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**Child Developmental MRI (CDM) Project: Protocol for a study on elucidating the pathophysiology of attention-deficit/hyperactivity disorder and autism spectrum disorder through a multi-institutional and -dimensional approach**

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| **Keywords:** Child & adolescent psychiatry < PSYCHIATRY, Paediatric neurology < PAEDIATRICS, Developmental neurology & neurodisability < PAEDIATRICS |
Child Developmental MRI (CDM) Project: Protocol for a study on elucidating the pathophysiology of attention-deficit/hyperactivity disorder and autism spectrum disorder through a multi-institutional and -dimensional approach

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Keywords: attention-deficit/hyperactivity disorder, autism spectrum disorder, neuroimaging, neurobiological markers, travelling subject  

Word count: 3750
ABSTRACT

Introduction

Neuroimaging studies on attention-deficit/hyperactivity disorder (ADHD) and autism spectrum disorder (ASD) have demonstrated differences in extensive brain structure, activity, and network. However, there remains heterogeneity and inconsistency across these findings, presumably because of the diversity of the disorders themselves, small sample sizes, and site and parameter differences in magnetic resonance imaging (MRI) scanners, and their overall pathogenesis remains unclear.

Methods and analysis

To address these gaps in the literature, we will apply the travelling-subject approach to correct site differences in MRI scanners and clarify brain structure and network characteristics of children with ADHD and ASD using large samples collected in a multi-centre collaboration. In addition, we will investigate the relationship between these characteristics and genetic, epigenetic, biochemical marker, and behavioural and psychological measures. We will collect resting-state functional MRI (fMRI) and T1- and diffusion-weighted MRI data from 15 healthy adults as travelling subjects and 300 children (ADHD, n=100; ASD, n=100; and typical development, n=100) with multi-dimensional assessments. We will also apply data from more than 1,000 samples acquired in our previous neuroimaging studies on ADHD and ASD.

Ethics and dissemination

The study protocol has been approved by the Research Ethics Committee of the University of Fukui Hospital (approval no.: 20220601). Our study findings will be submitted to scientific peer-reviewed journals and conferences.

STRENGTHS AND LIMITATIONS OF THIS STUDY

➢ We will multi-directionally compare neurobiological data using a large sample of children with neurodevelopmental disorders and typical development collected from multiple centres.

➢ We will apply the travelling-subject approach to correct site differences in MRI scanners.

➢ The multilateral approach, including corrections for site differences in MRI scanners, may contribute to elucidating the pathogenesis and establishing imaging biomarkers of ADHD and ASD.

➢ Longitudinal studies using a multilateral approach to ADHD and ASD may be needed following this cross-sectional study.
INTRODUCTION

Attention-deficit/hyperactivity disorder (ADHD) and autism spectrum disorder (ASD) are common neurodevelopmental disorders (NDDs) in child and adolescent psychiatry, with prevalence rates of >5% and >1.5%,[1,2] respectively. ADHD is characterised by age-appropriate symptoms of inattention, hyperactivity, and impulsivity, whereas ASD is characterised by difficulties in social communication/interaction and stereotypical repetitive behaviour.[3] Although the main characteristics of ADHD and ASD differ, current clinical assessments regarding accurate diagnosis and intervention based on pathology are difficult, due to clinical and neurobiological overlap between these disorders[4,5] and the high comorbidity rate.[6] In addition, several studies have reported that ADHD and ASD share various forms of behavioural impairment, particularly pertaining to executive function and motor skills.[7,8] ADHD and ASD are associated with an increased risk of depression, anxiety disorders, conduct disorders, severe central fatigue, and sleep disorders.[9-12] Therefore, it is important to establish early effective assessments and interventions for ADHD and ASD to prevent them from proceeding to secondary psychiatric comorbidities. Although many neuroimaging studies have been conducted to elucidate the underlying pathology and develop objective assessments for NDDs, these studies have yielded inconsistent findings.

Structurally, patients with ADHD show decreased grey matter volume (GMV) in the frontal lobe, amygdala, caudate nucleus, hippocampus, and putamen compared with control subjects.[13-16] Studies of patients with ADHD found a larger right GMV, as well as a larger GMV in the dorsolateral prefrontal, temporal, intracalcarine cortices, and parietal lobule.[17-19] Moreover, the largest study on volumetry in ADHD (conducted by the ENIGMA-ADHD Working Group) did not support previous findings regarding structural changes in some brain regions because of the small effect size.[20] In addition, resting-state functional magnetic resonance imaging (fMRI) studies have reported that compared with healthy subjects, patients with ADHD show more profound atypicality in the default mode, cognitive control, reward, attention, and amygdala-seeded network,[21-24] suggesting delays or alterations in the maturation of these connectors.[25-27] However, in contrast to previous studies, a meta-analysis of resting-state fMRI studies did not observe specific functional connectivity in ADHD.[28]

Previous studies on the brain structure of patients with ASD have found smaller GMV in the middle frontal gyrus, middle temporal gyrus, amygdala, hippocampus, putamen, cerebellum, and precentral gyrus compared with control subjects.[29-31] However, several studies did not find such a reduction in GMV in ASD.[32-35] In addition, numerous studies have attempted to clarify local resting-state differences between subjects with ASD and controls. Although increased local functional connectivity of the frontal, temporal, and occipital lobes in the resting state has been reported in subjects with ASD,[36-38] some other studies of subjects with ASD did not find such between-group differences in these regions.[39-41] Site and parameter differences in MRI scanners may explain the aforementioned observed discrepancies among these neuroimaging studies. These factors were not controlled between studies, which might have weakened the
essential characteristics of the disorders. There is also a paucity of evaluation criteria aimed at understanding the diversity of the disorder and its different underlying genetic backgrounds. To establish a distinct brain structure and network between ADHD and ASD, it is essential to evaluate these disorders from multiple perspectives via large-scale multi-site studies, while controlling for different MRI scanners. The travelling-subject (TS) approach is a candidate for such correction in MRI scanners because it can differentiate most of the sample variability resulting from measurement biases in brain structure and activity.[42-45]

In this exploratory study, we will focus on child development in subjects with NDDs at multiple levels, such as genetics, epigenetics, neurotransmitter and amino acid level, and behavioural measures. First, we will investigate site differences in MRI scanners using the TS approach. Second, we will investigate whether children with ADHD, ASD, and typical development show differences in the specificity of neurobiological functions. After correcting for site differences in MRI scanners, we will compare brain structure and resting-state functional connectivity among children with ADHD, ASD, and typical development. Subsequently, we will investigate associations between structural and functional changes, genetics, epigenetics, biochemical markers, and behavioural and psychological measurements in ADHD and ASD. In addition to these new data, we will use data from more than 1,000 existing samples collected from children with NDDs and typical development in our previous neuroimaging studies.[46-51]

METHODS AND ANALYSIS

Study design

This multi-centre cross-sectional study will be carried out at the Research Centre for Child Mental Development at the University of Fukui, Osaka University, and Chiba University. The study will be conducted in accordance with the Helsinki Declaration on ethical principles for medical research involving human subjects. The inclusion and exclusion criteria will be applied to identify individuals to be included in the study (Table 1). The study flow diagram is shown in Figure 1.

Table 1. Inclusion and exclusion criteria

<table>
<thead>
<tr>
<th>Neurodevelopmental disorder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inclusion criteria</td>
</tr>
<tr>
<td>1. Fulfil the diagnostic criteria for ADHD and ASD according to the DSM-5</td>
</tr>
<tr>
<td>2. Aged 6 to 18 years at the time of informed consent</td>
</tr>
<tr>
<td>Exclusion criteria</td>
</tr>
</tbody>
</table>
1. Full-scale intelligence quotient < 70
2. History of severe head trauma or neurological illness
3. Potential for hazards associated with MRI examination (such as the presence of metal on
the body surface or internal structures, pregnancy or possibility of pregnancy, claustrophobia,
and fear of the dark)

Typical development

Inclusion criteria
1. Aged 6 to 18 years at the time of informed consent
2. Does not receive special education

Exclusion criteria
1. Full-scale intelligence quotient < 70
2. History of severe head trauma, neurological illness or neurodevelopmental disorder
3. Potential for hazards associated with MRI examination (such as the presence of metal on
the body surface or internal structures, pregnancy or possibility of pregnancy, claustrophobia,
and fear of the dark)

Travelling subject

Inclusion criteria
1. Aged 20 to 65 years at the time of informed consent
2. Does not receive special education

Exclusion criteria
1. History of severe head trauma, neurological illness or neurodevelopmental disorder
2. Cognitive impairment
3. Potential for hazards associated with MRI examination (such as the presence of metal on
the body surface or internal structures, pregnancy or possibility of pregnancy, claustrophobia,
and fear of the dark)

The Japanese versions of the Wechsler Intelligence Scale for Children, fourth or fifth edition
(WISC-IV or WISC-V) will be used to assess full-scale intelligence quotient. WISC-IV and
WISC-V consist of verbal subtests (of information, similarities, arithmetic, vocabulary,
comprehension, and digit span) and performance subtests (of picture completion, coding/digit
symbols, picture arrangement, block design, and object assembly). DSM-5, Diagnostic and
Statistical Manual of Mental Disorders, Fifth Edition; MRI, magnetic resonance imaging

Recruitment of participants
Study participants will be recruited between 12 July 2022 and 31 March 2032. Children with
NDDs (ADHD and ASD) will be recruited from the University of Fukui Hospital, Chiba
University Hospital, and Osaka University Hospital. The diagnoses of ADHD and ASD will be
based on the criteria outlined in the Diagnostic and Statistical Manual of Mental Disorders, Fifth
Edition.[3] Clinicians at each hospital will introduce the study to patients who fulfil the inclusion
criteria. Children with typical development will be recruited from the community. In addition,
we will recruit healthy travelling community-dwelling adults to account for site differences in
MRI scanners, because long-term MRI scanning for scanner correction in a multi-site study may
not be suitable for children. Informed consent will be obtained from participants and/or their
legal guardians.

Patient and public involvement

Patients and/or the public were not involved in the study design and will not be involved in the
conduct, reporting, and dissemination of the findings of this study.

Outcomes

Primary endpoints:

➢ Brain structure and resting-state functional connectivity

The following secondary endpoints will be acquired to assess the correlation with the brain
measures of primary endpoints:

