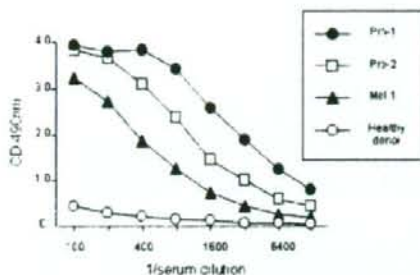and prostate cancer patients, as well as in melanoma patients. No antibody was detected in 39 sera samples from healthy donors.

Figure 4

Antibody response against NGO-Pr-54 in cancer patients. ELISA for sera from prostate cancer patients (Pro-1 and -2) and a melanoma patient (Mel-1) using recombinant protein. Control, healthy donor.

Table 2

Antibody response against NGO-Pr-54 in cancer patients.

Tumor	Positive / Total
Healthy donor	0/39
Bladder cancer	2/39 (5.1%)
Brain cancer	0/44
Cervical cancer	0/35
Colon cancer	1/65 (1.5%)
Endometrial cancer	0/26
Hepatocellular carcinoma	2/49 (4.1%)
Lung cancer	6/174 (3.4%)
Melanoma	7/53 (13.2%)
Ovarian cancer	2/36 (5.6%)
Prostate cancer	5/118 (4.2%)

Discussion

In this study, HERV-K gag-related NGO-Pr-54 antigen was identified by SEREX analysis in a prostate cancer using autologous patient serum. *NGO-Pr-54* mRNA was observed to be faintly expressed in normal prostate and strongly expressed in a variety of cancers including ovarian cancers (5/8), prostate cancers (6/9), and leukemias (5/14). A phage plaque assay showed that a strong reaction was consistently observed with clone ZH042 having *NGO-Pr-54* with a deleted 5' end, suggesting that it contained the sequence coding for the protein product. TI-35 mAb was produced using the recombinant protein (438 aa) deduced from the sequence of ZH042 as antigen for immunizing mice. Transfection of clone ZH042 into 293T cells resulted in the production of an approximately 50-kDa molecule visualized by Western blot. Natural

production of the molecule was confirmed in the SK-MEL-23 melanoma cell line. An indirect immunofluorescence assay showed NGO-Pr-54 protein is expressed on the cell surface as well as in the cytoplasm. Cell surface expression was confirmed by flow cytometry using TI-35 mAb. The antibody response against NGO-Pr-54 was observed in patients with bladder (5.1%), liver (4.1%), lung (3.4%), ovarian (5.6%), and prostate (4.2%) cancer, as well as in patients with malignant melanoma (13.2%).

Most endogenous retroviruses are defective due to the presence of stop codons and frameshifts in the genes (9, 22). *HERV-K* is the most conserved family including intact ORFs (9). However, the presence of ORFs did not always result in translation of the protein (23). *NGO-Pr-54* harbors an intact ORF spanning gag consisting of 715 aa. While the molecular size appeared to be consistent with that of the gag precursor determined previously (24), transfection of the full length sequence of *NGO-Pr-54* failed to produce protein in 293T cells. On the other hand, transfection of ZH042 that lacked the 5' region from *NGO-Pr-54* resulted in the production of a protein approximately 50 kDa in size that appeared to be the processed form of the gag protein. The exact reason why the transfection of the full length NGO-Pr-54 *gag* gene failed to produce a protein product, but transfection of the 5' region-deleted ZH042 construct resulted in the production of gag protein is unknown at present. The *HERV-K* genome has as a feature the nuclear retention of mRNAs, with their export being mediated by Rec proteins (25, 26). The lack of protein production by the full-length sequence could be due to the impairment of nuclear export of the *HERV-K* mRNA. An approximately 20-kDa protein was observed in ZH042-transfected 293T cells, which might be the mature gag processing product. However, the recombinant 438 aa protein used in this study contained no protease sequences adjacent to the gag protein. The absence of a 20-kDa molecule in the HERV-K-expressing melanoma cell line SK-MEL-23 might suggest that it is a degradation product due to cellular proteases in 293T cells.

