PEER REVIEW HISTORY

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ARTICLE DETAILS

<table>
<thead>
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<th>TITLE (PROVISIONAL)</th>
<th>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</th>
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<td>AUTHORS</td>
<td>Yamashita, Masatoshi; Kagitani-Shimono, Kuriko; Hirano, Yoshiyuki; Hamatani, Sayo; Nishitani, Shota; Yao, Akiko; Kurata, Sawa; Kosaka, Hidetaka; Jung, Minyoung; Yoshida, Tokiko; Sasaki, Tsuyoshi; Matsumoto, Koji; Kato, Yoko; Nakanishi, Mariko; Tachibana, Masaya; Mohri, Ikuko; Tsuchiya, Kenji J.; Tsujikawa, Tetsuya; Okazawa, Hidehiko; Shimizu, Eiji; Taniike, Masako; Tomoda, Akemi; Mizuno, Yoshifumi</td>
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VERSION 1 – REVIEW

<table>
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<tr>
<th>REVIEWER</th>
<th>Castellanos, Francisco X  Nathan S Kline Institute for Psychiatric Research, Center of Brain Imaging and Neumodulation</th>
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<tbody>
<tr>
<td>REVIEW RETURNED</td>
<td>11-Jan-2023</td>
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GENERAL COMMENTS

This is a protocol to examine the pathophysiology of ADHD and autism spectrum disorder (ASD) by recruiting participants across 3 sites. The protocol also includes the recruitment of 15 healthy adults (traveling subjects (TS)) who will undergo scans at each of the sites to facilitate accounting for scanner/site differences.

It is commendable to present a detailed protocol prior to conducting such a study. The inclusion of TS is also worthwhile, given the challenges of combining data across scanners and sites.

The protocol does raise some questions and issues.

First, the protocol plans for the 15 TS to each be scanned three times, but the Fukui site has 2 scanners – one of which is a PET/MRI scanner – which may introduce some additional uncontrolled variability. If it is necessary to use the PET/MRI scanner to study the children, then the TS should also be imaged on that machine.

The protocol does not specify the length of the resting state scans. The number of TR’s (or volumes) is a major determinant of the reliability of resting state measures – given that the scanning parameters are all single echo EPI sequences, which are inherently noisier than multi-echo sequences, it would be advisable to attempt to obtain longer sequences whenever possible. Doing so is difficult given the greater tendency of children to move – so it may be worthwhile considering also including
scanning during movie conditions as well as during open eye resting state.

A major omission is lack of specific head motion criteria – how will head motion be measured, and what threshold is unacceptable? How will excessive motion be handled?

The protocol mentions both eyes open and eyes closed conditions, at the foot of Table 3, but does not specify whether both are to be included, whether they would be counterbalanced, etc.

In general, eyes closed conditions are an invitation to sleep, which can systematically alter the amplitude of the signals – it’s a major challenge in the field, but it seems advisable to avoid including such a confound in a newly collected data set, if possible.

The phenotyping is broad, but only includes one measure that is related to ASD, and in a complex manner. The SRS (presumably 2nd edition?) has been extensively used in ASD research, but it reflects many conditions. It should be included, but the absence of more focused ASD measurements will make it difficult to link these data to those from other groups. It may not be feasible to obtain ADOS-2 or ADI-R interviews, but other autism indices should be included if they have been validated in Japanese samples.

The collection of urine to quantify monoamine metabolites and tryptophan is likely to be a waste of time. The references cited are either irrelevant or out-of-date. The urinary compartment is too distant from neuronal sources in general. To do this well, the authors would need to consider 24-hour collections, etc. Otherwise, the results will be meaningless, given variations in fluid intake, time of day, etc.

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A minor note: the authors state “multilateral” several times – this does not translate well into English. They likely mean “multisite” in most cases.

The sentence at the end of page 4, and start of page 5: “These factors were not controlled between studies, which might have weakened the essential characteristics of the disorders” does not make sense.

