Pre/Perinatal/Early-Life Exposures and Early Child Neurodevelopment

Results from the Japan Environment and Children's Study (JECS)

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Pre/Perinatal/Early-Life Exposures and Early Child Neurodevelopment: Results from the Japan Environment and Children's Study (JECS)

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To my family and my friends

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Results from the Japan Environment and Children's Study

Kahoko Yasumitsu-Lovell

Gillberg Neuropsychiatry Centre, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden, 2023

ABSTRACT

Background: Combinations of genetic and environmental factors contribute to aetiologies of neuropsychiatric problems and neurodevelopmental disorders (NDDs) in intricate manners. Among the environmental factors, exposures during the pre-/perinatal periods and early childhood are particularly important. Holistic assessment of additive risks during these periods on child neurodevelopment is crucial for prevention, early detection, and intervention. Equally, when concerns arise about a child's development, comprehensive/ holistic assessment of neurodevelopment is extremely important as comorbidities are the rule rather than the exception. **Aim**: The overall aim of this thesis is to examine possible associations between pre-/perinatal and early-life exposures and child neurodevelopment up until 3 years of age. The thesis focuses on the association between pre-/perinatal optimality and child development at 1 month and 3 years of age (*Study I*), between birth month and gross motor development at age 6 and 12 months (*Study II*), and between child vitamin D and neurodevelopment at age 2 years (*Study III*). The fourth study assesses the ability of the ESSENCE-Q used at child age 2.5 years as a screening tool to identify child overall neurodevelopmental problems and relate findings to NDDs diagnosed before 3 years of age (*Study IV*). **Methods**: Medical records, blood samples, and self-administered parental questionnaires from the Japan Environment and Children's Study (JECS), one of the world's largest ongoing national birth cohort studies (more than 100,000 mother-child dyads), were utilised throughout the four studies. **Results**: Obstetric reduced optimality scale scores showed dose-response associations with NDDs at child age 3 years (*Study I*). Summer-born babies lagged behind winter-born babies regarding gross motor development at ages 6 and 12 months (*Study II*). Low vitamin D level was negatively associated with cognitive and communication development in boys (*Study III)*. Parent-completed ESSENCE-Q was useful for screening out children without neurodevelopmental problems (*Study IV*). **Conclusions:** Child neurodevelopment by age 3 years was associated with negative pre-/perinatal factors, seasonality, and, in boys, with low vitamin D levels at age 2 years. These findings could be taken to indicate that better support should be provided for children who experienced adversities in their early life, as early as during the prenatal period. The ESSENCE-Q can probably be used for screening out children without major NDDs.

Keywords: ESSENCE-Q, JECS, Neurodevelopmental disorders/problems, Pre-/Perinatal factors, Vitamin D

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SAMMANFATTNING PÅ SVENSKA

Bakgrund:
Genetiska och olika medicinska pre- och perinatala riskfaktorer/icke-optimala faktorer – ofta i kombinationer – samt ogynnsamma faktorer under tidig barndom bidrar till det breda orsakspanorama som föreligger vid utvecklingsneurologiska/neuropsykiatriska funktionsnedsättningar/funktions-problem (numera ofta sammanfattade under begreppet ESSENCE) hos barn. För tidig upptäckt och för olika typer av stöd och insatser krävs en helhetsbedömning avseende barnets utveckling inom olika funktionsområden och en värdering av ärftliga och, inte minst, icke-optimala faktorer pre-/perinatalt och under tidig barndom. När oro uppstår för ett barns utveckling behövs en sådan helhetsbedömning av barnets olika funktioner; grov- och finmotorisk förmåga, en bedömning av barnets generella kognitiva förmågor (språkliga och icke-språkliga), av sociala färdigheter och avseende olika beteendemässiga svårigheter. En sådan bred bedömning är mycket viktig eftersom överlappning av olika problemtyper/ och funktionsnedsättningar är regel snarare än undantag. En helhetssyn är också avgörande för att på befolkningsnivå kunna utveckla förebyggande insatser, metoder för tidig upptäckt och intervention.
Syfte: Det övergripande syftet med denna avhandling är att undersöka möjliga samband mellan icke-optimala/risksituationer pre-/perinatalt/under tidig barndom å ena sidan och barns motoriska och sociala utveckling fram till 3 års ålder å den andra.
Metoder: Arbetet utgår från den stora, pågående prospektiva födelsekohortstudien ”Japan Environment and Children’s Study (JECS)”, som inkluderar flera än 100,000 gravida kvinnor och deras barn. Barnen kommer att följas till åtminstone 13 års ålder. Särskilda faktorer under den pre-och perinatala perioden och under tidig barndom har analyserats i detta avhandlingsarbete, som omfattar fyra delstudier. I den första av dessa undersöks eventuella samband mellan sådana pre- och perinatala faktorer och barnens utveckling vid 1 månad och 3 års ålder (*Studie I*). I den andra studien undersöks eventuella samband mellan födelsemånad och grovmotorisk utveckling vid 6 och 12 månaders ålder (*Studie II*), och i den tredje studien samband mellan nivåer av vitamin D och barns utveckling vid 2 års ålder (*Studie III*). I den fjärde studien utvärderas ett frågeformulär, ett ”screeningverktyg”, ESSENCE-Q, med 11 korta frågor om viktiga funktionsområden och symtom. Föräldrar har i studien besvarat formuläret när barnet är 2.5 år och angivit om de har oroat sig för barnets utveckling inom något eller flera av de funktionsområden som formuläret tar upp. Resultaten relaterades till senare, före 3 års ålder, ställda diagnoser avseende utvecklingsneurologiska funktionsnedsättningar, framförallt intellektuell

funktionsnedsättning, autism, motorisk utvecklingsförsening och försenad språklig utveckling (*Studie IV*). Data från barnens medicinska journaler har granskats och resultat från blodprover (vitamin D) har sammanställts. Utvecklingsbedömningar och föräldraenkäter från JECS, som är en av världens största pågående nationella födelsekohortstudier, har använts i studierna.

Resultat: De barn som vid 3 års ålder hade någon eller flera utvecklingsneurologiska diagnoser hade haft signifikant fler icke-optimala faktorer pre- och/eller perinatalt, jämfört med barm utan sådana riskfaktorer (*Studie I*). Barn födda under sommarmånaderna hade sämre grovmotorisk utveckling vid 6 och 12 månaders ålder jämfört med barn födda under vintermånaderna (*Studie II*). Låg D-vitaminnivå hos pojkar var tydligt negativt associerad med barnets kognitiva och kommunikativa utveckling (*Studie III*). Föräldraenkäten ESSENCE-Q, med frågor om föräldraoro relaterade till barnets utveckling visades ha relativt god förmåga att identifiera barn *utan* neuropsykiatriska/utvecklingsneurologiska funktionsnedsättningar eller problem (*Studie IV*).

Slutsatser: Utvecklingsproblem vid 3 års ålder var associerade med negativa, icke-optimala pre-och perinatala faktorer. Tidpunkt under året för barnets födelse hade samband med motorisk utveckling under första levnadsåret. För pojkar var låga nivåer av D-vitamin vid 2 års ålder associerade med sen kognitiv och kommunikativ utveckling. Sammantaget framkom således att pre-och perinatala faktorer, årstid för födelsen och hos pojkar nivån av vitamin D hade betydelse för utvecklingen upp till 3 års ålder. Fynden talar för att barn med riskfaktorer pre- och perinatal samt under tidig barndom behöver följas upp avseende behov av olika former av insatser. Formuläret ESSENCE-Q kan förmodligen användas som ett screeningverktyg för att framförallt identifiera barn *utan* utvecklingsneurologiska funktionsnedsättningar.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. Yasumitsu-Lovell K, Thompson L, Fernell E, Eitoku M. Suganuma N, Gillberg C. Pre-/perinatal reduced optimality and neurodevelopment at 1 month and 3 years of age: Results from the Japan Environment and Children's Study. PLoS One 2023; 18(1):e0280249.

II. Yasumitsu-Lovell K, Thompson L, Fernell E, Eitoku M. Suganuma N, Gillberg C. Birth month and infant gross motor development: Results from the Japan Environment and Children's Study. PLoS One 2021; 16(5):e0251581.

III. Yasumitsu-Lovell K, Thompson L, Fernell E, Eitoku M. Suganuma N, Gillberg C. Vitamin D and neurodevelopmental problems at age 2 years: Results from the JECS. *Submitted*.

IV. Yasumitsu-Lovell K, Thompson L, Fernell E, Eitoku M. Suganuma N, Gillberg C. Validity of the ESSENCE-Q neurodevelopmental screening tool among the participants of the Japan Environment and Children's Study (JECS). *Submitted*.

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ABBREVIATIONS

ADHD	Attention-Deficit/Hyperactivity Disorder
ASD	Autism Spectrum Disorder
AUC	Area Under the Curve
C-A	Cognitive-Adaptive (a domain of the KSPD)
DCD	Developmental Coordination Disorder
DLD	Developmental Language Delay
DNBC	Danish National Birth Cohort
DOHaD	Developmental Origins of Health and Disease
EPDS	Edinburgh Postnatal Depression Scale
ESSENCE	Early Symptomatic Syndromes Eliciting Clinical Examination
FFQ	Food Frequency Questionnaire
ID	Intellectual Disability / Intellectual Developmental Disorder (IDD)
IUGR	Intrauterine growth restriction
J-ASQ-3	Japanese translation of the Ages and Stages Questionnaire Third Edition
JECS	Japan Environment and Children's Study
KSPD	Kyoto Scale of Psychological Development 2001
L-S	Language-Social (a domain of the KSPD)
LD	Learning Disorders
MD	Motor Delay
MoBa	Norwegian Mother Infant Study
NDD	Neurodevelopmental Disorder
NDP	Neurodevelopmental Problem
OR/aOR	Odds Ratio/Adjusted Odds Ratio
P-M	Postural-Motor (a domain of the KSPD)
ROC	Receiver Operating Curve
RR/aRR	Relative Risk/Adjusted Risk Ratio
SCS	Sub-Cohort Study (of JECS)
SGA	Small for Gestational Age

1 INTRODUCTION

Neurodevelopmental disorders (NDDs) are conditions characterized by deviations in the child's expected development within areas of cognitive, social, emotional, motor, and behavioural functioning. NDDs typically emerge in childhood and have life-time effects on an individual's development and daily functioning. The most common forms of NDDs are autism spectrum disorder (ASD), attention-deficit/hyperactivity disorder (ADHD), Tourette's syndrome, intellectual disability (ID), speech and language disorder (SLD), and developmental coordination disorder (DCD). Reactive attachment disorder (RAD), disinhibited social engagement disorder (DSED), selective mutism, paediatric acute-onset neuropsychiatric syndrome (PANS), paediatric autoimmune neuropsychiatric disorders associated with streptococcal infection (PANDAS), behavioural phenotype syndromes (BPS), some variants of epilepsy, and avoidant/restrictive food intake disorder (ARFID) are also sometimes included in the broad group of childhood onset NDDs/neuropsychiatric disorders.[1,2]

Despite all these "different" diagnoses, from early life, individuals with NDDs show a variety of coexisting, pathological/atypical symptoms regarding communication, fine and gross motor skills, and sensory reactions, and NDDs are almost *always* comorbid with each other. It is estimated that approximately 10% of school-aged children and 5% of preschool children meet criteria for at least one NDD. The overlap of symptoms among different NDD diagnoses is the rule rather than exception, and different neurodevelopmental issues of the same individual surface in different occasions as one grows up.[1] For instance, prevalence of ADHD and specific learning disorders (specific LD), such as dyslexia, dysgraphia, and dyscalculia, typically *increase* at "school age" (i.e., starting around 5-7 years old). In Japan today, 10.4% of primary school pupils (age 6-12 years) and 5.6% of junior high school students (age 13-15 years) have been reported to need some special supports due to their ASD-/ADHD-like behaviour and specific LD.[3]

As awareness of the importance of early intervention and treatment has been growing, so has the need for early detection and for grasping the overlapping symptoms among different NDD diagnoses been emphasised.[4,5] The overlapping nature of NDDs and the increasing demand from society brought Gillberg to introduce the concept of **ESSENCE** – Early Symptomatic Syndromes Eliciting Neurodevelopmental Clinical Examinations in 2010.[6] This concept assumes that "comorbidity is *always* present", that different symptoms can be the most conspicuous/disabling within the same individual

at different time points in life.[1] The concept allows clinicians and researchers to observe individuals without putting them into one NDD box rigidly, and to support them flexibly throughout their life as a "multiprofessional team" consisting of a wide variety of specialists such as medical doctors, nurses, psychologists, teachers, and speech/language pathologists, even when different problems surface during different life stages which, as mentioned, is almost always the case.[1]

The aetiologies of NDDs vary, with genetic and/or environmental factors contributing in complex and interactive patterns. It is estimated that genetic factors contribute approximately 60 - 80%.[7] However, most genomic risks present not in the monogenic high penetrant alleles but in vulnerable alleles with low penetrance, and gene-environment interactions and offspring's sex affect the phenotypic expressions of NDDs.[8] Among environmental factors, those during the pre-/perinatal periods are most crucial, possibly shaping the life-time wellbeing, including neurodevelopment, of individuals, as explained by the Development Origin of Health and Disease (DOHaD) theory,[9,10] particularly because the central nervous system starts developing soon after the conception and throughout one's early adulthood.[11-14]

Among pre-/perinatal environmental factors, maternal systemic chronic and acute inflammation is increasingly recognised as an insult for offspring's immune and developmental epigenetic code programming, therefore, possibly increase a risk of NDDs.[8] Prenatal chronic and acute maternal immune activation caused by a variety of diseases and conditions, such as obesity, gestational diabetes mellitus, preeclampsia, stress, depression, smoking, pollution, autoimmune diseases, and infection, are now understood to affect offspring's life-long neurological systems. [15,16]

Similar to maternal immune activation, prenatal and early-life nutrition status, including anthropometry, macro/micronutrients such as fatty acid, protein, iron, copper, and vitamins like B12, D, A, E, K, and folate also plays a crucial role in child neurodevelopment, as maternal nutritional signals determine a foetus's epigenetic remodelling.[17] In animal models, prenatal non-optimal nutrition is strongly related to epigenetic changes in the foetal brain, affecting neurogenesis, synaptic plasticity, and hypothalamic-pituitary-adrenal (HPA) axis,[17] but for human, previous study results on maternal nutrient status have been still inconclusive and further studies are needed.[18]

1.1 Neurodevelopmental Disorders and related risks in early life

Prenatal genetic factors, inherited or new mutations, constitute a major part of the underlying causal factors for NDDs. Several numerical and structural chromosomal abnormalities and specific genes have been identified: trisomies, such as trisomy 21 (Down syndrome), the most common aneuploidy, trisomy18 and 13, and sex chromosome aneuploidies, such as XXY, XYY and XO (Turner syndrome). Fragile X syndrome is the leading single-gene cause of inherited intellectual disability and autism. Genome sequencing, now replacing chromosomal microarray and FMR1 analysis, has been found to be a sensitive first-line test to diagnose individuals with intellectual disability.[19] Through advances in genetics, over 100 genes have been identified to be associated with autism.[20] Several copy number variations (gain or loss of DNA); deletions, duplications are also associated with a range of neurodevelopmental disorders, e.g., 22q11.2 deletion syndrome and specific duplication syndromes.[21]

Prenatal infections affecting the foetus may be examples of other acquired prenatal causes of NDDs. Congenital cytomegalovirus infection (CMV) is a well-known example, being one of the many aetiologies underlying autism spectrum disorder with intellectual disability.[22]

Pre-/perinatal complications, such as premature birth and emergency caesarean section deliveries, have been shown to increase risks for NDDs.[23-25] In a UK study, for children who had been born at a gestational age of less than 32 weeks, the prevalence of special educational needs was 27%, three times higher than among children born at term.[26] Increasing numbers of children are surviving after being born extremely preterm (born before 28 gestational weeks) due to improved perinatal/neonatal intensive care. However, extreme prematurity is a major risk factor for neurodevelopmental disorders and long-term disability. There is increasing awareness of common cognitive and neuropsychiatric problems and their special needs at school among extremely preterm children. Early identification of infants at risk for neurodevelopmental impairment is crucial for early interventions.[27]

A well-known risk factor in near-term/term infants is perinatally acquired hypoxic-ischaemic encephalopathy (HIE) of mild, moderate, or severe level. From the pre-hypothermia era, it is known that a significant proportion of survivors, even when free of major neuromotor disability, suffer more subtle cognitive impairment, including executive difficulties. Therapeutic hypothermia (TH) is now standard of care in near-term/term infants with

moderate/severe hypoxic-ischemic encephalopathy (HIE) in most high-resource countries.[28]

In addition to pre-/perinatal medical conditions, chemical exposures during the periods, such as alcohol, heavy metal and human-made chemicals, such as lead, mercury, perfluorooctane sulfonate (PFOS), and polyfluoroalkyl substances (PFAS), are also likely to be hazardous environmental risks for neurodevelopment, particularly exposures during the foetal and infantile periods [29]. Exposure to maternal alcohol consumption during pregnancy may adversely impact the developing foetus resulting in a continuum of disabilities, i.e., foetal alcohol spectrum disorders (FASD). FASDs constitute the most common preventable cause of developmental disorders. There are clinical guidelines with diagnostic criteria to facilitate diagnosis and appropriate interventions, including counselling and support regarding upcoming pregnancies.[30]

Vitamin D has been studied as one of the essential nutrients necessary throughout life, starting from the pre-/perinatal and early childhood stages to adulthood.[31,32] In addition to its well-known role in calcium regulation and phosphate metabolism,[31] vitamin D has been also understood as a "neurosteroid", influencing neurodevelopment as early as foetal and infantile periods.[33-36] Both maternal and child vitamin D status and the efficacy of vitamin D supplementation on neurodevelopment have been studied, but not all published results have been consistent, and further research is needed to understand the underlying mechanisms.[37,38]

Birth month, a perinatal biometeorological factor, is also known to be one of the perinatal factors affecting lifetime disease risk, including cardiovascular, respiratory, reproductive, and neurological/psychiatric problems.[39] Many studies have investigated the association of birth month/season with neurological, neuropsychiatric, and neurodevelopmental disorders, including ASD, ADHD, epilepsy, LD, schizophrenia, and depression.[39-47] Nevertheless, previous study results have not been conclusive. For example, birth seasons/months with the highest risk of ASD are ranging from spring,[41,47] to summer/autumn,[48,49] or no association.[49]

The heterogeneity of previous study results is highly likely due to wide variety of study designs, sample sizes, study regions, diagnostic criteria, and insufficient information on various confounding factors.[7,9,23,50,51]. Large birth cohort studies, with prospectively collected information on various pre-/perinatal risk factors, enable us to overcome such challenges.[52] These studies require significant investment and so there are few available internationally. Four large national birth cohort studies focusing on environmental exposures

are currently being conducted: the Danish National Birth Cohort (DNBC, 100,000 participants, 1990 - ongoing); Norwegian Mother, Father and Child Cohort Study (MoBa, 90,000 participants, 1998 - ongoing); the Japan Environment and Children's Study (JECS, 100,000 participants, 2011-ongoing), and the China Birth Cohort Study (CBCS, aiming to recruit 500,000 participants, 2017 - ongoing).[53]

In addition to large sample size and appropriate study design, it is crucial to include all conceivable and possibly additive adverse effects of pre-/perinatal risk conditions, because several risk factors during the periods are known to contribute to offspring's NDDs and neurodevelopmental problems (NDPs) in a complex manner[23]. Some studies have used an "obstetric optimality scale score", in which each pre-/perinatal factors were weighted equally, typically 0 for optimal and 1 for suboptimal conditions[7,11-14]. Other studies handled each of the risk factors utilising multivariate models to identify specific aetiological association of individual factors for future NDDs and NDPs. [9,23,50,51] Both approaches have strengths and weaknesses: the former is applicable to examine the additive effects but not to specify the individual risk factors, and vice versa for the latter.

1.2 Early Symptomatic Syndromes Eliciting Neurodevelopmental Clinical Examinations (ESSENCE) and early detection

On the basis of the concept of ESSENCE, Gillberg designed the ESSENCE-Q, a simple one-page screening questionnaire in 2010, originally for children who show signs of neurodevelopmental deviations and problems that later might be diagnosed as specific NDDs.[6,54] The ESSENCE-Q has been now developed to be used from childhood to adulthood. It can be used as a short and structured interview by medical professionals or as a questionnaire completed by parents/caregivers. It consists of 11 questions that elicit concerns that have persisted for several months within the following 11 domains: (1) general development; (2) motor development; (3) sensory reactions; (4) communication/language/babble; (5) activity (overactivity/passivity) or impulsivity; (6) attention/concentration/"listening"; (7) social interaction with/interest in other children; (8) behaviour (e.g., repetitive, routine insistence); (9) mood (depressed, elated/manic, extreme irritability, crying spells); (10) sleep; and (11) feeding.[54] Three responses are available for each item: "yes", "maybe/a little", or "no", scored 0,1,2 respectively.[54] The total score range is 0-22, with higher scores indicating more concerns. The ESSENCE-Q has been translated into 15 languages to date, and has been used in a variety of context, including public health, clinical, and research settings.

The questionnaire has been used in combination with the diagnostic criteria – the International Statistical Classification of Diseases -10 (ICD-10) and the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV/DSM-5),[55-59] and standardized developmental tests and questionnaires, such as the Wechsler Preschool and Primary Scale of Intelligence (WPPSI),[58,60] the Wechsler Intelligence Scale for Children (WISC),[58,61] the Modified Checklist for Autism in Toddlers (M-CHAT),[62] the Strengths and Difficulties Questionnaire (SDQ),[55,56,63] and the Kyoto Scale of Psychological Development 2001 (KSPD)[55-57] in both community and research contexts. [7,8] As of today, 8 validation studies have been conducted in clinical and municipal public health settings in Japan, Sweden, Slavic-speaking countries, and India, with the number of participants in each ranging between 100 and 300 (*Table 1.1*),[9-16] but a large-scale validation study on a general population has not yet been performed.

Many studies have investigated early precursors of NDDs,[64] but to our best knowledge, no nationwide cohort studies have conducted analyses on data from mothers from the prenatal period in relation to children's neurodevelopment as early as age 1 month, and then followed them up prospectively. Likewise, no previous studies have investigated a large number of young children with focus on their overall neurodevelopment rather than on one specific NDD, such as ASD or ADHD, even though we now know that comorbidities are almost always the rule.[6] In addition, the importance of early support for children with NDPs has been increasing, so has more holistic early screening and detection of NDPs. The PhD project based on this background.

Table 1.1 ESSENCE-Q Validation Studies

	Publication year	Authors	Title	Country	Language	Number of participants	Child's age	Public health/ clinical settings	Respondents
1	2016	Hatakenaka et al.	ESSENCE-Q: first clinical validation study of a new screening questionnaire for young children with suspected neurodevelopmental problems in south Japan	Japan Kochi Pref.	Japanese	130	1 y 9 m–6 y (mean, 3 y 6 m)	Clinical (Children referred to the Kochi Prefectural Medical Welfare Centre)	Parents (caregivers)
2	2017	Hatakenaka et al.	ESSENCE-Q used as a screening tool for neurodevelopmental problems in public health check-ups for young children in south Japan	Japan Kami city, Kochi Pref.	Japanese	143 149	18 m 36 m	Public health	(1) Mothers (2) Public health nurses (3) Specialized psychologists
3	2018	Stevanovic et al.	ESSENCE-Q: Slavic language versions for developmental screening in young children	Serbia, Macedonia, Slovenia, Bulgaria, Bosnia & Herzegovina, Montenegro, Croatia	7 south Slavic languages	451	12 m–7 y	Clinical (children referred for diagnostic and/or therapeutic purposes)	Parents (caregivers)
4	2020	Hatakenaka et al.	ESSENCE-Q obtained in routine Japanese public child health check-ups may be a valuable tool in neurodevelopmental screening	Japan Aki city, Kochi Pref.	Japanese	99 108	20 m 40 m	Public health	(1) Mothers (2) Public health nurses (3) Specialized psychologists
5	2022	Kattimani et al.	Predictive validity of ESSENCE Q screening tool for early detection of neurodevelopmental disorder in children	India	Tamil English	100	12 m–5 y (mean, 26.3 m)	Recruited at outpatient department and immunization clinic (public)	Primary caregivers (75% mothers, 22% fathers, 3% grandmothers)
6	2022	Cederlund et al.	Parent questionnaires in the evaluation of pre-school children referred for neuropsychiatric assessment	Sweden	Swedish	124	Preschool age (mean, 54.4 m)	Clinical (referred consecutively for neuropsychiatric assessment to the child-and adolescent clinic at the NU hospital)	Parents/caregivers
7	2022	Landgren et al.	The ESSENCE-Q for neurodevelopmental problems: A Swedish school-based validation study in 11-year-old children	Sweden	Swedish	173	11 y	School-based (public)	Parents
8	2022	Landgren et al.	The ESSENCE-Questionnaire in medical records screening for neurodevelopmental symptoms/problems: Utility and clinical validity	Sweden	Swedish	169	11 y	School-based (public)	Medial record assessment with ESSENCE-Q

Pref: Prefecture, y: year, m: month

1.3 Birth cohort studies focusing on children's environmental health

Birth cohort studies follow up children from the pre-/peri-/neonatal periods to childhood/adulthood periods. It is regarded as the most appropriate type of study design to investigate the causal relationship between prenatal exposures and offspring's health outcomes.[65] Prospectively collected data enable researchers to accurately collect information, without recall biases. However, they are usually very time- and energy-consuming with the risk of selection-bias due to a loss of the participants to follow-up. The cancellation of the National Children's Study in the US (2000-2014) and the abandoned Life Study in the UK (2014-2015), aiming to recruit 100,000 and 80,000 pregnant women respectively, are examples of how challenging it can be to conduct large-scale birth cohort studies from recruitment, acquiring representative data, and follow-up.[66]

Children's environmental health was discussed as the central topic at the Environment Leaders' Summit of the G7 countries plus Russia, "the Eight", which resulted in the *1997 Declaration of the Environment Leaders of the Eight on Children's Environmental Health*, the 'Miami Declaration'.[67,68] It addressed 7 items for action to tackle collectively among the Eight as follow: (1) to take children's unique exposure patterns into accounts; (2) to phase out the use of lead in gasolines and other products; (3) to provide clean water; (4) to reduce air pollution; (5) to protect infant and children particularly from indoor exposure to tobacco smoke and to reduce youth access to tobacco smoking; (6) to establish an inventory of international research on endocrine-disrupting chemicals, linked to various health issues such as cancer, reproductive disorders, behaviour changes, and immune system problems; and (7) to consider global climate changes considering the specific vulnerability of children.[68]

Following the Miami Declaration, two national birth cohort studies focusing on child environmental health were launched. The Danish National Birth Cohort (DNBC), one of the ongoing largest national birth cohorts, was established in 1996 with the focus on children's environmental health. It recruited 100,000 pregnant women by 2002.[69] In addition to health, social, and economic information linked to unique personal identification number, prenatal maternal blood and umbilical cord blood samples were collected from DNBC participants. Exposure information was collected by computer-assisted telephone interviews twice during pregnancy.[70]

The Norwegian Mother, Father and Child Cohort Study (MoBa) was launched in 1998, and the recruitment continued till 2008. The cohort consists of more than 114,000 children, 95,000 mothers and 75,000 fathers.[71,72] Biological samples – blood, urine, and children's teeth – have been collected, and the unique identification numbers enable linkage to health registries, such as for birth records, diseases, death records, prescription, vaccination, and cancer.[71]

1.4 Japan Environment and Children's Study (JECS)

Prior to the Japan Environment and Children's Study (JECS), some smaller but significant birth cohort studies started, with the primary focus on children's environmental health in Japan. The Hokkaido Study and the Tohoku Study of Child Development (TSCD) started in 2001, with over 20,000 and 1,500 mother-child pairs respectively. In 2007, The Hamamatsu Birth Cohort for Mothers and Children (HBC Study) was launched with the primary objective to investigate neurodevelopmental trajectories, with the registration of 1,139 mothers and 1,258 children.[73] All these ongoing cohorts are highly localised and only representing some areas in Hokkaido, Miyagi, and Aichi prefectures.

