welfare restrict the open sharing of epidemiologic data. This means that researchers interested in using the data must collaborate with and participate in the JECS-A project. This project was recently started and is still ongoing. We are open to new proposals that fall within the nature of the JECS-A cohort.

Acknowledgements

We would like to express our gratitude to all the JECS-A cohort participants. We also express our sincere appreciation to the collaborating hospitals, clinics, local healthcare centers, local government agencies in Nagoya City and Ichinomiya City, and the JECS-A

staff members for their continuous assistance in promoting this study. The authors also specially thank Takaaki Hiraiwa, Hiroki Yoshizoe, Maiko Miyata, Hirohisa Kano and Takahiro Kurihara for the data management and their assistance in the data analysis.

Contributors

TE designed the JECS-A cohort architecture, developed the protocol, analyzed the data, and wrote the first draft of the manuscript. YY and MT organized the study team, obtained approvals and contributed to the development of the protocol, design, and data collection tools. TOm was in charge of coordination with relevant organizations and organized the JECS-A members. NS, SK, TMa and TOg performed the analysis and interpreted the data. YI, HS, NO and JU designed and developed the protocol for the JECS-A sub-cohorts A2, A3 and B and edited and analyzed the data. AN, MK, YO, TMi, SSu, MSO and SSa designed each adjunct study, supervised the data collection, and drafted the manuscript. MK was a member of the JECS Steering Committee, was responsible for the study design and protocol, supervised the data collection, and edited and drafted the manuscript. All the authors interpreted the data, contributed to the writing of the manuscript, revised it critically for important intellectual content, and agreed with the final version and the findings.

Funding The Japan Environment and Children's Study was funded by the Ministry of the Environment, Japan. This work was also supported by grants from the Environment Research and Technology Development Fund (ERTDF, No 5-1551 and 5-1851), Japan Society for the Promotion of Science (JSPS) KAKENHI Grant Number 16H03733, 25293151, 25285185, 19H01078 and Grants-in-aid for research of Nagoya City University (2011-6, 2012-4, 2013-2, 2013-10, 2014-4, 2015-10, 2016-4).

Competing Interests None declared

Ethical approval This study was approved by the Review Board of the Ministry of the Environment, by the Institutional Review Board of Nagoya City University Graduate School of Medical Sciences (No. 70-00-0134; No. 00000544-6, 00000574-2) and by the Ethics Committees of all participating institutions.

Data sharing statement The original data and specimens will be made available to investigators and stakeholders working within the JECS-A project.

Researchers interested in collaborations or further information should contact the JECS-A secretariat by e-mail at eisei@med.nagoya-cu.ac.jp.

Disclaimer The findings and conclusions of this article are solely the responsibility of the authors and do not represent the official views of the Japanese government.

REFERENCES

1. Ishitsuka K, Nakayama SF, Kishi R, et al. Japan Environment and Children's Study: backgrounds, activities, and future directions in global perspectives. *Environ Health Prev Med* 2017;22(1):61. doi: 10.1186/s12199-017-0667-y

2. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders (DSM–5): American Psychiatric Association Publishing 2013:xiii-xv.

3. Xiang S, Carrie A. A review of the prevalence of Autism Spectrum Disorder in Asia,

Research in Autism Spectrum Disorders. *Autism Spectrum Disorders* 2010;4(2):156-67. doi: http://dx.doi.org/10.1016/j.rasd.2009.10.003.

4. Hansen SN, Schendel DE, Parner ET. Explaining the increase in the prevalence of autism spectrum disorders: the proportion attributable to changes in reporting practices. *JAMA Pediatr* 2015;169(1):56-62. doi: 10.1001/jamapediatrics.2014.1893

5. Rodriguez-Barranco M, Lacasana M, Aguilar-Garduno C, et al. Association of arsenic, cadmium and manganese exposure with neurodevelopment and behavioural disorders in children: a systematic review and meta-analysis. *Sci Total Environ* 2013;454-455:562-77. doi: 10.1016/j.scitotenv.2013.03.047

6. Rodrigues EG, Bellinger DC, Valeri L, et al. Neurodevelopmental outcomes among 2- to 3-year-old children in Bangladesh with elevated blood lead and exposure to arsenic and manganese in drinking water. *Environ Health* 2016;15:44. doi: 10.1186/s12940-016-0127-y

7. Melchior M, Hersi R, van der Waerden J, et al. Maternal tobacco smoking in pregnancy and children's socio-emotional development at age 5: The EDEN mother-child birth cohort study. *Eur Psychiatry* 2015;30(5):562-8. doi: 10.1016/j.eurpsy.2015.03.005

8. Joelsson P, Chudal R, Talati A, et al. Prenatal smoking exposure and neuropsychiatric comorbidity of ADHD: a finnish nationwide population-based cohort study. *BMC Psychiatry* 2016;16:306. doi: 10.1186/s12888-016-1007-2

9. Han JY, Kwon HJ, Ha M, et al. The effects of prenatal exposure to alcohol and environmental tobacco smoke on risk for ADHD: a large population-based study. *Psychiatry Res* 2015;225(1-2):164-8. doi: 10.1016/j.psychres.2014.11.009

10. Padron A, Galan I, Garcia-Esquinas E, et al. Exposure to secondhand smoke in the home and mental health in children: a population-based study. *Tob Control*

2016;25(3):307-12. doi: 10.1136/tobaccocontrol-2014-052077

11. Polanska K, Ligocka D, Sobala W, et al. Phthalate exposure and child development: the Polish Mother and Child Cohort Study. *Early Hum Dev* 2014;90(9):477-85. doi: 10.1016/j.earlhumdev.2014.06.006

12. Kyriklaki A, Vafeiadi M, Kampouri M, et al. Prenatal exposure to persistent organic pollutants in association with offspring neuropsychological development at 4years of age: The Rhea mother-child cohort, Crete, Greece. *Environ Int* 2016;97:204-11. doi: 10.1016/j.envint.2016.09.012

13. Goudarzi H, Nakajima S, Ikeno T, et al. Prenatal exposure to perfluorinated chemicals and neurodevelopment in early infancy: The Hokkaido Study. *Sci Total Environ* 2016;541:1002-10. doi: 10.1016/j.scitotenv.2015.10.017

14. Yolton K, Cornelius M, Ornoy A, et al. Exposure to neurotoxicants and the development of attention deficit hyperactivity disorder and its related behaviors in childhood. *Neurotoxicol Teratol* 2014;44:30-45. doi: 10.1016/j.ntt.2014.05.003

15. Engel SM, Wetmur J, Chen J, et al. Prenatal exposure to organophosphates, paraoxonase 1, and cognitive development in childhood. *Environ Health Perspect* 2011;119(8):1182-8. doi: 10.1289/ehp.1003183

16. Bouchard MF, Chevrier J, Harley KG, et al. Prenatal exposure to organophosphate pesticides and IQ in 7-year-old children. *Environ Health Perspect* 2011;119(8):1189-95. doi: 10.1289/ehp.1003185

17. Stein LJ, Gunier RB, Harley K, et al. Early childhood adversity potentiates the adverse association between prenatal organophosphate pesticide exposure and child IQ: The CHAMACOS cohort. *Neurotoxicology* 2016;56:180-87. doi: 10.1016/j.neuro.2016.07.010

18. Yurumez E, Akca OF, Ugur C, et al. Mothers' alexithymia, depression and anxiety levels and their association with the quality of mother-infant relationship: a preliminary study. *Int J Psychiatry Clin Pract* 2014;18(3):190-6. doi: 10.3109/13651501.2014.940055

19. Li Y, Jiang WQ, Du YS, et al. Relationships between behavioral symptoms of non-medicated Chinese children with attention deficit hyperactivity disorder and parenting stress: Comparison of different subtypes and comorbidities. *Asia Pac Psychiatry* 2016;8(2):127-35. doi: 10.1111/appy.12213

20. Oya N, Ito Y, Hioki K, et al. Quantitative analysis of organophosphate insecticide metabolites in urine extracted from disposable diapers of toddlers in Japan. *Int J Hyg Environ Health* 2017;220(2 Pt A):209-16. doi: 10.1016/j.ijheh.2016.10.009

21. Saito S, Ueyama J, Kondo T, et al. A non-invasive biomonitoring method for assessing levels of urinary pyrethroid metabolites in diapered children by gas chromatography-mass spectrometry. *J Expo Sci Environ Epidemiol* 2014;24(2):200-7. doi: 10.1038/jes.2013.31

22. Robins DL, Casagrande K, Barton M, et al. Validation of the modified checklist for Autism in toddlers, revised with follow-up (M-CHAT-R/F). *Pediatrics* 2014;133(1):37-45. doi: 10.1542/peds.2013-1813

23. Manning JT, Baron-Cohen S, Wheelwright S, et al. The 2nd to 4th digit ratio and autism. *Dev Med Child Neurol* 2001;43(3):160-4.

