4.2. Differences between the HC and patient groups

Our results support the hypothesis that the ASDs group has lower activation in a broad prefrontal area (Figs. 4 and 5) relative to the HC group. The classification accuracy obtained using [oxy-Hb] changes between the ASDs and HC groups during both the SST and the [SST + post-SST] period was highest in the channels for the left VLPFC. These results are consistent with those of previous studies (Yamasaki et al., 2010; Abell et al., 1999). A previous fMRI study showed that compared to healthy adults, adults with ASDs had significantly lower task-related activation in the DLPFC during a spatial working memory task (Luna et al., 2002; Ohnishi et al., 2000; Smith et al., 2004; Rinehart et al., 2002). Further, compared to healthy children, children with autism also showed reduced activation in the right DLPFC and VLPFC during a novelty detection task (Gomot et al., 2006).

The fMRI study revealed that pre-SMA and PMA would show functional interconnectivity via the basal ganglia circuitry to mediate response execution or inhibition, whereas the VLPFC would influence the basal ganglia circuitry via connectivity with pre-SMA (Duann et al., 2009). The DLPFC, with its direct connections to the basal ganglia (Alexander et al., 1986), is part of a distributed neural network supporting the selection and suppression of motor responses (Garavan et al., 2002). The fMRI study on ASDs children showed that when using a go/no-go task, there was a significant negative correlation between age and 2 right VLPFC correlation pairs: right VLPFC–bilateral pre-SMA and right VLPFC–right caudate (Lee et al., 2009). Our study detected a dysfunction in the neural basis of inhibition in adults with ASDs in areas including the bilateral VLPFC, DLPFC, Pre-SMA, and PMA, when compared to healthy controls using the NIRS, although it was difficult to detect a dysfunction of the basal ganglia.

As hypothesized, compared to the HC group, the ADHD group showed underactivation of the right SMA, pre-SMA, and bilateral DLPFC during the SST. The classification accuracy obtained using [oxy-Hb] changes between the ADHD and the HC group during both the SST and the [SST + post-SST] period was highest in the channels for the right pre-SMA and right PMA. These results are consistent with those of previous studies on drug-naïve adult patients with ADHD (Cubillo et al., 2010; Rubia et al., 2011), which used fMRI to show reduced activation in the right PFC during the SST. We also found that the ADHD group had lesser activation than the HC group in the left DLPFC, which has been directly implicated in attention switching in normal adults (Smith et al., 2004). A recent meta-analysis showed that patients with ADHD have consistent functional abnormalities in 2 distinct domain-dissociated right hemispheric fronto-basal ganglia networks, the VLPFC, supplementary motor area, and anterior cingulate cortex for inhibition and the DLPFC, parietal, and cerebellar areas for attention (Hart et al., 2013). Regarding cortical surface areas that NIRS could measure, these results are consistent with our current study and a recent NIRS study of adults with ADHD, which used the SST to show reduced bilateral activation of the inferior frontal cortex in these individuals compared to healthy adults (Schecklmann et al., 2013).

Our observation of decreased frontal activation during the SST was in contrast to previous studies showing no abnormality of activation in patients with ADHD during a go/no-go task (Dibbets et al., 2009; Kooistra et al., 2010). This inconsistency may be related to task differences and MPH treatment history. Most of the patients included in those 2 previous studies (Dibbets et al., 2009; Kooistra et al., 2010) had been chronically medicated with MPH, and there is evidence of long-term effects of MPH on brain structure (Shaw et al., 2009) and brain function (Konrad et al., 2007).

4.3. Limitations

This study had several limitations. First, trying to differentiate two behaviorally defined psychiatric disorders by using biological markers may not be the final goal for the psychiatry, since the current diagnostic system is solely based on the categorization by behavior. Rather, future psychiatry should pursue comprehensive recapturing of the association between various dimensions of behavior and their biological basis. The importance of identified biomarkers in the current study should be interpreted in this context. For example, neuroimaging biomarkers may be more useful in making decision on the use of a certain pharmacological intervention for an individual patient, compared with the behaviorally categorized diagnosis per se, which should be clarified in future studies. Second, only 6 patients underwent structured interviews using the ADI-R, ADOS, and CAADID. Although we included other participants in this study only when at least 2 of the 3 trained child psychiatrists had seen patients and given consistent diagnoses, the inter-rater reliability for their evaluating psychiatrists was not established. Third, our study focused on adult subjects; thus, it is unclear whether our results can be extended to children with ASDs and ADHD. Fourth, the application of our results in clinical practice requires the replication of the findings in an independent sample. Fifth, the number of patients included in the subsample (patients having ASDs with ADHD symptoms: 10 participants) was smaller than the optimal sample size for neuroimaging (Carter et al., 2008). However, we found a significant difference in prefrontal activation between patients having ASDs with ADHD symptoms and those having ADHD. Although we also analyzed the correlation between [oxy-Hb] changes during the task or the [task + post-task] period and ASRS scores in addition to the analysis of this subsample using cut off score of ASRS, there were no significant correlations for either the ASDs or the ADHD group. Finally, because subjects were matched for IQ in each group and since IQ scores were relatively high, participants in our study may not be representative of all general patients. It is necessary that our data be replicated in a larger sample of participants.

4.4. Conclusions

In conclusion, the present study provides evidence of functional differences in activation in the left VLPFC between drug-naïve patients with ASDs and those with ADHD. Thus, the signal time course in the left VLPFC may be a diagnostic marker for distinguishing ADHD from ASDs. NIRS may be a candidate for an auxiliary diagnostic tool that is useful for both clinicians and patients.

Competing interests

Dr. Kiyoto Kasai reports the following financial relationship. The University of Tokyo and the Research and Developmental Center, Hitachi Medical Corporation, have had an official contract for a collaborative study on the clinical applications of near-infrared spectroscopy in psychiatric disorders, which has been approved by the Research Promotion Office, University of Tokyo Hospital. For the present study, the Hitachi Medical Corporation provided a project grant (JPY 300,000/year). Drs. Ayaka Ishii-Takahashi, Ryu Takizawa, Yuki Kawakubo, Hitoshi Kuwabara, and Kiyoto Kasai at the University of Tokyo and Shingo Kawasaki at the Hitachi Medical Corporation developed the "stimulus presentation device and stimulus task presentation method for optional measurement apparatus" described (patent no. 2008-146721, Japan; patent no. 12996190, United States of America; patent no. 09758336.3, European Union; and patent no. 20090120823.5, the People's Republic of China). The University of Tokyo transferred this patent to the Hitachi Medical Corporation, and the Hitachi Medical Corporation paid a transfer fee (JPY 100,000) to the University of Tokyo. The other authors report no conflicts of interest.

Role of the funding source

This study was supported in part by Grants-in-Aid for Scientific Research (innovative areas nos. 23118001 & 23118004 [Adolescent Mind & Self-Regulation] to KK; no. 23791309 to RT) and for the "Development

of Biomarker Candidates for Social Behavior" study carried out under the Strategic Research Program for Brain Sciences (to KK) by the MEXT. This study was also supported in part by Health and Labor Sciences Research Grants for Comprehensive Research on Disability Health and Welfare (H23-seishin-Ippan-002 to RT&YN&AI), the Japan Society for the Promotion of Science, KAKENHI (Grant-in-Aid for Young Scientists (B) no. 2479201 to AI)), an Intramural Research Grant for Neurological and Psychiatric Disorders of NCNP (no. 23-10 to RT&YN), and by the Japan Research Foundation for Clinical Pharmacology (to RT).

Author contributions

Ishii-Takahashi, M.D., Ph.D., had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Ishii-Takahashi, M.D., Ph.D., Kawakubo, Ph.D., Kasai, M.D., Ph.D. Acquisition of data: Ishii-Takahashi, M.D., Ph.D., Kawakubo, Ph.D., Kuwabara, M.D., Ph.D., Okuhata, Ph.D., Hamada, Med. Analysis and interpretation of data: Ishii-Takahashi, M.D., Ph.D., Hamada, Bed, Nishimura, Ph.D., Takizawa, M.D., Ph.D., Kawasaki, MS. Drafting of the manuscript: Ishii-Takahashi, M.D., Ph.D. Critical revision of the manuscript for important intellectual content: Nishimura, Ph.D. Takizawa, M.D., Ph.D., Matsubayashi, Ph.D., Yamasue, M.D., Ph.D., Kasai, M.D., Ph.D. Statistical analysis: Ishii-Takahashi, M.D., Ph.D., Nishimura, Ph.D., Takizawa, M.D., Ph.D., Kawasaki, MS. Obtained funding: Ishii-Takahashi, M.D., Ph.D., Takizawa, M.D., Ph.D., Nishimura, Ph.D., Kawakubo, Ph.D., Kasai, M.D., Ph.D. Administrative, technical, or material support: Kawasaki, MS. Study supervision: Kano, M.D., Ph.D., Kasai, M.D., Ph.D., Igarashi M.D., Ph.D. All contributors have approved the final version of the manuscript.

Acknowledgments

The authors would like to thank all the participants in this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.nicl.2013.10.002.

References

Abell, F., Krams, M., Ashburner, J., Passingham, R., Friston, K., Frackowiak, R., Happe, F., Frith, C., Frith, U., 1999. The neuroanatomy of autism: a voxel-based whole brain analysis of structural scans. Neuroreport 10, 1647–1651.

Alexander, G.E., DeLong, M.R., Strick, P.L., 1986. Parallel organization of functionally segregated circuits linking basal ganglia and cortex. Annu. Rev. Neurosci. 9, 357–381.

American Psychiatric Association, 2000. Diagnostic and Statistical Manual of Mental Disorders. Text Revision, 4th edn. American Psychiatric Association, Washington, DC 69–93.

Atkinson, M., Hollis, C., 2010. NICE guideline: attention deficit hyperactivity disorder. Arch. Dis. Child. Educ. Pract. Ed. 95, 24–27.

Autism Network (RUPP), 2005. Randomized, controlled, crossover trial of methylphenidate in pervasive developmental disorders with hyperactivity. Arch. Gen. Psychiatry 62, 1266–1274.

Barkley, R.A., Murphy, K.R., O'Connell, T., Connor, D.F., 2005. Effects of two doses of methylphenidate on simulator driving performance in adults with attention deficit hyperactivity disorder. J. Safety Res. 36, 121–131.

Baron-Cohen, S., Wheelwright, S., Skinner, R., Martin, J., Clubley, E., 2001. The autism-spectrum quotient (AQ): evidence from Asperger syndrome/high-functioning autism, males and females, scientists and mathematicians. J. Autism Dev. Disord. 31, 5–17.

Bookheimer, S.Y., Wang, A.T., Scott, A., Sigman, M., Dapretto, M., 2008. Frontal contributions to face processing differences in autism: evidence from fMRI of inverted face processing. J. Int. Neuropsychol. Soc. 14, 922–932.

Burbach, J.P., 2010. Neuropsychiatric connections of ADHD genes. Lancet 376, 1367–1368.

Carter, C.S., Heckers, S., Nichols, T., Pine, D.S., Strother, S., 2008. Optimizing the design and analysis of clinical functional magnetic resonance imaging research studies. Biol. Psychiatry 15, 842–849.

Casey, B.J., Epstein, J.N., Buhle, J., Liston, C., Davidson, M.C., Tonev, S.T., Spicer, J., Niogi, S., Millner, A.J., Reiss, A., Garrett, A., Hinshaw, S.P., Greenhill, L.L., Shafritz, K.M., Vitolo, A., Kotler, L.A., Jarrett, M.A., Glover, G., 2007. Frontostriatal connectivity and its role in cognitive control in parent–child dyads with ADHD. Am. J. Psychiatry 164, 1729–1736.

Christakou, A., Murphy, C.M., Chantiluke, K., Cubillo, A.I., Smith, A.B., Giampietro, V., Dal, Y.E., Ecker, C., Robertson, D., Murphy, D.G., Rubia, K., 2012. Disorder-specific functional abnormalities during sustained attention in youth with attention deficit hyperactivity disorder (ADHD) and with Autism. Mol. Psychiatry 18, 236–244.

Corbett, B.A., Constantine, L.J., Hendren, R., Rocke, D., Ozonoff, S., 2009. Examining executive functioning in children with autism spectrum disorder, attention deficit hyperactivity disorder and typical development. Psychiatry Res. 166, 210–222.

Cubillo, A., Halari, R., Ecker, C., Giampietro, V., Taylor, E., Rubia, K., 2010. Reduced activation and inter-regional functional connectivity of fronto-striatal networks in adults with childhood Attention-Deficit Hyperactivity Disorder (ADHD) and persisting symptoms during tasks of motor inhibition and cognitive switching. J. Psychiatr. Res. 44, 629–639.

Dapretto, M., Davies, M.S., Pfeifer, J.H., Scott, A.A., Sigman, M., Bookheimer, S.Y., Iacoboni, M., 2006. Understanding emotions in others: mirror neuron dysfunction in children with autism spectrum disorders. Nat. Neurosci. 9, 28–30.

Di Martino, A., Melis, G., Cianchetti, C., Zuddas, A., 2004. Methylphenidate for pervasive developmental disorders: safety and efficacy of acute single dose test and ongoing therapy: an open-pilot study. J. Child Adolesc. Psychopharmacol. 14, 207–218.

Dibbets, P., Evers, L., Hurks, P., Marchetta, N., Jolles, J., 2009. Differences in feedback- and inhibition-related neural activity in adult ADHD. Brain Cogn. 70, 73–83.

Duann, J.R., Ide, J.S., Luo, X., Li, C.S., 2009. Functional connectivity delineates distinct roles of the inferior frontal cortex and presupplementary motor area in stop signal inhibition. J. Neurosci. 29, 171–179.

Garavan, H., Ross, T.J., Murphy, K., Roche, R.A., Stein, E.A., 2002. Dissociable executive functions in the dynamic control of behavior: inhibition, error detection, and correction. Neuroimage 17, 1820–1829.

Geurts, H.M., Verté, S., Oosterlaan, J., Roeyers, H., Sergeant, J.A., 2004. How specific are executive functioning deficits in attention deficit hyperactivity disorder and autism? J. Child Psychol. Psychiatry 45, 836–854.

