Data supplement for Kato et al., Lower Availability of Mitochondrial Complex I in Anterior Cingulate Cortex of Autism: A Positron Emission Tomography Study. Am J Psychiatry (doi: 10.1176/appi.ajp.22010014)

CONTENTS

Supplemental Text

Supplemental Introduction

Our previous studies confirmed that the uptake of [^{18}F]BCPP-EF reflected the specific binding to cellular MC-I. By in vitro assay [^{18}F]BCPP-EF binding was inhibited by rotenone, a specific MC-I inhibitor, in a dose-dependent manner.[1,2] By in vivo assay in living rat and monkey using brain PET, the significant reduction in its uptake by rotenone was also observed.[1,2] Furthermore, we have demonstrated the capability of [^{18}F]BCPP-EF for diagnostic and therapeutic monitoring in monkey models of neuropsychiatric disorders known to have impaired brain mitochondrial function.[3-5]

Supplemental Results

Additional analyses to consider potential confounds

To control potential confounding effects of brain volume and subthreshold anxiety and depression, the ANOVAs were additionally conducted with total intracranial volume, regional brain volume where the significant diagnostic difference in SUVR was found, CES-D, or state / trait STAI as a covariate.

The additional analyses with treating total intracranial volume or ACC volume as a covariate also reached at the same statistical conclusion such as significant interaction between group status and ROI ($F_{5,39} = 8.44$, $P < 0.001$ with total intracranial volume; $F_{5,39} = 8.55$, $P < 0.001$ with ACC volume), while the main effects of ROI and diagnosis were not significant. Furthermore, the analysis with treating total intracranial volume or ACC volume as covariate also showed that the SUVR in ACC was significantly lower in the individuals with ASD than those in TD ($F_{1,43} = 9.73$, $P = 0.003$ with total intracranial volume; $F_{1,43} = 9.50$, $P = 0.004$ with ACC volume). The ACC volume did not show significant correlation with the SUVR in this region in ASD or TD subjects. There was no significant difference in the total intracranial volume or ACC volume between the participants with ASD and TD.

Furthermore, we tested potential confounding effects of subthreshold depression and anxiety. The additional analyses with treating CES-D or state / trait scores of STAI as a covariate also reached at the same statistical conclusion such as significant interaction between group status and ROI ($F_{5,40} = 7.28$, $P < 0.001$ with CES-D; $F_{1,43} = 6.72$, $P < 0.001$ with STAI state; $F_{1,43} = 6.30$, $P < 0.001$ with STAI trait) and significant main effect of ROI ($F_{5,40} = 18.87$, $P < 0.001$ with CES-D; $F_{1,43} = 3.64$, $P = 0.008$ with STAI state; $F_{1,43} = 2.90$, $P = 0.025$ with STAI trait), while the main effects of diagnosis were not significant. Furthermore, the analysis with treating CES-D or state / trait scores of STAI as covariate also showed that the SUVR in ACC was significantly lower in the individuals with ASD than those in TD ($F_{1,44} = 14.12$, $P = 0.001$ with CES-D; $F_{1,43} = 11.67$, $P = 0.001$ with STAI state; $F_{1,43} = 11.65$, $P = 0.001$ with STAI trait). There was no significant correlation of SUVR of ACC with CES-D or state / trait scores of STAI in TD or ASD groups.

Supplemental Discussion

The decreased [^{18}F]BCPP-EF binding is induced by the degradation and quantitative reduction of MC-I proteins in specific brain regions. However, a transit conformational change in MC-I between the active (A-form with higher affinity) and deactive (D-form with lower affinity) forms with different affinities for rotenone, which shares the same binding sites with [^{18}F]BCPP-EF,[1,6] was revealed.[7] This suggests that reductions in [^{18}F]BCPP-EF uptake by the brain may also reflect some transition from the A-form to D-form in the binding site of MC-I, and not just the degradation of the MC-I proteins. In general, such type of protein conformational change precedes the complete protein degradation after the onset of neuronal damage. Taken together, [^{18}F]BCPP-EF PET can detect not only quantity, but also functional activities of MC-I, which can contribute to the detection of region-specific brain dysfunction.[8,9]

