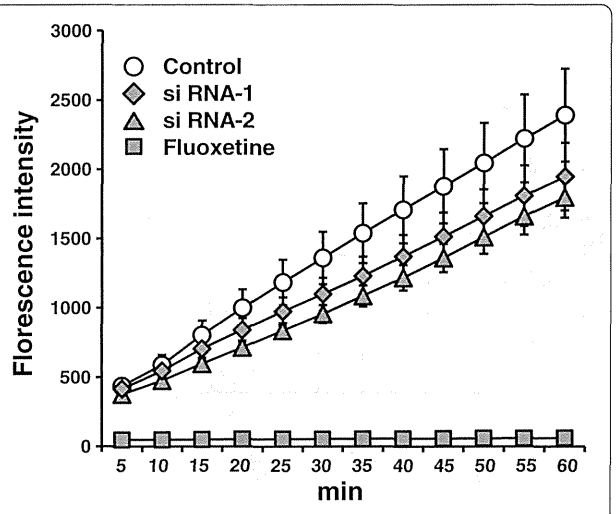

Figure 4 NSF knockdown results in decreased uptake function of SERT in HEK293-hSERT cells. Fluorescent substrate uptake activity was significantly decreased in HEK293-hSERT cells transfected with siRNAs targeting specific NSF sequences, siRNA-1 (♦) and siRNA-2 (▲), compared with negative control (o) (control vs siRNA-1 $P < 0.01$, and control vs siRNA-2 $P < 0.001$, one-way repeated measures ANOVA with Tukey's *post hoc* test). Nonspecific uptake was determined in the presence of 10 μM fluoxetine (■). Data are expressed as a percentage of the control level. Each point corresponds to the mean ± standard deviation, $n = 8$. siRNA, small interfering RNA.

patients were significantly lower than that in controls ($P = 0.0011$, Mann–Whitney *U* test) (Figure 8B). Moreover, there was a significantly negative correlation between *NSF* expression and ADI-R Domain A score, which quantified impairment in social interaction, in individuals with ASD ($r_s = 0.131$, $P = 0.0498$, Spearman's rank correlation coefficient test) (Figure 8C). There were no significant correlations between *NSF* expression levels and levels of *SLC6A4* and any other symptom profile or clinical variables (data not shown).

Discussion

In this study, NSF was identified as a novel SERT-binding protein interacting with the N-terminal region of SERT. NSF knockdown resulted in decreased membrane expression of SERT and decreased uptake of substrate. These results clearly show that NSF modulates SERT membrane trafficking, which is consistent with its uptake function. An immunoprecipitation assay using mouse brain and immunocytochemistry of cultured mouse raphe neurons clearly indicated that SERT–NSF complexes were formed under physiological conditions *in vivo*. In addition, a study of post-mortem brains revealed that the *SLC6A4* expression level was not affected in subjects with autism, but the *NSF* expression level in the raphe region tended to be decreased; however, this potential trend is not statistically significant. In lymphocytes, the *SLC6A4* expression level was also unchanged, but the *NSF* expression level was significantly decreased in subjects with ASD and correlated with the severity of clinical symptoms.

N-ethylmaleimide-sensitive factor functions and protein binding

NSF is a homohexameric ATPase [61,62], which is an essential component of the protein machinery responsible for various membrane fusion events, including intercisternal Golgi protein transport and the exocytosis of synaptic vesicles [63]. NSF binds to soluble NSF attachment protein–receptor (SNARE) complexes and mediates the recycling of spent SNARE complexes for subsequent rounds of membrane fusion [63,64]. While this is a major function of NSF, it also interacts with receptor proteins, such as AMPA, β2 adrenergic and $GABA_A$ receptors, and is thought to affect their trafficking patterns or recycling [49-57]. Additionally, an interaction between NSF and arrestin 1 regulates the expression of vesicular glutamate transporter 1 and excitatory amino acid transporter 5 in the photoreceptor synapse [58]. In the present study, we found, for the first time, that NSF binds the neurotransmitter transporter SERT and regulates its function in the CNS.

Serotonin transporter forms complexes with *N*-ethylmaleimide-sensitive factor *in vivo*

Several putative SERT-binding proteins have been reported [21-32]. However, almost all of these were identified using the yeast two-hybrid system and little is known regarding whether any of these proteins bind to SERT and regulate its function in the mammalian brain. Also, little is known about the involvement of these proteins in autism [65,66]. Therefore, in this study, we used a pull-down system together with mouse brain tissue to identify novel SERT-binding proteins. Moreover, we used the tcTPC method, which is an innovative tool for studying proteins in living tissues [40]. This method enabled us to preserve protein–protein interactions occurring under physiological conditions. This cross-linking also preserves membrane protein assemblies, which are degraded by solubilizing detergents. For instance, whereas most detergents cause rapid disintegration of the γ-secretase complex, three of four known components of the complex were purified and identified from harsh detergents and a high salt concentration by tcTPC [40]. Because NSF was not co-immunoprecipitated with SERT from non-tcTPC-treated brains (Figure 6A), it is likely that SERT–NSF complexes are sensitive to solubilizing detergents. The discovery of complexes including NSF and SERT, which form in the mammalian brain under physiological conditions, in the present study, is important from the viewpoint of their potential involvement in the pathophysiology of disorders such as autism. It is not yet