Finally, the authors may benefit from considering the work of Taki (Russell) Shinohara and colleagues on harmonizing data across sites (ComBat and its variations). This is a protocol to examine the pathophysiology of ADHD and autism spectrum disorder (ASD) by recruiting participants across 3 sites. The protocol also includes the recruitment of 15 healthy adults (traveling subjects (TS)) who will undergo scans at each of the sites to facilitate accounting for scanner/site differences.
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REVIEWER Soman, Shania Mereen
Deakin University
REVIEW RETURNED 21-Feb-2023

GENERAL COMMENTS Thank you for sending me this paper to review. In this work, Yamashita and colleagues used travelling-subject approach to correct site differences in MRI scanners and clarify brain structure and network characteristics of children with ADHD and ASD collected in a multi-center collaboration. This will be a great study. I have few comments.
1) I find that aim of the study is missing in the abstract section. Aim of the study should be mentioned under introduction section in the abstract.
2) The study focusses on child development in subjects with NDDs at multiple levels (genetics, epigenetics, neurotransmitter, amino acid level and behavioural measures). But the authors haven’t mentioned any information of prior studies that has examined these modalities in ADHD and ASD. It would be good to flush out this in the body of introduction to strengthen the aim of the paper.
3) The authors have mentioned primary outcomes of the study as brain structure and resting state functional connectivity. What are you measuring using brain structure? Is it gray matter volume? It would have been good to make this clear.

4) Under image acquisition, it is mentioned that DTI images will be acquired. But it is not clear in the outcomes whether this modality will be used for future analysis. Also, it would be good to add under data analysis plan the white matter tracts you will be focusing on.

5) Authors have mentioned under data analysis plan that they will be using TS approach to investigate site corrections in MRI scanners. It would be good to explain how this approach helps to differentiate most of the sample variability.

6) It would be good to include implication of each outcome of the main protocol in discussion.

**VERSION 1 – AUTHOR RESPONSE**

Reviewer 1 (Dr. Francisco X Castellanos)

Evaluation

This is a protocol to examine the pathophysiology of ADHD and autism spectrum disorder (ASD) by recruiting participants across 3 sites. The protocol also includes the recruitment of 15 healthy adults (travelling subjects (TS)) who will undergo scans at each of the sites to facilitate accounting for scanner/site differences. It is commendable to present a detailed protocol prior to conducting such a study. The inclusion of TS is also worthwhile, given the challenges of combining data across scanners and sites. The protocol does raise some questions and issues.

Reply: We greatly appreciate your review. We have revised the manuscript to address the reviewer comments as best as we could, although, as pointed out by the editor, we cannot alter the approved protocol. Nevertheless, the modified manuscript has been improved significantly thanks to your insightful comments.

Specific comments

1. The protocol plans for the 15 TS to each be scanned three times, but the Fukui site has 2 scanners – one of which is a PET/MRI scanner – which may introduce some additional uncontrolled variability. If it is necessary to use the PET/MRI scanner to study the children, then the TS should also be imaged on that machine.

Reply: Thank you for the comment. We will collect new imaging data for typically developing children and children with NDDs using a 3-T PET/MRI scanner at the University of Fukui, a 3-T Architect scanner at Osaka University, and a 3-T Discovery MR750 scanner at Chiba University. In addition to the abovementioned scanners, we will also collect imaging data for healthy travelling adults using a 3-T Discovery MR750 scanner at the University of Fukui, because some imaging data in existing samples were acquired using that scanner. It is important to use the 3-T PET/MRI scanner (at the University of Fukui) for healthy travelling adults, as this is needed for measurement bias correction for the scanners. We have revised the corresponding text in the Method and Analysis section (page 11, line 265 and lines 268-271) to clarify these aspects.

2. The protocol does not specify the length of the resting state scans. The number of TR's (or volumes) is a major determinant of the reliability of resting state measures – given that the scanning parameters are all single echo EPI sequences, which are inherently noisier than multi-echo sequences, it would be advisable to attempt to obtain longer sequences whenever possible. Doing so is difficult given the greater tendency of children to move – so it may be worthwhile considering also including scanning during movie conditions as well as during open-eye resting state.

Reply: We greatly appreciate these insightful suggestions. As you pointed out, it is important to acquire multiple echo times for each MRI volume during the resting state. Unfortunately, our MRI
scanners are not equipped with multi-echo EPI software. We have added the scan time for each scanner to Table 3 (pages 12-13). Although we agree with your opinion regarding longer sequences and scanning during movie-watching in principle, this may raise concerns about the psychosomatic burden due to long-term MRI scanning and the lack of generalizability regarding differences in preferences for movies. However, we do scan for structural imaging, such as T1-weighted imaging and DTI, during movie-watching.

