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

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

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5 **Strengths and limitations of this study**

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- The main strength of the J ECS-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.

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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

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4 childhood exposure. A cohort study of seven-year-old children suggested that social adversities
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6 including a poor learning environment and parent-child interactions were significantly
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8 associated with a moderate decrease in the intelligence quotient (IQ) score and its subset
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10 scores.¹⁷ Several recent studies have also discussed the effects of parent-child interactions,
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12 focusing on the effects of alexithymia in mothers¹⁸ and of depressive symptoms on parenting
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14 stress.¹⁹ These lines of evidence suggest that factors influencing children's neurodevelopment
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16 have multifaceted aspects extending from the prenatal period to early childhood. Accumulating
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18 knowledge gained from epidemiological studies suggests the need to investigate postnatal
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20 influences, such as the effects of interactions between mothers and their children, as well as
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22 prenatal exposures on the neurodevelopment of children.
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28 On the other hand, the establishment of a methodology for exposure assessments during
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30 early childhood is also urgently required. Although the central nervous system develops rapidly
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32 during early childhood, the amount of exposure to environmental chemical substances taken
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34 into the bodies of infants has rarely been investigated because most infants wear diapers that
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36 fully absorb all urine. Noninvasive biomonitoring using urine samples is required for exposure
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38 assessments during early childhood, and a methodology for extracting urine from used
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40 disposable diapers has also been studied.^{20 21}
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45 Furthermore, public health services in local communities must be capable of providing early
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47 intervention support for children with neurodevelopmental disorders. Preventive screening for
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49 the detection of disorders during the critical period of brain development during early
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51 childhood can help to minimize later difficulties and to improve the trajectory of subjects with
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53 ADHD in later life. A validated screening checklist for autism at the age of 2 years, called M-
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55 CHAT-R/F,²² is also available as part of early medical checkups, but this screening protocol
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57 requires a 2-step screening test that must be conducted by a physician. The development of
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4 secondary tools allowing easy and objective screening would provide a reliable means of
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6 detecting disabilities at earlier stages, enabling the provision of early intervention support for
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8 children with disabilities. One solution for earlier screening might be the second to fourth digit
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10 (2D:4D) ratio, which is defined as the proportion between the lengths of the index and ring
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12 fingers. Recent studies^{23 24} have investigated whether the 2D:4D ratio, a controversial but
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14 commonly used proxy marker of prenatal androgen concentrations based on the extreme male
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16 brain theory, is associated with ASD. If anthropometric differences in the 2D:4D ratio could
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18 be confirmed at an earlier stage of childhood, the 2D:4D ratio might be available as an
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20 alternative and indirect screening measure. Another recent trend for early intervention can be
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22 found in neonatal oral-motor assessments, such as sucking behavior. While contradictory
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24 findings have been obtained,²⁵ a recent longitudinal study also showed interesting evidence
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26 that some sucking behaviors in preterm infants are associated with later abnormal
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28 neurodevelopmental outcomes at the age of 2 years.²⁶ As the available early screening tools
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30 for the prediction of later neurodevelopmental outcomes are presently insufficient, further
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32 research on the development of screening tools for early intervention is needed.
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40 The JECS-A was established in 2011 and has four main objectives: (1) to ascertain the effects
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42 of prenatal and postnatal chemical exposures on pregnant women and their children; (2) to
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44 investigate potential exposures for which the neurodevelopmental outcomes were modified by
45
46 the mothers' interactions with their children during early childhood; (3) to develop a
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48 biomonitoring method using urine samples extracted from diapers for measuring internal
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50 exposure to chemical substances; and (4) to ascertain the availabilities of secondary tools such
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52 as the 2D:4D ratio or neonatal oral-motor assessments at earlier stages of childhood. This
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54 article outlines the JECS-A cohort and its baseline data to date.
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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

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4 recruitment period from January 2011 to March 2014, a total of 5,721 (70.3%) participants
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6 were enrolled as the baseline cohort of pregnant women.
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9 After recruitment, the registered JECS participants were also invited to participate in the
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11 JECS-A adjunct studies if they provided any of the following three types of consent: agreement
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13 to complete the adjunct questionnaire survey only (Sub-cohort A1, n = 3,426), agreement to
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15 provide maternal biological specimens in addition to agreeing to participate in Sub-cohort A1
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17 (Sub-cohort A2, n = 2,924), and agreement to undergo genetic analyses in addition to agreeing
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19 to participate in Sub-cohorts A1 and A2 (Sub-cohort A3, n = 1,753). Subsequently, of the 5,555
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21 children, including 49 pairs of twins, born from the enrolled mothers, 57 children dropped out
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23 of the study because of infant death (n = 7), mother death (n = 1), withdrawal of consent (n =
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25 48), or a change in residence to an area outside the surveyed area (n = 1). On the other hand,
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27 4 children who moved into the surveyed area from another area were included. As of 18 months
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29 after delivery, a total of 5,502 children remained within the JECS-A cohort, accounting for
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31 approximately 40% of the children born in the study area.
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38 A second recruitment for the JECS-A adjunct studies was also planned, focusing on children.
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40 We approached the guardians of targeted children at legal check-ups for 18-month-olds
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42 provided at regional public health centers and health consultation centers. The recruitment
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44 period was divided into two phases. Of the 5,502 children remaining in the JECS-A cohort at
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46 18 months of age, a total of 2,576 children had reached the age of 18 months during the second
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48 year of the 3-year recruitment period. During this second-year period, we conducted a survey
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50 of the anthropometric measurements of the hands of 18-month-old children to confirm the
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52 applicability of the 2D:4D ratio during early childhood. By gradually increasing the number
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54 of recruitment facilities capable of obtaining the consent of the children's guardians during
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56 this period, a total of 1,357 children (coverage of target samples: 52.7%, consent rate: 99.0%)
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4 were enrolled in Sub-cohort C. Subsequent to this recruitment, another recruitment was also
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6 set for 18-month-old children born from mothers registered during the last year of the pregnant
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8 women recruitment (Sub-cohort B). This cohort was specifically planned to collect biological
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10 samples from diapers, and written informed consent for the adjunct survey involving the
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12 provision of biological specimens from each child was obtained from 1,192 children (82.9%).
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14 All the enrolled children had reached the age of 1.5 years as of June 2016. The follow-up
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16 schedule for children beyond the age of 3 years has yet to be finalized, but these sub-cohorts
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18 will be followed until the children reach the age of 13 years. Note that the above figures were
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20 based on the data set jecs-ag-20160424, which was released in June, 2016, and on the
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22 provisional data set determined as of August 1, 2018.
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30 **Patient and Public Involvement**

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

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4 The measurements collected for the adjunct studies of JECS in the JECS-A are summarized
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6 in **Table 1**. Data acquisition for some socio-demographics and neurodevelopmental outcomes
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8 was planned within the main body of the JECS.
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11 The adjunct cohorts were designed for four specific main objectives. First, to challenge the
12
13 multifaceted problems surrounding children's development, Sub-cohort A1 was formed with
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15 the intention of collecting data on longitudinal changes in prenatal and postnatal exposures
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17 considered to affect neurodevelopmental outcomes. One of our special concerns is the effect
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19 of alexithymia in mothers on early child development. The 20-item Toronto Alexithymia Scale
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21 (TAS-20)³⁰ will be used to measure this trait as a primary factor, and studies will investigate
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23 the pregnancy and perinatal outcomes and/or the developmental outcomes of postnatal infants.
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25 As there are many potential determinants extending from the prenatal to early childhood period,
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27 the risks will be estimated using the mother's and child's demographic variables or other
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29 related factors as confounding factors.
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35 Sub-cohort A1 will also be used to examine the relationship between neonatal oral-motor
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37 assessments and subsequent abnormal neurodevelopmental outcomes. Using the short version
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39 of Infant Behavior Questionnaire (IBQ) and Early Childhood Behavior Questionnaire (ECBQ)
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41 as factors reflecting temperamental self-regulation, the utility of such assessments as early
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43 screening tools for predicting subsequent neurodevelopmental outcomes will be verified.
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48 Sub-cohorts A2 and A3 will mainly focus on studies using biological specimens (urine,
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50 serum, and/or whole blood) obtained from pregnant women. These biological samples will be
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52 used for exposure assessments, single nucleotide polymorphism analyses, and activity
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54 determinations of chemical metabolism-related enzymes, and so on. Data on the levels of
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56 urinary exposure biomarkers including chemical metabolites will be combined with those in
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58 sub-cohort B and, eventually, the longitudinal exposure trajectory and the relationships
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4 between exposure and health outcomes will also be analyzed. These cohorts will be used to
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6 derive human biomonitoring reference values (e.g., RV95) for chemicals in urine samples for
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8 the general population of Japanese pregnant women.
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11 Sub-cohort B will be used to clarify the chemical exposure levels in infants and toddlers.
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13 Biological monitoring data for chemical exposure during early childhood are limited because
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15 the collection of urine from non-toilet-trained children is difficult. To estimate the amount of
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17 exposure to environmental chemical substances in the bodies of infants, the sub-cohort will be
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19 used to study a noninvasive biomonitoring approach using urine samples extracted from used
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21 disposable diapers. This cohort will also be used to determine the reference values for
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23 chemicals in urine samples for the general population of Japanese young children.
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28 Sub-cohort C will be used to study anthropometric measurements of the lengths of digits on
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30 both hands in children. A previous study suggested that autistic children aged 2 to 14 years
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32 have a smaller 2nd digit to 4th digit (2D:4D) ratio than normal children of the same
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34 generation.³¹ However, little is known about whether the 2D:4D ratio in Japanese infants and
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36 children under the age of 2 years can be used for the early diagnosis of autism, and the results
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38 of studies are contradictory. Thus, Sub-cohort C was designed to investigate the longitudinal
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40 changes in 2D:4D ratios in 18-month-old and 36-month-old infants. As for the anthropometric
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42 measurements of the palms and digits of the children's hands, we have established a specialized
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44 protocol involving photographic records and digit measurements that has a high reliability
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46 (Intra/Inter-Class Correlations: $ICC_1 = 0.97$ [95% CI, 0.87-0.99], $ICC_3 = 0.93$ [0.83-0.98]). In
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48 short, our easy-to-use photocopying method, which involves placing the child's palm on a box
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50 composed of transparent acrylic thin plates and photographing it from a digital camera fixed
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52 inside the box, was devised for application in mandatory health checkup settings. All the
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54 2D:4D ratio data will be obtained using this protocol.
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4 The JECS-A sub-cohorts do not include any information regarding medical diagnoses
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6 affecting neurodevelopmental outcomes at this time, but such information will be available in
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8 the future through the main data of the JECS, which is being conducted under the same protocol
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10 across 15 regions in Japan.
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16 FINDINGS TO DATE

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18 **Table 2** shows the main socio-demographic characteristics of the pregnant women. To
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20 ascertain whether the JECS-A baseline profiles are representative of the pregnant women in
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22 the study areas in general, the national statistics for Aichi prefecture including Nagoya City
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24 and Ichinomiya City (since data for smaller areas were not available) were substituted and
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26 included in the table. Similar distribution trends for variables such as “Age at delivery” were
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28 confirmed when the JECS-A baseline cohort of pregnant women and the national statistics
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30 were compared; however, differences in “Education level” and “Household income” were
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32 observed. Regarding education level, the percentage of JECS participants who had graduated
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34 from high school was relatively low, compared with the national statistics (25.1% vs. 39.7%),
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36 while the percentage of college/junior college/technology college students was higher among
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38 the JECS participants, compared with the national statistics (35.3% vs. 20.8%). Moreover, the
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40 number of JECS-A participants with a household income of 2 million yen or less was about
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42 1/8 of the national statistics. Likewise, unemployed participants (mainly housewives)
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44 accounted for 45% of the JECS-A cohort. These results suggest a possible selection bias. We
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46 recruited pregnant women who had visited the obstetric facilities during the daytime; this might
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48 have resulted in a lower study participation rate among pregnant employees.
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57 As for the baseline cohort of children, a total of 5,502 (99.0%) of the 5,555 children remained
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59 in the JECS-A at 18 months after delivery (provisional figures as of August 1, 2018). **Table 3**
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4 compares the baseline data of the newborns with the national statistics. Basic demographics
5 including “Sex”, “Birth weight” and “Birth height” had similar distributions, indicating the
6 representativeness of the sample.
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11 Similarly, **Table 4** shows the fundamental characteristics of the pregnant women and their
12 children in each sub-cohort of the JECS-A. No notable differences in the descriptive statistics
13 were observed among the sub-cohorts. Furthermore, the drop-out rates for each sub-cohort, to
14 date, have been maintained at less than 10% relative to the baseline. However, 10% of the sub-
15 cohort values for household income are missing because many participants did not provide
16 information on the income. Therefore, caution is needed when using this variable as a
17 confounding factor in multiple regression analyses.
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30 **STRENGTHS AND LIMITATIONS**