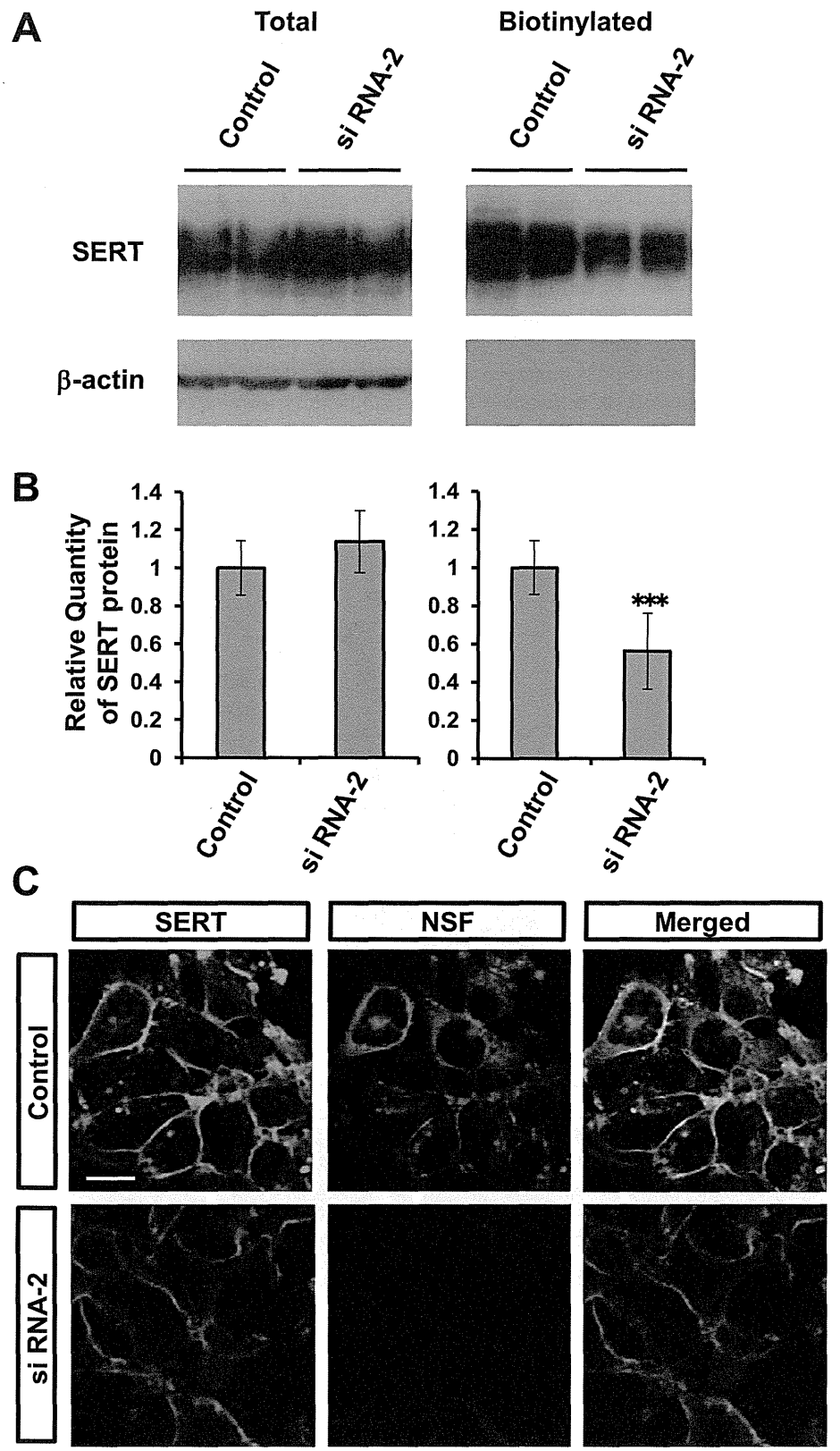

Figure 5 (See legend on next page.)

(See figure on previous page.)
Figure 5 NSF knockdown results in decreased SERT expression at the plasma membrane in HEK293-hSERT cells. (A) Biotinylation experiments in HEK293-hSERT cells transfected with siRNA-2 targeting a specific NSF sequence or negative control. Transfected cells were incubated with sulfo-NHS-SS-biotin, and labeled proteins were analyzed by immunoblotting using anti-SERT antibodies. **(B)** Quantitation of relative band densities for SERT was performed by scanning densitometry. Data are expressed as the means ± standard deviation, $n = 6$ to 9. $^{***}P < 0.001$ vs negative control (two-tailed unpaired *t*-test). **(C)** Double immunocytochemical staining for SERT (green) and NSF (red) in HEK293-hSERT cells transfected with control siRNA (upper panels) and siRNA for NSF (siRNA-2, lower panels). Scale bar: 10 μm. Results are representative of three independent experiments. NSF, *N*-ethylmaleimide-sensitive factor; SERT, serotonin transporter; siRNA, small interfering RNA.

clear whether NSF binds SERT directly or indirectly. In addition, the band for the SERT–NSF complex was smeared, suggesting that multiple types of SERT–NSF complexes exist. It is possible that SERT interacts with NSF through other proteins. Indeed, it is possible that $GABA_A$ receptors interact with NSF via $GABA_A$ receptor-associated protein, and regulate its intracellular distribution and recycling [56,67]. Detailed analyses of these SERT–NSF complexes are needed.

Serotonin transporter and *N*-ethylmaleimide-sensitive factor expressions in autism

Recently, Nakamura and colleagues reported that the levels of SERT based on its radioligand binding were significantly lower throughout the brain in autistic individuals compared with controls [17]. On the other hand, Azmitia and colleagues reported increased immunoreactivity to a SERT antibody of serotonin axons in the postmortem cortex of autism patients [18]. Our results show

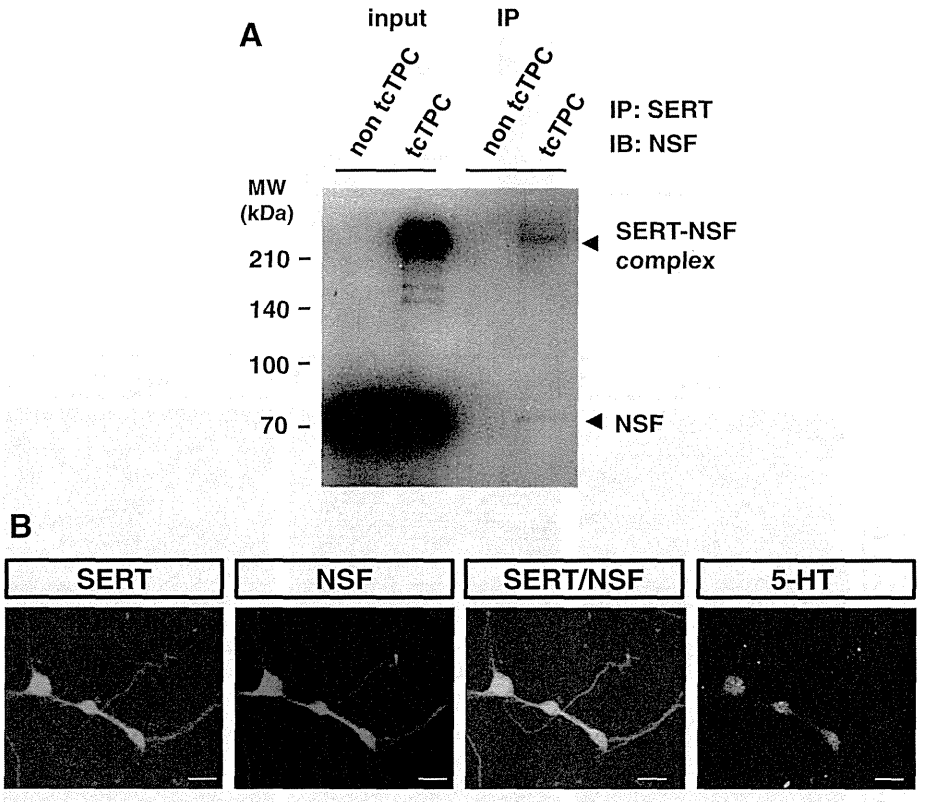

Figure 6 NSF interacts with SERT *in vivo*. (A) Interaction of SERT with NSF in mouse brain. Immunoblot of total proteins from non-tcTPC- and tcTPC-treated mouse brains (as input, lanes 1 and 2, respectively). Proteins from non-tcTPC- or tcTPC-treated mouse brains were immunoprecipitated with SERT antibodies (lane 3 and 4), and the resulting immunoblot was probed for NSF. In immunoprecipitated samples using tcTPC-treated mouse brains, SERT–NSF complexes and free NSF were identified (lane 4). Results are representative of three independent experiments. **(B)** NSF co-localizes with SERT in primary cultures of mouse raphe nuclei neurons. Triple immunocytochemical staining for SERT (green), NSF (red) and 5-HT (blue) in primary cultures of mouse raphe nuclei neurons. The third panel (merged) shows that NSF co-localizes with SERT primary cultures of mouse raphe nuclei neurons. These neurons are 5-HT-positive serotonergic neurons (as shown in the fourth panel). Scale bars: 10 μm. Results are representative of three independent experiments. 5-HT, 5-hydroxytryptamine; IB, immunoblotting; IP, immunoprecipitation; MW, molecular weight; NSF, *N*-ethylmaleimide-sensitive factor; SERT, serotonin transporter; tcTPC, time-controlled transcardiac perfusion cross-linking.