3. A major omission is lack of specific head motion criteria – how will head motion be measured, and what threshold is unacceptable? How will excessive motion be handled?
Reply: Thank you for the questions. Excessive head motion during resting-state fMRI will be addressed by exclusion (over 3.0 mm, 3.0 degree) [2-4] and scrubbing as necessary [5]. We have added this explanation and the corresponding references to the Method and Analysis section (page 14, lines 310-311). In addition, we have removed the overlapping text in the Methods and Analysis section and revised the Reference list (added references ([63],[64],[81]).

4. The protocol mentions both eyes open and eyes closed conditions, at the foot of Table 3, but does not specify whether both are to be included, whether they would be counterbalanced, etc. In general, eyes closed conditions are an invitation to sleep, which can systematically alter the amplitude of the signals – it’s a major challenge in the field, but it seems advisable to avoid including such a confound in a newly collected data set, if possible.
Reply: We greatly appreciate the comment. Resting-state fMRI scanning for healthy travelling adults at the University of Fukui will be conducted using PET/MR and MR750 scanners under both open-eye and closed-eye conditions, because some of our existing sample data were acquired under closed-eye conditions at the University of Fukui. Therefore, such conditions are essential to correct measurement biases in MRI scanners when using the travelling-subject approach. Also, we will not deal with closed-eye conditions during resting-state fMRI in the newly collected data. We have revised the corresponding text in the footnote of Table 3 (page 13, lines 275-281).

5. The phenotyping is broad, but only includes one measure that is related to ASD, and in a complex manner. The SRS (presumably 2nd edition?) has been extensively used in ASD research, but it reflects many conditions. It should be included, but the absence of more focused ASD measurements will make it difficult to link these data to those from other groups. It may not be feasible to obtain ADOS-2 or ADI-R interviews, but other autism indices should be included if they have been validated in Japanese samples.
Reply: We greatly appreciate the comments. As you pointed out, it would be better to conduct ADOS-2 and/or ADI-R interviews, but it was not feasible with regard to the present protocol in terms of the study design and time management, because we already plan to use several questionnaires for our multi-dimensional approach. Instead, we diagnose ASD based on the DSM-5 criteria and additionally use the SRS-2 and the Short Sensory Profile (SSP), both of which have been validated in Japanese samples, to assess the core symptoms of ASD. The SRS-2 can assess social communication and interaction and restricted interests and repetitive behaviours according to the diagnostic criteria of the DSM-5 and includes five subdomains: social awareness, social cognition, social communication, social motivation, and restricted interests and repetitive behaviours. The SSP can assess sensory sensitivity for ASD risk across seven subdomains: tactile sensitivity, taste/smell sensitivity, movement sensitivity, under-responsive/seeks sensation, auditory filtering, low energy/weak, and visual/auditory sensitivity. The DSM-5 added sensory abnormalities as a core symptom to the diagnostic criteria for ASD [6], because these abnormalities are associated with intelligence quotient [7], daily functioning [8], and academic performance [9]. Moreover, the Japanese versions of these questionnaires are accepted as powerful tools for quantifying the clinical features of ASD [10-12]. We have revised the information regarding SRS-2 and SSP in Table 2 (page 8). In addition, we have added explanations regarding these as a limitation in the Discussion section (page 16, lines 381-391). The References section has also been revised accordingly (added references [89],[90],[91]).

6. The collection of urine to quantify monoamine metabolites and tryptophan is likely to be a waste of time. The references cited are either irrelevant or out-of-date. The urinary compartment is too distant from neuronal sources in general. To do this well, the authors would need to consider 24-hour
collections, etc. Otherwise, the results will be meaningless, given variations in fluid intake, time of day, etc.