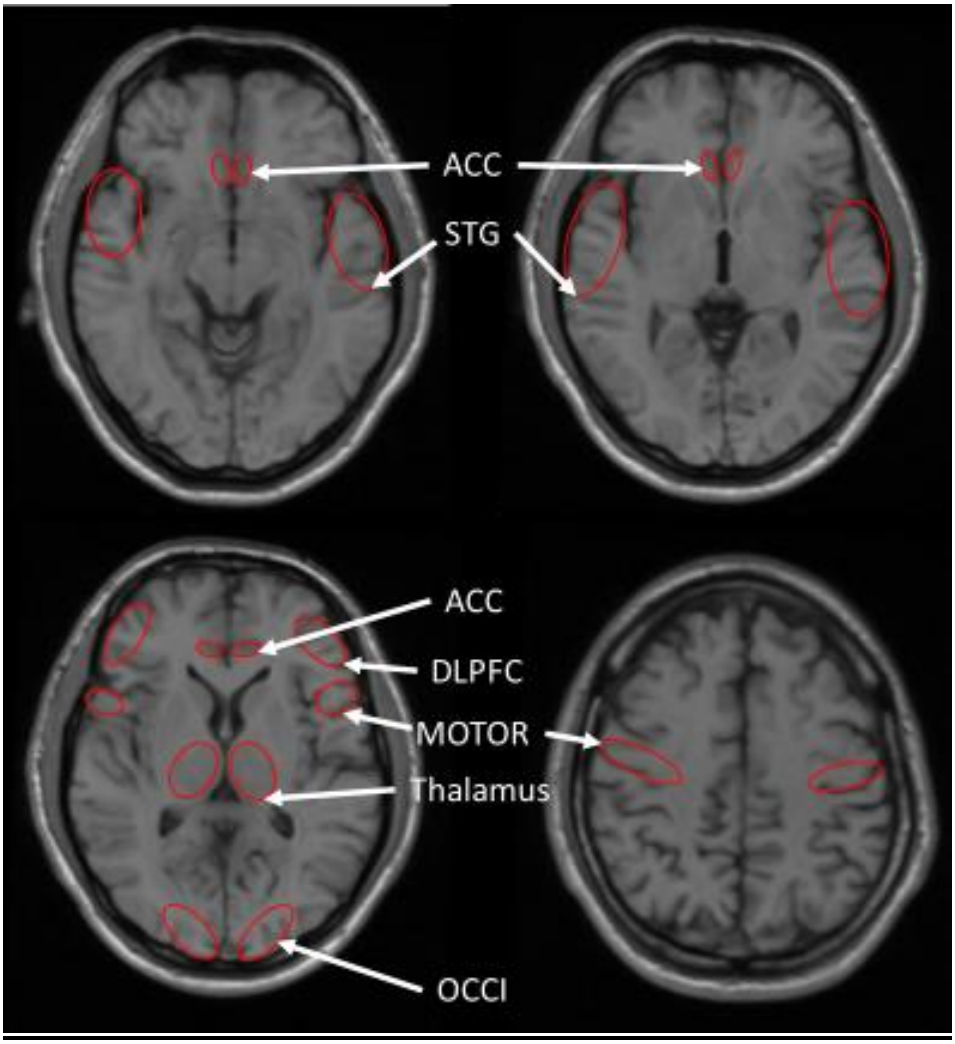
Supplemental References

1. Tsukada H, Nishiyama S, Fukumoto D, Kanazawa M, Harada N. Novel PET probes ^{18}F-BCPP-EF and ^{18}F-BCPP-BF for mitochondrial complex I: a PET study in comparison with ^{18}F-BMS-747158-02 in rat brain. *J Nucl Med*. 2014;55(3):473-480.
2. Kazami S, Nishiyama S, Kimura Y, Itoh H, Tsukada H. BCPP compounds, PET probes for early therapeutic evaluations, specifically bind to mitochondrial complex I. *Mitochondrion*. 2019;46:97-102.
3. Tsukada H, Ohba H, Nishiyama S, Kanazawa M, Kakiuchi T, Harada N. PET imaging of ischemia-induced impairment of mitochondrial complex I function in monkey brain. *J Cereb Blood Flow Metab*. 2014;34(4):708-714.
4. Tsukada H, Nishiyama S, Ohba H, Kanazawa M, Kakiuchi T, Harada N. Comparing amyloid-β deposition, neuroinflammation, glucose metabolism, and mitochondrial complex I activity in brain: a PET study in aged monkeys. *Eur J Nucl Med Mol Imaging.* 2014;41(11):2127-2136.
5. Kanazawa M, Ohba H, Nishiyama S, Kakiuchi T, Tsukada H. Effect of MPTP on Serotonergic Neuronal Systems and Mitochondrial Complex I Activity in the Living Brain: A PET Study on Conscious Rhesus Monkeys. *J Nucl Med*. 2017;58(7):1111-1116.
6. Tsukada H, Ohba H, Kanazawa M, Kakiuchi T, Harada N. Evaluation of ^{18}F-BCPP-EF for mitochondrial complex 1 imaging in the brain of conscious monkeys using PET. *Eur J Nucl Med Mol Imaging.* 2014;41(4):755-763.
7. Grivennikova VG, Maklashina EO, Gavrikova EV, Vinogradov AD. Interaction of the mitochondrial NADH-ubiquinone reductase with rotenone as related to the enzyme active/inactive transition. *Biochim Biophys Acta*. 1997;1319(2-3):223-232.
8. Terada T, Obi T, Bunai T, et al. In vivo mitochondrial and glycolytic impairments in patients with Alzheimer disease. *Neurology*. 2020;94(15):e1592-e1604.
9. Terada T, Therriault J, Kang MSP, et al. Mitochondrial complex I abnormalities is associated with tau