The JECS is the ongoing first nationwide birth cohort study aiming to elucidate environmental factors, particularly early-life chemical exposures, and their impact on children's health and development.[74] A total of 104,065 foetal records were registered at 15 Regional Centres throughout Japan, from Hokkaido to Okinawa, between January 2011 and March 2014, with 100,303 live births (51,396 boys, 48,889 girls, and 18 cases with missing sex information) (*Figure 1.1*).[74] In 2022, the Ministry of the Environment announced its plan to extend the follow-up period – originally up until 13 years of age – to around 40 years of age, aiming to investigate further health outcomes appearing during/after adolescence such as psycho-neurologic and lifestyle-related diseases, and infertility of the current participants as well as health of the next generation.[75]

In the Main Study of the JECS, maternal information was collected from the time of recruitment through questionnaires, medical record transcription, and biospecimens (maternal blood, urine, hair, and breast milk, umbilical cord blood, child hair). Paternal participation was voluntarily and was ultimately approximately 50% of maternal participation. After age 1 month until 8 years, the follow-up has been conducted by biannual questionnaires.

In the Sub Cohort Study (SCS), which is conducted with 5% of the Main Study participants, more in-depth data collection has taken place, such as home visits to collect environmental samples at age 1.5 and 3 years, and face-to-face follow-up, including blood sample collection, neurodevelopmental assessment, and clinical examination at 2,4, 6, and 8 years of age.

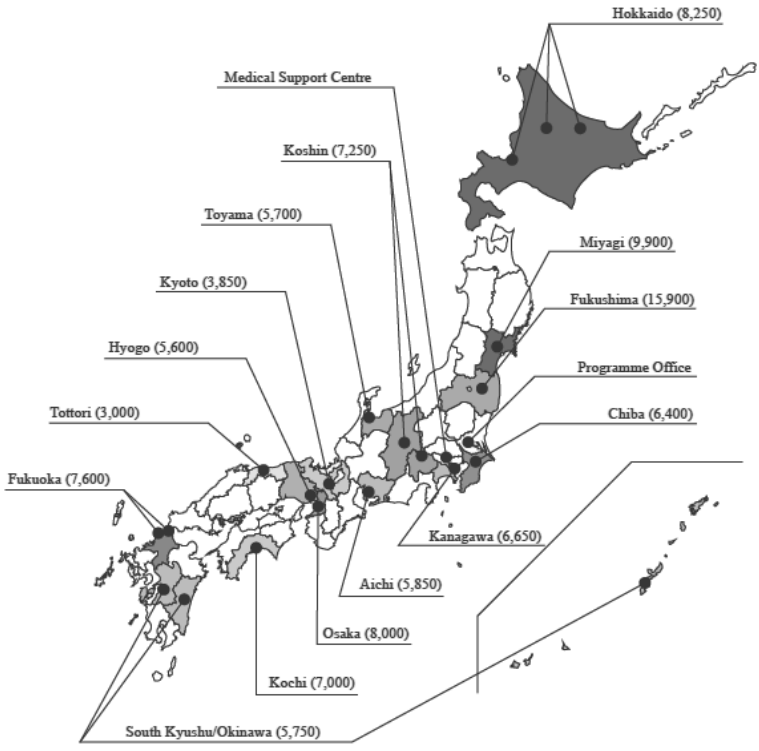

Figure 1.1 JECS 15 Regional Centres (from Kawamoto et. al. BMC Public Health 2014, 14:25)

1.5 JECS and the Gillberg Neuropsychiatry Centre

The Gillberg Neuropsychiatry Centre (GNC) has collaborated with the JECS team shortly after the launch of the JECS, and the PhD student herself also worked intensively in all the collaborations. First, the ESSENCE-Q, a one-page neurodevelopmental screening tool developed by Gillberg was incorporated in the 2.5-year JECS questionnaire with other sets of validated questionnaires, such as the Japanese translation of the Ages and Stages Questionnaire Third Edition (J-ASQ-3),[76] and the Japanese version of Parenting Stress Index (PSI),[77] and some other questions on family's daily life.

Gillberg, Thompson, and Fernell at the GNC in Gothenburg, Hatakenaka at the Kochi Gillberg Neuropsychiatry Centre (KGNC), and Suganuma at the Kochi Regional Centre (one of the 15 JECS Regional Centres) collaborated with the JECS Core Centre and the Medical Support Centre, both of which oversee the operation of the whole JECS. The 2.5-year questionnaire with the ESSENCE-Q was sent out to all the participants of the Main Study between 2014 and 2017. *Study IV* in this PhD project is the first large-scale validation study, utilising the JECS data.

Second, for the 2-year check-up by paediatricians for the SCS, approximately 5,000 participants (5% of the Main Study), the same group – the GNC, the KGNC, the Kochi Regional Centre have contributed to develop the neuromotor five-minute exam 2-year-old version (N5E2) to assess children's neurodevelopment.[78] The N5E2 consists of 11 items: (1) retrieving a rolling ball; (2) gait; (3) toe-walking; (4) asymmetries of posture and/or movement; (5) age at unsupported walking; (6) speaking in two-rode understandable sentences; (7) hypotonus; (8) hypertonus; (9) eye movement; (10) vision problem; and (11) hearing problem.[78] The Medical Support Centre made an instruction video to distribute to all the 15 Regional Centres while consulting with the GNC to enhance the consistency among the paediatricians throughout Japan. The pilot study of the N5E2, with the results also published, showed good agreement among 11 items and good inter-rater reliability for each of the 11 items as well as the total score.[78] The further analyses utilising the N5E2 in the JECS SCS are forthcoming in the near future.

Third, at the Kochi Regional Centre, three additional questionnaire surveys were conducted with participants in Kochi Prefecture: two studies on ARFID and one study utilising ESSENCE-Q and M-CHAT.[79] An initial validation study of a screening tool for ARFID was conducted to 3728 parents of 4-7-year-old children in Kochi prefecture who participated in the JECS, with the finding that point prevalence of children screening positive for ARFID 1.3%.[2] The second ARFID study, utilising the same data as the first one, found the overrepresentation of NDPs among ARFID screen positive children. In this second ARFID analysis, the results of the ESSENCE-Q and the J-ASQ-3 from the JECS Main Study were also incorporated to assess children's NDPs between 1-3 years of age, comparing to the later-conducted ARFID screening test, indicating the necessity of careful follow-up for those with NDPs for a possible ARFID risk later.[80] The questionnaire survey using ESSENCE-Q and M-CHAT were collected from 1178 children of 2 – 3 years of age, and the preliminary analysis has been conducted for the future publication.

2 AIMS

The overarching aim of this PhD project is to scrutinise pre-/perinatal factors and child neurodevelopment up until 3 years of age (*Figure 2.1*). The following aims will be addressed in the studies included in this work:

Study I: Examine the possible association between reduced optimality in the pre-/perinatal periods and possible early signs of neurodevelopmental problems at 1 month and NDD diagnosis at 3 years of age

Study II: Assess the possible association between birth month and child gross motor development at 6 and 12 months of age

Study III: Investigate if there is any association between vitamin D and identified neurodevelopmental problems at 2 years of age

Study IV: Evaluate the validity of the ESSENCE-Q, a new screening tool for common symptoms shared among children with NDDs, utilised when the cohort was 2.5 years of age

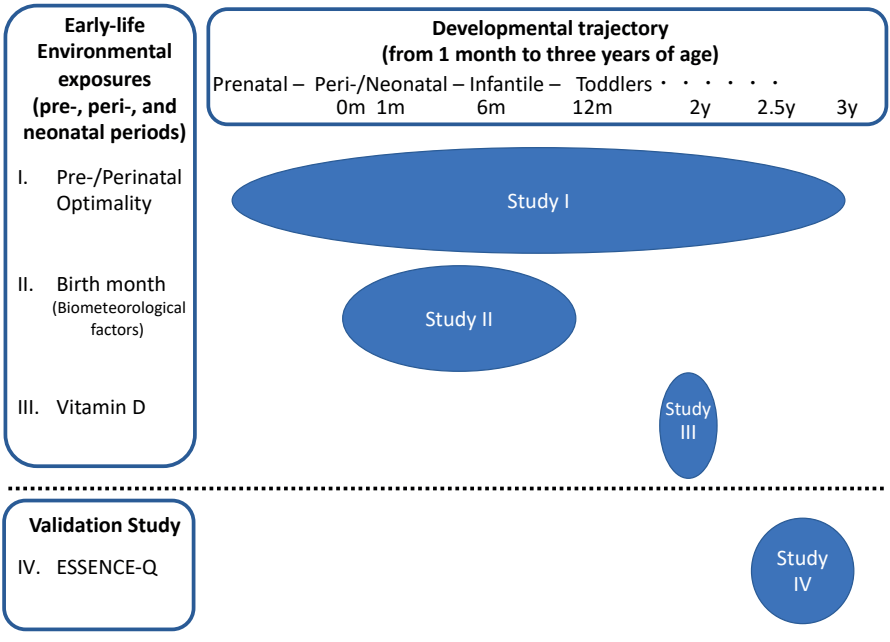

Figure 2.1 Overview of studies included in this PhD project

3 MATERIALS AND METHODS

The data for all four studies were part of the JECS, with variations regarding the number of participants and eligibility criteria, such as age, exposure, outcome, study design, in each analysis (*Table3.1*).

As the JECS is an ongoing study, the data are periodically cleaned, fixed, and made available for analysis as the children grow up. To date, the data up to age 4 years are available both for the Main and Sub Cohorts. For this PhD project, the data up until 3 years of age were analysed.

Table 3.1 Overview of studies and methods

Study	***I***	***II***	***III***	***IV***
Cohort	JECS Main Cohort		JECS Sub Cohort	JECS Main Cohort
Sample size	71,682 (Boys: 36,714 / Girls: 34,968)	72,203 (Boys: 36,784/Girls: 35,419)	4,653 (Boys: 2,363/Girls: 2,290)	77,612 (Boys: 39,690 / Girls: 37,922)
Research Question	Pre-/perinatal optimality and Neurodevelopment	Birth month and Gross motor development	Vitamin D and Neurodevelopment	ESSENCE-Q and NDDs
Study design	Prospective birth cohort study		Cross sectional study	Validation study
Exposure (*I,II,III*) Screening tools (*IV*)	Pre-/perinatal optimality (25 items)	Birth month	Vitamin D (serum 25OHD) (2 y)	ESSENCE-Q (2.5y) J-ASQ-3 (2.5y)
Outcome	Parental observation of child development (1m) NDD diagnosis (3y)	Gross motor development assessed with J-ASQ-3 (6m & 12m)	KSPD (2y)	NDD diagnosis (3y)

ESSENCE-Q: Early Symptomatic Syndromes Eliciting Neurodevelopmental Clinical Examinations Questionnaire; J-ASQ-3: Japanese translation of the Ages and Stages Questionnaire Third Edition; JECS: Japan Environment and Children's Study; KSPD: Kyoto Scale of Psychological Development; NDDs: Neurodevelopmental Disorders

3.1 Study Populations

The participants of all the four studies were the JECS participants: those of the JECS Main Study (*Study I, II, IV*) and the Sub-Cohort Study, which was 5% of the Main Study participants (*Study III*). For each study, different inclusion/exclusion criteria were applied respectively, depending on the exposure and outcome variables.

3.1.1 JECS Main Cohort (Study I, II, IV)

Between January 2011 and March 2014, 104,062 foetal records were registered throughout Japan, and 100,303 live births were recorded by December 2014 (jecs-ta-20190930 dataset released in October 2019 and supplementary dataset, ageof03_comparisontable001_ver003). Paternal participation was voluntary, and 50,170 fathers enrolled (the same dataset as above).

To ensure the generalizability, 15 Regional Centres were selected from the northern (Hokkaido) to southern (Okinawa) ends of Japan, covering all four major islands as well as prefectures with history of serious pollutions of mercury and cadmium – Kumamoto and Toyama. Soon after recruitment started, the Great East Japan Earthquake hit the country on March 11, 2011, which lead to the Fukushima Daiichi Nuclear Power Plant accident. Due to increased national concerns over the impact of radioactivity on children's health, the study area in Fukushima, which had been originally only 3 cities, was expanded to all the 59 municipalities across the whole prefecture.[81]

Participants were recruited at cooperating health care providers and/or local government offices where pregnant women register themselves, with a targeted coverage rate more than 50% in the study areas [74]. The inclusion criteria were as follows: *1*) residents of the study areas at the time of recruitment; *2*) due date between 1 August 2011 and mid-2014; and *3*) sufficient comprehension of the Japanese language [74]. The baseline characteristics of the JECS mother-child dyads were comparable with those obtained in the national survey in 2013 [82]. Detailed information on the JECS protocol and its representativeness can be found elsewhere. [74,82]

Study I included 71,682 children (36,714 boys and 34,968 girls) out of 100,303 live births, who had complete data on: (1) 25 suboptimal pre-/perinatal factors (exposure); (2) 1-month development (6 items, outcome 1); and (3) NDD diagnosis at age 3 (outcome 2).

Study II included 72,203 children (36,784 boys and 35.419 girls) meeting the eligibility criteria: full-term birth (gestational weeks ≥ 37) with a complete set of data on birth month, maternal vitamin D intake, and the J-ASQ-3 at 6 and 12 months of age.

Study IV included 77,612 children (39,690 boys and 37,922 girls), whose questionnaires were returned at age 2.5 and 3 years and with all 11 items of the ESSENCE-Q were answered.

3.1.2 JECS Sub Cohort (Study III)

Approximately 5% of the JECS Main Study participants in the Study Area of each Regional Centre were registered in the SCS. The eligibility criteria for the SCS were: 1) children were born after April 1, 2013; 2) complete data up to 6 months of age (at enrolment in the 1st trimester, once during 2nd or 3rd trimester, at 1 month and 6 months after birth); and (2) all the biospecimens except umbilical cord blood – maternal blood (twice during pregnancy and at birth), maternal urine (twice during pregnancy), hair (both for mothers and children), infant capillary blood sample, and breastmilk – were collected.[83]

Among those who met the inclusion criteria, 10,302 mothers were invited for the SCS with the acceptance number of 5,017 children (4,986 pregnancies).[83] The profiles of the SCS participants – mothers, fathers, and children, were not substantially different from those of the Main Study.[83]

Study III included 4,653 children (2,363 boys and 2,290 girls) out of the 5,017 SCS participants, with the information on both serum 25(OH)D concentration levels and the KSPD DQ scores at age 2 years.

3.2 Measures by study

3.2.1 Outcome Measurements

Parental observation of child development (*Study I*, at age 1 month)

Based on previous findings that individuals with NDDs frequently show coexisting pathological/atypical signs in communication, motor function, and sensory reactions,[6] six questions on child development on gross motor function, vision, hearing, crying, and reactions when being held were extracted from the parent-completed questionnaire 1 month after their children were born (*Table 3.2*).[52,84] As no validated questionnaire was included in the JECS Main Study questionnaire at 1 month of age, an experienced child psychiatrist (Gillberg) and child neurologist (Fernell) finalised these six questions and dichotomised the responses to them to create a developmental scale. The higher score indicates more concerns with the highest possible score being 6. The 1-year developmental scale scores were one of the two outcome measurements.

Table 3.2 Parental observation scale at 1 month of age

	Questions in 1-month JECS questionnaire	Interpretation	Answer & Score (Typical: 0 / Concern: 1)
1	Ability of baby to move his/her right and left limbs equally well	"Gross motor function"	yes = 0; no/uncertain = 1
2	Reaction of baby to sound (e.g. parent's voice)	"Hearing"	yes = 0; no/uncertain = 1
3	Apparent ability of baby to see things	"Vision"	yes = 0; no/uncertain = 1
4	Frequency of difficulty holding the baby because of issues with his/her attachment or behaviour, or both (e.g. crying, bending backwards)	"Difficulty holding"	sometimes/seldom/ never = 0; often = 1
5	Intensity and frequency of crying	"Intense/frequent crying"	sometimes but short = 0; quite often and long/hardly ever = 1
6	Trouble calming the crying baby	"Trouble calming"	no = 0; yes = 1

Diagnosis of Neurodevelopmental disorders (*Study I* & *IV*, at age 3 years)

NDD diagnosis information was collected from a single question in the 3-year questionnaire to the parents: "Has your child been diagnosed by doctors since turning 2 years with any of the following conditions? Please include the earlier diagnoses being followed up to date." The NDDs listed along with other diseases such as cancer and allergies were motor delay (MD), intellectual disability and/or developmental language disorder (ID/DLD), and autism spectrum disorder (ASD). Parent-reported NDD diagnosis at age 3 years was utilised as the second outcome in *Study I*, and for the validation of a screening tool, ESSENCE-Q, in *Study IV*.

Japanese Translation of the Ages and Stages Questionnaire Third Edition (J-ASQ-3, *Study II* and *IV*)

In the JECS main study, J-ASQ-3, a recently-validated screening tool in Japanese but widely-used in English (ASQ-3),[76] was included in the biannual questionnaire from 6 months until 5 years of age. The J-ASQ-3 assesses development in 5 domains: communication, gross motor, fine motor, problem

solving, and personal-social. Each domain consists of 6 items and 3 responses are available for each item (scores in the brackets): Yes (10), Sometimes (5), and Not yet (0). It is possible to score between 0 and 60 in each domain, with higher scores indicating more healthy development.

For *Study II*, the association between gross motor skills at 6 and 12 months, measured by J-ASQ-3, and birth month was assessed. As the J-ASQ-3 was still in the process of validation, gross motor scores were dichotomised, following the cut-off values for the validated English version: 22.25 and 21.49 at 6 and 12 months respectively.[85]

In *Study IV*, the 5 domains of the J-ASQ-3 at age 2.5 years and the 3-year NDD diagnosis were utilised to assess the validity of the ESSENCE-Q, completed by the parents/caregivers when the children were 2.5 years of age. Unlike *Study II*, the J-ASQ-3 scores were observed as they were as a continuous variable. We compared mean scores of each of the 5 J-ASQ-3 domains between those responding Yes/No to each of the 11 ESSENCE-Q items.

Kyoto Scale of Psychological Development 2001 (*Study III*, at age 2 years)

The JECS Sub-Cohort participants, 5% of the Main Study Cohort, participated in thorough follow-up examination, including face-to-face check-ups and biospecimen collection every other year since 2 years of age. At age 2, the Kyoto Scale of Psychological Development (KSPD), the most common standardised developmental measurement in clinical practice and a widely-used tool in research in Japan, was conducted by JECS-trained testers, including nurses and psychologists among the JECS Group.[86-89] The KSPD consists of 3 subdomains: Postural-Motor (P-M), Cognitive-Adaptive (C-A), and Language-Social (L-S).[89] The developmental quotient (DQ) was calculated by dividing developmental age by chronological age, then multiplying by 100. Overall DQ as well as DQ for each of the 3 domains were calculated.[86] The KSPD DQs were treated both as continuous variables (the DQs as they were) and as categorical variables after having dichotomised the DQ scores at the cut-off value of < 70, the value most commonly-used in Japan to determine public service eligibility.[86]

ESSENCE-Q (*Study IV*, at age 3 years)

ESSENCE-Q, a simple one-page screening questionnaire now consisting of 11 items, was developed by Gillberg in 2010 - 2012, on the basis of an overarching umbrella concept of ESSENCE (Early Symptomatic Syndromes Eliciting Neurodevelopmental Clinical Examinations) explained in the earlier section (1.2).

The Japanese version of ESSENCE-Q was created through a collaboration between Christopher Gillberg, Yuhei Hatakenaka, an experienced Japanese child psychiatrist, and Loren Waller, a native English scholar with excellent knowledge of the Japanese language and culture.[57]

The ESSENCE-Q was included in the JECS questionnaire survey when the participating children were 2.5 years of age. It consisted of 11 items, the same as the latest version of the ESSENCE-Q, but with one less item (i.e. absences/"funny spells") in the Japanese version: (1) general development; (2) motor development; (3) sensory reactions; (4) communication/language/babble; (5) activity (overactivity/passivity) or impulsivity; (6) attention/concentration/ "listening"; (7) social interaction with/interest in other children; (8) behaviour (e.g., repetitive, routine insistence); (9) mood (depressed, elated/manic, extreme irritability, crying spells); (10) sleep; (11) feeding. The responses in the JECS version were modified from the original three options (Yes, Maybe/A little, and No) to two (Yes/No). According to a researcher in the committee in charge of developing the JECS questionnaires, the rationale behind the modifications were: (1) the committee expressed concern that the item on absences/"funny spells" might not be comprehended adequately by the participants without a more explicit explanation; (2) there was also apprehension that a majority of participants would choose maybe/a little, in line with prevalent cultural norms and communication style which incline to be subtle, ambiguous, and nuanced. In *Study IV*, each item was scored on a 0,1 scale, and total score range was 0-11, with higher scores indicating more concerns.

3.2.2 Exposure Measurements

3.2.2.1 Pre-/Perinatal optimality Scale (Study I)

To investigate the additive non-optimal effects on child neurodevelopment during pre-/perinatal periods, the "reduced optimality" concept was applied. The concept was introduced by Prechtl in 1968, and has been used by many researchers studying a range of outcomes.[90] On the basis of the reduced optimality concept, while accommodating the items available in the JECS data, we created the suboptimality scale consisted of 25 items (*Table 3.3*).

In addition to the suboptimality scale total scores, *Study I* also investigated the effect of each item of the scale after separating maternal epilepsy, diabetes, and thyroid disorders under the "Maternal disorder" item.

3.2.2.2 Birth month (Study II)

The information on birth month was collected from the medical record transcripts at medical institutes. After confirming no significant differences between the 4 birth years, 2011–2014, regarding outcome (J-ASQ-3 gross motor scores), general characteristics of the participants, and meteorological agency information, the data were collapsed across birth years.

3.2.2.3 Serum 25-hydroxyvitamin D (Study III)

Among the Sub-Cohort of the JECS, the serum 25(OH)D2 and 25(OH)D3 concentrations of 4,655 children were measured when the children were 2 years of age by using liquid chromatography tandem mass spectrometry (LC-MS/MS), calibrated with 6PLUS1 Multilevel Serum Calibrator Set 25-OH-Vitamin D3/D2.[91] Values for 25(OH)D3 and 25(OH)D2 concentrations below 4 ng/mL were truncated as 4 ng/mL. The total serum 25(OH)D level was defined as the sum of the 25(OH)D3 and 25(OH)D2 concentrations. The values were treated as it is – continuous – and categorical variables: "deficiency" ($<$ 20 ng/mL); "insufficiency" ($\geq$ 20 ng/mL and $<$ 30 ng/mL); and "sufficiency" ($\geq$ 30 ng/mL). These cut-offs were decided following the previous international studies and most-commonly used cut-offs.[91,92]

Table3.3 Reduced optimality scale index: pre-/perinatal conditions and their optimal values.

Pre-/perinatal factor	Optimal value
1. Maternal age	20–35
2. Parity	1 or 2
3. History of spontaneous abortion	0–2
4. Assisted reproductive technologies (ARTs)[a]	No
5. Threatened abortion/premature labour	Absent
6. Antibiotic intake during pregnancy	Absent
7. Pregnancy-induced hypertension (PIH) and hypertension	Absent
8. Psychiatric specialist care	Absent
9. Maternal disorders[b]	Absent
10. Neuropsychotropic medication use	Absent
11. Gestational age (weeks)	37–40
12. Intrauterine growth restriction (IUGR)	No
13. Small for gestational age (SGA)	No
14. Twin or multiple birth	No
15. Breech, foot, or other abnormal presentation	No
16. Vacuum/forceps extraction	No
17. Induced delivery	No
18. Caesarean section delivery	No
19. Epidural analgesia	No
20. Length of labour (h)	0–24
21. Apgar score (5 min)	9 or 10
22. Umbilical cord/placental problems	No
23. Meconium staining	No
24. Neonatal transportation	No
25. Hyperbilirubinemia	Absent

[a]ARTs include ovulation induction, artificial insemination with the husband's sperm, in vitro fertilization, intracytoplasmic sperm injection, fresh embryo transfer, frozen embryo transfer, and blastocyst transfer.
[b]Maternal disorders include diabetes/gestational diabetes, epilepsy, and hyper- or hypothyroidism.

3.3 Analytical and statistical methods

Data analyses were performed in Stata/MP versions 15.0 for *Study II*, 16.0 for *Study I*, and 17.0 for *Studies III* and *IV* (StataCorp LLC. College Station, TX, USA). The statistical significance level was set to $p < 0.05$ in all studies.

3.3.1 Study I

Binomial regression with a log-link function was used to assess the associations between reduced optimality scores and developmental concerns at 1 month/NDDs at 3 years.