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33 The main strength of the JECS-A of children is its large sample size, reflecting a
34 representative population. Approximately 40% of the children born in the study area have been
35 included in the study to date. The participating children will be followed until they reach the
36 age of 13 years. The study protocol³² of the JECS decided on a target retention rate of 80% or
37 higher at the age of 13 years. The linkage between the JECS-A sub-cohort and the main data
38 of the JECS study will further allow novel and challenging studies with a high generalizability.
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47 The main weakness of the JECS-A cohort concerns the pregnant women participants. A
48 relatively large proportion of single-income, middle-class households were included in the
49 JECS-A; many low-income households refused to participate in the surveys, and some
50 participants did not provide information concerning their household income. Regarding the
51 latter, the impact of the missing values can be adjusted to some extent using the multiple
52 imputation method. However, researchers must take into account the effect of response biases
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4 when conducting statistical analyses using paired data for pregnant women and their children
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6 and when interpreting these results.
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10 **COLLABORATION**

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

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59 TE designed the JECS-A cohort architecture, developed the protocol, analyzed the data, and
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4 wrote the first draft of the manuscript. YY and MT organized the study team, obtained
5
6 approvals and contributed to the development of the protocol, design, and data collection tools.
7
8 TOm was in charge of coordination with relevant organizations and organized the JECS-A
9
10 members. NS, SK, TMa and TOg performed the analysis and interpreted the data. YI, HS, NO
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16 study, supervised the data collection, and drafted the manuscript. MK was a member of the
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18 JECS Steering Committee, was responsible for the study design and protocol, supervised the
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20 data collection, and edited and drafted the manuscript. All the authors interpreted the data,
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4 Committees of all participating institutions.
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8 **Data sharing statement** The original data and specimens will be made available to
9
10 investigators and stakeholders working within the JECS-A project.
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12
13 Researchers interested in collaborations or further information should contact the JECS-A
14
15 secretariat by e-mail at eisei@med.nagoya-cu.ac.jp.
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20 **Disclaimer** The findings and conclusions of this article are solely the responsibility of the
21
22 authors and do not represent the official views of the Japanese government.
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26 27 28 29 30 **REFERENCES**

- 31
32
33 1. Ishitsuka K, Nakayama SF, Kishi R, et al. Japan Environment and Children's Study:
34
35 backgrounds, activities, and future directions in global perspectives. *Environ Health*
36
37 *Prev Med* 2017;22(1):61. doi: 10.1186/s12199-017-0667-y
38
39
40 2. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders
41
42 (DSM–5): American Psychiatric Association Publishing 2013:xiii-xv.
43
44
45 3. Xiang S, Carrie A. A review of the prevalence of Autism Spectrum Disorder in Asia,
46
47 Research in Autism Spectrum Disorders. *Autism Spectrum Disorders* 2010;4(2):156-67.
48
49 doi: <http://dx.doi.org/10.1016/j.rasd.2009.10.003>.
50
51
52 4. Hansen SN, Schendel DE, Parner ET. Explaining the increase in the prevalence of autism
53
54 spectrum disorders: the proportion attributable to changes in reporting practices. *JAMA*
55
56 *Pediatr* 2015;169(1):56-62. doi: 10.1001/jamapediatrics.2014.1893
57
58
59 5. Rodriguez-Barranco M, Lacasana M, Aguilar-Garduno C, et al. Association of arsenic,
60

- 1
2
3
4 cadmium and manganese exposure with neurodevelopment and behavioural disorders in
5
6 children: a systematic review and meta-analysis. *Sci Total Environ* 2013;454-455:562-
7
8 77. doi: 10.1016/j.scitotenv.2013.03.047
9
10
11 6. Rodrigues EG, Bellinger DC, Valeri L, et al. Neurodevelopmental outcomes among 2- to 3-
12
13 year-old children in Bangladesh with elevated blood lead and exposure to arsenic and
14
15 manganese in drinking water. *Environ Health* 2016;15:44. doi: 10.1186/s12940-016-
16
17 0127-y
18
19
20
21 7. Melchior M, Hersi R, van der Waerden J, et al. Maternal tobacco smoking in pregnancy and
22
23 children's socio-emotional development at age 5: The EDEN mother-child birth cohort
24
25 study. *Eur Psychiatry* 2015;30(5):562-8. doi: 10.1016/j.eurpsy.2015.03.005
26
27
28 8. Joelsson P, Chudal R, Talati A, et al. Prenatal smoking exposure and neuropsychiatric
29
30 comorbidity of ADHD: a finnish nationwide population-based cohort study. *BMC*
31
32 *Psychiatry* 2016;16:306. doi: 10.1186/s12888-016-1007-2
33
34
35 9. Han JY, Kwon HJ, Ha M, et al. The effects of prenatal exposure to alcohol and environmental
36
37 tobacco smoke on risk for ADHD: a large population-based study. *Psychiatry Res*
38
39 2015;225(1-2):164-8. doi: 10.1016/j.psychres.2014.11.009
40
41
42 10. Padron A, Galan I, Garcia-Esquinas E, et al. Exposure to secondhand smoke in the home
43
44 and mental health in children: a population-based study. *Tob Control* 2016;25(3):307-
45
46 12. doi: 10.1136/tobaccocontrol-2014-052077
47
48
49
50 11. Polanska K, Ligocka D, Sobala W, et al. Phthalate exposure and child development: the
51
52 Polish Mother and Child Cohort Study. *Early Hum Dev* 2014;90(9):477-85. doi:
53
54 10.1016/j.earlhumdev.2014.06.006
55
56
57 12. Kyriklaki A, Vafeiadi M, Kampouri M, et al. Prenatal exposure to persistent organic
58
59 pollutants in association with offspring neuropsychological development at 4years of
60

- 1
2
3
4 age: The Rhea mother-child cohort, Crete, Greece. *Environ Int* 2016;97:204-11. doi:
5
6 10.1016/j.envint.2016.09.012
7
8
9 13. Goudarzi H, Nakajima S, Ikeno T, et al. Prenatal exposure to perfluorinated chemicals and
10
11 neurodevelopment in early infancy: The Hokkaido Study. *Sci Total Environ*
12
13 2016;541:1002-10. doi: 10.1016/j.scitotenv.2015.10.017
14
15
16 14. Yolton K, Cornelius M, Ornoy A, et al. Exposure to neurotoxicants and the development
17
18 of attention deficit hyperactivity disorder and its related behaviors in childhood.
19
20 *Neurotoxicol Teratol* 2014;44:30-45. doi: 10.1016/j.ntt.2014.05.003
21
22
23 15. Engel SM, Wetmur J, Chen J, et al. Prenatal exposure to organophosphates, paraoxonase
24
25 1, and cognitive development in childhood. *Environ Health Perspect* 2011;119(8):1182-
26
27 8. doi: 10.1289/ehp.1003183
28
29
30 16. Bouchard MF, Chevrier J, Harley KG, et al. Prenatal exposure to organophosphate
31
32 pesticides and IQ in 7-year-old children. *Environ Health Perspect* 2011;119(8):1189-
33
34 95. doi: 10.1289/ehp.1003185
35
36
37 17. Stein LJ, Gunier RB, Harley K, et al. Early childhood adversity potentiates the adverse
38
39 association between prenatal organophosphate pesticide exposure and child IQ: The
40
41 CHAMACOS cohort. *Neurotoxicology* 2016;56:180-87. doi:
42
43 10.1016/j.neuro.2016.07.010
44
45
46
47 18. Yurumez E, Akca OF, Ugur C, et al. Mothers' alexithymia, depression and anxiety levels
48
49 and their association with the quality of mother-infant relationship: a preliminary study.
50
51 *Int J Psychiatry Clin Pract* 2014;18(3):190-6. doi: 10.3109/13651501.2014.940055
52
53
54 19. Li Y, Jiang WQ, Du YS, et al. Relationships between behavioral symptoms of non-
55
56 medicated Chinese children with attention deficit hyperactivity disorder and parenting
57
58 stress: Comparison of different subtypes and comorbidities. *Asia Pac Psychiatry*
59
60

- 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

- 1
2
3
4 27. Kawamoto T, Nitta H, Murata K, et al. Rationale and study design of the Japan environment
5
6 and children's study (JECS). *BMC Public Health* 2014;14:25. doi: 10.1186/1471-2458-
7
8 14-25
9
10
11 28. Michikawa T, Nitta H, Nakayama SF, et al. The Japan Environment and Children's Study
12
13 (JECS): A Preliminary Report on Selected Characteristics of Approximately 10 000
14
15 Pregnant Women Recruited During the First Year of the Study. *J Epidemiol*
16
17 2015;25(6):452-8. doi: 10.2188/jea.JE20140186
18
19
20
21 29. Michikawa T, Nitta H, Nakayama SF, et al. Baseline Profile of Participants in the Japan
22
23 Environment and Children's Study (JECS). *J Epidemiol* 2018;28(2):99-104. doi:
24
25 10.2188/jea.JE20170018
26
27
28 30. Moriguchi Y, Maeda M, Igarashi T, et al. Age and gender effect on alexithymia in large,
29
30 Japanese community and clinical samples: a cross-validation study of the Toronto
31
32 Alexithymia Scale (TAS-20). *Biopsychosoc Med* 2007;1:7. doi: 10.1186/1751-0759-1-
33
34 7
35
36
37
38 31. Manning JT, Baron-Cohen S, Wheelwright S, et al. The 2nd to 4th digit ratio and autism.
39
40 *Dev Med Child Neurol* 2001;43(3):160-4.
41
42
43 32. National Centre for Japan Environment and Children's study. Japan Environment and
44
45 Children's study (JECS), Study Protocol (ver. 1.4) [Available from:
46
47 https://www.env.go.jp/en/chemi/hs/jecs/data/about/jecs-study_protocol_14_en.pdf.
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Table 1 Summary of measurements collected for the adjunct JECS-A study

Main items	Sub-cohort covered					Timing of measurement					
	A1	A2	A3	B	C	prenatal (weeks)		postnatal (months after birth)			
						<22	≥22	1 m	6 m	18 m	24 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)

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4 **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
5 Revised Short Form; **ECBQ-SF**, Early Childhood Behavior Questionnaire Short Form; **DCDQ**, Developmental Coordination Disorder Questionnaire, **2D:4D**, the second to
6 fourth digit ratio; **PARS**, Pervasive Developmental Disorders Autism Society Japan Rating Scale; **ASD**, autism spectrum disorder; **ADHD**, attention deficit hyperactivity
7 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
8 JECS is a gestational age of more than 22 weeks.
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11 **Sub cohort A1**, Agreement on the adjunct questionnaire survey only; **Sub cohort A2**, Agreement on the adjunct questionnaire survey and provision of mother's
12 biological specimen (urine); **Sub cohort A3**, Agreement on the adjunct questionnaire survey and provision of mother's biological specimen (urine) with gene analysis;
13 **Sub cohort B**, Obtaining informed consent for provision of child's biological specimen from guardian (legally acceptable representatives); **Sub cohort C**, Obtaining
14 informed consent for anthropometry measurement on child hands from guardian (legally acceptable representatives)
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For peer review only

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 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 2017

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

For peer review only

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	118 7	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	118 2	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	306 2.3	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