Reply: We greatly appreciate these insightful suggestions and apologize for not having included the details of the urine collection protocol in the Methods and Analysis section. Biochemical biomarkers in biological fluids can help diagnose neurodevelopmental disorders at a younger age and without requiring the use of invasive methods, which in turn could help clinicians to provide earlier and more reliable diagnoses. Urine is a considered to be a candidate biological fluid for identifying such markers because it has already been associated with the prediction of various diseases [13-15]. Biochemically and pharmaceutically, MHPG (one of the final metabolites of norepinephrine metabolism) excreted in the urine is thought to have been transported from the central nervous system to tubular epithelial cells via a transporter system in the blood-brain barrier along with urinary HVA (a dopamine metabolite) and 5-HIAA (a serotonin metabolite) [16,17]. Moreover, in a previous study we discovered that rats injected intraventricularly with 5,7-dihydroxytryptamine (a serotonin neurotoxin) had lower serotonin content compared with vehicle-treated rats, and importantly, this was associated with lower 5-HIAA excretion in the urine [18], suggesting a brain-urine coupling of neurotransmitters. Thus, many researchers believe that urinary biochemical biomarkers have great value in terms of the development of cost-effective and non-invasive methods of assessment. Nevertheless, urine samples are susceptible to oxidation, and their hydrogen-ion concentration rapidly becomes unstable. This may lead to the underestimation of monoamine metabolite concentrations in case of long-term sample storage, such as 24-h collection. Moreover, it is difficult to control factors such as dietary composition, exercise, and immobilization stress during a 24-h period for all participants. To overcome these difficulties, and based on the estimated time taken for the transport of monoamine metabolites from the brain to tubular epithelial cells [16,17], fresh urine will be collected after a 30 min rest, as previously reported [19]. Further, we will instruct the participants to refrain from the intake of food and beverages such as alcohol, coffee, fish, red beef, and blue cheese, which can be difficult to digest, and intense physical activity for 24 h before the experiment. Water will be the only beverage participants will be allowed to have on the test day. These explanations have been added to the Methods and Analysis section of the revised manuscript (page 10, lines 234-239).

7. The protocol does not mention the incorporation of any method of habituating or training the children to the process of undergoing a scan. Without such a process, the yield is likely to be less than desirable. Purchasing a full-size mock scanner may be impractical and is expensive, but there are protocols now available on the web in which families can expose their children to the sounds of the scanner before being in the machine itself. Similarly, habituating to the amount of time they must remain still is an important pre-scan measure which improves yield. Too much effort will be expended for naught if this is not considered now.

Reply: We greatly appreciate these insightful suggestions. As you pointed out, we think that it is important to carefully habituate children to the scanner sounds before the actual MRI. We have now added a relevant explanation in the Methods and Analysis section (page 11, lines 264-265).

Minor comments
1. The authors state “multilateral” several times – this does not translate well into English. They likely mean “multisite” in most cases.

Reply: Thank you for the comment. As you pointed out, we have modified the corresponding term throughout the manuscript.

2. The sentence at the end of page 4, and start of page 5: “These factors were not controlled between studies, which might have weakened the essential characteristics of the disorders” does not make sense.

Reply: Thank you for the comment. We have removed the corresponding text in the Introduction section.

3. The authors may benefit from considering the work of Taki (Russell) Shinohara and colleagues on harmonizing data across sites (ComBat and its variations).

Reply: We greatly appreciate the suggested references and have referred to the recommended literature [20-22]. Recently, Maikusa and colleagues [23] reported that travelling-subject (TS)-GLM
and TS-ComBat reduced Cohen’s d by up to 85 and 81.3%, respectively, while ComBat showed a reduction of only 59.0%. This suggests that TS-based harmonization could provide more effective bias correction. Therefore, our approved protocol will use TS-based harmonization.

Reviewer 2 (Dr. Shania Mereen Soman)
Evaluation
Thank you for sending me this paper to review. In this work, Yamashita and colleagues used travelling-subject approach to correct site differences in MRI scanners and clarify brain structure and network characteristics of children with ADHD and ASD collected in a multi-center collaboration. This will be a great study. I have few comments.

Reply: We greatly appreciate your review. We have revised the manuscript to address your comments, although we cannot alter the approved protocol, as the editor has also pointed out. Nevertheless, the modified manuscript has been refined considerably thanks to your insightful comments.

Specific comments
1. I find that aim of the study is missing in the abstract section. Aim of the study should be mentioned under introduction section in the abstract.

Reply: Thank you for the comment. As you pointed out, we have added the corresponding text in the Abstract (page 3, lines 50-54), and removed the overlapping text from the Abstract.

2. The study focuses on child development in subjects with NDDs at multiple levels (genetics, epigenetics, neurotransmitter, amino acid level and behavioural measures). But the authors haven’t mentioned any information of prior studies that has examined these modalities in ADHD and ASD. It would be good to flush out this in the body of introduction to strengthen the aim of the paper.

Reply: We greatly appreciate the suggestion. Multi-dimensional approaches may provide a means to link pathogenesis and brain functions as assessed based on neuroimaging techniques, as previously reported [24]. Therefore, we have revised certain portions of the text in the Introduction (page 5, lines 119-138) and the Methods and Analysis section (page 10, line 233; page 11, line 253) and removed overlapping text from the Methods and Analysis section. Additionally, we have also revised the References section to include new references ([46],[47],[48],[49],[50],[51],[52],[53],[54],[55],[56]).