and clinical symptoms in mild Alzheimer's disease. *Mol Neurodegener*. 2021;16(1):28.

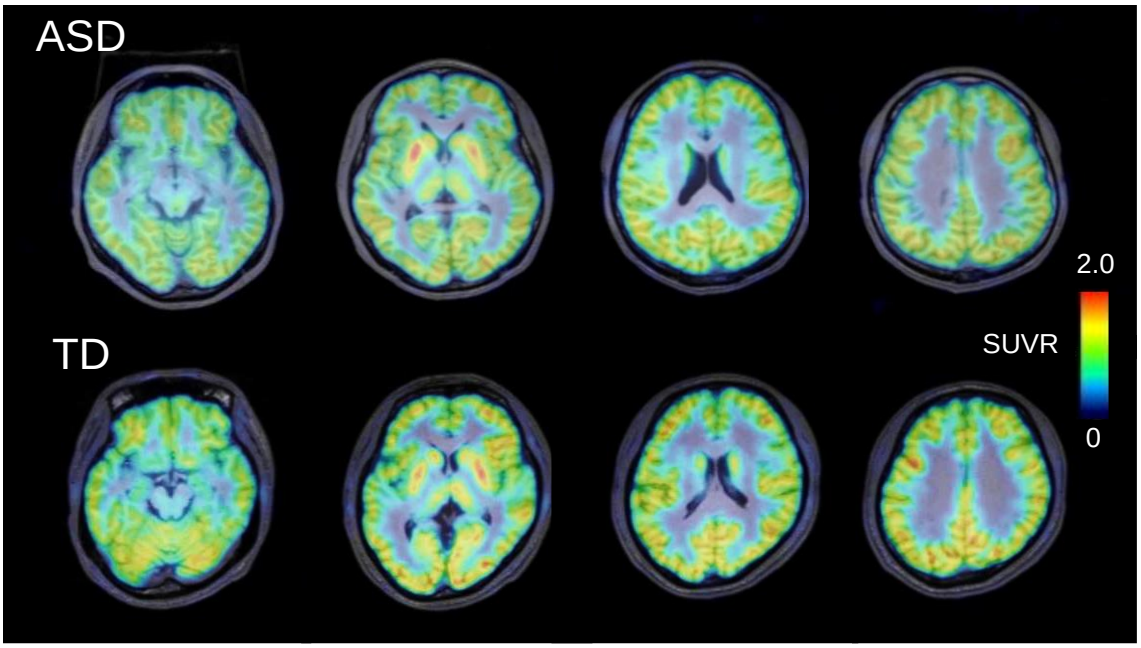
10. Wechsler D. The psychometric tradition: Developing the Wechsler Adult Intelligence Scale. Contemporary Educational Psychology. 1981;
11. Lord C, Rutter M, Le Couteur A. Autism Diagnostic Interview-Revised: a revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. J Autism Dev Disord. 1994;24(5):659-685.
12. Lord C, Rutter M, Goode S, et al. Austism diagnostic observation schedule: A standardized observation of communicative and social behavior. J Autism Dev Disord. 1989;19(2):185-212.
13. First MB, Williams JB, Karg RS, Spitzer RL, eds. Structured clinical interview for DSM-5—Research version. Arlington, VA: American Psychiatric Association; 2015:1-94.
14. Baron-Cohen S, Wheelwright S, Skinner R, Martin J, Clubley E. The autism-spectrum quotient (AQ): evidence from Asperger syndrome/high-functioning autism, males and females, scientists and mathematicians. J Autism Dev Disord. 2001;31(1):5-17.
15. Hollingshead AB. Two factor index of social position. New Haven. Yale University; 1957.
16. Radloff L. The CES-D Scale: A Self-Report Depression Scale for Research in the General Population. Appl Psychol Meas. 1977;1:385-401.
17. Spielberger CD, Gorsuch RL, Lushene R, Vagg PR, Jacobs GA, eds. Manual for the State-Trait Anxiety Inventory. Palo Alto, CA: Consulting Psychologists Press; 1983.
18. Tsukada H, Nishiyama S, Ohba H, Kanazawa M, Kakiuchi T, Harada N. Comparing amyloid-β deposition, neuroinflammation, glucose metabolism, and mitochondrial complex I activity in brain: a PET study in aged monkeys. Eur J Nucl Med Mol Imaging. 2014;41(11):2127-2136.
19. Tsukada H, Ohba H, Kanazawa M, Kakiuchi T, Harada N. Evaluation of 18F-BCPP-EF for mitochondrial complex 1 imaging in the brain of conscious monkeys using PET. Eur J Nucl Med Mol Imaging. 2014;41(4):755-763.