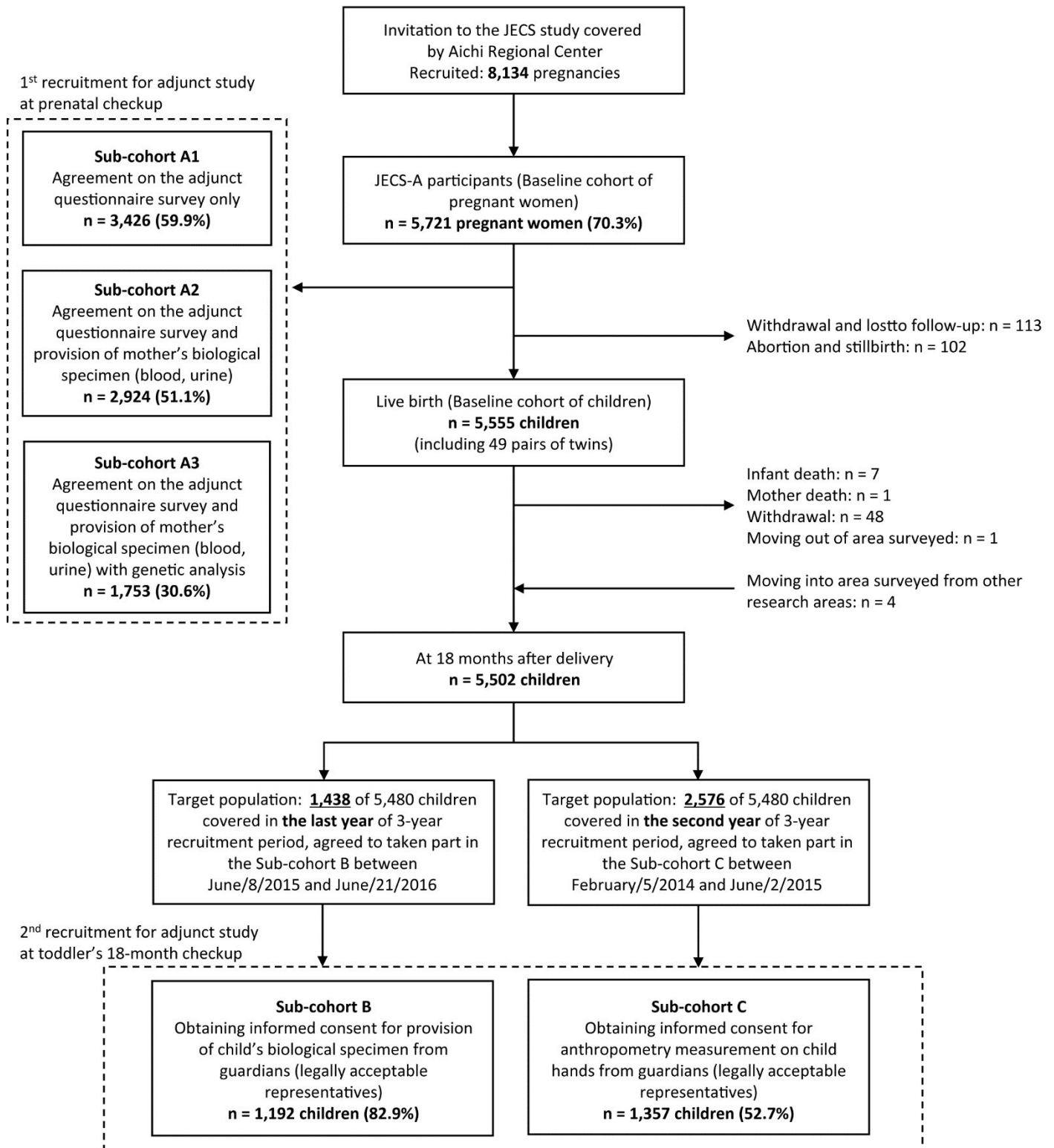


Figure 1. Flowchart showing each stage of study recruitment

BMJ Open

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

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Cohort profile: Aichi regional sub-cohort of the Japan Environment and Children's

Study (JECS-A)

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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)

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5 **Strengths and limitations of this study**

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- The main strength of the J ECS-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.

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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 disorders² 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} Furthermore, interest is growing regarding the potential role of social

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4 stressors in modifying the relationship between the above early childhood exposure and the
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6 neurodevelopmental outcomes. A cohort study of seven-year-old predominantly Mexican
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8 American children in California's Salinas Valley suggested that social adversities including a
9
10 poor learning environment and parent-child interactions were significantly associated with a
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12 moderate decrease in the intelligence quotient (IQ) score and its subset scores.¹⁷ Several
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14 recent studies have also discussed the effects of parent-child interactions, focusing on the
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16 effects of alexithymia in mothers¹⁸ and of depressive symptoms on parenting stress.¹⁹ Thus,
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18 there is the need to investigate postnatal influences, such as the effects of interactions
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20 between mothers and their children, as well as prenatal and postnatal toxicant exposures on
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22 the neurodevelopment of children. The above research questions will be addressed to
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24 complement those of the main study of the JECS.
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31 The second theme of the adjunct studies conducted in JECS-A is the exposure assessments
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33 using urine collected during early childhood when the central nervous system develops
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35 rapidly. So far, the amount of exposure to environmental chemical substances taken into the
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37 bodies of infants has rarely been investigated because most infants wear diapers that fully
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39 absorb all urine. Noninvasive biomonitoring using urine samples is thus required especially
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41 for exposure assessments of chemicals with short biological half lives, and a methodology
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43 for extracting urine from used disposable diapers has been investigated in our previous
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45 studies.^{20 21} Since the urine during early childhood was not collected in the main study of the
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47 JECS, the adjunct studies using such urine in JECS-A will address research questions
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49 regarding environmental exposure in that period.
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55 The third theme of the adjunct studies is the investigation of objective screening tools to
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57 detect neurodevelopmental disorders at earlier stages. Public health services in local
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59 communities must be capable of providing early intervention support for children with such
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4 disorders. Preventive screening during early childhood can help to minimize later difficulties
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6 and to improve the trajectory of subjects with neurodevelopmental disorders in later life. For
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8 example, a validated screening checklist for autism at the age of 2 years, called
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10 M-CHAT-R/F,²² is available as part of early medical checkups, but this screening protocol
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12 requires a 2-step screening test that must be conducted by a physician. As a secondary tool
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14 allowing easy and objective screening the second to fourth digit (2D:4D) ratio, which is
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16 defined as the proportion between the lengths of the index and ring fingers, might be useful.
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18 A previous study suggested that autistic children aged 2 to 14 years have a smaller 2D:4D
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20 ratio than normal children of the same generation.²³ Recent studies^{24 25} have also investigated
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22 whether the 2D:4D ratio, a controversial but commonly used proxy marker of prenatal
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24 androgen concentrations based on the extreme male brain theory, is associated with ASD.
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26 However, this hypothetical screening method needs further scientific evidence in different
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28 epidemiological settings in different part of the globe. Another recent trial aiming to early
29
30 intervention for neurodevelopmental problems can be found in neonatal oral-motor
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32 assessments, such as sucking behavior. While contradictory findings have been obtained,²⁶ a
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34 recent longitudinal study also showed interesting evidence that some sucking behaviors in
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36 preterm infants are associated with later abnormal neurodevelopmental outcomes at the age
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38 of 2 years.²⁷ As such, further research on the development of screening tools for early
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40 intervention is needed.

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49 The JECS-A was established in 2011 as a sub-cohort of the JECS and has three main
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51 objectives: (1) to clarify the effects of prenatal and postnatal chemical exposures and social
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53 stressors on pregnant women and/or their children, especially neurodevelopmental outcomes
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55 of the children; (2) to develop a biomonitoring method using urine samples extracted from
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57 diapers for measuring internal exposure to chemical substances; and (3) to develop secondary
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4 tools such as the 2D:4D ratio or neonatal oral-motor assessments to screen future
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6 neurodevelopmental problems at earlier stages of childhood. This article outlines the JECS-A
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8 and its baseline data to date.
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10 11 12 13 **COHORT DESCRIPTION**

14 15 16 **Setting**

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18 The JECS is an ongoing nationwide birth cohort study with a total of 100,000 children and
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20 their parents (father's participation is optional, but suggested) across 15 regions in Japan.^{28 29}
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23 ³⁰ To identify risk factors in the environment affecting children's growth and health, the
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25 participating children are being followed from their fetal stage until the age of 13 years. The
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27 JECS has 15 regional sub-cohorts. At Aichi Regional Center of the JECS, an
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29 interdisciplinary team composed of toxicologists, psychologists, epidemiologists, public
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31 health specialists, pediatricians, obstetricians and gynecologists, biostatisticians, and
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33 ergonomists designed the JECS-A as part of the JECS cohort. The study areas (**Figure 1**)
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35 covered by the Aichi Regional Center of the JECS consist of Ichinomiya City (population of
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37 387,000 in 2012) and Kita-ward in Nagoya City (population of 165,000 in 2012). Nagoya
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39 City consists of 16 wards with a population of over 2 million and is Japan's third largest
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41 industrial metropolis, next to Tokyo and Osaka. Kita-ward is in the northern part of Nagoya
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43 City. Ichinomiya City neighbors Nagoya City and traditionally was known as an area
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45 involved in textile production but is now a regional commercial and residential area with a
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47 mixed economy of manufacturing and agriculture. Both areas are relatively urban and widely
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49 known in the automobile and ceramics industries.
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56 57 **Enrollment strategy**