➢ Clinical information

➢ Psychological measurements

➢ Cognitive measurements

➢ Genomic and epigenomic data

➢ Urine levels of monoamine metabolites and tryptophan

Psychological measurements

We will use the Japanese-translated versions of many psychological questionnaires to assess
psychological characteristics and lifestyles in both children with NDDs and typical development.
The questionnaires are shown in Table 2.
<table>
<thead>
<tr>
<th>Questionnaire</th>
<th>Item</th>
<th>Assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRS</td>
<td>65-item; 4-point Likert scale (1 = not at all to 4 = very)</td>
<td>Social communication and restricted repetitive behaviours</td>
</tr>
<tr>
<td>SP-short form</td>
<td>38-item; 5-point Likert scale (1 = not at all to 5 = very)</td>
<td>Hyperesthesia and insensitive</td>
</tr>
<tr>
<td>Conners 3</td>
<td>108-item (parents) and 99-item (children); 4-point Likert scale (0 = not at all to 3 = very)</td>
<td>Inattentiveness and hyperactivity/impulsivity</td>
</tr>
<tr>
<td>DSRSC</td>
<td>18-item; 3-point Likert scale (0 = not at all to 2 = very)</td>
<td>Depression</td>
</tr>
<tr>
<td>SCAS</td>
<td>38-item; 4-point Likert scale (0 = never to 3 = always)</td>
<td>Symptoms of anxiety disorders</td>
</tr>
<tr>
<td>ACE</td>
<td>10-item; Yes or No response</td>
<td>Physical, verbal, or sexual abuse, mental illness, or substance abuse in the nuclear family</td>
</tr>
<tr>
<td>CTQ</td>
<td>28-item; 5-point Likert scale (1 = never to 5 = always)</td>
<td>Childhood maltreatment and its severity</td>
</tr>
<tr>
<td>SES</td>
<td>5-item; Single answer</td>
<td>Parental education, occupation, and monthly income</td>
</tr>
<tr>
<td>PSI-short form</td>
<td>19-item; 5-point Likert scale (1 = strongly disagree to 5 = strongly agree)</td>
<td>Level of anxiety in interaction with their children and parental stress related to their children’s temperament and behaviour</td>
</tr>
<tr>
<td>PS</td>
<td>30-item; 7-point Likert scale (1 = effective discipline to 7 = dysfunctional discipline)</td>
<td>Discipline style to child misbehaviours</td>
</tr>
<tr>
<td>Kid-KINDL R questionnaire</td>
<td>30-item; 5-point Likert scale (1 = not at all to 5 = very)</td>
<td>Physical health, mental health, self-esteem, family, friends, and school life</td>
</tr>
<tr>
<td>Kiddo-KINDOL R questionnaire</td>
<td>30-item; 5-point Likert scale (1 = not at all to 5 = very)</td>
<td>Physical health, mental health, self-esteem, family, friends, and school life</td>
</tr>
<tr>
<td>JSQ-ES</td>
<td>38-item; 6-point Likert scale (1 = not at all to 6 = very)</td>
<td>Sleep disturbance and problematic sleep habits</td>
</tr>
<tr>
<td>JSQ-JH</td>
<td>38-item; 6-point Likert scale (1 = not at all to 6 = very)</td>
<td>Sleep disturbance and problematic sleep habits</td>
</tr>
<tr>
<td>Test</td>
<td>Description</td>
<td>Measurement</td>
</tr>
<tr>
<td>-------</td>
<td>-----------------------------------------------------------------------------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>EHI</td>
<td>10-item; Typing a “+” or “++” in the appropriate column (right or left)</td>
<td>Degree of hand laterality in daily activities</td>
</tr>
<tr>
<td>BRIEF</td>
<td>86-item; 3-point Likert scale (1 = never to 3 = often)</td>
<td>Assessment of ability to inhibit, shift, control emotions, working memory, and plan/organize</td>
</tr>
<tr>
<td>FCV-19S</td>
<td>7-item; 5-point Likert scale (1 = strongly disagree to 5 = strongly agree)</td>
<td>Severity of individuals’ fear of COVID-19</td>
</tr>
<tr>
<td>DCDQ</td>
<td>15-item; 5-point Likert scale (1 = not at all like your child to 5 = extremely like your child)</td>
<td>Child’s gross- and fine-motor coordination</td>
</tr>
<tr>
<td>CFS</td>
<td>14-item; 4-point Likert scale (0 = less than usual to 3 = much more than usual)</td>
<td>Mental and physical fatigue</td>
</tr>
</tbody>
</table>

SRS, Social Responsiveness Scale; SP, Sensory Profile; DSRSC, Depression Self-Rating Scale for Children; SCAS, Spence Children’s Anxiety Scale; ACE, Adverse Childhood Experiences; CTQ, Childhood Trauma Questionnaire; SES, Socioeconomic Status; PSI, Parenting Stress Index; PS, Parenting Scale; JSQ-ES, Japanese Sleep Questionnaire for Elementary Schoolers; JSQ-JH, Japanese Sleep Questionnaire for Junior High Schoolers; EHI, Edinburgh Handedness Inventory; BRIF, Behaviour Rating Inventory of Executive Function; FCV-19S, Fear of Coronavirus-19 Scale; DCDQ, Developmental Coordination Disorder Questionnaire; CFS, Chalder Fatigue Scale

**Behavioural measurements**

Cognitive and eye-tracking functions will be assessed in children with ADHD, ASD, and typical development. The cognitive test will consist of the Cambridge Neuropsychological Tasks Automated Battery (CANTAB; [http://www.cambridgecognition.com/cantab/](http://www.cambridgecognition.com/cantab/)).[52-54] The stop signal task of the CANTAB will be utilised to assess the inhibition response. Participants will be asked to quickly respond to an arrow stimulus by selecting one of two options depending on the direction in which the arrow is pointing. Thereafter, participants will be instructed to withhold their behavioural response when an auditory signal is present. The spatial working memory task of CANTAB will be utilised to assess the retention of spatial information and retrieval of retained items from the working memory. Participants will be asked to find blue tokens hidden inside a number of coloured boxes on the screen and place them in an empty column on the side of the screen. Since the colour and position of the boxes will be changed to avoid the stereotyped search strategy in each trial, participants will be instructed not to return to a box where a token has previously been found.

The Gazefinder (JVC Kenwood Co. Ltd., Yokohama, Japan) task in the eye-tracking test will be used to assess eye gaze patterns allocated to specific objects (e.g., the human face with or
without mouth motion, the biological motion of a human, the preference paradigm for people or
gometry, and a screenshot of finger-pointing to social and geometry areas) on a video
monitor.[55-57] A previous cross-sectional study showed that children with ASD have a lower
gaze ratio at the people region in the preference paradigm compared to children with typical
development, suggesting that developmental characteristics of ASD can be assessed using the
gaze ratio.[57] However, little is known about the characteristic feature of gaze patterns in
ADHD, that is, differences in eye-tracking characteristics between ADHD and ASD. Thus, we
will compare children with ADHD, ASD, and typical development to investigate the specificity
of eye tracking.

Urine collection and high-performance liquid chromatography assay

Previous studies have shown that monoamines and amino acids affect cognitive and brain
functions in ADHD[58,59] and ASD;[60,61] thus, these molecular markers may be suitable for
detecting NDD pathologies. Urinary levels of monoamine metabolites and tryptophan, which are
predictive and non-invasive biomarkers of brain function,[62-64] will be measured in children
with ADHD, ASD, and typical development. Collected urine samples will be diluted with 6.7
mM hydrochloric acid and 2.5% perchloric acid to separate albumin, as previously reported.[64]
The obtained supernatant will be stored at -78°C until high-performance liquid chromatography
(Nanospace SI-2 3001; Shiseido Japan Co. Ltd., Tokyo, Japan) assay with an electrochemical
detector (Nanospace SI-2 3005; Shiseido Japan Co. Ltd.) and a chromate recorder (C-R8A;
Shimadzu Corporation, Kyoto, Japan). The mobile phase will consist of 15% methanol in a
solution (pH, 4.13) containing 30 mM citric acid, 10 mM disodium hydrogen phosphate, 0.5 mM
sodium octyl sulphate, 50 mM sodium chloride, and 0.05 mM ethylenediaminetetraacetic acid, as
previously reported.[64-66] This will be pumped through a 5 μM C18 column (150 mm × 4.6
mm) at a flow rate of 0.7 mL/min.

Genetic polymorphism and epigenetic assays of saliva samples

To assess neural structural and functional impairment-related pathophysiological processes in
NDDs, it is essential to understand how genetic and epigenetic risk factors are associated with
such atypical characteristics. Genetic polymorphisms (single-nucleotide polymorphisms) and
epigenetics (DNA methylation) will be assessed in children with ADHD, ASD, and typical
development. Saliva samples (which do not require invasive collection)[67] will be directly
collected using Oragene Discover OGR-675 kits (DNA Genotek Inc., Ottawa, ON, Canada).
Saliva DNA will be extracted using preplT®/L2P reagent (DNA Genotek Inc.) and quantified
using Qubit™ dsDNA HS assay kits (Thermo Fisher Scientific Inc., Pittsburgh, PA, USA), as
previously reported.[67-69] Thereafter, we plan to characterise vulnerable genetic and epigenetic
factors at both genome-wide and candidate gene levels (e.g., oxytocin and glutamate receptors, catechol-O-methyltransferase, and branched-chain aminotransferase).

**Image acquisition**

Scanning will be performed using a 3-T GE Signa PET/MR scanner (General Electric HealthCare, Chicago, IL, USA) or a 3-T GE Discovery MR750 scanner (General Electric HealthCare) at the University of Fukui, a 3-T GE Signa Architect scanner (General Electric HealthCare) at Osaka University, and a 3-T GE Discovery MR750 scanner (General Electric HealthCare) at Chiba University. Imaging orders will be placed for resting-state fMRI, T1-weighted, and diffusion-weighted (DTI) images. Scanning parameters are shown in Table 3.
<table>
<thead>
<tr>
<th>Site</th>
<th>Head coil</th>
<th>TR (ms)</th>
<th>TE (ms)</th>
<th>FA (deg)</th>
<th>FOV (mm)</th>
<th>Matrix</th>
<th>Voxel dimension (mm)</th>
<th>Slice thickness (mm)</th>
<th>Slice Direction</th>
<th>b-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>University of Fukui I</td>
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</tr>
<tr>
<td>Resting-state fMRI</td>
<td>2300</td>
<td>30</td>
<td>81</td>
<td>192 × 192</td>
<td>64 × 64</td>
<td>3 × 3 × 3.5</td>
<td>3.5 (gap 0.5)</td>
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<tr>
<td>T1WI</td>
<td>8.5</td>
<td>3.2</td>
<td>11</td>
<td>256 × 256</td>
<td>256 × 256</td>
<td>1 × 1 × 1</td>
<td>1 (gap 0)</td>
<td></td>
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<tr>
<td>DTI</td>
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<td>74.6</td>
<td>N/A</td>
<td>240 × 240</td>
<td>128 × 128</td>
<td>1.9 × 1.9 × 3</td>
<td>3.0 (gap 0)</td>
<td></td>
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<tr>
<td>University of Fukui II</td>
<td>32 ch</td>
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<td>T1WI</td>
<td>6.4</td>
<td>2</td>
<td>11</td>
<td>256 × 256</td>
<td>256 × 256</td>
<td>1 × 1 × 1</td>
<td>1 (gap 0)</td>
<td></td>
<td></td>
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<tr>
<td>DTI</td>
<td>8400</td>
<td>84.4</td>
<td>N/A</td>
<td>256 × 256</td>
<td>128 × 128</td>
<td>2 × 2 × 2</td>
<td>2 (gap 0)</td>
<td></td>
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<tr>
<td>Resting-state fMRI</td>
<td>1200</td>
<td>30</td>
<td>90</td>
<td>220 × 220</td>
<td>64 × 64</td>
<td>3.438 × 3.438 × 3.536</td>
<td>3.6</td>
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<tr>
<td>T1WI</td>
<td>876.332</td>
<td>0.02</td>
<td>5</td>
<td>240 × 240</td>
<td>240 × 240</td>
<td>0.938 × 0.938 × 0.5</td>
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<tr>
<td>DTI</td>
<td>6000</td>
<td>75</td>
<td>N/A</td>
<td>260 × 260</td>
<td>128 × 128</td>
<td>1.016 × 1.016 × 3</td>
<td>3</td>
<td></td>
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<tr>
<td>Chiba University</td>
<td>32 ch</td>
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<td>Resting-state fMRI</td>
<td>2300</td>
<td>30</td>
<td>81</td>
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<tr>
<td>T1WI</td>
<td>8.124</td>
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<td>1.875 × 1.875 × 2</td>
<td>2 (gap 0)</td>
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</tbody>
</table>
During resting-state fMRI under open-eye conditions, participants will be instructed to think about nothing in particular, stay awake, and keep their eyes open and fixated on the crosshair on the screen. During resting-state fMRI under closed-eye conditions, participants will be instructed to think about nothing in particular, stay awake, and keep their eyes closed. fMRI, functional magnetic resonance imaging; T1WI, T1-weighted images; DTI, diffusion-weighted images; TR, repetition time; TE, echo time; FA, flip angle; FOV, field of view; N/A, not applicable; ch, channels
Data analysis plan

As previously reported, we will investigate site corrections in MRI scanners using the TS approach.[42-45] This approach can differentiate most of the sample variability resulting from measurement biases in fMRI[41] and structural MRI.[68] Thereafter, we will investigate whether differences in brain structure and activity exist among children with ADHD, ASD, and typical development. Whole-brain voxel-based GMV analyses will be performed using the statistical parametric mapping software SPM12 (Wellcome Centre for Human Neuroimaging, London, UK). Based on differences in GMV among the groups, regions of interest (ROIs) will be extracted using the MarsBaR software[69] to investigate the correlation between GMV and behavioural or biological measures. DTI analyses will be performed using the FSL software package (http://www.fmrib.ox.ac.uk/fsl) and the TRActs Constrained by UnderLying Anatomy (TRACULA) tool in FreeSurfer (http://www.surfer.nmr.mgh.harvard.edu/). Based on the differences in fractional anisotropy among the groups, the correlation between fractional anisotropy of each white matter pathway and behavioural or biological measures will be determined. The resting-state functional connectivity will be performed using the CONN toolbox[70] (http://www.nitrc.org/projects/conn, PRID: SCR_009550). A seed-to-voxel analysis will be performed using the ROIs obtained from the GMV analysis. In addition, we will investigate forms of functional connectivity such as the default mode network, salience network, dorsal attention network, cognitive control network, and affective network. Further, we will analyse the correlation between the functional connectivity value and behavioural or biological measures.