Table 2 Information for post-mortem brain tissues

Sample ID	Diagnosis	Age (years)	Gender	Post-mortem interval (hours)	Race	Cause of death
1065	Control	15	M	12	Caucasian	Multiple injuries
1297	Control	15	M	16	African-American	Multiple injuries
1407	Control	9	F	20	African-American	Asthma
1541	Control	20	F	19	Caucasian	Head injuries
1708	Control	8	F	20	African-American	Asphyxia, multiple injuries
1790	Control	14	M	18	Caucasian	Multiple injuries
1793	Control	12	M	19	African-American	Drowning
1860	Control	8	M	5	Caucasian	Cardiac arrhythmia
4543	Control	29	M	13	Caucasian	Multiple injuries
4638	Control	15	F	5	Caucasian	Chest injuries
4722	Control	14	M	16	Caucasian	Multiple injuries
797	Autism	9	M	13	Caucasian	Drowning
1638	Autism	20	F	50	Caucasian	Seizure
4231	Autism	8	M	12	African-American	Drowning
4721	Autism	8	M	16	African-American	Drowning
4899	Autism	14	M	9	Caucasian	Drowning
5000	Autism	27	M	8.3	NA	NA
6294	Autism	16	M	NA	NA	NA

F, female; M, male; NA, not available.

that, at least, *SLC6A4* mRNA expression is normal in the raphe region of post-mortem brains from subjects with autism. Our findings and previous results lead us to two suggestions. First, although the transcription of *SLC6A4* is normal in subjects with autism, the level of SERT protein at the pre-synaptic membrane is decreased because of an impairment of the trafficking system. Second, SERT protein that is not delivered to the pre-synaptic membrane accumulates in axon fibers in the brains of subjects with autism. In lymphocytes, we found that *SLC6A4* expression was not changed in subjects with ASD. In contrast with our finding, Hu *et al.* previously reported that there was a significant decrease in the expression in the more severely affected twin for autistic twin pairs studied using lymphoblastoid cell lines [68]. This study used lymphoblastoid cell lines, not lymphocytes, from only three sets of discordant twins, and *SLC6A4* expression was not compared with normal controls [68]. These differences may be the cause of the discrepancies between the present study and that report.

We found that the *NSF* expression levels tended to decrease in the raphe region of post-mortem brains from subjects with autism; however, this trend was not statistically significant ($n = 11$ control and $n = 7$ autism). Further studies with larger numbers of post-mortem brains are needed to clarify *NSF* expression status in the brain of autism patients. In lymphocytes, we found, for the first time, that *NSF* expression was significantly lower in subjects with ASD and lower *NSF* expression correlated with the severity of impairments in social interaction. Our findings suggest that peripheral NSF mRNA levels may serve as a reliable peripheral biological marker of ASD.

Sullivan *et al.* reported that the expression levels of a number of biologically relevant genes are statistically similar between lymphocytes and CNS tissues including the brain, and suggested that the cautious and thoughtful

Table 3 Demographic data associated with raphe brain-tissue samples

	Control ($n = 11$)	Autism ($n = 7$)	*P* value
Age (years) (range)	14.45 (8–29)	14.57 (8–27)	NS[a]
Race, *n* (%)	Caucasian 7 (63.6), African-American 4 (36.4)	Caucasian 3 (42.9), African-American 2 (28.6), NA 2 (28.6)	NS[b]
Gender, *n* (%)	Male 7 (63.6), Female 4 (36.4)	Male 6 (85.7), Female 1 (14.3)	NS[b]
Post-mortem interval (hours) (range)	14.82 (5–20)	18.05 (8.3-50)	NS[a]

[a]Derived from Mann–Whitney *U* test, [b]Derived from Fisher's exact test.
NA, not available; NS, not significant.

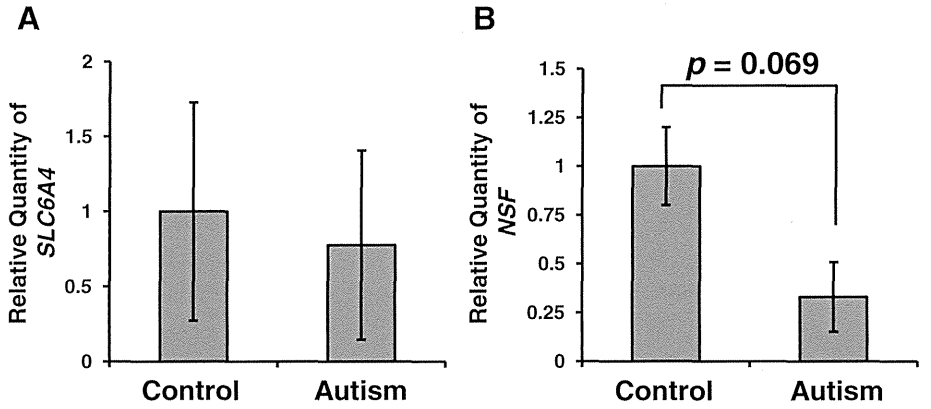

Figure 7 *SLC6A4* and *NSF* expression in the raphe region of post-mortem brains. Comparison of *SLC6A4* **(A)** and *NSF* **(B)** expression levels in the raphe region of post-mortem brains from control and autistic subjects. The Mann–Whitney *U* test was used to compare gene expression levels between autism and control groups. Data are presented as the means ± standard error of the mean. $n = 11$ control and $n = 7$ autism. NSF, *N*-ethylmaleimide-sensitive factor.

use of lymphocytic gene expression may be a useful surrogate for gene expression in the CNS when it has been determined that the gene is expressed in both [69]. In support of previous findings [59,60], the expressions of *SLC6A4* and *NSF* were detected in both tissues, and it is likely that levels of *SLC6A4* and *NSF* in the peripheral lymphocytes may reflect the levels in post-mortem brains, although further study is needed.