Relative risks (RRs) were calculated for 27 individual pre-/perinatal factors by using the log-binomial model, after the "Maternal disorders" in the scale had been broken down to 3 diseases: epilepsy, diabetes, and thyroid disease. Household income and maternal education were added to the final model as socioeconomic status to calculate adjusted RRs (aRRs). No multicollinearity among explanatory variables was found in the model. As 4,947 participants (7.0%) were not included in the final model due to the missed values (socioeconomic status), multiple imputation by chained equations was conducted to confirm that the results of the final model were reliable.

Finally, we scrutinised whether the parental observation scores for child 1-month development predicted NDDs at age 3 years by conducting receiver operating characteristic (ROC) curve analysis and calculating the area under the curve (AUC). By using binomial regression with a log-link function, the RRs of each of the 6 developmental concern items at 1 month for 3-year NDDs were calculated.

3.3.2 Study II

Gross motor development scores measured by J-ASQ-3 were dichotomised at the cut-off values of 22.25 and 21.49 at 6 and 12 months respectively. Modified Poisson regression analysis with a robust variance estimator was utilised to investigate the association between birth month and gross motor development (pass or fail at the cut-off) at age 6 and 12 months (with January as the reference).[93-95] Covariates included in the final model to calculate adjusted Relative Risk (aRR) had been selected based on whether bivariate testing (chi-squared or two-tailed independent samples t-tests) showed any significant association with gross motor development at either time point (Table S1 of *Study II*). Adjusts RRs were also calculated separately for boys and girls, after stratifying the participants by their sex.

Multiple imputation by chained equation was conducted as 23,398 participants (32.5%) were not included in the final adjusted model. The results were unchanged in the imputed results.

Finally, we divided the participants into four groups by the outcome results at 6 and 12 months as follows: BEST (passed at both age 6 and 12 months), IMPROVED (failed at 6 months but passed at 12 months), WORSENED (passed at 6 months but failed at 12 months), and WORST (failed at both time points).

3.3.3 Study III

All the analyses were stratified by sex, since both serum vitamin D concentration levels (exposure) and the KSPD DQs (outcome) were significantly different, with boys' vitamin D levels higher and girls' KSPD DQs higher. To investigate the association, both 25(OH)D concentrations and the KSPD DQs were treated as continuous and categorical variables with the four patterns of combination: (1) continuous – continuous, (2) continuous – categorical, (3) categorical – continuous, and (4) categorical – categorical. Statistical analyses conducted were shown as *Table 3.4*.

Table 3.4. Statistical analyses for study III

		KSPD DQ (P-M, C-A, L-S, Overall)	
		Continuous	Categorical (cut-off <70)
25(OH)D2 + 25(OH)D3	Continuous (ng/mL)	Spearman correlation	Wilcoxon Kolmogorov-Smirnov independent sample t-test
	Categorical Deficient (< 20ng/mL) Insufficient (≥ 20 & < 30ng/mL) Sufficient (>30ng/mL)	Kruskal-Wallis	Logistic regression

After interaction and multicollinearity (variance inflation factor 1.14) had been excluded, test month (month when the 2-year-old check-up was conducted), latitude, and major known medical risk factors for neurodevelopmental delay – small for gestational age (SGA) and maternal age – as well as a common social factor – daycare attendance at the age 2 – were added in the final analysis to calculate adjusted Odds Ratio (aOR). Maternal education was not included, since it showed no association with the KSPD DQ scores in the bivariate analysis.

3.3.4 Study IV

Each of the 11 ESSENCE-Q items was rated as 0 (“no”) or 1 (“yes”), with the total score ranging from 0 to 11 and higher scores indicating more neurodevelopmental concerns/problems.

First, the total ESSENCE-Q scores at 2.5 years of age were treated as they were, i.e., as a continuous variable. A two-sample *t*-test was conducted to compare ESSENCE-Q scores between NDD and non-NDD groups at age 3 years. To investigate the association between ESSENCE-Q scores and number of NDD diagnoses/comorbidities, the Kruskal–Wallis test was performed.

Second, to assess the validity of the ESSENCE-Q, ROC curve analysis was conducted, and the AUC for NDDs and each of the 3 diagnostic groups (MD, ID/DLD, and ASD) were calculated. An optimal cut off was decided, after the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), Youden index (sensitivity + specificity – 1), and likelihood ratio (LR) of ESSENCE-Q scores were evaluated.

Third, the parental response to each of the 11 questions and its association with NDD diagnoses was assessed by using a chi-squared test.

Finally, the correlation between ESSENCE-Q and J-ASQ-3, a standardised questionnaire internationally and domestically, was assessed with Spearman’s correlation. A two-sample *t*-test was used to compare the mean total score for each of the five J-ASQ-3 domains with each ESSENCE-Q question (yes or no regarding parental concern).

3.4 Ethical Considerations

The JECS protocol was reviewed and approved by the institutional review board (IRB) on Epidemiological Studies of the Ministry of the Environment (approved no. 100910001) and all the collaborating institutions. Written informed consent was obtained from all the JECS participants at the recruitment of the JECS Main Study and Sub-Cohort Study.

4 RESULTS

4.1 Study I

4.1.1 NDD diagnosis at age 3

Among 71,682 participants (36,714 boys and 34,968 girls) who had met the eligibility criteria (*Figure 4.1.1*), 750 children (1.05%) had at least 1 NDD diagnosis of either motor delay (MD), intellectual disability and/or developmental language delay (ID/DLD), and autism spectrum disorder (ASD) (*Table 4.1.1*) More boys (n = 542) than girls (n = 206) received NDD diagnoses. Most common NDDs were ID/DLD (*n* = 487), which was observed in all the multiple diagnoses, followed by ASD (n = 329) and MD (n = 172). The majority of children were diagnosed with 1 NDD (n = 530), followed by 2 NDD diagnoses (n = 202), and 3 NDD diagnoses (n =18). (*Table 4.1.1*).

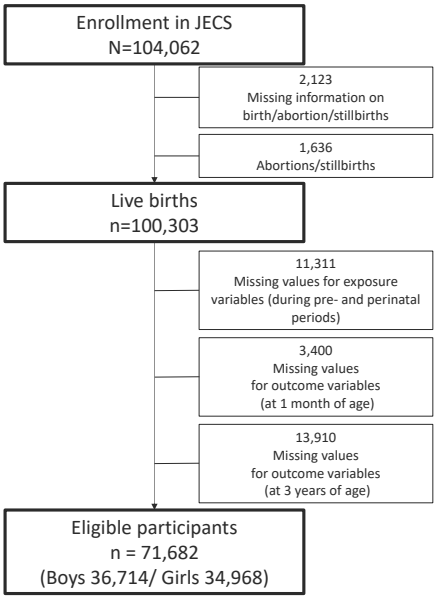

Figure 4.1.1 Flow chart showing the enrolment of eligible participants

Table 4.1.1 Prevalence of NDDs

NDD Diagnosis	Number of diagnosis 1	2	3	Number of Each diagnosis	%
Motor Delay	32			172	0.24%
ID and/or DLD	267			487	0.68%
ASDs	231			329	0.46%
Motor delay + ID and/or DLD		122			
Motor delay + ASDs		0			
ID and/or DLD + ASDs		80			
Motor delay + ID and/or DLD + ASDs			18		
Number of children with any NDDs	530	202	18	750	1.05%

4.1.2 Reduced optimality scale scores and developmental concerns at 1 month/NDDs at 3 years of age

Those with NDDs at age 3 years were significantly more likely to endorse one or more of 16 reduced optimality scale items than those without NDDs (*Table 4.1.2*). The reduced optimality scale scores were skewed to the right, with a median of 2. At age 1 month, mean reduced optimality scale scores among the "concern" group were significantly higher than that of the "typical" group (3.11 vs. 2.55, respectively; $p < 0.001$). Likewise, at age 3, mean reduced optimality scale scores among children with NDDs were significantly higher than that of children without NDDs (3.48 vs. 2.65, respectively; $p < 0.001$) (*Table 4.1.3*).

Table 4.1.2 Prevalence of pre-/perinatal factors among participants with/without NDD at age 3

Pre- and Perinatal Factors	Optimal	Total n = 71,682	NDD diagnosis n = 750	(%) (1.05%)	No NDD diagnosis n = 70,932	(%) (98.95%)	*p*-value
1. Maternal age	20-35	16,284	239	31.87	16,045	22.62	**<0.001**
2. Parity	1-2	32,604	382	50.93	32,222	45.43	**0.003**
3. Spontaneous abortion in history	0-2	613	8	1.06	605	0.85	0.527
4. ARTs	No	5,259	80	10.67	5,179	7.30	**<0.001**
5. Threatened abortion/premature labour	Absent	19,453	232	30.93	19,221	27.10	**0.019**
6. Antibiotic during pregnancy	Absent	15,829	169	22.53	15,660	22.08	0.765
7. PIH and hypertension	Absent	2,555	42	5.59	2,513	3.54	**0.003**
8. Psychiatric problems	No	538	12	1.60	526	0.74	**0.007**
9-1. Maternal diabetes /GDM	Absent	2,313	28	3.72	2,285	3.22	0.430
9-2. Maternal epilepsy	Absent	178	3	0.40	175	0.25	0.401
9-3. Maternal thyroidism (hyper-/ hypo-)	Absent	1,020	18	2.39	1,002	1.41	**0.023**
10. Neuropsychotropic drugs	No	781	14	1.86	767	1.08	**0.039**
11. Gestational week	37-40	10,065	156	20.74	9,909	13.97	**<0.001**
12. SGA	No	8,678	153	20.35	8,525	12.02	**<0.001**
13. Twins or multiple birth	No	1,221	19	2.53	1,202	1.69	0.077
14. Abnormal foetal presentations	No	2,881	40	5.33	2,841	4.01	0.065
15. Vacuum/forceps extraction	No	4,228	48	6.38	4,180	5.89	0.558
16. Induced delivery	No	12,500	140	18.67	12,360	17.44	0.373
17. Caesarean section delivery	No	13,608	207	27.60	13,401	18.89	**<0.001**
18. Epidural anaesthesia	No	1,530	24	3.19	1,506	2.12	**0.042**
19. Labour > 24h	<24	2,337	22	2.93	2,315	3.26	0.612
20. Apgar score (5 minutes)	9-10	3,727	82	10.93	3,645	5.14	**<0.001**
21. Umbilical cord/placenta problems	No	16,924	175	23.33	16,749	23.61	0.858
22. Meconium staining	No	2,447	34	4.53	2,413	3.40	0.090
23. Neonatal transportation	No	4,064	124	16.53	3,940	5.55	**<0.001**
24. Hyperbilirubinemia	Absent	7,899	133	17.73	7,766	10.95	**<0.001**
25. IUGR	No	1,428	32	4.27	1,396	1.97	**<0.001**

Table 4.1.3 Reduced optimality scale scores and outcomes at age 1 month and 3 years

Suboptimality Scale Scores	no. of participants	At 1 month of age[a]				At 3 years of age			
		"Typical"	%	"Concerns'	%	No NDD diagnosis	%	NDD diagnosis	%
0	6,930	6,105	88.10	825	11.90	6,897	99.52	33	0.48
1	14,773	12,449	84.27	2,324	15.73	14,662	99.25	111	0.75
2	16,340	13,129	80.35	3,211	19.65	16,205	99.17	135	0.83
3	13,505	10,396	76.98	3,109	23.02	13,356	98.90	149	1.10
4	9,054	6,835	75.49	2,219	24.51	8,940	98.74	114	1.26
5	5,329	3,841	72.08	1,488	27.92	5,247	98.46	82	1.54
6	2,857	2,056	71.96	801	28.04	2,802	98.07	55	1.93
7	1,435	1,014	70.66	421	29.34	1,409	98.19	26	1.81
≥8	1,459	988	67.72	471	32.28	1,414	96.92	45	3.08
Total	71,682	56,813	79.26	14,869	20.74	70,932	98.95	750	1.05
Mean[b]		3.11		2.55		3.48		2.65	

With the reduced optimality scale score 0 as reference, Risk ratios (RRs) of the reduced optimality scale scores increased in a dose-dependent manner for both 1-month and 3-year outcomes (*Table 4.1.4*).

Table 4.1.4 RR of suboptimality scale scores for 1-month/3-year outcomes

Suboptimality Scale Scores	Outcome at 1 month of age				NDD diagnosis at 3 years of age			
	RR	*p* value	95%CI		RR	*p* value	95%CI	
0	Reference				Reference			
1	1.32	**<0.001**	1.23	1.42	1.58	**0.021**	1.07	2.32
2	1.65	**<0.001**	1.54	1.77	1.74	**0.004**	1.19	2.54
3	1.93	**<0.001**	1.80	2.08	2.32	**<0.001**	1.59	3.37
4	2.06	**<0.001**	1.91	2.22	2.64	**<0.001**	1.80	3.89
5	2.35	**<0.001**	2.17	2.53	3.23	**<0.001**	2.16	4.83
6	2.36	**<0.001**	2.16	2.57	4.04	**<0.001**	2.63	6.21
7	2.46	**<0.001**	2.22	2.73	3.80	**<0.001**	2.28	6.34
8≥	2.71	**<0.001**	2.46	2.99	6.48	**<0.001**	4.15	10.11

4.1.3 Each item of the suboptimality scale and outcomes at 3 years

Among all the items of the suboptimality scale, 6 items showed statistically significant associations with 3-year NDD diagnosis – neonatal transportation, epidural analgesia, young/advanced maternal age, caesarean section delivery, Apgar score ≤ 8, and hyperbilirubinemia, listed from aRR highest to lowest (*Table 4.1.5*). All these 6 items except Apgar score had been among 14 significant risk factors for 1-month outcome (*Table 4.1.5*).

When the aRRs of the suboptimality scale items for each of the 3 NDDs (MD, ID/DLD, and ASD) were also examined in addition to the aRRs of all NDDs combined, some differences were found depending on the NDD diagnosis. Young/advanced maternal age and Caesarean section delivery were risk factors for all the three NDD diagnoses, and aRRs of total NDDs were the third and fourth highest among the list of risk factors. Epidural analgesia and nulliparity/high parity were significant risks only for ASD. Neonatal transportation, SGA, and Apgar score ≤ 8 were risks for MD and ID and/or DLD. Maternal hyper-/hypothyroidism was a risk factor only for MD. Hyperbilirubinemia was a risk factor only when all the NDDs were combined (*Table 4.1.5*).

As a sub-analysis, maternal age was recategorized into 3 groups: < 20, ≥ 20 to ≤ 35, and > 35 years, since the optimal maternal age range was defined as 20 to 35 years inclusive (n = 55,398; 77.28%) and the non-optimal maternal age included both older (n = 403; 0.56%) and younger (n = 15,881; 22.5%) than the optimal range. Only the aRR of the oldest group showed a significant association (aRR 1.50; 95% CI 1.27–1.77; $p < 0.001$). Likewise, gestational age at birth was also recategorized into 3 groups: < 37 weeks (pre-term), ≥ 37 to < 41 weeks (term), and ≥ 41 weeks (post-term), since non-optimal gestational age included both pre-term (< 37 weeks, n = 3,448) and post-term (≥ 41 weeks n = 6,577). Adjusted RRs of the pre- and post-term groups in the final analysis showed no statistically significant association (pre-term: aRR 0.81, 95%CI 0.56–1.17, $p = 0.259$; post-term: aRR 1.21, 95%CI 0.95–1.56, $p = 0.113$).

Table 4.1.5 Adjusted relative risk of each pre-/perinatal factor for NDDs at age 3

	Outcome at age 1 month (n = 14,869)			Any NDD diagnosis at age 3 (n = 750)			MD (n = 172)			ID/DLD (n = 487)			ASD (n = 329)		
	aRR	*p* value	95% CI	aRR	*p* value	95% CI	aRR	*p* value	95% CI	aRR	*p* value	95% CI	aRR	*p* value	95% CI
1. Young/advanced maternal age	1.08	**<0.001**	1.05 1.12	1.48	**<0.001**	1.26 1.75	1.46	**0.028**	1.04 2.04	1.53	**<0.001**	1.25 1.87	1.33	**0.029**	1.03 1.72
2. Nulliparity/high parity	2.08	**<0.001**	2.02 2.15	1.16	0.064	0.99 1.35	1.08	0.627	0.78 1.50	0.96	0.718	0.79 1.17	1.51	**0.001**	1.19 1.92
3. History of spontaneous abortion	0.94	0.473	0.80 1.11	1.10	0.782	0.55 2.21	1.58	0.434	0.50 4.92	0.60	0.384	0.19 1.88	0.70	0.619	0.17 2.82
4. ARTs	1.25	**<0.001**	1.19 1.31	1.13	0.357	0.88 1.45	1.09	0.745	0.66 1.77	1.18	0.287	0.87 1.61	1.03	0.870	0.69 1.54
5. Threatened abortion/premature Labour	1.04	**0.032**	1.00 1.07	1.09	0.321	0.92 1.28	1.14	0.448	0.81 1.59	1.13	0.222	0.93 1.39	0.93	0.587	0.72 1.21
6. Antibiotic during pregnancy	0.98	0.176	0.94 1.01	0.98	0.855	0.82 1.18	1.13	0.512	0.79 1.60	1.05	0.645	0.85 1.31	0.93	0.586	0.70 1.22
7. PIH and hypertension	0.97	0.420	0.90 1.04	1.00	0.984	0.72 1.40	0.71	0.332	0.36 1.42	1.22	0.318	0.83 1.78	0.89	0.673	0.50 1.56
8. Psychiatric problems	1.07	0.372	0.92 1.24	1.76	0.085	0.92 3.36	1.05	0.940	0.28 3.92	1.84	0.114	0.86 3.93	1.41	0.547	0.46 4.27
9-1. Diabetes /GDM	0.97	0.489	0.90 1.05	0.87	0.495	0.58 1.30	0.59	0.252	0.24 1.45	0.82	0.440	0.50 1.35	1.56	0.078	0.95 2.55
9-2. Epilepsy	1.36	**0.004**	1.10 1.68	1.30	0.671	0.39 4.31	0.87	0.900	0.11 7.21	1.70	0.401	0.49 5.82	1.07	0.951	0.13 8.56
9-3. Thyroidism (hyper- & hypo-)	1.00	0.979	0.89 1.12	1.34	0.241	0.82 2.20	2.15	**0.046**	1.01 4.57	1.28	0.442	0.68 2.38	1.05	0.914	0.43 2.54
10. Neuropsychotropic drugs	1.27	**<0.001**	1.11 1.44	1.18	0.602	0.63 2.24	2.39	0.089	0.88 6.51	1.46	0.299	0.72 2.98	1.14	0.803	0.40 3.25
11. Gestational age	0.96	0.049	0.92 1.00	1.08	0.504	0.87 1.34	0.84	0.439	0.54 1.31	0.95	0.695	0.72 1.25	1.11	0.552	0.79 1.55
12. IUGR	1.08	0.125	0.98 1.18	1.33	0.165	0.89 1.98	1.82	0.055	0.99 3.36	1.41	0.138	0.89 2.24	0.71	0.454	0.29 1.75
13. SGA	1.08	**0.002**	1.03 1.13	1.17	0.198	0.92 1.48	1.76	**0.014**	1.12 2.75	1.46	**0.008**	1.10 1.94	0.97	0.888	0.66 1.44
14. Twins or multiple birth	1.11	**0.039**	1.01 1.22	0.73	0.214	0.44 1.20	0.36	**0.052**	0.13 1.01	0.66	0.187	0.36 1.22	0.84	0.685	0.36 1.97
15. Abnormal foetal presentations	0.99	0.703	0.92 1.06	0.82	0.290	0.57 1.18	0.67	0.269	0.33 1.36	0.82	0.377	0.52 1.28	0.86	0.595	0.49 1.50
16. Vacuum/forceps extraction	1.11	**<0.001**	1.05 1.17	1.19	0.290	0.86 1.63	1.65	0.120	0.88 3.10	1.12	0.590	0.74 1.68	1.09	0.732	0.67 1.75
17. Induced delivery	1.02	0.316	0.98 1.06	1.19	0.103	0.97 1.47	1.37	0.169	0.87 2.16	1.07	0.626	0.82 1.40	1.15	0.369	0.84 1.58
18. Caesarean section delivery	1.09	**<0.001**	1.04 1.14	1.41	**0.001**	1.16 1.73	1.69	**0.010**	1.14 2.53	1.35	**0.018**	1.05 1.72	1.56	**0.004**	1.15 2.11
19. Epidural analgesia	1.21	**<0.001**	1.11 1.32	1.55	**0.037**	1.03 2.33	1.66	0.230	0.73 3.78	1.33	0.320	0.76 2.31	1.99	**0.013**	1.15 3.42
20. Labour > 24h	1.06	0.114	0.99 1.13	0.89	0.605	0.57 1.38	0.57	0.344	0.18 1.82	0.89	0.683	0.49 1.59	0.95	0.857	0.51 1.75
21. Apgar Score (5 minutes)	1.00	0.938	0.94 1.06	1.38	**0.014**	1.07 1.79	1.86	**0.004**	1.22 2.85	1.46	**0.016**	1.07 1.99	1.06	0.823	0.66 1.69
22. Umbilical cord/placenta problems	0.99	0.573	0.96 1.02	0.91	0.279	0.76 1.08	0.86	0.394	0.60 1.22	0.83	0.108	0.67 1.04	0.98	0.874	0.75 1.28
23. Meconium Staining	1.11	**0.003**	1.04 1.19	1.21	0.288	0.85 1.74	1.62	0.132	0.86 3.02	1.38	0.149	0.89 2.13	1.13	0.674	0.64 1.98
24. Neonatal transportation	1.07	**0.028**	1.01 1.14	2.30	**<0.001**	1.81 2.92	6.32	**<0.001**	4.25 9.40	2.39	**0.000**	1.79 3.20	1.19	0.454	0.75 1.88
25. Hyperbilirubinemia	1.05	**0.037**	1.00 1.09	1.24	**0.040**	1.01 1.53	0.95	0.809	0.63 1.44	1.26	0.078	0.97 1.63	1.31	0.104	0.95 1.81

Adjusted for family income and maternal education

4.1.4 Association between developmental concerns at 1 month and NDDs at 3 years of age

Parental observation scale scores at age 1 month showed the association with 3-year NDDs, with a RR of 1.56 (95% CI 1.33–1.82; $p < 0.001$). However, the AUC was 0.5593, indicating that the scale scores may not predict NDDs at 3 years of age.

When the associations between each item of the 1-month parental observation scale (i.e., gross motor function, hearing, vision, difficulty holding, intense/frequent crying, and trouble calming) and each NDD at 3 years of age were examined, some differences were identified in MD and ASD. Children with MD had already been observed to have gross motor function problems at age 1 month (RR2.43; 95% CI 1.52–3.86; $p < 0.001$) (*Table 4.1.6*). All the 6 items except gross motor function showed significant associations with ASD, with the "difficulty holding" showing the highest RR (2.08; 95% CI 1.48–2.91; $p < 0.001$), followed by "trouble calming," "hearing," "intense/frequent crying," and "vision" (*Table 4.1.6*).

Table 4.1.6 Relative risk (RR) of each of the 6 items at age 1 month for NDDs at age 3

	Any NDD diagnosis				MD				ID/DLD				ASD			
	RR	*p* value	95%CI		RR	*p* value	95%CI		RR	*p* value	95%CI		RR	*p* value	95%CI	
1. Gross motor function	1.63	**<0.001**	1.26	2.12	2.43	**<0.001**	1.52	3.86	1.69	**0.001**	1.23	2.33	1.13	0.604	0.71	1.79
2. Hearing	1.76	**<0.001**	1.43	2.17	1.95	**0.002**	1.28	2.97	1.80	**<0.001**	1.39	2.32	1.72	**0.001**	1.25	2.37
3. Vision	1.29	**0.002**	1.10	1.52	1.40	0.050	1.00	1.96	1.28	**0.017**	1.05	1.57	1.38	**0.010**	1.08	1.76
4. Difficulty in holding	1.48	**0.001**	1.15	1.91	0.98	0.956	0.52	1.86	1.16	0.418	0.81	1.64	2.08	**<0.001**	1.48	2.91
5. Intence/frequent crying	1.42	**<0.001**	1.21	1.67	1.59	**0.005**	1.15	2.21	1.35	**0.003**	1.11	1.65	1.45	**0.002**	1.14	1.84
6. Trouble calming down	1.36	**<0.001**	1.15	1.60	1.48	**0.025**	1.05	2.08	1.03	0.812	0.82	1.29	1.77	**<0.001**	1.40	2.24
Tptal coutcome scores	1.56	**<0.001**	1.33	1.82	1.89	**<0.001**	1.38	2.60	1.42	**0.001**	1.16	1.73	1.62	**<0.001**	1.28	2.05

4.2 Study II

Among 72,203 children (36,784 boys and 35,419 girls), 14,960 (20.7%) and 10,260 (14.2%) infants scored below the cut-off for the J-ASQ-3 gross motor domain at 6 and 12 months of age respectively (*Figure 4.2.1*). At age 6 months, August- and September-born babies showed the highest percentages (32.0% and 31.1%, respectively) below the cut-off in gross motor development, while February- and March-born babies showed the lowest percentages (9.6% each) (*Table 4.2.1*). At 12 months, the peak shifted to earlier months of the year, to the June- and July-born (18.4% each), as did the trough to the December-, January-, and February-born (11.6%, 10.6%, and 11.6%).

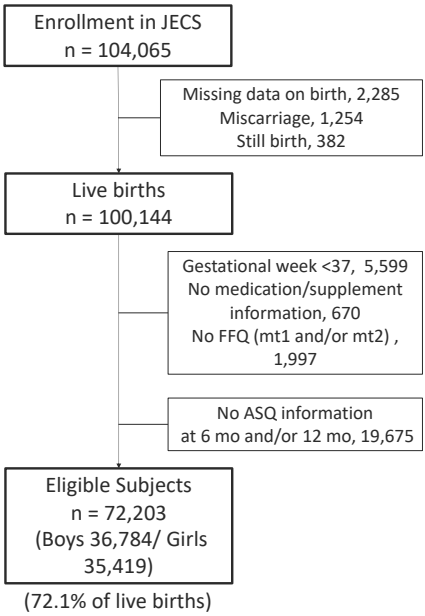

Figure 4.2.1 Flow chart showing the enrolment of eligible participants

Both crude and adjusted relative risks (RR, aRR) of birth month for gross motor development at age 6 and 12 months showed a cyclical trend of being highest in summer (August/September-born) and lowest in winter (February/March-born) at 6-month motor development (January as reference). Adjusted RR for gross motor development at age 12 month showed a similar trend, with the peak and the trough shifted to earlier months (June/July-born). The discrepancy of the aRR peaks and troughs became narrower at age 12 months (*Table 4.2.2*).