24. Teatero ML, Netley C. A critical review of the research on the extreme male brain theory and digit ratio (2D:4D). *J Autism Dev Disord* 2013;43(11):2664-76. doi: 10.1007/s10803-013-1819-6

25. Guyatt AL, Heron J, Knight Ble C, et al. Digit ratio and autism spectrum disorders in the

Avon Longitudinal Study of Parents and Children: a birth cohort study. *BMJ Open* 2015;5(8):e007433. doi: 10.1136/bmjopen-2014-007433

26. Slattery J, Morgan A, Douglas J. Early sucking and swallowing problems as predictors of neurodevelopmental outcome in children with neonatal brain injury: a systematic review. *Dev Med Child Neurol* 2012;54(9):796-806. doi: 10.1111/j.1469-8749.2012.04318.x

27. Wolthuis-Stigter MI, Luinge MR, da Costa SP, et al. The association between sucking behavior in preterm infants and neurodevelopmental outcomes at 2 years of age. *J Pediatr* 2015;166(1):26-30. doi: 10.1016/j.jpeds.2014.09.007

28. Kawamoto T, Nitta H, Murata K, et al. Rationale and study design of the Japan environment and children's study (JECS). *BMC Public Health* 2014;14:25. doi: 10.1186/1471-2458-14-25

29. Michikawa T, Nitta H, Nakayama SF, et al. The Japan Environment and Children's Study (JECS): A Preliminary Report on Selected Characteristics of Approximately 10 000 Pregnant Women Recruited During the First Year of the Study. *J Epidemiol* 2015;25(6):452-8. doi: 10.2188/jea.JE20140186

30. Michikawa T, Nitta H, Nakayama SF, et al. Baseline Profile of Participants in the Japan Environment and Children's Study (JECS). *J Epidemiol* 2018;28(2):99-104. doi: 10.2188/jea.JE20170018

31. Booker CL, Harding S, Benzeval M. A systematic review of the effect of retention methods in population-based cohort studies. *BMC Public Health* 2011;11:249. doi: 10.1186/1471-2458-11-249

32. Moriguchi Y, Maeda M, Igarashi T, et al. Age and gender effect on alexithymia in large, Japanese community and clinical samples: a cross-validation study of the Toronto

Alexithymia Scale (TAS-20). *Biopsychosoc Med* 2007;1:7. doi: 10.1186/1751-0759-1-7

33. National Centre for Japan Environment and Children's study. Japan Environment and Children's study (JECS), Study Protocol (ver. 1.4) [Available from: https://www.env.go.jp/en/chemi/hs/jecs/data/about/jecs-study_protocol_14_en.pdf.

34. Flacking R, Nyqvist KH, Ewald U. Effects of socioeconomic status on breastfeeding duration in mothers of preterm and term infants. *Eur J Public Health* 2007;17(6):579-84. doi: 10.1093/eurpub/ckm019

35. Ogden CL, Lamb MM, Carroll MD, et al. Obesity and socioeconomic status in children and adolescents: United States, 2005-2008. *NCHS Data Brief* 2010(51):1-8.

36. Eckenrode J, Smith EG, McCarthy ME, et al. Income inequality and child maltreatment in the United States. *Pediatrics* 2014;133(3):454-61. doi: 10.1542/peds.2013-1707

37. Violato M, Petrou S, Gray R, et al. Family income and child cognitive and behavioural development in the United Kingdom: does money matter? *Health Econ* 2011;20(10):1201-25. doi: 10.1002/hec.1665

Figure legend/caption

Figure 1 Study areas covered by the JECS-A

Figure 2. Flowchart showing each stage of study recruitment for adjunct studies in the JECS-A

Table 1 Summary of measurements collected for the adjunct studies conducted in JECS-A

Main items		Sub-cohort covered					Timing of measurement							
							prenatal (trimester)		postnatal (months after birth)					
		A1	A2	A3	B	C	1st	2nd/3rd	1 m	6 m	18 m	24 m	36 m
Pregnant women														
Risk and confounding factors														
Socio-demographic	Age, Maternal age, Marital status, Household income, Education level	○	○	○	○	○	○							
Lifestyle	Smoking, Second-hand smoking, Drinking habits, Dietary status, Sleeping habits	○	○	○		○	○	○	○	○	○	○	○	
Psychological and psychiatric	TAS–20	●					●	●						
	POMS-SF	●					●							
	Readiness of Parenthood Scale	●					●	●	●	●				
Obstetric	History of pregnancy, infertility treatment, prenatal diagnosis and gestational duration	○												
Biomonitoring data	Urine (up to 50 ml, mothers only), parental blood (up to 1.5ml), cord blood (up to 1.5ml)		●	●	●	●	●							
Children														
Risk and confounding factors														
Demographic	Height, Weight, BMI, Sex	○	○	○	○	○								
Behavioral	Neonatal oral-motor assessment	●							●					
	DCDQ	●									●		●	
Childcare	Breast feeding and weaning food status	●									●			
Psychological and psychiatric	Mary Rothbart's Temperament Questionnaires	●												
	・IBQ-R-SF										●			
	・ECBQ-SF											●		
Biomonitoring data	Overnight urine extracted from disposable diapers (up to 30ml)				●						●		●	
Anthropometric data	2D:4D				●						●		●	
Outcome examples to be analyzed														
LBW			○	○					○					
ASD		○	○	○	○	○							○	
ADHD		○	○	○		○							○	

○: Refer to the main study of the Japan Environment and Children's Study (JECS), ●: Measurements conducting solely in the JECS-A

BMI, body mass index; **TAS-20**, the 20–item Toronto Alexithymia Scale; **POMS-SF**, Short form of the Profile of Mood States; **IBQ-R-SF**, The Infant Behavior Questionnaire Revised Short Form; **ECBQ-SF**, Early Childhood Behavior Questionnaire Short Form; **DCDQ**, Developmental Coordination Disorder Questionnaire, **2D:4D**, the second to fourth digit ratio; **PARS**, Pervasive Developmental Disorders Autism Society Japan Rating Scale; **ASD**, autism spectrum disorder; **ADHD**, attention deficit hyperactivity disorder; LBW, low birth weight.

Sub cohort A1, Agreement on the adjunct questionnaire survey only; **Sub cohort A2**, Agreement on the adjunct questionnaire survey and provision of mother's biological specimen (blood and urine); **Sub cohort A3**, Agreement on the adjunct questionnaire survey and provision of mother's biological specimen (blood and urine) with genetic analyses; **Sub cohort B**, Informed consent obtained from guardians (legally acceptable representatives) for provision of child's biological specimen; **Sub cohort C**, Informed consent obtained from guardians (legally acceptable representatives) for anthropometry measurement on child hands

Table 2 Comparison of the JECS-A baseline data with national statistics in Aichi prefecture for pregnant women: verification of representativeness of the sample

	JECS-A baseline cohort of pregnant women (95% CI)	National statistics (Aichi pref.)
Pregnant women [a] (n)	5721	65218
Age at Delivery [a]		
15-19 (%)	0.7 (0.5-0.9)	1.3
20-24	6.7 (6.0-7.3)	8.2
25-29	25.3 (24.1-26.4)	28.3
30-34	36.4 (35.1-37.6)	36.6
35-39	23.2 (22.1-24.3)	21.4
40-44	4.9 (4.4-5.5)	4.2
45-49	0.1 (0.0-0.2)	0.1
50-	0.0	0.0
Missing (n)	157	
Education level [b] (n)		2397000
Junior high school/High school (%)	29.9 (28.7-31.1)	47.2
College/Junior college/Technology college	35.3 (34.1-36.6)	20.8
University	27.6 (26.4-28.8)	28.4
Graduate school	1.6 (1.3-2.0)	3.6
Missing (n)	315	
Household income [b c d] (n)		3018900
<2,000 (%)	2.4 (2.0-2.8)	18.1
2,000-<4,000	24.4 (23.3-25.5)	26.7
4,000-<6,000	33.1 (31.9-34.3)	20.2
6,000-<8,000	17.2 (16.2-18.2)	13.4
8,000-<10,000	7.4 (6.7-8.1)	8.3
≥10,000	4.3 (3.7-4.8)	5.0
Missing (n)	643	

[a] Data of Aichi prefecture as reference, provided by national vital statistics, Ministry of Health, Labour and Welfare in 2014.
[b] Data of Aichi prefecture provided by Ministry of Internal Affairs and Communications in 2012.
[c] Population of Aichi Prefecture including single-person households and families
[d] Household income shows thousand Japanese Yen(JPY), 110 JPY = 1US$ as of 2019

-: no available data provided; 95%CI: 95% Confidence Interval

Table 3 Comparison of the JECS-A baseline data with national statistics in Aichi prefecture for children: verification of representativeness of the sample

	JECS-A baseline cohort of children (n = 5457)			National statistics (Aichi pref.) [a]		
	Total	Male	Female	Total	Male	Female
Children (n) [b]	5457	2793	2659	65218	33649	31569
Sex (Singleton births)						
(%)		51.2	48.7		51.6	48.4
95% CI		(50.5-51.9)	(48.0-49.4)			
Missing (n)	5					
Birth weight, g (Singleton births)						
Mean	3034.3	3076.1	2990.7	3000.0	3040.0	2950.0
95%CI	(3022.9-3045.7)	(3059.7-3092.5)	(2975.1-3006.3)	-	-	-
Missing (n)	43	23	16			
Low birth weight (Singleton births)						
<2500 g (%)	7.8	6.6	9.0	9.8	8.5	11.1
95%CI	(7.4-8.2)	(6.3-6.9)	(8.7-9.3)			
Missing (n)	43	23	16			
Birth length, cm (Singleton births)						
Mean	49.7	49.9	49.5	49.3	49.6	49.1
95%CI	(49.6-49.8)	(49.8-50.0)	(49.4-49.6)	-	-	-
Missing (n)	51	28	19			

[a] Data of Aichi prefecture as reference, provided by national vital statistics, Ministry of Health, Labour and Welfare in 2014.

[b] Multiple births (49 pairs of twins) were excluded.