Goldberg, M.C., Mostofsky, S.H., Cutting, L.E., Mahone, E.M., Astor, B.C., Denckla, M.B., Landa, R.J., 2005. Subtle executive impairment in children with autism and children with ADHD. J. Autism Dev. Disord. 35, 279–293.

Gomot, M., Bernard, F.A., Davis, M.H., Belmonte, M.K., Ashwin, C., Bullmore, E.T., Baron-Cohen, S., 2006. Change detection in children with autism: an auditory event-related fMRI study. Neuroimage 29, 475–484.

Groen, Y., Wijers, A.A., Mulder, L.J., Waggeveld, B., Minderaa, R.B., Althaus, M., 2008. Error and feedback processing in children with ADHD and children with autistic spectrum disorder: an EEG event-related potential study. Clin. Neurophysiol. 119, 2476–2493.

Happé, F., Booth, R., Charlton, R., Hughes, C., 2006. Executive function deficits in autism spectrum disorders and attention-deficit/hyperactivity disorder: examining profiles across domains and ages. Brain Cogn. 61, 25–39.

Hart, H., Radua, J., Nakao, T., Mataix-Cols, D., Rubia, K., 2013. Meta-analysis of functional magnetic resonance imaging studies of inhibition and attention in attention-deficit/hyperactivity disorder: exploring task-specific, stimulant medication, and age effects. JAMA Psychiatry 70, 185–198.

Hofvander, B., Delorme, R., Chaste, P., Nydén, A., Wentz, E., Ståhlberg, O., Herbrecht, E., Stopin, A., Anckarsäter, H., Gillberg, C., Råstam, M., Leboyer, M., 2009. Psychiatric and psychosocial problems in adults with normal-intelligence autism spectrum disorders. BMC Psychiatry 9, 35.

Hoshi, Y., Tamura, M., 1993. Dynamic changes in cerebral oxygenation in chemically induced seizures in rats: study by near-infrared spectrophotometry. Brain Res. 603, 215–221.

Hoshi, Y., Kobayashi, N., Tamura, M., 2001. Interpretation of near-infrared spectroscopy signals: a study with a newly developed perfused rat brain model. J. Appl. Physiol. 90, 1657–1662.

Jensen, P.S., Arnold, L.E., Swanson, J.M., Vitiello, B., Abikoff, H.B., Greenhill, L.L., Hechtman, L., Hinshaw, S.P., Pelham, W.E., Wells, K.C., Conners, C.K., Elliott, G.R., Epstein, J.N., Hoza, B., March, J.S., Molina, B.S., Newcorn, J.H., Severe, J.B., Wigal, T., Gibbons, R.D., Hur, K., 2007. 3-year follow-up of the NIMH MTA study. J. Am. Acad. Child Adolesc. Psychiatry 46, 989–1002.

Johnston, K., Madden, A.K., Bramham, J., Russell, A.J., 2011. Response inhibition in adults with autism spectrum disorder compared to attention deficit/hyperactivity disorder. J. Autism Dev. Disord. 41, 903–912.

Kameyama, M., Fukuda, M., Uehara, T., Mikuni, M., 2004. Sex and age dependencies of cerebral blood volume changes during cognitive activation: a multichannel near-infrared spectroscopy study. Neuroimage 22, 1715–1721.

Kemner, C., Verbaten, M.N., Cuperus, J.M., Camfferman, G., van Engeland, H., 1995. Auditory event-related brain potentials in autistic children and three different control groups. Biol. Psychiatry 38, 150–165.

Konrad, K., Neufang, S., Fink, G.R., Herpertz-Dahlmann, B., 2007. Long-term effects of methylphenidate on neural networks associated with executive attention in children with ADHD: results from a longitudinal functional MRI study. J. Am. Acad. Child Adolesc. Psychiatry 46 (12), 1633–1641.

Kooij, S.J., Bejerot, S., Blackwell, A., Caci, H., Casas-Brugue, M., Carpentier, P.J., Edvinsson, D., Fayyad, J., Foeken, K., Fitzgerald, M., Gaillac, V., Ginsberg, Y., Henry, C., Krause, J., Lensing, M.B., Manor, I., Niederhofer, H., Nunes-Filipe, C., Ohlmeier, M.D., Oswald, P., Pallanti, S., Pehlivanidis, A., Ramos-Quiroga, J.A., Rastam, M., Ryffel-Rawak, D., Stes, S., Asherson, P., 2010. European consensus statement on diagnosis and treatment of adult ADHD: the European Network Adult ADHD. BMC Psychiatry 10, 67.

Kooistra, L., van der Meere, J.J., Edwards, J.D., Kaplan, B.J., Crawford, S., Goodyear, B.G., 2010. Preliminary fMRI findings on the effects of event rate in adults with ADHD. J. Neural Transm. 117, 655–662.

Lang, R., Regester, A., Lauderdale, S., Ashbaugh, K., Haring, A., 2010. Treatment of anxiety in autism spectrum disorders using cognitive behaviour therapy: a systematic review. Dev. Neurorehabil. 13, 53–63.

Lee, P.S., Yerys, B.E., Della Rosa, A., Foss-Feig, J., Barnes, K.A., James, J.D., VanMeter, J., Vaidya, C.J., Gaillard, W.D., Kenworthy, L.E., 2009. Functional connectivity of the inferior frontal cortex changes with age in children with autism spectrum disorders: a fcMRI study of response inhibition. Cereb. Cortex 19, 1787–1794.

Lehnhardt, F.G., Gawronski, A., Volpert, K., Schilbach, L., Tepest, R., Vogeley, K., 2012. Psychosocial functioning of adults with late diagnosed autism spectrum disorders—a retrospective study. Fortschr. Neurol. Psychiatr. 80, 88–97.

Lijffijt, M., Kenemans, J.L., Verbaten, M.N., van Engeland, H., 2005. A meta-analytic review of stopping performance in attention-deficit/hyperactivity disorder: deficient inhibitory motor control? J. Abnorm. Psychol. 114, 216–222.

Luna, B., Minshew, N.J., Garver, K.E., Lazar, N.A., Thulborn, K.R., Eddy, W.F., Sweeney, J.A., 2002. Neocortical system abnormalities in autism: an fMRI study of spatial working memory. Neurology 59, 834–840.

Makris, N., Biederman, J., Valera, E.M., Bush, G., Kaiser, J., Kennedy, D.N., Caviness, V.S., Faraone, S.V., Seidman, L.J., 2007. Cortical thinning of the attention and executive function networks in adults with attention-deficit/hyperactivity disorder. Cereb. Cortex 17, 1364–1375.

Malisza, K.L., Clancy, C., Shiloff, D., Holden, J., Jones, C., Paulson, K., Yu, D.C., Summers, R., Chudley, A.E., 2011. Functional magnetic resonance imaging of facial information processing in children with autistic disorder, attention deficit hyperactivity disorder and typically developing controls. Int. J. Adolesc. Med. Health 23, 269–277.

Marumo, K., Takizawa, R., Kinou, M., Kawasaki, S., Kawakubo, Y., Fukuda, M., Kasai, K., 2013. Functional abnormalities in the left ventrolateral prefrontal cortex during a semantic fluency task, and their association with thought disorder in patients with schizophrenia. Neuroimage (S1053-8119(13)00397-2).

Matsumoto, T., Yamaguchi, A., Asami, T., Kamijo, A., Iseki, E., Hirayasu, Y., Wada, K., 2005. Drug preferences in illicit drug abusers with a childhood tendency of attention deficit/hyperactivity disorder: a study using the Wender Utah Rating Scale in a Japanese prison. Psychiatry Clin. Neurosci. 59, 311–318.

Matsuoka, K., Uno, M., Kasai, K., Koyama, K., Kim, Y., 2006. Estimation of premorbid IQ in individuals with Alzheimer's disease using Japanese ideographic script (Kanji) compound words: Japanese version of National Adult Reading Test. Psychiatry Clin. Neurosci. 60, 332–339.

McDougle, C.J., Holmes, J.P., Carlson, D.C., Pelton, G.H., Cohen, D.J., Price, L.H., 1998. A double-blind, placebo-controlled study of risperidone in adults with autistic disorder and other pervasive developmental disorders. Arch. Gen. Psychiatry 55, 633–641.

Michielsen, M., Comijs, H.C., Semeijn, E.J., Beekman, A.T., Deeg, D.J., Kooij, J.J., 2013. The comorbidity of anxiety and depressive symptoms in older adults with attention-deficit/hyperactivity disorder: a longitudinal study. J. Affect. Disord. 148, 220–227.

Newcorn, J.H., Kratochvil, C.J., Allen, A.J., Casat, C.D., Ruff, D.D., Moore, R.J., Michelson, D., 2008. Atomoxetine and osmotically released methylphenidate for the treatment of attention deficit hyperactivity disorder: acute comparison and differential response. Am. J. Psychiatry 165, 721–730.

Nishimura, Y., Takizawa, R., Muroi, M., Marumo, K., Kinou, M., Kasai, K., 2011. Prefrontal cortex activity during response inhibition associated with excitement symptoms in schizophrenia. Brain Res. 1370, 194–203.

Nylander, L., Holmqvist, M., Gustafson, L., Gillberg, C., 2013. Attention-deficit/hyperactivity disorder (ADHD) and autism spectrum disorder (ASD) in adult psychiatry. A 20-year register study. Nord. J. Psychiatry 67, 344–350.

Ohnishi, T., Matsuda, H., Hashimoto, T., Kunihiro, T., Nishikawa, M., Uema, T., Sasaki, M., 2000. Abnormal regional cerebral blood flow in childhood autism. Brain 123, 1838–1844.

Oldfield, R.C., 1971. The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia 9, 97–113.

Overmeyer, S., Bullmore, E.T., Suckling, J., Simmons, A., Williams, S.C., Santosh, P.J., Taylor, E., 2001. Distributed grey and white matter deficits in hyperkinetic disorder: MRI evidence for anatomical abnormality in an attentional network. Psychol. Med. 31, 1425–1435.

Ozonoff, S., Jensen, J., 1999. Brief report: specific executive function profiles in three neurodevelopmental disorders. J. Autism Dev. Disord. 29, 171–177.

Ozonoff, S., Strayer, D.L., 1997. Inhibitory function in nonretarded children with autism. J. Autism Dev. Disord. 27, 59–77.

Ponseti, J., Granert, O., Jansen, O., Wolff, S., Beier, K., Neutze, J., Deuschl, G., Mehdorn, H., Siebner, H., Bosinski, H., 2012. Assessment of pedophilia using hemodynamic brain response to sexual stimuli. Arch. Gen. Psychiatry 69, 187–194.

Raymaekers, R., Antrop, I., van der Meere, J.J., Wiersema, J.R., Roeyers, H., 2007. HFA and ADHD: a direct comparison on state regulation and response inhibition. J. Clin. Exp. Neuropsychol. 29, 418–427.

Rinehart, N.J., Bradshaw, J.L., Brereton, A.V., Tonge, B.J., 2002. Lateralization in individuals with high-functioning autism and Asperger's disorder: a frontostriatal model. J. Autism Dev. Disord. 32, 321–331.

Rubia, K., Overmeyer, S., Taylor, E., Brammer, M., Williams, S.C., Simmons, A., Bullmore, E.T., 1999. Hypofrontality in attention deficit hyperactivity disorder during higher-order motor control: a study with functional MRI. Am. J. Psychiatry 156, 891–896.

Rubia, K., Russell, T., Overmeyer, S., Brammer, M.J., Bullmore, E.T., Sharma, T., Simmons, A., Williams, S.C., Giampietro, V., Andrew, C.M., Taylor, E., 2001. Mapping motor inhibition: conjunctive brain activations across different versions of go/no-go and stop tasks. Neuroimage 13, 250–261.

Rubia, K., Halari, R., Cubillo, A., Smith, A.B., Mohammad, A.-M., Brammer, M., Taylor, E., 2011. Methylphenidate normalizes fronto-striatal underactivation during interference inhibition in medication-naïve boys with attention-deficit hyperactivity disorder. Neuropsychopharmacology 36, 1–12.

Schecklmann, M., Ehlis, A.C., Plichta, M.M., Dresler, T., Heine, M., Boreatti-Hummer, A., Romanos, M., Jacob, C., Pauli, P., Fallgatter, A.J., 2013. Working memory and response inhibition as one integral phenotype of adult ADHD? A behavioral and imaging correlational investigation. J. Atten. Disord. 17, 470–482.

Schmitz, N., Rubia, K., Daly, E., Smith, A., Williams, S., Murphy, D.G., 2006. Neural correlates of executive function in autistic spectrum disorders. Biol. Psychiatry 59, 7–16.

Shaw, P., Sharp, W.S., Morrison, M., Eckstrand, K., Greenstein, D.K., Clasen, L.S., Evans, A.C., Rapoport, J.L., 2009. Psychostimulant treatment and the developing cortex in attention deficit hyperactivity disorder. Am. J. Psychiatry 166, 58–63.

Singh, A.K., Dan, I., 2006. Exploring the false discovery rate in multichannel NIRS. Neuroimage 33, 542–549.

Smith, A.B., Taylor, E., Brammer, M., Rubia, K., 2004. Neural correlates of switching set as measured in fast, event-related functional magnetic resonance imaging. Hum. Brain Mapp. 21, 247–256.

Stigler, K.A., McDonald, B.C., Anand, A., Saykin, A.J., McDougle, C.J., 2011. Structural and functional magnetic resonance imaging of autism spectrum disorders. Brain Res. 1380, 146–161.

Strangman, G., Boas, D.A., Sutton, J.P., 2002. Non-invasive neuroimaging using near-infrared light. Biol. Psychiatry 52, 679–693.

Suto, T., Fukuda, M., Ito, M., Uehara, T., Mikuni, M., 2004. Multichannel near-infrared spectroscopy in depression and schizophrenia: cognitive brain activation study. Biol. Psychiatry 55, 501–511.

Takizawa, R., Kasai, K., Kawakubo, Y., Marumo, K., Kawasaki, S., Yamasue, H., Fukuda, M., 2008. Reduced frontopolar activation during verbal fluency task in schizophrenia: a multi-channel near-infrared spectroscopy study. Schizophr. Res. 99, 250–262.