The serotonin transporter–*N*-ethylmaleimide-sensitive factor binding and implications for pathophysiology in autism

Sanyal and Krishnan reported a lethal mutation in the *Drosophila* homolog of NSF [70]. Intriguingly, mutant adult survivors show abnormal seizure-like paralytic behavior [70]. Additionally, Matveeva and colleagues reported that decreased production of NSF is associated with epilepsy in rats [71]. Importantly, a high rate of co-occurrence of autism and epilepsy has been described [72-76]. Approximately 30% of children with autism have epilepsy and 30% of children with epilepsy have autism [77]. Interestingly, an abnormal status for SERT has been reported in epileptic patients as follows. Autoradiography experiments have revealed that the temporal neocortex surrounding the epileptic focus of patients with mesial temporal lobe epilepsy presents diminished SERT binding in all cortical layers [78]. A significant decrease was found in the SERT density in the platelet membranes from epileptic patients having undergone an epileptic seizure [79,80]. Additionally, it has been shown that epileptic patients who had been treated with inhibitors of serotonin reuptake, such as fluoxetine and citalopram, in addition to their ongoing antiepileptic therapy displayed remarkable clinical improvements [81,82]. This indirect evidence implies the relationship between SERT and NSF in neurological disorders, such

Table 4 Demographic data associated with lymphocyte samples

	Control ($N = 30$)[b]	Autism ($N = 30$)[b]	*P* value
Age (years)	11.1 ± 2.3 (6–16)	11.6 ± 2.7 (7–16)	NS[a]
ADI-R			
Domain A score		20.0 ± 5.3 (10–30)	
Domain BV score		14.3 ± 4.0 (8–23)	
Domain C score		8.5 ± 3.4 (3–9)	
Domain D score		3.1 ± 1.1 (1–5)	
WISC-III			
Verbal IQ	99.1 ± 10.3 (77–120)	90.4 ± 28.7 (44–153)	NS[a]
Performance IQ	97.0 ± 10.2 (76–114)	89.8 ± 22.9 (47–131)	NS[a]
Full-scale IQ	97.8 ± 9.5 (82–115)	89.0 ± 26.9 (42–140)	NS[a]

[a]Derived from Mann–Whitney *U* test; [b]values are expressed as mean ± standard deviation (range).
ADI-R, Autism Diagnostic Interview-Revised; IQ, intelligence quotient; NS, not significant; WISC-III, the third edition of the Wechsler Intelligence Scale for Children.

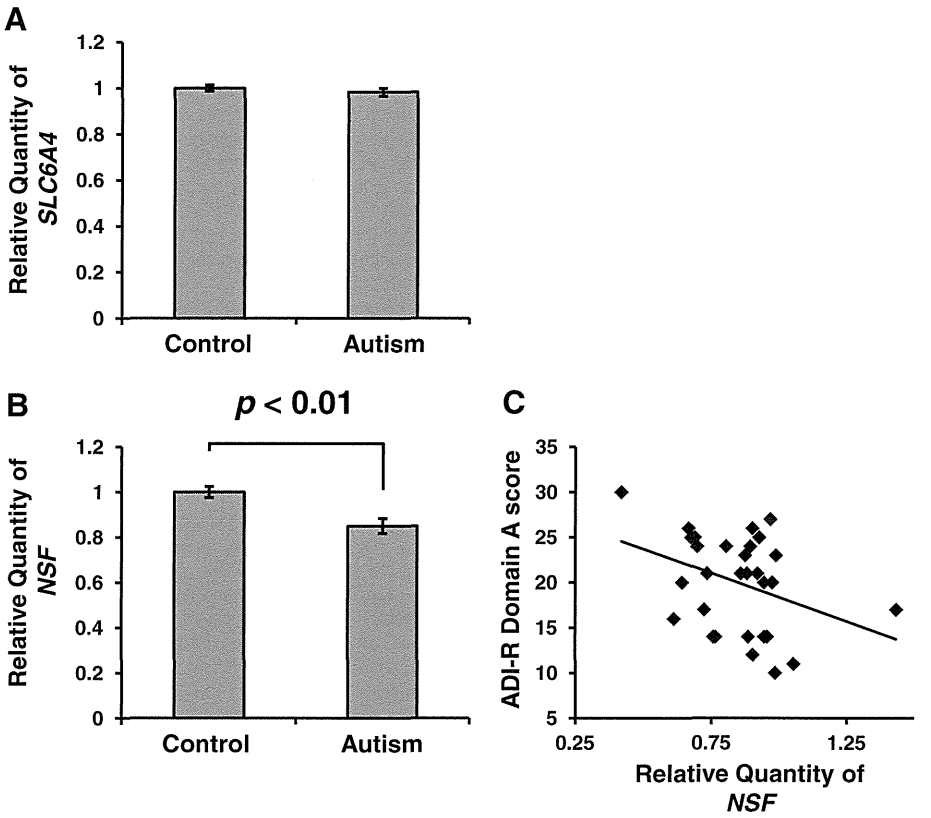

Figure 8 *SLC6A4* and *NSF* expression in lymphocytes. Comparison of *SLC6A4* **(A)** and *NSF* **(B)** expression levels in lymphocytes from control and ASD subjects. The Mann–Whitney *U* test was used to compare gene expression levels between autism and control groups. Data are presented as the means ± standard error of the mean. $n = 30$ control and $n = 30$ autism. The *NSF* expression levels in ASD patients were significantly lower than in controls ($P = 0.0011$). **(C)** Correlation between lymphocyte *NSF* expression levels and Autism Diagnostic Interview-Revised (ADI-R) domain A scores in autistic subjects. There was a negative correlation between lymphocyte *NSF* expression levels and ADI-R domain A scores ($r_s = 0.131$, $P = 0.0498$), $n = 30$ autism. ADI-R, autism diagnostic interview-revised; NSF, *N*-ethylmaleimide-sensitive factor.

as autism. Further investigations of the status of SERT–NSF binding in the brain of autism patients would be useful for understanding the mechanisms that underlie autism. In addition, an animal model, such as an NSF conditional knockout mouse, would be a useful tool for understanding the mechanisms that underlie ASD.

As mentioned above, NSF interacts with neurotransmitter receptors such as AMPA, β2 adrenergic and $GABA_A$ receptors, and regulates the membrane trafficking and recycling of these receptors [49-57]. An abnormal status of many of these receptors has been reported in autism. Binding of $GABA_A\alpha5$ and its radioligand was significantly lower throughout the brains of participants with ASDs compared with controls [83]. The mRNA levels of AMPA receptor were significantly increased in the post-mortem cerebellum of autistic individuals, while the receptor density was slightly decreased in people with autism [84]. It is possible that NSF may contribute to the pathophysiology of autism through these known interactions with relevant molecules.

Conclusions

This study showed that dysfunctional trafficking of SERT mediated by NSF may be linked with the pathophysiology of autism. The identification of SERT-binding proteins provides new opportunities not only to dissect the accessory components involved in SERT function and regulation, but also to elucidate the pathophysiology of psychiatric disorders or developmental disorders, such as autism. Future studies should examine the pathophysiological implications of SERT–NSF interactions for autism.

Additional files

Additional file 1: Figure S1. N-tail-specific binding of syntaxin-1A to SERT was confirmed by Western blot analysis.

Additional file 2: Figure S2. SERT is transported to the plasma membrane in HEK293-hSERT cells. **(A, B)** Double immunocytochemical staining for SERT (green) and the membrane maker cadherin (red) in HEK293-hSERT cells. **(C)** SERT was mainly co-localized with the membrane

maker (cadherin) (merged). Scale bar: 10 μm. Results are representative of three independent experiments.