Endogenous retroviral sequences have been shown to be expressed in human cancers. Increased expression of a HERV-K *env* transcript was shown in melanoma (27) and in breast (28) and ovarian (29) cancers as compared to the expression in normal tissues. *HERV-E* mRNA expression was shown in prostate cancer, but not in normal prostate (30). *HERV-H* expression was shown in gastrointestinal cancer (31). This study showed HERV-K *gag* expression in prostate cancer, ovarian cancer, and leukemia. Furthermore, cell surface expression of HERV-K gag was shown, something which could be useful for antibody therapy of cancer, as seen for HER2/neu (32) or NY-BR-1 (33). Localization of the HERV-K gag protein in the cell membrane has been shown by Western blotting using purified membrane and rabbit anti-HERV-K gag serum (34). Recently, *HERV-K* 22q11.23 was shown to be fused to *ETV1*, creating oncogenic fusions in prostate cancer (35). *HERV-K* 22q11.23 was expressed in normal prostate at higher levels than in other normal tissues. This gene was shown to be overexpressed in the LNCap prostate cancer cell line in response to synthetic androgen.

The antigenicity of HERV elements has been shown serologically and by CD8 T cell recognition. Antibodies against HERV-K gag and env have been detected in patients with germ cell tumor (24, 36), ovarian cancer (29), leukemia (37), and malignant melanoma (27). Moreover, the association of antibody response to better prognosis was observed (38). On the other hand, Schiavetti *et al.* (39) identified the antigenic peptide

presented by HLA-A2 molecules encoded by a very short open reading frame present in the *env* region of the gene belonging to HERV-K. The gene *HERV-K-MEL* was expressed in most malignant melanoma samples. It was also expressed in most naevi and as a part of carcinomas and sarcomas. However, in normal tissues, it is expressed only in testis and some skin samples. *HERV-K-MEL* is a pseudogene that does not code for a retroviral envelope protein. Because of the presence of many mutations, a main ORF can not be defined. Thus, the antigenic peptide can be encoded by a small ORF. Takahashi *et al.* (40) obtained donor-derived CD8 CTLs recognizing recipient renal cell cancer (RCC) in hematopoietic stem cell transplantation (HSCT) and identified the peptide epitope presented on HLA-A11. The peptide was derived from the gene belonging to HERV-E, which was expressed in renal cancer cell lines and tissues, but not in normal tissues including kidney.

The antibody response to HERV products and CTL recognition of HERV-derived peptides in cancer patients suggests that HERVs are immunogenic in cancer patients and can be utilized as target antigens for cancer immunotherapy.

Abbreviations

HERV, human endogenous retrovirus; ORF, open reading frame; SEREX, serological recombinant cDNA expression cloning

Acknowledgements

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Materials and methods

Mice

BALB/c and SCID mice were purchased from Japan SLC (Shizuoka, Japan). The mice were bred at the Laboratory Animal Center in Okayama University. The experiments were conducted according to the Guidelines for Animal Experiments at Okayama University, the Japanese Government Animal Protection and Management Law (No. 105) and the Japanese Government Notification on Feeding and Safekeeping of Animals (No. 6).

Tissues and sera

Prostate cancer specimens used for SEREX were obtained surgically from a patient at Aichi Cancer Center (Nagoya, Japan). Tumor specimens used for reverse transcription (RT)-PCR analysis were obtained surgically from patients at Okayama University Hospital (Okayama, Japan) and Osaka University Hospital (Osaka, Japan). Sera were obtained from 39 healthy donors and cancer patients at Okayama University Hospital. Informed consent was obtained from each healthy donor and each patient for the use of specimens and sera.