Takizawa, R., Tochigi, M., Kawakubo, Y., Marumo, K., Sasaki, T., Fukuda, M., Kasai, K., 2009. Association between catechol-*O*-methyltransferase Val108/158Met genotype and prefrontal hemodynamic response in schizophrenia. PLoS ONE 4, e5495.

Tsuzuki, D., Jurcak, V., Singh, A.K., Okamoto, M., Watanabe, E., Dan, I., 2007. Virtual spatial registration of stand-alone fNIRS data to MNI space. Neuroimage 34, 1506–1518.

Tzourio-Mazoyer, N., Landeau, B., Papathanassiou, D., Crivello, F., Etard, O., Delcroix, N., Mazoyer, B., Joliot, M., 2002. Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. Neuroimage 15, 273–289.

Verté, S., Geurts, H.M., Roeyers, H., Oosterlaan, J., Sergeant, J.A., 2006. The relationship of working memory, inhibition, and response variability in child psychopathology. J. Neurosci. Methods 151, 5–14.

Villringer, A., Planck, J., Hock, C., Schleinkofer, L., Dirnagl, U., 1993. Near infrared spectroscopy (NIRS): a new tool to study hemodynamic changes during activation of brain function in human adults. Neurosci. Lett. 154, 101–104.

Wakabayashi, A., Baron-Cohen, S., Wheelwright, S., Tojo, Y., 2006. The Autism-Spectrum Quotient (AQ) in Japan: a cross-cultural comparison. J. Autism Dev. Disord. 36, 263–270.

Whalen, C.K., Henker, B., Buhrmester, D., Hinshaw, S.P., Huber, A., Laski, K., 1989. Does stimulant medication improve the peer status of hyperactive children? J. Consult. Clin. Psychol. 57, 545–549.

White, S.W., Ollendick, T., Scahill, L., Oswald, D., Albano, A.M., 2009. Preliminary efficacy of a cognitive-behavioral treatment program for anxious youth with autism spectrum disorders. J. Autism Dev. Disord. 39, 1652–1662.

Willcutt, E.G., Doyle, A.E., Nigg, J.T., Faraone, S.V., Pennington, B.F., 2005. Validity of the executive function theory of attention-deficit/hyperactivity disorder: a meta-analytic review. Biol. Psychiatry 57, 1336–1346.

Williams, K., Wheeler, D.M., Silove, N., Hazell, P., 2010a. Selective serotonin reuptake inhibitors (SSRIs) for autism spectrum disorders (ASD). Cochrane Database Syst. Rev. CD004677.

Williams, N.M., Zaharieva, I., Martin, A., Langley, K., Mantripragada, K., Fossdal, R., Stefansson, H., Stefansson, K., Magnusson, P., Gudmundsson, O.O., Gustafsson, O., Holmans, P., Owen, M.J., O'Donovan, M., Thapar, A., 2010b. Rare chromosomal deletions and duplications in attention-deficit hyperactivity disorder: a genome-wide analysis. Lancet 376, 1401–1408.

Xiao, T., Xiao, Z., Ke, X., Hong, S., Yang, H., Su, Y., Chu, K., Xiao, X., Shen, J., Liu, Y., 2012. Response inhibition impairment in high functioning autism and attention deficit hyperactivity disorder: evidence from near-infrared spectroscopy data. PLoS ONE 7, e46569.

Yamasaki, S., Yamasue, H., Abe, O., Suga, M., Yamada, H., Inoue, H., Kuwabara, H., Kawakubo, Y., Yahata, N., Aoki, S., Kano, Y., Kato, N., Kasai, K., 2010. Reduced gray matter volume of pars opercularis is associated with impaired social communication in high-functioning autism spectrum disorders. Biol. Psychiatry 68, 1141–1147.

Kuroda et al. Trials 2013, 14:231
http://www.trialsjournal.com/content/14/1/231

TRIALS

STUDY PROTOCOL Open Access

A cognitive-behavioral intervention for emotion regulation in adults with high-functioning autism spectrum disorders: study protocol for a randomized controlled trial

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Abstract

Background: Adults with high-functioning autism spectrum disorders (ASD) have difficulties in social communication; thus, these individuals have trouble understanding the mental states of others. Recent research also suggests that adults with ASD are unable to understand their own mental states, which could lead to difficulties in emotion-regulation. Some studies have reported the efficacy of cognitive-behavioral therapy (CBT) in improving emotion-regulation among children with ASD. The current study will investigate the efficacy of group-based CBT for adults with ASD.

Methods/Design: The study is a randomized, waitlist controlled, single-blinded trial. The participants will be 60 adults with ASD; 30 will be assigned to a CBT group and 30 to a waitlist control group. Primary outcome measures are the 20-item Toronto Alexithymia Scale, the Coping Inventory for Stressful Situations, the Motion Picture Mind-Reading task, and an ASD questionnaire. The secondary outcome measures are the Center for Epidemiological Studies Depression Scale, the World Health Organization Quality of Life Scale 26-item version, the Global Assessment of Functioning, State-trait Anxiety Inventory, Social Phobia and Anxiety Inventory, and Liebowitz Social Anxiety Scale. All will be administered during the pre- and post-intervention, and 12 week follow-up periods. The CBT group will receive group therapy over an 8 week period (one session per week) with each session lasting approximately 100 minutes. Group therapy will consist of four or five adults with ASD and two psychologists. We will be using visual materials for this program, mainly the Cognitive Affective Training kit.

Discussion: This trial will hopefully indicate the efficacy of group-based CBT for adults with high- functioning ASD.

Trial registration: This trial was registered in The University Hospital Medical Information Network Clinical Trials Registry No. UMIN000006236.

Keywords: Autism spectrum disorders, Emotion regulation, High-functioning adults, Cognitive-behavioral therapy, Randomized controlled trial

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Background

Autism spectrum disorders (ASD) are a group of developmental disorders that include qualitative impairment in interpersonal communication as a core symptom. Even for an adult with high-functioning ASD, whose intellectual development is within the normal range, it is difficult to overcome difficulties in understanding the thoughts and emotions of others; this leads to impairments in interpersonal communication [1-3]. In recent years, studies have shown that an individual may not only find it difficult to recognize the emotions of others but also struggle with identifying one's own emotions and matching the nature of those emotions with the appropriate strength and language given the current context; this can lead to difficulties in identifying or expressing their own mental states [4,5]. Some studies have shown that 50% of ASD adults have alexithymia, which is a personality construct characterized by a sub-clinical inability to identify and describe one's own emotions [6,7]. This inability to identify or express one's own mental states, coupled with a lack of emotion recognition, makes it even more difficult to establish mutual relationships. Failure to adapt to a group may become seriously affected and lead to interpersonal difficulties. Adults with ASD often present with one or more co-morbid disorders, such as anxiety or depression [8,9]. In many cases, a combination of mood disorders and anxiety arises due to chronic stress within a group. Hence, the treatment of patients with underlying ASD is a major issue for the mental health field. Even if symptomatic treatment is successful in relieving psychiatric symptoms, adults with ASD still find it difficult to adapt to society due to interpersonal communication difficulties. Recent research has also suggested that there are many adults with undiagnosed ASD among individuals who receive treatment for other psychiatric disorders. Many are diagnosed with ASD in adulthood without noticeable ASD symptoms during childhood [10].

Cognitive-behavioral therapy (CBT) interventions are being implemented within small group or individual therapy, with the aim of improving the regulation of emotions associated with ASD difficulties [11-15]. Sofronoff and colleagues [12] examined 71 children, aged 10 to 12 years, diagnosed with Asperger's syndrome (AS). In some cases, the children's parents were randomly assigned to one of three conditions (child-only intervention, child and parent intervention, and waitlist control). Small-group CBT was carried out, and the results for the three groups were compared among the three groups. Each of the intervention groups contained three participants, matched on sex and age. Two graduate student therapists conducted CBT for each group. There were 23 participants, over eight groups, in the child-only intervention group. Although there was no direct parental involvement, activities were explained after the sessions, and parents were instructed to have their children perform tasks at home. There were 25 participants, over nine groups, in the child and parent intervention condition. The interventions for the children in this condition were the same as those in the child-only intervention condition, with one psychotherapist for each group, which also included two parents. Twenty-three individuals were assigned to the control group. Six 2 hour sessions, during which participants studied how to be emotionally aware and use appropriate methods for coping with emotions, were conducted over 1 week. Results were examined via children's self-assessments using the "James and the Maths Test", a story describing anxiety about a math test. The parents also completed an assessment using the Spence Child Anxiety Scale and Social Worries Questionnaire–Parent. These assessments were performed at pre- and post-intervention, and also during a follow-up session (6 weeks later). A significant intervention effect was observed when both children and parents took part in the intervention; the child-only intervention was the next most effective treatment. Furthermore, a randomized comparative trial conducted by Sofronoff and colleagues [13] revealed similar results for a small-group CBT intervention to help with anger control. Based on the efficacy of the emotion-regulation which these studies showed, the Cognitive Affective Training Kit (The Cat-kit) was developed [16]. It is designed to help individuals with ASD become aware of how their thoughts, feelings and actions all interact and, in the process of using the various visual components, they share their insights with others.

White and colleagues [17] developed a manual-based CBT program to target anxiety symptoms as well as social skill deficit in adolescents with ASD. Their treatment program includes 12 to 16 individual sessions of 50 to 75 minutes with session content tailored to the individual. Small-group CBT starts approximately 3 weeks after the start of the individual sessions. The small group sessions continue over five, 60 minute sessions, during alternate weeks. Parental participation during the intervention occurs for the last 10 to 20 minutes of their child's individual sessions. This treatment program was carried out with four children (aged 12 to 14 years) with ASD with a co-current anxiety disorder. The Child and Adolescent symptom Inventory-20, a brief parent-report scale, was used to assess anxiety symptoms. The Anxiety Disorders Interview Schedule for Children/Parents, a clinician rating, was used to assess anxiety. The Social Responsiveness Scale was a parent-report scale that measures their child's social disability, and the self-reported Multidimensional Anxiety Scale for Children was completed by the children. All measures were administered at baseline, midpoint, endpoint, and 6 months following treatment. The treatment program was effective in

reducing anxiety in three of the four subjects and improving the social skills in all four subjects.

To our knowledge, the only detailed report on the efficacy of CBT intervention among adults with ASD comes from Cardaciotto and colleagues [11]. In this study, the subject was a 23-year-old male with AS and co-morbid social anxiety disorder. The intervention included individual CBT over 14 weeks; a clinician who did not administer the CBT examined the effects of the therapy. The subject was assessed at the initial examination (6 months before the intervention), 2 weeks before the intervention, immediately before the initial intervention, during the intervention, immediately after the intervention, and 2 months after the intervention, using the Social Phobia and Anxiety Inventory (SPAI), Liebowitz Social Anxiety Scale (LSAS), and Beck Depression Inventory II. The subject showed improvements across all three measures.

Objectives

The purpose of this study is to investigate the efficacy of group-based CBT for adults with ASD. Our primary hypothesis is that, through group-based CBT focusing on emotion-regulation and psychoeducation about ASD, adults with ASD can understand their own and others' emotions and thoughts, exercise emotion-regulation, and increase their knowledge of ASD and self-awareness, especially of their own strengths and weaknesses related to ASD. A small-group adult CBT study protocol will be prepared with reference to previous CBT studies. As noted above, parent training, as well as other forms of intervention, can be carried out along with a child's CBT. Thus, parents gain a greater understanding of ASD and the necessary modifications to a child's environment, which is highly effective in enabling a parent being able to adapt to a child with ASD. However, unlike in children, adults with ASD need to understand more about their own strengths and weaknesses, as it is more desirable and practical that they are able to modify their own environment instead of his/her parents. Therefore, this study, which attempts to help improve social adaptation among adults with ASD, comprises two programs: (1) increasing the individual's emotional awareness and allowing them to acquire appropriate coping skills, and (2) increasing self-awareness through ASD psychoeducation, by learning about the symptoms and biological cause of ASD and the individual's strengths and weakness associated with it. These programs will be provided regularly, and the effects of these programs will be assessed.

Methods/Design

Trial design

This study is a randomized controlled trial. It follows a waitlist control, single-blinded (participants and psychologists who conduct the group-based CBT are not blinded and the assessors of all measures are blinded) design. The allocation of participants will be equal (1:1) across a CBT group (intervention group) and a waitlist control group. All assessment will be administrated by the blinded assessors at the enrolment, post-intervention, and at 12 weeks follow-up. The entire trial design is illustrated in Figure 1. First, an assessment for eligibility will be performed. For individuals who meet the inclusion

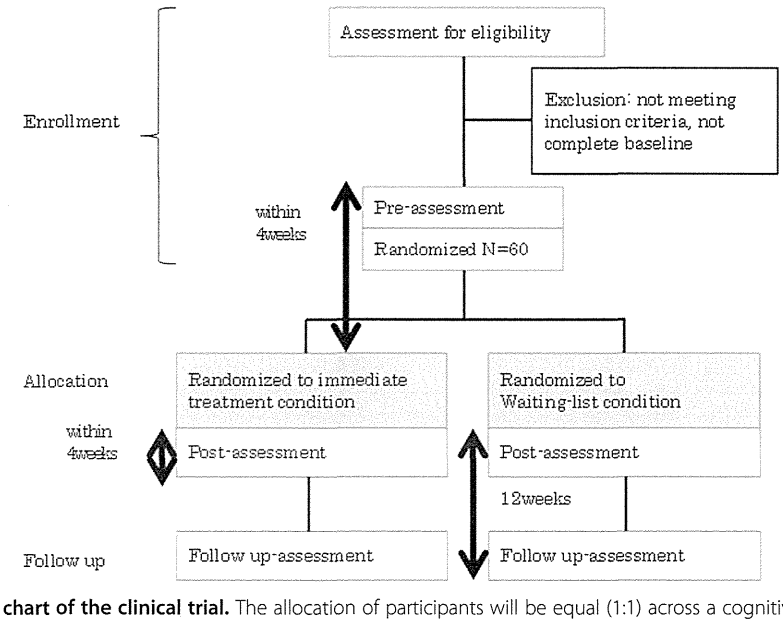

Figure 1 CONSORT flow chart of the clinical trial. The allocation of participants will be equal (1:1) across a cognitive-behavioral therapy group and a waitlist control group. All assessment will be administrated at the enrolment, post-intervention, and at 12 weeks follow-up. Both pre- and post-assessment will be performed at 4 weeks before or after the treatment.

criteria, a pre-assessment will be performed no more than 4 weeks before the treatment. After pre-assessment, participants will be allocated into the immediate treatment condition or the waitlist condition. After the completion of treatment, post-assessment will be performed within 4 weeks. After an additional 12 weeks, a follow-up assessment will be performed. The pre-assessment and intervention will be conducted at the Department of Child and Adolescent Mental Health, the National Institute of Mental Health, National Center of Neurology and Psychiatry, and the Department of Child Psychiatry, the University of Tokyo Hospital. The allocation and post- and follow-up assessments will be conducted at the Department of Child Psychiatry, the University of Tokyo Hospital.