Additional file 3: Figure S3. Transfection efficacy of siRNA in HEK293-hSERT cells. We determined the proportion of siRNA-transfected HEK293-hSERT cells using a commercially available fluor-oligo kit (TYE 563 DS, Integrated DNA Technologies). The proportion of siRNA-transfected cells was 90%. Upper panels show untreated cells and lower panels show red fluorescent oligo-transfected cells. Left panels show phase-contrast images and right panels show the images obtained by fluorescence microscopy (excitation: 546 nm, emission: 590 nm). Scale bar: 50 μm. Results are representative of three independent experiments.

Additional file 4: Figure S4. CBB staining of membranes from biotinylated fractions. Biotinylation experiments in HEK293-hSERT cells transfected with siRNA-2 targeting a specific NSF sequence or negative control. Transfected cells were incubated with sulfo-NHS-SS-biotin. After Western blot analysis, the membrane was stained with CBB as a protein-loading control.

Additional file 5: Figure S5. Confirmation of tcTPC efficacy. **(A)** Western blotting of total proteins from non-tcTPC- or tcTPC-treated mouse brains (lanes 1 and 2, respectively) using anti-SERT antibodies. Results are representative of three independent experiments. It was confirmed that SERT-containing cross-linked complexes were retained by the tcTPC method (lane 2). **(B)** Proteins from non-tcTPC- or tcTPC-treated mouse brains were immunoprecipitated with rat immunoglobulin G (IgG) as a negative control (lanes 1 and 5) and SERT antibodies (lanes 2 to 4 and 6 to 8), and the resulting Western blot was probed for SERT. In immunoprecipitated samples using tcTPC-treated mouse brains, SERT-containing cross-linked complexes were identified (lanes 6 to 8) in a dose-dependent manner. Results are representative of three independent experiments.

Abbreviations

5-HT: 5-hydroxytryptamine; ADI-R: autism diagnostic interview-revised; ANOVA: analysis of variance; ASD: autism spectrum disorder; cDNA: complementary DNA; CNS: central nervous system; C-SERT: C-terminal domain of SERT; DMEM: Dulbecco's modified Eagle's medium; GST: glutathione S-transferase; HBSS: Hank's balanced salt solution; Hic-5: hydrogen peroxide-inducible clone 5 protein; hSERT: human serotonin transporter; IQ: intelligence quotient; LC-MS/MS: liquid chromatography-tandem mass spectrometry; MacMARCKS: macrophage myristoylated alanine-rich C kinase substrate; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; MW: molecular weight; nNOS: nitric oxide synthase; NSF: *N*-ethylmaleimide-sensitive factor; N-SERT: N-terminal domain of SERT; PBS: phosphate-buffered saline; PCR: polymerase chain reaction; PMI: post-mortem interval; PP2A: phosphatase 2A; qRT-PCR: quantitative real-time reverse-transcription-polymerase chain reaction; RT: room temperature; RT-PCR: reverse-transcription-polymerase chain reaction; SCAMP2: secretory carrier membrane protein 2; SCID: structured clinical interview for DSM-IV; SERT: serotonin transporter; siRNA: small interfering RNA; SLC6A4: member 4 of solute carrier family 6 (neurotransmitter transporter); SNARE: soluble NSF attachment protein–receptor; tcTPC: time-controlled transcardiac perfusion cross-linking.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

HM and TK co-designed the study. KI and HM collected blood samples; collected, analyzed and interpreted the data and prepared the manuscript. TT and SY produced the SERT antibody. KO, HT, KY and SM collected, analyzed and interpreted the data. KN recruited participants, collected blood samples and obtained post-mortem brain samples. KJT and KM collected blood samples and undertook clinical evaluations. MT recruited participants. TS recruited participants and diagnosed ASD. TK collected, analyzed and interpreted the data and prepared the manuscript. NM analyzed and interpreted the data, and prepared the manuscript. All authors read and approved the final manuscript.

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ORIGINAL ARTICLE

Evaluation of Motor Coordination in Boys with High-Functioning Pervasive Developmental Disorder Using the Japanese Version of the Developmental Coordination Disorder Questionnaire

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Abstract Children with high-functioning pervasive developmental disorder (HFPDD) often have motor coordination dysfunction. However, there is no assessment tool for screening developmental coordination disorder (DCD) in Japan, which makes it difficult to evaluate the actual motor impairments of children with HFPDD. We evaluated the motor coordination function of 54 school-age boys with HFPDD using the Japanese version of the Developmental Coordination Disorder Questionnaire (DCDQ-J). We subsequently assessed the relationship between DCDQ-J scores and the results of the Japanese version of the Autism Diagnostic Interview-Revised (ADI-R) of 48 boys. The

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total and subscale DCDQ-J scores of the boys with HFPDD were significantly lower than the population means in the same grade: 37.0 % were below 2 standard deviations for the total score, 38.9 % for control during movement, 26.0 % for fine motor/handwriting, and 37.0 % for general coordination. Furthermore, the scores of Qualitative Abnormalities in Communication in the ADI-R were negatively correlated with control during movement, fine motor/handwriting, and total scores in the DCDQ-J. This study is the first to show Japanese children with HFPDD frequently exhibit considerably poor motor coordination according to the DCDQ-J. The screening or assessment of motor dysfunction in HFPDD using assessment tools such as the DCDQ could aid the development of interventions for these underestimated problems in Japan.

Keywords High-functioning pervasive developmental disorder (HFPDD) · Developmental coordination disorder (DCD) · Developmental coordination disorder questionnaire (DCDQ) · Motor coordination dysfunction · Autism diagnostic interview-revised (ADI-R) · Questionnaire

Introduction

Clinically, children with high-functioning pervasive developmental disorder (HFPDD) often have motor coordination dysfunction, which is often referred to as "clumsiness" (Sturm et al. 2004). This motor coordination problem is applicable to Developmental Coordination Disorder (DCD) in the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR) (American Psychiatric Association 2000). From their meta-analysis, Fournier et al. (2010) conclude motor coordination deficits are a cardinal feature of autism spectrum disorders (ASD) including HFPDD. However, there is currently no assessment tool to facilitate the screening of DCD in Japan, which makes it difficult to evaluate the actual motor impairments of children with HFPDD. The Developmental Coordination Disorder Questionnaire (DCDQ) is a parent-rated scale for screening for pediatric DCD (Wilson et al. 2000, 2009). The DCDQ has already been translated into many languages, and the European Academy for Childhood Disability (EACD) guideline recommends it as the best-evaluated questionnaire (Blank et al. 2012). We recently developed the Japanese version of the DCDQ (DCDQ-J) for Japanese children and investigated its reliability and applicability as a screening tool for DCD in Japanese children (Nakai et al. 2011). Green et al. (2009) investigated the degree of movement skill impairments in children ASD using the Movement Assessment Battery for Children (M-ABC) (Henderson and Sugden 1992) and DCDQ. They report the DCDQ performs moderately well as a tool for screening possible motor difficulties in children with ASD. In the present study, we investigated the degree of motor coordination dysfunction in Japanese children with HFPDD using the DCDQ-J. We also assessed the relationships of DCDQ scores with ASD symptoms and cognitive functions using the Autism Diagnostic Interview-Revised (ADI-R) and Wechsler Intelligence Scale for Children, 3rd edition (WISC-III).