-: no available data provided, 95%CI: 95% Confidence Interval

Table 4 Baseline characteristics of paired data of pregnant women and their children in the JECS-A cohort

	Sub-cohort				
	A1	A2	A3	B	C
Pregnant women (n)	3426	2924	1753	1352	1187
Age at Delivery					
Mean	31.6	31.7	31.8	31.8	32.0
SD	4.9	4.9	5.0	4.8	4.7
Missing (n)	44	14	7	0	0
Smoking habits					
Never smoked (%)	62.8	63.7	63.7	64.2	64.6
Ex-smokers who quit before pregnancy	21.6	22.0	23.1	21.8	23.1
Smokers during early pregnancy	9.0	9.0	8.3	8.4	7.7
Smokers	2.5	2.5	2.1	2.8	2.2
Missing (n)	144	82	51	38	29
Second-hand smoking					
Rarely (%)	67.5	69.1	69.6	68.6	71.9
A few days a week	18.6	18.6	18.0	19.1	17.1
Daily	10.4	10.1	10.3	10.1	9.6
Missing (n)	124	66	36	30	17
Household income (JPY)					
<2,000 (%)	2.3	2.1	2.5	1.8	2.1
2,000-<4,000	25.8	26.0	25.6	24.4	24.3
4,000-<6,000	33.4	33.7	34.2	35.1	35.9
6,000-<8,000	17.5	18.1	17.5	18.6	18.6
8,000-<10,000	7.3	7.5	7.8	7.5	8.3
≥10,000	4.5	4.7	4.5	3.9	4.5
Missing (n)	314	233	141	116	76

Children (n)[a]	3336	2884	1726	1346	1182
Sex (Singleton births)					
Male (%)	50.5	50.4	50.1	49.9	50.4
Female	49.5	49.6	49.8	50.1	49.6
Missing (n)	1	1	1	0	0
Birth weight, g (Singleton births)					
Mean	3041.6	3052.3	3054.1	3039.1	3062.3
SD	432.8	419.9	430.4	410.0	416.7
Missing (n)	22	15	9	4	7
Birth height, cm (Singleton births)					
Mean	49.6	49.7	49.7	49.7	49.7
SD	2.3	2.2	2.2	2.1	2.1
Missing (n)	28	18	11	6	8

[a] Multiple births (twin pairs) were excluded; A1: 20 (n = 40), A2: 16 (n = 32), A3: 12 (n = 24), B: 5 (n = 10), and C: 6 (n = 12).

Sub cohort A1, Agreement on the adjunct questionnaire survey only; **Sub cohort A2**, Agreement on the adjunct questionnaire survey and provision of mother's biological specimen (blood and urine); **Sub cohort A3**, Agreement on the adjunct questionnaire survey and provision of mother's biological specimen (blood and urine) with genetic analysis; **Sub cohort B**, Informed consent obtained for provision of child's biological specimen from guardian (legally acceptable representatives); **Sub cohort C**, Informed consent obtained for anthropometry measurement on child hands from guardians (legally acceptable representatives). JPY: Japanese Yen, 110 JPY = 1 US$ as of 2019

Figure 1 Study areas covered by the JECS-A

Figure 1 Study areas covered by the JECS-A

65x82mm (300 x 300 DPI)

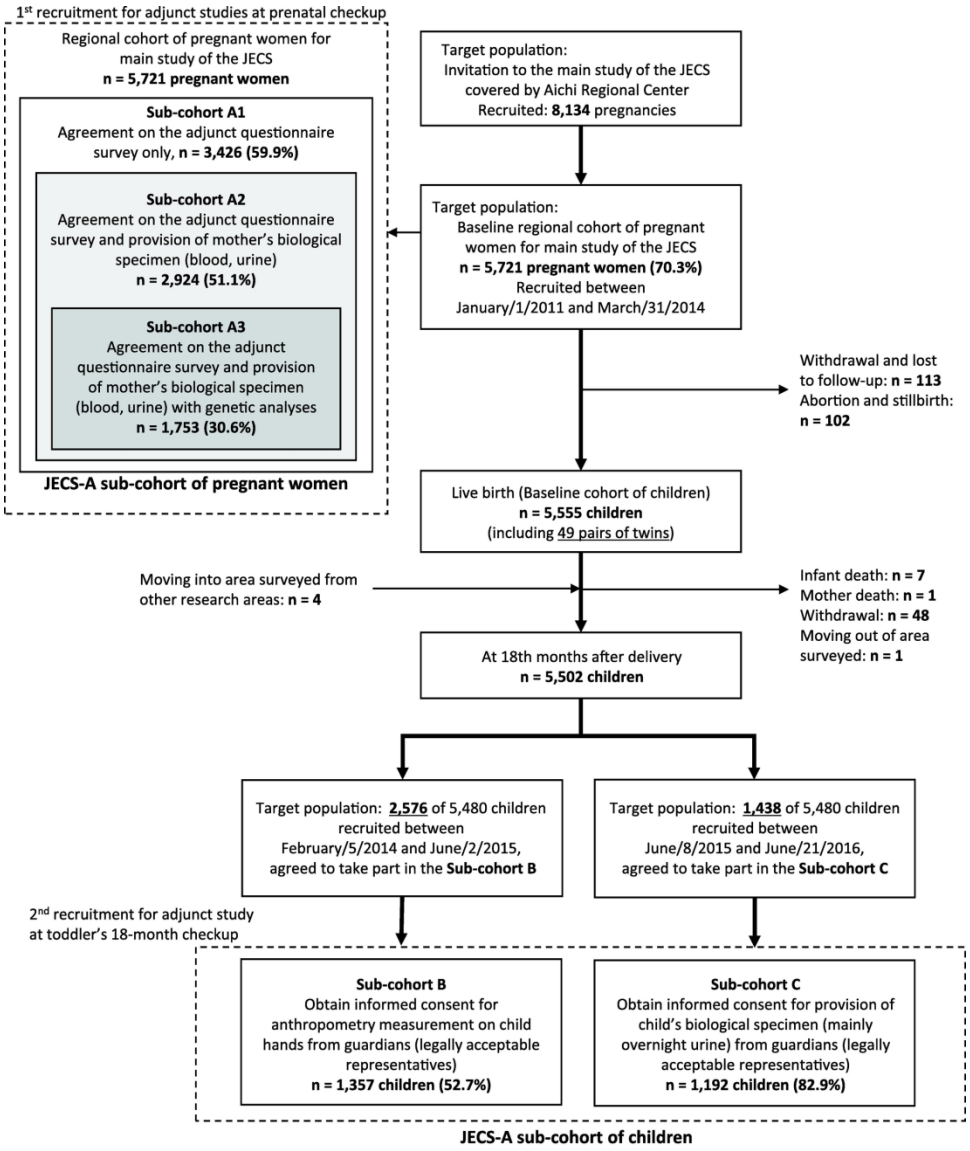

Figure 2. Flowchart showing each stage of study recruitment for adjunct studies in the JECS-A

Figure 2. Flowchart showing each stage of study recruitment for adjunct studies in the JECS-A

178x223mm (300 x 300 DPI)

BMJ Open

Cohort profile: Aichi regional sub-cohort of the Japan Environment and Children's Study (JECS-A)

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-028105.R2
Article Type:	Cohort profile
Date Submitted by the Author:	10-Oct-2019
Complete List of Authors:	Ebara, Takeshi; Nagoya City University Graduate School of Medical Sciences and Medical School, Department of Occupational and Environmental Health Yamada, Yasuyuki; Nagoya City University Graduate School of Medical Sciences and Medical School, Department of Occupational and Environmental Health; Juntendo University School of Health and Sports Science Graduate School of Health and Sports Science Shoji, Naoto; Nagoya City University Graduate School of Medical Sciences and Medical School, Department of Occupational and Environmental Health; Asahi University, School of Health Sciences Ito, Yuki; Nagoya City University Graduate School of Medical Sciences and Medical School, Department of Occupational and Environmental Health Nakagawa, Atsuko; Nagoya City University Faculty of Humanities and Social Sciences Graduate School of Humanities and Social Sciences Miyachi, Taishi; Nagoya City University Graduate School of Medical Sciences and Medical School, Pediatrics and Neonatology; Nagoya West District Care Center for Disabled Children Ozaki, Yasuhiko; Nagoya City University Graduate School of Medical Sciences and Medical School, Obstetrics and Gynecology Omori, Toyonori; Nagoya City University Graduate School of Medical Sciences and Medical School, Health Care Policy and Management Suzuki, Sadao; Nagoya City University Graduate School of Medical Sciences and Medical School, Department of Public Health Kojima, Masayo ; Nagoya City University Graduate School of Medical Sciences and Medical School, Medical Education Ueyama, Jun; Nagoya University Graduate School of Medicine Faculty of Medicine, Department of Pathophysiological Laboratory Sciences, Field of Radiological and Medical Laboratory Sciences Tomizawa, Motohiro; Nagoya City University Graduate School of Medical Sciences and Medical School, Department of Occupational and Environmental Health; Tokyo University of Agriculture Kato, Sayaka; Nagoya City University Graduate School of Medical Sciences and Medical School, Department of Occupational and Environmental Health; Nagoya City University Graduate School of Medical Sciences and Medical School, Pediatrics and Neonatology Oguri, Tomoko; Nagoya City University Graduate School of Medical Sciences and Medical School, Department of Occupational and Environmental Health; National Institute of Advanced Industrial Science and Technology (AIST) Matsuki, Taro; Nagoya City University Graduate School of Medical

	Sciences and Medical School, Department of Occupational and Environmental Health Sato, Hirotaka; Nagoya City University Graduate School of Medical Sciences and Medical School, Department of Occupational and Environmental Health Oya, Naoko; Nagoya City University Graduate School of Medical Sciences and Medical School, Department of Occupational and Environmental Health Sugiura-Ogasawara , M; Nagoya City University Graduate School of Medical Sciences and Medical School, Obstetrics and Gynecology Saitoh, Shinji; Nagoya City University Graduate School of Medical Sciences and Medical School, Pediatrics and Neonatology Kamijima, Michihiro; Nagoya City University Graduate School of Medical Sciences and Medical School, Department of Occupational and Environmental Health
Primary Subject Heading:	Epidemiology
Secondary Subject Heading:	Epidemiology, Paediatrics
Keywords:	JECS, birth cohort, EPIDEMIOLOGY, JECS-A

Cohort profile: Aichi regional sub-cohort of the Japan Environment and Children's Study (JECS-A)