The target sample size is 60, and registration began on September 1, 2011. This trial was registered in the University Hospital Medical Information Network Clinical Trials Registry and approved by the International Committee of Medical Journal Editors (No. UMIN000006236).

Ethical consideration

The Ethics Committee of the National Institute of Neurology and Psychiatry (No. A2010-022) and Graduate School of Medicine and Faculty of Medicine at the University of Tokyo have approved the study protocol (No. 2702). All participants will be asked to sign a written informed consent, as approved by the ethical committee of each site, according to the Declaration of Helsinki after receiving a complete explanation of the trial.

Participants

The participants will be individuals diagnosed with pervasive developmental disorder based on the text revision of the diagnostic and statistical manual of mental disorders, fourth edition (DSM-IV-TR) criteria [18]. In our study, the Cat-kit [16], which was used in the studies of Sofronoff and colleagues, will be used, along with the procedure of those studies. Sofronoff and colleagues had 23 to 25 participants in each of their intervention groups. Therefore we decided to use 25 participants each for the intervention and control groups, recruiting 30 individuals per group to account for potential dropouts.

The inclusion criteria are as follows: (1) age between 18 and 50 years; (2) a full intelligence quotient (IQ) of at least 85 and a verbal IQ of 100 or above (IQ will be evaluated using the Wechsler Adult Intelligence Scale, Third Edition [19]); (3) a specific diagnosis based on the Autism Diagnostic Schedule (ADOS) [20] or the Autism Diagnostic Interview, Revised (ADI-R) [21] with a score above the ASD cut-off; (4) high school graduate or above; (5) knowledge of one's diagnosis, (6) realization of one's poor

Table 1 All measures assessed at enrollment, pre-intervention, post-intervention, and follow-up, including primary and secondary outcomes

Measures	Time required (minutes)	Dx data	Pre-intervention	Post-intervention	Follow-up
DSM-IV-TR		○			
M.I.N.I	30		○		
WAIS-III	60	○			
AQ	10	○			
E-SQ	15	○			
ADI-R	(120: parent)	○			
ADOS	90	○			
SRS-A	15	○			
SCQ	(20: parent)	○			
MPMR	15		○	○	○
TAS-20	5		○	○	○
CISS	10		○	○	○
ASD	5		○	○	○
Questionnaire					
WHO-QOL26	10		○	○	○
GAF	30		○	○	○
STAI	10		○	○	○
SPAI	25		○	○	○
LSAS	10		○	○	○
CES-D	5		○	○	○

emotional self-awareness/ability to express emotion and poor understanding of others' emotions and thoughts, and (7) willingness to participate. Individuals with a comorbid psychiatric and/or unstable condition will be excluded (Table 1). The Mini-International Neuropsychiatric Interview [22] will be used to evaluate psychiatric comorbidity. Any individual who fails to attend more than three sessions will be regarded as 'dropping out', but supplementary instruction will be regarded as equivalent to attendance and will be offered up to three times. Current medication doses should not be increased greatly during the trial. Furthermore, current individual psychological therapy and regular medical treatment will be continued during the intervention. A detailed ASD assessment and diagnosis will be carried out for all intervention candidates. Recruitment of individuals with an ASD diagnosis will be conducted through the Department of Child Psychiatry or Neuropsychiatry at the University of Tokyo Hospital or an advertisement on the University of Tokyo Hospital web site.

Inclusion criteria

- Aged 18–50 years
- Primary diagnosis of autism spectrum disorders (based on criteria by psychiatrists from the text revision of the diagnostic and statistical manual of mental disorders, fourth edition)
- A full intelligence quotient of at least 85 and a verbal intelligence quotient of 100 or above
- Autism Diagnostic Schedule or Autism Diagnostic Interview, Revised score above the autism spectrum disorders cut-off point
- Educational qualifications: high school graduate or beyond
- Informed of his or her diagnosis
- Aware that he or she has poor emotional self-awareness or ability to express emotion and difficulty understanding others' emotions and thoughts
- Willing to participate in the study

Exclusion criteria

- A psychiatric comorbid and/or unstable condition

As shown in Table 1, candidates will be provided with a detailed diagnostic confirmation using the Autism-Spectrum Quotient, Japanese version [23], which is a self-report questionnaire measuring the degree to which any adult with a normal IQ possesses traits related to the autistic spectrum. Additional measures will include the Social Responsiveness Scale for Adults, Japanese version [24], which measures the severity of autism spectrum symptoms (completed by a relative), and the Empathizing- Systemizing Quotient, Japanese version [25], which assesses a person's strength of interest in empathy (defined as the drive to identify with a person's thoughts and feelings and respond with an appropriate emotion). A person's strength of interest in systems is defined as the drive to analyze or construct a system. Additionally, interviews using the ADOS for individuals with ASD and interviews with parents using the ADI-R will be conducted. Final participation will be decided after confirmation that the subject meets participation criteria.

ADI-R, Autism Diagnostic Interview, Revised; ADOS, Autism Diagnostic Schedule; ASD, autism spectrum disorders; AQ, Autism-Spectrum Quotient; CES-D, Center for Epidemiological Studies Depression Scale; CISS, Coping Inventory for Stressful Situations; DSM-IV-TR, text revision of the diagnostic and statistical manual of mental disorders, fourth edition; Dx, Diagnosis; E-SQ, Empathizing-Systemizing Quotient; GAF, Global Assessment of Functioning; LSAS, Liebowitz Social Anxiety Scale; M.I.N.I., Mini-International Neuropsychiatric Interview; MPMR, Motion Picture Mind-Reading task; SCQ, Social Communication Questionnaire; SPAI, Social Phobia and Anxiety Inventory; SRS-A, Social responsiveness scale for adults; STAI, State-trait Anxiety Inventory; TAS-20, 20-item Toronto Alexithymia Scale; WAIS-III, Wechsler Adult Intelligence Scale- Third Edition; WHO-QOL 26, World Health Organization Quality of Life 26-item version.

Assessments/measures

Primary outcomes

Our hypothesis is that CBT will help adults with ASD to understand their own and others' emotions and thoughts and to exercise emotion regulation, by increasing their knowledge of ASD and self-awareness, especially their own strength and weakness related to ASD. Therefore, primary outcome measures will be the 20-item Toronto Alexithymia Scale, Japanese version (TAS-20) [26] scores at post-intervention to evaluate the ability to understand one's own mind and the percentage of correct response on the Motion Picture Mind-Reading task (MPMR) [27] at post-intervention to evaluate the ability of understanding the minds of others. As other primary outcome measures, we will also adopt the Coping Inventory for Stressful Situations, Japanese version (CISS) [28] scores at post-intervention to assess coping skills during stressful situations and the ASD questionnaire scores at post-intervention to assess the knowledge about ASD and the attitude for ASD. These measures are described in greater detail below.

The TAS-20 is one of the most commonly used measures of alexithymia. Alexithymia is characterized by a difficulty in identifying and describing emotions and the tendency to minimize emotional experience and focus

attention externally. This measure is a self-report one and consists of 20 items and three factors: difficulty in identifying feeling, difficulty in describing feeling, externally oriented thinking. Each items is rated from 1 (strongly disagree) to 5 (strongly agree) and the total score ranges from 20 to 100. The time required for this test is about 5 minutes.

The MPMR, developed by Wakabayashi and Katsumata [27], involves advanced theory of mind tasks. Tasks are based on the scenes from a television drama. A total of 41 video clips (each 3 to 11 seconds in length) are included from the television drama series, *Shiroi Kyotō* [The White Tower], which depicts malpractice in a famous Japanese medical school. The participant is asked to judge whether the word or phrase presented on the screen aptly describes the person in each scene. The time required for this test is about 15 minutes.

The CISS determines the preferred coping style of an individual and assesses the relationship between the individual's coping style and his or her personality. Its results are useful for treatment and intervention planning. The CISS measures three types of coping styles: task-oriented, emotion-oriented, and avoidance coping. This measure is also based on self-reports. The CISS consists of 48 items and each item is rated from 1 (not at all) to 5 (very much). The total score of each of the three coping styles ranges from 16 to 80. The time required for this test is about 10 minutes.

The ASD questionnaire that assesses the knowledge about ASD and the attitude to ASD was developed for this study. The questionnaire involved 10 knowledge-based questions (1 = true to 3 = not true) and five attitude-based questions (1 = disagree to 5 = agree) regarding ASD. The time required for this test is about 5 minutes.

Secondary outcomes

We anticipate that they will experience improvement in anxiety and depressive symptoms and their adaptation to their lives as a result of their improved awareness of their own and others' mind, increased knowledge about ASD, and enhanced coping skills for emotion-regulation. Thus, secondary outcome measures will be the scores of the TAS-20, the CISS and the ASD questionnaire and the percentage of correct response on the MPMR at 12 weeks follow-up and the scores of the State-trait Anxiety Inventory (STAI) [29], the LSAS [30], the SPAI [31], the Center for Epidemiological Studies Depression Scale (CES-D) [32], the Global Assessment of Functioning, Japanese version (GAF) [18] and the World Health Organization Quality of Life 26-item version, Japanese version (WHO-QOL 26) [33] at post-intervention and 12 weeks follow-up. These measures are described in greater detail below.

The STAI is a self-report questionnaire that includes separate measures for state and trait of anxiety. The STAI consists of 20 items each for state and trait of anxiety. Each item for state anxiety is rated from 1 (not at all) to 4 (very much so) and each item for trait anxiety is rated from 1 (almost never) to 4 (almost always). The total score for each ranges from 20 to 80 The time required for this test is about 10 minutes.

The SPAI is a self-report questionnaire that assesses specific somatic symptoms, cognitions, and behaviors across a wide range of potentially fear-inducing situations to measure social anxiety and fear. The SPAI consists of 109 items and two domains: social phobia and agoraphobia. Each item is rated from 0 (never) to 6 (always). The social phobia score ranges from 0 to 192 and the agoraphobia score ranges from 0 to 78. The time required for this test is about 25 minutes.

The LSAS is a questionnaire designed to assess the range of social interactions and performance situations that individuals with social phobia may fear and/or avoid. This measure was designed as a self-report questionnaire, but we use it here in the form of an interview. The LSAS comprises 24 social situations that are each rated for level of fear (0 = none to 3 = severe) and avoidance (0 = none to 3 = usually). The total score ranges from 0 to 144. The time required for the interview is about 10 minutes.

The CES-D is a self-report screening tool for depression and consists of 20 items. Each item is rated from 1 (absent) to 4 (five or more times a week), and the total score ranges from 0 to 60. The time required for this test is about 5 minutes.

The GAF is used by clinicians to make a global assessment of an individual's adaptive level of functioning on a scale from 0 (poor) to 100 (good). The time required for this interview is about 30 minutes.

The WHO-QOL 26 is used to measure an individual's subjective sense of wellbeing and quality of life, rather than determining the possible presence of an illness. The WHO-QOL 26 consists of 26 items and four domains: physical health, psychological health, social relationships, and environment. Each item is rated from 1 (poor) to 5 (good) and presented as an average score. The time required for this test is about 5 minutes.

It takes about 1 hour for the participant to fill out all of the questionnaires; therefore, they will be sent via mail to their home 7 to 10 days before the assessment date with careful consideration of the participants' burden. During the pre-, post-, and follow-up assessments, the GAF and LSAS interviews will take about 30 minutes. The theory of mind tasks, MPMR (done on a PC) will take about 15 minutes.

Details of the intervention program

The CBT group will receive group therapy over an 8 week period (1 session/week) with each session lasting approximately 100 minutes. Each session will include a short

Table 2 Timetable of one session

Schedule		
5 minutes	Greeting	
30 minutes	Psychoeducation on autism spectrum disorders	
10 minutes	Relaxation	
40 minutes	Work and discussion	
	<Topics>	
	Session 1 Autism spectrum disorders	Session 2 Relaxation
	Session 3 Happiness	Session 4 Comfort
	Session 5 Affection	Session 6 Anxiety
	Session 7 Anger	Session 8 Coping skills
5 minutes	Relaxation	

period of relaxation between topics (Table 2). Group therapy consists of four to five adults with ASD and two therapists. The therapist who conducts the group therapy as the leader is the certified developmental psychologist who has a PhD and over 10 years experience working with individuals with ASD. The other therapist, the sub-leader, is also a psychologist and has a Masters degree. One or two typical-development volunteers will also join the group and do the same program as the participants with ASD.

The program has two parts; one is the psychoeducation on ASD and the other is the emotion-regulation program. Materials for the psychoeducation on ASD prepared for this study will be used for learning and understanding the nature of ASD. The Cat-kit [16] will be used for the emotion-regulation program. The titles of each session are as follows: (1) the characteristics of autism; (2) relaxation 1 and happiness; (3) relaxation 2 and comfort; (4) differences from others and sadness; (5) strengths and anxiety; (6) weaknesses and coping with anxiety; (7) anger and coping methods; (8) summary: autism characteristics and conveying emotions.

During each session, participants will be asked to do some written work. For the part of psychoeducation on ASD, they will describe and present their own preferences, strengths, weaknesses, *et cetera*. In the emotion-regulation program, they will present their experience and physiological changes associated with emotion. The typical-development volunteers will also perform the same tasks. Finally, after completion of the intervention, the individuals with ASD will make out an original notebook containing descriptions of the nature of ASD and emotion-regulation learning and they will be encouraged to use this for continued study.