Methods

Participants

The participants of this study were drawn from 176 school-age children who were members of a nonprofit organization for families with children with PDD. The participants were diagnosed with PDD by child and adolescent psychiatrists on the basis of the DSM-IV-TR criteria. Questionnaires were sent to the parents. We collect responses from 104 respondents. The exclusion criteria were epilepsy, psychiatric disorders (e.g., depression, bipolar disorder, and schizophrenia), genetic and chromosomal disorders, and hearing and visual impairments. Comorbid disorders were assessed by clinical interview. We excluded 7 cases because of faulty answer, the cases of 16 girls, 14 cases with mental retardation, and 13 cases missing results for the full-scale IQ of the Japanese version of the WISC-III (Japanese WISC-III Publication Committee 1998). We ultimately enrolled 54 boys with HFPDD (IQ>70). The mean participant age was 11.5 years (range: 6 years 10 months to 15 years 5 months). Thirty-six participants were elementary school students: 5, 3, 8, 6, 6, and 8 in the 1st through 6th years, respectively. Eighteen boys were junior high school students: 8, 6, and 4 in the 1st, 2nd, and 3rd years, respectively. The mean full IQ was 106.6 (range: 72–146). Among the 54 participants, 15 took medications: 7 took risperidone, 4 took selective serotonin reuptake inhibitors (SSRIs), 3 took methylphenidate, 3 took anti-epileptic drugs (carbamazepine: 2, valproate: 1) as mood stabilizers, 1 took haloperidol, and 1 took alprazolam.

Forty-eight boys underwent ADI-R interviews performed by Japanese interviewers who had undergone a 3-day ADI-R training workshop in the US (Lord et al. 1994). They created a Japanese translation of the ADI-R and received permission from the original author and publisher to use it after validating it on a Japanese sample (Tsuchiya et al. 2013). According to the ADI-R scores, 39, 3, and 6 participants were diagnosed with autistic disorder, Asperger disorder, and PDD not otherwise specified.

This study was approved by the Ethics Committee of the Hamamatsu University School of Medicine. Written informed consent was obtained from all parents of the participants prior to participation.

DCDQ-J

The DCDQ is a parent-rated questionnaire designed to screen for coordination disorders in children aged 5–15 years. It comprises the following 15 items in 3 subscales: "control during movement" (CDM, 6 sub-items), "fine motor/handwriting" (FM, 4 sub-items), and "general coordination" (GC, 5 sub-items). Each item is scored on a 5-point scale based on a comparison between the child and other children as follows: "not at all like your child" (1 point), "a bit like your child" (2 points), "somewhat like your child" (3 points), "quite a bit like your child" (4 points), and "very much like your child" (5 points); higher scores indicate better coordination. We recently developed the DCDQ-J and conducted a preliminary investigation of its reliability and psychometric properties using relatively large population samples (Nakai et al. 2011). The results indicate the DCDQ-J is a useful screening tool for DCD in Japan. In the present study, we used the population mean scores of Japanese children at each school level from preschool (i.e., 5 years old) to the 3rd year of junior high school (i.e., 15 years old) (Nakai et al. 2011).

Statistical Analysis

The differences between the mean scores of the boys with HFPDD and population means of Japanese boys at each grade were compared using the Z statistic. The level of significance was set at $P<0.05$. Spearman rank correlation coefficients were calculated to evaluate the correlations of DCDQ-J with and WISC-III and ADI-R scores. PASW Statistics 18.0 (SPSS Inc.) was used for all statistical analyses.

Results

Among all boys with HFPDD, 37.0 %, 38.9 %, 26.0 %, and 37.0 % had total, CDM, FM, and GC scores below 2 standard deviations (SDs) of the population mean, respectively (Table 1). The mean total DCDQ-J scores of the boys with HFPDD were significantly lower than the mean scores of the test standardization population of Japanese boys at the same school level (Figs. 1, 2, 3 and 4). However, the CDM and FM subscale scores in the second year of elementary school and the FM subscale score in the third year of junior high school were not significantly different between the boys with HFPDD and the population mean. Our previous study revealed that in Japanese children, the total, CDM, and FM scores increase linearly with increasing grade while GC scores exhibit non-linear changes (Nakai et al. 2011). In contrast, in the present study, the total, CDM, and FM scores of the boys with HFPDD remained low in all grades, except FM scores in the third grade of junior high school (Fig. 3). None of the DCDQ-J scores of the 15 boys who took medication differed significantly from those of other boys without medication.

The correlations of the subscale and total DCDQ-J scores with Verbal IQ (VIQ; $n=50$), Performance IQ (PIQ; $n=50$), and Full-scale IQ (FIQ; $n=54$) in the WISC-III as well as the ADI-R domain scores ($n=48$) are shown in Table 2. However, no correlations were found between the subscale and total DCDQ-J scores with VIQ or FIQ in the WISC-III. However, the PIQ score of the WISC-III was moderately correlated with the FM score in the DCDQ-J ($r=0.30$, $P=0.034$). Furthermore, the score of Qualitative Abnormalities in Communication in the ADI-R was moderately negatively

Table 1 Number (percentage) of participants with total and DCDQ-J subscale scores according to SD

−3 SD[a]	−3–−2 SD	−2–−1.5 SD	−1.5–−1 SD	−1–1 SD	1 SD+	
CDM[b]	4 (7.4 %)	17 (31.5 %)	7 (13.0 %)	8 (14.8 %)	17 (31.5 %)	1 (1.9 %)
FM[c]	1 (1.9 %)	13 (24.1 %)	9 (16.7 %)	6 (11.1 %)	23 (42.6 %)	2 (3.7 %)
GC[d]	0 (0.0 %)	20 (37.0 %)	13 (24.1 %)	6 (11.1 %)	14 (25.9 %)	1 (1.9 %)
Total[e]	2 (3.7 %)	18 (33.3 %)	14 (25.9 %)	5 (9.3 %)	14 (25.9 %)	1 (1.9 %)

[a] Standard deviation

[b] Control during movement

[c] Fine motor/handwriting

[d] General coordination

[e] Total score

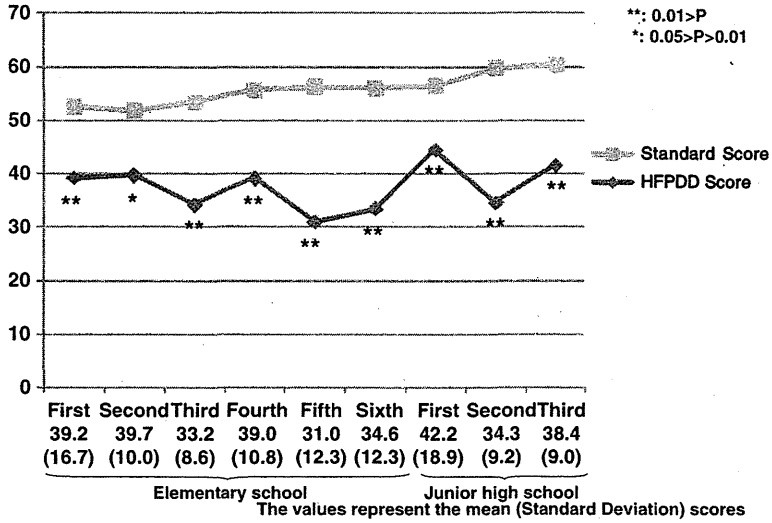

Fig. 1 Mean total DCDQ-J score at each school level. Values represent mean (SD) (** $P<0.01$, * $0.05>P>0.01$)

correlated with the CDM ($r=-0.32$, $P=0.031$), FM ($r=-0.31$, $P=0.034$), and total scores ($r=-0.35$, $P=0.016$) in the DCDQ-J.