At age 6 months, the aRRs for the February-, March-, and April-born were less than 1 and $p < 0.05$, indicating that these birth months were "protective" for gross motor development at 6 months with January as reference. No similar protective birth months were observed for the 12-month gross motor development outcome (*Table 4.2.2*).

The aRRs of latitude were also significant after adjustment. All the 3 corresponding groups with higher latitudes than the reference group (latitude group of ≥ 25˚N,

< 30˚N) resulted in higher aRRs, with the highest aRRs observed by the group of latitudes ≥ 35˚N, < 40˚N, the second highest latitude group.

Table 4.2.1 Participant Characteristics

Independent variables	TOTAL	ASQ @ 6m, Gross Motor score < 22.25		P value	ASQ @ 12m, Gross Motor score < 21.49		P value
	N=72,203	n=14,960 (20.7)			n=10,260 (14.2)		
Birth month				**<0.001** *			**<0.001** *
January	5,594	704	(12.6)		592	(10.6)	
February	5,109	491	(9.6)		591	(11.6)	
March	5,571	537	(9.6)		715	(12.8)	
April	5,539	591	(10.7)		826	(14.9)	
May	5,781	896	(15.5)		949	(16.4)	
June	5,518	1,316	(23.9)		1,017	(18.4)	
July	6,408	1,799	(28.1)		1,178	(18.4)	
August	7,275	2,329	(32.0)		1,169	(16.1)	
September	7,467	2,322	(31.1)		1,012	(13.6)	
October	6,849	1,869	(27.3)		895	(13.1)	
November	5,622	1,146	(20.4)		684	(12.2)	
Latitude				**<0.001** *			**<0.001** *
≥ 40˚N, < 45˚N	5,907	906	(15.3)		720	(12.2)	
≥ 35˚N, < 40˚N	42,540	9,433	(22.2)		6,322	(14.9)	
≥ 30˚N, < 35˚N	23,164	4,556	(19.7)		3,179	(13.7)	
≥ 25˚N, < 30˚N	592	65	(11.0)		39	(6.6)	
December	5,470	960	(17.6)		632	(11.6)	
Vitamin D < 7μg (Daily intake during 1st trimester) *				0.734			0.971
< 7μg	55,641	11,544	(20.8)		7,908	(14.2)	
≥ 7μg	16,562	3,416	(20.6)		2,352	(14.2)	
Vitamin D < 7μg (Daily intake during 2nd/3rd trimes				0.710			0.708
< 7μg	58,404	12,085	(20.7)		8,313	(14.2)	
≥ 7μg	13,799	2,875	(20.8)		1,947	(14.1)	
Prenatal Vitamin D supplement				0.112			0.136
No	72,014	14,912	(20.7)		10,226	(14.2)	
Yes	189	48	(25.4)		34	(18.0)	
Prenatal multivitamin supplement				0.929			0.384
No	68,550	14,201	(20.7)		9,723	(14.2)	
Yes	3,653	759	(20.8)		537	(14.7)	
Prenatal multi supplement				0.197			0.091
No	68,501	14,162	(20.7)		9,699	(14.2)	
Yes	3,702	798	(21.6)		561	(15.2)	

* The cut-off for vitamin D: in accordance with a guideline by the Ministry of Health, Labour, and Welfare. https://www.mhlw.go.jp/file/04-Houdouhappyou-10904750-Kenkoukyoku-Gantaisakukenkouzoushinka/0000041955.pdf

Table 4.2.2 Gross motor development at age 6 and 12 months by birth month and latitude

	Unadjusted model at 6 months			Adjusted model at 6 months *			Unadjusted MODEL at 12 months			Adjusted model at 12 months		
	RR	[95% CI]	*p*	aRR	[95% CI]	*p*	RR	[95% CI]	*p*	aRR	[95% CI]	*p*
Birth month												
January	Reference			Reference			Reference			Reference		
February	0.77	(0.69 0.85)	**< 0.001**	0.75	(0.66 0.86)	**< 0.001**	1.09	(0.98 1.22)	0.098	1.13	(0.99 1.29)	0.077
March	0.77	(0.69 0.85)	**< 0.001**	0.77	(0.68 0.87)	**< 0.001**	1.21	(1.10 1.34)	**< 0.001**	1.21	(1.06 1.37)	**0.004**
April	0.85	(0.77 0.94)	**0.002**	0.82	(0.72 0.93)	**0.002**	1.41	(1.28 1.56)	**< 0.001**	1.49	(1.32 1.68)	**< 0.001**
May	1.23	(1.12 1.35)	**< 0.001**	1.21	(1.08 1.35)	**0.001**	1.55	(1.41 1.71)	**< 0.001**	1.63	(1.45 1.83)	**< 0.001**
June	1.90	(1.75 2.07)	**< 0.001**	1.83	(1.66 2.03)	**< 0.001**	1.74	(1.59 1.92)	**< 0.001**	1.84	(1.64 2.07)	**< 0.001**
July	2.23	(2.06 2.41)	**< 0.001**	2.13	(1.94 2.35)	**< 0.001**	1.74	(1.58 1.90)	**< 0.001**	1.78	(1.59 1.99)	**< 0.001**
August	2.54	(2.35 2.74)	**< 0.001**	2.50	(2.28 2.74)	**< 0.001**	1.52	(1.38 1.66)	**< 0.001**	1.57	(1.40 1.76)	**< 0.001**
September	2.47	(2.29 2.67)	**< 0.001**	2.43	(2.21 2.66)	**< 0.001**	1.28	(1.16 1.41)	**< 0.001**	1.34	(1.19 1.51)	**< 0.001**
October	2.18	(2.01 2.36)	**< 0.001**	2.10	(1.91 2.31)	**< 0.001**	1.24	(1.12 1.36)	**< 0.001**	1.24	(1.10 1.39)	**0.001**
November	1.63	(1.49 1.78)	**< 0.001**	1.61	(1.46 1.79)	**< 0.001**	1.15	(1.04 1.28)	**0.007**	1.18	(1.04 1.34)	**0.010**
December	1.40	(1.28 1.53)	**< 0.001**	1.35	(1.21 1.51)	**< 0.001**	1.09	(0.98 1.22)	0.096	1.11	(0.98 1.27)	0.105
Latitude												
≥ 25˚N, < 30˚N	Reference			Reference			Reference			Reference		
≥ 30˚N, < 35˚N	1.75	(1.39 2.20)	**< 0.001**	1.85	(1.41 2.43)	**< 0.001**	2.10	(1.55 2.85)	**< 0.001**	1.79	(1.29 2.49)	**0.001**
≥ 35˚N, < 40˚N	1.97	(1.57 2.47)	**< 0.001**	2.09	(1.59 2.74)	**< 0.001**	2.26	(1.67 3.07)	**< 0.001**	1.86	(1.34 2.59)	**< 0.001**
≥ 40˚N, < 45˚N	1.36	(1.07 1.72)	**0.011**	1.53	(1.16 2.03)	**0.003**	1.87	(1.37 2.55)	**< 0.001**	1.60	(1.14 2.25)	**0.007**

Total number for the unadjusted and the adjusted models (logistic regression) were 72,203 and 48,805 respectively.

* Adjusted model: Multivariate analyses of birth month adjusted for latitude, time spent outside per day during pregnancy, pre-pregnancy BMI, maternal age, parity, Assisted Reproductive Treatments, threatened abortion/premature labour, neuropsychotropic drugs, smallness for gestational age, twins or multiple birth, breech/foot/other abnormal presentation, induced delivery, C-section, labour > 24h, Apgar Score (5 minutes), umbilical cord/placenta problems, meconium staining, neonatal transportation, respiratory distress, hyperbilirubinemia, intrauterine growth restriction, maternal smoking and drinking during pregnancy, EPDS scores at 6 months, maternal education, and family income

When the participants were divided into the 4 groups by gross motor development trajectory at age 6 and 12 months (BEST, IMPROVED, WORSENED, and WORST), the similar tendency of "the risk for gross motor problems/delay being highest for the summer-born and lowest for the winter-born" was also confirmed. In the BEST group, the birth months of February, March, and January showed the highest percentages (82.5%, 81.4%, and 81.3%), and the lowest percentages in the WORST group (3.7%, 3.9%, and 4.4%). Conversely, in the WORST group, the birth months of July, August, and June showed the highest percentage (10.9%, 10.3%, and 9.4%, respectively), but those of August, September, and July showed the lowest percentages in the BEST group (62.2%, 63.8%, and 64.5%) (Table 4.2.3).

The IMPROVED and WORSENED groups also had a peak and a trough each, although their months of occurrence were different. In the IMPROVED group, the birth months of September-, August-, and October improved most (22.7%, 21.8%, and 19.5%, respectively), whereas those of March-, February -, and April improved least (5.7%, 6.0%, and 6.0%). In the WORSENED group, the birth months of April-, May-, and June showed the highest percentage (10.2%, 9.4%, 9.0%), while those of November, October, and September were the lowest (5.2%, 5.3%, and 5.6%) (Table 4.2.3).

Table 4.2.3 Gross Motor at age 6 and 12 months by birth month/latitude

	≥ cutoff at 6m and 12m (BEST)		< cutoff at 6m ≥ cutoff at 12m (IMPROVED)		≥ cutoff at 6m < cutoff at 12m (WORSENED)		< cutoff at 6m and 12m (WORST)		TOTAL
Birth Month									
January	4,546	(81.3)	456	(8.2)	344	(6.2)	248	(4.4)	5,594
February	4,214	(82.5)	304	(6.0)	404	(7.9)	187	(3.7)	5,109
March	4,537	(81.4)	319	(5.7)	497	(8.9)	218	(3.9)	5,571
April	4,381	(79.1)	332	(6.0)	567	(10.2)	259	(4.7)	5,539
May	4,341	(75.1)	491	(8.5)	544	(9.4)	405	(7.0)	5,781
June	3,703	(67.1)	798	(14.5)	499	(9.0)	518	(9.4)	5,518
July	4,132	(64.5)	1098	(17.1)	477	(7.4)	701	(10.9)	6,408
August	4,523	(62.2)	1,583	(21.8)	423	(5.8)	746	(10.3)	7,275
September	4,760	(63.8)	1,695	(22.7)	385	(5.2)	627	(8.4)	7,467
October	4,617	(67.4)	1,337	(19.5)	363	(5.3)	532	(7.8)	6,849
November	4,159	(74.0)	779	(13.9)	317	(5.6)	367	(6.5)	5,622
December	4,176	(76.3)	662	(12.1)	334	(6.1)	298	(5.5)	5,470
Latitude									
≥ 25°N, < 30°N	504	(85.1)	49	(8.3)	23	(3.9)	16	(2.7)	592
≥ 30°N, < 35°N	16,994	(73.4)	2,991	(12.9)	1,614	(7.0)	1,565	(6.8)	23,164
≥ 35°N, < 40°N	29,986	(70.5)	6,232	(14.7)	3,121	(7.3)	3,201	(7.5)	42,540
≥ 40°N, < 45°N	4,605	(78.0)	582	(9.9)	396	(6.7)	324	(5.5)	5,907
Total	52,089	(72.1)	9,854	(13.7)	5,154	(7.1)	5,106	(7.1)	72,203

Regarding latitude, the southernmost (≥ 25°N, < 30°N) group – participants in Okinawa – performed best in gross motor development, followed by those of the northernmost (≥ 40°N, < 40˚N). As the number of Okinawa participants was relatively small (n = 592), we designated each of the other latitude groups as the reference group. However, no changes in the order of aRR values were observed.

At age 6 and 12 months, girls significantly underperformed boys in gross motor development ($p < 0.001$): 7,370 boys (20.0%) and 7,590 girls (21.4%) scored below the cut-off at age 6 months, and 5,037 boys (13.7%) and 5,223 girls (14.8%) failed at age 12 months. 2,437 boys (6.6%) and 2,669 girls (7.5%) failed at both time points. The aRR by sex showed a similar trend to that of the total participants, while the aRR for girls was slightly higher than that for boys except for the birth month of February (*Figure 4.2.2*)

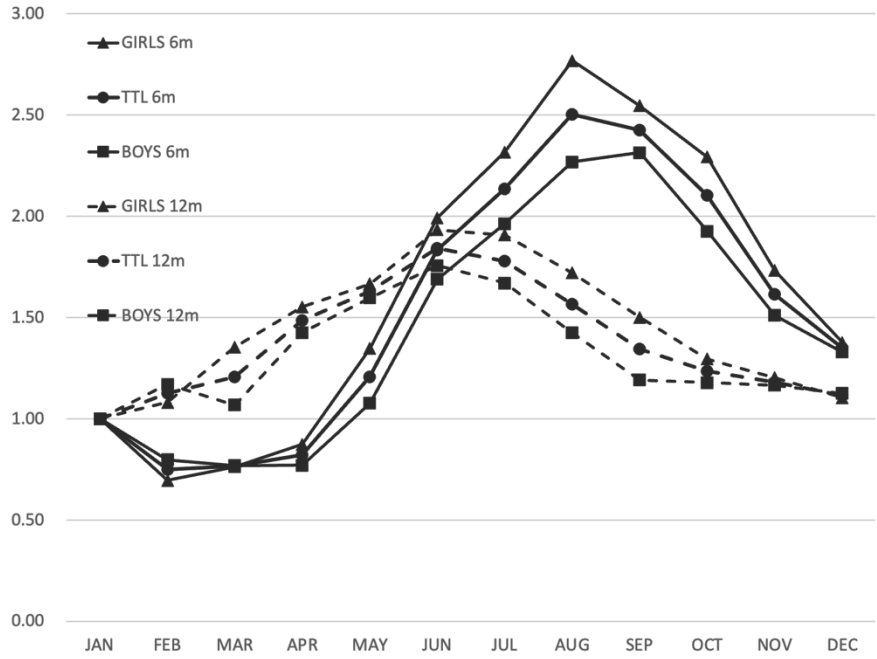

Figure 4.2.2 Adjusted Risk Ration for possible gross motor delay by birth month by gender

January as reference.

For outcome at age 6 months, all months except April for girls and May for boys were statistically significant. For outcome at age 12 months, between March and November for total and girls, and between April and September for boys were statistically significant ($p < 0.05$).

4.3 Study III

4.3.1 Serum 25(OH)D concentration

Among 4,653 participants (2,363 boys and 2,290 girls), serum 25(OH)D3 concentration levels fluctuated by test month, with the highest levels observed in August and the lowest in February. Boys' mean 25(OH)D3 levels were consistently higher than those of girls throughout the year (*Figure 4.3.1*, *Figure S4.3.1.* See APPENDIX for supplementary tables and figures). In contrast, 25(OH)D2 levels were 4 ng/mL, which was the truncated value, for all except three participants: 5.5 ng/mL (boy), 5.4 ng/mL (girl), and 10.3 ng/mL (girl), meaning that almost all 25(OH)D2 levels were ≤ 4.0 ng/mL. As the total vitamin D were the addition of 25(OH)D3 and 25(OH)D2, the total 25(OH)D levels fluctuated by season. Likewise, mean of the total 25(OH)D levels were significantly higher among boys than girls, with means of 25.6 ng/mL (95%CI (25.4 - 26.0)) and 24.6 ng/mL (95%CI (24.3 - 24.8)) ($p < 0.001$) respectively. This significant difference by sex remained after making the vitamin D variable a categorical one – deficient (< 20ng/mL), insufficient (≥ 20, < 30ng/mL), and sufficient (≥ 30 ng/mL) levels -–, with a higher proportion of boys in the sufficient group and a higher proportion of girls in the deficient group (*Table 4.3.1*). The 25(OH)D concentrations also differed significantly depending on test month, latitude, and daycare attendance (*Table S4.3.1*).

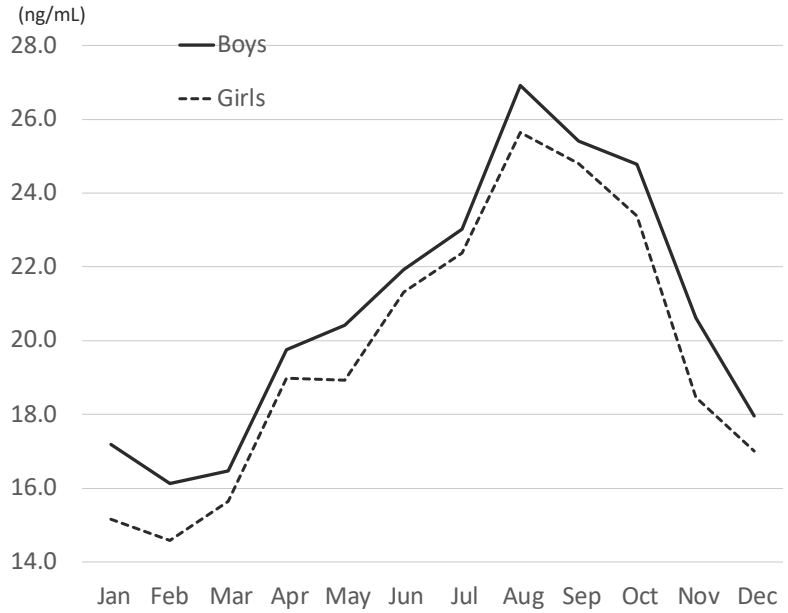

Figure 4.3.1 Mean 25(OH)D3 by month by child sex

Table 4.3.1 Categorised 25(OH)D concentrations and KSPD DQ (< 70)

		KSPD DQ											
		P-M < 70		*p*	C-A < 70		*p*	L-S < 70		*p*	Overall < 70		*p*
(Boys)		(n = 167, 7.1%)			(n = 113, 4.8%)			(n = 207, 8.8%)			(n = 108, 4.6%)		
25(OH)D (ng/mL)				0.476			**0.030**			**0.029**			**0.007**
< 20	(n = 541,22.9%)	38	(7.0)		37	(6.8)		62	(11.5)		38	(7.0)	
≥ 20 and < 30	(n = 1,196, 50.6%)	91	(7.6)		53	(4.4)		100	(8.4)		48	(4.0)	
30 ≥	(n = 626, 26.5%)	38	(6.1)		23	(3.7)		45	(7.2)		22	(3.5)	
(Girls)		(n = 139, 6.1%)			(n = 91, 4.0%)			(n = 92, 4.0%)			(n = 80, 3.5%)		
25(OH)D (ng/mL)				0.567			0.150			0.853			0.250
< 20	(n = 607, 26.5%)	39	(6.4)		21	(3.5)		24	(4.0)		20	(3.3)	
≥ 20 and < 30	(n = 1,189, 51.9%)	75	(6.3)		56	(4.7)		46	(3.9)		48	(4.0)	
30 ≥	(n = 494, 21.6%)	25	(5.1)		14	(2.8)		22	(4.5)		12	(2.4)	

4.3.2 KSPD DQ scores

Mean and median DQs of the 3 domain and the Overall DQ were significantly higher for girls, with the largest discrepancy for L-S and the narrowest for P-M (*Table S4.3.2 (a)*). When KSPD DQs were dichotomised at 70, 2,045 boys (86.5%) and 2,083 girls (91.0%) passed the cut off (DQs ≥ 70) in all the 3 domains and the Overall DQs ($p < 0.001$). More boys failed the cut off values than girls in every domain and Overall DQs, but this difference was significant only for the L-S domain (8.8% vs 4.0%, $p < 0.001$, *Table 4.3.1*).

Regarding failure (DQs < 70) overlaps among the 3 domains, L-S only was most common among boys (n = 113), followed by P-M only (n = 76) and all 3 domains (n = 59) while among girls, P-M only was most common (n = 73), followed by all 3 domains (n = 41). Those who failed in all the 3 domains accounted for more than a half of those with Overall DQs < 70 (108 boys and 80 girls). (*Table S4.3.2 (b)*).

4.3.3 Association between Serum 25(OH)D levels and KSPD DQs

Serum 25(OH)D concentration showed weak but statistically significant positive correlation with KSPD DQ in the L-S domain for both boys (0.0914, $p < 0.001$) and girls (0.0576, $p < 0.01$), meaning that the higher serum 25(OH)D concentration levels, the higher their L-S DQs became. Boys also showed a weak but significant positive correlation between 25(OH)D and the KSPD Overall DQ (0.0510, $p < 0.05$)) (*Table S4.3.3 (a) (b)*).

When the children were divided into 3 groups by 25(OH)D concentration level, significant differences in L-S DQ (both with mean and median) were observed

in a dose-dependent manner, both for boys and girls: the vitamin D "sufficient" group scored highest and gradually decreased in the "insufficient" and the "deficient" groups (*Table S4.3.4*).

Regarding the association between dichotomised KSPD DQs (< 70 and ≥ 70) and 25(OH)D concentration levels, only boys showed the association: 25(OH)D concentration was significantly lower (both mean and median) among those scoring < 70 in all except the P-M domain (*Table S4.3.5*). No such significant association was observed among girls (*Table S4.3.5*). The significant associations among boys remained the same after adjustment: aOR of 25(OH)D concentrations were < 1.00 – indicating that the higher 25(OH)D concentration, the less likely their DQs < 70 for all but the P-M domain (aOR = 0.96 (p = 0.015, 95%CI (0.93, 0.99)) for C-A; aOR = 0.97 (p = 0.012, 95%CI (0.95, 0.99)) for L-S; and aOR = 0.95 (p = 0.005, 95%CI (0.93, 0.99)) for Overall DQ < 70 (*Table S4.3.6*).

Finally, when both variables were treated categorically, a similar tendency was observed. Only boys showed statistically significant differences among the 3 different vitamin D level groups: the lower the vitamin D level group boys belong to, the more likely their DQs were < 70 in all the KSPD domains except P-M (*Table 4.3.1*). With the vitamin D sufficient group (≥ 30 ng/mL) as reference, boys' aOR of vitamin D deficiency (< 20 ng/mL) were more likely to score below the cut-off for all the domains but P-M: 2.33 for Overall DQ, 1.91 for C-A, and 1.69 for L-S (*Table 4.3.2*). Only girls' insufficient group (≥ 20 & < 30 ng/mL) showed a significant aOR 1.97 for C-A; and 1.95 for the Overall DQ (*Table 4.3.2*).

Table 4.3.2 Association between 25(OH)D and KSPD DQ

(Boys)	P-M < 70				C-A < 70				L-S < 70				Overall < 70			
25(OH)D (ng/mL)	OR	*p*	95% CI		OR	*p*	95% CI		OR	*p*	95% CI		OR	*p*	95% CI	
< 20	1.17	0.511	0.73	1.86	1.92	0.016	1.13	3.28	1.67	0.012	1.12	2.50	2.07	0.008	1.21	3.55
≥ 20 and < 30	1.27	0.225	0.86	1.89	1.22	0.443	0.74	2.00	1.18	0.380	0.82	1.70	1.15	0.599	0.69	1.92
30 ≥	Reference															

(Boys)	P-M < 70				C-A < 70				L-S < 70				Overall < 70			
25(OH)D (ng/mL)	aOR	*p*	95% CI		aOR	*p*	95% CI		aOR	*p*	95% CI		aOR	*p*	95% CI	
< 20	1.47	0.150	0.87	2.50	1.91	0.037	1.04	3.50	1.69	0.024	1.07	2.67	2.33	0.006	1.27	4.29
≥ 20 and < 30	1.41	0.106	0.93	2.13	1.25	0.393	0.75	2.10	1.15	0.463	0.79	1.69	1.23	0.445	0.72	2.09
30 ≥	Reference															

(Girls)	P-M < 70				C-A < 70				L-S < 70				Overall < 70			
25(OH)D (ng/mL)	OR	*p*	95% CI		OR	*p*	95% CI		OR	*p*	95% CI		OR	*p*	95% CI	
< 20	1.29	0.337	0.77	2.16	1.23	0.557	0.62	2.44	0.88	0.680	0.49	1.60	1.37	0.397	0.66	2.83
≥ 20 and < 30	1.26	0.325	0.79	2.01	1.69	0.082	0.93	3.07	0.86	0.579	0.51	1.45	1.69	0.109	0.89	3.21
30 ≥	Reference															

(Girls)	P-M < 70				C-A < 70				L-S < 70				Overall < 70			
25(OH)D (ng/mL)	aOR	*p*	95% CI		aOR	*p*	95% CI		aOR	*p*	95% CI		aOR	*p*	95% CI	
< 20	1.53	0.155	0.85	2.74	1.79	0.133	0.84	3.80	0.92	0.815	0.46	1.83	1.75	0.175	0.78	3.92
≥ 20 and < 30	1.32	0.261	0.81	2.14	1.97	0.031	1.07	3.63	0.88	0.659	0.51	1.53	1.95	0.048	1.01	3.77
30 ≥	Reference															

OR: Odds ratio, aOR: adjusted odds ratio (adjusted for test month, latitude, SGA, maternal age, and daycare attendance)

4.4 Study IV

4.4.1 NDDs diagnosed at age 3

Among 77,612 studied participants (39,690 boys and 37,922 girls), 854 children (625 boys and 229 girls, 1.1%) had received at least one NDD diagnosis out of MD; ID and/or DLD; and ASD by 3 years (*Table 4.4.1, Figure S4.4.1*). ID/DLD were diagnosed most often, followed by ASD and MD (*Table 4.4.1*). The majority of children (n = 587) had been diagnosed with one NDD, and multiple NDD diagnoses always included ID/DLD, except for 1 child with MD and ASD (*Table 4.4.2*).

Table 4.4.1 Prevalence of NDDs according to child's gender

	Male (n = 39,690)		Female (n = 37,922)		Total (n = 77,612)	
NDDs	625	(1.6)	229	(0.6)	854	(1.1)
MD	145	(0.4)	79	(0.2)	224	(0.3)
ID/DLD	415	(1.1)	152	(0.4)	567	(0.7)
ASD	273	(0.7)	76	(0.2)	349	(0.4)

ASD, autism spectrum disorder; ID/DLD, intellectual disability and/or developmental language disorder; MD, motor delay; NDDs, neurodevelopmental disorders
Data given as n (%)

Table4.4.2 Prevalence of NDD

NDD diagnosis	Number of diagnoses 1	2	3	Number of cases diagnosed	(%) (Total, 77,612)
MD	44	-	-	224	(0.3)
ID/DLD	301	-	-	567	(0.7)
ASD	242	-	-	349	(0.4)
MD + ID/DLD	-	160	-	-	-
MD + ASD	-	1	-	-	-
ID/DLD + ASD	-	87	-	-	
MD + ID/DLD + ASD	-	-	19	-	
Number of children with any NDDs	587	248	19	854	(1.1)

4.4.2 ESSENCE-Q scores and NDD diagnoses

The percentages for score 0, meaning no parental concerns for all the 11 items, in the NDD and non-NDD groups were 3.6% (n = 31) and 50.1% (n = 38,430) respectively (*Table 4.4.3*). The score distributions were also very different between the two groups (*Figure 4.4.1*). In the NDD group, the ESSENCE-Q score distribution was skewed only slightly, whereas that of the non-NDD group was right-skewed (*Figure 4.4.1*).