3. The authors have mentioned primary outcomes of the study as brain structure and resting state functional connectivity. What are you measuring using brain structure? Is it gray matter volume? It would have been good to make this clear.

Reply: We greatly appreciate the comment. We plan to analyse grey matter volume, cortical thickness, and surface area using Statistical Parametric Mapping 12 (SPM12) or FreeSurfer. We have revised the corresponding text in the Methods and Analysis section to clarify this (page 8, line 186; page 14, lines 295-301).

4. Under image acquisition, it is mentioned that DTI images will be acquired. But it is not clear in the outcomes whether this modality will be used for future analysis. Also, it would be good to add under data analysis plan the white matter tracts you will be focusing on.

Reply: We apologize for not having included concrete measures of DTI in the Methods and Analysis section and greatly appreciate the suggestion. A meta-analysis of DTI studies reported that compared with those with typical development, individuals with ASD show abnormal fractional anisotropy in language-related tracts in the inferior frontal-occipital fasciculus and longitudinal fasciculus [25], while those with ADHD show abnormal fractional anisotropy in motor control-related tracts in the capsula interna, anterior corona radiata, and cerebellum [26]. These we plan to analyse these white matter tracts as this may help to explain the neurobiological distinctions between these disorders. We have revised the corresponding text in the Methods and Analysis section (page 8, line 186; page 14, lines 302-308).

5. Authors have mentioned under data analysis plan that they will be using TS approach to investigate site corrections in MRI scanners. It would be good to explain how this approach helps to differentiate most of the sample variability.
Reply: We greatly appreciate this insightful suggestion. Site differences result in two types of biases: measurement bias (related to imaging variables, field strength, MRI manufacturers, and scanner models) and sampling bias (related to differences in participant groups), as previously reported [27]. Since our travelling-subject dataset includes only healthy participant data, and they will be the same across all sites, we will correct measurement bias only to quantitatively investigate the site differences in the resting-state fMRI data and brain structure. Briefly, we will calculate the coefficients for the scanner, head coil, fMRI manufacturer, and phase encoding at each site and use them to correct imaging data using regression models and clustering algorithms. We have now added explanations regarding these aspects in the Methods and Analysis section (page 14, lines 286-294).

6. It would be good to include implication of each outcome of the main protocol in discussion.

Reply: We greatly appreciate the suggestion. We have added explanations regarding the usefulness of the various multi-dimensional approaches to the Discussion section (pages 15-16, lines 348-378) and revised the References section accordingly ([82],[83],[84],[85],[86],[87],[88],[92],[93]).

References


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**VERSION 2 – REVIEW**

| REVIEWER | Castellanos, Francisco X  
Nathan S Kline Institute for Psychiatric Research, Center of Brain Imaging and Neuromodulation |
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**GENERAL COMMENTS**

The manuscript has been greatly improved and is recommended for acceptance. A couple of minor flaws should be addressed.
On line 169, “Study participants will be recruited between 12 July 2022 and 31 March 2032.” Presumably they meant March 2023.

In line 429, what does “Tsuyoshi Sasaki received grants or contracts from any entity in Shionogi” mean?

Finally, please recheck the references. I noted ref 64 is ”broken”

I end by congratulating the authors - this is a landmark project, and I wish them well. I hope my pessimism about the urinary metabolites is wrong - they've done a good job of at least providing a reasonable rationale for pursuing such measures.

VERSION 2 – AUTHOR RESPONSE

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Reply: We greatly appreciate further review. We have revised the manuscript to address the minor comments as best as we could, although, as pointed out by the editor, we cannot alter the approved protocol.

1. On line 169, “Study participants will be recruited between 12 July 2022 and 31 March 2032.” Presumably they meant March 2023.

Reply: Thank you for the comment. As approved by the Research Ethics Committee of the University of Fukui Hospital, the recruitment period is correct. Actually, we expect to be able to recruit participants sooner than the time frame we have set, but due to the large sample size, we have set such a recruitment period with plenty of time to spare, just in case.

2. In line 429, what does “Tsuyoshi Sasaki received grants or contracts from any entity in Shionogi” mean?

Reply: We apologize for the errors in the Competing Interests Statement. The corrected statement is as follows: Tsuyoshi Sasaki received grants or contracts from Shionogi. We have added this revision in the Competing Interests Statement section (page 17, line 429).

3. Finally, please recheck the references. I noted ref 64 is ”broken”

Reply: We apologize for the errors in the title of ref 64. We have revised the corresponding title in the References section ([64]).