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59 **Figure 2** shows a flowchart depicting each stage of study recruitment. A
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4 community-based recruitment strategy was adopted at designated 32 obstetric facilities
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6 including hospitals, clinics or midwifery units providing medical care for pregnant women in
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8 the study areas. The participants living in the areas can reach any of the 32 facilities within
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10 30 minutes by car or public transportation such as subway or train. Women in the early stage
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12 of pregnancy who visited an obstetrics facility for prenatal health care were invited to
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14 participate in the JECS if they met the following criteria: (1) residence within the study
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16 areas, (2) an estimated delivery date after August 2011, and (3) an ability to read and write
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18 the Japanese language so as to complete the self-administered questionnaire. In addition to
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20 this recruitment, we asked public health centers of the local governments to help us approach
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22 hard-to-reach groups such as low socio-economic status (SES) or reluctant pregnant women.
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24 All pregnant women receive the Maternal and Child Health Handbook at the public health
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26 centers to get complimentary municipal maternal care for pregnancy, delivery, and childcare
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28 under the Maternal and Child Health Law in Japan. Taking advantages of the occasions
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30 enabling face-to-face communication with all pregnant women in the study area, our staffs
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32 carefully explained the importance of the JECS to them and requested their cooperation for
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34 the study at the obstetric facilities.
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43 Of the 8,134 pregnant women who were invited as eligible recruits during the recruitment
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45 period from January 2011 to March 2014, a total of 5,721 (70.3%) participants from
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47 cooperating 32 obstetrics facilities were enrolled as the baseline regional cohort of pregnant
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49 women for main study of the JECS. The study area has 4,400 pregnant women annually on
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51 average, suggesting that 5,721 participants accounted for about 40% of the target population
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53 in the area.
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57 After the recruitment to the main study of the JECS, the registered 5,721 JECS participants
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59 were also invited to participate in adjunct studies conducted solely in the JECS-A and asked
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4 if they could provide any of the following three types of consent: agreement to complete the
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6 adjunct questionnaire survey only (Sub-cohort A1, n = 3,426), agreement to provide maternal
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8 biological specimens in addition to agreeing to participate in Sub-cohort A1 (Sub-cohort A2,
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10 n = 2,924), and agreement to undergo genetic analyses in addition to agreeing to participate
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12 in Sub-cohorts A1 and A2 (Sub-cohort A3, n = 1,753). Sub-cohorts A1, A2 and A3 were
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14 designed as a hierarchy, that is, the participants in sub-cohort A3 agreed to participate in
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16 studies conducted in sub-cohorts A1 and A2. Each participant in the adjunct studies gave
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18 written informed consent before any surveys began.
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23 Adjunct questionnaires for Sub-cohort A1 were distributed three times at the obstetric
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25 facilities during 1st and 2nd/3rd trimesters, in principle, and one month after the delivery,
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27 and mailed thereafter. Selective attrition caused by low SES may result in the estimates of
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29 findings being biased ³¹, so that enrollment and retention strategies play an important role in
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31 longitudinal cohort studies. We sent 1,000 Japanese yen (JPY, 110 JPY = 1US\$ as of 2019)
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33 worth of a prepaid card for every adjunct mail survey as monetary incentive to respondents.
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35 The prepaid card called 'Quo card' is familiar and can be used at all kinds of restaurants,
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37 convenience stores, gas stations, bookstores etc. in Japan. In addition to the monetary
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39 incentives to participate, we also conducted reminder calls or letters to retain participants
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41 who were likely to drop out of the study, focusing on non-respondents of the postal surveys.
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47 In Sub-cohorts A2 and A3, the parental blood (up to 1.5 ml) and urine (up to 50 ml,
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49 mothers only) during 1st and 2nd/3rd trimesters in principle and the cord blood (up to 1.5 ml)
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51 were collected and stored at around -80 °C. Participants in the JECS-A were then followed
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53 regardless of whether or not they took part in the above adjunct studies. Subsequently, of the
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55 5,555 children, including 49 pairs of twins, born from the enrolled mothers, 57 children
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57 dropped out of the JECS-A because of infant death (n = 7), mother death (n = 1), withdrawal
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4 of consent (n = 48), or a change in residence to an area outside the surveyed area (n = 1). On
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6 the other hand, 4 children who moved into the surveyed area from another one were included.
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8 As of 18 months after delivery, a total of 5,502 children remained within the JECS-A,
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10 accounting for approximately 40% of the children born in the study area.
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14 A second recruitment for the adjunct studies in the JECS-A was also conducted during the
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16 follow-up of the study participants of the main study of the JECS, focusing on children. We
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18 approached the guardians of targeted children at legal check-ups for 18-month-olds provided
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20 at regional public health centers and health consultation centers. The recruitment period was
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22 divided into two phases. Of the 5,502 children remaining in the JECS-A at 18 months of age,
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24 a total of 2,576 children had reached the age until June 2015. We conducted a survey of the
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26 anthropometric measurements of the 2D:4D ratio of the children on both hands (Sub-cohort
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28 B). A total of 1,357 children (coverage of target samples: 52.7%, consent rate: 99.0%) were
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30 enrolled in Sub-cohort B. Subsequent to this recruitment, another recruitment was also set for
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32 18-month-old children born from mothers registered during the last year of the pregnant
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34 women recruitment (Sub-cohort C). This cohort was specifically planned to collect biological
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36 samples (mainly overnight urine) from diapers, and written informed consent for the adjunct
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38 survey was obtained from 1,192 children (82.9%). All the enrolled children had reached the
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40 age of 1.5 years as of June 2016. The follow-up schedule for children beyond the age of 3
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42 years has yet to be finalized, but these sub-cohorts within the JECS-A will be followed until
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44 the children reach the age of 13 years. Informed parental consent from the legally authorized
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46 representative was obtained for all the enrollment of children in the studies. Note that the
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48 above figures were based on the data set jecs-ag-20160424, which was released in June 2016,
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50 and on the provisional data set determined as of August 1, 2018.
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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

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4 potential determinants extending from the prenatal to early childhood period, the risks will
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6 be estimated using the mother's and child's demographic variables or other related factors as
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8 confounding factors. Sub-cohorts A2 and A3 will mainly focus on studies using biological
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10 specimens (urine and/or blood) obtained from pregnant women and their spouses when
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12 available. These biological samples will be used for exposure assessments, genetic and
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14 epigenetic analyses, and activity determinations of chemical metabolism-related enzymes,
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16 and so on. Data on the levels of urinary exposure biomarkers including chemical metabolites
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18 will be combined with those in sub-cohort C and, eventually, the longitudinal exposure
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20 trajectory and the relationships between exposure and health outcomes will also be analyzed.
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22 Second, Sub-cohort C was designed to clarify the chemical exposure levels in toddlers. To
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24 estimate their amount of exposure to environmental chemicals, a noninvasive biomonitoring
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26 approach was adopted in which a paper diaper worn overnight was collected under
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28 refrigerated conditions at the age of 18 and 36 months. This cohort will also be used to
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30 derive human biomonitoring reference values (e.g., RV95) of urinary chemical
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32 concentrations in the Japanese children.
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40 Third, sub-cohort B was designed to develop secondary tools such as the 2D:4D ratio or
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42 neonatal oral-motor assessments to screen future neurodevelopmental problems at earlier
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44 stages of childhood. One of our major interest is the lengths of digits on both hands in
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46 children especially the longitudinal changes in 2D:4D ratios between 18-month-old and
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48 36-month-old infants. As for the anthropometric measurements of the palms and digits of the
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50 children's hands, we have established a specialized protocol involving photographic records
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52 and digit measurements that has a high reliability (Intra/Inter-Class Correlations: $ICC_1 = 0.97$
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54 [95% CI, 0.87-0.99], $ICC_3 = 0.93$ [0.83-0.98]). In short, our easy-to-use photocopying
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56 method, which involves placing the child's palm on a box composed of transparent acrylic
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4 thin plates and photographing it with a digital camera fixed inside the box, was devised for
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6 application in health checkup settings enforced by law. All the 2D:4D ratio data will be
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8 obtained using this protocol. Sub-cohort A1 will also be used to examine the relationship
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10 between neonatal oral-motor assessments and subsequent abnormal neurodevelopmental
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12 outcomes. Using the short version of Infant Behavior Questionnaire (IBQ) and Early
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14 Childhood Behavior Questionnaire (ECBQ) as factors reflecting temperamental
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16 self-regulation, the utility of such assessments as early screening tools for predicting
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18 subsequent neurodevelopmental outcomes will be verified.
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23 The JECS-A do not include any information regarding medical diagnoses affecting
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25 neurodevelopmental outcomes at this time, but such information will be available in the
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27 future through the main study of the JECS, which is being conducted under the same protocol
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29 across 15 regions in Japan.
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33 34 35 **FINDINGS TO DATE**

36 37 **Participant characteristics of pregnant women**

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39 **Table 2** shows the main socio-demographic characteristics of the pregnant women. To
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41 ascertain whether the JECS-A baseline profiles are representative of the pregnant women in
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43 the study areas in general, the national statistics for Aichi prefecture including Nagoya City
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45 and Ichinomiya City (since data for each city were not available) were included in the table.
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47 Similar distribution trends for variables such as “Age at delivery” were confirmed when the
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49 JECS-A baseline cohort of pregnant women and the national statistics were compared;
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51 however, differences in “Education level” and “Household income” were observed.
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53 Regarding education level, the percentage of JECS participants who had graduated from high
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55 school was relatively low, compared with the national statistics (25.1% vs. 39.7%), while the
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4 percentage of college/junior college/technology college students was higher among the JECS
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6 participants, compared with the national statistics (35.3% vs. 20.8%). Moreover, the number
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8 of JECS-A participants with a household income of 2 million yen or less was about 1/8 of the
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10 national statistics. Likewise, unemployed participants (mainly housewives) accounted for
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12 45% of the JECS-A cohort. These results suggest a possible selection bias. We recruited
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14 pregnant women who had visited the obstetric facilities during the daytime; this might have
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16 resulted in a lower study participation rate among pregnant employees.
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23 **Participant characteristics of children**

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25 As for the baseline cohort of children, a total of 5,502 (99.0%) of the 5,555 children
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27 remained in the JECS-A at 18 months after delivery (provisional figures as of August 1,
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29 2018). **Table 3** compares the baseline data of the newborns with the national statistics. Basic
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31 demographics including “Sex”, “Birth weight” and “Birth height” had similar distributions,
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33 indicating the representativeness of the sample.
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37 Similarly, **Table 4** shows the fundamental characteristics of the pregnant women and their
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39 children in each sub-cohort of the JECS-A. No notable differences in the descriptive
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41 statistics were observed among the sub-cohorts. Furthermore, the drop-out rates for each
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43 sub-cohort, to date, have been maintained at less than 10% relative to the baseline. However,
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45 10% of the sub-cohort values for household income are missing because many participants
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47 did not provide information on the income. Therefore, caution is needed when using this
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49 variable as a confounding factor in multiple regression analyses.
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57 **STRENGTHS AND LIMITATIONS**

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4 The main strength of the JECS-A of children is its large sample size, reflecting a
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6 representative population. Approximately 40% of the children born in the study area have
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8 been included in the study to date. The participating children will be followed until they
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10 reach the age of 13 years. The study protocol³³ of the JECS decided on a target retention rate
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12 of 80% or higher at the age of 13 years. The linkage between the JECS-A sub-cohort and the
13
14 main data of the JECS study will further allow novel and challenging studies with a high
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16 generalizability.
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21 The main weakness of the JECS-A cohort concerns the pregnant women participants
22
23 related to a selective attrition. A relatively large proportion of single-income, middle-class
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25 households were included in the JECS-A; many low-income households refused to
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27 participate in the surveys, and some participants did not provide information concerning their
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29 household income. As enrollment strategies, though we set about recruiting from the first
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31 trimester of pregnancy (<12-week gestation), it was hard to get their cooperation in earlier
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33 weeks of a pregnancy, when the risk of miscarriage is higher. Most of pregnant women
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35 registered were actually after 10th week of pregnancy. Furthermore, we had no choice but to
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37 call on pregnant women for participation who had visited the obstetric facilities mainly
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39 during the daytime, owing to limited number of staffs during the evening shift at hospitals.
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41 Employed participants including all non-regular employees (part-time, temporary or contract
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43 employees) only accounted for 33% in the group of annual household income with 2 million
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45 Japanese yen or less. This means a selective attrition that working pregnant women of low
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47 SES might have been excluded in the cohorts. Such family socioeconomic status will have
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49 possibilities affecting several outcomes related to child development such as breastfeeding
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51 duration ³⁴, obesity in children ³⁵ and child maltreatment ³⁶. A British study ³⁷, however,
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53 suggests that cognitive and behavioural development has a weak or absent direct effect of
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4 income inequality after controlling potential confounders. Thus, possible biases as a result of
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6 selective attrition and direct SES effects for child should be carefully examined in future
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8 studies when using the JECS-A cohort data. The impact of the missing values can be adjusted
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10 to some extent by comparing estimates resulting from the multiple imputation and from
11
12 complete case analysis, using the data of main study of the JECS as reference. However,
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14 researchers must take into account the effect of response biases when conducting statistical
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16 analyses using paired data for pregnant women and their children and when interpreting these
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18 results.
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25 **COLLABORATION**

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28 The original data and specimens will be made available to investigators and stakeholders
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30 working within the JECS-A project. The study must adhere to the JECS policy on the
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32 availability of research results, publications, intellectual property rights, and data sharing. At
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34 the moment, the Ethical Guidelines for Medical and Health Research Involving Human
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36 Subjects enforced by the Japan Ministry of Education, Culture, Sports, Science and
37
38 Technology and the Ministry of Health, Labour and Welfare restrict the open sharing of
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40 epidemiologic data. This means that researchers interested in using the data must collaborate
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42 with and participate in the JECS-A project. This project was recently started and is still
43
44 ongoing. We are open to new proposals that fall within the nature of the JECS-A cohort.
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9