Discussion

In this study, almost all subscale and the total DCDQ-J scores of Japanese boys with HFPDD were significantly lower than the standard scores of boys at the same school level. However,.the CDM and FM scores in the second year of elementary school and

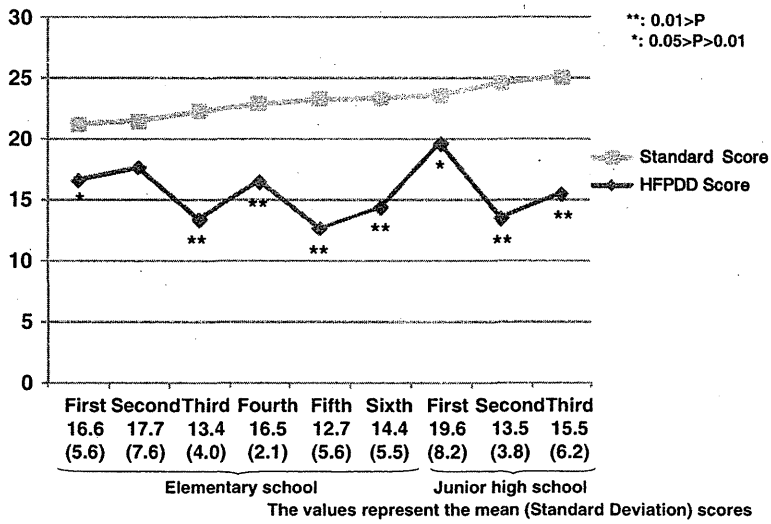

Fig. 2 Mean CDM DCDQ-J score at each school level. Values represent mean (SD) (** $P<0.01$, * $0.05>P>0.01$)

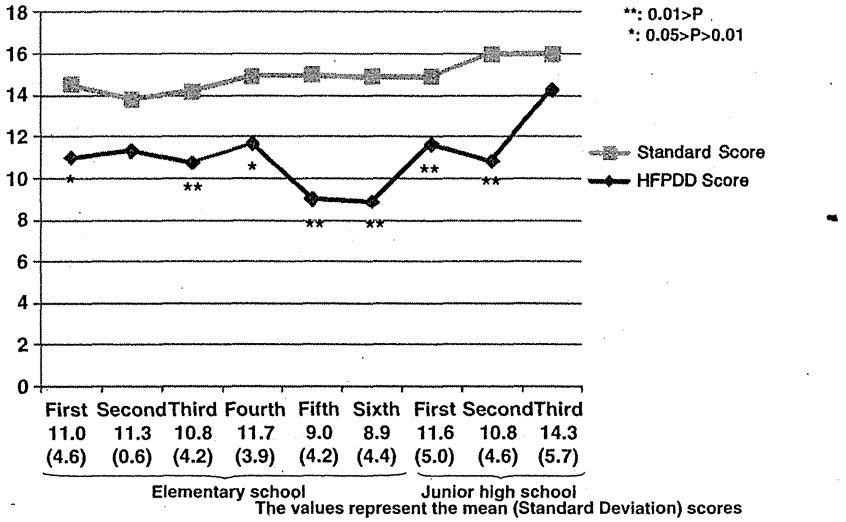

Fig. 3 Mean FM DCDQ-J score at each school level. Values represent mean (SD) (** $P<0.01$, * $0.05>P>0.01$)

the FM score in the third year of junior high school were not significantly different between the HFPDD boys and population means, because the sample size might have been too small.

Social and communication impairments are the core features of PDD. Criterion C of DCD in the DSM-IV-TR specifies the disturbance does not meet the criteria for PDD (American Psychiatric Association 2000). However, clumsiness in PDD is often clinically recognized by parents or practitioners (Sturm et al. 2004). Movement problems are common in children with autism, Asperger syndrome, or PDD not otherwise specified (Ghaziuddin and Butler 1998). Moreover, a recent meta-analysis

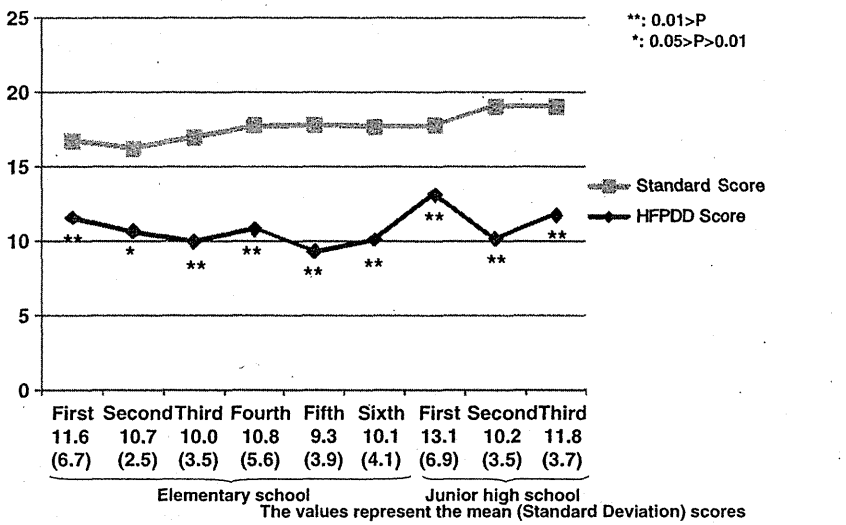

Fig. 4 Mean GC DCDQ-J score at each school level. Values represent mean (SD) (** $P<0.01$, * $0.05>P>0.01$)

Table 2 Correlation between DCDQ-J scores, and WISC-III and ADI-R scores

WISC-III			ADI-R			
VIQ	PIQ	FIQ	Social interaction[a]	Communication[b]	Stereotyped behavior[c]	
[d]CDM	0.07	0.22	0.14	−0.06	−0.32*	−0.27
[e]FM	0.04	0.30*	0.19	−0.09	−0.31*	0.03
[f]GC	−0.18	0.13	−0.03	−0.11	−0.25	−0.02
[g]Total	−0.02	0.23	0.11	−0.09	−0.35*	−0.05

* $0.05>P>0.01$

[a] Qualitative abnormalities in reciprocal social interaction

[b] Qualitative abnormalities in communication

[c] Restricted, repetitive, and stereotyped patterns of behavior

[d] Control during movement

[e] Fine Motor/handwriting

[f] General coordination

[g] Total score

demonstrates motor coordination deficits are a cardinal feature of ASD (Fournier et al. 2010). Indeed, the Australian scale for Asperger syndrome contains 2 items for movement skills such as poor motor coordination, catching a ball, gait, and running (Garnett and Attwood 1998). Furthermore, Gillberg's Criteria for Asperger's Disorder also include motor clumsiness (Gillberg and Gillberg 1989). Children with HFPDD frequently exhibit poor motor coordination from both clinical and scientific perspectives. The present study is the first report using the DCDQ and showing Japanese children with HFPDD frequently have motor coordination impairments. Furthermore, the study provides some evidence supporting the validity of the DCDQ-J for use in Japanese populations, because the results are corroborated by those in non-Japanese populations.