In the NDD group, 31 parents scored 0, meaning that they had no concerns regarding their children's developmental status at age 2.5 years even though their NDD diagnosis was given by 3 years. In the non-NDD group, 131 (0.2%) scored 11, meaning that their parents had concerns in every domain even though no NDDs had been diagnosed and 97 (74.0%) of them scored as "typically developing" on the ASQ-3 at age 2.5 years, at the same time as the ESSENCE-Q survey was conveyed in the same JECS questionnaire.

Mean score differences between NDD and non-NDD groups were statistically significant ($p < 0.001$) (*Table 4.4.3*). In addition, the mean score was significantly higher among boys (1.34; 95% CI: 1.32–1.35) than girls (1.01; 95% CI: 1.00–1.03) ($p < 0.001$). Mean scores increased with number of NDD diagnoses: 5.06, 6.14, and 7.32 for those with 1 NDD diagnosis (n = 587), 6.14, 2 diagnoses (n = 248), and 3 diagnoses (n = 19) ($p < 0.001$).

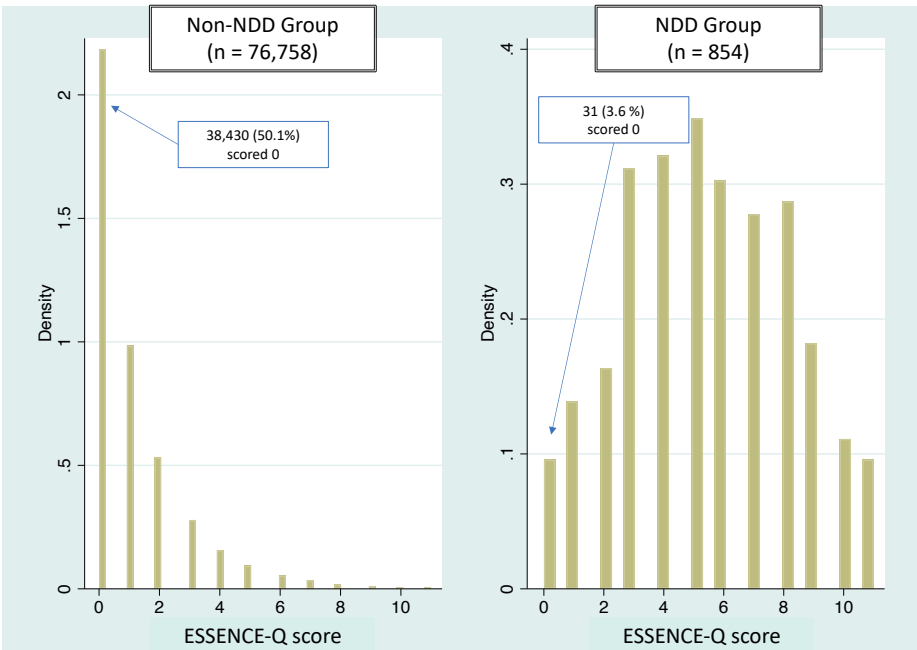

Figure 4.4.1. Distribution of ESSENCE-Q scores in Non-NDD and NDD groups

4.4.3 ROC curve analysis

Figure 4.4.2 shows ROC curves of 2.5-year ESSENCE-Q scores as predictors of 3-year NDD diagnoses. AUCs for NDDs exceeded 0.90 in all cases: 0.91 (NDDs total), 0.91 (MD), 0.90 (ID/DLD), and 0.93 (ASD) (*Table 4.4.4*). For NDD total as well as each MD, ID/DLD, and ASD, the Youden index was highest at total ESSENCE-Q score ≥ 3, with sensitivity between 84.83% and 89.97%, with the highest sensitivity observed with ASD, and specificity between 84.27% and 84.83% (*Table 4.4.5*). PPVs (NDDs: 5.86%; MD: 1.60%; ID/DLD: 2.54%; ASD: 2.54%) were much lower than NPVs (NDDs: 99.80%; MD: 99.96%; ID/DLD: 99.95%; ASD: 99.95%) (*Table 4.4.4*). AUC, sensitivity, NPV, LR (-) were similar for boys and girls (*Table S4.4.1*, *Table S4.4.2*).

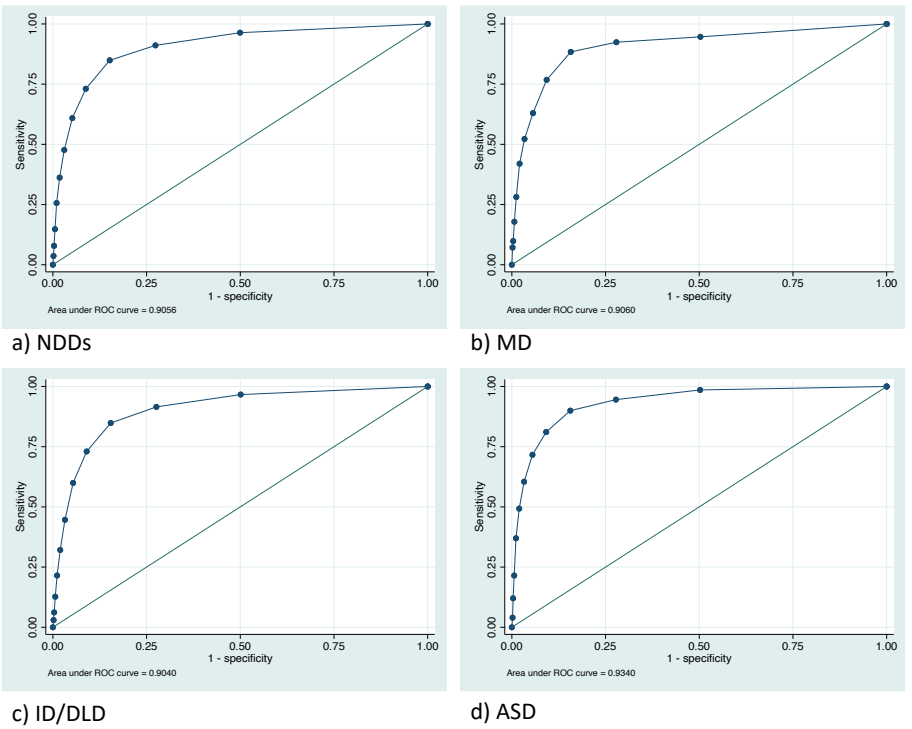

Figure 4.4.2 ROC curves for ESSENCE-Q scores at 2.5 years according to NDD diagnosis at 3 years of age

4.4.4 Individual questions of ESSENCE-Q

When examining each of the 11 ESSENCE-Q items, responses differed significantly between the NDD and non-NDD groups ($p < 0.001$) (*Table 4.4.6*). Particularly, "4. Communication" was the major concern (89.5%) in the NDD, ID/DLD, and ASD groups, followed by "1. General development" (80.2%), with wide discrepancies compared with the respective non-NDD group (Communication: 14.2%; General development: 7.4%). Communication remained a key concern, even for the children with NDDs whose ESSENCE-Q scores were 1 or 2 and below the optimal cut-off (≥ 3). Among the 98 children with NDDs with a score of 1 (n = 45) or 2 (n = 53), 74 parents (75.5%; score 1, n = 32; score 2, n = 42) were concerned about child communication. Among the 26,682 non-NDD participants scored 1 (17,317) or 2 (9,365), 4,821 parents (18.3%; score 1, n = 2,448; score 2, n = 2,373) scored "yes" for concerns on communication (*Table S4.4.3*). In the non-NDD group, "11. Feeding" was the most common concern (n = 23,540, 30.7%) and was a concern for a majority (53.8%) of parents/caregivers in the NDD group (*Table S4.4.3*).

4.4.5 Correlation between J-ASQ-3 and ESSENCE-Q

ESSENCE-Q total scores were negatively correlated with J-ASQ-3 overall scores (–0.36; $p < 0.001$) and J-ASQ-3 total and each of the 5 domain scores (communication, –0.31; gross motor, –0.20; fine motor, –0.24; problem solving, –0.30; and social–personal, –0.32, with $p < 0.001$ for all domains). Furthermore, the total scores for each of the J-ASQ-3 domains were significantly different between the "with concern" and "no concern" groups for each of the 11 ESSENCE-Q questions ($p < 0.001$), with the "no concern" group scoring higher than the "with concern" group (*Table S4.4.4*).

Table 4.4.3 ESSENCE-Q scores and NDD diagnoses

ESSENCE-Q score	Participants (n = 77,612)		NDDs				MD				ID/DLD				ASD			
			- (n = 76,758)		+ (n = 854)		- (n = 77,388)		+ (n = 224)		- (n = 77,045)		+ (n = 567)		- (n = 77,263)		+ (n = 349)	
0	38,461	(49.6)	38,430	(50.1)	31	(3.6)	38,449	(49.7)	12	(5.4)	38,442	(49.9)	19	(3.4)	38,456	(49.8)	5	(1.4)
1	17,362	(22.4)	17,317	(22.6)	45	(5.3)	17,357	(22.4)	5	(2.2)	17,333	(22.5)	29	(5.1)	17,348	(22.5)	14	(4.0)
2	9,418	(12.1)	9,365	(12.2)	53	(6.2)	9,409	(12.2)	9	(4.0)	9,380	(12.2)	38	(6.7)	9,402	(12.2)	16	(4.6)
3	5,009	(6.5)	4,908	(6.4)	101	(11.8)	4,983	(6.4)	26	(11.6)	4,942	(6.4)	67	(11.8)	4,978	(6.4)	31	(8.9)
4	2,864	(3.7)	2,760	(3.6)	104	(12.2)	2,833	(3.7)	31	(13.8)	2,790	(3.6)	74	(13.1)	2,831	(3.7)	33	(9.5)
5	1,765	(2.3)	1,652	(2.2)	113	(13.2)	1,741	(2.2)	24	(10.7)	1,678	(2.2)	87	(15.3)	1,726	(2.2)	39	(11.2)
6	1,042	(1.3)	944	(1.2)	98	(11.5)	1,019	(1.3)	23	(10.3)	971	(1.3)	71	(12.5)	1,003	(1.3)	39	(11.2)
7	709	(0.9)	619	(0.8)	90	(10.5)	678	(0.9)	31	(13.8)	649	(0.8)	60	(10.6)	666	(0.9)	43	(12.3)
8	418	(0.5)	325	(0.4)	93	(10.9)	395	(0.5)	23	(10.3)	368	(0.5)	50	(8.8)	364	(0.5)	54	(15.5)
9	262	(0.3)	203	(0.3)	59	(6.9)	244	(0.3)	18	(8.0)	225	(0.3)	37	(6.5)	229	(0.3)	33	(9.5)
10	140	(0.2)	104	(0.1)	36	(4.2)	134	(0.2)	6	(2.7)	122	(0.2)	18	(3.2)	112	(0.1)	28	(8.0)
11	162	(0.2)	131	(0.2)	31	(3.6)	146	(0.2)	16	(7.1)	145	(0.2)	17	(3.0)	148	(0.2)	14	(4.0)
ESSSENE-Q mean score (95% CI)	1.18 (1.17–1.19)		1.13 (1.12–1.14)		5.42 (5.23–5.61)		1.17 (1.16–1.18)		5.72 (5.34–6.10)		1.15 (1.14–1.16)		5.26 (5.04–5.48)		1.16 (1.15–1.17)		6.20 (5.91–6.49)	
Median	1		0		5		1		6		1		5		1		6	

Table 4.4.4 Area under the curve (AUC), sensitivity, specificity, Youden index, positive predictive value (PPV), and negative predictive value (NPV) at the cut-off of ≥ 3

	NDDs	MD	ID/DLD	ASD
AUC	0.91	0.91	0.90	0.93
Sensitivity	84.89%	88.39%	84.83%	89.97%
Specificity	84.83%	84.27%	84.57%	84.39%
Youden index	69.72%	72.66%	69.40%	74.37%
PPV	5.86%	1.60%	2.54%	2.54%
NPV	99.80%	99.96%	99.95%	99.95%
LR (+)	5.60	5.62	5.50	5.77
LR (-)	0.18	0.14	0.18	0.12

Table 4.4.5 Sensitivity and specificity by each ESSENCE-Q score for NDDs

Cut point	Sensitivity	Specificity	LR+	LR-	Youden Index
(≥ 0)	100.00%	0.00%	1.00		0.00%
(≥ 1)	96.37%	50.07%	1.93	0.07	46.44%
(≥ 2)	91.10%	72.63%	3.33	0.12	63.73%
(≥ 3)	84.89%	84.83%	5.60	0.18	69.72%
(≥ 4)	73.07%	91.22%	8.32	0.30	64.29%
(≥ 5)	60.89%	94.82%	11.75	0.41	55.71%
(≥ 6)	47.66%	96.97%	15.73	0.54	44.63%
(≥ 7)	36.18%	98.20%	20.10	0.65	34.38%
(≥ 8)	25.64%	99.01%	25.80	0.75	24.65%
(≥ 9)	14.75%	99.43%	25.86	0.86	14.18%
(≥ 10)	7.85%	99.69%	25.63	0.92	7.54%
(≥ 11)	3.63%	99.83%	21.27	0.97	3.46%

Table 4.4.6 Number of "yes" responses for each question of ESSENCE-Q

ESSENCE-Q question	Total		NDD (–)		NDD (+)		MD (–)		MD (+)		ID/DLD (–)		ID/DLD (+)		ASD (–)		ASD (+)	
1. General development	6,399	(8.2)	5,714	(7.4)	685	(80.2)	6,203	(8.0)	196	(87.5)	5,931	(7.7)	468	(82.5)	6,113	(7.9)	286	(81.9)
2. Motor development	3,282	(4.2)	2,917	(3.8)	365	(42.7)	3,096	(4.0)	186	(83.0)	3,021	(3.9)	261	(46.0)	3,170	(4.1)	112	(32.1)
3. Sensory reactions	1,468	(1.9)	1,217	(1.6)	251	(29.4)	1,373	(1.8)	95	(42.4)	1,314	(1.7)	154	(27.2)	1,341	(1.7)	127	(36.4)
4. Communication	11,659	(15.0)	10,895	(14.2)	764	(89.5)	11,468	(14.8)	191	(85.3)	11,130	(14.4)	529	(93.3)	11,342	(14.7)	317	(90.8)
5. Activity	10,818	(13.9)	10,340	(13.5)	478	(56.0)	10,716	(13.9)	102	(45.5)	10,527	(13.7)	291	(51.3)	10,566	(13.7)	252	(72.2)
6. Attention	7,335	(9.5)	6,859	(8.9)	476	(55.7)	7,230	(9.3)	105	(46.9)	7,043	(9.1)	292	(51.5)	7,084	(9.2)	251	(71.9)
7. Social interaction	6,515	(8.4)	6,084	(7.9)	431	(50.5)	6,408	(8.3)	107	(47.8)	6,244	(8.1)	271	(47.8)	6,291	(8.1)	224	(64.2)
8. Behaviour	4,032	(5.2)	3,750	(4.9)	282	(33.0)	3,969	(5.1)	63	(28.1)	3,879	(5.0)	153	(27.0)	3,865	(5.0)	167	(47.9)
9. Mood	8,217	(10.6)	7,960	(10.4)	257	(30.1)	8,156	(10.5)	61	(27.2)	8,067	(10.5)	150	(26.5)	8,081	(10.5)	136	(39.0)
10. Sleep	7,881	(10.2)	7,700	(10.0)	181	(21.2)	7,824	(10.1)	57	(25.4)	7,766	(10.1)	115	(20.3)	7,800	(10.1)	81	(23.2)
11. Feeding	23,999	(30.9)	23,540	(30.7)	459	(53.8)	23,880	(30.8)	119	(53.1)	23,700	(30.8)	299	(52.7)	23,788	(30.8)	211	(60.5)
Total	77,612	(100.0)	76,758	(100.0)	854	(100.0)	77,388	(100.0)	224	(100.0)	77,045	(100.0)	567	(100.0)	77,263	(100.0)	349	(100.0)

5 DISCUSSION

Studies I, II, and *III* investigated whether NDPs of children participating in the JECS (at age 1 months, 6 months, 12 months, 2 years, 2.5 years, and 3 years) were associated with pre-/perinatal factors, birth month, and serum vitamin D concentration levels. *Study I* investigated the pre-/perinatal factors by using suboptimality scale scores as well as the individual scale items as possible risk factors. Parental observation was also utilised as a screening tool, at 1 month (*Study I*, 6 questions) and at 2.5 years of age (*Study IV*, ESSENCE-Q), for both of which the total scores and the individual scale items were investigated as possible predictors of NDDs at 3 years of age.

5.1 Study I

The findings of the study were that: (1) pre-/perinatal reduced optimality scale scores were associated with child neurodevelopment in a dose-dependent manner; (2) advanced maternal age (> 35 years) and caesarean section delivery were shared risk factors for MD, ID/DLD, and ASD, whereas other factors were unique to each of the NDDs; and (3) as early as 1 month after birth, parents seemed able to perceive some signs of NDDs diagnosed by age 3.

Our findings align with the previous studies on the association between pre-/perinatal reduced optimality and offspring's NDDs, particularly in perinatal factors, such as neonatal transportation, epidural analgesia, caesarean section delivery, Apgar scores ≤ 8, and hyperbilirubinemia.[11,23,50,96,97] Direct causal associations cannot be confirmed, but the optimality concept provides useful guidelines for the pathogenetic classification of NDDs. The study results re-emphasise the importance of medical care for expectant mothers > 35 years of age and for the perinatal period, as well as the need to careful follow up children who experienced adversities at birth.

Advanced maternal age and caesarean section delivery were the shared risk factors for MD, ID and/or DLD, and ASD. Advanced maternal age is known to be a detrimental biological risk factor due to the rapid decline in healthy oocytes among women over 35 years, and to be a risk factor for pre-/perinatal complications.[96-108] Paternal ages are highly correlated with maternal age, and advanced paternal age itself is also a biological risk factor in many studies.[103] Although parental young age was also a possible risk for child NDDs such as ADHD in previous studies,[104] young maternal age (< 20) was not a significant risk factor in the present study. Possible reasons for the results are the relatively small number of mothers < 20 years of age, and/or no data on ADHD diagnosis

at this early stage of life. The second shared risk, caesarean section delivery, may not have been a risk itself in some cases, but the reasons behind the need for caesarean section delivery – maternal factors including maternal emotional vulnerability, and obstetric factors such as foetal distress and stalled labour – may have contributed as a more fundamental risk of NDDs.[106,109] Unfortunately, the collapsed record on *planned* and *emergency c*aesarean section delivery in the JECS hindered any further investigations within this study.

The high aRR for epidural analgesia for ASD and NDDs must be interpreted carefully, and requires further investigation. Recent studies have investigated the risk of ASD posed by epidural analgesia, particularly after the finding by Qiu et al.[110] that epidural analgesia increased the risk of ASD by 37% in California. Subsequent studies, from Canadian and Danish cohort study data, concluded that there was no increased risk of ASD,[111,112] and other researchers have questioned the statistical methods used by Qiu et al.—particularly that they did not sufficiently account for residual confounding factors.[112-115] In Japan, epidural analgesia is not a standard procedure, and it was chosen by only 2.13% of women in the present sample. Therefore, similar to some cases of caesarean section delivery, the choice to use epidural analgesia could be an indication that this represents a group with specific risk factors contributing to a decision to request epidural analgesia. For instance, they could have an extreme fear of pain during delivery due to their own neurodevelopmental/ psychiatric problems, a tendency that was also found in a Danish cohort study.[112] Thus it may be the underlying NDD in the mother is a common factor in this association, and the epidural analgesia merely coincidental. Another possibility is that medical professionals may choose epidural analgesia because of perinatal complications which themselves could be major risk factors for NDDs.

Although the total scores of the developmental concern scale at 1 month did not accurately predict NDD diagnoses at 3 years of age, each of the six items in the scale may have been able to indicate some NDPs. Our findings suggest that parental observations could be a useful source of information for the early detection of, and support in, neurodevelopmental problems. People with ASD are known to have sensory and emotion regulation issues, which possibly explain the high RR among children with ASD for all except the gross motor function item at age 1 month.

5.2 Study II

Gross motor development at 6 and 12 months of age was significantly associated with birth month. The cyclical pattern of "higher risk for the summer-born and lower risk for the winter-born" was observed both at 6 and 12 months of age, with a much wider discrepancy between the aRR peak and the trough at 6 months old, and with the peak shifted slightly earlier at 12 months. The study findings may indicate "disadvantages in the winter" and "advantages in the summer" affecting brain development during early pregnancy—the most crucial period for central nervous system development.[116,117]

The results also indicate the continued seasonal effects and the plasticity of brain development during the first 12 months of postnatal life. One study suggested a positive summer climatic effect, with warmer weather offering a more preferable environment for infants to explore their environment, as well as more nutritious food when they start weaning.[118] The narrowed discrepancy between the peak and the trough of the cyclical aRR trends at age 12 months may also indicate that gestational negative effects on summer-born infants gradually decrease, or that gestational positive effects on winter-born infants slowly diminish due to the proliferation of a range environmental influences after birth.

The study results align with previous smaller studies in Japan, the U.S.A., and China. A Japanese study (n = 742 infants) found that March/April-born children performed the best at gross motor development at age 6 and 10 months, but the 1-year cyclical variations disappeared at age 14 months.[119] In the study in Denver, USA, the locomotor onset of summer/fall-born children lagged behind winter/spring-born (n = 425) by 3 weeks.[120] In China, winter-born children scored higher on cognitive and psychomotor development tests at 8 to 10 months of age (n = 650).[118] Likewise, a larger and older cohort study in the US (n = 22,123) reported that winter/spring-born children showed superior motor development at 8 months and 4 years but not at 7 years of age.[46] However, a study in Canada (n = 145) found no significant seasonal differences at age 7 months.[121]

The possible mechanism of our findings could be explained with critical windows of brain development and seasonality. Human brain development starts soon after conception and continues into early adulthood.[122] In particular, the period from conception to the end of infancy is known as a critical window of developmental brain plasticity and growth, with the first trimester believed to be crucial for the central nervous system.[123,124] For instance, if a child was

born in the summer (August), the critical window of brain development would have been in late autumn to winter (November to February). Likewise, the critical window of brain development for the February-born children, who performed the best in this study, would have been in the summer (June to September). The finding that winter-born children accounted for the highest percentage in the "BEST" group, and the summer-born accounted for highest in the "WORST" group was also a possible indication that more risks may exist in winter and so did more protective factors in summer in terms of brain development.

Birth month can represent various biometeorological factors, such as sunlight, temperature, humidity, physical activity, nutritional intake, infection, and metabolic/endocrinological status. As most of the vitamin D we obtain requires being exposed to the sun, vitamin D deficiency could be a possible major risk factor in the winter. Among various benefits, Vitamin D is known to be crucial for neurodevelopment, including gross motor development.[38,125-129] Clear seasonality of vitamin D concentration levels has been found in previous studies in Japan.[130,131]

Another possible seasonal risk factor characteristic of winter is influenza virus infection. Offspring exposed to influenza during between 0 and 8 gestational weeks showed a slight delay in their psychomotor development at 6 months in the Norwegian Influenza Pregnancy Cohort Study.[132]

Only the gross motor domain out of the five J-ASQ-3 domains showed clear seasonality and outperformance by male infants at both time points. Given the fact that more boys generally receive NDD diagnoses than girls, such as ASD, ADHD, intellectual disability, and language delay,[133,134] and several studies have reported that winter/spring birth is a risk factor for ASD,[41,47,135,136] and the fact that various combinations between genetic and environmental factors contribute to the aetiology of neurodevelopmental disorders, it is likely that most children will catch up in gross motor development. Further follow-up will confirm the implication of infant gross motor problems by age 12 months.

5.3 Study III

The major finding of *Study III* was that, only among boys, vitamin D deficiency (< 20 ng/mL) showed a clear association with delayed cognitive and communication development. Future investigation is necessary for girls, since only the aRR of the vitamin D insufficient group showed an association with low cognitive function (C-A) when both vitamin D and KSPD scores were treated as categorical variables, but no clear patterns nor significant association were observed throughout other analyses.

Most previous studies measured maternal blood during pregnancy or cord blood, rather than the child's blood. Some measured children's own serum 25-hydroxyvitamin D, but the outcome was usually dichotomised – with/without NDD – and no detailed information on neurodevelopment assessed by developmental tests was given [4,5,11.] The results of the only study we could find using a similar methodology to ours, based on a subcohort of the Avon Longitudinal Study of Parents and Children (ALSPAC), concluded that no association was found between vitamin D deficiency and neurodevelopment. The different results from the present study are most likely due to several reasons: the ALSPAC sample excluded those with previous behavioural problems, there was no analysis by sex, a smaller sample size (approximately 2,500), and testing occurred at a much older age (blood test at mean age of 10, neurodevelopmental assessment at mean age of 11.7 years).[18]

Any causal relationship between boys' vitamin D deficiency and cognitive/social development problems cannot be elucidated in this study only, even though the association and the pattern were significant throughout the analyses. Vitamin D deficiency could be a risk factor for neurodevelopmental problems for some individuals. Conversely, some children with neurodevelopmental challenges could have abnormalities in their steroid metabolism, including vitamin D, a neurosteroid.[6,19] Both directions of causality may coexist as various types of neurodevelopmental problems AND a variety of aetiologies among even very similar neurodevelopmental diagnoses have been observed. One study found that mothers with Somali origin with children with autism had an approximately 30% lower mean value of vitamin D during spring compared with mothers without a child with autism.[137] Another Swedish sibling study showed that children with ASD had had significantly lower vitamin D than their sibling without ASD soon after birth, which indicates that vitamin D deficiency could be an early marker.[22] In addition experimental studies showed that low vitamin D adversely impacted brain development, such as alteration of the forebrain's dopaminergic turnover.[6,23] Animal studies also show an association between vitamin D

deficiency and elevated testosterone levels in the foetal brain, indicating that increased foetal exposure to testosterone, which leads to increased androgenisation of the brain, may explain a possible pathogenetic process for higher prevalence of autism among males.[6,24] The finding in our study, that boys with deficient vitamin D levels had poorer outcomes on cognitive and social developmental tests, could be considered in the context of these findings, indicating boys' possible specific vulnerability to vitamin D deficiency.