10 11 12 13 **Contributors**

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15
16 TE designed the JECS-A cohort architecture, developed the protocol, analyzed the data,
17
18 and wrote the first draft of the manuscript. YY and MT organized the study team, obtained
19
20 approvals and contributed to the development of the protocol, design, and data collection
21
22 tools. TOm was in charge of coordination with relevant organizations and organized the
23
24 JECS-A members. NS, SK, TMa and TOg performed the analysis and interpreted the data.
25
26 YI, HS, NO and JU designed and developed the protocol for the JECS-A sub-cohorts A2, A3
27
28 and B and edited and analyzed the data. AN, MK, YO, TMi, SSu, MSO and SSa designed
29
30 each adjunct study, supervised the data collection, and drafted the manuscript. MK was a
31
32 member of the JECS Steering Committee, was responsible for the study design and protocol,
33
34 supervised the data collection, and edited and drafted the manuscript. All the authors
35
36 interpreted the data, contributed to the writing of the manuscript, revised it critically for
37
38 important intellectual content, and agreed with the final version and the findings.
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15 Committees of all participating institutions.
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23 **Data sharing statement** The original data and specimens will be made available to
24 investigators and stakeholders working within the JECS-A project.
25
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27
28 Researchers interested in collaborations or further information should contact the JECS-A
29 secretariat by e-mail at eisei@med.nagoya-cu.ac.jp.
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35 **Disclaimer** The findings and conclusions of this article are solely the responsibility of the
36 authors and do not represent the official views of the Japanese government.
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45 REFERENCES

- 46
47 1. Ishitsuka K, Nakayama SF, Kishi R, et al. Japan Environment and Children's Study:
48 backgrounds, activities, and future directions in global perspectives. *Environ Health*
49 *Prev Med* 2017;22(1):61. doi: 10.1186/s12199-017-0667-y
50
51
52 2. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders
53 (DSM–5): American Psychiatric Association Publishing 2013:xiii-xv.
54
55
56
57 3. Xiang S, Carrie A. A review of the prevalence of Autism Spectrum Disorder in Asia,
58
59
60

- 1
2
3
4 Research in Autism Spectrum Disorders. *Autism Spectrum Disorders*
5
6 2010;4(2):156-67. doi: <http://dx.doi.org/10.1016/j.rasd.2009.10.003>.
7
8
9 4. Hansen SN, Schendel DE, Parner ET. Explaining the increase in the prevalence of autism
10
11 spectrum disorders: the proportion attributable to changes in reporting practices.
12
13 *JAMA Pediatr* 2015;169(1):56-62. doi: 10.1001/jamapediatrics.2014.1893
14
15
16 5. Rodriguez-Barranco M, Lacasana M, Aguilar-Garduno C, et al. Association of arsenic,
17
18 cadmium and manganese exposure with neurodevelopment and behavioural disorders
19
20 in children: a systematic review and meta-analysis. *Sci Total Environ*
21
22 2013;454-455:562-77. doi: 10.1016/j.scitotenv.2013.03.047
23
24
25
26 6. Rodrigues EG, Bellinger DC, Valeri L, et al. Neurodevelopmental outcomes among 2- to
27
28 3-year-old children in Bangladesh with elevated blood lead and exposure to arsenic
29
30 and manganese in drinking water. *Environ Health* 2016;15:44. doi:
31
32 10.1186/s12940-016-0127-y
33
34
35
36 7. Melchior M, Hersi R, van der Waerden J, et al. Maternal tobacco smoking in pregnancy
37
38 and children's socio-emotional development at age 5: The EDEN mother-child birth
39
40 cohort study. *Eur Psychiatry* 2015;30(5):562-8. doi: 10.1016/j.eurpsy.2015.03.005
41
42
43
44 8. Joelsson P, Chudal R, Talati A, et al. Prenatal smoking exposure and neuropsychiatric
45
46 comorbidity of ADHD: a finnish nationwide population-based cohort study. *BMC*
47
48 *Psychiatry* 2016;16:306. doi: 10.1186/s12888-016-1007-2
49
50
51
52 9. Han JY, Kwon HJ, Ha M, et al. The effects of prenatal exposure to alcohol and
53
54 environmental tobacco smoke on risk for ADHD: a large population-based study.
55
56 *Psychiatry Res* 2015;225(1-2):164-8. doi: 10.1016/j.psychres.2014.11.009
57
58
59
60 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 4 years 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

- 1
2
3
4 18. Yurumez E, Akca OF, Ugur C, et al. Mothers' alexithymia, depression and anxiety levels
5
6 and their association with the quality of mother-infant relationship: a preliminary
7
8 study. *Int J Psychiatry Clin Pract* 2014;18(3):190-6. doi:
9
10 10.3109/13651501.2014.940055
11
12
13 19. Li Y, Jiang WQ, Du YS, et al. Relationships between behavioral symptoms of
14
15 non-medicated Chinese children with attention deficit hyperactivity disorder and
16
17 parenting stress: Comparison of different subtypes and comorbidities. *Asia Pac*
18
19 *Psychiatry* 2016;8(2):127-35. doi: 10.1111/appy.12213
20
21
22
23 20. Oya N, Ito Y, Hioki K, et al. Quantitative analysis of organophosphate insecticide
24
25 metabolites in urine extracted from disposable diapers of toddlers in Japan. *Int J Hyg*
26
27 *Environ Health* 2017;220(2 Pt A):209-16. doi: 10.1016/j.ijheh.2016.10.009
28
29
30
31 21. Saito S, Ueyama J, Kondo T, et al. A non-invasive biomonitoring method for assessing
32
33 levels of urinary pyrethroid metabolites in diapered children by gas
34
35 chromatography-mass spectrometry. *J Expo Sci Environ Epidemiol* 2014;24(2):200-7.
36
37 doi: 10.1038/jes.2013.31
38
39
40 22. Robins DL, Casagrande K, Barton M, et al. Validation of the modified checklist for
41
42 Autism in toddlers, revised with follow-up (M-CHAT-R/F). *Pediatrics*
43
44 2014;133(1):37-45. doi: 10.1542/peds.2013-1813
45
46
47 23. Manning JT, Baron-Cohen S, Wheelwright S, et al. The 2nd to 4th digit ratio and autism.
48
49 *Dev Med Child Neurol* 2001;43(3):160-4.
50
51
52 24. Teatero ML, Netley C. A critical review of the research on the extreme male brain theory
53
54 and digit ratio (2D:4D). *J Autism Dev Disord* 2013;43(11):2664-76. doi:
55
56 10.1007/s10803-013-1819-6
57
58
59 25. Guyatt AL, Heron J, Knight Ble C, et al. Digit ratio and autism spectrum disorders in the
60

- 1
2
3
4 Avon Longitudinal Study of Parents and Children: a birth cohort study. *BMJ Open*
5
6 2015;5(8):e007433. doi: 10.1136/bmjopen-2014-007433
7
8
9 26. Slattery J, Morgan A, Douglas J. Early sucking and swallowing problems as predictors of
10
11 neurodevelopmental outcome in children with neonatal brain injury: a systematic
12
13 review. *Dev Med Child Neurol* 2012;54(9):796-806. doi:
14
15 10.1111/j.1469-8749.2012.04318.x
16
17
18 27. Wolthuis-Stigter MI, Luinge MR, da Costa SP, et al. The association between sucking
19
20 behavior in preterm infants and neurodevelopmental outcomes at 2 years of age. *J*
21
22 *Pediatr* 2015;166(1):26-30. doi: 10.1016/j.jpeds.2014.09.007
23
24
25 28. Kawamoto T, Nitta H, Murata K, et al. Rationale and study design of the Japan
26
27 environment and children's study (JECS). *BMC Public Health* 2014;14:25. doi:
28
29 10.1186/1471-2458-14-25
30
31
32 29. Michikawa T, Nitta H, Nakayama SF, et al. The Japan Environment and Children's Study
33
34 (JECS): A Preliminary Report on Selected Characteristics of Approximately 10 000
35
36 Pregnant Women Recruited During the First Year of the Study. *J Epidemiol*
37
38 2015;25(6):452-8. doi: 10.2188/jea.JE20140186
39
40
41
42 30. Michikawa T, Nitta H, Nakayama SF, et al. Baseline Profile of Participants in the Japan
43
44 Environment and Children's Study (JECS). *J Epidemiol* 2018;28(2):99-104. doi:
45
46 10.2188/jea.JE20170018
47
48
49 31. Booker CL, Harding S, Benzeval M. A systematic review of the effect of retention
50
51 methods in population-based cohort studies. *BMC Public Health* 2011;11:249. doi:
52
53 10.1186/1471-2458-11-249
54
55
56 32. Moriguchi Y, Maeda M, Igarashi T, et al. Age and gender effect on alexithymia in large,
57
58 Japanese community and clinical samples: a cross-validation study of the Toronto
59
60

- 1
2
3
4 Alexithymia Scale (TAS-20). *Biopsychosoc Med* 2007;1:7. doi:
5
6 10.1186/1751-0759-1-7
7
8
9 33. National Centre for Japan Environment and Children's study. Japan Environment and
10
11 Children's study (JECS), Study Protocol (ver. 1.4) [Available from:
12
13 https://www.env.go.jp/en/chemi/hs/jecs/data/about/jecs-study_protocol_14_en.pdf.
14
15
16 34. Flacking R, Nyqvist KH, Ewald U. Effects of socioeconomic status on breastfeeding
17
18 duration in mothers of preterm and term infants. *Eur J Public Health*
19
20 2007;17(6):579-84. doi: 10.1093/eurpub/ckm019
21
22
23 35. Ogden CL, Lamb MM, Carroll MD, et al. Obesity and socioeconomic status in children
24
25 and adolescents: United States, 2005-2008. *NCHS Data Brief* 2010(51):1-8.
26
27
28 36. Eckenrode J, Smith EG, McCarthy ME, et al. Income inequality and child maltreatment
29
30 in the United States. *Pediatrics* 2014;133(3):454-61. doi: 10.1542/peds.2013-1707
31
32
33 37. Violato M, Petrou S, Gray R, et al. Family income and child cognitive and behavioural
34
35 development in the United Kingdom: does money matter? *Health Econ*
36
37 2011;20(10):1201-25. doi: 10.1002/hec.1665
38
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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

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

Main items	Sub-cohort covered					Timing of measurement						
	A1	A2	A3	B	C	prenatal (trimester)		postnatal (months after birth)				
						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	○	○	○		○						○	

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6 ○: Refer to the main study of the Japan Environment and Children's Study (JECS), ●: Measurements conducting solely in the JECS-A
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10 **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
11 Revised Short Form; **ECBQ-SF**, Early Childhood Behavior Questionnaire Short Form; **DCDQ**, Developmental Coordination Disorder Questionnaire, **2D:4D**, the second to
12 fourth digit ratio; **PARS**, Pervasive Developmental Disorders Autism Society Japan Rating Scale; **ASD**, autism spectrum disorder; **ADHD**, attention deficit hyperactivity
13 disorder; **LBW**, low birth weight.
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16 **Sub cohort A1**, Agreement on the adjunct questionnaire survey only; **Sub cohort A2**, Agreement on the adjunct questionnaire survey and provision of mother's
17 biological specimen (blood and urine); **Sub cohort A3**, Agreement on the adjunct questionnaire survey and provision of mother's biological specimen (blood and urine)
18 with genetic analyses; **Sub cohort B**, Informed consent obtained from guardians (legally acceptable representatives) for provision of child's biological specimen; **Sub**
19 **cohort C**, Informed consent obtained from guardians (legally acceptable representatives) for anthropometry measurement on child hands
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Table 2 Comparison of the J ECS-A baseline data with national statistics in Aichi prefecture for pregnant women: verification of representativeness of the sample

	J ECS-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

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

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

	J ECS-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			

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^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

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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)