Takeshi Ebara[1], Yasuyuki Yamada[1,9], Naoto Shoji[1,10], Yuki Ito[1], Atsuko Nakagawa[7], Taishi Miyachi[2,11], Yasuhiko Ozaki[3], Toyonori Omori[4], Sadao Suzuki[5], Masayo Kojima[6], Jun Ueyama[8], Motohiro Tomizawa[1†], Sayaka Kato[1,2], Tomoko Oguri[1††], Taro Matsuki[1], Hirotaka Sato[1], Naoko Oya[1], Mayumi Sugiura-Ogasawara[3], Shinji Saitoh[2], Michihiro Kamijima[1]

Department of [1]Occupational and Environmental Health, [2]Pediatrics and Neonatology, [3]Obstetrics and Gynecology, [4]Health Care Policy and Management, [5]Public Health, [6]Medical Education, Nagoya City University, Graduate School of Medical Sciences, Nagoya, Japan
[7]School of Humanities and Social Sciences, Nagoya City University, Nagoya, Japan
[8]Department of Pathophysiological Laboratory Sciences, Field of Radiological and Medical Laboratory Sciences, Nagoya University Graduate School of Medicine, Japan.
[9]Juntendo University Graduate School of Health and Sports Science, Chiba, Japan
[10] Asahi University, School of Health Sciences
[11]Nagoya West District Care Center for Disabled Children, Nagoya, Japan

Present affiliation: [†]Tokyo University of Agriculture, [††]National Institute of Advanced Industrial Science and Technology (AIST)

Corresponding Author:
Takeshi Ebara, PhD.
Department of Occupational and Environmental Health, Nagoya City University, Graduate School of Medical Sciences. Mizuho-ku, Nagoya, 4678601, Japan
TEL: 81-52-853-8171 FAX: 81-52-859-1228
e-mail: ebara@med.nagoya-cu.ac.jp

ABSTRACT

Purpose: Effects of fetal, perinatal and childhood environment on the health of children at birth and during later life have become a topic of concern. The Aichi regional sub-cohort of the Japan Environment and Children's Study (JECS-A) is an ongoing birth cohort of pregnant women and their children that has been used to provide unique data, as adjunct studies of JECS, on multifaceted potential factors affecting children's health.

Participants: The JECS-A is part of the Japan Environment and Children's Study (JECS) which follows a total of 100,000 pairs of children and their mothers (fathers' participation is optional) across 15 regions in Japan. In JECS-A, of the 8,134 pregnant women living in Ichinomiya City and Nagoya City, Japan, a total of 5,721 pregnant women and their 5,555 children were included. Sociodemographic and psychological data as well as biological specimens were collected from the pregnant women and their spouses (if available) in the cohort during their pregnancy. Information on children included in the JECS-A was collected from their mothers and includes demographic, behavioral, childcare, psychological and psychiatric data. Urine extracted from disposable diapers and anthropometric data were also obtained from the children.

Findings to date: A similar distribution trend for age at delivery was confirmed between the pregnant women enrolled in the JECS-A and the national statistics of the relevant areas. However, differences in education level and household income were observed. A total of 5,502 children remained in the cohort at 18 months after delivery. Compared with the national statistics, the basic demographics of the children in the cohort represented the population in the study areas.

Future plans: The enrolled children in the JECS-A will be followed until the age of 13 years. The studies that come from JECS-A will complement JECS and bring novel results with a high level of generalizability.

(300 words)

Strengths and limitations of this study

- The main strength of the JECS-A of children is its large sample size, reflecting a representative population.
- Another strength can be found in considering multifaceted potential factors affecting children's health in the prospective birth cohort over two generations.
- The main limitation of the cohort is that the population with low-income households is not included.

INTRODUCTION

The effects of fetal, perinatal and childhood environmental factors on the health of children at birth and during later life are a topic of concern. A number of birth cohort studies have thus been conducted worldwide to address this issue, and the Japan Environment and Children's Study (JECS), which focuses on the effects of environmental chemical pollutants, is one of the largest. The JECS was launched in 2011 by the Ministry of the Environment, Japan, after 3 years of planning.[1] The JECS consists of nationwide regional sub-cohorts that can be used to conduct studies, so called as adjunct studies of JECS, which are independent of the main study of the JECS. This article describes the cohort profile of a regional sub-cohort of the JECS, the Aichi regional sub-cohort of the JECS (JECS-A), containing 5,721 pregnant women and their 5,555 children. The following research themes are the main focuses of the adjunct studies conducted in JECS-A.

The first one is neurodevelopmental disorders[2] including intellectual disability, communication disorders, autism spectrum disorder (ASD), attention deficit hyperactivity disorder (ADHD), specific learning disorder, and motor disorders. A review has indicated that the estimated prevalence of ASD in Asia before 1980 was around 1.9/10,000, but this figure has recently increased by ten times.[3] Though a previous study conducted in Denmark claimed that 33% of the increase in the prevalence of ASD in recent years can be accounted for by changes in the diagnostic criteria and reporting methods,[4] this increased prevalence remains a complex and highly controversial issue that needs to be addressed. Recent epidemiological studies have linked neurodevelopmental outcomes to prenatal exposure to environmental toxicants such as heavy metals,[5,6] prenatal tobacco exposure,[7,8] environmental tobacco exposure,[9,10] phthalates,[11] persistent organic pollutants,[12,13] and organophosphate pesticides (OPs).[14,15 16] Furthermore, interest is growing regarding the potential role of social

stressors in modifying the relationship between the above early childhood exposure and the neurodevelopmental outcomes. A cohort study of seven-year-old predominantly Mexican American children in California's Salinas Valley suggested that social adversities including a poor learning environment and parent-child interactions were significantly associated with a moderate decrease in the intelligence quotient (IQ) score and its subset scores.[17] Several recent studies have also discussed the effects of parent-child interactions, focusing on the effects of alexithymia in mothers[18] and of depressive symptoms on parenting stress.[19] Thus, there is the need to investigate postnatal influences, such as the effects of interactions between mothers and their children, as well as prenatal and postnatal toxicant exposures on the neurodevelopment of children. The above research questions will be addressed to complement those of the main study of the JECS.

The second theme of the adjunct studies conducted in JECS-A is the exposure assessments using urine collected during early childhood when the central nervous system develops rapidly. So far, the amount of exposure to environmental chemical substances taken into the bodies of infants has rarely been investigated because most infants wear diapers that fully absorb all urine. Noninvasive biomonitoring using urine samples is thus required especially for exposure assessments of chemicals with short biological half lives, and a methodology for extracting urine from used disposable diapers has been investigated in our previous studies.[20 21] Since the urine during early childhood was not collected in the main study of the JECS, the adjunct studies using such urine in JECS-A will address research questions regarding environmental exposure in that period.

The third theme of the adjunct studies is the investigation of objective screening tools to detect neurodevelopmental disorders at earlier stages. Public health services in local communities must be capable of providing early intervention support for children with such

disorders. Preventive screening during early childhood can help to minimize later difficulties and to improve the trajectory of subjects with neurodevelopmental disorders in later life. For example, a validated screening checklist for autism at the age of 2 years, called M-CHAT-R/F,[22] is available as part of early medical checkups, but this screening protocol requires a 2-step screening test that must be conducted by a physician. As a secondary tool allowing easy and objective screening the second to fourth digit (2D:4D) ratio, which is defined as the proportion between the lengths of the index and ring fingers, might be useful. A previous study suggested that autistic children aged 2 to 14 years have a smaller 2D:4D ratio than normal children of the same generation.[23] Recent studies[24 25] have also investigated whether the 2D:4D ratio, a controversial but commonly used proxy marker of prenatal androgen concentrations based on the extreme male brain theory, is associated with ASD. However, this hypothetical screening method needs further scientific evidence in different epidemiological settings in different part of the globe. Another recent trial aiming to early intervention for neurodevelopmental problems can be found in neonatal oral-motor assessments, such as sucking behavior. While contradictory findings have been obtained,[26] a recent longitudinal study also showed interesting evidence that some sucking behaviors in preterm infants are associated with later abnormal neurodevelopmental outcomes at the age of 2 years.[27] As such, further research on the development of screening tools for early intervention is needed.

The JECS-A was established in 2011 as a sub-cohort of the JECS and has three main objectives: (1) to clarify the effects of prenatal and postnatal chemical exposures and social stressors on pregnant women and/or their children, especially neurodevelopmental outcomes of the children; (2) to develop a biomonitoring method using urine samples extracted from diapers for measuring internal exposure to chemical substances; and (3) to develop secondary

tools such as the 2D:4D ratio or neonatal oral-motor assessments to screen future neurodevelopmental problems at earlier stages of childhood. This article outlines the JECS-A and its baseline data to date.

COHORT DESCRIPTION

Setting

The JECS is an ongoing nationwide birth cohort study with a total of 100,000 children and their parents (father's participation is optional, but suggested) across 15 regions in Japan.[28 29 30] To identify risk factors in the environment affecting children's growth and health, the participating children are being followed from their fetal stage until the age of 13 years. The JECS has 15 regional sub-cohorts. At Aichi Regional Center of the JECS, an interdisciplinary team composed of toxicologists, psychologists, epidemiologists, public health specialists, pediatricians, obstetricians and gynecologists, biostatisticians, and ergonomists designed the JECS-A as part of the JECS cohort. The study areas (**Figure 1**) covered by the Aichi Regional Center of the JECS consist of Ichinomiya City (population of 387,000 in 2012) and Kita-ward in Nagoya City (population of 165,000 in 2012). Nagoya City consists of 16 wards with a population of over 2 million and is Japan's third largest industrial metropolis, next to Tokyo and Osaka. Kita-ward is in the northern part of Nagoya City. Ichinomiya City neighbors Nagoya City and traditionally was known as an area involved in textile production but is now a regional commercial and residential area with a mixed economy of manufacturing and agriculture. Both areas are relatively urban and widely known in the automobile and ceramics industries.