Sample size

We finally aim to build the largest database of NDDs in Japan, comparable to other international databases. Although cross-sectional large-sample databases containing neuroimaging data of persons with NDDs exist (the ADHD-200 database comprising the data of 285 individuals with ADHD and 491 persons with typical development aged 7–21 years and the Autism Brain Imaging Data Exchange [ABIDE- II ] database comprising the data of 521 persons with ASD and 593 individuals with typical development aged 5–64 years), we already possess the data of more than 1,000 MRI data with bioinformatic, behavioural, and psychological information on children with NDDs and typical development.[46-51] Moreover, we will acquire multi-dimensional data from 300 children (100 with ADHD, 100 with ASD, and 100 with typical development) and scan 15 healthy travelling adults to correct for site differences in MRI scanners according to the previously described TS approach.[42-45]

ETHICS AND DISSEMINATION
The study protocol has been approved by the Research Ethics Committee of the University of Fukui Hospital (approval no. 20220601). Informed consent will be sought from all participants and/or their legal guardians. The results will be disseminated in academic journals, conferences, and databases as well as social media.

DISCUSSION

The Child Developmental MRI Project aims to clarify the pathogenesis underlying NDDs and establish relevant neurobiological markers by constructing the largest database of NDDs in Japan. The main protocol pertains to (i) a comparative analysis of brain structure and network among children with ADHD, ASD, and typical development in conjunction with more than 1,000 existing samples; (ii) a multi-dimensional approach to these NDDs and typical development using a genetic, epigenetic, neurotransmitter and amino acid markers, and cognitive and psychological measures; and (iii) a TS approach to correct for differences in MRI scanners at multiple sites. Although a high rate of comorbidity and neurobiological commonalities between ADHD and ASD is thought to complicate their differential diagnosis, our project will be the first to demonstrate neurobiological distinctions between these disorders. This large-sample multilateral study will shed new light on the diversity of NDDs and the establishment of accurate diagnoses based on their pathophysiology.

Our project has some limitations. The geographic area is not entirely controlled, which will limit the generalisability of our results. However, we will construct the largest database that multidimensionally assesses the neurobiological bases of NDDs in Japan. Finally, our study is cross-sectional, and longitudinal studies of the multi-dimensional approach to ADHD and ASD are needed, as previous longitudinal studies have reported differences in atypical brain structures at the developmental stage.[71,72]

Acknowledgements

We are grateful to Hiroshi Oikawa, Eiji Kidoya, and Masayuki Kanamoto of the University of Fukui for supporting us with the MRI scans. We thank Ayumu Yamashita and Shinsuke Koike at the University of Tokyo for supporting us in the TS approach.

Authors’ contributions

YM designed the research. MY and YM wrote the manuscript. All authors contributed to the experimental procedures and revised the manuscript.
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Competing interests statement

Tsuyoshi Sasaki received grants or contracts from any entity in Shionogi. Tsuyoshi Sasaki received payment or honoraria for lectures, presentations, speaking, manuscript writing, and educational events from Sumitomo Pharma, Takeda, Otsuka, Meiji Seika, Nobel Pharma, Kyowa, Towa, Eisai, Janssen, Yoshitomi, and Shionogi. Tsuyoshi Sasaki participated on the Data Safety Monitoring Board and Advisory Board of Mochida Pharmaceutical Co., Ltd. The other authors report no financial relationships with commercial interests. None of the authors have financial disclosures with regard to the subject of this protocol.

References


Figure legend
Figure 1. Flow diagram for study on neurodevelopmental disorders (a) and the travelling-subject (TS) approach (b). In the TS approach, all participants will undergo MRI scans at 3 sites within 3 months. ADHD, attention-deficit/hyperactivity disorder, ASD, autism spectrum disorder; TD, typical development; TS, travelling-subject; MRI, magnetic resonance imaging
Recruitment (ADHD, ASD, and TD)

Eligibility screening

Exclude participants who do not meet the criteria

Questionnaire response

Urine sample collection

MRI scanning

Behavioural measurements

Saliva sample collection

Recruitment (TS)

Eligibility screening

MRI scanning at the University of Fukui

MRI scanning at the Osaka University

MRI scanning at the Chiba University
# Child Developmental MRI (CDM) Project: Protocol for a multi-centre, cross-sectional study on elucidating the pathophysiology of attention-deficit/hyperactivity disorder and autism spectrum disorder through a multi-dimensional approach

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---|---|
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Child Developmental MRI (CDM) Project: Protocol for a multi-centre, cross-sectional study on elucidating the pathophysiology of attention-deficit/hyperactivity disorder and autism spectrum disorder through a multi-dimensional approach

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Keywords: attention-deficit/hyperactivity disorder, autism spectrum disorder, neuroimaging, neurobiological markers, travelling subject

Word count: 3262
ABSTRACT

Introduction

Neuroimaging studies on attention-deficit/hyperactivity disorder (ADHD) and autism spectrum disorder (ASD) have demonstrated differences in extensive brain structure, activity, and network. However, there remains heterogeneity and inconsistency across these findings, presumably because of the diversity of the disorders themselves, small sample sizes, and site and parameter differences in magnetic resonance imaging (MRI) scanners, and their overall pathogenesis remains unclear. To address these gaps in the literature, we will apply the travelling-subject approach to correct site differences in MRI scanners and clarify brain structure and network characteristics of children with ADHD and ASD using large samples collected in a multi-centre collaboration. In addition, we will investigate the relationship between these characteristics and genetic, epigenetic, biochemical markers, and behavioural and psychological measures.

Methods and analysis

We will collect resting-state functional MRI (fMRI) and T1- and diffusion-weighted MRI data from 15 healthy adults as travelling subjects and 300 children (ADHD, n=100; ASD, n=100; and typical development, n=100) with multi-dimensional assessments. We will also apply data from more than 1,000 samples acquired in our previous neuroimaging studies on ADHD and ASD.

Ethics and dissemination

The study protocol has been approved by the Research Ethics Committee of the University of Fukui Hospital (approval no.: 20220601). Our study findings will be submitted to scientific peer-reviewed journals and conferences.

STRENGTHS AND LIMITATIONS OF THIS STUDY

➢ We will multi-directionally compare neurobiological data using a large sample of children with neurodevelopmental disorders and typical development collected from multiple centres.

➢ We will apply the travelling-subject approach to correct site differences in MRI scanners.

➢ The multisite approach, including corrections for site differences in MRI scanners, may contribute to elucidating the pathogenesis and establishing imaging biomarkers of ADHD and ASD.

➢ Longitudinal studies using a multisite approach to ADHD and ASD may be needed following this cross-sectional study.
INTRODUCTION

Attention-deficit/hyperactivity disorder (ADHD) and autism spectrum disorder (ASD) are common neurodevelopmental disorders (NDDs) in child and adolescent psychiatry, with prevalence rates of >5% and >1.5%,\[1,2\] respectively. ADHD is characterised by age-inappropriate symptoms of inattention, hyperactivity, and impulsivity, whereas ASD is characterised by difficulties in social communication/interaction and stereotypical repetitive behaviour.\[3\] Although the main characteristics of ADHD and ASD differ, clinical assessments regarding accurate diagnosis and intervention based on pathology are currently difficult due to considerable clinical and neurobiological overlap between these disorders\[4,5\] and the high comorbidity rate.\[6\] In addition, several studies have reported that ADHD and ASD share various forms of behavioural impairment, particularly pertaining to executive function and motor skills\[7,8\] ADHD and ASD are associated with an increased risk of depression, anxiety disorders, conduct disorders, severe central fatigue, and sleep disorders.\[9-12\] Therefore, it is important to establish early effective assessments and interventions for ADHD and ASD to prevent them from proceeding to secondary psychiatric comorbidities. Although many neuroimaging studies have been conducted to elucidate the underlying pathology and develop objective assessments for NDDs, these studies have yielded inconsistent findings.

Structurally, patients with ADHD show decreased grey matter volume (GMV) in the frontal lobe, amygdala, caudate nucleus, hippocampus, and putamen compared with control subjects.\[13-16\] Other studies of patients with ADHD found a larger right GMV, as well as a larger GMV in the dorsolateral prefrontal, temporal, intracalcarine cortices, and parietal lobule.\[17-19\] However, the largest study on volumetry in ADHD (conducted by the ENIGMA-ADHD Working Group) did not support previous findings regarding structural changes in some brain regions because of the small effect size.\[20\] In addition, resting-state functional magnetic resonance imaging (fMRI) studies have reported that compared with healthy subjects, patients with ADHD show more profound atypicality in the default mode, cognitive control, reward, attention, and amygdala-seeded network,\[21-24\] suggesting delays or alterations in the maturation of these connectors.\[25-27\] However, in contrast to previous studies, a meta-analysis of resting-state fMRI studies did not observe specific functional connectivity in ADHD.\[28\]

Previous studies on the brain structure of patients with ASD have found a smaller GMV in the middle frontal gyrus, middle temporal gyrus, amygdala, hippocampus, putamen, cerebellum, and precentral gyrus compared with control subjects.\[29-31\] However, several other studies did not find such reductions in GMV in ASD.\[32-35\] In addition, numerous studies have attempted to clarify local resting-state differences between subjects with ASD and controls. Although increased local functional connectivity of the frontal, temporal, and occipital lobes in the resting state has been reported in subjects with ASD,\[36-38\] some other studies of subjects with ASD did not find such between-group differences in these regions.\[39-41\]
Site and parameter differences in MRI scanners may explain the aforementioned observed discrepancies among neuroimaging studies. To clarify the distinct brain structure and network differences between ADHD and ASD, it is essential to evaluate these disorders from multiple perspectives via large-scale multi-site studies, while controlling for different MRI scanners. The travelling-subject (TS) approach is a promising candidate strategy for MRI scanner correction, because it can account for most of the sample variability resulting from measurement biases in brain structure and activity.[42-45]

In addition to the issue of MRI scanner measurement biases, there is also a paucity of evaluation criteria aimed at understanding the diversity of the disorders and their different underlying genetic backgrounds. A previous cross-sectional study on the Gazefinder system showed that children with ASD had a lower gaze ratio at the people region in the preference paradigm compared with children with typical development, suggesting that developmental characteristics of ASD can be assessed using the gaze ratio.[46] Nevertheless, it remains unclear whether specific gaze motions are cross-sectionally evident in children with ASD relative to ADHD. Neurochemical measures, some genetic polymorphisms and epigenetic alterations in risk genes related to monoamine metabolism, have been implicated in both of ADHD[47-49] and ASD,[50] highlighting their association with such genes and synaptic regulation of neurotransmitter binding and release. Additionally, it has been reported that functional connectivities of the caudate nucleus-parietal cortex and nucleus accumbens-occipital cortex are correlated with both polygenetic risk score and diagnostic status in ADHD.[51] Although these findings suggest that candidate single nucleotide polymorphisms (SNPs) and DNA methylation may contribute to identifying the neural consequences of risk genes in NDDs, evidence in the literature is scarce. Furthermore, previous studies on the molecular basis of NDDs focused on monoamines and tryptophan (an essential amino acid and a precursor of serotonin) metabolism [52-56] and lacked information about their associations with brain structures and activity. Thus, multi-dimensional approaches may provide a means to link pathogenesis and imaging biomarkers of NDDs.