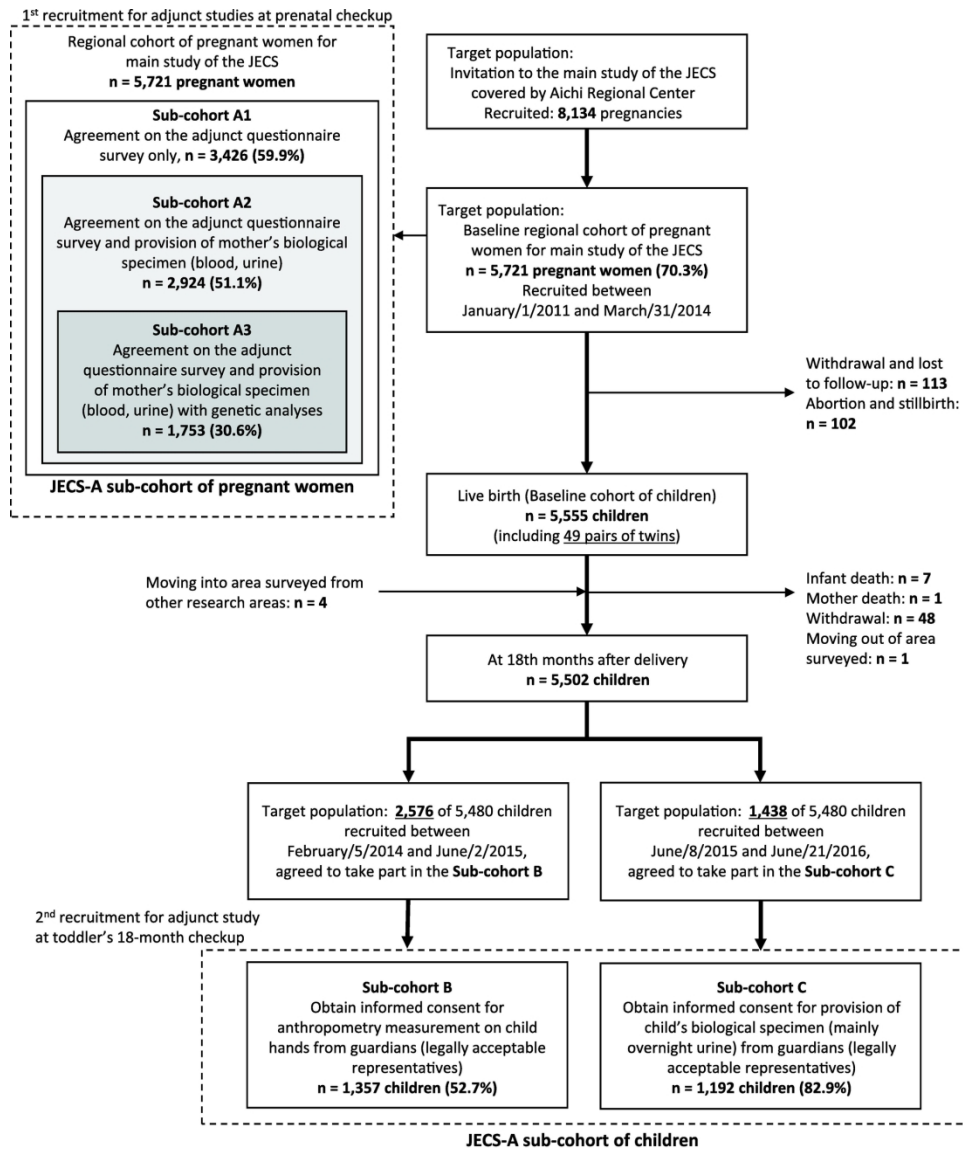


Figure 2. Flowchart showing each stage of study recruitment for adjunct studies in the JECs-A

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)

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Manuscripts

Cohort profile: Aichi regional sub-cohort of the Japan Environment and Children's

Study (JECS-A)

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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)

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5 **Strengths and limitations of this study**

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- 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.

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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 disorders² 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} Furthermore, interest is growing regarding the potential role of social

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4 stressors in modifying the relationship between the above early childhood exposure and the
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6 neurodevelopmental outcomes. A cohort study of seven-year-old predominantly Mexican
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8 American children in California's Salinas Valley suggested that social adversities including a
9
10 poor learning environment and parent-child interactions were significantly associated with a
11
12 moderate decrease in the intelligence quotient (IQ) score and its subset scores.¹⁷ Several
13
14 recent studies have also discussed the effects of parent-child interactions, focusing on the
15
16 effects of alexithymia in mothers¹⁸ and of depressive symptoms on parenting stress.¹⁹ Thus,
17
18 there is the need to investigate postnatal influences, such as the effects of interactions
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20 between mothers and their children, as well as prenatal and postnatal toxicant exposures on
21
22 the neurodevelopment of children. The above research questions will be addressed to
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24 complement those of the main study of the JECS.
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31 The second theme of the adjunct studies conducted in JECS-A is the exposure assessments
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33 using urine collected during early childhood when the central nervous system develops
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35 rapidly. So far, the amount of exposure to environmental chemical substances taken into the
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37 bodies of infants has rarely been investigated because most infants wear diapers that fully
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39 absorb all urine. Noninvasive biomonitoring using urine samples is thus required especially
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41 for exposure assessments of chemicals with short biological half lives, and a methodology
42
43 for extracting urine from used disposable diapers has been investigated in our previous
44
45 studies.^{20 21} Since the urine during early childhood was not collected in the main study of the
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47 JECS, the adjunct studies using such urine in JECS-A will address research questions
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49 regarding environmental exposure in that period.
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55 The third theme of the adjunct studies is the investigation of objective screening tools to
56
57 detect neurodevelopmental disorders at earlier stages. Public health services in local
58
59 communities must be capable of providing early intervention support for children with such
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4 disorders. Preventive screening during early childhood can help to minimize later difficulties
5
6 and to improve the trajectory of subjects with neurodevelopmental disorders in later life. For
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8 example, a validated screening checklist for autism at the age of 2 years, called
9
10 M-CHAT-R/F,²² is available as part of early medical checkups, but this screening protocol
11
12 requires a 2-step screening test that must be conducted by a physician. As a secondary tool
13
14 allowing easy and objective screening the second to fourth digit (2D:4D) ratio, which is
15
16 defined as the proportion between the lengths of the index and ring fingers, might be useful.
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18 A previous study suggested that autistic children aged 2 to 14 years have a smaller 2D:4D
19
20 ratio than normal children of the same generation.²³ Recent studies^{24 25} have also investigated
21
22 whether the 2D:4D ratio, a controversial but commonly used proxy marker of prenatal
23
24 androgen concentrations based on the extreme male brain theory, is associated with ASD.
25
26 However, this hypothetical screening method needs further scientific evidence in different
27
28 epidemiological settings in different part of the globe. Another recent trial aiming to early
29
30 intervention for neurodevelopmental problems can be found in neonatal oral-motor
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32 assessments, such as sucking behavior. While contradictory findings have been obtained,²⁶ a
33
34 recent longitudinal study also showed interesting evidence that some sucking behaviors in
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36 preterm infants are associated with later abnormal neurodevelopmental outcomes at the age
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38 of 2 years.²⁷ As such, further research on the development of screening tools for early
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40 intervention is needed.
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50 The JECS-A was established in 2011 as a sub-cohort of the JECS and has three main
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52 objectives: (1) to clarify the effects of prenatal and postnatal chemical exposures and social
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54 stressors on pregnant women and/or their children, especially neurodevelopmental outcomes
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56 of the children; (2) to develop a biomonitoring method using urine samples extracted from
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58 diapers for measuring internal exposure to chemical substances; and (3) to develop secondary
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4 tools such as the 2D:4D ratio or neonatal oral-motor assessments to screen future
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6 neurodevelopmental problems at earlier stages of childhood. This article outlines the JECS-A
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8 and its baseline data to date.
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10 11 12 13 **COHORT DESCRIPTION**

14 15 16 **Setting**

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18 The JECS is an ongoing nationwide birth cohort study with a total of 100,000 children and
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20 their parents (father's participation is optional, but suggested) across 15 regions in Japan.^{28 29}
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22
23 ³⁰ To identify risk factors in the environment affecting children's growth and health, the
24
25 participating children are being followed from their fetal stage until the age of 13 years. The
26
27 JECS has 15 regional sub-cohorts. At Aichi Regional Center of the JECS, an
28
29 interdisciplinary team composed of toxicologists, psychologists, epidemiologists, public
30
31 health specialists, pediatricians, obstetricians and gynecologists, biostatisticians, and
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33 ergonomists designed the JECS-A as part of the JECS cohort. The study areas (**Figure 1**)
34
35 covered by the Aichi Regional Center of the JECS consist of Ichinomiya City (population of
36
37 387,000 in 2012) and Kita-ward in Nagoya City (population of 165,000 in 2012). Nagoya
38
39 City consists of 16 wards with a population of over 2 million and is Japan's third largest
40
41 industrial metropolis, next to Tokyo and Osaka. Kita-ward is in the northern part of Nagoya
42
43 City. Ichinomiya City neighbors Nagoya City and traditionally was known as an area
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45 involved in textile production but is now a regional commercial and residential area with a
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47 mixed economy of manufacturing and agriculture. Both areas are relatively urban and widely
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49 known in the automobile and ceramics industries.
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56 57 **Enrollment strategy**

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59 **Figure 2** shows a flowchart depicting each stage of study recruitment. A
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4 community-based recruitment strategy was adopted at designated 32 obstetric facilities
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6 including hospitals, clinics or midwifery units providing medical care for pregnant women in
7
8 the study areas. The participants living in the areas can reach any of the 32 facilities within
9
10 30 minutes by car or public transportation such as subway or train. Women in the early stage
11
12 of pregnancy who visited an obstetrics facility for prenatal health care were invited to
13
14 participate in the JECS if they met the following criteria: (1) residence within the study
15
16 areas, (2) an estimated delivery date after August 2011, and (3) an ability to read and write
17
18 the Japanese language so as to complete the self-administered questionnaire. In addition to
19
20 this recruitment, we asked public health centers of the local governments to help us approach
21
22 hard-to-reach groups such as low socio-economic status (SES) or reluctant pregnant women.
23
24 All pregnant women receive the Maternal and Child Health Handbook at the public health
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26 centers to get complimentary municipal maternal care for pregnancy, delivery, and childcare
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28 under the Maternal and Child Health Law in Japan. Taking advantages of the occasions
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30 enabling face-to-face communication with all pregnant women in the study area, our staffs
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32 carefully explained the importance of the JECS to them and requested their cooperation for
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34 the study at the obstetric facilities.
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43 Of the 8,134 pregnant women who were invited as eligible recruits during the recruitment
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45 period from January 2011 to March 2014, a total of 5,721 (70.3%) participants from
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47 cooperating 32 obstetrics facilities were enrolled as the baseline regional cohort of pregnant
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49 women for main study of the JECS. The study area has 4,400 pregnant women annually on
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51 average, suggesting that 5,721 participants accounted for about 40% of the target population
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53 in the area.
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57 After the recruitment to the main study of the JECS, the registered 5,721 JECS participants
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59 were also invited to participate in adjunct studies conducted solely in the JECS-A and asked
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4 if they could provide any of the following three types of consent: agreement to complete the
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6 adjunct questionnaire survey only (Sub-cohort A1, n = 3,426), agreement to provide maternal
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8 biological specimens in addition to agreeing to participate in Sub-cohort A1 (Sub-cohort A2,
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10 n = 2,924), and agreement to undergo genetic analyses in addition to agreeing to participate
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12 in Sub-cohorts A1 and A2 (Sub-cohort A3, n = 1,753). Sub-cohorts A1, A2 and A3 were
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14 designed as a hierarchy, that is, the participants in sub-cohort A3 agreed to participate in
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16 studies conducted in sub-cohorts A1 and A2. Each participant in the adjunct studies gave
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18 written informed consent before any surveys began.
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23 Adjunct questionnaires for Sub-cohort A1 were distributed three times at the obstetric
24
25 facilities during 1st and 2nd/3rd trimesters, in principle, and one month after the delivery,
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27 and mailed thereafter. Selective attrition caused by low SES may result in the estimates of
28
29 findings being biased ³¹, so that enrollment and retention strategies play an important role in
30
31 longitudinal cohort studies. We sent 1,000 Japanese yen (JPY, 110 JPY = 1US\$ as of 2019)
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33 worth of a prepaid card for every adjunct mail survey as monetary incentive to respondents.
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35 The prepaid card called 'Quo card' is familiar and can be used at all kinds of restaurants,
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37 convenience stores, gas stations, bookstores etc. in Japan. In addition to the monetary
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39 incentives to participate, we also conducted reminder calls or letters to retain participants
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41 who were likely to drop out of the study, focusing on non-respondents of the postal surveys.
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47 In Sub-cohorts A2 and A3, the parental blood (up to 1.5 ml) and urine (up to 50 ml,
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49 mothers only) during 1st and 2nd/3rd trimesters in principle and the cord blood (up to 1.5 ml)
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51 were collected and stored at around -80 °C. Participants in the JECS-A were then followed
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53 regardless of whether or not they took part in the above adjunct studies. Subsequently, of the
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55 5,555 children, including 49 pairs of twins, born from the enrolled mothers, 57 children
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57 dropped out of the JECS-A because of infant death (n = 7), mother death (n = 1), withdrawal
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4 of consent (n = 48), or a change in residence to an area outside the surveyed area (n = 1). On
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6 the other hand, 4 children who moved into the surveyed area from another one were included.
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8 As of 18 months after delivery, a total of 5,502 children remained within the JECS-A,
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10 accounting for approximately 40% of the children born in the study area.
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14 A second recruitment for the adjunct studies in the JECS-A was also conducted during the
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16 follow-up of the study participants of the main study of the JECS, focusing on children. We
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18 approached the guardians of targeted children at legal check-ups for 18-month-olds provided
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20 at regional public health centers and health consultation centers. The recruitment period was
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22 divided into two phases. Of the 5,502 children remaining in the JECS-A at 18 months of age,
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24 a total of 2,576 children had reached the age until June 2015. We conducted a survey of the
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26 anthropometric measurements of the 2D:4D ratio of the children on both hands (Sub-cohort
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28 B). A total of 1,357 children (coverage of target samples: 52.7%, consent rate: 99.0%) were
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30 enrolled in Sub-cohort B. Subsequent to this recruitment, another recruitment was also set for
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32 18-month-old children born from mothers registered during the last year of the pregnant
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34 women recruitment (Sub-cohort C). This cohort was specifically planned to collect biological
35
36 samples (mainly overnight urine) from diapers, and written informed consent for
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38 participation of 1,192 children (82.9%) of the adjunct survey was obtained from their
39
40 guardians. All the enrolled children had reached the age of 1.5 years as of June 2016. The
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42 follow-up schedule for children beyond the age of 3 years has yet to be finalized, but these
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44 sub-cohorts within the JECS-A will be followed until the children reach the age of 13 years.
45
46 Informed parental consent from the legally authorized representative was obtained for all the
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48 enrollment of children in the studies. Note that the above figures were based on the data set
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50 `jecs-ag-20160424`, which was released in June 2016, and on the provisional data set
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52 determined as of August 1, 2018.
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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