Enrollment strategy

Figure 2 shows a flowchart depicting each stage of study recruitment. A

community-based recruitment strategy was adopted at designated 32 obstetric facilities including hospitals, clinics or midwifery units providing medical care for pregnant women in the study areas. The participants living in the areas can reach any of the 32 facilities within 30 minutes by car or public transportation such as subway or train. Women in the early stage of pregnancy who visited an obstetrics facility for prenatal health care were invited to participate in the JECS if they met the following criteria: (1) residence within the study areas, (2) an estimated delivery date after August 2011, and (3) an ability to read and write the Japanese language so as to complete the self-administered questionnaire. In addition to this recruitment, we asked public health centers of the local governments to help us approach hard-to-reach groups such as low socio-economic status (SES) or reluctant pregnant women. All pregnant women receive the Maternal and Child Health Handbook at the public health centers to get complimentary municipal maternal care for pregnancy, delivery, and childcare under the Maternal and Child Health Law in Japan. Taking advantages of the occasions enabling face-to-face communication with all pregnant women in the study area, our staffs carefully explained the importance of the JECS to them and requested their cooperation for the study at the obstetric facilities.

Of the 8,134 pregnant women who were invited as eligible recruits during the recruitment period from January 2011 to March 2014, a total of 5,721 (70.3%) participants from cooperating 32 obstetrics facilities were enrolled as the baseline regional cohort of pregnant women for main study of the JECS. The study area has 4,400 pregnant women annually on average, suggesting that 5,721 participants accounted for about 40% of the target population in the area.

After the recruitment to the main study of the JECS, the registered 5,721 JECS participants were also invited to participate in adjunct studies conducted solely in the JECS-A and asked

if they could provide any of the following three types of consent: agreement to complete the adjunct questionnaire survey only (Sub-cohort A1, n = 3,426), agreement to provide maternal biological specimens in addition to agreeing to participate in Sub-cohort A1 (Sub-cohort A2, n = 2,924), and agreement to undergo genetic analyses in addition to agreeing to participate in Sub-cohorts A1 and A2 (Sub-cohort A3, n = 1,753). Sub-cohorts A1, A2 and A3 were designed as a hierarchy, that is, the participants in sub-cohort A3 agreed to participate in studies conducted in sub-cohorts A1 and A2. Each participant in the adjunct studies gave written informed consent before any surveys began.

Adjunct questionnaires for Sub-cohort A1 were distributed three times at the obstetric facilities during 1st and 2nd/3rd trimesters, in principle, and one month after the delivery, and mailed thereafter. Selective attrition caused by low SES may result in the estimates of findings being biased [31], so that enrollment and retention strategies play an important role in longitudinal cohort studies. We sent 1,000 Japanese yen (JPY, 110 JPY = 1US$ as of 2019) worth of a prepaid card for every adjunct mail survey as monetary incentive to respondents. The prepaid card called 'Quo card' is familiar and can be used at all kinds of restaurants, convenience stores, gas stations, bookstores etc. in Japan. In addition to the monetary incentives to participate, we also conducted reminder calls or letters to retain participants who were likely to drop out of the study, focusing on non-respondents of the postal surveys.

In Sub-cohorts A2 and A3, the parental blood (up to 1.5 ml) and urine (up to 50 ml, mothers only) during 1st and 2nd/3rd trimesters in principle and the cord blood (up to 1.5 ml) were collected and stored at around -80 ˚C. Participants in the JECS-A were then followed regardless of whether or not they took part in the above adjunct studies. Subsequently, of the 5,555 children, including 49 pairs of twins, born from the enrolled mothers, 57 children dropped out of the JECS-A because of infant death (n = 7), mother death (n = 1), withdrawal

of consent (n = 48), or a change in residence to an area outside the surveyed area (n = 1). On the other hand, 4 children who moved into the surveyed area from another one were included. As of 18 months after delivery, a total of 5,502 children remained within the JECS-A, accounting for approximately 40% of the children born in the study area.

A second recruitment for the adjunct studies in the JECS-A was also conducted during the follow-up of the study participants of the main study of the JECS, focusing on children. We approached the guardians of targeted children at legal check-ups for 18-month-olds provided at regional public health centers and health consultation centers. The recruitment period was divided into two phases. Of the 5,502 children remaining in the JECS-A at 18 months of age, a total of 2,576 children had reached the age until June 2015. We conducted a survey of the anthropometric measurements of the 2D:4D ratio of the children on both hands (Sub-cohort B). A total of 1,357 children (coverage of target samples: 52.7%, consent rate: 99.0%) were enrolled in Sub-cohort B. Subsequent to this recruitment, another recruitment was also set for 18-month-old children born from mothers registered during the last year of the pregnant women recruitment (Sub-cohort C). This cohort was specifically planned to collect biological samples (mainly overnight urine) from diapers, and written informed consent for participation of 1,192 children (82.9%) of the adjunct survey was obtained from their guardians. All the enrolled children had reached the age of 1.5 years as of June 2016. The follow-up schedule for children beyond the age of 3 years has yet to be finalized, but these sub-cohorts within the JECS-A will be followed until the children reach the age of 13 years. Informed parental consent from the legally authorized representative was obtained for all the enrollment of children in the studies. Note that the above figures were based on the data set jecs-ag-20160424, which was released in June 2016, and on the provisional data set determined as of August 1, 2018.

Patient and Public Involvement

To develop the JECS-A cohort, we have established a system for public involvement in research, organizing an annual advisory committee consisting of the representatives from local government, medical association, nursing association, women's group, lawyers and mass media. The role of the committee offers advice and research questions from public concern, as members of an external project supporting group. Moreover, the committee has great contribution not only to sharing knowledge or engaging and creating a dialogue with the public, but also to playing a valuable role in advising on recruitment of participants and suggesting ideas for conducting the research. We also have held an open lecture for the participants every year since 2012, as an opportunity in which information and knowledge about the research is provided and disseminated.

Data collection and measurements for adjunct studies of the JECS-A

The data collected for the adjunct studies in the JECS-A are summarized in **Table 1**. Data acquisition for some socio-demographics and neurodevelopmental outcomes was planned within the main study of the JECS.

Each of sub-cohorts in JECS-A was designed for the three main objectives as stated in the Introduction. First, to challenge the multifaceted problems surrounding children's development, Sub-cohort A1 was formed with the intention of collecting data on longitudinal changes in prenatal and postnatal exposures considered to affect neurodevelopmental outcomes. One of our special concerns is the effect of alexithymia in mothers on early child development. The 20-item Toronto Alexithymia Scale (TAS-20)[32] will be used to measure this trait as a primary factor, and studies will investigate the pregnancy and perinatal

outcomes and/or the developmental outcomes of postnatal infants. As there are many potential determinants extending from the prenatal to early childhood period, the risks will be estimated using the mother's and child's demographic variables or other related factors as confounding factors. Sub-cohorts A2 and A3 will mainly focus on studies using biological specimens (urine and/or blood) obtained from pregnant women and their spouses when available. These biological samples will be used for exposure assessments, genetic and epigenetic analyses, and activity determinations of chemical metabolism-related enzymes, and so on. Data on the levels of urinary exposure biomarkers including chemical metabolites will be combined with those in sub-cohort C and, eventually, the longitudinal exposure trajectory and the relationships between exposure and health outcomes will also be analyzed. Second, Sub-cohort C was designed to clarify the chemical exposure levels in toddlers. To estimate their amount of exposure to environmental chemicals, a noninvasive biomonitoring approach was adopted in which a paper diaper worn overnight was collected under refrigerated conditions at the age of 18 and 36 months. This cohort will also be used to derive human biomonitoring reference values (e.g., RV95) of urinary chemical concentrations in the Japanese children.

Third, sub-cohort B was designed to develop secondary tools such as the 2D:4D ratio or neonatal oral-motor assessments to screen future neurodevelopmental problems at earlier stages of childhood. One of our major interest is the lengths of digits on both hands in children especially the longitudinal changes in 2D:4D ratios between 18-month-old and 36-month-old infants. As for the anthropometric measurements of the palms and digits of the children's hands, we have established a specialized protocol involving photographic records and digit measurements that has a high reliability (Intra/Inter-Class Correlations: $ICC_1 = 0.97$ [95% CI, 0.87-0.99], $ICC_3 = 0.93$ [0.83-0.98]). In short, our easy-to-use photocopying

method, which involves placing the child's palm on a box composed of transparent acrylic thin plates and photographing it with a digital camera fixed inside the box, was devised for application in health checkup settings enforced by law. All the 2D:4D ratio data will be obtained using this protocol. Sub-cohort A1 will also be used to examine the relationship between neonatal oral-motor assessments and subsequent abnormal neurodevelopmental outcomes. Using the short version of Infant Behavior Questionnaire (IBQ) and Early Childhood Behavior Questionnaire (ECBQ) as factors reflecting temperamental self-regulation, the utility of such assessments as early screening tools for predicting subsequent neurodevelopmental outcomes will be verified.

The JECS-A do not include any information regarding medical diagnoses affecting neurodevelopmental outcomes at this time, but such information will be available in the future through the main study of the JECS, which is being conducted under the same protocol across 15 regions in Japan.

FINDINGS TO DATE

Participant characteristics of pregnant women

Table 2 shows the main socio-demographic characteristics of the pregnant women. To ascertain whether the JECS-A baseline profiles are representative of the pregnant women in the study areas in general, the national statistics for Aichi prefecture including Nagoya City and Ichinomiya City (since data for each city were not available) were included in the table. Similar distribution trends for variables such as "Age at delivery" were confirmed when the JECS-A baseline cohort of pregnant women and the national statistics were compared; however, differences in "Education level" and "Household income" were observed. Regarding education level, the percentage of JECS participants who had graduated from high

school was relatively low, compared with the national statistics (25.1% vs. 39.7%), while the percentage of college/junior college/technology college students was higher among the JECS participants, compared with the national statistics (35.3% vs. 20.8%). Moreover, the number of JECS-A participants with a household income of 2 million yen or less was about 1/8 of the national statistics. Likewise, unemployed participants (mainly housewives) accounted for 45% of the JECS-A cohort. These results suggest a possible selection bias. We recruited pregnant women who had visited the obstetric facilities during the daytime; this might have resulted in a lower study participation rate among pregnant employees.