Randomization

Enrollment and random allocation will be performed through central registration at the University Hospital Clinical Trial Alliance Clinical Research Supporting System (UHCT ACReSS) at the University of Tokyo. A minimization method will be used with sex as the allocation factor. A third party, who is not involved in this trial, will enroll participants after examining their eligibility and informed consent. Owing to allocation concealment, the random allocation sequence will be provided by UHCT ACReSS and will not be revealed to any researchers or staff until the end of the enrollment period. As this is a single-blinded trial, all assessments will be conducted by raters without knowledge of whether the participant is in the CBT or waitlist conditions.

Statistical methods

All analyses will be performed using SPSS 20 J (SPSS Inc., Chicago, IL, USA). All data will be analyzed under the intent-to-treat principle. For the primary outcomes, independent *t*-tests will be used to compare changes in scores between the pre-assessment and post-assessment periods between the CBT group and the waitlist control group. The primary outcomes will be analyzed controlling for potential confounds (for example, age, gender, IQ, and clinical characteristics) using regression models. Secondary outcomes will be analyzed using relevant tests at each assessment, controlling for possible confounds as described above. Subgroup analyses will be performed for any possible confounds to differentiate the efficacy of CBT at follow-up.

Discussion

Expected results are that adults with ASD will be able to identify their own and understand others' mental states. We further predict that our CBT group will improve their coping skills. Furthermore, secondary symptoms, such as anxiety or depression, should reduce and adaptive behaviors should improve. The novelty of this trial lies in the utilization of CBT for improving emotion regulation among adults with ASD, in contrast with previous studies, which have included children and adolescents (up to age 16) as participants. Previous studies have also focused on the management of anxiety or anger whereas one of our study objectives is to manage the self-regulation of emotion in general. Moreover, previous studies have implemented CBT for children with ASD in addition to parental training. The current study will focus on psychoeducation regarding ASD for adult patients; that is, the current study does not include parent training as a means of the subject's understanding ASD. Thus, our design should help to determine the efficacy of CBT for adults with ASD. This CBT intervention is the first step in understanding ASD and emotion-regulation in adulthood, especially for persons diagnosed with ASD in adulthood. Our results will hopefully provide promising avenues for developing services for adults with high-functioning ASD in Japan.

Trial status

At the time of submission, 88% of the participants have been included in the trial. Of these participants, 68% have been tested at follow-up.

Abbreviations

ADOS: Autism Diagnostic Schedule; ADI-R: Autism Diagnostic Interview, Revised; AS: Asperger's syndrome; ASD: autism spectrum disorders; Cat-kit: Cognitive Affective Training Kit; CBT: cognitive-behavioral therapy; CES-D: Center for Epidemiological Studies Depression Scale; CISS: Coping Inventory for Stressful Situations; DSM-IV-TR: text revision of the diagnostic and statistical manual of mental disorders, fourth edition; GAF: Global Assessment of Functioning; IQ: intelligence quotient; LSAS: Liebowitz Social Anxiety Scale; MPMR: Motion Picture Mind-Reading task; STAI: State-trait Anxiety Inventory; SPAI: Social Phobia and Anxiety Inventory; TAS-20: 20-item Toronto Alexithymia Scale; UHCT ACReSS: University Hospital Clinical Trial Alliance Clinical Research Supporting System; WHO-QOL 26: World Health Organization Quality of Life 26-item version.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

MK and YK equally contributed to the design and management of this trial and wrote most of the manuscript. HK made substantial contributions to the conception and design of this trial. KY contributed to the development of the CISS-Japanese version. YK and YK are the directors of each site and made substantial contributions to revising the design and management of this trial. All authors have read and approved the final manuscript.

Acknowledgements

This study is supported by a Grant-in-Aid for Scientific Research(C) (No 22531078), the Japan Society for the Promotion of Science (JSPS), Japan; Intramural Research Grant (23–1) for Neurological and Psychiatric Disorders of the National Center of Neurology and Psychiatry (NCNP); and a Grant-in -Aid for Scientific Research on Innovative Areas (No. 23119706), Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan.

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Received: 16 January 2013 Accepted: 2 July 2013
Published: 23 July 2013

References

1. Baron-Cohen S, Wheelwright S, Hill J, Raste Y, Plumb I: **The "Reading the Mind in the Eyes" test revised version: a study with normal adults, and adults with Asperger syndrome or high-functioning autism.** *J Child Psychol Psychiatry* 2001, **42**:241–251.
2. Roeyers H, Buysee A, Ponnet K, Pichal B: **Advancing advanced mind-reading test: empathic accuracy in adults with a pervasive developmental disorder.** *J Child Psychol Psychiatry* 2001, **42**:271–278.
3. Golan O, Baron-Cohen S, Hill J: **The Cambridge Mindreading (CAM) face-voice battery: testing complex emotion recognition in adults with and without Asperger Syndrome.** *J Autism Dev Disord* 2006, **36**:169–183.
4. Attwood T: **Cognitive behaviour therapy for children and adults with Asperger's syndrome.** *Behav Change* 2004, **21**:147–161.
5. Williams D: **Theory of own mind in autism: evidence of a specific deficit in self awareness?** *Autism* 2010, **14**:474–494.
6. Hill E, Berthoz S, Frith U: **Brief report: cognitive processing of own emotions in individuals with autistic spectrum disorder and in their relatives.** *J Autism Dev Disord* 2004, **34**:229–235.
7. Berthoz S, Hill EL: **The validity of using self-reports to assess emotion regulation abilities in adults with autism spectrum disorders.** *Eur Psychiatry* 2005, **20**:291–298.
8. Joshi G, Petty C, Wozniak J, Henin A, Fried R, Galdo M, Kotarski M, Walls S, Biederman J: **The heavy burden of psychiatric comorbidity in youth with autism spectrum disorders: a large comparative study of a psychiatrically referred population.** *J Autism Dev Disord* 2010, **40**:1361–1370.
9. Lugnegård T, Hallerbäck MU, Gillberg C: **Psychiatric comorbidity in young adults with a clinical diagnosis of Asperger syndrome.** *Res Dev Disabil* 2011, **32**:1910–1917.
10. Lehnhardt FG, Gawronski A, Volpert K, Schilbach L, Tepest R, Vogeley K: **Psychosocial functioning of adults with late diagnosed autism spectrum disorders – a retrospective study.** *Fortschr Neurol Psychiatr* 2012, **80**:88–97.
11. Cardaciotto L, Herbert JD: **Cognitive behavior therapy for social anxiety disorder in the context of Asperger's syndrome: a single-subject report.** *Cogn Behav Pract* 2004, **11**:75–81.
12. Sofronoff K, Attwood T, Hinton S: **A randomized controlled trial of a CBT intervention for anxiety in children with Asperger syndrome.** *J Child Psychol Psychiatry* 2005, **46**:1152–1160.
13. Sofronoff K, Attwood T, Hinton S: **A randomized controlled trial of a cognitive behavioural intervention for anger management in children diagnosed with Asperger's syndrome.** *J Autism Dev Disord* 2007, **37**:1203–1214.
14. Wood JJ, Drahota A, Sze K, Har K, Chiu A, Langer DA: **Cognitive behavioral therapy for anxiety in children with autism spectrum disorders: a randomized, controlled trial.** *J Child Psychol Psychiatry* 2009, **50**:224–234.
15. White SW, Ollendick T, Scahill L, Oswald D, Albano AM: **Preliminary efficacy of a cognitive-behavioral treatment program for anxious youth with autism spectrum disorders.** *J Autism Dev Disord* 2009, **39**:1652–1662.
16. Attwood T: *The Cognitive Affective Training-kit (The Cat-kit).* Arlington TX: Future Horizons; 2008 (Japanese version: The Cognitive Affective Training-kit (The Cat-kit). Saga: Village Shuppan; 2009).
17. White SW, Albano AM, Johnson CR, Kasari C, Ollendick T, Klin A, Oswald D, Scahill L: **Development of a cognitive-behavioral intervention program to treat anxiety and social deficits in teens with high-functioning autism.** *Clin Child Fam Psychol Rev* 2010, **13**:77–90.
18. American Psychiatric Association: *Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR).* Washington, DC: American Psychiatric Association; 2011.
19. Wechsler D: *Wechsler Adult Intelligence Scale - Third Edition (WAIS-III).* New York, Bloomington, MN: NCS Pearson Inc; 1997 (Japanese version: Fujita, K., Maekawa, H., Dairoku, K., Yamanaka, K. Wechsler Adult Intelligence Scale, Third Edition. Tokyo: Nihon Bunka Kagakusha; 2006).
20. Lord C, Rutter M: *DiLavore PC, Risi S: Autism Diagnostic Observation Schedule.* Los Angeles: Western Psychological Services; 2002.
21. Rutter M, Lord C, Le Couteur A: *Autism Diagnostic Interview Revised Manual.* Torrance, CA: Wester Psychological Services; 2003.
22. Otsubo T, Tanaka K, Koda R, Shinoda J, Sano N, Tanaka S, Aoyama H, Mimura M, Kamijima K: **Reliability and validity of Japanese version of the mini-international neuropsychiatric interview.** *Psychiatry Clin Neurosci* 2005, **59**:517–526.
23. Kurita H, Koyama T, Osada H: **Autism-spectrum quotient-Japanese version and its short forms for screening normally intelligent persons with pervasive developmental disorders.** *Psychiatry Clin Neurosci* 2005, **59**:490–496.
24. Constantino JN: *Social Responsiveness Scale, Second Edition (SRS-2).* Los Angeles, CA: Western Psychological Services; 2012.
25. Wakabayashi A, Baron-Cohen S, Uchiyama T, Yoshida Y, Kuroda M, Wheelwright S: **The Empathy Quotient (EQ) and Systemizing Quotient (SQ) in Japan: an investigation of adults with Asperger syndrome or high-functioning autism, and normal sex differences.** *J Autism Dev Disord* 2007, **37**:1823–1832.
26. Moriguchi Y, Maeda M, Igarashi T, Ishikawa T, Shoji M, Kubo C, Komaki G: **Age and gender effect on alexithymia in large, Japanese community and clinical samples: a cross-validation study of the Toronto Alexithymia Scale (TAS-20).** *Biopsychosoc Med* 2007, **1**:1–15.
27. Wakabayashi A, Katsumata A: **The motion picture mind-reading test: measuring individual differences of social cognitive ability in a young adult population in Japan.** *J Individual Differences* 2011, **32**:55–64.
28. Endler NS, Parker JDA: *Coping Inventory for Stressful Situations (CISS)-Second Edition. NY: Multi-Health Systems, Inc; 1999. (Japanese version: Yokoyama K.,*

Furukawa, T, Watanabe K: Coping Inventory for Stressful Situations (CISS). Kaneko shobo: Tokyo; 2012.

29. Suzuki T, Tsukamoto K, Abe K: **Characteristics factor structures of the Japanese version of the State-Trait Anxiety Inventory: coexistence of positive–negative and state-trait factor structures.** *J Pers Assess* 2000, **74**:447–458.
30. Asakura S, Inoue S, Sasaki F, Sasaki Y, Kitagawa N, Inoue T, Denda K, Itou M, Matsubara R, Koyama T: **The validation and reliability of Japanese version of Liebowitz Social Anxiety Scale (LSAS).** *Seishin Igaku* 2002, **44**:1077–1084.
31. Turner SM, Beidel CC, Dance CV: ***Social Phobia and Anxiey Inventory (SPAI).*** **New York: Multi-Health Systems, Inc; 1996. (Japanese version: Okajima I, Kanai Y, Sasagawa S, Kanazawa J, Akita K, Chen J, Sakano Y: Development of the Japanese Version of the Social Phobia and Anxiety Inventory.** *Japanese J Res Behavior Therapy* 2008, **34**:297–309.
32. Radloff LS, Radloff LS: **The CES-D scale: a self- report depression scale for research in the general population.** *Appl Psychol Meas* 1977, **11**:385–401. Japanese version: Shima S, Shikano T, Kitamura T, Asai M: New Self-Rating Scale for Depression. *Seishinigaku* 1985, 27: 717–723.
33. Organization TWH: *WHO Quality of Life 26.* Geneva: The World Health Organization; 1991 (Japanese version: Tazaki M, Nakane Y: WHO Quality of Life 26 Japanese Version. Tokyo: Kaneko shobo; 1997).

doi:10.1186/1745-6215-14-231
Cite this article as: Kuroda *et al.*: A cognitive-behavioral intervention for emotion regulation in adults with high-functioning autism spectrum disorders: study protocol for a randomized controlled trial. *Trials* 2013 14:231.

PLOS ONE

Altered Metabolites in the Plasma of Autism Spectrum Disorder: A Capillary Electrophoresis Time-of-Flight Mass Spectroscopy Study

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Abstract

Clinical diagnosis and severity of autism spectrum disorders (ASD) are determined by trained clinicians based on clinical evaluations of observed behaviors. As such, this approach is inevitably dependent on the expertise and subjective assessment of those administering the clinical evaluations. There is a need to identify objective biological markers associated with diagnosis or clinical severity of the disorder. To identify novel candidate metabolites as potential biomarkers for ASD, the current study applied capillary electrophoresis time-of-flight mass spectroscopy (CE-TOFMS) for high-throughput profiling of metabolite levels in the plasma of 25 psychotropic-naïve adult males with high-functioning ASD and 28 age-matched typically-developed control subjects. Ten ASD participants and ten age-matched controls were assigned in the first exploration set, while 15 ASD participants and 18 controls were included in the second replication set. By CE-TOFMS analysis, a total of 143 metabolites were detected in the plasma of the first set. Of these, 17 metabolites showed significantly different relative areas between the ASD participants and the controls ($p<0.05$). Of the 17 metabolites, we consistently found that the ASD participants had significantly high plasma levels of arginine ($p=0.024$) and taurine ($p=0.018$), and significantly low levels of 5-oxoproline ($p<0.001$) and lactic acid ($p=0.031$) compared with the controls in the second sample set. Further confirmatory analysis using quantification of absolute metabolite concentrations supported the robustness of high arginine ($p=0.001$) and low lactic acid ($p=0.003$) in the combined sample (n = 53). The present study identified deviated plasma metabolite levels associated with oxidative stress and mitochondrial dysfunction in individuals with ASD.