Study III found no association between vitamin D and the KSPD P-M domain for both sexes. Possible reasons are: (1) gross motor development delay does not cause a difference in the amount of time spent outside (therefore the children get similar amounts of vitamin D by being exposed to sunlight); (2) low vitamin D levels do not affect gross motor development as they do cognitive and communication development; and/or (3) vitamin D metabolism does not differ between children with and without gross motor development problems. Since comorbidities between motor and cognitive/social developmental delays are common, it is necessary to confirm the role of vitamin D in different domains of child neurodevelopment.

Many studies have found that inadequate levels of vitamin D in early life negatively affect neurodevelopment and vitamin D supplementation may alleviate ASD symptoms, although efficacy of supplementation has been inconsistent. [22,25-28] The fact that fewer than 25% of the children in this study were in the vitamin D sufficient group and another 25% were in the deficient group might be seen to be alarming from a public health perspective, particularly for Japan where food is not systematically fortified with vitamin D as it is in the Nordic and North American countries.[12,13] Reconsideration of an appropriate amount of sun exposure and vitamin D supplementation/fortification could benefit Japanese children's overall health.

5.4 Study IV

The ESSENCE-Q was found to be valid in identifying children without NDDs. The low PPV for NDDs was possibly due to the low prevalence of reported NDDs at this early stage of life. Participants with missed responses (≥ 1) on ESSENCE-Q (n = 2,338), therefore excluded from our final analysis, were much fewer than those with missed responses on J-ASQ-3 (n = 6,507) in the same JECS questionnaire at age 2.5 year, indicating the usability of the ESSENCE-Q. This difference may derive from the fewer questions (11 vs 30) and non age-specific approach of the ESSENCE-Q. In addition, unlike most developmental tests,[21] it asks parents whether they have concerns on their child's development, instead of what their child can achieve, which may provide parents with a safer environment to respond candidly without pressuring them that their child is possibly behind their peers developmentally.

ESSENCE-Q achieved AUC ≥ 0.90 for overall NDDs and each NDD diagnosis; these values are similar to previous validations[11,13] and can be considered to be "excellent to outstanding".[22] The AUC in the study was much greater than those of two previous Japanese studies, regardless of who completed the questionnaire—mothers, public health nurses, or psychologists.[10,12]

The optimal cut off ≥ 3 obtained in the study is consistent with the results of some previous validation studies,[10,11] although a direct comparison is not possible mainly due to the ESSENCE-Q in the present study having only two response options (yes/no), rather than the original three. In addition, the sample (child age, cultural background) and settings (clinical, public health, health check-up at school) also differ from previous studies. [10,11] In this study, ROC analysis showed that, with a cut-off of ≥ 3, the ESSENCE-Q achieved high sensitivity (84.89%), specificity (84.83%), NPV (99.80%), and LR(-) (0.18; < 0.1 is considered "to provide strong evidence to rule out diagnoses")[23], even though the PPV was low highly likely due to the low prevalence at this stage of life.[24] Taking both the importance of early support for children with NDPs and the adequacy as well as efficiency in public health services into consideration, the lower cut-off of the ESSENCE-Q scores, such as the score ≥ 2 while compromising specificity, might be worth considering to safely rule out only those with highly-likely "no concerns of NDPs" at this early life stage.

Analysis of individual responses to each of the 11 ESSENCE-Q questions showed that a much higher percentage of parents/caregivers of children with NDDs had concerns regarding communication (89.5% vs 14.2%) and general development (80.2% vs 7.4%) than those in the non-NDD group. Feeding was

the most common concern (30.7%) in the non-NDD group, whereas the percentage endorsing feeding concerns (53.8%), despite being higher than that of non-NDD group, ranked fifth within the NDD group, after communication, general development, activity, and attention. In two previous studies on individual responses to ESSENCE-Q items, concerns regarding attention and mood were most common among the parents of 11-year-old Swedish children,[15] and communication, activity, attention, and social interaction were the most common concerns for preschool-age children (mean age 54.4 months) referred for neuropsychiatric assessment.[14] The differences between the current and previous studies can most likely be attributed to differences in children's age and study settings (general population vs. referred children), indicating that different problems surface within the same individual through their life course and that incorporating other factors, such as referral status and cultural context, is important. Nevertheless, further investigation on each item and the patterns of responses in relation to the total scores could be worthwhile. In our case, the results indicate that careful follow-up may be necessary for children whose total scores fall below the cut-off but where parental concerns exist in a particular domain (e.g. communication and general development).

Both ESSENCE-Q total scores and individual items were consistent with those of J-ASQ-3. The total scores of ESSENCE-Q were negatively correlated with those of J-ASQ-3, given that higher ESSENCE-Q scores indicate greater parental concern, whereas lower J-ASQ-3 scores indicate developmental delays. Parental concern regarding communication and general development in ESSENCE-Q were reflected in the communication domain in J-ASQ-3, as was motor development ESSENCE-Q concern in the J-ASQ-3 gross motor domain.

5.5 Strengths and Limitations

The strengths of all the studies lie in the design of JECS: a nationally representative very large dataset with prospectively collected information from the prenatal period until child age 3 years. The JECS Main Cohort basic characteristics were confirmed to be similar to those of Japan's 2013 Vital Statistics Survey, and the Sub-Cohort was not substantially different from the Main Cohort.[82,83] In particular, data of children's own serum vitamin D measured by LC-MS/MS and the standardized neurodevelopment test (KSPD), the most widely used in Japan for clinical and research settings, conducted by nationally trained JECS testers enabled us to thoroughly investigate the association between vitamin D and child neurodevelopment at age 2 (*Study II*). The J-ASQ-3, a validated developmental questionnaire, added strength to *Studies II* and *III*.

Five major limitations need to be taken into consideration, in addition to possible other non-genetic risk factors not included in the four studies, such as maternal alcohol intake and cigarette smoking during pregnancy. First, not all the NDDs were diagnosed at this young age and the NDD diagnosis information was based upon parent-completed questionnaire, not medical registry database. Most of the participants analysed in this PhD project were too young to receive a diagnosis except severe cases. More NDD diagnoses are highly likely to be given as they grow up and start going to school, particularly for ADHD and LD. The reported NDDs are unlikely to be overreported, because the question specifically asked about "diagnoses *by medical doctors*." Nonetheless, the possibility of underreporting cannot be completely excluded, given the stigma and secrecy that can accompany NDD diagnosis in Japan, particularly at this early stage of life (*Studies I and IV*).

Second, no genetic information was available, nor was parental NDD diagnosis included in the analyses for adjustment in the final models, even though it has been understood that genetic factors contribute to child NDDs in 60 - 80% of all cases (*Studies I, II*, and *III*).

Third, 25(OH)D2 concentration levels were truncated at the value < 4ng/mL, which hindered us from conducting more detailed investigation on the association between child 23(OH)D and neurodevelopment (*Study III*).

Fourth, since 22-28% of the JECS Main Cohort were excluded from the analyses after having applied the inclusion and exclusion criteria, the representativeness of the eligible participants within the JECS Main Cohort may not be fully guaranteed (*Studies I, II,* and *IV*). In *Study I*, mean maternal

age at delivery — one of the major characteristics as well as a risk factor for offspring's neurodevelopmental problems — of the included and excluded groups were 31.2 and 30.8 years respectively. The small discrepancy in maternal age suggests that it would be safe to assume that the two groups were similar to each other, and the eligible participants were representative of the JECS participants as a whole. On the contrary, the fact that those excluded in *Study IV* due to missing ESSENCE-Q responses had slightly higher prevalence of NDDs indicates that information bias cannot be eliminated with self-administered questionnaires.

Finally, as all the studies were observational studies, we were only able to speculate about possible mechanisms of the associations found and about causal relationships by rereferring to previous observational studies and experimental studies.

6 CONCLUSIONS

The studies included in this thesis found that:

I. Pre-/perinatal reduced optimality was associated, in a dose-dependent manner, with NDDs diagnosed in children at 3 years of age. Risk factors, except advanced maternal age and caesarean section delivery, vary for different NDDs. In addition, parental developmental concerns as early as 1 month after birth may be able to predict later diagnoses of NDDs.

II. Summer-born infants underperformed and winter-born infants overperformed as regard motor development at 6 months of age. The discrepancy decreased but the seasonal tendency remained at age 12 months. The results could be taken to indicate positive biometeorological effects of summer and negative effects of winter during not only the prenatal but also the postnatal period.

III. Boys with vitamin D deficiency (25(OH)D < 20 ng/mL) had neurodevelopmental delay as measured by the KSPD in all the domains except P-M. Serum 25(OH)D concentrations were measured only once around the second birthday, further studies are necessary to replicate the study results and to acquire longitudinal data before firm conclusions can be drawn about the role of vitamin D and possible need for extra supplementation in early child development.

IV. The ESSENCE-Q might be a useful tool, particularly for *screening out* neurotypically developing children at age 2.5 years. Concerns on communication and general development were high among the NDD group, indicating that the tool's accuracy might improve by assessing not only the total scores but also responses to each of the 11 items of the questionnaire.

Study I and *Study IV* utilised both the total scores and the responses to each of the individual items of some scales and questionnaires – the reduced optimality scale for pre-/perinatal periods, the 1-month developmental scale by parental observation, and the ESSENCE-Q. The total scores showed their utility in predicting future NDD risks and screening out for ongoing NDPs. Information from the individual items made it possible to investigate further regarding possible associations specific to particular NDDs, such as the shared prenatal

risks across different NDDs (e.g. advanced maternal age and caesarean section delivery). Similarly, the association between parental concerns at age 1 month on child's sensory and emotional regulation and 3-year ASD was significant, and the ESSENCE-Q items on communication and general development were more prevalent among the NDD group. The implication of these results is that some risk factors could be associated with particular NDDs and that some early manifestations could be signs for certain NDDs. Given that a wide variety of aetiologies exist behind even "specific" NDPs, and overlap is the rule, further investigation is required.

Birth month was associated with gross motor development at 6 and 12 months of age in the Main Study (*Study II*). However, it was not associated with the KSPD P-M domain among the SCS children (*Study III*). These results may indicate that gross motor developmental delay associated with birth month could "catch-up" as the children grow older. Nonetheless, further follow-up of children who showed motor development delays in early life is needed even if they catch up with their peers, as gross motor development delay is common among children with NDPs, and different NDPs may surface in the same individuals later in their life.

Finally, as the studies analysed findings from children in the JECS only up until 3 years of age, and considering the very low prevalence of reported NDDs, it is highly likely that more NDDs will be diagnosed as the cohort grows older. As different NDPs tend to surface at different stages of an individual's life, further follow-up on these children from the perspective of ESSENCE is crucial.

In summary, the present thesis found that delays in child neurodevelopment up to age 3 years were associated with pre-/perinatal non-optimal factors, seasonality, and at least in boys with vitamin D deficiency at age 2 years. The relevance of these findings is that a careful follow-up of children who have experienced adversities in the pre-/perinatal periods or who have very low serum 25(OH)D in early life is needed.

7 FUTURE PERSPECTIVES

Given that approximately10% of school children are estimated to have some form of ESSENCE, it is highly likely that the prevalence (1.1%) reported in the JECS at 3 years of age included only very severe cases and that more problems/diagnoses will be reported as the cohort grows up. From the 7-year JECS questionnaire, we ask parents/caregivers to provide us with the contacts of the medical professionals who had given the diagnosis of some common diseases including NDDs, to confirm and register the diagnoses with the JECS database. In addition, other NDDs which were not included in our analyses, such as ADHD, have been added to the JECS questionnaires from age 5 years. As of July 2023, the JECS children are between 8.5 years to 12 years, and the data sets are available up until age 4 years. Knowing that the number of children with NDDs is almost certainly going to increase, it is likely that the PPV of the ESSENCE-Q, which was very low in *Study IV*, will increase with age of children in the JECS.

The accuracy of NDD diagnosis information from the questionnaire was a major challenge throughout the four studies, as the information was based on parental response on the questionnaires. From the 7-year questionnaire, the contacts of the medical professionals who had given the diagnoses are also added in the questionnaire (ASD, ADHD, LD, DCD, and ID). This additional information is expected to improve the "validity" of a reported NDD diagnosis.

In addition to the questionnaire survey twice a year, the JECS has conducted a face-to-face examination of the 8-year-old children, with the aim to achieve 50% participation rate, and a similar follow-up examination has just started for 12-year-old children, which is expected to compensate the limitation of the questionnaire survey. Furthermore, the SCS (5% of the whole JECS participants) is also continuously followed up with clinical and neurodevelopmental examinations every two years.

In 2022, the Japanese Ministry of the Environment announced the extension of the follow-up period, from 13 years old to at least around age 40 years, and the acquisition of the participants' consent has just started in June 2023[67]. The consent will be acquired from the parents for now until their children turn 18, then another consent from the children has to be requested. This extension of follow-up period allows us to find more NDD cases for further investigation.

In Japan, a personalized identification number system "My Number System" has been introduced for tax and social security purposes since 2016, and the

Japanese government is currently encouraging the citizens and residents to link the My Numbers to national health insurance cards. In the long run, the JECS may be able to have access to health information similar to that of the DNBC and the MoBa studies.

Studies I, II, and *III* did not cover a wider range of non-genetic pre-/perinatal risk factors, other risk/beneficial factors, such as maternal alcohol intake during pregnancy, and those during periconceptional period, such as folic acid supplementation, but they could – and should – be included in future studies. Also, other environmental factors, such as chemical/heavy metal exposures, need to be incorporated for more holistic assessment of aetiology of NDDs/NDPs.

Considering that the heritability of NDDs is approximately 60-80%, parental NDDs/NDPs information must be included in more complete analyses. Unfortunately, all the analyses did not adjust for genetic factors, because parental history of NDD diagnosis was clearly underreported even though the JECS did ask participants about such factors. The underreporting could derive from the constantly-developing nature of diagnosis in this field which overlooked individuals with NDDs a generation ago, or/and from the stigma connected with NDD diagnosis in Japan – a country which tends to value uniformity and conformity. In the JECs, as genetic analyses using parents' blood sample are to be conducted, and more opportunities exist to make more detailed enquiry in person, in addition to the questionnaires, more holistic analyses could become possible to tackle the intricate mechanisms of aetiology of NDDs. Alternatively, the results from the Autism Spectrum Quotient (AQ-10), which was included in a JECS prenatal questionnaire about the parents, could be included in the future analysis as an alternative proxy for some genetic factors.

Sex differences regarding both susceptibility to environment and neurodevelopment were observed throughout the studies. At 6 and 12 months of age, boys outperformed girls in gross motor development, and girls seemed to be more "susceptible" to birth month as regards gross motor performance Interestingly, the gross motor domain was the only domain out of the five J-ASQ-3 domains in which boys outperformed girls, but that significant difference disappeared at age 2 when child neurodevelopment was assessed with the KSPD. In the SCS, girls significantly outperformed boys in all the domains even in the P-M domain at age 2 years, and more boys were given NDD diagnoses at age 3 years. It could be that girls' overall neurodevelopment exceeds that of boys in every way, including in the gross motor domain, but it is also possible that sex differences do exist regarding manifestations and

trajectories of NDDs, therefore, further research on sex differences in the susceptibility to environmental factors as well as in the field of NDDs is important.

The public health implications of the results of *Study IV* on the ESSENCE-Q are that the simple, cost-effective, and age-neutral questionnaire, completed by parents, could be a useful tool not only to *rule out* children without NDPs but also to holistically *monitor* individuals with possible NDPs in the long run.

Since the introduction of the Act on Support for Persons with Developmental Disabilities in 2004, the support in the Japanese society for individuals with NDPs/NDDs, including among municipal, medical, and educational organisations, has been progressing. Concurrently, the importance of early detection and early intervention has started to be increasingly recognised from various perspectives – from the individual's well-being to the reduction of medical/societal costs by preventing possible further problems caused if individuals with NDPs/NDDs are left unsupported.

The Ministry of Health, Labour, and Welfare reported (2009) that each Japanese municipality started to incorporate more careful assessment regarding child neurodevelopment in the current well-child check-ups at age 1.5 and 3 years all over the country, while aiming not to make parents feel "judged" about their parenting but to make them feel "heard" regarding their concerns,[138] which, the ESSENCE-Q is all about. Health and education resources to support children with NDPs/NDDs in Japan are still very limited and usually most parents need to wait to see a specialist for NDD diagnosis when a routine check-up raises a flag. Therefore, the most important of the Wilson and Jungner criteria for screening have not been met in most parts of the country, and screening procedures need to be carefully reviewed and implemented.[139]

Despite this slowly developing situation in Japan, and probably in many other countries, the ESSENCE-Q is likely to provide a "safe" environment for parents to express concerns about their child's development, and to help public health nurses interview children in a structured way as earlier studies have identified.[55,56]

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REFERENCES

1. Gillberg C. The ESSENCE of Autism and Other Neurodevelopmental Conditions. Jessica Kingsley Publishers; 2021:141.
2. Dinkler L, Yasumitsu-Lovell K, Eitoku M, Fujieda M, et al. Development of a parent-reported screening tool for avoidant/restrictive food intake disorder (ARFID): Initial validation and prevalence in 4-7-year-old Japanese children. *Appetite* 2022;168:105735.
3. Ministry of Education, Science, and Technology (MEXT). Survey on children with special educational needs enrolled in regular classes. Accessed 9 July, 2023 https://www.mext.go.jp/content/20230524-mext-tokubetu01-000026255_01.pdf (In Japanese)
4. Magan-Maganto M, Bejarano-Martin A, Fernandez-Alvarez C, Narzisi A, et al. Early Detection and Intervention of ASD: A European Overview. *Brain Sciences* 2017;7(12):_159.
5. Harris J. Leo Kanner and autism: a 75-year perspective. *International Review of Psychiatry* 2018;30(1):3-17.
6. Gillberg C. The ESSENCE in child psychiatry: Early Symptomatic Syndromes Eliciting Neurodevelopmental Clinical Examinations. *Research in Developmental Disabilities* 2010;31(6):1543-1551.
7. Gómez-Vallejo S, Leoni M, Ronald A, Colvert E, et al. Autism spectrum disorder and obstetric optimality: a twin study and meta-analysis of sibling studies. *Journal of Child Psychology and Psychiatry* 2021;62(11):1353-1362.
8. Han VX, Patel S, Jones HF, Dale RC. Maternal immune activation and neuroinflammation in human neurodevelopmental disorders. *Nature Reviews Neurology* 2021;17(9):564- 579.
9. Bilder DA, Pinborough-Zimmerman J, Bakian AV, Miller JS, et al. Prenatal and perinatal factors associated with intellectual disability. *American Journal on Intellectual and Developmental Disabilities* 2013;118(2):156-176.
10. Barker DJ, Winter PD, Osmond C, Margetts B. Weight in infancy and death from ischaemic heart disease. *Lancet* 1989;2(8663):577-580.
11. Dodds L, Fell DB, Shea S, Armson BA. The role of prenatal, obstetric and neonatal factors in the development of autism. *Journal of Autism and Developmental Disorders* 2011;41(7):891-902.
12. Gillberg C, Gillberg C, Steffenburg S. Reduced optimality in the pre-, peri-, and neonatal periods is not equivalent to severe peri- or neonatal risk: a rejoinder to Goodman's technical note. *Journal of Child Psychology and Psychiatry* 1990;31(5):813-815.
13. Gillberg C, Enerskog I, Johansson SE. Mental retardation in urban children: a population study of reduced optimality in the pre-, peri- and

neonatal periods. *Developmental Medicine and Child Neurology* 1990;32(3):230-237.
14. Gillberg C, Gillberg IC. Infantile autism: a total population study of reduced optimality in the pre-, peri-, and neonatal period. *Journal of Autism and Developmental Disorders* 1983;13(2):153-166.
15. Han VX, Patel S, Jones HF, Nielsen TC, et al. Maternal acute and chronic inflammation in pregnancy is associated with common neurodevelopmental disorders: a systematic review. *Translational Psychiatry* 2021;11(1):71.
16. Veena SR, Krishnaveni GV, Srinivasan K, Thajna KP, et al. Association between maternal vitamin D status during pregnancy and offspring cognitive function during childhood and adolescence. *Asia Pacific Journal* of *Clinical Nutrition* 2017;26(3):438-449.
17. Cortés-Albornoz MC, García-Guáqueta DP, Velez-van-Meerbeke A, Talero-Gutiérrez C. Maternal Nutrition and Neurodevelopment: A Scoping Review. *Nutrients* 2021;13(10):3530.
18. Veena SR, Gale CR, Krishnaveni GV, Kehoe SH, et al. Association between maternal nutritional status in pregnancy and offspring cognitive function during childhood and adolescence; a systematic review. *BMC Pregnancy and Childbirth* 2016;16:220.
19. Lindstrand A, Ek M, Kvarnung M, Anderlid BM, et al. Genome sequencing is a sensitive first-line test to diagnose individuals with intellectual disability. *Genetics in Medicine* 2022;24(11):2296-2307.
20. Rolland T, Cliquet F, Anney RJL, Moreau C, et al. Phenotypic effects of genetic variants associated with autism. *Nature Medicine* 2023;29(7):1671-1680.
21. Coleman M, Gillberg C. *The Autisms*. 4 ed. Oxford University Press; 2012.
22. Engman ML, Sundin M, Miniscalco C, Westerlund J, et al. Prenatal acquired cytomegalovirus infection should be considered in children with autism. *Acta Paediatrica* 2015;104(8):792-795.
23. Gardener H, Spiegelman D, Buka SL. Perinatal and neonatal risk factors for autism: a comprehensive meta-analysis. *Pediatrics* 2011;128(2):344-355.
24. Gardener H, Spiegelman D, Buka SL. Prenatal risk factors for autism: comprehensive meta-analysis. *British Journal of Psychiatry* 2009;195(1):7-14.
25. Kim JY, Son MJ, Son CY, Radua J, et al. Environmental risk factors and biomarkers for autism spectrum disorder: an umbrella review of the evidence. *Lancet Psychiatry.* 2019;6(7):590-600.
26. Alterman N, Johnson S, Carson C, Petrou S, et al. Gestational age at birth and child special educational needs: a UK representative birth cohort study. *Archives of Disease in Childhood* 2021;106(9):842-848.

27. Örtqvist M, Einspieler C, Ådén U. Early prediction of neurodevelopmental outcomes at 12 years in children born extremely preterm. *Pediatric Research* 2022;91(6):1522-1529.
28. Robertsson Grossmann K, Eriksson Westblad M, Blennow M, Lindström K. Outcome at early school age and adolescence after hypothermia-treated hypoxic-ischaemic encephalopathy: an observational, population-based study. *Archives of Disease in Childhood, Fetal and Neonatal Edition* 2023;108(3):295-301.
29. Shaffer RM, Sellers SP, Baker MG, de Buen Kalman R, et al. Improving and expanding estimates of the global burden of disease due to environmental health risk factors. *Environmental Health Perspectives* 2019;127(10):105001.
30. Hoyme HE, Kalberg WO, Elliott AJ, Blankenship J, et al. Updated Clinical Guidelines for Diagnosing Fetal Alcohol Spectrum Disorders. *Pediatrics* 2016;138(2):e20154256.
31. Antonucci R, Locci C, Clemente MG, Chicconi E, Antonucci L. Vitamin D deficiency in childhood: old lessons and current challenges. *Journal of Pediatric Endocrinology and Metabolism* 2018;31(3):247-260.
32. Charoenngam N, Holick MF. Immunologic Effects of Vitamin D on Human Health and Disease. *Nutrients* 2020;12(7):2097.
33. Cui X, Eyles DW. Vitamin D and the Central Nervous System: Causative and Preventative Mechanisms in Brain Disorders. *Nutrients* 2022;14(20):4353.
34. Gáll Z, Székely O. Role of Vitamin D in Cognitive Dysfunction: New Molecular Concepts and Discrepancies between Animal and Human Findings. *Nutrients* 2021;13(11):3672.
35. Voltas N, Canals J, Hernández-Martínez C, Serrat N, et al. Effect of Vitamin D Status during Pregnancy on Infant Neurodevelopment: The ECLIPSES Study. *Nutrients* 2020;12(10):3196.
36. Wang H, Yu XD, Huang LS, Chen Q, et al. Fetal vitamin D concentration and growth, adiposity and neurodevelopment during infancy. *European Journal of Clinical Nutrition* 2018;72(10):1396-1403.
37. Braithwaite VS, Crozier SR, D'Angelo S, Prentice A, et al. The Effect of Vitamin D Supplementation on Hepcidin, Iron Status, and Inflammation in Pregnant Women in the United Kingdom. *Nutrients* 2019;11(1):190.
38. Wicklow B, Gallo S, Majnemer A, Vanstone C, et al. Impact of Vitamin D Supplementation on Gross Motor Development of Healthy Term Infants: A Randomized Dose-Response Trial. *Physical and Occupational Therapy in Pediatrics* 2016;36(3):330-342.

39. Boland MR, Shahn Z, Madigan D, Hripcsak G, et al. Birth month affects lifetime disease risk: a phenome-wide method. *Journal* of the *American Medical Informatics Association* 2015;22(5):1042-1053.
40. Procopio M, Marriott PK, Davies RJ. Seasonality of birth in epilepsy: a Southern Hemisphere study. *Seizure* 2006;15(1):17-21.
41. Gillberg C. Do children with autism have March birthdays? *Acta Psychiatrica Scandinavica* 1990;82(2):152-156.
42. Landau EC, Cicchetti DV, Klin A, Volkmar FR. Season of birth in autism: a fiction revisited. *Journal of Autism and Developmental Disorders* 1999;29(5):385-393.
43. Mackay DF, Smith GC, Cooper SA, Wood R, et al. Month of Conception and Learning Disabilities: A Record-Linkage Study of 801,592 Children. *American Journal of Epidemiology* 2016;184(7):485-493.
44. Gadow KD, DeVincent CJ. Comparison of children with autism spectrum disorder with and without schizophrenia spectrum traits: gender, season of birth, and mental health risk factors. *Journal of Autism and Developmental Disorders* 2012;42(11):2285-2296.
45. Disanto G, Morahan JM, Lacey MV, DeLuca GC, et al. Seasonal distribution of psychiatric births in England. *PLoS One* 2012;7(4):e34866.
46. McGrath JJ, Saha S, Lieberman DE, Buka S. Season of birth is associated with anthropometric and neurocognitive outcomes during infancy and childhood in a general population birth cohort. *Schizophrenia Research* 2006;81(1):91-100.
47. Stevens MC, Fein DH, Waterhouse LH. Season of birth effects in autism. *Journal of Clinical and Experimental Neuropsychology* 2000;22(3):399-407.
48. Shalev H, Solt I, Chodick G. Month of birth and risk of autism spectrum disorder: a retrospective cohort of male children born in Israel. *BMJ Open* 2017;7(11):e014606.
49. Lee BK, Gross R, Francis RW, Karlsson H, et al. Birth seasonality and risk of autism spectrum disorder. *European Journal of Epidemiology* 2019;34(8):785-792.
50. Glasson EJ, Bower C, Petterson B, de Klerk N, et al. Perinatal factors and the development of autism: a population study. *Archives of General Psychiatry* 2004;61(6):618-627.
51. Haglund NG, Källen KB. Risk factors for autism and Asperger syndrome. Perinatal factors and migration. *Autism* 2011;15(2):163-183.
52. Thompson L, Kemp J, Wilson P, Pritchett R, et al. What have birth cohort studies asked about genetic, pre- and perinatal exposures and child and adolescent onset mental health outcomes? A systematic review. *European Child & Adolescent Psychiatry* 2010;19(1):1-15.