In this exploratory study, we will focus on the development of NDDs in children at multiple levels, including the behavioural measure, genetic, epigenetic, and neurotransmitter and amino acid levels. First, we will investigate site differences in MRI scanners using the TS approach. Second, we will investigate whether children with ADHD, ASD, and typical development show differences in the specificity of neurobiological functions. After correcting for site differences in MRI scanners, we will compare brain structure and resting-state functional connectivity among children with ADHD, ASD, and typical development. Subsequently, we will investigate associations between structural and functional changes; genetic, epigenetic, biochemical markers; and behavioural and psychological measurements in ADHD and ASD. In addition to these new data, we will use data from more than 1,000 existing samples collected from children with NDDs and typical development in our previous neuroimaging studies.[57-64]
METHODS AND ANALYSIS

Study design

This multi-centre cross-sectional study will be carried out at the Research Centre for Child Mental Development at the University of Fukui, Osaka University, and Chiba University. The study will be conducted in accordance with the Helsinki Declaration on ethical principles for medical research involving human subjects. The inclusion and exclusion criteria will be applied to identify individuals to be included in the study (Table 1). The study flow diagram is shown in Figure 1.

Table 1. Inclusion and exclusion criteria

<table>
<thead>
<tr>
<th>Neurodevelopmental disorder</th>
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<td>Inclusion criteria</td>
</tr>
<tr>
<td>1. Fulfil the diagnostic criteria for ADHD and ASD according to the DSM-5</td>
</tr>
<tr>
<td>2. Aged 6 to 18 years at the time of informed consent</td>
</tr>
<tr>
<td>Exclusion criteria</td>
</tr>
<tr>
<td>1. Full-scale intelligence quotient &lt; 70</td>
</tr>
<tr>
<td>2. History of severe head trauma or neurological illness</td>
</tr>
<tr>
<td>3. Potential for hazards associated with MRI examination (such as the presence of metal on the body surface or internal structures, pregnancy or possibility of pregnancy, claustrophobia, and fear of the dark)</td>
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<table>
<thead>
<tr>
<th>Typical development</th>
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<td>Inclusion criteria</td>
</tr>
<tr>
<td>1. Aged 6 to 18 years at the time of informed consent</td>
</tr>
<tr>
<td>2. Does not receive special education</td>
</tr>
<tr>
<td>Exclusion criteria</td>
</tr>
<tr>
<td>1. Full-scale intelligence quotient &lt; 70</td>
</tr>
<tr>
<td>2. History of severe head trauma, neurological illness, or neurodevelopmental disorder</td>
</tr>
<tr>
<td>3. Potential for hazards associated with MRI examination (such as the presence of metal on the body surface or internal structures, pregnancy or possibility of pregnancy, claustrophobia, and fear of the dark)</td>
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</table>

<table>
<thead>
<tr>
<th>Travelling subject</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inclusion criteria</td>
</tr>
</tbody>
</table>
1. Aged 20 to 65 years at the time of informed consent
2. Does not receive special education

Exclusion criteria
1. History of severe head trauma, neurological illness, or neurodevelopmental disorder
2. Cognitive impairment
3. Potential for hazards associated with MRI examination (such as the presence of metal on the body surface or internal structures, pregnancy or possibility of pregnancy, claustrophobia, and fear of the dark)

The Japanese versions of the Wechsler Intelligence Scale for Children, fourth or fifth edition (WISC-IV or WISC-V) will be used to assess full-scale intelligence quotient. WISC-IV and WISC-V consist of verbal subtests (of information, similarities, arithmetic, vocabulary, comprehension, and digit span) and performance subtests (of picture completion, coding/digit symbols, picture arrangement, block design, and object assembly). DSM-5, Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition; MRI, magnetic resonance imaging

Recruitment of participants
Study participants will be recruited between 12 July 2022 and 31 March 2032. Children with NDDs (ADHD and ASD) will be recruited from the University of Fukui Hospital, Chiba University Hospital, and Osaka University Hospital. The diagnoses of ADHD and ASD will be based on the criteria outlined in the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition. Clinicians at each hospital will introduce the study to patients who fulfil the inclusion criteria. Children with typical development will be recruited from the community. In addition, we will recruit healthy travelling community-dwelling adults to account for site differences in MRI scanners, because long-term MRI scanning for scanner correction in a multi-site study may not be suitable for children. Informed consent will be obtained from participants and/or their legal guardians.

Patient and public involvement
Patients and/or the public were not involved in the study design and will not be involved in the conduct, reporting, and dissemination of the findings of this study.

Outcomes
Primary endpoints:
Brain structure (GMV, cortical thickness, surface area, white matter fractional anisotropy) and resting-state functional connectivity

The following secondary endpoints will be assessed to investigate the correlations with the primary endpoint brain measures:

- Clinical information
- Psychological measurements
- Cognitive measurements
- Genomic and epigenomic data
- Urine levels of monoamine metabolites and tryptophan

**Psychological measurements**

We will use the Japanese-translated versions of various psychological questionnaires to assess psychological characteristics and lifestyles in both children with NDDs and typical development. The questionnaires are shown in **Table 2**.

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<tr>
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<tr>
<td>SRS-2</td>
<td>65-item; 4-point Likert scale (1 = not at all to 4 = very)</td>
<td>Social communication and restricted repetitive behaviours</td>
</tr>
<tr>
<td>SSP</td>
<td>38-item; 5-point Likert scale (1 = not at all to 5 = very)</td>
<td>Hyperesthesia and insensitive</td>
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<tr>
<td>Conners 3</td>
<td>108-item (parents) and 99-item (children); 4-point Likert scale (0 = not at all to 3 = very)</td>
<td>Inattentiveness and hyperactivity/impulsivity</td>
</tr>
<tr>
<td>DSRSC</td>
<td>18-item; 3-point Likert scale (0 = not at all to 2 = very)</td>
<td>Depression</td>
</tr>
<tr>
<td>SCAS</td>
<td>38-item; 4-point Likert scale (0 = never to 3 = always)</td>
<td>Symptoms of anxiety disorders</td>
</tr>
<tr>
<td>ACE</td>
<td>10-item; Yes or No response</td>
<td>Physical, verbal, or sexual abuse, mental illness, or substance abuse in the nuclear family</td>
</tr>
<tr>
<td>CTQ</td>
<td>28-item; 5-point Likert scale (1 = never to 5 = always)</td>
<td>Childhood maltreatment and its severity</td>
</tr>
<tr>
<td>Questionnaire</td>
<td>Items</td>
<td>Scale</td>
</tr>
<tr>
<td>--------------</td>
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<tr>
<td>SES</td>
<td>5-item; Single answer</td>
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<tr>
<td>PSI-short form</td>
<td>19-item; 5-point Likert scale (1 = strongly disagree to 5 = strongly agree)</td>
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<td>PS</td>
<td>30-item; 7-point Likert scale (1 = effective discipline to 7 = dysfunctional discipline)</td>
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<td>30-item; 5-point Likert scale (1 = not at all to 5 = very)</td>
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<td>Kiddo-KINDOL questionnaire</td>
<td>30-item; 5-point Likert scale (1 = not at all to 5 = very)</td>
<td></td>
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<tr>
<td>JSQ-ES</td>
<td>38-item; 6-point Likert scale (1 = not at all to 6 = very)</td>
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<td>38-item; 6-point Likert scale (1 = not at all to 6 = very)</td>
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<td>EHI</td>
<td>10-item; Typing a “+” or “++” in the appropriate column (right or left)</td>
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<tr>
<td>BRIEF</td>
<td>86-item; 3-point Likert scale (1 = never to 3 = often)</td>
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<tr>
<td>FCV-19S</td>
<td>7-item; 5-point Likert scale (1 = strongly disagree to 5 = strongly agree)</td>
<td></td>
</tr>
<tr>
<td>DCDQ</td>
<td>15-item; 5-point Likert scale (1 = not at all like your child to 5 = extremely like your child)</td>
<td></td>
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<tr>
<td>CFS</td>
<td>14-item; 4-point Likert scale (0 = less than usual to 3 = much more than usual)</td>
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201 SRS-2, Social Responsiveness Scale, Second Edition; SSP, Short Sensory Profile; DSRSC, Depression Self-Rating Scale for Children; SCAS, Spence Children’s Anxiety Scale; ACE, Adverse Childhood Experiences; CTQ, Childhood Trauma Questionnaire; SES, Socioeconomic Status; PSI, Parenting Stress Index; PS, Parenting Scale; JSQ-ES, Japanese Sleep Questionnaire for Elementary Schoolers; JSQ-JH, Japanese Sleep Questionnaire for Junior High Schoolers; EHI, Edinburgh Handedness Inventory; BRIEF, Behaviour Rating Inventory of Executive Function; FCV-19S, Fear of Coronavirus-19 Scale; DCDQ, Developmental Coordination Disorder Questionnaire; CFS, Chalder Fatigue Scale

Behavioural measurements

Cognitive and eye-tracking functions will be assessed in children with ADHD, ASD, and typical development. The cognitive test will consist of the Cambridge Neuropsychological Tasks Automated Battery (CANTAB; http://www.cambridgecognition.com/cantab/). The stop signal task of the CANTAB will be utilised to assess the inhibition response. Participants will be asked to quickly respond to an arrow stimulus by selecting one of two options depending on the direction in which the arrow is pointing. Thereafter, participants will be instructed to withhold their behavioural response when an auditory signal is present. The spatial working memory task of CANTAB will be utilised to assess the retention of spatial information and retrieval of retained items from the working memory. Participants will be asked to find blue tokens hidden inside a number of coloured boxes on the screen and place them in an empty column on the side of the screen. Since the colour and position of the boxes will be changed to avoid the stereotyped search strategy in each trial, participants will be instructed not to return to a box where a token has previously been found.

The Gazefinder (JVC Kenwood Co. Ltd., Yokohama, Japan) task in the eye-tracking test will be used to assess eye gaze patterns allocated to specific objects (e.g., the human face with or without mouth motion, the biological motion of a human, the preference paradigm for people or geometry, and a screenshot of finger-pointing to social and geometry areas) on a video monitor.[46,68,69]

Urine collection and high-performance liquid chromatography assay

Urinary levels of monoamine metabolites and tryptophan, which have been proposed as predictive, cost-effective, and non-invasive biomarkers of brain function,[52,56,70-72] will be measured in children with ADHD, ASD, and typical development. Prior to testing, the participants will be instructed to refrain from intense physical activity and the intake of food and beverages such as alcohol, coffee, high-fat fish, red beef, and blue cheese, which can be difficult to digest, for 24 h. Additionally, water will be the only beverage they will be allowed to have on the day of sample collection. On the test day, fresh urine will be collected after participants are made to rest for 30 min. Freshly collected urine samples will be diluted with 6.7 mM hydrochloric acid and 2.5% perchloric acid to separate albumin, as previously reported.[72] The obtained supernatant will be stored at -78 °C until high-performance liquid chromatography (Nanospace SI-2 3001; Shiseido Japan Co. Ltd., Tokyo, Japan) assay with an electrochemical detector (Nanospace SI-2 3005; Shiseido Japan Co. Ltd.) and a chromate recorder (C-R8A; Shimadzu Corporation, Kyoto, Japan). The mobile phase will consist of 15% methanol in a solution (pH, 4.13) containing 30 mM citric acid, 10 mM disodium hydrogen phosphate, 0.5 mM sodium octyl sulphate, 50 mM sodium chloride, and 0.05 mM ethylenediaminetetraacetic acid, as
previously reported.[72-74] This will be pumped through a 5 μM C_{18} column (150 mm × 4.6 mm) at a flow rate of 0.7 mL/min.