Citation: Kuwabara H, Yamasue H, Koike S, Inoue H, Kawakubo Y, et al. (2013) Altered Metabolites in the Plasma of Autism Spectrum Disorder: A Capillary Electrophoresis Time-of-Flight Mass Spectroscopy Study. PLoS ONE 8(9): e73814. doi:10.1371/journal.pone.0073814

Editor: Kenji Hashimoto, Chiba University Center for Forensic Mental Health, Japan

Received June 29, 2013; **Accepted** July 26, 2013; **Published** September 18, 2013

Funding: A part of this study was supported by CREST, Japan Science and Technology Agency, and the "Development of biomarker candidates for social behavior" carried out under the Strategic Research Program for Brain Sciences by the Ministry of Education, Culture, Sports, Science and Technology of Japan. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. to funding.

Competing Interests: The authors have declared that no competing interests exist.

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Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by social deficits, communication deficits and restricted and repetitive behaviors [1]. Diagnosis or clinical severity of ASD is mostly determined by trained clinicians based on clinical evaluations of observed behaviors [2,3]. As such, this approach is inevitably dependent on the expertise of those administering the diagnostic tests [4]. Thus, more objective methodologies for evaluating diagnosis or clinical severity of the disorder has been sought after [5–9]. These studies have revealed a higher mRNA expression in lymphocytes [5], metabolite alterations in the hippocampal formation [7], atypical brain responses to social stimuli [8], and increased serum levels of anterior pituitary hormones [9]. However, to the best of our knowledge, there are no reliable and practical biological markers for assessing clinical diagnosis or severity of ASD.

Metabolomics is a high-throughput metabolic profiling method to evaluate the concentration of metabolites in a given sample, such as plasma and urine. Because the status of metabolites is considered to be affected by pathophysiological processes, this approach can identify candidate molecules for representing the pathophysiology of developmental and psychiatric disorders [10–12]. To date, previous metabolomics studies using nuclear magnetic resonance (NMR), liquid chromatography mass spectroscopy (LC-MS), and gas chromatography MS (GC-MS) have reported possible biomarkers, such as amino acids, antioxidants, gut bacterial metabolites, and sulfur in urine from children with ASD [13,14]. Although urine collection is totally non-invasive and holds promise for the disease biomarkers, a portion of the plasma metabolites are biotransformed in the kidneys before production of

urine [4,15]. Actually, different candidate biological markers for other disorders have been detected from urine and plasma samples [15,16]. Previous literature suggests that changes in potential biomarkers in cerebrospinal fluid might be correlated with those in plasma [6]. However, to our knowledge, there was no previous study reporting metabolomic profiles quantified from plasma in individuals with ASD.

Capillary electrophoresis time-of-flight mass spectroscopy (CE-TOFMS) is a relatively new MS method that can measure metabolites of higher ionization and lower molecular weight with easy preparation and high throughput [17,18]. While the efficacy of CE–TOFMS has been demonstrated in various human clinical studies [19,20], no previous application of this method for ASD subjects has been reported.

To identify novel candidate metabolites as potential biomarkers for ASD, the current study applied CE-TOFMS to plasma samples from subjects with ASD and age-matched typically-developed control subjects. To minimize potential confounding effects of medications and neuropsychiatric comorbidities, including mental retardation and epilepsy, on metabolite levels, the participants with ASD, as well as those with typical development, were confined to psychotropic naïve subjects and free from neuropsychiatric comorbidities.

Methods

Ethics Statement

All the participants provided written informed consent after they were given a complete explanation of the study as approved by the ethics committee of The University of Tokyo Hospital (No. 2094-[5]). Using longitudinal clinical assessments, a trained psychiatrist (H.Y.) confirmed that all of these adult participants had no intellectual disabilities and no need for psychotropic medication, and were capable of providing informed consent. Therefore, no participant needed for someone else to provide consent on their behalf.

Study Participants

A total of 53 Japanese adults (all Asian ethnicity) participated in this study. Considering the relatively low analytical reproducibility of capillary electrophoresis [11], we randomly enrolled two independent sets of samples for exploration and replication. The participants were recruited from April 2010 to July 2012 at the outpatient clinic of The University of Tokyo Hospital. All the study participants were male, since previous studies have indicated the potential contribution of sexual dimorphism to the pathophysiology of ASD [21,22]. Ten ASD participants and ten age-matched controls were assigned in the first set (exploration set), while 15 ASD participants and 18 age-matched control subjects were included in the second set (replication set) (Table 1). All the ASD participants were psychotropic-naïve. The diagnostic protocols and clinical assessments were described detail in our previous papers [23,24]. Briefly, an experienced psychiatrist (H.Y.) carefully diagnosed the participants with ASD on the basis of DSM-IV-TR [1] criteria after more than 2 months of follow-up examinations. Another trained psychiatrist (H.K.) confirmed the clinical diagnoses of the ASD participants using the Japanese version of the Autism Diagnostic Interview–Revised (ADI-R) [3,25]. For the six participants who did not meet the threshold in the ADI-R social domain, the ASD diagnosis was confirmed using the Autism Diagnostic Observation Schedule - Generic [2] by a trained psychologist (M.K.). Moreover the Global Assessment of Functioning (GAF) [1] was evaluated in all the ASD participants. The verbal Intelligence quotients (IQ) of the control group (all the participants in the first set and eight participants in the second set) was estimated using a Japanese version of the National Adult Reading Test [26,27]. Although the National Adult Reading Test can measure IQ accurately in controls, the test is problematic for ASD participants because of the well-known imbalances in their intellectual abilities. Therefore, the IQs of the ASD participants were assessed using the Wechsler Adult Intelligent Scale-revised [28] or Wechsler Adult Intelligent Scale third edition [29].

The exclusion criteria were: neurological illness, traumatic brain injury with any known cognitive consequences or loss of consciousness for more than 5 min, low IQ (i.e. below 80), previous alcohol dependence, previous continuous illegal substance use (e.g., cannabis). Additional exclusion criteria for the control group were any past and present psychiatric disease detected by screening with the modified Mini-International Neuropsychiatric Interview [30] or a family history of axis I disorder within the first-degree relatives.

CE-TOFMS Analysis

Peripheral blood samples were drawn by experienced physicians from a peripheral vein while the participant was fasting (>3 h without any meal and/or nutritious drink). Plasma samples were isolated via centrifugation at 1200 g for 10 min, and then stored at −80°C until use.

Plasma samples (100 μL) were immersed into 0.45 mL methanol containing 10 μM each methionine sulfone and 10-camphorsulfonic acid, and mixed well. Then, 200 μL deionized water and 0.5 mL chloroform were added and the solution was centrifuged at 2,300 g for 5 min at 4°C. The upper aqueous layer was centrifugally filtered through a 5-kDa cutoff filter (Human Metabolome Technologies Inc., Tsuruoka, Japan) to remove proteins. The filtrate was lyophilized and dissolved in 50 μL of ultrapure water containing reference compounds prior to mass spectrometry analysis. The water was produced by a Milli-Q Academic A10 (EMD Millipore, Billerica, MA, USA).

Samples were applied to a capillary electrophoresis system equipped with an Agilent 6210 time-of-flight mass spectrometer (CE-TOFMS, Agilent Technologies, Santa Clara, CA, USA), as previously described [31]. Raw data files from CE-TOFMS were processed using customized proprietary software written in Java (An extended version of MathDAMP that has been developed in Keio University) [32]. The software performs (1) peak picking, and (2) peak alignment. For (1), all peaks potentially corresponding to metabolites were extracted. After peak picking, the migration time of electrophoresis was normalized using those of the internal standards. For (2), an alignment was applied according to similar mass-to-charge ratios (m/z) and normalized migration times. The tolerance was set to 100 ppm (m/z) and 0.5 min (Normalized migration time). The peak matrix was matched with the annotation table of the metabolomics library (The Basic Scan metabolomics service of Human Metabolome Technologies Inc.) described previously based on their m/z and migration times [33]. All peak areas were divided by the area of the internal standard (i.e. Relative area) to normalize the signal intensities, and to avoid injection-volume bias and mass-spectrometry detector sensitivity bias among multiple measurements.

Statistical Analysis

Comparisons for relative metabolite concentrations between individuals with ASD and controls. All statistical analyses were conducted using the SPSS version 17.0 (IBM Inc., Armonk, NY, USA). As the current sample sizes were relatively small, normal distributions of the present data sets across all metabolites were not warranted in advance. Therefore, non-

Table 1. Demographic characteristics of the study participants.

	First sample set		Second sample set	
	ASD (n = 10)	Controls (n = 10)	ASD (n = 15)	Controls (n = 18)
Age (years)	32.2±7.0	32.9±3.6	28.6±5.3	28.7±4.0
ADI-R S	12.3±5.4	NA	16.9±6.8	NA
ADI-R C	9.5±2.0	NA	12.8±4.6	NA
ADI-R R	3.3±2.0	NA	4.8±1.8	NA
GAF	45.5±8.8	NA	46.7±6.8	NA
Full Scale IQ	102.5±11.2	NA	109.3±9.5	NA
Verbal IQ	111.3±11.7	115.2±4.7	113.0±12.9	108.8±9.3
Performance IQ	90.3±14.1	NA	100.9±17.0	NA

Values are given in mean ± SD, except for the number of participants.
Abbreviations: ASD, autism spectrum disorders; ADI-R, autism diagnostic interview revised; S, social domain; C, communication domain; R, restricted and repetitive behavior domain; GAF, global assessment of functioning; NA, not applicable.
doi:10.1371/journal.pone.0073814.t001

parametric statistics were employed for the statistical analyses. The obtained relative area values of each metabolite were regarded as their relative concentration. We tested the differences of these relative concentrations between the ASD participants and the controls using two-tailed Mann-Whitney U test in the first set. For the purpose of exploration, significance levels were set at $p<0.05$ for each metabolite without correction for multiple comparisons. For the purpose of replication, we compared the metabolite levels, which showed significant differences due to diagnosis in the first set, between the participants with ASD and the controls in the second set using one-tailed Mann-Whitney U test. As analyses on the second set were aimed to replicate findings in the first set, the directions of expected effects were known *a priori*. Thus, one-tailed tests ($p<0.05$ were set as significance) were used without correction for multiple comparisons [34].

Discriminant analyses between ASD and controls based on the identified metabolites. To preliminarily test the possibility of the detected metabolites as a diagnostic tool, we conducted discriminant analyses using metabolite levels that were significantly and consistently different in the ASD participants in the first and the second sets. Since the discriminant analyses were conducted separately on the first and second sets, the significance levels were set at $p<0.025$ with Bonferroni correction for two data sets.

Comparisons for absolute metabolites concentrations between ASD individuals and controls. Analysis by CE-TOFMS in our system enables measurement of the absolute quantities of pre-determined 108 major metabolites, based on the peak area of internal controls of each metabolite. Since the quantity of these metabolites can be reliably compared across different experimental batches, absolute concentrations were quantified for these metabolites for the purpose of further verification in the combined sample of the first and second sets. We compared absolute concentrations of the major metabolites using two-tailed U test between the 25 ASD individuals and 28 controls. To test the robustness of findings from comparisons using relative metabolite measures, statistical significance was set at $p<0.05/N$ (number of metabolites absolutely quantified among the metabolites consistently showed significant differences at relative concentrations in both the first and second sets) employing Bonferroni correction.

Correlation analyses between metabolites and clinical measures. To explore clinical significance of the deviated metabolites in the plasma from ASD subjects, we calculated Spearman's rank correlation coefficients between their clinical characteristics (i.e. ADI-R, GAF, IQ) and absolute value of significant metabolites in the combined sample set. Since the correlation analyses were implicated as exploratory analyses, we adopted absolute values in the combined sample set to increase the statistical power, and then statistical significance was defined as $p<0.05$.

Results

By the CE-TOFMS analysis, a total of 143 metabolites were detected in the plasma samples of the first sample set (79 and 64 metabolites for the cation and anion modes, respectively). Of these, 17 metabolites showed significantly different relative areas between the ASD participants and the controls. Of the 17 metabolites which showed significant difference in the first sample set, 14 metabolites were successfully quantified in the second sample set, although a total of 141 metabolites were measurable (76 and 65 metabolites for cation and anion modes, respectively). The lists of detected metabolites in the first and the second sample sets were available in Table S1. For the purpose of validating the findings in the first sample set, we conducted a one-tailed Mann-Whitney U test for the relative areas of the 14 metabolites. Then, we found that the ASD participants had significantly high levels of arginine ($p=0.024$) and taurine ($p=0.018$), low levels of 5-oxoproline ($p<0.001$) and lactic acid ($p=0.031$) compared with the controls in the second sample set (Table 2 and Figure 1).

Discriminant analysis in the first sample set showed that diagnosis of 80.0% (16 out of 20) of the subjects was correctly classified with the four used metabolites (arginine, taurine, 5-oxoproline, and lactic acid; Wilks's $\lambda=0.447$, $p<0.012$, Figure 2). These four metabolites were differentiated by participants with ASD and controls with an area under the receiver-operating characteristic curve (AUC) value of 0.940 ($p=0.001$). These discriminant analyses in the second sample set showed that diagnosis of 78.8% (26 out of 33) of the subjects was correctly classified ($\lambda=0.437$, $p<0.001$, Figure 2), and AUC value was 0.957 ($p<0.001$).

Among pre-determined 108 major metabolites, which could measure absolute quantities, 39 metabolites were identified in all the 53 participants (25 ASD participants and 28 controls). Among the four metabolites listed in Table 2, two metabolites (arginine, lactic acid) were included in the 39 major metabolites identified.