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4 outcomes and/or the developmental outcomes of postnatal infants. As there are many
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6 potential determinants extending from the prenatal to early childhood period, the risks will
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8 be estimated using the mother's and child's demographic variables or other related factors as
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10 confounding factors. Sub-cohorts A2 and A3 will mainly focus on studies using biological
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12 specimens (urine and/or blood) obtained from pregnant women and their spouses when
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14 available. These biological samples will be used for exposure assessments, genetic and
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16 epigenetic analyses, and activity determinations of chemical metabolism-related enzymes,
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18 and so on. Data on the levels of urinary exposure biomarkers including chemical metabolites
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20 will be combined with those in sub-cohort C and, eventually, the longitudinal exposure
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22 trajectory and the relationships between exposure and health outcomes will also be analyzed.
23
24 Second, Sub-cohort C was designed to clarify the chemical exposure levels in toddlers. To
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26 estimate their amount of exposure to environmental chemicals, a noninvasive biomonitoring
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28 approach was adopted in which a paper diaper worn overnight was collected under
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30 refrigerated conditions at the age of 18 and 36 months. This cohort will also be used to
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32 derive human biomonitoring reference values (e.g., RV95) of urinary chemical
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34 concentrations in the Japanese children.
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43 Third, sub-cohort B was designed to develop secondary tools such as the 2D:4D ratio or
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45 neonatal oral-motor assessments to screen future neurodevelopmental problems at earlier
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47 stages of childhood. One of our major interest is the lengths of digits on both hands in
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49 children especially the longitudinal changes in 2D:4D ratios between 18-month-old and
50
51 36-month-old infants. As for the anthropometric measurements of the palms and digits of the
52
53 children's hands, we have established a specialized protocol involving photographic records
54
55 and digit measurements that has a high reliability (Intra/Inter-Class Correlations: $ICC_1 = 0.97$
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57 [95% CI, 0.87-0.99], $ICC_3 = 0.93$ [0.83-0.98]). In short, our easy-to-use photocopying
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4 method, which involves placing the child's palm on a box composed of transparent acrylic
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6 thin plates and photographing it with a digital camera fixed inside the box, was devised for
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8 application in health checkup settings enforced by law. All the 2D:4D ratio data will be
9
10 obtained using this protocol. Sub-cohort A1 will also be used to examine the relationship
11
12 between neonatal oral-motor assessments and subsequent abnormal neurodevelopmental
13
14 outcomes. Using the short version of Infant Behavior Questionnaire (IBQ) and Early
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16 Childhood Behavior Questionnaire (ECBQ) as factors reflecting temperamental
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18 self-regulation, the utility of such assessments as early screening tools for predicting
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20 subsequent neurodevelopmental outcomes will be verified.
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26 The JECS-A do not include any information regarding medical diagnoses affecting
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28 neurodevelopmental outcomes at this time, but such information will be available in the
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30 future through the main study of the JECS, which is being conducted under the same protocol
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32 across 15 regions in Japan.
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37 38 **FINDINGS TO DATE**

39 40 **Participant characteristics of pregnant women**

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42 **Table 2** shows the main socio-demographic characteristics of the pregnant women. To
43
44 ascertain whether the JECS-A baseline profiles are representative of the pregnant women in
45
46 the study areas in general, the national statistics for Aichi prefecture including Nagoya City
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48 and Ichinomiya City (since data for each city were not available) were included in the table.
49
50 Similar distribution trends for variables such as "Age at delivery" were confirmed when the
51
52 JECS-A baseline cohort of pregnant women and the national statistics were compared;
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54 however, differences in "Education level" and "Household income" were observed.
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59 Regarding education level, the percentage of JECS participants who had graduated from high
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4 school was relatively low, compared with the national statistics (25.1% vs. 39.7%), while the
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6 percentage of college/junior college/technology college students was higher among the JECS
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8 participants, compared with the national statistics (35.3% vs. 20.8%). Moreover, the number
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10 of JECS-A participants with a household income of 2 million yen or less was about 1/8 of the
11
12 national statistics. Likewise, unemployed participants (mainly housewives) accounted for
13
14 45% of the JECS-A cohort. These results suggest a possible selection bias. We recruited
15
16 pregnant women who had visited the obstetric facilities during the daytime; this might have
17
18 resulted in a lower study participation rate among pregnant employees.
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26 **Participant characteristics of children**

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28 As for the baseline cohort of children, a total of 5,502 (99.0%) of the 5,555 children
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30 remained in the JECS-A at 18 months after delivery (provisional figures as of August 1,
31
32 2018). **Table 3** compares the baseline data of the newborns with the national statistics. Basic
33
34 demographics including “Sex”, “Birth weight” and “Birth height” had similar distributions,
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36 indicating the representativeness of the sample.
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40 Similarly, **Table 4** shows the fundamental characteristics of the pregnant women and their
41
42 children in each sub-cohort of the JECS-A. No notable differences in the descriptive
43
44 statistics were observed among the sub-cohorts. Furthermore, the drop-out rates for each
45
46 sub-cohort, to date, have been maintained at less than 10% relative to the baseline. However,
47
48 10% of the sub-cohort values for household income are missing because many participants
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50 did not provide information on the income. Therefore, caution is needed when using this
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52 variable as a confounding factor in multiple regression analyses.
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60 **STRENGTHS AND LIMITATIONS**

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4 The main strength of the JECS-A of children is its large sample size, reflecting a
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6 representative population. Approximately 40% of the children born in the study area have
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8 been included in the study to date. The participating children will be followed until they
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10 reach the age of 13 years. The study protocol³³ of the JECS decided on a target retention rate
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12 of 80% or higher at the age of 13 years. The linkage between the JECS-A sub-cohort and the
13
14 main data of the JECS study will further allow novel and challenging studies with a high
15
16 generalizability.
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20
21 The main weakness of the JECS-A cohort concerns the pregnant women participants
22
23 related to a selective attrition. A relatively large proportion of single-income, middle-class
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25 households were included in the JECS-A; many low-income households refused to
26
27 participate in the surveys, and some participants did not provide information concerning their
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29 household income. As enrollment strategies, though we set about recruiting from the first
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31 trimester of pregnancy (<12-week gestation), it was hard to get their cooperation in earlier
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33 weeks of a pregnancy, when the risk of miscarriage is higher. Most of pregnant women
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35 registered were actually after 10th week of pregnancy. Furthermore, we had no choice but to
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37 call on pregnant women for participation who had visited the obstetric facilities mainly
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39 during the daytime, owing to limited number of staffs during the evening shift at hospitals.
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41 Employed participants including all non-regular employees (part-time, temporary or contract
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43 employees) only accounted for 33% in the group of annual household income with 2 million
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45 Japanese yen or less. This means a selective attrition that working pregnant women of low
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47 SES might have been excluded in the cohorts. Such family socioeconomic status will have
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49 possibilities affecting several outcomes related to child development such as breastfeeding
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51 duration ³⁴, obesity in children ³⁵ and child maltreatment ³⁶. A British study ³⁷, however,
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53 suggests that cognitive and behavioural development has a weak or absent direct effect of
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4 income inequality after controlling potential confounders. Thus, possible biases as a result of
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6 selective attrition and direct SES effects for child should be carefully examined in future
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8 studies when using the JECS-A cohort data. The impact of the missing values can be adjusted
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10 to some extent by comparing estimates resulting from the multiple imputation and from
11
12 complete case analysis, using the data of main study of the JECS as reference. However,
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14 researchers must take into account the effect of response biases when conducting statistical
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16 analyses using paired data for pregnant women and their children and when interpreting these
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18 results.
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25 **COLLABORATION**

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28 The original data and specimens will be made available to investigators and stakeholders
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30 working within the JECS-A project. The study must adhere to the JECS policy on the
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32 availability of research results, publications, intellectual property rights, and data sharing. At
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34 the moment, the Ethical Guidelines for Medical and Health Research Involving Human
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36 Subjects enforced by the Japan Ministry of Education, Culture, Sports, Science and
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38 Technology and the Ministry of Health, Labour and Welfare restrict the open sharing of
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40 epidemiologic data. This means that researchers interested in using the data must collaborate
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42 with and participate in the JECS-A project. This project was recently started and is still
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44 ongoing. We are open to new proposals that fall within the nature of the JECS-A cohort.
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52
53
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55
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57
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59
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9

10 11 12 13 **Contributors**

14
15
16 TE designed the JECS-A cohort architecture, developed the protocol, analyzed the data,
17
18 and wrote the first draft of the manuscript. YY and MT organized the study team, obtained
19
20 approvals and contributed to the development of the protocol, design, and data collection
21
22 tools. T_{Om} was in charge of coordination with relevant organizations and organized the
23
24 JECS-A members. NS, SK, T_{Ma} and T_{Og} performed the analysis and interpreted the data.
25
26 YI, HS, NO and JU designed and developed the protocol for the JECS-A sub-cohorts A2, A3
27
28 and B and edited and analyzed the data. AN, M_{Ko}, YO, T_{Mi}, S_{Su}, M_{SO} and S_{Sa} designed
29
30 each adjunct study, supervised the data collection, and drafted the manuscript. M_{Ka} was a
31
32 member of the JECS Steering Committee, was responsible for the study design and protocol,
33
34 supervised the data collection, and edited and drafted the manuscript. All the authors
35
36 interpreted the data, contributed to the writing of the manuscript, revised it critically for
37
38 important intellectual content, and agreed with the final version and the findings.
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10
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21 **Data sharing statement** The original data and specimens will be made available to
22 investigators and stakeholders working within the JECS-A project.
23

24
25 Researchers interested in collaborations or further information should contact the JECS-A
26 secretariat by e-mail at eisei@med.nagoya-cu.ac.jp.
27
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33 **Disclaimer** The findings and conclusions of this article are solely the responsibility of the
34 authors and do not represent the official views of the Japanese government.
35
36