FIGURE S1. Examples of regions of interest



Abbreviations: ACC, anterior cingulate cortex; STG, superior temporal gyrus; OCCI, occipital cortex; DLPFC, dorsolateral prefrontal cortex; MOTOR, primary motor cortex.

FIGURE S2. Representative PET images of SUVR of [^{18}F] BCPP-EF



The top four images represent those of a participant with autism spectrum disorder (ASD), while the bottom four were those of a typically developed (TD) participant.

TABLE S1. SUVR of [^{18}F] BCPP-EF in the regions of interest of the participants with ASD and TD included in the correlational analyses

	Participants with ASD (N=22)		Participants with TD (N=24)		t-value	*P*-value	Cohen's *d*
Regions	Mean	SD	Mean	SD			
ACC	0.77	0.12	0.87	0.07	3.66	0.0010*	1.08
Thalamus	0.98	0.13	1.04	0.13	1.51	0.137	0.45
STG	0.84	0.08	0.85	0.05	0.80	0.437	0.24
OCCI	1.03	0.18	1.11	0.10	1.78	0.091	0.53
DLPFC	0.93	0.13	0.92	0.08	-0.43	0.680	0.13
MOTOR	0.90	0.11	0.89	0.07	-0.34	0.739	0.10

Abbreviations: ASD, autism spectrum disorder; TD, typically developed; ACC, anterior cingulate cortex; STG, superior temporal gyrus; OCCI, occipital cortex; DLPFC, dorsolateral prefrontal cortex; MOTOR, primary motor cortex.

*Statistically significant after Bonferroni correction.

TABLE S2. Correlations between [^{18}F]BCPP-EF SUVR in the anterior cingulate cortex and Autism Diagnostic Observation Schedule-2 subscales

(N = 22)	Correlation coefficient	*P*-value
Autism Diagnostic Observation Schedule-2		
Reciprocity	-0.406	0.061
Communication	-0.537	0.0099*
Restricted and Repetitive Behaviors	0.076	0.738

*Statistically significant after Bonferroni correction