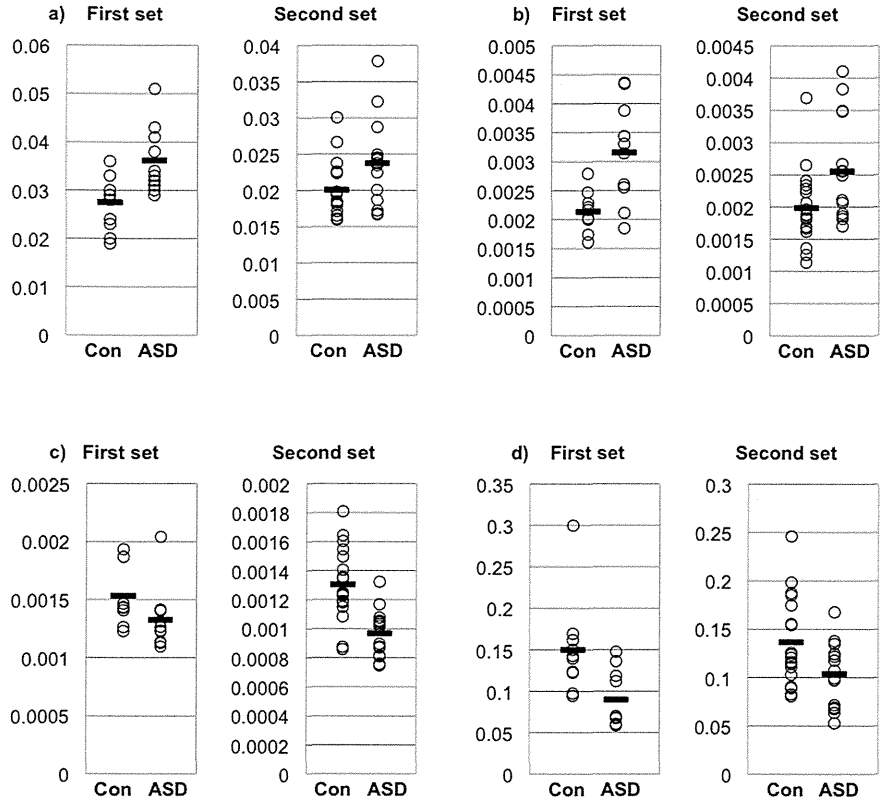

Figure 1. Metabolites with significantly deviated relative concentrations in the ASD participants. Plots show relative areas of arginine (a), taurine (b), 5-oxoproline (c), lactic acid (d), in the first (left) and the second (right) sample sets. Y-axis indicates relative concentrations. Bars indicate mean concentration in the group. Con, controls; ASD, participants with autistic spectrum disorders.
doi:10.1371/journal.pone.0073814.g001

We thus compared their absolute concentrations using the combined dataset. The significance level was defined at $p<0.025$ with Bonferroni correction for two metabolites. Absolute concentration of arginine was significantly higher ($p=0.001$), while that of lactic acid was significantly lower ($p=0.003$) in the ASD group than in the controls (Table 3).

Correlation analysis using Spearman's rank correlation coefficients in the combined sample showed significant negative correlation between the concentration of arginine and GAF score (rho = −0.413, $p=0.040$, Figure 3). There was no significant correlation between metabolites and ADI-R or IQ scores.

Discussion

The current metabolomic analysis using CE-TOFMS revealed that plasma levels of several metabolites were significantly different between the participants with ASD and controls. We analyzed two independent sample sets, and consistently identified high levels of arginine and taurine, and low levels of lactic acid and 5-oxoproline in the participants with ASD compared with the controls. Taking advantage of the function of absolute concentration quantification of major metabolites, altered concentrations of arginine and lactic acid in the participants with ASD were further confirmed. To the best of our knowledge, this is the first study that elucidated possible

Table 2. Results from comparisons for relative metabolite concentrations between individuals with ASD and controls.

Direction of change					First sample set[a]			Second sample set[a]		
	Metabolites	Mode	m/z	MT (min)	ASD	Controls	p value[b]	ASD	Controls	p value[c]
Higher	Arginine	Cation	175.12	7.12	0.036 (0.0070)	0.028 (0.0060)	0.011	0.024 (0.0059)	0.020 (0.0037)	0.024
	Taurine	Cation	126.02	23.31	0.0031 (0.00088)	0.0021 (0.00034)	0.007	0.0026 (0.00081)	0.0020 (0.00059)	0.018
Lower	5-Oxoproline	Anion	128.03	9.50	0.0013 (0.00027)	0.0015 (0.00026)	0.035	0.00097 (0.00016)	0.0013 (0.00025)	<0.001
	Lactic acid	Anion	89.02	10.93	0.090 (0.035)	0.15 (0.058)	0.009	0.10 (0.033)	0.14 (0.046)	0.031

[a]Mean relative area value and its SD are given.
[b]Two- and [c]one-tailed Mann-Whitney U tests.
Abbreviations: m/z, mass-to-charge ratio; MT, migration time; ASD, participants with autism spectrum disorder; NA, not applicable; ND not detected.
doi:10.1371/journal.pone.0073814.t002

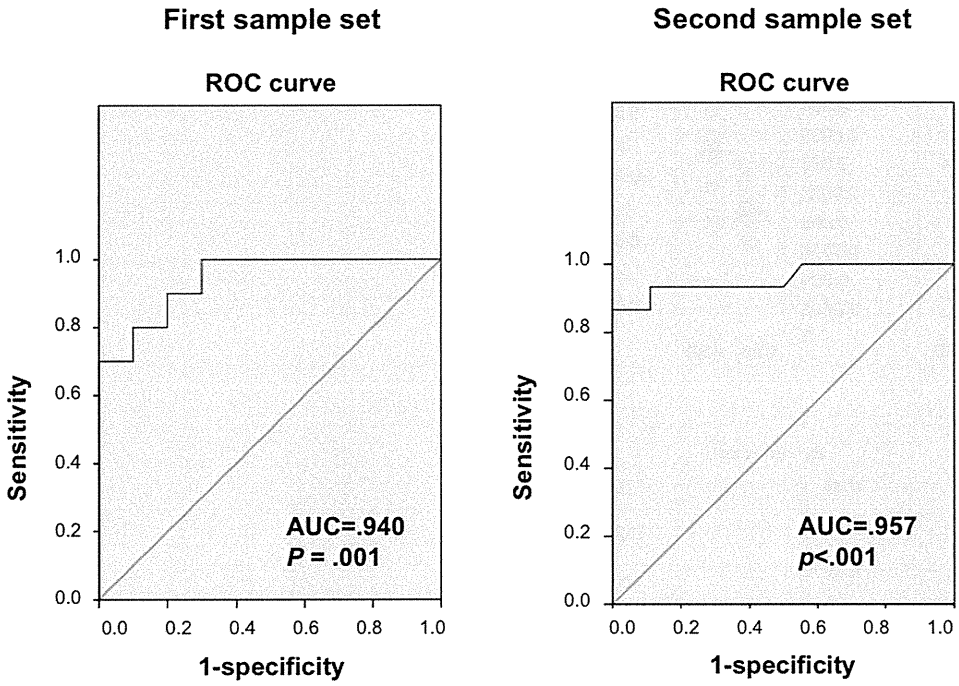

Figure 2. Results of discriminant analyses. A receiver-operating characteristic (ROC) curve and area under the curve (AUC) according to the results of discrimination analysis in the first (left) and the second sample sets (right) are presented. The results of discriminant analyses between the subjects with autism spectrum disorders and controls are indicated with blue lines.
doi:10.1371/journal.pone.0073814.g002

biomarkers in peripheral blood plasma to evaluate ASD using metabolomics analysis.

Arginine is an essential precursor for the synthesis of proteins and other molecules of enormous biological importance, including nitric oxide (NO). Excessive arginine is thought to induce oxidative stress via NO production [35]. Though the present CE-TOFMS analyses were not able to measure nitric oxide concentration, NO level was previously reported to be high in ASD children [36].

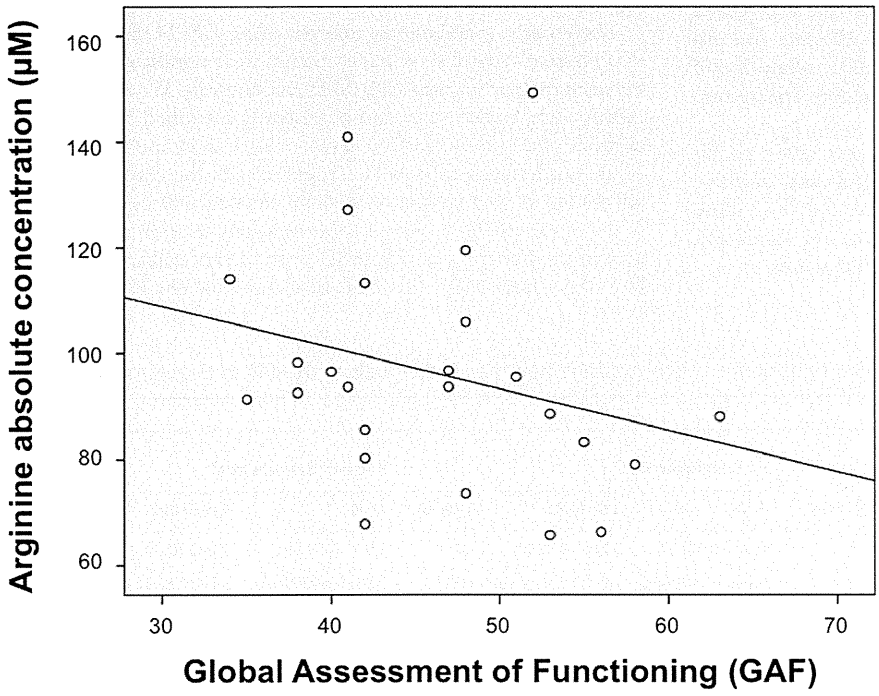

Figure 3. Relationship between arginine absolute concentration and GAF score. Scatter plot shows correlation coefficients between arginine absolute concentration and GAF score ($rho = -0.413$, $p = 0.040$).
doi:10.1371/journal.pone.0073814.g003

Table 3. Absolute quantities of the metabolites in the ASD and control groups.

	Concentration (μM)[a]		
Metabolites	**ASD**	**Controls**	**p value[b]**
Arginine	96.3 (21.6)	78.3 (15.2)	0.001
Lactic acid	1678.3 (661.6)	2406.0 (868.3)	0.003

[a]Mean concentration and its SD are given. [b]Two-tailed Mann-Whitney U tests.
Abbreviation: ASD, participants with autism spectrum disorders.
doi:10.1371/journal.pone.0073814.t003

However, children with ASD were reported to have no difference in plasma arginine concentration [37], and there is no report that examined arginine concentration in adults with ASD other than the present study. On the other hand, research on other psychiatric disorders, such as schizophrenia, have reported excess [38] or reduced plasma arginine levels [12]. The precise nature of the relationship between arginine and oxidative stress in neuropsychiatric disorders is unclear; however, the common susceptibility genes for ASD and schizophrenia, TCF4 [39] and NOS1 [40,41] has been suggested to be involved in the arginine-NO pathway. The arginine concentration was negatively correlated with GAF score in the current participants with ASD, although there was no significant correlation with ADI-R scores. Therefore, the current study could not draw the conclusion that arginine was a specific biomarker for the pathogenesis of ASD rather than that for general difficulties due to psychiatric disorders. Besides, since ADI-R scores are calculated mainly based on behavioral characteristics during childhood, a possibility that arginine concentration reflect the current ASD severity during adulthood rather than that during childhood remains. Future studies should address these issues.

The inhibitory amino acid taurine is an osmoregulator and neuromodulator, also exerting protective actions in neural tissue, including under conditions of oxidative stress [42]. Plasma taurine concentration in children with ASD was reported to be high compared with typically developing children [37,43], and other researches has reported contradictory results that showed low concentrations of taurine in ASD children [44–46]. Similarly, contradictory changes in urine concentrations of taurine in ASD children were reported, with elevated [13] and low concentrations [14]. Although the reason for the contradictory findings in plasma and urine concentrations remains unclear, taurine is hypothesized to be protective for autistic symptoms [47,48]. The present result, which showed elevated plasma taurine level in adults with ASD, might be due to compensatory regulation against pathogenesis of ASD, such as oxidative stress.

The endogenous molecule 5-oxoproline is derived from L-glutamate, being a major intermediate in the γ-glutamyl cycle. This cycle is necessary for the synthesis and breakdown of glutathione, and also for the intracellular transport of free amino acids [49]. Although the pathological role of 5-oxoproline in the human brain is not clarified, it has been presumed from animal studies that 5-oxoproline elicits oxidative stress, which may represent a pathophysiological mechanism in the neuro-pathological disorder in which this metabolite accumulates [50,51]. Direct contribution of 5-oxoproline down-regulation in ASD pathology is not clear, but like taurine and arginine, 5-oxoproline might be a putative biomarker for ASD that has oxidative stress alterations.

Lactic acid is thought to be one of the biochemical markers that may indicate mitochondrial function [52]. Previous studies have suggested that mitochondrial dysfunction and altered energy metabolism may influence the social and cognitive deficits in autism [53–56]. A school-based study of 69 children aged 11 to 14 years with ASD found mitochondrial respiratory chain dysfunction and hyperlactacidemia [57]. The present results showed that the lower lactic acid concentration in ASD participants was contradictory to the results of Oliveira et al. (2005). A possible explanation for this inconsistency is that the present sample sets consisted of adults with ASD. In conjunction with the reported high lactic acid concentration in children with ASD, low lactic acid concentration in adults with ASD in the present results might suggest altered regulation of mitochondrial function during development. There was no significant correlation between lactic acid level and clinical measures, therefore further studies were needed to scrutinize clinical significance of low lactic acid concentration in adults with ASD.

It should be noted that this study has several potential methodological limitations. First, the present study consisted of male subjects only. Though we were able to avoid confounding sex influence on the results, the present findings might not be generalizable to females. Second, longitudinal metabolite measurements with detailed clinical investigations from childhood will be needed to determine if the identified metabolites showed development-dependent changes. Third, the current discriminant analyses successfully classified the participants with ASD and controls using concentrations of the detected four metabolites. However, since the current discriminant analyses used the same sample sets as those for detection of the four metabolites owing to the limited sample size of the present study, the possibility for discrimination remains to be validated in a future study with independent samples. In addition, other psychiatric disorders (e.g. schizophrenia) have to be considered as a clinical control group in future studies, because the pathophysiology of these psychiatric disorders is thought to be shared [58,59]. Comparison among other psychiatric disorders may clarify the pathogenesis more clearly. Fourth, CE-TOFMS is not very effective for the separation of neutral metabolites and large molecules. Concomitant analysis of the same sample by LC-MS, GC-MS, and NMR approaches has the potential to greatly expand the coverage of target metabolites. Finally, the total sample size of the present study ($n = 53$) was relatively small, although, by conducting power analyses based on the effect sizes of the four altered metabolites detected in the current study ($d = 0.72–1.24$), the present study provides useful information about required total sample sizes in future studies ($n = 58–158$) to detect the deviated metabolites with more conservative threshold for statistical significance with power of 80% (e.g. Bonferroni correction).