Participant characteristics of children

As for the baseline cohort of children, a total of 5,502 (99.0%) of the 5,555 children remained in the JECS-A at 18 months after delivery (provisional figures as of August 1, 2018). **Table 3** compares the baseline data of the newborns with the national statistics. Basic demographics including "Sex", "Birth weight" and "Birth height" had similar distributions, indicating the representativeness of the sample.

Similarly, **Table 4** shows the fundamental characteristics of the pregnant women and their children in each sub-cohort of the JECS-A. No notable differences in the descriptive statistics were observed among the sub-cohorts. Furthermore, the drop-out rates for each sub-cohort, to date, have been maintained at less than 10% relative to the baseline. However, 10% of the sub-cohort values for household income are missing because many participants did not provide information on the income. Therefore, caution is needed when using this variable as a confounding factor in multiple regression analyses.

STRENGTHS AND LIMITATIONS

The main strength of the JECS-A of children is its large sample size, reflecting a representative population. Approximately 40% of the children born in the study area have been included in the study to date. The participating children will be followed until they reach the age of 13 years. The study protocol[33] of the JECS decided on a target retention rate of 80% or higher at the age of 13 years. The linkage between the JECS-A sub-cohort and the main data of the JECS study will further allow novel and challenging studies with a high generalizability.

The main weakness of the JECS-A cohort concerns the pregnant women participants related to a selective attrition. A relatively large proportion of single-income, middle-class households were included in the JECS-A; many low-income households refused to participate in the surveys, and some participants did not provide information concerning their household income. As enrollment strategies, though we set about recruiting from the first trimester of pregnancy (<12-week gestation), it was hard to get their cooperation in earlier weeks of a pregnancy, when the risk of miscarriage is higher. Most of pregnant women registered were actually after 10th week of pregnancy. Furthermore, we had no choice but to call on pregnant women for participation who had visited the obstetric facilities mainly during the daytime, owing to limited number of staffs during the evening shift at hospitals. Employed participants including all non-regular employees (part-time, temporary or contract employees) only accounted for 33% in the group of annual household income with 2 million Japanese yen or less. This means a selective attrition that working pregnant women of low SES might have been excluded in the cohorts. Such family socioeconomic status will have possibilities affecting several outcomes related to child development such as breastfeeding duration [34], obesity in children [35] and child maltreatment [36]. A British study [37], however, suggests that cognitive and behavioural development has a weak or absent direct effect of

income inequality after controlling potential confounders. Thus, possible biases as a result of selective attrition and direct SES effects for child should be carefully examined in future studies when using the JECS-A cohort data. The impact of the missing values can be adjusted to some extent by comparing estimates resulting from the multiple imputation and from complete case analysis, using the data of main study of the JECS as reference. However, researchers must take into account the effect of response biases when conducting statistical analyses using paired data for pregnant women and their children and when interpreting these results.

COLLABORATION

The original data and specimens will be made available to investigators and stakeholders working within the JECS-A project. The study must adhere to the JECS policy on the availability of research results, publications, intellectual property rights, and data sharing. At the moment, the Ethical Guidelines for Medical and Health Research Involving Human Subjects enforced by the Japan Ministry of Education, Culture, Sports, Science and Technology and the Ministry of Health, Labour and Welfare restrict the open sharing of epidemiologic data. This means that researchers interested in using the data must collaborate with and participate in the JECS-A project. This project was recently started and is still ongoing. We are open to new proposals that fall within the nature of the JECS-A cohort.

Acknowledgements

We would like to express our gratitude to all the JECS-A cohort participants. We also express our sincere appreciation to the collaborating hospitals, clinics, local healthcare centers, local government agencies in Nagoya City and Ichinomiya City, and the JECS-A

staff members for their continuous assistance in promoting this study. The authors also specially thank Takaaki Hiraiwa, Hiroki Yoshizoe, Maiko Miyata, Hirohisa Kano and Takahiro Kurihara for the data management and their assistance in the data analysis.

Contributors

TE designed the JECS-A cohort architecture, developed the protocol, analyzed the data, and wrote the first draft of the manuscript. YY and MT organized the study team, obtained approvals and contributed to the development of the protocol, design, and data collection tools. TOm was in charge of coordination with relevant organizations and organized the JECS-A members. NS, SK, TMa and TOg performed the analysis and interpreted the data. YI, HS, NO and JU designed and developed the protocol for the JECS-A sub-cohorts A2, A3 and B and edited and analyzed the data. AN, MKo, YO, TMi, SSu, MSO and SSa designed each adjunct study, supervised the data collection, and drafted the manuscript. MKa was a member of the JECS Steering Committee, was responsible for the study design and protocol, supervised the data collection, and edited and drafted the manuscript. All the authors interpreted the data, contributed to the writing of the manuscript, revised it critically for important intellectual content, and agreed with the final version and the findings.

Funding The Japan Environment and Children's Study was funded by the Ministry of the Environment, Japan. This work was also supported by grants from the Environment Research and Technology Development Fund (ERTDF, No 5-1551 and 5-1851), Japan Society for the Promotion of Science (JSPS) KAKENHI Grant Number 16H03733, 25293151, 25285185, 19H01078 and Grants-in-aid for research of Nagoya City University (2011-6, 2012-4, 2013-2, 2013-10, 2014-4, 2015-10, 2016-4).

Competing Interests None declared

Ethical approval This study was approved by the Review Board of the Ministry of the Environment, by the Institutional Review Board of Nagoya City University Graduate School of Medical Sciences (No. 70-00-0134; No. 00000544-6, 00000574-2).

Data sharing statement The original data and specimens will be made available to investigators and stakeholders working within the JECS-A project.

Researchers interested in collaborations or further information should contact the JECS-A secretariat by e-mail at eisei@med.nagoya-cu.ac.jp.

Disclaimer The findings and conclusions of this article are solely the responsibility of the authors and do not represent the official views of the Japanese government.

REFERENCES

1. Ishitsuka K, Nakayama SF, Kishi R, et al. Japan Environment and Children's Study: backgrounds, activities, and future directions in global perspectives. *Environ Health Prev Med* 2017;22(1):61. doi: 10.1186/s12199-017-0667-y
2. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders (DSM–5): American Psychiatric Association Publishing 2013:xiii-xv.
3. Xiang S, Carrie A. A review of the prevalence of Autism Spectrum Disorder in Asia, Research in Autism Spectrum Disorders. *Autism Spectrum Disorders*

2010;4(2):156-67. doi: http://dx.doi.org/10.1016/j.rasd.2009.10.003.

4. Hansen SN, Schendel DE, Parner ET. Explaining the increase in the prevalence of autism spectrum disorders: the proportion attributable to changes in reporting practices. *JAMA Pediatr* 2015;169(1):56-62. doi: 10.1001/jamapediatrics.2014.1893

5. Rodriguez-Barranco M, Lacasana M, Aguilar-Garduno C, et al. Association of arsenic, cadmium and manganese exposure with neurodevelopment and behavioural disorders in children: a systematic review and meta-analysis. *Sci Total Environ* 2013;454-455:562-77. doi: 10.1016/j.scitotenv.2013.03.047

6. Rodrigues EG, Bellinger DC, Valeri L, et al. Neurodevelopmental outcomes among 2- to 3-year-old children in Bangladesh with elevated blood lead and exposure to arsenic and manganese in drinking water. *Environ Health* 2016;15:44. doi: 10.1186/s12940-016-0127-y

7. Melchior M, Hersi R, van der Waerden J, et al. Maternal tobacco smoking in pregnancy and children's socio-emotional development at age 5: The EDEN mother-child birth cohort study. *Eur Psychiatry* 2015;30(5):562-8. doi: 10.1016/j.eurpsy.2015.03.005

8. Joelsson P, Chudal R, Talati A, et al. Prenatal smoking exposure and neuropsychiatric comorbidity of ADHD: a finnish nationwide population-based cohort study. *BMC Psychiatry* 2016;16:306. doi: 10.1186/s12888-016-1007-2

9. Han JY, Kwon HJ, Ha M, et al. The effects of prenatal exposure to alcohol and environmental tobacco smoke on risk for ADHD: a large population-based study. *Psychiatry Res* 2015;225(1-2):164-8. doi: 10.1016/j.psychres.2014.11.009

10. Padron A, Galan I, Garcia-Esquinas E, et al. Exposure to secondhand smoke in the home and mental health in children: a population-based study. *Tob Control* 2016;25(3):307-12. doi: 10.1136/tobaccocontrol-2014-052077

11. Polanska K, Ligocka D, Sobala W, et al. Phthalate exposure and child development: the Polish Mother and Child Cohort Study. *Early Hum Dev* 2014;90(9):477-85. doi: 10.1016/j.earlhumdev.2014.06.006

12. Kyriklaki A, Vafeiadi M, Kampouri M, et al. Prenatal exposure to persistent organic pollutants in association with offspring neuropsychological development at 4years of age: The Rhea mother-child cohort, Crete, Greece. *Environ Int* 2016;97:204-11. doi: 10.1016/j.envint.2016.09.012

13. Goudarzi H, Nakajima S, Ikeno T, et al. Prenatal exposure to perfluorinated chemicals and neurodevelopment in early infancy: The Hokkaido Study. *Sci Total Environ* 2016;541:1002-10. doi: 10.1016/j.scitotenv.2015.10.017