**Genetic polymorphism and epigenetic assays of saliva samples**

To assess neural structural and functional impairment-related pathophysiological processes in NDDs, it is essential to understand how genetic and epigenetic risk factors are associated with such atypical characteristics. SNPs and DNA methylation will be assessed in children with ADHD, ASD, and typical development. Saliva samples (which do not require invasive collection)[75] will be directly collected using Oragene Discover OGR-675 kits (DNA Genotek Inc., Ottawa, ON, Canada). Saliva DNA will be extracted using prepIT®/L2P reagent (DNA Genotek Inc.) and quantified using Qubit™ dsDNA HS assay kits (Thermo Fisher Scientific Inc., Pittsburgh, PA, USA), as previously reported.[75-77] Thereafter, we plan to characterise vulnerable genetic and epigenetic factors at both genome-wide and candidate gene levels (e.g., oxytocin and glutamate receptors, catechol-O-methyltransferase, and branched-chain aminotransferase).

**Image acquisition**

Prior to scanning, children will be carefully habituated to the MRI scanner sounds, which may improve yield. Children with NDDs and typical development will be scanned using a 3-T GE Signa PET/MR scanner (General Electric HealthCare, Chicago, IL, USA) at the University of Fukui, a 3-T GE Signa Architect scanner (General Electric HealthCare) at Osaka University, and a 3-T GE Discovery MR750 scanner (General Electric HealthCare) at Chiba University. In addition to the abovementioned scanners, a 3-T GE Discovery MR750 scanner (General Electric HealthCare) at the University of Fukui will also be used for scanning healthy travelling adults, because some of the imaging data in our existing samples were acquired using this scanner. Imaging orders will be placed for resting-state fMRI, T1-weighted, and diffusion-weighted (DTI) images. Scanning parameters are shown in **Table 3**.
Table 3. Scanning parameters for multi-site studies

<table>
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<tr>
<th>Site</th>
<th>Head coil</th>
<th>Scan time (min:sec)</th>
<th>TR (ms)</th>
<th>TE (ms)</th>
<th>FA (deg)</th>
<th>FOV (mm)</th>
<th>Matrix</th>
<th>Voxel dimension (mm)</th>
<th>Slice thickness (mm)</th>
<th>Slice Direction</th>
<th>b-values</th>
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<td>8 ch</td>
<td>07:42</td>
<td>2300</td>
<td>30</td>
<td>81</td>
<td>192 × 192</td>
<td>64 × 64</td>
<td>3 × 3 × 3.5</td>
<td>3.5 (gap 0.5)</td>
<td>40</td>
<td>N/A</td>
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<tr>
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<td>8.5</td>
<td>3.2</td>
<td>11</td>
<td>256 × 256</td>
<td>256 × 256</td>
<td>1 × 1 × 1</td>
<td>1 (gap 0)</td>
<td></td>
<td>176</td>
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<tr>
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<td>128 × 128</td>
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<td>3.0 (gap 0)</td>
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<td>45</td>
<td>30</td>
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<tr>
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<td>07:42</td>
<td>2300</td>
<td>30</td>
<td>81</td>
<td>192 × 192</td>
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<td>3 × 3 × 3.5</td>
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<td>64</td>
<td>30</td>
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<tr>
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<td>1200</td>
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<td>90</td>
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<td>876.332</td>
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<td>5</td>
<td>240 × 240</td>
<td>240 × 240</td>
<td>0.938 × 0.938 × 0.5</td>
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<td>480</td>
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<td>DTI</td>
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<td>50</td>
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<tr>
<td>Chiba University (MR750)</td>
<td>32 ch</td>
<td>05:00</td>
<td>1200</td>
<td>30</td>
<td>90</td>
<td>220 × 220</td>
<td>64 × 64</td>
<td>3.438 × 3.438 × 3.536</td>
<td>3.6</td>
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<td>T1WI</td>
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<td>3</td>
<td>50</td>
<td>25</td>
<td>0, 1000</td>
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</table>
Resting-state fMRI scanning for healthy travelling adults at the University of Fukui will be performed using PET/MR and MR750 scanners under both open-eye and closed-eye conditions, because some of our existing sample data were acquired under the closed-eye condition using the MR750 scanner at the University of Fukui. During resting-state fMRI under open-eye conditions, participants will be instructed to think about nothing in particular, stay awake, and keep their eyes open and fixated on the crosshair on the screen. During resting-state fMRI under closed-eye conditions, participants will be instructed to think about nothing in particular, stay awake, and keep their eyes closed. Resting-state fMRI scanning for children with NDDs and typical development will be performed under open-eye conditions. fMRI, functional magnetic resonance imaging; T1WI, T1-weighted images; DTI, diffusion-weighted images; min, minutes; sec, seconds; TR, repetition time; TE, echo time; FA, flip angle; FOV, field of view; N/A, not applicable; ch, channels; NDDs, neurodevelopmental disorders.
Data analysis plan

We will investigate site corrections in MRI scanners using the TS approach. This approach can account for most of the sample variability resulting from measurement biases (due to differences in imaging variables, field strength, manufacturers, and scanner models) and sampling bias (due to differences in participant groups), as previously reported. Since our TS dataset will include only healthy participants, we plan to correct measurement bias only to quantitatively investigate the site effects in brain structure and activity. Briefly, we will calculate the coefficients for the scanner, head coil, fMRI manufacturer, and phase encoding at each site and correct imaging data on the basis of these coefficients using regression models and clustering algorithms. Thereafter, we will investigate whether differences in brain structure and activity exist among children with ADHD, ASD, and typical development. The GMV analyses will be performed using Statistical Parametric Mapping 12 (https://www.fil.ion.ucl.ac.uk/spm/software/spm12/) or FreeSurfer (http://www.surfer.nmr.mgh.harvard.edu/). The cortical thickness and surface area analyses will be performed using FreeSurfer. Based on differences in GMV, cortical thickness, and surface area among the groups, the extracted regions of interest (ROIs) will be used to investigate the correlations between the corresponding structures and behavioural or neurochemical measures. The white matter fractional anisotropy (FA) analyses will be performed using the FSL software package (http://www.fmrib.ox.ac.uk/fsl) and the TRActs Constrained by UnderLying Anatomy (TRACULA) tool in FreeSurfer. In particular, we plan to examine the FA values in the inferior frontal-occipital fasciculus, superior/inferior longitudinal fasciculus, capsula interna, anterior corona radiata, and cerebellum, because these regions are known to be associated with the pathogenesis of ADHD[78] and ASD.[79] Moreover, based on the group differences, the correlations between FA values and behavioural or neurochemical measures will be determined. Resting-state functional connectivity will be analysed using the CONN toolbox[80] (http://www.nitrc.org/projects/conn, PRID: SCR_009550). Excessive head motion will be addressed by exclusion (over 3.0 mm, 3.0 degree) and scrubbing as necessary.[63,64,81] A seed-to-voxel analysis will be performed using the ROIs obtained from the structural analysis. In addition, we will investigate forms of functional connectivity such as the default mode network, salience network, dorsal attention network, cognitive control network, and affective network. Further, we will analyse the correlation between the functional connectivity value and behavioural or biological measures.

Sample size

We finally aim to build the largest database of NDDs in Japan, comparable to other international databases. Although cross-sectional large-sample databases containing neuroimaging data of persons with NDDs exist (the ADHD-200 database comprising the data of 285 individuals with ADHD and 491 persons with typical development aged 7–21 years and the Autism Brain...
Imaging Data Exchange [ABIDE-II] database comprising the data of 521 persons with ASD and 593 individuals with typical development aged 5–64 years), we already possess the data of more than 1,000 MRI data with bioinformatic, behavioural, and psychological information on children with NDDs and typical development.[57-64] Moreover, we will acquire multi-dimensional data from 300 children (100 with ADHD, 100 with ASD, and 100 with typical development) and scan 15 healthy travelling adults to correct for site differences in MRI scanners according to the previously described TS approach.[42-45]

ETHICS AND DISSEMINATION

The study protocol has been approved by the Research Ethics Committee of the University of Fukui Hospital (approval no. 20220601). Informed consent will be sought from all participants and/or their legal guardians. The results will be disseminated in academic journals, conferences, and databases as well as social media.

DISCUSSION

The Child Developmental MRI Project aims to clarify the pathogenesis underlying NDDs and establish relevant neurobiological markers by constructing the largest database of NDDs in Japan. The main protocol pertains to (i) a comparative analysis of brain structure and network among children with ADHD, ASD, and typical development in conjunction with more than 1,000 existing samples; (ii) a multi-dimensional approach to these NDDs and typical development using genetic, epigenetic, neurotransmitter and amino acid markers, and cognitive and psychological measures; and (iii) a TS approach to correct for differences in MRI scanners at multiple sites. Although a high rate of comorbidity and neurobiological commonalities between ADHD and ASD is thought to complicate their differential diagnosis, our project will be the first to demonstrate neurobiological distinctions between these disorders.

In addition, outcomes based on multi-dimensional approaches may provide additional information regarding the specificities of brain functions associated with the diversity of these NDDs. A cross-sectional behavioural study showed that children with ASD had a lower gaze ratio during social stimuli compared with children with typical development.[46] Since the orbitofrontal-striatal-amygdala circuit, which responds to social stimuli such as faces and social approval, has been implicated in abnormal social behaviour in ASD,[82] it is natural to anticipate, together with the association of neural level, whether gaze processing in social information is impaired in ADHD and compare it with deficits reported in ASD. A recent meta-analysis of genome-wide association studies (GWAS), which analysed the data for 20,183 patients diagnosed with ADHD and 35,191 controls, aimed to establish the causal relationships between genetic variants and the disorder and 304 identified genetic variants of several potential
ADHD-specific genes (e.g., forkhead box P2, artemin, and dual specificity phosphatase 6).\cite{83} Moreover, a GWAS meta-analysis of the data for 18,381 individuals with ASD and 27,969 controls identified 5 risk loci, including those corresponding to neuronal growth regulator 1, polypyrimidine tract binding protein 2, and calcium-dependent secretion activator.\cite{84} These findings indicate that the majority of NDD cases may involve the pathogenic convergence of multiple variants, and not just a single gene defect. A few of the abovementioned genes are also thought to play key roles in the regulation of neurotransmitter levels\cite{84,85} and brain development.\cite{86,87} In particular, several studies have pointed out that NDDs are associated with more profound abnormalities in dopamine, norepinephrine, and serotonin metabolism.\cite{53,54,56} Furthermore, several recent studies have demonstrated that changes in tryptophan metabolism are associated with the pathology of ADHD\cite{52} and ASD.\cite{55} This was also supported by the demonstration of associations between chronic enhancement of free tryptophan and reduced branched-chain amino acid levels and hyperactivity/impulsivity in a rat model of ADHD.\cite{88} However, there is a lack of sufficient information about the associations between such neurochemical indexes and neuroimaging data in ADHD and ASD. Thus, our multi-dimensional analysis may help to clarify the diversity of NDDs and their pathogenesis, which hypothesizes multifactorial mechanisms at several different levels, from gene-molecular to brain and cognition. Moreover, the data collected in this large-sample multisite study will shed new light on the diversity of NDDs and could help in the establishment of criteria for more accurate diagnoses based on their underlying pathophysiology.