In conclusion, the present study measured small ionic metabolites in peripheral blood plasma using the CE-TOFMS system, and found elevated and reduced metabolites in plasma samples from ASD participants associated with markers of oxidative stress and mitochondrial dysfunction. These metabolites could become possible biomarkers for differential diagnosis, determination of severity, and prediction of drug response, which could promote better treatment options and result in better prognosis.

Supporting Information

Table S1 Detected metabolites list. Metabolites detected in both the first and second sets, those in the first set only, and those in the second set only were listed respectively.
(DOCX)

Acknowledgments

We would like to thank Dr. Fumiko Sunaga and Dr. Miki Bundo for substantial support of sample preparation and maintenance. We also thank Dr. Kazuya Iwamoto for substantial scientific support.

Author Contributions

Conceived and designed the experiments: HK HY KK. Performed the experiments: HY HI Y. Kawakubo MK YT NI TN YA. Analyzed the data: HK HY. Contributed reagents/materials/analysis tools: SK. Wrote the paper: HK HY SK Y. Kano KK.

References

1. American Psychiatric Association (2000) Diagnostic and Statistical Manual of Mental Disorders. Fourth edition. Text revision. Washington DC: American Psychiatric Association.
2. Lord C, Risi S, Lambrecht L, Cook EH, Jr., Leventhal BL, et al. (2000) The autism diagnostic observation schedule-generic: a standard measure of social and communication deficits associated with the spectrum of autism. J Autism Dev Disord 30: 205–223.
3. Tsuchiya KJ, Matsumoto K, Yagi A, Inada N, Kuroda M, et al. (2013) Reliability and validity of autism diagnostic interview-revised, Japanese version. J Autism Dev Disord 43: 643–662.
4. Emond P, Mavel S, Aidoud N, Nadal-Desbarats L, Montigny F, et al. (2013) GC-MS-based urine metabolic profiling of autism spectrum disorders. Anal Bioanal Chem 405: 5291–5300.
5. Nakamura K, Anitha A, Yamada K, Tsujii M, Iwayama Y, et al. (2008) Genetic and expression analyses reveal elevated expression of syntaxin 1A (STX1A) in high functioning autism. Int J Neuropsychopharmacol 11: 1073–1084.
6. Quinones MP, Kaddurah-Daouk R (2009) Metabolomics tools for identifying biomarkers for neuropsychiatric diseases. Neurobiol Dis 35: 165–176.
7. Suzuki K, Nishimura K, Sugihara G, Nakamura K, Tsuchiya KJ, et al. (2010) Metabolite alterations in the hippocampus of high-functioning adult subjects with autism. Int J Neuropsychopharmacol 13: 529–534.
8. Kaiser MD, Hudac CM, Shultz S, Lee SM, Cheung C, et al. (2010) Neural signatures of autism. Proc Natl Acad Sci U S A 107: 21223–21228.
9. Iwata K, Matsuzaki H, Miyachi T, Shimmura C, Suda S, et al. (2011) Investigation of the serum levels of anterior pituitary hormones in male children with autism. Mol Autism 2: 16.
10. Zheng P, Gao HC, Li Q, Shao WH, Zhang ML, et al. (2012) Plasma metabonomics as a novel diagnostic approach for major depressive disorder. J Proteome Res 11: 1741–1748.
11. Xu XH, Huang Y, Wang G, Chen SD (2012) Metabolomics: a novel approach to identify potential diagnostic biomarkers and pathogenesis in Alzheimer's disease. Neurosci Bull 28: 641–648.
12. Pilling S, Baron-Cohen S, Megnin-Viggars O, Lee R, Taylor C (2012) Recognition, referral, diagnosis, and management of adults with autism: summary of NICE guidance. BMJ 344: e4082.
13. Yap IK, Angley M, Veselkov KA, Holmes E, Lindon JC, et al. (2010) Urinary metabolic phenotyping differentiates children with autism from their unaffected siblings and age-matched controls. J Proteome Res 9: 2996–3004.
14. Ming X, Stein TP, Barnes V, Rhodes N, Guo L (2012) Metabolic perturbance in autism spectrum disorders: a metabolomics study. J Proteome Res 11: 5856–5862.
15. Cai HL, Li HD, Yan XZ, Sun B, Zhang Q, et al. (2012) Metabolomic analysis of biochemical changes in the plasma and urine of first-episode neuroleptic-naive schizophrenia patients after treatment with risperidone. J Proteome Res 11: 4338–4350.
16. Schicho R, Shaykhutdinov R, Ngo J, Nazyrova A, Schneider C, et al. (2012) Quantitative Metabolomic Profiling of Serum, Plasma, and Urine by (1)H NMR Spectroscopy Discriminates between Patients with Inflammatory Bowel Disease and Healthy Individuals. J Proteome Res.
17. Soga T, Baran R, Suematsu M, Ueno Y, Ikeda S, et al. (2006) Differential metabolomics reveals ophthalmic acid as an oxidative stress biomarker indicating hepatic glutathione consumption. J Biol Chem 281: 16768–16776.
18. Sugimoto M, Hirayama A, Robert M, Abe S, Soga T, et al. (2010) Prediction of metabolite identity from accurate mass, migration time prediction and isotopic pattern information in CE-TOFMS data. Electrophoresis 31: 2311–2318.
19. Hirayama A, Kami K, Sugimoto M, Sugawara M, Toki N, et al. (2009) Quantitative metabolome profiling of colon and stomach cancer microenvironment by capillary electrophoresis time-of-flight mass spectrometry. Cancer Res 69: 4918–4925.
20. Soga T, Sugimoto M, Honma M, Mori M, Igarashi K, et al. (2011) Serum metabolomics reveals gamma-glutamyl dipeptides as biomarkers for discrimination among different forms of liver disease. J Hepatol 55: 896–905.
21. Yamasue H, Kuwabara H, Kawakubo Y, Kasai K (2009) Oxytocin, sexually dimorphic features of the social brain, and autism. Psychiatry Clin Neurosci 63: 129–140.
22. Baron-Cohen S, Lombardo MV, Auyeung B, Ashwin E, Chakrabarti B, et al. (2011) Why are autism spectrum conditions more prevalent in males? PLoS Biol 9: e1001081.
23. Aoki Y, Abe O, Yahata N, Kuwabara H, Natsubori T, et al. (2012) Absence of age-related prefrontal NAA change in adults with autism spectrum disorders. Transl Psychiatry 2: e178.
24. Watanabe T, Yahata N, Abe O, Kuwabara H, Inoue H, et al. (2012) Diminished medial prefrontal activity behind autistic social judgments of incongruent information. PLoS One 7: e39561.
25. Lecavalier L, Aman MG, Scahill L, McDougle CJ, McCracken JT, et al. (2006) Validity of the autism diagnostic interview-revised. Am J Ment Retard 111: 199–215.
26. Matsuoka K, Uno M, Kasai K, Koyama K, Kim Y (2006) Estimation of premorbid IQ in individuals with Alzheimer's disease using Japanese ideographic script (Kanji) compound words: Japanese version of National Adult Reading Test. Psychiatry Clin Neurosci 60: 332–339.
27. Matsuoka K, Kim Y (2006) Japanese Adult Reading Test. Tokyo: Shinko-Igaku Publishers.
28. Wechsler D (1981) Wechsler Adult Intelligence Scale-Revised. New York: Psychological Corporation.
29. Wechsler D (1997) Wechsler Adult Intelligence Scale, 3rd edition. San Antonio: The Psychological Corporation.
30. Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, et al. (1998) The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J Clin Psychiatry 59 Suppl 20: 22–33;quiz 34–57.
31. Ooga T, Sato H, Nagashima A, Sasaki K, Tomita M, et al. (2011) Metabolomic anatomy of an animal model revealing homeostatic imbalances in dyslipidaemia. Mol Biosyst 7: 1217–1223.
32. Baran R, Kochi H, Saito N, Suematsu M, Soga T, et al. (2006) MathDAMP: a package for differential analysis of metabolite profiles. BMC Bioinformatics 7: 530.
33. Ohashi Y, Hirayama A, Ishikawa T, Nakamura S, Shimizu K, et al. (2008) Depiction of metabolome changes in histidine-starved Escherichia coli by CE-TOFMS. Mol Biosyst 4: 135–147.
34. Johannesen JK, O'Donnell BF, Shekhar A, McGrew JH, Hetrick WP (2012) Diagnostic Specificity of Neurophysiological Endophenotypes in Schizophrenia and Bipolar Disorder. Schizophr Bull.
35. Delwing D, Bavaresco CS, Wyse AT (2008) Protective effect of nitric oxide synthase inhibition or antioxidants on brain oxidative damage caused by intracerebroventricular arginine administration. Brain Res 1193: 120–127.
36. Zoroglu SS, Yurekli M, Meram I, Sogut S, Tutkun H, et al. (2003) Pathophysiological role of nitric oxide and adrenomedullin in autism. Cell Biochem Funct 21: 55–60.
37. Shimmura C, Suda S, Tsuchiya KJ, Hashimoto K, Ohno K, et al. (2011) Alteration of plasma glutamate and glutamine levels in children with high-functioning autism. PLoS One 6: e25340.
38. Macciardi F, Lucca A, Catalano M, Marino C, Zanardi R, et al. (1990) Amino acid patterns in schizophrenia: some new findings. Psychiatry Res 32: 63–70.
39. Talkowski ME, Rosenfeld JA, Blumenthal I, Pillalamarri V, Chiang C, et al. (2012) Sequencing chromosomal abnormalities reveals neurodevelopmental loci that confer risk across diagnostic boundaries. Cell 149: 525–537.
40. Kim HW, Cho SC, Kim JW, Cho IH, Kim SA, et al. (2009) Family-based association study between NOS-I and -IIA polymorphisms and autism spectrum disorders in Korean trios. Am J Med Genet B Neuropsychiatr Genet 150B: 300–306.
41. O'Donovan MC, Craddock N, Norton N, Williams H, Peirce T, et al. (2008) Identification of loci associated with schizophrenia by genome-wide association and follow-up. Nat Genet 40: 1053–1055.
42. Saransaari P, Oja SS (2000) Taurine and neural cell damage. Amino Acids 19: 509–526.
43. Moreno-Fuenmayor H, Borjas L, Arrieta A, Valera V, Socorro-Candanoza L (1996) Plasma excitatory amino acids in autism. Invest Clin 37: 113–128.
44. Geier DA, Kern JK, Garver CR, Adams JB, Audhya T, et al. (2009) A prospective study of transsulfuration biomarkers in autistic disorders. Neurochem Res 34: 386–393.
45. Kern JK, Geier DA, Adams JB, Garver CR, Audhya T, et al. (2011) A clinical trial of glutathione supplementation in autism spectrum disorders. Med Sci Monit 17: CR677–682.
46. Tu WJ, Chen H, He J (2012) Application of LC-MS/MS analysis of plasma amino acids profiles in children with autism. J Clin Biochem Nutr 51: 248–249.
47. Good P (2011) Does fever relieve autistic behavior by improving brain blood flow? Neuropsychol Rev 21: 66–67.
48. Good P (2011) Do salt cravings in children with autistic disorders reveal low blood sodium depleting brain taurine and glutamine? Med Hypotheses 77: 1015–1021.
49. Meister A (1991) Glutathione deficiency produced by inhibition of its synthesis, and its reversal; applications in research and therapy. Pharmacol Ther 51: 155–194.
50. Pederzolli CD, Sgaravatti AM, Braum CA, Prestes CC, Zorzi GK, et al. (2007) 5-Oxoproline reduces non-enzymatic antioxidant defenses in vitro in rat brain. Metab Brain Dis 22: 51–65.

51. Pederzolli CD, Mescka CP, Zandona BR, de Moura Coelho D, Sgaravatti AM, et al. (2010) Acute administration of 5-oxoproline induces oxidative damage to lipids and proteins and impairs antioxidant defenses in cerebral cortex and cerebellum of young rats. Metab Brain Dis 25: 145–154.
52. Rossignol DA, Frye RE (2012) Mitochondrial dysfunction in autism spectrum disorders: a systematic review and meta-analysis. Mol Psychiatry 17: 290–314.
53. Fillano JJ, Goldenthal MJ, Rhodes CH, Marin-Garcia J (2002) Mitochondrial dysfunction in patients with hypotonia, epilepsy, autism, and developmental delay: HEADD syndrome. J Child Neurol 17: 435–439.
54. Filipek PA, Juranek J, Smith M, Mays LZ, Ramos ER, et al. (2003) Mitochondrial dysfunction in autistic patients with 15q inverted duplication. Ann Neurol 53: 801–804.
55. Correia C, Coutinho AM, Diogo L, Grazina M, Marques C, et al. (2006) Brief report: High frequency of biochemical markers for mitochondrial dysfunction in autism: no association with the mitochondrial aspartate/glutamate carrier SLC25A12 gene. J Autism Dev Disord 36: 1137–1140.
56. Giulivi C, Zhang YF, Omanska-Klusek A, Ross-Inta C, Wong S, et al. (2010) Mitochondrial dysfunction in autism. JAMA 304: 2389–2396.
57. Oliveira G, Diogo L, Grazina M, Garcia P, Ataide A, et al. (2005) Mitochondrial dysfunction in autism spectrum disorders: a population-based study. Dev Med Child Neurol 47: 185–189.
58. Weiss LA, Shen Y, Korn JM, Arking DE, Miller DT, et al. (2008) Association between microdeletion and microduplication at 16p11.2 and autism. N Engl J Med 358: 667–675.
59. McCarthy SE, Makarov V, Kirov G, Addington AM, McClellan J, et al. (2009) Microduplications of 16p11.2 are associated with schizophrenia. Nat Genet 41: 1223–1227.