53. Yue W, Zhang E, Liu R, Zhang Y, et al. The China birth cohort study (CBCS). European Journal of Epidemiology 2022;37(3):295-304.
54. Gillberg Neuropsychiatry Centre. University of Gothenburg. ESSENCE-Q screening questionnaire. Accessed 29 July, 2023 https://www.gu.se/en/gnc/gncs-resources/screening-questionnaires/essence-q-screening-questionnaire
55. Hatakenaka Y, Maeda M, Ninomiya H, Hachiya K, et al. ESSENCE-Q obtained in routine Japanese public child health check-ups may be a valuable tool in neurodevelopmental screening. *Acta Paediatrica* 2020;109(4):764-773.
56. Hatakenaka Y, Ninomiya H, Billstedt E, Fernell E, et al. ESSENCE-Q – used as a screening tool for neurodevelopmental problems in public health checkups for young children in south Japan. *Neuropsychiatric Disease and Treatment* 2017;13:1271-1280.
57. Hatakenaka Y, Fernell E, Sakaguchi M, Ninomiya H, et al. ESSENCE-Q – a first clinical validation study of a new screening questionnaire for young children with suspected neurodevelopmental problems in south Japan. *Neuropsychiatric Disease and Treatment* 2016;12:1739-1746.
58. Cederlund M. Parent Questionnaires in the Evaluation of Pre-School Children Referred for Neuropsychiatric Assessment. *Journal of Autism and Developmental Disorders* 2022;52(4):1742-1751.
59. Stevanovic D, Knez R, Zorcec T, Anderluh M, et al. ESSENCE-Q: Slavic language versions for developmental screening in young children. *Neuropsychiatric Disease and Treatment* 2018;14:2141-2148.
60. Wechsler Preschool and Primary Scale of Intelligence | Fourth Edition (WPPSI-IV). Accessed 20 July, 2023.
61. Alan S. Kaufman SER, Diane L. Coalson. Intelligent Testing with the WISC-V. Wiley; 2016.
62. Robins DL, Casagrande K, Barton M, Chen CM, 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.
63. Landgren V, Svensson L, Knez R, Theodosiou M, et al. The ESSENCE-Questionnaire for Neurodevelopmental Problems – A Swedish School-Based Validation Study in 11-Year-Old Children. *Neuropsychiatric Disease and Treatment* 2022;18:2055-2067.
64. Ozonoff S. Editorial: Early detection of mental health and neurodevelopmental disorders: the ethical challenges of a field in its infancy. *Journal of Child Psychology and Psychiatry* 2015;56(9):933-935.
65. Canova C, Cantarutti A. Population-Based Birth Cohort Studies in Epidemiology. *International Journal of Environmental Research* and *Public Health* 2020;17(15):5276.

66. Sweeney S. Born to fail: The rise and fall of birth cohort studies. *TLS. Times Literary Supplement*, no. 5948, 2017, pp. 28+. Accessed 29 July, 2023.
67. Group HaEESW. Report of the Health and Environment Epidemiological Study Working Group The Future of Japan Environment and Children's Study (JECS). Accessed 29 July, 2023 (https://www.env.go.jp/content/000128616.pdf)
68. Floride M. 1997 declaration of the environment leaders of the Eight on children's environmental health. *Canadian Journal of Public Health* 1998;89 Suppl 1(Suppl 1):S5-8, s5-9.
69. Olsen J, Melbye M, Olsen SF, Sørensen TI, et al. The Danish National Birth Cohort--its background, structure and aim. *Scandinavian Journal of Public Health* 2001;29(4):300-307.
70. Danish National Birth Cohort. Accessed 29 July, 2023 https://www.dnbc.dk
71. Magnus P, Birke C, Vejrup K, Haugan A, et al. Cohort Profile Update: The Norwegian Mother and Child Cohort Study (MoBa). *International Journal of Epidemiology* 2016;45(2):382-388.
72. Magnus P, Irgens LM, Haug K, Nystad W, et al. Cohort profile: the Norwegian Mother and Child Cohort Study (MoBa). *International Journal of Epidemiology* 2006;35(5):1146-1150.
73. Takagai S, Tsuchiya KJ, Itoh H, Kanayama N, et al. Cohort Profile: Hamamatsu Birth Cohort for Mothers and Children (HBC Study). *International Journal of Epidemiology* 2016;45(2):333-342.
74. Kawamoto T, Nitta H, Murata K, Toda E, et al. Rationale and study design of the Japan environment and children's study (JECS). *BMC Public Health* 2014;14:25.
75. Group HaEESW. Report of the Health and Environment Epidemiological Study Working Group. The Future of Japan Environment and Children's Study (JEC). Accessed 29 July, 2023, https://www.env.go.jp/content/000128616.pdf (in Japanese)
76. Mezawa H, Aoki S, Nakayama SF, Nitta H, et al. Psychometric profile of the Ages and Stages Questionnaires, Japanese translation. *Pediatrics International: Official Journal of the Japan Pediatric Society* 2019;61(11):1086-1095.
77. Hatakeyama T, Matsumura K, Tsuchida A, Inadera H. Factor structure of the Parenting Stress Index-Short Form used in the Japan Environment and Children's Study. *Scientific Reports* Nov 9 2022;12(1):19123.
78. Aoki S, Hashimoto K, Mezawa H, Hatakenaka Y, et al. Development of a new screening tool for neuromotor development in children aged two – the neuromotor 5 min exam 2-year-old version (N5E2). *Brain & Development* 2018;40(6):445-451.

79. Kamio Y. Early detection of and diagnostic tools for Asperger's disorder. *Nihon Rinsho Japanese Journal of Clinical Medicine.* 2007;65(3):477-80.
80. Dinkler L, Yasumitsu-Lovell K, Eitoku M, Fujieda M, et al. Early neurodevelopmental problems and risk for avoidant/restrictive food intake disorder (ARFID) in 4-7-year-old children: A Japanese birth cohort study. *JCPP Advances* 2022;2(3):e12094.
81. Hashimoto K, Yasumura S, Fujimori K, Kyozuka H, et al. The Japan Environment and Children's Study (JECS) in Fukushima Prefecture-A progress report on the enrollment stage. *Fukushima Journal of Medical Science* 2017;63(2):57-63.
82. Michikawa T, Nitta H, Nakayama SF, Yamazaki S, et al. Baseline Profile of Participants in the Japan Environment and Children's Study (JECS). *Journal of Epidemiology* 2018;28(2):99-104.
83. Sekiyama M, Yamazaki S, Michikawa T, Nakayama S, et al. Study Design and Participants' Profile in the Sub-Cohort Study in the Japan Environment and Children's Study (JECS). *Journal of Epidemiology* 2022;32(5):228-236.
84. Williams CJ, Kessler D, Fernyhough C, Lewis G, et al. The association between maternal-reported responses to infant crying at 4 weeks and 6 months and offspring depression at 18: a longitudinal study. *Archives of Women's Mental Health* 2016;19(2):401-408.
85. Yasumitsu-Lovell K, Thompson L, Fernell E, Eitoku M, et al. Birth month and infant gross motor development: Results from the Japan Environment and Children's Study (JECS). *PLoS One*.2021;16(5):e0251581.
86. Aoki S, Hashimoto K, Ikeda N, Takekoh M, et al. Comparison of the Kyoto Scale of Psychological Development 2001 with the parent-rated Kinder Infant Development Scale (KIDS). *Brain & Development* 2016;38(5):481-490.
87. Koyama T, Osada H, Tsujii H, Kurita H. Utility of the Kyoto Scale of Psychological Development in cognitive assessment of children with pervasive developmental disorders. *Psychiatry and Clinical Neurosciences* 2009;63(2):241-243.
88. Tatsuta N, Murata K, Iwai-Shimada M, Yaginuma-Sakurai K, et al. Psychomotor Ability in Children Prenatally Exposed to Methylmercury: The 18-Month Follow-Up of Tohoku Study of Child Development. *Tohoku Journal of Experimental Medicine* 2017;242(1):1-8.
89. Ikuzawa M, Matsushita Y. Nakase A. Kyoto Scale of Psychological Development 2001. Kyoto International Social Welfare Exchange Centre.
90. Prechtl HF. The optimality concept. *Early Human Development* 1980;4(3):201-205.

91. Yang L, Sato M, Saito-Abe M, Irahara M, et al. 25-Hydroxyvitamin D levels among 2-year-old children: findings from the Japan environment and Children's study (JECS). *BMC Pediatrics* 2021;21(1):539.
92. Holick MF. Vitamin D status: measurement, interpretation, and clinical application. *Annals of Epidemiology* 2009;19(2):73-78.
93. Chen W, Qian L, Shi J, Franklin M. Comparing performance between log-binomial and robust Poisson regression models for estimating risk ratios under model misspecification. *BMC Medical Research Methodology* 2018;18(1):63.
94. Yelland LN, Salter AB, Ryan P. Performance of the modified Poisson regression approach for estimating relative risks from clustered prospective data. *American Journal of Epidemiology* 2011;174(8):984-992.
95. Zou G. A modified poisson regression approach to prospective studies with binary data. *American Journal of Epidemiology* 2004;159(7):702-706.
96. Bilder D, Pinborough-Zimmerman J, Miller J, McMahon W. Prenatal, perinatal, and neonatal factors associated with autism spectrum disorders. *Pediatrics* 2009;123(5):1293-1300.
97. Guinchat V, Thorsen P, Laurent C, Cans C, et al. Pre-, peri- and neonatal risk factors for autism. *Acta Obstetricia et Gynecologica Scandinavica* 2012;91(3):287-300.
98. Rieske RD, Matson JL. Parental Age at Conception and the Relationship with Severity of Autism Symptoms. *Developmental Neurorehabilitation* 2020;23(5):265-270.
99. Janecka M, Hansen SN, Modabbernia A, Browne HA, et al. Parental age and differential estimates of risk for neuropsychiatric disorders: Findings from the Danish Birth Cohort. *Journal of the American Academy of Child and Adolescent Psychiatry* 2019;58(6):618-627.
100. Wang C, Geng H, Liu W, Zhang G. Prenatal, perinatal, and postnatal factors associated with autism: A meta-analysis. *Medicine* 2017;96(18):e6696.
101. Sandin S, Schendel D, Magnusson P, Hultman C, et al. Autism risk associated with parental age and with increasing difference in age between the parents. *Molecular Psychiatry* 2016;21(5):693-700.
102. Quinlan CA, McVeigh KH, Driver CR, Govind P, et al. Parental age and autism spectrum disorders among New York City children 0-36 months of Age. *Maternal and Child Health Journal* 2015;19(8):1783-1790.
103. D'Onofrio BM, Rickert ME, Frans E, Kuja-Halkola R, et al. Paternal age at childbearing and offspring psychiatric and academic morbidity. *JAMA Psychiatry* 2014;71(4):432-438.

104. Parner ET, Baron-Cohen S, Lauritsen MB, Jørgensen M, et al. Parental age and autism spectrum disorders. *Annals of Epidemiology* 2012;22(3):143-150.
105. Kinzler WL, Ananth CV, Smulian JC, Vintzileos AM. Parental age difference and adverse perinatal outcomes in the United States. *Paediatric and Perinatal Epidemiology* 2002;16(4):320-327.
106. Eriksson MA, Westerlund J, Anderlid BM, Gillberg C, et al. First-degree relatives of young children with autism spectrum disorders: some gender aspects. *Research in Developmental Disabilities* 2012;33(5):1642-1648.
107. Gillberg C. Maternal age and infantile autism. *Journal of Autism and Developmental Disorders*. 1980;10(3):293-7.
108. Gillberg C, Rasmussen P, Wahlström J. Neuropsychiatric problems among children born to older mothers. *Acta Paedopsychiatrica*. Sep 1980;46(1-2):57-65.
109. da Silva GAP, da Cunha A. Environment and child health. *Jornal de Pediatria (Rio J)*. 2022;98 Suppl 1:S1-S3.
110. Qiu C, Lin JC, Shi JM, Chow T, et al. Association between epidural analgesia during labor and risk of autism spectrum disorders in offspring. *JAMA Pediatrics* 2020;174(12):1168-1175.
111. Wall-Wieler E, Bateman BT, Hanlon-Dearman A, Roos LL, et el. Association of epidural labor analgesia with offspring risk of autism spectrum disorders. *JAMA Pediatrics* 2021; 75(7):698-705.
112. Mikkelsen AP, Greiber IK, Scheller NM, Lidegaard Ø. Association of labor epidural analgesia with autism spectrum disorder in children. *JAMA* 2021;326(12):1170-1177.
113. McKeen DM, Zaphiratos V. Lack of evidence that epidural pain relief during labour causes autism spectrum disorder: a position statement of the Canadian Anesthesiologists' Society. *Canadian Journal of Anesthesia* 2021;68(2):180-182.
114. Glezerson BA, Trivedi V, McIsaac DI. On the stated association between labour epidural analgesia and risk of autism spectrum disorder in offspring. *Canadian Journal of Anesthesia* 2021;68(3):428-429.
115. Saito M, Nakamura K, Hirota K. Concerns for labor analgesia and autism spectrum disorders. *Journal of Anesthesia* 2021;35(2):319-320.
116. Volpe JJ. Overview: normal and abnormal human brain development. *Mental Retardation and Developmental Disabilities Research Reviews* 2000;6(1):1-5.
117. Lagercrantz H. Key milestones in the history of developmental brain research. *Acta Paediatrica* 2021;110(6):1722-1729.
118. Bai Y, Shang G, Wang L, Sun Y, et al. The relationship between birth season and early childhood development: Evidence from northwest rural China. *PLoS One* 2018;13(10):e0205281.

119. Tsuchiya KJ, Tsutsumi H, Matsumoto K, Takei N, et al. Seasonal variations of neuromotor development by 14 months of age: Hamamatsu Birth Cohort for mothers and children (HBC Study). *PLoS One* 2012;7(12):e52057.
120. Benson JB. Season of birth and onset of locomotion: Theoretical and methodological implications. *Infant Behavior and Development* 1993;16(1):69-81.
121. Bartlett DJ. The influence of geographic region on the seasonality of early motor development. *Infant Behavior and Development* 1998;21(4):591-601.
122. Sandman CA. Mysteries of the human fetus revealed. *Monographs of the Society for Research in Child Dev*elopment 2015;80(3):124-137.
123. Said-Mohamed R, Pettifor JM, Norris SA. Life History theory hypotheses on child growth: Potential implications for short and long-term child growth, development and health. *American Journal of Physical Anthropology* 2018;165(1):4-19.
124. Selevan SG, Kimmel CA, Mendola P. Identifying critical windows of exposure for children's health. *Environmental Health Perspectives* 2000;108 Suppl 3:451-455.
125. Whitehouse AJ, Holt BJ, Serralha M, Holt PG, et al. Maternal serum vitamin D levels during pregnancy and offspring neurocognitive development. *Pediatrics* 2012;129(3):485-493.
126. Kocovska E, Fernell E, Billstedt E, Minnis H, Gillberg C. Vitamin D and autism: clinical review. *Research in Developmental Disabilities* 2012;33(5):1541-1550.
127. Tous M, Villalobos M, Iglesias L, Fernandez-Barres S, et al. Vitamin D status during pregnancy and offspring outcomes: a systematic review and meta-analysis of observational studies. *European Journal of Clinical Nutrition* 2020;74(1):36-53.
128. Tavakolizadeh R, Ardalani M, Shariatpanahi G, Mojtahedi SY, et al. Is There Any Relationship between Vitamin D Deficiency and Gross Motor Development in 12-Month-Old Children? *Iranian Journal of Child Neurology* 2019;13(3):55-60.
129. Filteau S, Rehman AM, Yousafzai A, Chugh R, et al. Associations of vitamin D status, bone health and anthropometry, with gross motor development and performance of school-aged Indian children who were born at term with low birth weight. *BMJ Open* 2016;6(1):e009268.
130. Ando E, Morisaki N, Asakura K, Sasaki S, et al. Serum 25-hydroxyvitamin D levels showed strong seasonality but lacked association with vitamin D intake in 3-year-old Japanese children. *British Journal of Nutrition* 2018;120(9):1034-1044.
131. Ayabe T, Yamamoto-Hanada K, Mezawa H, Konishi M, et al. Regional differences in infant 25-Hydroxyvitamin D: Pilot study of the Japan

Environment and Children's Study. *Pediatrics International* 2018;60(1):30-34.
132. Borren I, Tambs K, Gustavson K, Schjølberg S, et al. Early prenatal exposure to pandemic influenza A (H1N1) infection and child psychomotor development at 6months – A population-based cohort study. *Early Human Development* 2018;122:1-7.
133. Fombonne E. Epidemiology of autistic disorder and other pervasive developmental disorders. *Journal of Clinical Psychiatry* 2005;66 Suppl 10:3-8.
134. Andersson GW, Gillberg C, Miniscalco C. Pre-school children with suspected autism spectrum disorders: do girls and boys have the same profiles? *Research in Developmental Disabilities* 2013;34(1):413-422.
135. Zerbo O, Iosif AM, Delwiche L, Walker C, et al. Month of conception and risk of autism. *Epidemiology* 2011;22(4):469-475.
136. Hebert KJ, Miller LL, Joinson CJ. Association of autistic spectrum disorder with season of birth and conception in a UK cohort. *Autism Research* 2010;3(4):185-190.
137. Fernell E, Barnevik-Olsson M, Bågenholm G, Gillberg C, et al. Serum levels of 25-hydroxyvitamin D in mothers of Swedish and of Somali origin who have children with and without autism. *Acta Paediatrica* 2010;99(5):743-747.
138. Ministry of Health Labour, and Welfare. Research findings on screening and early support for developmental disorders related to infant health check-up. Ministry of Health, Labour, and Welfare. Accessed 25 July, 2023 https://www.mhlw.go.jp/bunya/kodomo/boshi-hoken15/dl/15.pdf (in Japanese)
139. Wilson JMG, Jungner G. Principles and practice of screening for disease. World Health Organization. Geneva: World Health Organization;1968. Accessed 25 July, 2023 https://apps.who.int/iris/handle/10665/37650

APPENDIX

ESSENCE-Q questionnaire (*Study IV*)

ESSENCE-Q
in the JECS 2.5-year-questionnaire

Have you (or anybody else) been concerned for more than a few months regarding child's development below?

		Yes	No
1	General Development	☐	☐
2	Motor Development	☐	☐
3	Sensory reactions (e.g. touch, sound, light, smell, taste, heat, cold, pain)	☐	☐
4	Communication/language/babble	☐	☐
5	Activity (overactivity/passivity) or impulsivity	☐	☐
6	Attention/concentration/"listening"	☐	☐
7	Social interaction/interest in other children	☐	☐
8	Behaviour (e.g. repetitive, routine insistence)	☐	☐
9	Mood (depressed, elated/manic, extreme irritability, crying spells)	☐	☐
10	Sleep	☐	☐
11	Feeding	☐	☐

Supplementary Tables and Figures

Study III

Table S 4.3.1 General characteristics of the participants by 25(OH)D status

	Total	25 (OH) D (ng/mL)							KSPD DQ											
		< 20		≥ 20 & < 30				p	P-M < 70		p	C-A < 70		p	L-S < 70		p	TOTAL < 70		p
	(n = 4,653)	(n = 1,148, 24.7%)		(n = 2,385, 51.3%)		(n = 1,120, 24.1%)			(n = 306, 6.6%)			(n = 204, 4.4%)			(n = 299, 6.4%)			(n = 188, 4.0%)		
Gender								**<0.001**			0.170			0.178			**<0.001**			0.062
Boys	2,363	541	(22.9)	1,196	(50.6)	626	(26.5)		167	(7.1)		113	(4.8)		207	(8.8)		108	(4.6)	
Girls	2,290	607	(26.5)	1,189	(51.9)	494	(21.6)		139	(6.1)		91	(4.0)		92	(4.0)		80	(3.5)	
Test month								**<0.001**			0.341			0.223			0.439			0.216
January	230	129	(56.1)	78	(33.9)	23	(10.0)		11	(4.8)		7	(3.0)		13	(5.7)		6	(2.6)	
February	230	141	(61.3)	81	(35.2)	8	(3.5)		16	(7.0)		11	(4.8)		15	(6.5)		11	(4.8)	
March	270	143	(53.0)	118	(43.7)	9	(3.3)		10	(3.7)		8	(3.0)		12	(4.4)		5	(1.9)	
April	314	102	(32.5)	160	(51.0)	52	(16.6)		21	(6.7)		20	(6.4)		22	(7.0)		16	(5.1)	
May	401	105	(26.2)	245	(61.1)	51	(12.7)		32	(8.0)		21	(5.2)		30	(7.5)		21	(5.2)	
June	497	91	(18.3)	288	(57.9)	118	(23.7)		44	(8.9)		26	(5.2)		28	(5.6)		25	(5.0)	
July	504	54	(10.7)	310	(61.5)	140	(27.8)		32	(6.3)		24	(4.8)		39	(7.7)		21	(4.2)	
August	429	24	(5.6)	194	(45.2)	211	(49.2)		27	(6.3)		13	(3.0)		25	(5.8)		13	(3.0)	
September	494	40	(8.1)	239	(48.4)	215	(43.5)		36	(7.3)		22	(4.5)		32	(6.5)		21	(4.3)	
October	473	45	(9.5)	256	(54.1)	172	(36.4)		33	(7.0)		29	(6.1)		33	(7.0)		26	(5.5)	
November	395	108	(27.3)	225	(57.0)	62	(15.7)		20	(5.1)		11	(2.8)		20	(5.1)		10	(2.5)	
December	256	119	(46.5)	110	(43.0)	27	(10.5)		18	(7.0)		11	(4.3)		25	(9.8)		12	(4.7)	
Latitude								**<0.001**			0.377			0.817			0.571			0.929
≥ 25˚N, < 30˚N (Okinawa)	40	1	(2.5)	25	(62.5)	14	(35.0)		4	(10.0)		2	(5.0)		3	(7.5)		1	(2.5)	
≥ 30˚N, < 40˚N (Main island)	4,253	1,006	(23.7)	2,198	(51.7)	1,049	(24.7)		283	(6.7)		188	(4.4)		269	(6.3)		174	(4.1)	
≥ 40˚N, < 45˚N (Hokkaido)	360	141	(39.2)	162	(45.0)	57	(15.8)		19	(5.3)		14	(3.9)		27	(7.5)		13	(3.6)	
SGA								**0.009**			**0.016**			**0.017**			0.085			**0.001**
Yes	611	176	(28.8)	280	(45.8)	155	(25.4)		54	(8.8)		38	(6.2)		49	(8.0)		40	(6.5)	
No	4,042	972	(24.0)	2,105	(52.1)	965	(23.9)		252	(6.2)		166	(4.1)		250	(6.2)		148	(3.7)	
Gestational week								0.050			0.070			0.179			0.446			0.098
<37 weeks	208	66	(31.7)	99	(47.6)	43	(20.7)		20	(9.6)		13	(6.3)		16	(7.7)		13	(6.3)	
≥37 & <42 weeks	4,445	1,082	(24.3)	2,286	(51.4)	1,077	(24.2)		286	(6.4)		191	(4.3)		283	(6.4)		175	(3.9)	
Maternal age at birth								**0.030**			0.187			0.077			0.081			0.071
<20 years old	14	5	(35.7)	8	(57.1)	1	(7.1)		2	(14.3)		2	(14.3)		3	(21.4)		2	(14.3)	
≥20 & <35 years old	3,119	794	(25.5)	1,608	(51.6)	717	(23.0)		196	(6.3)		128	(4.1)		195	(6.3)		118	(3.8)	
≥35 years old	1,519	349	(23.0)	768	(50.6)	402	(26.5)		108	(7.1)		74	(4.9)		100	(6.6)		68	(4.5)	
Daycare attendance								**<0.001**			0.345			0.132			**<0.001**			**0.010**
Yes	2,209	481	(21.8)	1,166	(52.8)	562	(25.4)		138	(6.2)		87	(3.9)		100	(4.5)		73	(3.3)	
No	2,348	646	(27.5)	1,166	(49.7)	536	(22.8)		163	(6.9)		114	(4.9)		195	(8.3)		113	(4.8)	

Table S4.3.2 (a) KSPD DQ by sex

KSPD DQ	Sex	Mean	Median	(95% CI)		p
Overall	Boys	90.20	92.00	89.64	90.77	< 0.001
	Girls	94.48	96.00	93.88	95.08	
P-M	Boys	91.06	84.00	90.22	91.89	0.001
	Girls	92.82	87.00	92.00	93.64	
C-A	Boys	91.65	92.00	91.01	92.28	< 0.001
	Girls	95.86	96.00	95.19	96.54	
L-S	Boys	87.32	88.00	86.62	88.03	< 0.001
	Girls	93.65	94.00	92.94	94.36	

Table S4.3.2 (b) Details of the failure (DQ < 70) in the 3 domains and overlaps

	Boys (n = 2,363)			*Girls (n = 2,290)*			*Total (N = 4,653)*		
	n	%	Also failed in Overall (n)	n	%	Also failed in Overall (n)	n	%	Also failed in Overall (n)
P-M only	76	3.2%	(3)	73	3.2%	(1)	149	3.2%	(4)
C-A only	19	0.8%	(9)	24	1.0%	(12)	43	0.9%	(21)
L-S only	113	4.8%	(2)	36	1.6%	(0)	149	3.2%	(2)
P-M and C-A	16	0.7%	(14)	18	0.8%	(16)	34	0.7%	(30)
P-M and L-S	16	0.7%	(3)	7	0.3%	(3)	23	0.5%	(6)
C-A and L-S	19	0.8%	(18)	8	0.3%	(7)	27	0.6%	(25)
P-M, C-A, and L-S	59	2.5%	(59)	41	1.8%	(41)	100	2.1%	(100)