Coordination dysfunction is likely to induce delayed motor development, clumsiness, and poor posture. Children with coordination impairments also tend to exhibit delayed acquisition of skills for performing daily living and school activities (Missiuna et al. 2006; Polatajko and Cantin 2005); therefore, such children tend to be less eager, pay less attention to these activities, and tire more easily. However, such motor coordination problems are likely to be mistakenly attributed to a lack of parental discipline or poor motivation of the child. If parents and teachers continually use inappropriate approaches to such problems, the child may develop emotional difficulties or self-distrust (Piek et al. 2006; Skinner and Piek 2001), which can strain their relationships with the child (Stephanson and Chesson 2008; Rivard et al. 2007). Therefore, support provided to children with HFPDD should include attention to coordination and motor problems in an effort to deliver comprehensive treatment. In addition, the DSM-5 (American Psychiatric Association 2013), which allows the co-occurrence of DCD and ASD, is more clinically applicable in this aspect than the DSM-IV-TR.

The present results show fewer boys with HFPDD had FM difficulties than difficulties in the other DCDQ-J subscales. These findings might be clinically attributable to

the fact that the FM questions in the DCDQ-J are limited to inquiring if a child is able or unable to write and use scissors. Meanwhile, the results of 2 meta-analyses suggest motor coordination deficits such as gait and balance, arm motor function, movement planning, and handwriting are more prevalent in children with PDD than normal children (Fournier et al. 2010; Kushki et al. 2011).

Although children with an IQ<70 are reported to have greater impairment in movement skills than those with an IQ>70, there was no correlation between the subscale or total DCDQ-J scores with FIQ in the WISC-III in boys with HFPDD in the present study. The moderate but significant correlation between PIQ in the WISC-III and the FM score in the DCDQ-J is thought to be attributable to the fact that some subtests in the WISC-III, such as Object Assembly, Mazes, and Picture Arrangement, involve fine motor coordination or manipulation. Meanwhile, the levels of impairment in CDM, FM, and total DCDQ-J scores were significantly correlated with the score of Qualitative Abnormalities in Communication in the ADI-R. Dziuk et al. (2007) report the level of impairment in praxis performance is significantly correlated with total the Autism Diagnosis Observation Schedule-Generic (ADOS-G) score. They state this suggests the impaired performance of skilled gestures may contribute to impaired social interaction and communication in autism. Moreover, dyspraxia may be a core feature of autism or a marker of the neurological deficits that underlie the broad features of the disorder. Some authors state motor coordination is closely related to a child's cognitive and social development; this is because coordination increases a child's ability to explore and manipulate their environment, motivating them to participate in social activities (Missiuna et al. 2006; Piek et al. 2006). The frequent coordination dysfunction observed in children with PDD is believed to be due to impairments in social function and coordination dysfunction caused by the observed coordination dysfunction during development. Haswell et al. (2009) measured generalization patterns as children learned to control a novel tool. Their findings raise the possibility that common problems exist in the brains of people with autism. In addition, they found the brains of children with autism develop a stronger-than-normal association between self-generated motor commands and proprioceptive feedback; furthermore, the greater the reliance on proprioception, the greater the child's social functioning and imitation impairments. Moreover, a recent meta-analysis of structural brain imaging studies revealed the total volume of the brain and volumes of specific regions such as the cerebral hemispheres, caudate nucleus, and cerebellum are greater in people with autism (Stanfield et al. 2008). The cerebellum is considered to play an important role in the modulation of not only motor functions, but also linguistic, cognitive, and empathic functions (Murdoch 2010; Vakalopoulos 2013). Abnormal movement-related potentials in autism, which implicate basal ganglia, thalamus, and supplementary motor area involvement, are a likely source of motor dysfunction in autism (Enticott et al. 2009). Thus, the abovementioned brain regions play crucial roles in the development of motor coordination and social communication in PDD. However, the present and previous results indicate not all patients with PDD suffer from motor impairments and vice versa. Although the reasons for these differences are not well understood, our results help clarify the motor functions and possible heterogeneous neuropathology of PDD in Japan. Indeed, several brain regions are reported to be structurally abnormal in PDD (Fournier et al. 2010). Nevertheless, additional studies are required to clarify the source(s) of motor impairments apparent in PDD.

Limitations

This study has some limitations that should be mentioned. First, few boys with HFPDD were analyzed. Although this study was limited to boys with HFPDD, a previous study reports that girls with ASD also have considerable coordination problems very often (Kopp et al. 2010). Thus, larger studies and comparative studies of boys and girls are needed. Second, it is necessary to examine the factors influencing the motor function of children with HFPDD, such as medication. In the present study, there was no significant difference between children who took medication and those who did not. However, risperidone (Aman et al. 2009), SSRIs (Loubinoux et al. 2005), and methylphenidate (Bart et al. 2013) are reported to have beneficial effects on motor functions. Furthermore, carbamazepine (Braathen et al. 1997) and valproate (Farkas et al. 2010) might be related to motor impairments in children with epilepsy. Third, it is also necessary to examine the relationship between motor coordination and social impairments in normal children. Finally, in the present study, the motor coordination of the boys with HFPDD was only assessed by a questionnaire, i.e., the DCDQ-J, which does not involve neurological examinations.

However, Wilson et al. (2000) confirm the DCDQ is a valid clinical screening tool for DCD; correlations between DCDQ scores, and M-ABC and Test of Visual–Motor Integration scores support concurrent validity. Concordantly, the EACD (2012) states a questionnaire may be useful as an initial diagnostic tool and that the DCDQ is currently the best-evaluated questionnaire. Future studies should investigate the predictive validity of the DCDQ-J and develop a psychometrically sound and culturally appropriate standardized international test (American Psychiatric Association 2013) in Japan. In fact, we are currently developing the Japanese version of the M-ABC2 (Hirata et al. 2014).

Conclusion

This study is the first report indicating Japanese children with HFPDD frequently have motor coordination impairments according to the Japanese version of the DCDQ. The levels of impairment according to the CDM, FM, and total DCDQ-J scores are significantly correlated with the score of Qualitative Abnormalities in Communication in the ADI-R. On the other hand, not all patients with PDD have motor coordination dysfunction. Therefore, additional studies are required to clarify the relationships among the development of motor coordination, cognition, and socialization and between PDD and DCD. Clinicians should consider the screening and assessment of movement impairments as part of the routine investigation for children with PDD. Screening for or assessing motor dysfunctions in HFPDD using tools such as the DCDQ-J could lead to the development of treatments and new pathophysiologic concepts.

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