Our project has some limitations. First, the geographic area is not entirely controlled, which will limit the generalisability of our results. However, we will construct the largest database that multidimensionally assesses the neurobiological bases of NDDs in Japan. Second, the present study does not apply Autism Diagnostic Observation Schedule, Second Edition and Autism Diagnostic Interview–Revised assessments for ASD, because it will be difficult to include these measures given the design and time management aspects of the protocol. Alternatively, we diagnose ASD based on the DSM-5 and additionally use the Social Responsiveness Scale, Second Edition and the Short Sensory Profile, both of which have been validated in Japanese samples, to identify the core symptoms of ASD. These measurements are accepted as powerful tools for quantifying the clinical features of ASD.\cite{89-91} Moreover, we believe that our multi-dimensional approach involving neuroimaging, genetic, molecular, and behavioural data will help to adequately predict the specificity of neurobiological functions in children with ADHD and ASD. Third, our study is cross-sectional, and longitudinal studies on the multi-dimensional approach to ADHD and ASD will also be needed in the future, as previous longitudinal studies have reported differences in atypical brain structures at the developmental stage.\cite{92,93}

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Authors’ contributions

**Masatoshi Yamashita:** Conceptualization, Methodology, Writing – Original Draft, Writing – Review & Editing, Funding acquisition. **Kuriko Kagitani-Shimono:** Conceptualization, Methodology, Writing – Review & Editing, Funding acquisition. **Yoshiyuki Hirano:** Conceptualization, Methodology, Writing – Review & Editing, Funding acquisition. **Sayo Hamatani:** Methodology, Writing – Review & Editing. **Akiko Yao:** Methodology, Writing – Review & Editing. **Sawa Kurata:** Methodology, Writing – Review & Editing. **Hirotaka Kosaka:** Methodology, Writing – Review & Editing. **Minyoung Jung:** Methodology, Writing – Review & Editing. **Koji Matsumoto:** Methodology, Writing – Review & Editing. **Sawako Kurata:** Methodology, Writing – Review & Editing. **Yoko Kato:** Methodology, Writing – Review & Editing. **Masaya Tachibana:** Methodology, Writing – Review & Editing. **Ikuko Mohri:** Methodology, Writing – Review & Editing. **Kenji J. Tsuchiya:** Methodology, Writing – Review & Editing. **Tetsuya Tsujikawa:** Methodology, Writing – Review & Editing. **Eiji Shimizu:** Methodology, Writing – Review & Editing. **Masako Taniike:** Methodology, Writing – Review & Editing. **Akemi Tomoda:** Methodology, Writing – Review & Editing. **Yoshifumi Mizuno:** Conceptualization, Methodology, Writing – Original Draft, Writing – Review & Editing, Project administration, Funding acquisition.

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**Competing interests statement**

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Phenomenology, Diagnostic Issues, and Treatment Considerations. Curr Psychiatry Rep


64 Mizuno Y, Cai W, Supekar K, et al. Methylphenidate enhances spontaneous fluctuations in reward and cognitive control networks in children with attention-


**Figure legend**

Figure 1. Flow diagram for study on neurodevelopmental disorders (a) and the travelling-subject (TS) approach (b). In the TS approach, all participants will undergo MRI scans at 3 sites within 3 months. ADHD, attention-deficit/hyperactivity disorder, ASD, autism spectrum disorder; TD, typical development; TS, travelling-subject; MRI, magnetic resonance imaging.
Recruitment (ADHD, ASD, and TD)

Eligibility screening

Questionnaire response

Urine sample collection

MRI scanning

Behavioural measurements

Exclude participants who do not meet the criteria

MRI scanning at the University of Fukui

MRI scanning at the Osaka University

MRI scanning at the Chiba University

Saliva sample collection
Child Developmental MRI (CDM) Project: Protocol for a multi-centre, cross-sectional study on elucidating the pathophysiology of attention-deficit/hyperactivity disorder and autism spectrum disorder through a multi-dimensional approach

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<b>Primary Subject Heading</b>: Mental health

Secondary Subject Heading: Paediatrics, Radiology and imaging

Keywords: Child & adolescent psychiatry < PSYCHIATRY, Paediatric neurology < PAEDIATRICS, Developmental neurology & neurodisability < PAEDIATRICS
Child Developmental MRI (CDM) Project: Protocol for a multi-centre, cross-sectional study on elucidating the pathophysiology of attention-deficit/hyperactivity disorder and autism spectrum disorder through a multi-dimensional approach

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ABSTRACT

Introduction

Neuroimaging studies on attention-deficit/hyperactivity disorder (ADHD) and autism spectrum disorder (ASD) have demonstrated differences in extensive brain structure, activity, and network. However, there remains heterogeneity and inconsistency across these findings, presumably because of the diversity of the disorders themselves, small sample sizes, and site and parameter differences in magnetic resonance imaging (MRI) scanners, and their overall pathogenesis remains unclear. To address these gaps in the literature, we will apply the travelling-subject approach to correct site differences in MRI scanners and clarify brain structure and network characteristics of children with ADHD and ASD using large samples collected in a multi-centre collaboration. In addition, we will investigate the relationship between these characteristics and genetic, epigenetic, biochemical markers, and behavioural and psychological measures.

Methods and analysis

We will collect resting-state functional MRI (fMRI) and T1- and diffusion-weighted MRI data from 15 healthy adults as travelling subjects and 300 children (ADHD, n=100; ASD, n=100; and typical development, n=100) with multi-dimensional assessments. We will also apply data from more than 1,000 samples acquired in our previous neuroimaging studies on ADHD and ASD.

Ethics and dissemination

The study protocol has been approved by the Research Ethics Committee of the University of Fukui Hospital (approval no.: 20220601). Our study findings will be submitted to scientific peer-reviewed journals and conferences.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- We will multi-directionally compare neurobiological data using a large sample of children with neurodevelopmental disorders and typical development collected from multiple centres.
- We will apply the travelling-subject approach to correct site differences in MRI scanners.
- The multisite approach, including corrections for site differences in MRI scanners, may contribute to elucidating the pathogenesis and establishing imaging biomarkers of ADHD and ASD.
- Longitudinal studies using a multisite approach to ADHD and ASD may be needed following this cross-sectional study.
INTRODUCTION

Attention-deficit/hyperactivity disorder (ADHD) and autism spectrum disorder (ASD) are common neurodevelopmental disorders (NDDs) in child and adolescent psychiatry, with prevalence rates of >5% and >1.5%,[1,2] respectively. ADHD is characterised by age-inappropriate symptoms of inattention, hyperactivity, and impulsivity, whereas ASD is characterised by difficulties in social communication/interaction and stereotypical repetitive behaviour.[3] Although the main characteristics of ADHD and ASD differ, clinical assessments regarding accurate diagnosis and intervention based on pathology are currently difficult due to considerable clinical and neurobiological overlap between these disorders[4,5] and the high comorbidity rate.[6] In addition, several studies have reported that ADHD and ASD share various forms of behavioural impairment, particularly pertaining to executive function and motor skills.[7,8] ADHD and ASD are associated with an increased risk of depression, anxiety disorders, conduct disorders, severe central fatigue, and sleep disorders.[9-12] Therefore, it is important to establish early effective assessments and interventions for ADHD and ASD to prevent them from proceeding to secondary psychiatric comorbidities. Although many neuroimaging studies have been conducted to elucidate the underlying pathology and develop objective assessments for NDDs, these studies have yielded inconsistent findings.

Structurally, patients with ADHD show decreased grey matter volume (GMV) in the frontal lobe, amygdala, caudate nucleus, hippocampus, and putamen compared with control subjects.[13-16] Other studies of patients with ADHD found a larger right GMV, as well as a larger GMV in the dorsolateral prefrontal, temporal, intracalcarine cortices, and parietal lobule.[17-19] However, the largest study on volumetry in ADHD (conducted by the ENIGMA-ADHD Working Group) did not support previous findings regarding structural changes in some brain regions because of the small effect size.[20] In addition, resting-state functional magnetic resonance imaging (fMRI) studies have reported that compared with healthy subjects, patients with ADHD show more profound atypicality in the default mode, cognitive control, reward, attention, and amygdala-seeded network,[21-24] suggesting delays or alterations in the maturation of these connectors.[25-27] However, in contrast to previous studies, a meta-analysis of resting-state fMRI studies did not observe specific functional connectivity in ADHD.[28]

Previous studies on the brain structure of patients with ASD have found a smaller GMV in the middle frontal gyrus, middle temporal gyrus, amygdala, hippocampus, putamen, cerebellum, and precentral gyrus compared with control subjects.[29-31] However, several other studies did not find such reductions in GMV in ASD.[32-35] In addition, numerous studies have attempted to clarify local resting-state differences between subjects with ASD and controls. Although increased local functional connectivity of the frontal, temporal, and occipital lobes in the resting state has been reported in subjects with ASD,[36-38] some other studies of subjects with ASD did not find such between-group differences in these regions.[39-41]
Site and parameter differences in MRI scanners may explain the aforementioned observed discrepancies among neuroimaging studies. To clarify the distinct brain structure and network differences between ADHD and ASD, it is essential to evaluate these disorders from multiple perspectives via large-scale multi-site studies, while controlling for different MRI scanners. The travelling-subject (TS) approach is a promising candidate strategy for MRI scanner correction, because it can account for most of the sample variability resulting from measurement biases in brain structure and activity.[42-45]

In addition to the issue of MRI scanner measurement biases, there is also a paucity of evaluation criteria aimed at understanding the diversity of the disorders and their different underlying genetic backgrounds. A previous cross-sectional study on the Gazefinder system showed that children with ASD had a lower gaze ratio at the people region in the preference paradigm compared with children with typical development, suggesting that developmental characteristics of ASD can be assessed using the gaze ratio.[46] Nevertheless, it remains unclear whether specific gaze motions are cross-sectionally evident in children with ASD relative to ADHD. Neurochemical measures, some genetic polymorphisms and epigenetic alterations in risk genes related to monoamine metabolism, have been implicated in both of ADHD[47-49] and ASD,[50] highlighting their association with such genes and synaptic regulation of neurotransmitter binding and release. Additionally, it has been reported that functional connectivities of the caudate nucleus-parietal cortex and nucleus accumbens-occipital cortex are correlated with both polygenic risk score and diagnostic status in ADHD.[51] Although these findings suggest that candidate single nucleotide polymorphisms (SNPs) and DNA methylation may contribute to identifying the neural consequences of risk genes in NDDs, evidence in the literature is scarce. Furthermore, previous studies on the molecular basis of NDDs focused on monoamines and tryptophan (an essential amino acid and a precursor of serotonin) metabolism [52-56] and lacked information about their associations with brain structures and activity. Thus, multi-dimensional approaches may provide a means to link pathogenesis and imaging biomarkers of NDDs.

In this exploratory study, we will focus on the development of NDDs in children at multiple levels, including the behavioural measure, genetic, epigenetic, and neurotransmitter and amino acid levels. First, we will investigate site differences in MRI scanners using the TS approach. Second, we will investigate whether children with ADHD, ASD, and typical development show differences in the specificity of neurobiological functions. After correcting for site differences in MRI scanners, we will compare brain structure and resting-state functional connectivity among children with ADHD, ASD, and typical development. Subsequently, we will investigate associations between structural and functional changes; genetic, epigenetic, biochemical markers; and behavioural and psychological measurements in ADHD and ASD. In addition to these new data, we will use data from more than 1,000 existing samples collected from children with NDDs and typical development in our previous neuroimaging studies.[57-64]
METHODS AND ANALYSIS

Study design

This multi-centre cross-sectional study will be carried out at the Research Centre for Child Mental Development at the University of Fukui, Osaka University, and Chiba University. The study will be conducted in accordance with the Helsinki Declaration on ethical principles for medical research involving human subjects. The inclusion and exclusion criteria will be applied to identify individuals to be included in the study (Table 1). The study flow diagram is shown in Figure 1.