37 38 39 40 41 42 **REFERENCES**

- 43
44
45 1. Ishitsuka K, Nakayama SF, Kishi R, et al. Japan Environment and Children's Study:
46 backgrounds, activities, and future directions in global perspectives. *Environ Health*
47 *Prev Med* 2017;22(1):61. doi: 10.1186/s12199-017-0667-y
48
49
50
51
52 2. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders
53 (DSM-5): American Psychiatric Association Publishing 2013:xiii-xv.
54
55
56
57 3. Xiang S, Carrie A. A review of the prevalence of Autism Spectrum Disorder in Asia,
58
59
60 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

- 1
2
3
4 11. Polanska K, Ligocka D, Sobala W, et al. Phthalate exposure and child development: the
5
6 Polish Mother and Child Cohort Study. *Early Hum Dev* 2014;90(9):477-85. doi:
7
8 10.1016/j.earlhumdev.2014.06.006
9
- 10
11 12. Kyriklaki A, Vafeiadi M, Kampouri M, et al. Prenatal exposure to persistent organic
12
13 pollutants in association with offspring neuropsychological development at 4years of
14
15 age: The Rhea mother-child cohort, Crete, Greece. *Environ Int* 2016;97:204-11. doi:
16
17 10.1016/j.envint.2016.09.012
18
- 19
20 21 13. Goudarzi H, Nakajima S, Ikeno T, et al. Prenatal exposure to perfluorinated chemicals
22
23 and neurodevelopment in early infancy: The Hokkaido Study. *Sci Total Environ*
24
25 2016;541:1002-10. doi: 10.1016/j.scitotenv.2015.10.017
26
27
- 28 28 14. Yolton K, Cornelius M, Ornoy A, et al. Exposure to neurotoxicants and the development
29
30 of attention deficit hyperactivity disorder and its related behaviors in childhood.
31
32 *Neurotoxicol Teratol* 2014;44:30-45. doi: 10.1016/j.ntt.2014.05.003
33
34
- 35 35 15. Engel SM, Wetmur J, Chen J, et al. Prenatal exposure to organophosphates, paraoxonase
36
37 1, and cognitive development in childhood. *Environ Health Perspect*
38
39 2011;119(8):1182-8. doi: 10.1289/ehp.1003183
40
41
- 42 42 16. Bouchard MF, Chevrier J, Harley KG, et al. Prenatal exposure to organophosphate
43
44 pesticides and IQ in 7-year-old children. *Environ Health Perspect*
45
46 2011;119(8):1189-95. doi: 10.1289/ehp.1003185
47
48
- 49 49 17. Stein LJ, Gunier RB, Harley K, et al. Early childhood adversity potentiates the adverse
50
51 association between prenatal organophosphate pesticide exposure and child IQ: The
52
53 CHAMACOS cohort. *Neurotoxicology* 2016;56:180-87. doi:
54
55 10.1016/j.neuro.2016.07.010
56
57
- 58 58 18. Yurumez E, Akca OF, Ugur C, et al. Mothers' alexithymia, depression and anxiety levels
59
60

- 1
2
3
4 and their association with the quality of mother-infant relationship: a preliminary
5
6 study. *Int J Psychiatry Clin Pract* 2014;18(3):190-6. doi:
7
8 10.3109/13651501.2014.940055
9
10
11 19. Li Y, Jiang WQ, Du YS, et al. Relationships between behavioral symptoms of
12
13 non-medicated Chinese children with attention deficit hyperactivity disorder and
14
15 parenting stress: Comparison of different subtypes and comorbidities. *Asia Pac*
16
17 *Psychiatry* 2016;8(2):127-35. doi: 10.1111/appy.12213
18
19
20
21 20. Oya N, Ito Y, Hioki K, et al. Quantitative analysis of organophosphate insecticide
22
23 metabolites in urine extracted from disposable diapers of toddlers in Japan. *Int J Hyg*
24
25 *Environ Health* 2017;220(2 Pt A):209-16. doi: 10.1016/j.ijheh.2016.10.009
26
27
28 21. Saito S, Ueyama J, Kondo T, et al. A non-invasive biomonitoring method for assessing
29
30 levels of urinary pyrethroid metabolites in diapered children by gas
31
32 chromatography-mass spectrometry. *J Expo Sci Environ Epidemiol* 2014;24(2):200-7.
33
34 doi: 10.1038/jes.2013.31
35
36
37 22. Robins DL, Casagrande K, Barton M, et al. Validation of the modified checklist for
38
39 Autism in toddlers, revised with follow-up (M-CHAT-R/F). *Pediatrics*
40
41 2014;133(1):37-45. doi: 10.1542/peds.2013-1813
42
43
44 23. Manning JT, Baron-Cohen S, Wheelwright S, et al. The 2nd to 4th digit ratio and autism.
45
46 *Dev Med Child Neurol* 2001;43(3):160-4.
47
48
49 24. Teatero ML, Netley C. A critical review of the research on the extreme male brain theory
50
51 and digit ratio (2D:4D). *J Autism Dev Disord* 2013;43(11):2664-76. doi:
52
53 10.1007/s10803-013-1819-6
54
55
56 25. Guyatt AL, Heron J, Knight Ble C, et al. Digit ratio and autism spectrum disorders in the
57
58 Avon Longitudinal Study of Parents and Children: a birth cohort study. *BMJ Open*
59
60

- 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:

1
2
3
4 10.1186/1751-0759-1-7
5

- 6 33. National Centre for Japan Environment and Children's study. Japan Environment and
7
8 Children's study (JECS), Study Protocol (ver. 1.4) [Available from:
9
10 https://www.env.go.jp/en/chemi/hs/jecs/data/about/jecs-study_protocol_14_en.pdf.
11
12
- 13 34. Flacking R, Nyqvist KH, Ewald U. Effects of socioeconomic status on breastfeeding
14
15 duration in mothers of preterm and term infants. *Eur J Public Health*
16
17 2007;17(6):579-84. doi: 10.1093/eurpub/ckm019
18
19
- 20 35. Ogden CL, Lamb MM, Carroll MD, et al. Obesity and socioeconomic status in children
21
22 and adolescents: United States, 2005-2008. *NCHS Data Brief* 2010(51):1-8.
23
24
- 25 36. Eckenrode J, Smith EG, McCarthy ME, et al. Income inequality and child maltreatment
26
27 in the United States. *Pediatrics* 2014;133(3):454-61. doi: 10.1542/peds.2013-1707
28
29
- 30 37. Violato M, Petrou S, Gray R, et al. Family income and child cognitive and behavioural
31
32 development in the United Kingdom: does money matter? *Health Econ*
33
34 2011;20(10):1201-25. doi: 10.1002/hec.1665
35
36
37
38
39
40
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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

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

Main items	Sub-cohort covered					Timing of measurement						
	A1	A2	A3	B	C	prenatal (trimester)		postnatal (months after birth)				
						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	○	○	○		○						○	

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6 ○: Refer to the main study of the Japan Environment and Children's Study (JECS), ●: Measurements conducting solely in the JECS-A
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10 **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
11 Revised Short Form; **ECBQ-SF**, Early Childhood Behavior Questionnaire Short Form; **DCDQ**, Developmental Coordination Disorder Questionnaire, **2D:4D**, the second to
12 fourth digit ratio; **PARS**, Pervasive Developmental Disorders Autism Society Japan Rating Scale; **ASD**, autism spectrum disorder; **ADHD**, attention deficit hyperactivity
13 disorder; **LBW**, low birth weight.
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16 **Sub cohort A1**, Agreement on the adjunct questionnaire survey only; **Sub cohort A2**, Agreement on the adjunct questionnaire survey and provision of mother's
17 biological specimen (blood and urine); **Sub cohort A3**, Agreement on the adjunct questionnaire survey and provision of mother's biological specimen (blood and urine)
18 with genetic analyses; **Sub cohort B**, Informed consent obtained from guardians (legally acceptable representatives) for provision of child's biological specimen; **Sub**
19 **cohort C**, Informed consent obtained from guardians (legally acceptable representatives) for anthropometry measurement on child hands
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Table 2 Comparison of the J ECS-A baseline data with national statistics in Aichi prefecture for pregnant women: verification of representativeness of the sample

	J ECS-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

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

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

	J ECS-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			

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^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

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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

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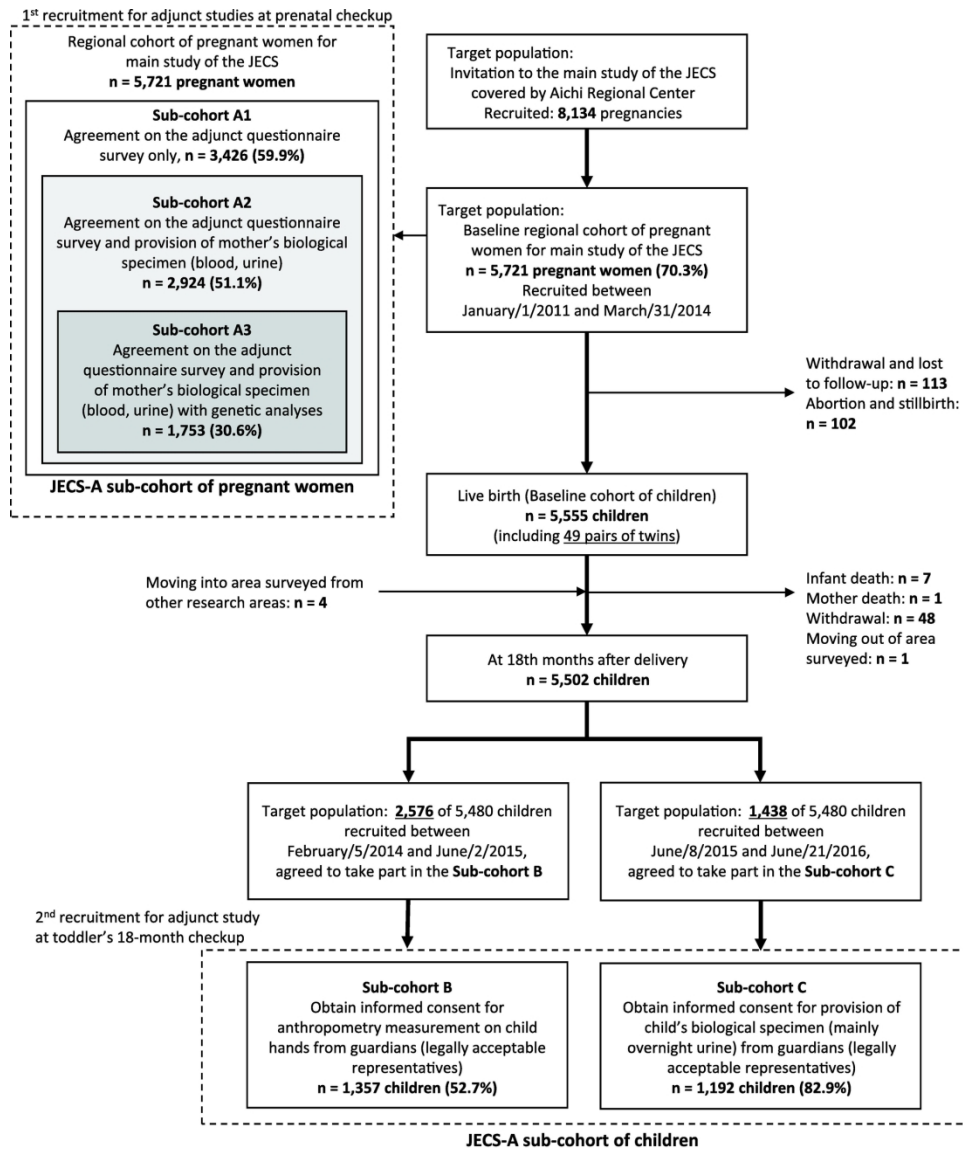


Figure 2. Flowchart showing each stage of study recruitment for adjunct studies in the JECs-A

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

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