14. Yolton K, Cornelius M, Ornoy A, et al. Exposure to neurotoxicants and the development of attention deficit hyperactivity disorder and its related behaviors in childhood. *Neurotoxicol Teratol* 2014;44:30-45. doi: 10.1016/j.ntt.2014.05.003

15. Engel SM, Wetmur J, Chen J, et al. Prenatal exposure to organophosphates, paraoxonase 1, and cognitive development in childhood. *Environ Health Perspect* 2011;119(8):1182-8. doi: 10.1289/ehp.1003183

16. Bouchard MF, Chevrier J, Harley KG, et al. Prenatal exposure to organophosphate pesticides and IQ in 7-year-old children. *Environ Health Perspect* 2011;119(8):1189-95. doi: 10.1289/ehp.1003185

17. Stein LJ, Gunier RB, Harley K, et al. Early childhood adversity potentiates the adverse association between prenatal organophosphate pesticide exposure and child IQ: The CHAMACOS cohort. *Neurotoxicology* 2016;56:180-87. doi: 10.1016/j.neuro.2016.07.010

18. Yurumez E, Akca OF, Ugur C, et al. Mothers' alexithymia, depression and anxiety levels

and their association with the quality of mother-infant relationship: a preliminary study. *Int J Psychiatry Clin Pract* 2014;18(3):190-6. doi: 10.3109/13651501.2014.940055

19. Li Y, Jiang WQ, Du YS, et al. Relationships between behavioral symptoms of non-medicated Chinese children with attention deficit hyperactivity disorder and parenting stress: Comparison of different subtypes and comorbidities. *Asia Pac Psychiatry* 2016;8(2):127-35. doi: 10.1111/appy.12213
20. Oya N, Ito Y, Hioki K, et al. Quantitative analysis of organophosphate insecticide metabolites in urine extracted from disposable diapers of toddlers in Japan. *Int J Hyg Environ Health* 2017;220(2 Pt A):209-16. doi: 10.1016/j.ijheh.2016.10.009
21. Saito S, Ueyama J, Kondo T, et al. A non-invasive biomonitoring method for assessing levels of urinary pyrethroid metabolites in diapered children by gas chromatography-mass spectrometry. *J Expo Sci Environ Epidemiol* 2014;24(2):200-7. doi: 10.1038/jes.2013.31
22. Robins DL, Casagrande K, Barton M, et al. Validation of the modified checklist for Autism in toddlers, revised with follow-up (M-CHAT-R/F). *Pediatrics* 2014;133(1):37-45. doi: 10.1542/peds.2013-1813
23. Manning JT, Baron-Cohen S, Wheelwright S, et al. The 2nd to 4th digit ratio and autism. *Dev Med Child Neurol* 2001;43(3):160-4.
24. Teatero ML, Netley C. A critical review of the research on the extreme male brain theory and digit ratio (2D:4D). *J Autism Dev Disord* 2013;43(11):2664-76. doi: 10.1007/s10803-013-1819-6
25. Guyatt AL, Heron J, Knight Ble C, et al. Digit ratio and autism spectrum disorders in the Avon Longitudinal Study of Parents and Children: a birth cohort study. *BMJ Open*

2015;5(8):e007433. doi: 10.1136/bmjopen-2014-007433

26. Slattery J, Morgan A, Douglas J. Early sucking and swallowing problems as predictors of neurodevelopmental outcome in children with neonatal brain injury: a systematic review. *Dev Med Child Neurol* 2012;54(9):796-806. doi: 10.1111/j.1469-8749.2012.04318.x

27. Wolthuis-Stigter MI, Luinge MR, da Costa SP, et al. The association between sucking behavior in preterm infants and neurodevelopmental outcomes at 2 years of age. *J Pediatr* 2015;166(1):26-30. doi: 10.1016/j.jpeds.2014.09.007

28. Kawamoto T, Nitta H, Murata K, et al. Rationale and study design of the Japan environment and children's study (JECS). *BMC Public Health* 2014;14:25. doi: 10.1186/1471-2458-14-25

29. Michikawa T, Nitta H, Nakayama SF, et al. The Japan Environment and Children's Study (JECS): A Preliminary Report on Selected Characteristics of Approximately 10 000 Pregnant Women Recruited During the First Year of the Study. *J Epidemiol* 2015;25(6):452-8. doi: 10.2188/jea.JE20140186

30. Michikawa T, Nitta H, Nakayama SF, et al. Baseline Profile of Participants in the Japan Environment and Children's Study (JECS). *J Epidemiol* 2018;28(2):99-104. doi: 10.2188/jea.JE20170018

31. Booker CL, Harding S, Benzeval M. A systematic review of the effect of retention methods in population-based cohort studies. *BMC Public Health* 2011;11:249. doi: 10.1186/1471-2458-11-249

32. Moriguchi Y, Maeda M, Igarashi T, et al. Age and gender effect on alexithymia in large, Japanese community and clinical samples: a cross-validation study of the Toronto Alexithymia Scale (TAS-20). *Biopsychosoc Med* 2007;1:7. doi:

10.1186/1751-0759-1-7

33. National Centre for Japan Environment and Children's study. Japan Environment and Children's study (JECS), Study Protocol (ver. 1.4) [Available from: https://www.env.go.jp/en/chemi/hs/jecs/data/about/jecs-study_protocol_14_en.pdf.

34. Flacking R, Nyqvist KH, Ewald U. Effects of socioeconomic status on breastfeeding duration in mothers of preterm and term infants. *Eur J Public Health* 2007;17(6):579-84. doi: 10.1093/eurpub/ckm019

35. Ogden CL, Lamb MM, Carroll MD, et al. Obesity and socioeconomic status in children and adolescents: United States, 2005-2008. *NCHS Data Brief* 2010(51):1-8.

36. Eckenrode J, Smith EG, McCarthy ME, et al. Income inequality and child maltreatment in the United States. *Pediatrics* 2014;133(3):454-61. doi: 10.1542/peds.2013-1707

37. Violato M, Petrou S, Gray R, et al. Family income and child cognitive and behavioural development in the United Kingdom: does money matter? *Health Econ* 2011;20(10):1201-25. doi: 10.1002/hec.1665

Figure legend/caption

Figure 1 Study areas covered by the JECS-A

Figure 2. Flowchart showing each stage of study recruitment for adjunct studies in the JECS-A

Table 1 Summary of measurements collected for the adjunct studies conducted in JECS-A

Main items		Sub-cohort covered					Timing of measurement							
							prenatal (trimester)		postnatal (months after birth)					
		A1	A2	A3	B	C	1st	2nd/3rd	1 m	6 m	18 m	24 m	36 m
Pregnant women														
Risk and confounding factors														
Socio-demographic	Age, Maternal age, Marital status, Household income, Education level	○	○	○	○	○	○							
Lifestyle	Smoking, Second-hand smoking, Drinking habits, Dietary status, Sleeping habits	○	○	○		○	○	○	○	○	○	○	○	
Psychological and psychiatric	TAS–20	●					●	●						
	POMS-SF	●					●							
	Readiness of Parenthood Scale	●					●	●	●	●				
Obstetric	History of pregnancy, infertility treatment, prenatal diagnosis and gestational duration	○												
Biomonitoring data	Urine (up to 50 ml, mothers only), parental blood (up to 1.5ml), cord blood (up to 1.5ml)		●	●	●	●	●							
Children														
Risk and confounding factors														
Demographic	Height, Weight, BMI, Sex	○	○	○	○	○								
Behavioral	Neonatal oral-motor assessment	●							●					
	DCDQ	●									●		●	
Childcare	Breast feeding and weaning food status	●									●			
Psychological and psychiatric	Mary Rothbart's Temperament Questionnaires	●												
	・IBQ-R-SF										●			
	・ECBQ-SF											●		
Biomonitoring data	Overnight urine extracted from disposable diapers (up to 30ml)				●						●		●	
Anthropometric data	2D:4D				●						●		●	
Outcome examples to be analyzed														
LBW			○	○					○					
ASD		○	○	○	○	○							○	
ADHD		○	○	○		○							○	

○: Refer to the main study of the Japan Environment and Children's Study (JECS), ●: Measurements conducting solely in the JECS-A

BMI, body mass index; **TAS-20**, the 20–item Toronto Alexithymia Scale; **POMS-SF**, Short form of the Profile of Mood States; **IBQ-R-SF**, The Infant Behavior Questionnaire Revised Short Form; **ECBQ-SF**, Early Childhood Behavior Questionnaire Short Form; **DCDQ**, Developmental Coordination Disorder Questionnaire, **2D:4D**, the second to fourth digit ratio; **PARS**, Pervasive Developmental Disorders Autism Society Japan Rating Scale; **ASD**, autism spectrum disorder; **ADHD**, attention deficit hyperactivity disorder; LBW, low birth weight.