Cell lines

293T is human embryonic kidney cell line 293 transfected with the SV40 T gene (41). NS-1 is a myeloma cell line derived from BALB/c MOPC-21 cells (42). COS7 is a cell line derived from SV40-transformed African green monkey kidney cells (43). Colon cancer cell lines SK-CO-1, WiDr, LS 174T, SW480, COLO 201, COLO 320DM, and LoVo were obtained from the American Type Culture Collection (ATCC, Rockville, MD). DLD-1 was obtained from the RIKEN BioResource Center (Ibaraki, Japan). Lung cancer cell lines OU-LC-MS, OU-LU-5, 1-87, 11-18, PC-9, LK-87, QG-56, EBC-2, and LC-1sq were described previously (44, 45, 46). OU-LU-17 was provided by Dr. M. Shimono (Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan). Prostate cancer cell lines LNCap, DU 145, and PC-3 were obtained from the American Type Culture Collection. Mesothelioma cell lines C-13 and YM were established in our laboratory. ACC-MESO-1 and ACC-MESO-4 were obtained from the RIKEN BioResource Center (47). Melanoma cell lines SK-MEL-19, SK-MEL-23, SK-MEL-27, SK-MEL-28, SK-MEL-37, SK-MEL-64, CLL-MEL were provided Dr. R. Ueda (Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan). The acute T cell leukemia cell line Jurkat was obtained from the American Type Culture Collection. These cell lines were maintained in RPMI 1640 supplemented with 10% FCS.

Total RNA isolation and cDNA synthesis

Total RNA was isolated from frozen tumor specimens and pellets of washed cell lines using an RNeasy Mini Kit (QIAGEN, Hilden, Germany). Isolated RNA was treated with DNase (TURBO DNA-free, Ambion, Austin, TX, USA) to remove genomic DNA contamination. Treated RNA (2 μg) was reverse transcribed into single-stranded cDNA using Moloney murine leukemia virus reverse transcriptase (Ready-To-Go You-Prime

First-Strand Beads, Amersham Biosciences, Piscataway, NJ, USA) and oligo (dT)$_{15}$ as a primer. Complementary DNA was tested for integrity by amplifying the glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) transcript for over 30 cycles. An RNA panel from normal tissues was purchased from Ambion and treated with DNase as described above.

Reverse transcription (RT)-PCR analysis

To amplify the *NGO-Pr-54* cDNA segment, primers specific for *NGO-Pr-54* were designed. Primers for RT-PCR were: 5'-CGTCTAATTCACCAGCAACAC-3' (forward), 5'-TAGACTTTTGAGCAGCATCTTG-3' (reverse). The amplification program for *NGO-Pr-54* was 10 s at 98°C, 30 s at 55°C, and 1 min at 72°C for 35 cycles after denaturing at 98°C for 2 min. These cycles were followed by a 10-min elongation step at 72°C. The PCR products (702 bp) were analyzed on a 0.8% agarose gel.

Quantitative real-time RT-PCR

Two-step real-time RT-PCR was run on an Applied Biosystems 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The gene-specific primers and TaqMan probe for *NGO-Pr-54* were designed using Primer Express software (version 1.5) (Applied Biosystems). The forward primer was 5'-CAGCGATGGCGTCTAATTCA-3' and the reverse primer was 5'-AGTGGGCGGCTGAGGATAC-3'. The TaqMan probe was 5'-FAM-AGCAACACAGGACGCGGCGC-TAMRA-3'. PCR was performed with TaqMan Universal PCR Master Mix (Applied Biosystems), the primer pair, TaqMan probe, and çDNA solution (corresponding to 60 ng total RNA). The thermal cycling conditions comprised an initial denaturation step at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min.

Construction of cDNA libraries

Messenger RNA was isolated from prostate cancer specimens using a Fast Track mRNA purification kit (Invitrogen, Life Technologies, Carlsbad, CA, USA). Complementary DNA expression libraries were prepared in a λZAP expression vector using a cDNA library kit (Stratagene, La Jolla, CA, USA).

Immunoscreening of cDNA expression libraries

Complementary DNA expression libraries were screened with autologous patient serum as described previously (48). Briefly, the serum was diluted 1:10 and preabsorbed with phage-transfected *Escherichia coli* lysate. Nitrocellulose membranes containing the phage plaques at a density of 4,000-5,000 pfu per 130 mm plate were incubated overnight at room temperature with the serum diluted 1:200. After washing, the filters were incubated with alkaline phosphatase-conjugated goat anti-human Fcγ. The reactive clones were visualized with 5-bromo-4-chloro-3-indolyl-phosphatase and nitroblue tetrazolium.