KSPD: Kyoto Scale of Psychological Development; P-M: Postural-Motor, C-A: Cognitive-Adaptive, L-S: Language-Social, DQ: Developmental quotient

Table S4.3.3 (a) Correlation between 25(OH)D, KSPD DQ (Boys)

	25(OH)D	KSPD P-M	KSPD C-A	KSPD L-S	KSPD Overall
25(OH)D	1				
KSPD P-M		1			
KSPD C-A		0.2590***	1		
KSPD L-S	0.0914***	0.2897***	0.4630***	1	
KSPD Overall	0.0510*	0.4627***	0.8545***	0.7920***	1

Table S4.3.3 (b) Correlation between 25(OH)D, KSPD DQ (Girls)

	25(OH)D	KSPD P-M	KSPD C-A	KSPD L-S	KSPD Overall
25(OH)D	1				
KSPD P-M		1			
KSPD C-A		0.2590***	1		
KSPD L-S	0.0576**	0.2814***	0.4508***	1	
KSPD Overall		0.4565***	0.8391***	0.8069***	1

*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$

KSPD: Kyoto Scale of Psychological Development; P-M: Postural-Motor, C-A: Cognitive-Adaptive, L-S: Language-Social

Table S4.3.4 KSPD DQ as continuous variable and 25(OH)D in 3 categories

		P-M			C-A			L-S			Overall		
25(OH)D (ng/mL)	n	Mean	Median	*p*	Mean	Median	*p*	Mean	Median	*p*	Mean	Median	*p*
(Boys)													
< 20	541	89.17	83	0.121	90.41	92	0.349	84.51	87	<0.001	88.45	91	0.064
≥ 20 & < 30	1,196	91.23	84		92.19	92.5		87.78	88		90.62	92	
≥ 30	626	92.37	84		91.67	92		88.89	90		90.92	92	
Total	2,363	91.06	84		91.65	92		87.32	88		90.20	92	
(Girls)													
< 20	607	92.03	85	0.356	96.18	96	0.991	92.51	93	0.031	94.23	95	0.341
≥ 20 & < 30	1,189	92.82	86		95.64	96		93.95	94		94.42	95	
≥ 30	494	93.79	95		96.02	96		94.32	95.5		94.94	96	
Total	2,290	92.82	87		95.86	96		93.65	94		94.48	96	

KSPD: Kyoto Scale of Psychological Development; P-M: Postural-Motor, C-A: Cognitive-Adaptive, L-S: Language-Social, DQ: Developmental quotient

TABLE S4.3.5 KSPD DQ (Dichotomised at 70) and 25(OH)D concentration as continuous variable

	n	Mean (ng/mL)	Median (ng/mL)	95% CI (ng/mL)		*p*
(Boys)						
P-M ≥ 70	2,196	25.69	25.45	25.37	26.00	0.494
P-M < 70	167	25.16	24.90	24.15	26.17	
C-A ≥ 70	2,250	25.74	25.50	25.43	26.04	0.005
C-A < 70	113	23.89	23.30	22.56	25.22	
L-S ≥ 70	2,156	25.78	25.50	25.47	26.09	0.004
L-S < 70	207	24.30	23.80	23.26	25.34	
Overall ≥ 70	2,255	25.74	25.50	25.43	26.04	0.004
Overall < 70	108	23.80	23.15	22.46	25.15	
(Girls)						
P-M ≥ 70	2,151	24.56	24.10	24.27	24.86	0.473
P-M < 70	139	24.43	23.70	23.26	25.60	
C-A ≥ 70	2,199	24.54	24.10	24.25	24.84	0.772
C-A < 70	91	24.87	23.90	23.30	26.44	
L-S ≥ 70	2,198	24.51	24.00	24.22	24.80	0.237
L-S < 70	92	25.62	25.05	23.96	27.28	
Overall ≥ 70	2,210	24.54	24.05	24.25	24.83	0.715
Overall < 70	80	25.03	24.00	23.33	26.73	

KSPD: Kyoto Scale of Psychological Development; P-M: Postural-Motor, C-A: Cognitive-Adaptive, L-S: Language-Social,
DQ: Developmental quotient

Table S4.3.6 Crude and adjusted ORs of 25(OH)D as continuous variable for KSPD DQ < 70

	KSPD P-M < 70				KSPD C-A < 70				KSPD L-S < 70				KSPD Overall < 70			
	OR aOR	*p*	95% CI		OR aOR	*p*	95% CI		OR aOR	*p*	95% CI		OR aOR	*p*	95% CI	
(Boys)																
	0.99	0.372	0.97	1.01	0.97	**0.010**	0.94	0.99	0.97	**0.006**	0.95	0.99	0.96	**0.008**	0.94	0.99
	1.00	0.068	0.95	1.00	0.96	**0.015**	0.93	0.99	0.97	**0.012**	0.95	0.99	0.95	**0.005**	0.93	0.99
(Girls)																
	1.00	0.824	0.97	1.02	1.01	0.661	0.98	1.04	1.02	0.136	0.99	1.05	1.01	0.539	0.98	1.04
	0.99	0.588	0.96	1.02	0.99	0.589	0.96	1.03	1.03	0.125	0.99	1.06	1.00	0.898	0.97	1.04

OR: Crude Odds Ratio, aOR: adjusted for test month, latitude, SGA, maternal age, and daycare attendance

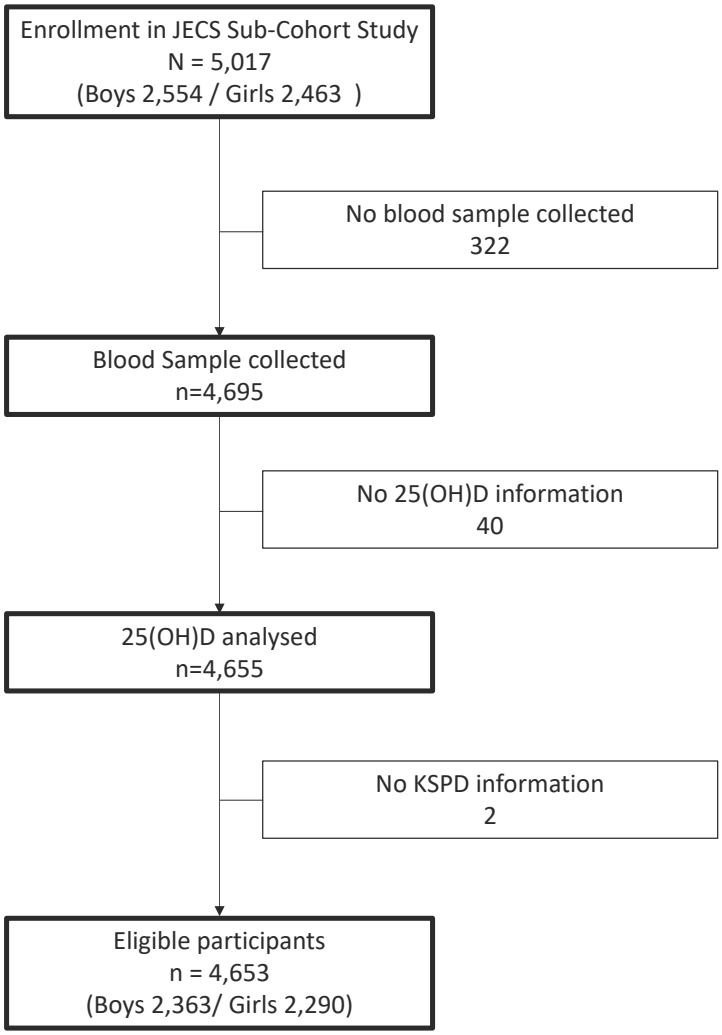

Figure S4.3.1 Flow chart showing the enrolment of eligible participants

Study IV

Table S4.4.1 ESSENCE-Q at the cut-off ≥ 3 and NDD diagnosis (total participants and according to gender)

	Total participants			NDDs Boys			Girls		
ESSENCE-Q	–	+	Total	–	+	Total	–	+	Total
Negative	65,112	129	65,241	32,139	93	32,232	32,973	36	33,009
Positive	11,646	725	12,371	6,926	532	7,458	4,720	193	4,913
Total	76,758	854	77,612	39,065	625	39,690	37,693	229	37,922

					MD				
ESSENCE-Q	–	+	Total	–	+	Total	–	+	Total
Negative	65,215	26	65,241	32,216	16	32,232	32,999	10	33,009
Positive	12,173	198	12,371	7,329	129	7,458	4,844	69	4,913
Total	77,388	224	77,612	39,545	145	39,690	37,843	79	37,922

						ID/DLD			
ESSENCE-Q	–	+	Total	–	+	Total	–	+	Total
Negative	65,155	86	65,241	32,169	63	32,232	32,986	23	33,009
Positive	11,890	481	12,371	7,106	352	7,458	4,784	129	4,913
Total	77,045	567	77,612	39,275	415	39,690	37,770	152	37,922

						ASD			
ESSENCE-Q	–	+	Total	–	+	Total	–	+	Total
Negative	65,206	35	65,241	32,205	27	32,232	33,001	8	33,009
Positive	12,057	314	12,371	7,212	246	7,458	4,845	68	4,913
Total	77,263	349	77,612	39,417	273	39,690	37,846	76	37,922

ASD, autism spectrum disorder; DLD, developmental language disorder; ID, intellectual disability; MD, motor delay; NDDs, neurodevelopmental disorders

Table S4.4.2 AUC, sensitivity, specificity, Youden index, PPV, and NPV at the cut-off of ≥ 3 according to child's gender

a) Boys (n = 39,690)

	NDDs	MD	ID/DLD	ASD
AUC	0.90	0.90	0.89	0.92
Sensitivity	85.12%	88.97%	84.82%	90.11%
Specificity	82.27%	81.47%	81.91%	81.70%
Youden index	67.39%	70.43%	66.73%	71.81%
PPV	7.13%	1.73%	4.72%	3.30%
NPV	99.71%	99.95%	99.80%	99.92%
LR (+)	4.80	4.80	4.69	4.92
LR (–)	0.18	0.14	0.19	0.12

b) Girls (n = 37,922)

	NDDs	MD	ID/DLD	ASD
AUC	0.91	0.91	0.91	0.95
Sensitivity	84.28%	87.34%	84.87%	89.47%
Specificity	87.48%	87.20%	87.33%	87.20%
Youden index	71.76%	74.54%	72.20%	76.67%
PPV	3.93%	1.40%	2.63%	1.38%
NPV	99.89%	99.97%	99.93%	99.98%
LR (+)	6.73	6.82	6.70	6.99
LR (–)	0.18	0.15	0.17	0.12

ASD, autism spectrum disorder; DLD, developmental language disorder; ID, intellectual disability; MD, motor delay; NDDs, neurodevelopmental disorders; PPV, positive predictive value; NPV, negative predictive value; LR (+), positive likelihood ratio; LR (-), negative likelihood ratio

Table S4.4.3 Answers to the 11 questions of ESSENCE-Q according to NDD diagnosis

ESSENCE-Q score	NDD (+) 1. General development			NDD (–) 1. General development		
	No	Yes	Total	No	Yes	Total
0	31	0	31	38,430	0	38,430
1	44	1	45	17,069	248	17,317
2	31	22	53	8,527	838	9,365
3	32	69	101	3,907	1,001	4,908
4	10	94	104	1,819	941	2,760
5	12	101	113	842	810	1,652
6	2	96	98	318	626	944
7	4	86	90	105	514	619
8	3	90	93	19	306	325
9	0	59	59	8	195	203
10	0	36	36	0	104	104
11	0	31	31	0	131	131
Total	169	685	854	71,044	5,714	76,758

ESSENCE-Q score	NDD (+) 5. Activity			NDD (–) 5. Activity		
	No	Yes	Total	No	Yes	Total
0	31	0	31	38,430	0	38,430
1	44	1	45	16,132	1,185	17,317
2	45	8	53	7,117	2,248	9,365
3	81	20	101	2,847	2,061	4,908
4	72	32	104	1,113	1,647	2,760
5	45	68	113	468	1,184	1,652
6	24	74	98	201	743	944
7	20	70	90	76	543	619
8	12	81	93	24	301	325
9	2	57	59	9	194	203
10	0	36	36	1	103	104
11	0	31	31	0	131	131
Total	376	478	854	66,418	10,340	76,758

ESSENCE-Q score	NDD (+) 9. Mood			NDD (–) 9. Mood		
	No	Yes	Total	No	Yes	Total
0	31	0	31	38,430	0	38,430
1	45	0	45	16,299	1,018	17,317
2	52	1	53	7,612	1,753	9,365
3	97	4	101	3,298	1,610	4,908
4	94	10	104	1,626	1,134	2,760
5	93	20	113	830	822	1,652
6	74	24	98	391	553	944
7	52	38	90	210	409	619
8	41	52	93	73	252	325
9	15	44	59	26	177	203
10	3	33	36	3	101	104
11	0	31	31	0	131	131
Total	597	257	854	68,798	7,960	76,758

ESSENCE-Q score	NDD (+) 2. Motor Development			NDD (–) 2. Motor Development		
	No	Yes	Total	No	Yes	Total
0	31	0	31	38,430	0	38,430
1	42	3	45	17,020	297	17,317
2	51	2	53	8,972	393	9,365
3	67	34	101	4,443	465	4,908
4	58	46	104	2,368	392	2,760
5	71	42	113	1,328	324	1,652
6	53	45	98	695	249	944
7	46	44	90	369	250	619
8	46	47	93	145	180	325
9	18	41	59	58	145	203
10	6	30	36	13	91	104
11	0	31	31	0	131	131
Total	489	365	854	73,841	2,917	76,758

ESSENCE-Q score	NDD (+) 6. Attention			NDD (–) 6. Attention		
	No	Yes	Total	No	Yes	Total
0	31	0	31	38,430	0	38,430
1	44	1	45	16,892	425	17,317
2	45	8	53	8,244	1,121	9,365
3	85	16	101	3,544	1,364	4,908
4	65	39	104	1,572	1,188	2,760
5	54	59	113	693	959	1,652
6	21	77	98	288	656	944
7	27	63	90	155	464	619
8	4	89	93	59	266	325
9	2	57	59	18	185	203
10	0	36	36	4	100	104
11	0	31	31	0	131	131
Total	378	476	854	69,899	6,859	76,758

ESSENCE-Q score	NDD (+) 10. Sleep			NDD (–) 10. Sleep		
	No	Yes	Total	No	Yes	Total
0	31	0	31	38,430	0	38,430
1	44	1	45	15,901	1,416	17,317
2	51	2	53	7,025	2,340	9,365
3	92	9	101	3,574	1,334	4,908
4	98	6	104	1,877	883	2,760
5	93	20	113	1,052	600	1,652
6	83	15	98	562	382	944
7	69	21	90	338	281	619
8	61	32	93	179	146	325
9	35	24	59	83	120	203
10	16	20	36	37	67	104
11	0	31	31	0	131	131
Total	673	181	854	69,058	7,700	76,758

ESSENCE-Q score	NDD (+) 3. Sensory reactions			NDD (–) 3. Sensory reactions		
	No	Yes	Total	No	Yes	Total
0	31	0	31	38,430	0	38,430
1	45	0	45	17,187	130	17,317
2	51	2	53	9,227	138	9,365
3	95	6	101	4,783	125	4,908
4	92	12	104	2,636	124	2,760
5	87	26	113	1,538	114	1,652
6	82	16	98	850	94	944
7	47	43	90	511	108	619
8	44	49	93	238	87	325
9	20	39	59	111	92	203
10	9	27	36	30	74	104
11	0	31	31	0	131	131
Total	603	251	854	75,541	1,217	76,758

ESSENCE-Q score	NDD (+) 7. Social interaction			NDD (–) 7. Social interaction		
	No	Yes	Total	No	Yes	Total
0	31	0	31	38,430	0	38,430
1	43	2	45	16,657	660	17,317
2	48	5	53	8,228	1,137	9,365
3	80	21	101	3,842	1,066	4,908
4	79	25	104	1,899	861	2,760
5	64	49	113	943	709	1,652
6	38	60	98	397	547	944
7	21	69	90	186	433	619
8	11	82	93	65	260	325
9	8	51	59	23	180	203
10	0	36	36	4	100	104
11	0	31	31	0	131	131
Total	423	431	854	70,674	6,084	76,758

ESSENCE-Q score	NDD (+) 11. Feeding			NDD (–) 11. Feeding		
	No	Yes	Total	No	Yes	Total
0	31	0	31	38,430	0	38,430
1	41	4	45	8,149	9,168	17,317
2	42	11	53	3,540	5,825	9,365
3	66	35	101	1,622	3,286	4,908
4	62	42	104	764	1,996	2,760
5	58	55	113	380	1,272	1,652
6	37	61	98	176	768	944
7	23	67	90	97	522	619
8	24	69	93	35	290	325
9	11	48	59	20	183	203
10	0	36	36	5	99	104
11	0	31	31	0	131	131
Total	395	459	854	53,218	23,540	76,758

ESSENCE-Q score	4. Communication			4. Communication		
	No	Yes	Total	No	Yes	Total
0	31	0	31	38,430	0	38,430
1	13	32	45	14,869	2,448	17,317
2	11	42	53	6,992	2,373	9,365
3	16	85	101	3,094	1,814	4,908
4	7	97	104	1,393	1,367	2,760
5	6	107	113	636	1,016	1,652
6	4	94	98	271	673	944
7	1	89	90	126	493	619
8	1	92	93	34	291	325
9	0	59	59	16	187	203
10	0	36	36	2	102	104
11	0	31	31	0	131	131
Total	90	764	854	65,863	10,895	76,758

ESSENCE-Q score	8. Behaviour			8. Behaviour		
	No	Yes	Total	No	Yes	Total
0	31	0	31	38,430	0	38,430
1	45	0	45	16,995	322	17,317
2	50	3	53	8,801	564	9,365
3	97	4	101	4,310	598	4,908
4	91	13	104	2,253	507	2,760
5	95	18	113	1,202	450	1,652
6	72	26	98	571	373	944
7	50	40	90	303	316	619
8	32	61	93	104	221	325
9	7	52	59	34	169	203
10	2	34	36	5	99	104
11	0	31	31	0	131	131
Total	572	282	854	73,008	3,750	76,758

ASD, autism spectrum disorder; DLD, developmental language disorder; ID, intellectual disability; MD, motor delay; NDDs, neurodevelopmental disorders

Table S4.4.4 Mean scores of each of the five J-ASQ-3 domains relative to the concern noted for each ESSENCE-Q question

		a. Communication				b. Gross motor				c. Fine motor				d. Problem solving				e. Personal-Social			
ESSENCE-Q	Concerns	No. of children	Mean	95% CI		No. of children	Mean	95% CI		No. of children	Mean	95% CI		No. of children	Mean	95% CI		No. of children	Mean	95% CI	
1. General Development	No	66,051	54.30	54.23	54.37	66,070	55.35	55.29	55.41	65,789	48.12	48.03	48.21	65,923	51.77	51.69	51.84	65,987	51.06	51.00	51.13
	Yes	5,880	36.60	36.11	37.08	5,884	46.80	46.42	47.18	5,858	36.74	36.34	37.15	5,871	35.75	35.32	36.19	5,882	38.79	38.41	39.17
2.Motor development	No	68,901	53.35	53.27	53.43	68,924	55.29	55.23	55.34	68,626	47.63	47.54	47.72	68,769	50.96	50.88	51.05	68,838	50.54	50.47	50.61
	Yes	3,030	41.51	40.79	42.23	3,030	40.15	39.54	40.76	3,021	37.22	36.62	37.83	3,025	38.89	38.23	39.56	3,031	39.09	38.53	39.66
3. Sensory reactions	No	70,587	53.09	53.01	53.18	70,608	54.83	54.77	54.89	70,311	47.38	47.29	47.48	70,454	50.69	50.60	50.77	70,525	50.28	50.21	50.35
	Yes	1,344	40.23	39.09	41.37	1,346	45.24	44.31	46.18	1,336	37.17	36.22	38.12	1,340	38.37	37.32	39.43	1,344	38.59	37.66	39.52
4. Communication	No	61,188	55.62	55.56	55.68	61,200	55.49	55.44	55.55	60,947	48.60	48.51	48.70	61,077	52.85	52.77	52.92	61,125	51.60	51.53	51.67
	Yes	10,743	37.08	36.76	37.39	10,754	49.85	49.62	50.09	10,700	39.17	38.88	39.45	10,717	36.83	36.54	37.12	10,744	41.32	41.06	41.57
5. Activity (overactivity/ passivity) or impulsivity	No	61,955	53.73	53.64	53.81	61,970	55.00	54.93	55.06	61,729	47.99	47.90	48.09	61,847	51.32	51.23	51.40	61,893	50.87	50.80	50.95
	Yes	9,976	47.43	47.12	47.74	9,984	52.50	52.29	52.71	9,918	42.21	41.93	42.49	9,947	45.11	44.82	45.40	9,976	45.03	44.78	45.28
6. Attention/concentration/ "listening"	No	65,216	53.69	53.61	53.77	65,230	55.01	54.95	55.08	64,966	47.93	47.83	48.02	65,094	51.26	51.17	51.34	65,151	50.76	50.68	50.83
	Yes	6,715	44.69	44.27	45.11	6,724	51.13	50.84	51.41	6,681	40.07	39.71	40.43	6,700	42.66	42.28	43.04	6,718	43.31	42.99	43.63
7. Social interaction with / interest in other children	No	65,918	53.50	53.42	53.58	65,932	55.04	54.98	55.10	65,658	47.75	47.66	47.85	65,797	51.12	51.03	51.21	65,853	50.71	50.63	50.78
	Yes	6,013	45.79	45.35	46.22	6,022	50.36	50.05	50.67	5,989	41.06	40.68	41.44	5,997	43.17	42.76	43.58	6,016	42.99	42.65	43.34
8, Behaviour (repetitive, routine insistence)	No	68,247	53.21	53.13	53.30	68,267	54.86	54.80	54.93	67,974	47.51	47.41	47.60	68,115	50.82	50.73	50.90	68,188	50.40	50.32	50.47
	Yes	3,684	46.20	45.65	46.76	3,687	50.71	50.32	51.10	3,673	41.40	40.91	41.89	3,679	43.75	43.23	44.26	3,681	43.85	43.40	44.29
9. Mood	No	64,318	53.24	53.16	53.33	64,334	54.87	54.80	54.93	64,072	47.63	47.54	47.73	64,200	50.86	50.77	50.95	64,259	50.46	50.38	50.54
	Yes	7,613	49.56	49.24	49.88	7,620	52.84	52.61	53.06	7,575	43.48	43.17	43.79	7,594	47.00	46.69	47.32	7,610	46.69	46.42	46.96
10. Sleep	No	64,605	53.04	52.95	53.13	64,625	54.83	54.76	54.89	64,358	47.47	47.38	47.57	64,485	50.72	50.63	50.81	64,554	50.35	50.28	50.43
	Yes	7,326	51.18	50.88	51.48	7,329	53.08	52.85	53.31	7,289	44.72	44.41	45.03	7,309	48.15	47.84	48.45	7,315	47.48	47.22	47.75
11. Feeding	No	49,633	53.74	53.64	53.83	49,645	55.23	55.16	55.30	49,453	48.32	48.21	48.43	49,545	51.50	51.40	51.60	49,592	51.45	51.37	51.53
	Yes	22,298	50.89	50.71	51.06	22,309	53.37	53.25	53.50	22,194	44.68	44.51	44.86	22,249	48.13	47.96	48.31	22,277	46.96	46.81	47.11
Total		71,931	52.85	52.77	52.94	71,954	54.65	54.59	54.71	71,647	47.19	47.10	47.29	71,794	50.46	50.37	50.54	71,869	50.06	49.99	50.13

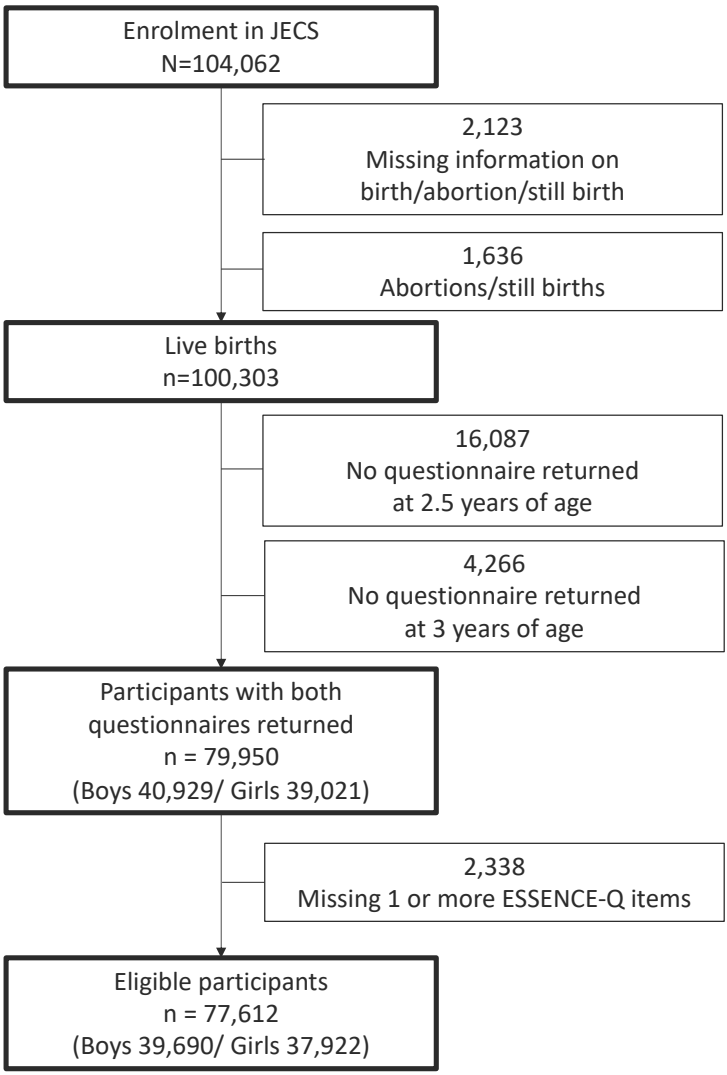

Figure S4.4.1 Flow chart showing the eligible participants