Table 1. Inclusion and exclusion criteria

<table>
<thead>
<tr>
<th>Neurodevelopmental disorder</th>
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<tbody>
<tr>
<td><strong>Inclusion criteria</strong></td>
</tr>
<tr>
<td>1. Fulfil the diagnostic criteria for ADHD and ASD according to the DSM-5</td>
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<tr>
<td>2. Aged 6 to 18 years at the time of informed consent</td>
</tr>
<tr>
<td><strong>Exclusion criteria</strong></td>
</tr>
<tr>
<td>1. Full-scale intelligence quotient &lt; 70</td>
</tr>
<tr>
<td>2. History of severe head trauma or neurological illness</td>
</tr>
<tr>
<td>3. Potential for hazards associated with MRI examination (such as the presence of metal on the body surface or internal structures, pregnancy or possibility of pregnancy, claustrophobia, and fear of the dark)</td>
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<th>Typical development</th>
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<td><strong>Inclusion criteria</strong></td>
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<tr>
<td>1. Aged 6 to 18 years at the time of informed consent</td>
</tr>
<tr>
<td>2. Does not receive special education</td>
</tr>
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<td><strong>Exclusion criteria</strong></td>
</tr>
<tr>
<td>1. Full-scale intelligence quotient &lt; 70</td>
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<tr>
<th>Travelling subject</th>
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<tr>
<td><strong>Inclusion criteria</strong></td>
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</table>
1. Aged 20 to 65 years at the time of informed consent
2. Does not receive special education

Exclusion criteria
1. History of severe head trauma, neurological illness, or neurodevelopmental disorder
2. Cognitive impairment
3. Potential for hazards associated with MRI examination (such as the presence of metal on
the body surface or internal structures, pregnancy or possibility of pregnancy, claustrophobia,
and fear of the dark)

The Japanese versions of the Wechsler Intelligence Scale for Children, fourth or fifth edition
(WISC-IV or WISC-V) will be used to assess full-scale intelligence quotient. WISC-IV and
WISC-V consist of verbal subtests (of information, similarities, arithmetic, vocabulary,
comprehension, and digit span) and performance subtests (of picture completion, coding/digit
symbols, picture arrangement, block design, and object assembly). DSM-5, Diagnostic and
Statistical Manual of Mental Disorders, Fifth Edition; MRI, magnetic resonance imaging

Recruitment of participants
Study participants will be recruited between 12 July 2022 and 31 March 2032. Children with
NDDs (ADHD and ASD) will be recruited from the University of Fukui Hospital, Chiba
University Hospital, and Osaka University Hospital. The diagnoses of ADHD and ASD will be
based on the criteria outlined in the Diagnostic and Statistical Manual of Mental Disorders, Fifth
Edition.[3] Clinicians at each hospital will introduce the study to patients who fulfil the inclusion
criteria. Children with typical development will be recruited from the community. In addition,
we will recruit healthy travelling community-dwelling adults to account for site differences in
MRI scanners, because long-term MRI scanning for scanner correction in a multi-site study may
not be suitable for children. Informed consent will be obtained from participants and/or their
legal guardians.

Patient and public involvement
Patients and/or the public were not involved in the study design and will not be involved in the
conduct, reporting, and dissemination of the findings of this study.

Outcomes
Primary endpoints:
Brain structure (GMV, cortical thickness, surface area, white matter fractional anisotropy) and resting-state functional connectivity

The following secondary endpoints will be assessed to investigate the correlations with the primary endpoint brain measures:

- Clinical information
- Psychological measurements
- Cognitive measurements
- Genomic and epigenomic data
- Urine levels of monoamine metabolites and tryptophan

Psychological measurements

We will use the Japanese-translated versions of various psychological questionnaires to assess psychological characteristics and lifestyles in both children with NDDs and typical development. The questionnaires are shown in Table 2.

Table 2. Psychological measurements

<table>
<thead>
<tr>
<th>Questionnaire</th>
<th>Item</th>
<th>Assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRS-2</td>
<td>65-item; 4-point Likert scale (1 = not at all to 4 = very)</td>
<td>Social communication and restricted repetitive behaviours</td>
</tr>
<tr>
<td>SSP</td>
<td>38-item; 5-point Likert scale (1 = not at all to 5 = very)</td>
<td>Hyperesthesia and insensitive</td>
</tr>
<tr>
<td>Conners 3</td>
<td>108-item (parents) and 99-item (children); 4-point Likert scale (0 = not at all to 3 = very)</td>
<td>Inattentiveness and hyperactivity/impulsivity</td>
</tr>
<tr>
<td>DSRSC</td>
<td>18-item; 3-point Likert scale (0 = not at all to 2 = very)</td>
<td>Depression</td>
</tr>
<tr>
<td>SCAS</td>
<td>38-item; 4-point Likert scale (0 = never to 3 = always)</td>
<td>Symptoms of anxiety disorders</td>
</tr>
<tr>
<td>ACE</td>
<td>10-item; Yes or No response</td>
<td>Physical, verbal, or sexual abuse, mental illness, or substance abuse in the nuclear family</td>
</tr>
<tr>
<td>CTQ</td>
<td>28-item; 5-point Likert scale (1 = never to 5 = always)</td>
<td>Childhood maltreatment and its severity</td>
</tr>
<tr>
<td>Questionnaire</td>
<td>Description</td>
<td></td>
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<tr>
<td>SES</td>
<td>5-item; Single answer</td>
<td>Parental education, occupation, and monthly income</td>
</tr>
<tr>
<td>PSI-short form</td>
<td>19-item; 5-point Likert scale (1 = strongly disagree to 5 = strongly agree)</td>
<td>Level of anxiety in interaction with their children and parental stress related to their children’s temperament and behaviour</td>
</tr>
<tr>
<td>PS</td>
<td>30-item; 7-point Likert scale (1 = effective discipline to 7 = dysfunctional discipline)</td>
<td>Discipline style in response to child misbehaviour</td>
</tr>
<tr>
<td>Kid-KINDL questionnaire</td>
<td>30-item; 5-point Likert scale (1 = not at all to 5 = very)</td>
<td>Physical health, mental health, self-esteem, family, friends, and school life</td>
</tr>
<tr>
<td>Kiddo-KINDOL questionnaire</td>
<td>30-item; 5-point Likert scale (1 = not at all to 5 = very)</td>
<td>Physical health, mental health, self-esteem, family, friends, and school life</td>
</tr>
<tr>
<td>JSQ-ES</td>
<td>38-item; 6-point Likert scale (1 = not at all to 6 = very)</td>
<td>Sleep disturbance and problematic sleep habits</td>
</tr>
<tr>
<td>JSQ-JH</td>
<td>38-item; 6-point Likert scale (1 = not at all to 6 = very)</td>
<td>Sleep disturbance and problematic sleep habits</td>
</tr>
<tr>
<td>EHI</td>
<td>10-item; Typing a “+” or “++” in the appropriate column (right or left)</td>
<td>Degree of hand laterality in daily activities</td>
</tr>
<tr>
<td>BRIEF</td>
<td>86-item; 3-point Likert scale (1 = never to 3 = often)</td>
<td>Assessment of ability to inhibit, shift, control emotions, working memory, and plan/organize</td>
</tr>
<tr>
<td>FCV-19S</td>
<td>7-item; 5-point Likert scale (1 = strongly disagree to 5 = strongly agree)</td>
<td>Severity of individuals’ fear of COVID-19</td>
</tr>
<tr>
<td>DCDQ</td>
<td>15-item; 5-point Likert scale (1 = not at all like your child to 5 = extremely like your child)</td>
<td>Child’s gross- and fine-motor coordination</td>
</tr>
<tr>
<td>CFS</td>
<td>14-item; 4-point Likert scale (0 = less than usual to 3 = much more than usual)</td>
<td>Mental and physical fatigue</td>
</tr>
</tbody>
</table>

References:
- SRS-2, Social Responsiveness Scale, Second Edition; SSP, Short Sensory Profile; DSRSC, Depression Self-Rating Scale for Children; SCAS, Spence Children’s Anxiety Scale; ACE, Adverse Childhood Experiences; CTQ, Childhood Trauma Questionnaire; SES, Socioeconomic Status; PSI, Parenting Stress Index; PS, Parenting Scale; JSQ-ES, Japanese Sleep Questionnaire for Elementary Schoolers; JSQ-JH, Japanese Sleep Questionnaire for Junior High Schoolers; EHI, Edinburgh Handedness Inventory; BRIEF, Behaviour Rating Inventory of Executive Function; FCV-19S, Fear of Coronavirus-19 Scale; DCDQ, Developmental Coordination Disorder Questionnaire; CFS, Chalder Fatigue Scale
Behavioural measurements

Cognitive and eye-tracking functions will be assessed in children with ADHD, ASD, and typical development. The cognitive test will consist of the Cambridge Neuropsychological Tasks Automated Battery (CANTAB; http://www.cambridgecognition.com/cantab/).[65-67] The stop signal task of the CANTAB will be utilised to assess the inhibition response. Participants will be asked to quickly respond to an arrow stimulus by selecting one of two options depending on the direction in which the arrow is pointing. Thereafter, participants will be instructed to withhold their behavioural response when an auditory signal is present. The spatial working memory task of CANTAB will be utilised to assess the retention of spatial information and retrieval of retained items from the working memory. Participants will be asked to find blue tokens hidden inside a number of coloured boxes on the screen and place them in an empty column on the side of the screen. Since the colour and position of the boxes will be changed to avoid the stereotyped search strategy in each trial, participants will be instructed not to return to a box where a token has previously been found.

The Gazefinder (JVC Kenwood Co. Ltd., Yokohama, Japan) task in the eye-tracking test will be used to assess eye gaze patterns allocated to specific objects (e.g., the human face with or without mouth motion, the biological motion of a human, the preference paradigm for people or geometry, and a screenshot of finger-pointing to social and geometry areas) on a video monitor.[46,68,69]

Urine collection and high-performance liquid chromatography assay

Urinary levels of monoamine metabolites and tryptophan, which have been proposed as predictive, cost-effective, and non-invasive biomarkers of brain function,[52,56,70-72] will be measured in children with ADHD, ASD, and typical development. Prior to testing, the participants will be instructed to refrain from intense physical activity and the intake of food and beverages such as alcohol, coffee, high-fat fish, red beef, and blue cheese, which can be difficult to digest, for 24 h. Additionally, water will be, the only beverage they will be allowed to have on the day of sample collection. On the test day, fresh urine will be collected after participants are made to rest for 30 min. Freshly collected urine samples will be diluted with 6.7 mM hydrochloric acid and 2.5% perchloric acid to separate albumin, as previously reported.[72] The obtained supernatant will be stored at -78 °C until high-performance liquid chromatography (Nanospace SI-2 3001; Shiseido Japan Co. Ltd., Tokyo, Japan) assay with an electrochemical detector (Nanospace SI-2 3005; Shiseido Japan Co. Ltd.) and a chromate recorder (C-R8A; Shimadzu Corporation, Kyoto, Japan). The mobile phase will consist of 15% methanol in a solution (pH, 4.13) containing 30 mM citric acid, 10 mM disodium hydrogen phosphate, 0.5 mM sodium octyl sulphate, 50 mM sodium chloride, and 0.05 mM ethylenediaminetetraacetic acid, as
previously reported.[72-74] This will be pumped through a 5 μM C₁₈ column (150 mm × 4.6 mm) at a flow rate of 0.7 mL/min.

Genetic polymorphism and epigenetic assays of saliva samples

To assess neural structural and functional impairment-related pathophysiological processes in NDDs, it is essential to understand how genetic and epigenetic risk factors are associated with such atypical characteristics. SNPs and DNA methylation will be assessed in children with ADHD, ASD, and typical development. Saliva samples (which do not require invasive collection)[75] will be directly collected using Oragene Discover OGR-675 kits (DNA Genotek Inc., Ottawa, ON, Canada). Saliva DNA will be extracted using preplT®/L2P reagent (DNA Genotek Inc.) and quantified using Qubit™ dsDNA HS assay kits (Thermo Fisher Scientific Inc., Pittsburgh, PA, USA), as previously reported.[75-77] Thereafter, we plan to characterise vulnerable genetic and epigenetic factors at both genome-wide and candidate gene levels (e.g., oxytocin and glutamate receptors, catechol-O-methyltransferase, and branched-chain aminotransferase).

Image acquisition

Prior to scanning, children will be carefully habituated to the MRI scanner sounds, which may improve yield. Children with NDDs and typical development will be scanned using a 3-T GE Signa PET/MR scanner (General Electric HealthCare, Chicago, IL, USA) at the University of Fukui, a 3-T GE Signa Architect scanner (General Electric HealthCare) at Osaka University, and a 3-T GE Discovery MR750 scanner (General Electric HealthCare) at Chiba University. In addition to the abovementioned scanners, a 3-T GE Discovery MR750 scanner (General Electric HealthCare) at the University of Fukui will also be used for scanning healthy travelling adults, because some of the imaging data in our existing samples were acquired using this scanner. Imaging orders will be placed for resting-state fMRI, T1-weighted, and diffusion-weighted (DTI) images. Scanning parameters are shown in Supplementary Table 1.

