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 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.

Methods and analysis We will collect resting-state functional MRI (fMRI) and T1-weighted 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 multidimensional assessments. We will also apply data from more than 1000 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 attention-deficit/hyperactivity disorder (ADHD) and autism spectrum disorder (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%,12 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 and the high comorbidity rate. In addition, several studies have reported that ADHD and ASD share various forms of behavioural impairment, particularly pertaining to executive function and motor skills. ADHD and ASD are associated with an increased risk of depression, anxiety disorders, conduct disorders, severe central fatigue and sleep disorders. 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. Other studies of patients with ADHD found a larger right GMV, as well as a larger GMV in the dorsolateral prefrontal, temporal, intralacarne cortex and parietal lobule. 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. In addition, resting-state functional MRI (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, suggesting delays or alterations in the maturation of these connectors. However, in contrast to previous studies, a meta-analysis of resting-state fMRI studies did not observe specific functional connectivity in ADHD.

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. However, several other studies did not find such reductions in GMV in ASD. 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, some other studies of subjects with ASD did not find such between-group differences in these regions.

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 multisite 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.

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. 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 and ASD, 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. Although these findings suggest that candidate single nucleotide polymorphisms (SNPs) and DNA methylation 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 and lacked information about their associations with brain structure and activity. Thus, multidimensional 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 1000 existing samples collected from children with NDDs and typical development in our previous neuroimaging studies.

## METHODS AND ANALYSIS

### Study design

This multi-centre cross-sectional study will be carried out at the Research Centre for Child Mental Development...
Box 1  Inclusion and exclusion criteria

<table>
<thead>
<tr>
<th>Neurodevelopmental disorder</th>
<th>Inclusion criteria</th>
<th>Exclusion criteria</th>
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<tbody>
<tr>
<td>1. Fulfil the diagnostic criteria for ADHD and ASD according to the DSM-5</td>
<td>1. Full-scale intelligence quotient &lt;70</td>
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<tr>
<td>2. Aged 6 to 18 years at the time of informed consent</td>
<td>2. History of severe head trauma or neurological illness</td>
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<tr>
<td>2. Aged 6 to 18 years at the time of informed consent</td>
<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>
<th>Inclusion criteria</th>
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<tr>
<td>1. Aged 6 to 18 years at the time of informed consent</td>
<td>1. Full-scale intelligence quotient &lt;70</td>
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<td>2. Does not receive special education</td>
<td>2. History of severe head trauma, neurological illness or neurodevelopmental disorder</td>
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<th>Travelling subject</th>
<th>Inclusion criteria</th>
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<tr>
<td>1. Aged 20 to 65 years at the time of informed consent</td>
<td>1. Full-scale intelligence quotient &lt;70</td>
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<td>2. Does not receive special education</td>
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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). ADHD, attention-deficit/hyperactivity disorder; ASD, autism spectrum disorder; DSM-5, Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition.

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 (box 1). The study flow diagram is shown in figure 1.

Recruitment of participants

Study participants will be recruited between 12 July 2022 and 31 March 2023. 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 (DSM-5). Clinicians at each hospital will introduce the study to patients who fulfill 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 multisite 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 1.

**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 the 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, Yokohama, Japan) task in the eye-tracking test will be used to assess eye gaze patterns allocated to specific objects (eg, 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 hours. 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, Tokyo, Japan) assay with an electrochemical detector (Nanospace SI-2 3005; Shiseido Japan Co) and a chromatograph 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 EDTA, 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, Ottawa, Ontario, Canada). Saliva DNA will be extracted using preplT/L2P reagent (DNA Genotek) and quantified using Qubit dsDNA HS assay kits (Thermo Fisher Scientific, Pittsburgh, Pennsylvania, USA), as previously reported.75–77 Thereafter, we plan to characterise vulnerable genetic and epigenetic factors at both genome-wide and candidate gene levels (eg, 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 3T GE Signa PET/MR scanner (General Electric Healthcare, Chicago, Illinois, USA) at the University of Fukui, a 3T GE Signa Architect scanner (General Electric Healthcare) at Osaka University and a 3T GE Discovery MR750 scanner (General Electric Healthcare) at Chiba University. In addition to the abovementioned scanners, a 3T GE Discovery MR750 scanner (General Electric

Figure 1 Flow diagram for study on neurodevelopmental disorders (A) and the TS approach (B). In the TS approach, all participants will undergo MRI scans at three sites within 3 months. ADHD, attention-deficit/hyperactivity disorder; ASD, autism spectrum disorder; TD, typical development; TS, travelling subject.
we will correct measurement bias only to quantitatively investigate the site effects as previously reported. Since our TS dataset will include 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 field strength, manufacturers and scanner models) and phase encoding at each site and correct imaging data in brain structure and activity. Briefly, we will calculate the 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

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

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<th>Table 1 Psychological measurements</th>
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<td><strong>Questionnaire</strong></td>
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<td>PSI-short form</td>
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<td>Kid-KINDL R questionnaire</td>
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<td>FCV-19S</td>
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<td>DCDQ</td>
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<td>CFS</td>
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ACE, Adverse Childhood Experiences; BRIEF, Behaviour Rating Inventory of Executive Function; CFS, Chalder Fatigue Scale; CTQ, Childhood Trauma Questionnaire; DCDQ, Developmental Coordination Disorder Questionnaire; DSRSC, Depression Self-Rating Scale for Children; EHI, Edinburgh Handedness Inventory; FCV-19S, Fear of Coronavirus-19 Scale; JSQ-ES, Japanese Sleep Questionnaire for Elementary Schoolers; JQ-JH, Japanese Sleep Questionnaire for Junior High Schoolers; PS, Parenting Scale; PSI, Parenting Stress Index; SCAS, Spence Children’s Anxiety Scale; SES, Socio-Economic Status; SRS-2, Social Responsiveness Scale, Second Edition; SSP, Short Sensory Profile.
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\(^7\) and ASD.\(^8\) 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\(^8\) (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.\(^63\) 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 1000 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.\(^42-45\)

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 (1) a comparative analysis of brain structure and network among children with ADHD, ASD and typical development in conjunction with more than 1000 existing samples; (2) a multi-dimensional approach to these NDDs and typical development using genetic, epigenetic, neurotransmitter and amino acid markers, and cognitive and psychological measures; and (3) 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 (eg, 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 five 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\(^84\) and brain development.\(^86\) \(^87\) In particular, several studies have pointed out

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.
that NDDs are associated with more profound abnormalities in dopamine, norepinephrine and serotonin metabolism.\textsuperscript{53, 54, 56} Furthermore, several recent studies have demonstrated that changes in tryptophan metabolism are associated with the pathology of ADHD\textsuperscript{52} and ASD.\textsuperscript{50} 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.\textsuperscript{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 hypothesises 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.\textsuperscript{86–91} 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.\textsuperscript{92, 93}

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Contributors MF: conceptualisation, methodology, writing—original draft, writing—review and editing, funding acquisition; KK-S and YH: conceptualisation, methodology, writing—review and editing, funding acquisition; SH: methodology, writing—review and editing, funding acquisition; SN, AY, SK, HK, MJ, TYS, KM, YK, MN, MT, IM, KJJ, TT, HO, ES, MF and AT: methodology, writing—review and editing; YM: conceptualisation, methodology, writing—original draft, writing—review and editing, project administration, funding acquisition.

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Patient consent for publication Not applicable.

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