Sub cohort A1, Agreement on the adjunct questionnaire survey only; **Sub cohort A2**, Agreement on the adjunct questionnaire survey and provision of mother's biological specimen (blood and urine); **Sub cohort A3**, Agreement on the adjunct questionnaire survey and provision of mother's biological specimen (blood and urine) with genetic analyses; **Sub cohort B**, Informed consent obtained from guardians (legally acceptable representatives) for provision of child's biological specimen; **Sub cohort C**, Informed consent obtained from guardians (legally acceptable representatives) for anthropometry measurement on child hands

Table 2 Comparison of the JECS-A baseline data with national statistics in Aichi prefecture for pregnant women: verification of representativeness of the sample

	JECS-A baseline cohort of pregnant women (95% CI)	National statistics (Aichi pref.)
Pregnant women [a] (n)	5721	65218
Age at Delivery [a]		
15-19 (%)	0.7 (0.5-0.9)	1.3
20-24	6.7 (6.0-7.3)	8.2
25-29	25.3 (24.1-26.4)	28.3
30-34	36.4 (35.1-37.6)	36.6
35-39	23.2 (22.1-24.3)	21.4
40-44	4.9 (4.4-5.5)	4.2
45-49	0.1 (0.0-0.2)	0.1
50-	0.0	0.0
Missing (n)	157	
Education level [b] (n)		2397000
Junior high school/High school (%)	29.9 (28.7-31.1)	47.2
College/Junior college/Technology college	35.3 (34.1-36.6)	20.8
University	27.6 (26.4-28.8)	28.4
Graduate school	1.6 (1.3-2.0)	3.6
Missing (n)	315	
Household income [b c d] (n)		3018900
<2,000 (%)	2.4 (2.0-2.8)	18.1
2,000-<4,000	24.4 (23.3-25.5)	26.7
4,000-<6,000	33.1 (31.9-34.3)	20.2
6,000-<8,000	17.2 (16.2-18.2)	13.4
8,000-<10,000	7.4 (6.7-8.1)	8.3
≥10,000	4.3 (3.7-4.8)	5.0
Missing (n)	643	

[a] Data of Aichi prefecture as reference, provided by national vital statistics, Ministry of Health, Labour and Welfare in 2014.
[b] Data of Aichi prefecture provided by Ministry of Internal Affairs and Communications in 2012.
[c] Population of Aichi Prefecture including single-person households and families
[d] Household income shows thousand Japanese Yen(JPY), 110 JPY = 1US$ as of 2019

-: no available data provided; 95%CI: 95% Confidence Interval

Table 3 Comparison of the JECS-A baseline data with national statistics in Aichi prefecture for children: verification of representativeness of the sample

	JECS-A baseline cohort of children (n = 5457)			National statistics (Aichi pref.) [a]		
	Total	Male	Female	Total	Male	Female
Children (n) [b]	5457	2793	2659	65218	33649	31569
Sex (Singleton births)						
(%)		51.2	48.7		51.6	48.4
95% CI		(50.5-51.9)	(48.0-49.4)			
Missing (n)	5					
Birth weight, g (Singleton births)						
Mean	3034.3	3076.1	2990.7	3000.0	3040.0	2950.0
95%CI	(3022.9-3045.7)	(3059.7-3092.5)	(2975.1-3006.3)	-	-	-
Missing (n)	43	23	16			
Low birth weight (Singleton births)						
<2500 g (%)	7.8	6.6	9.0	9.8	8.5	11.1
95%CI	(7.4-8.2)	(6.3-6.9)	(8.7-9.3)			
Missing (n)	43	23	16			
Birth length, cm (Singleton births)						
Mean	49.7	49.9	49.5	49.3	49.6	49.1
95%CI	(49.6-49.8)	(49.8-50.0)	(49.4-49.6)	-	-	-
Missing (n)	51	28	19			

[a] Data of Aichi prefecture as reference, provided by national vital statistics, Ministry of Health, Labour and Welfare in 2014.

[b] Multiple births (49 pairs of twins) were excluded.

-: no available data provided, 95%CI: 95% Confidence Interval

Table 4 Baseline characteristics of paired data of pregnant women and their children in the JECS-A cohort

	Sub-cohort				
	A1	A2	A3	B	C
Pregnant women (n)	3426	2924	1753	1352	1187
Age at Delivery					
Mean	31.6	31.7	31.8	31.8	32.0
SD	4.9	4.9	5.0	4.8	4.7
Missing (n)	44	14	7	0	0
Smoking habits					
Never smoked (%)	62.8	63.7	63.7	64.2	64.6
Ex-smokers who quit before pregnancy	21.6	22.0	23.1	21.8	23.1
Smokers during early pregnancy	9.0	9.0	8.3	8.4	7.7
Smokers	2.5	2.5	2.1	2.8	2.2
Missing (n)	144	82	51	38	29
Second-hand smoking					
Rarely (%)	67.5	69.1	69.6	68.6	71.9
A few days a week	18.6	18.6	18.0	19.1	17.1
Daily	10.4	10.1	10.3	10.1	9.6
Missing (n)	124	66	36	30	17
Household income (JPY)					
<2,000 (%)	2.3	2.1	2.5	1.8	2.1
2,000-<4,000	25.8	26.0	25.6	24.4	24.3
4,000-<6,000	33.4	33.7	34.2	35.1	35.9
6,000-<8,000	17.5	18.1	17.5	18.6	18.6
8,000-<10,000	7.3	7.5	7.8	7.5	8.3
≥10,000	4.5	4.7	4.5	3.9	4.5
Missing (n)	314	233	141	116	76

Children (n)[a]	3336	2884	1726	1346	1182
Sex (Singleton births)					
Male (%)	50.5	50.4	50.1	49.9	50.4
Female	49.5	49.6	49.8	50.1	49.6
Missing (n)	1	1	1	0	0
Birth weight, g (Singleton births)					
Mean	3041.6	3052.3	3054.1	3039.1	3062.3
SD	432.8	419.9	430.4	410.0	416.7
Missing (n)	22	15	9	4	7
Birth height, cm (Singleton births)					
Mean	49.6	49.7	49.7	49.7	49.7
SD	2.3	2.2	2.2	2.1	2.1
Missing (n)	28	18	11	6	8

[a] Multiple births (twin pairs) were excluded; A1: 20 (n = 40), A2: 16 (n = 32), A3: 12 (n = 24), B: 5 (n = 10), and C: 6 (n = 12).

Sub cohort A1, Agreement on the adjunct questionnaire survey only; **Sub cohort A2**, Agreement on the adjunct questionnaire survey and provision of mother's biological specimen (blood and urine); **Sub cohort A3**, Agreement on the adjunct questionnaire survey and provision of mother's biological specimen (blood and urine) with genetic analysis; **Sub cohort B**, Informed consent obtained for provision of child's biological specimen from guardian (legally acceptable representatives); **Sub cohort C**, Informed consent obtained for anthropometry measurement on child hands from guardians (legally acceptable representatives). JPY: Japanese Yen, 110 JPY = 1 US$ as of 2019

Figure 1 Study areas covered by the JECS-A

Figure 1 Study areas covered by the JECS-A

65x82mm (300 x 300 DPI)

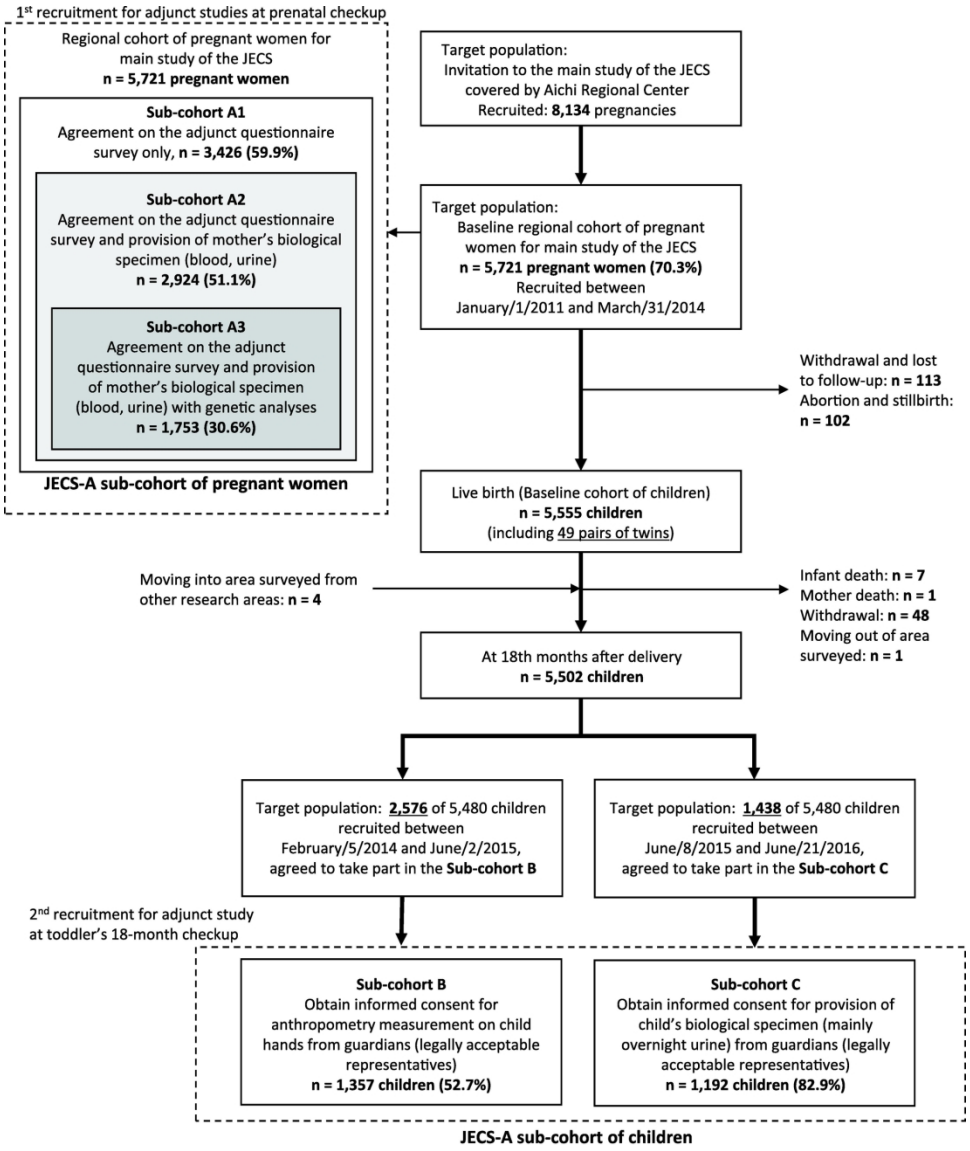

Figure 2. Flowchart showing each stage of study recruitment for adjunct studies in the JECS-A

Figure 2. Flowchart showing each stage of study recruitment for adjunct studies in the JECS-A

178x223mm (300 x 300 DPI)