Data analysis plan

We will investigate site corrections in MRI scanners using the TS approach.[42-45] This approach can account for most of the sample variability resulting from measurement biases (due to differences in imaging variables, field strength, manufacturers, and scanner models) and sampling bias (due to differences in participant groups), as previously reported.[42] Since our TS dataset will include only healthy participants, we plan to correct measurement bias only to quantitatively investigate the site effects in brain structure and activity. Briefly, we will calculate
the coefficients for the scanner, head coil, fMRI manufacturer, and phase encoding at each site and correct imaging data on the basis of these coefficients using regression models and clustering algorithms. Thereafter, we will investigate whether differences in brain structure and activity exist among children with ADHD, ASD, and typical development. The GMV analyses will be performed using Statistical Parametric Mapping 12 (https://www.fil.ion.ucl.ac.uk/spm/software/spm12/) or FreeSurfer (http://www.surfer.nmr.mgh.harvard.edu/). The cortical thickness and surface area analyses will be performed using FreeSurfer. Based on differences in GMV, cortical thickness, and surface area among the groups, the extracted regions of interest (ROIs) will be used to investigate the correlations between the corresponding structures and behavioural or neurochemical measures. The white matter fractional anisotropy (FA) analyses will be performed using the FSL software package (http://www.fmrib.ox.ac.uk/fsl) and the TRActs Constrained by UnderLying Anatomy (TRACULA) tool in FreeSurfer. In particular, we plan to examine the FA values in the inferior frontal-occipital fasciculus, superior/inferior longitudinal fasciculus, capsula interna, anterior corona radiata, and cerebellum, because these regions are known to be associated with the pathogenesis of ADHD[78] and ASD.[79] Moreover, based on the group differences, the correlations between FA values and behavioural or neurochemical measures will be determined. Resting-state functional connectivity will be analysed using the CONN toolbox[80] (http://www.nitrc.org/projects/conn, PRID: SCR_009550). Excessive head motion will be addressed by exclusion (over 3.0 mm, 3.0 degree) and scrubbing as necessary.[63,64,81] A seed-to-voxel analysis will be performed using the ROIs obtained from the structural analysis. In addition, we will investigate forms of functional connectivity such as the default mode network, salience network, dorsal attention network, cognitive control network, and affective network. Further, we will analyse the correlation between the functional connectivity value and behavioural or biological measures.

**Sample size**

We finally aim to build the largest database of NDDs in Japan, comparable to other international databases. Although cross-sectional large-sample databases containing neuroimaging data of persons with NDDs exist (the ADHD-200 database comprising the data of 285 individuals with ADHD and 491 persons with typical development aged 7–21 years and the Autism Brain Imaging Data Exchange [ABIDE- II ] database comprising the data of 521 persons with ASD and 593 individuals with typical development aged 5–64 years), we already possess the data of more than 1,000 MRI data with bioinformatic, behavioural, and psychological information on children with NDDs and typical development.[57-64] Moreover, we will acquire multi-dimensional data from 300 children (100 with ADHD, 100 with ASD, and 100 with typical development) and scan 15 healthy travelling adults to correct for site differences in MRI scanners according to the previously described TS approach.[42-45]
ETHICS AND DISSEMINATION

The study protocol has been approved by the Research Ethics Committee of the University of Fukui Hospital (approval no. 20220601). Informed consent will be sought from all participants and/or their legal guardians. The results will be disseminated in academic journals, conferences, and databases as well as social media.

DISCUSSION

The Child Developmental MRI Project aims to clarify the pathogenesis underlying NDDs and establish relevant neurobiological markers by constructing the largest database of NDDs in Japan. The main protocol pertains to (i) a comparative analysis of brain structure and network among children with ADHD, ASD, and typical development in conjunction with more than 1,000 existing samples; (ii) a multi-dimensional approach to these NDDs and typical development using genetic, epigenetic, neurotransmitter and amino acid markers, and cognitive and psychological measures; and (iii) a TS approach to correct for differences in MRI scanners at multiple sites. Although a high rate of comorbidity and neurobiological commonalities between ADHD and ASD is thought to complicate their differential diagnosis, our project will be the first to demonstrate neurobiological distinctions between these disorders.

In addition, outcomes based on multi-dimensional approaches may provide additional information regarding the specificities of brain functions associated with the diversity of these NDDs. A cross-sectional behavioural study showed that children with ASD had a lower gaze ratio during social stimuli compared with children with typical development.[46] Since the orbitofrontal-striatal-amygdala circuit, which responds to social stimuli such as faces and social approval, has been implicated in abnormal social behaviour in ASD,[82] it is natural to anticipate, together with the association of neural level, whether gaze processing in social information is impaired in ADHD and compare it with deficits reported in ASD. A recent meta-analysis of genome-wide association studies (GWAS), which analysed the data for 20,183 patients diagnosed with ADHD and 35,191 controls, aimed to establish the causal relationships between genetic variants and the disorder and 304 identified genetic variants of several potential ADHD-specific genes (e.g., forkhead box P2, artemin, and dual specificity phosphatase 6).[83] Moreover, a GWAS meta-analysis of the data for 18,381 individuals with ASD and 27,969 controls identified 5 risk loci, including those corresponding to neuronal growth regulator 1, polypyrimidine tract binding protein 2, and calcium-dependent secretion activator.[84] These findings indicate that the majority of NDD cases may involve the pathogenic convergence of multiple variants, and not just a single gene defect. A few of the abovementioned genes are also thought to play key roles in the regulation of neurotransmitter levels[84,85] and brain development.[86,87] In particular, several studies have pointed out that NDDs are associated
with more profound abnormalities in dopamine, norepinephrine, and serotonin metabolism.[53,54,56] Furthermore, several recent studies have demonstrated that changes in tryptophan metabolism are associated with the pathology of ADHD[52] and ASD.[55] This was also supported by the demonstration of associations between chronic enhancement of free tryptophan and reduced branched-chain amino acid levels and hyperactivity/impulsivity in a rat model of ADHD.[88] However, there is a lack of sufficient information about the associations between such neurochemical indexes and neuroimaging data in ADHD and ASD. Thus, our multi-dimensional analysis may help to clarify the diversity of NDDs and their pathogenesis, which hypothesizes multifactorial mechanisms at several different levels, from gene-molecular to brain and cognition. Moreover, the data collected in this large-sample multisite study will shed new light on the diversity of NDDs and could help in the establishment of criteria for more accurate diagnoses based on their underlying pathophysiology.

Our project has some limitations. First, the geographic area is not entirely controlled, which will limit the generalisability of our results. However, we will construct the largest database that multidimensionally assesses the neurobiological bases of NDDs in Japan. Second, the present study does not apply Autism Diagnostic Observation Schedule, Second Edition and Autism Diagnostic Interview-Revised assessments for ASD, because it will be difficult to include these measures given the design and time management aspects of the protocol. Alternatively, we diagnose ASD based on the DSM-5 and additionally use the Social Responsiveness Scale, Second Edition and the Short Sensory Profile, both of which have been validated in Japanese samples, to identify the core symptoms of ASD. These measurements are accepted as powerful tools for quantifying the clinical features of ASD.[89-91] Moreover, we believe that our multi-dimensional approach involving neuroimaging, genetic, molecular, and behavioural data will help to adequately predict the specificity of neurobiological functions in children with ADHD and ASD. Third, our study is cross-sectional, and longitudinal studies on the multi-dimensional approach to ADHD and ASD will also be needed in the future, as previous longitudinal studies have reported differences in atypical brain structures at the developmental stage.[92,93]

Acknowledgements

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Authors’ contributions

Masatoshi Yamashita: Conceptualization, Methodology, Writing – Original Draft, Writing – Review & Editing, Funding acquisition. Kuriko Kagitani-Shimono: Conceptualization,
Methodology, Writing – Review & Editing, Funding acquisition. **Yoshiyuki Hirano:** Conceptualization, Methodology, Writing – Review & Editing, Funding acquisition. **Sayo Hamatani:** Methodology, Writing – Review & Editing, Funding acquisition. **Shota Nishitani:** Methodology, Writing – Review & Editing. **Akiko Yao:** Methodology, Writing – Review & Editing. **Sawa Kurata:** Methodology, Writing – Review & Editing. **Hirotaka Kosaka:** Methodology, Writing – Review & Editing. **Minyoung Jung:** Methodology, Writing – Review & Editing. **Tsuyoshi Sasaki:** Methodology, Writing – Review & Editing. **Tokiko Yoshida:** Methodology, Writing – Review & Editing. **Koji Matsumoto:** Methodology, Writing – Review & Editing. **Yoko Kato:** Methodology, Writing – Review & Editing. **Masaya Tachibana:** Methodology, Writing – Review & Editing. **Ikuko Mohri:** Methodology, Writing – Review & Editing. **Kenji J. Tsuchiya:** Methodology, Writing – Review & Editing. **Tetsuya Tsujikawa:** Methodology, Writing – Review & Editing. **Hidehiko Okazawa:** Methodology, Writing – Review & Editing. **Eiji Shimizu:** Methodology, Writing – Review & Editing. **Masako Taniike:** Methodology, Writing – Review & Editing. **Yoshifumi Mizuno:** Conceptualization, Methodology, Writing – Original Draft, Writing – Review & Editing, Project administration, Funding acquisition.

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**Competing interests statement**

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**References**


Figure legend

Figure 1. Flow diagram for study on neurodevelopmental disorders (a) and the travelling-subject (TS) approach (b). In the TS approach, all participants will undergo MRI scans at 3 sites within 3 months. ADHD, attention-deficit/hyperactivity disorder; ASD, autism spectrum disorder; TD, typical development; TS, travelling-subject; MRI, magnetic resonance imaging
Recruitment (ADHD, ASD, and TD)

Eligibility screening

Exclude participants who do not meet the criteria

Questionnaire response

Urine sample collection

MRI scanning

Behavioural measurements

Saliva sample collection

a

Recruitment (TS)

Eligibility screening

b

MRI scanning at the University of Fukui

MRI scanning at the Osaka University

MRI scanning at the Chiba University
Child Developmental MRI (CDM) Project: Protocol for a multi-centre, cross-sectional study on elucidating the pathophysiology of attention-deficit/hyperactivity disorder and autism spectrum disorder through a multi-dimensional approach

Supplementary Table 1. Scanning parameters for multi-site studies

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<th>Site</th>
<th>Head coil</th>
<th>Scan time (min:sec)</th>
<th>TR (ms)</th>
<th>TE (ms)</th>
<th>FA (deg)</th>
<th>FOV (mm)</th>
<th>Matrix</th>
<th>Voxel dimension (mm)</th>
<th>Slice Direction</th>
<th>Slice thickness (mm)</th>
<th>Slice b-values</th>
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Resting-state fMRI scanning for healthy travelling adults at the University of Fukui will be performed using PET/MR and MR750 scanners under both open-eye and closed-eye conditions, because some of our existing sample data were acquired under the closed-eye condition using the MR750 scanner at the University of Fukui. During resting-state fMRI under open-eye conditions, participants will be instructed to think about nothing in particular, stay awake, and keep their eyes open and fixated on the crosshair on the screen. During resting-state fMRI under closed-eye conditions, participants will be instructed to think about nothing in particular, stay awake, and keep their eyes closed. Resting-state fMRI scanning for children with NDDs and typical development will be performed under open-eye conditions. fMRI, functional magnetic resonance imaging; T1WI, T1-weighted images; DTI, diffusion-weighted images; min, minutes; sec, seconds; TR, repetition time; TE, echo time; FA, flip angle; FOV, field of view; N/A, not applicable; ch, channels; NDDs, neurodevelopmental disorders.