Sequence analysis of reactive clones

pBK-CMV phagemids were excised *in vivo* from positive-staining phage. cDNA inserts were subjected to DNA sequencing using an ABI PRISM Model 377 automated sequencer (Perkin Elmer, Norwalk, CT, USA). The sequence alignments of the clones were analyzed for similarity using BLAST software, Genbank, EST, and SEREX databases.

Preparation of plasmid vectors

The *NGO-Pr-54* cDNA was excised from pBK-CMV phagemids and ligated into a pcDNA3.1(+) vector (Invitrogen)

and a p3xFLAG-CMV-14 expression vector (Sigma-Aldrich, Munich, Germany). TOP10 *Escherichia coli* cells were transformed with the recombinant vector. Plasmid DNA was purified using a QIAprep Spin Miniprep Kit (QIAGEN). Insertion of cDNA was confirmed by DNA sequencing.

Production of the recombinant NGO-Pr-54 protein

The *NGO-Pr-54* cDNA corresponding to the C-terminal 438 amino acids of the putative ORF was amplified by PCR. The amplified DNA was ligated into the histidine-tag-containing vector pQE30 (QIAGEN). NGO-Pr-54/pQE30 was introduced into M13. His-tagged recombinant NGO-Pr-54 protein was purified by nickel ion affinity chromatography (HisTrap HP, GE Healthcare, Uppsala, Sweden).

Generation of a monoclonal anti-NGO-Pr-54 antibody, TI-35

BALB/c mice were immunized intramuscularly with NGO-Pr-54/pcDNA3.1 (100 μg) four times at 2-week intervals using an electric pulse generator (CUY-21, BEX, Tokyo, Japan). Mice were boosted intraperitoneally with recombinant NGO-Pr-54 protein (100 μg) twice at a 2-week interval. Spleen cells from immunized mice were fused with NS-1 myeloma cells. Hybridoma cells were cultured in ClonaCell-HY Medium D (StemCell Technologies, Vancouver, BC, Canada). The production of NGO-Pr-54 specific monoclonal antibody from the hybridoma was assessed by ELISA using recombinant NGO-Pr-54 protein. SCID mice were administered intraperitoneally with hybridoma cells. The monoclonal antibody was purified from ascites of the mice via protein G affinity chromatography (Amersham Biosciences).

ELISA

Recombinant NGO-Pr-54 protein (1 μg/ml) in 0.05 mol/l carbonate buffer (pH 9.6) was adsorbed onto 96-well plates (Nunc, Rochester, NY, USA) at 4°C overnight. Plates were washed with 0.05% Tween-20/PBS and blocked with 200 μl/well of 5% FCS/PBS for 1 h at room temperature. After washing, sera serially diluted with 5% FCS/PBS were added to each well and incubated for 2 h at room temperature. After washing, diluted goat anti-human Fcγ or goat anti-mouse IgG, labeled with horseradish peroxidase (Jackson ImmunoResearch, Baltimore, PA, USA) was added and incubated for 1 h at room temperature. After washing, the substrate solution [50 mmol/l citric acid, 100 mmol/l Na$_2$HPO$_4$, 0.03% ortho-phenylenediamine, and 0.1% H$_2$O$_2$ in distilled water (pH 5.0)] was added to each well. After adding 3 mol/l H$_2$SO$_4$, the absorbance was read at 490 nm.

Western blot analysis

293T cells were transiently transfected with plasmids using Lipofectamine 2000 (Invitrogen). The lysate of transfectants and SK-MEL-23 was prepared with RIPA Lysis Buffer (Santa Cruz Biotechnology, CA, USA). The cell lysate was separated by electrophoresis under reducing conditions and transferred to a polyvinylidene difluoride (PVDF) membrane (Hybond-P; Amersham Biosciences). The membrane was incubated with TI-35 mAb (1 μg/ml) or anti-FLAG M2 mAb (Sigma-Aldrich) (1 μg/ml) for 1 h at room temperature. Bound antibody was detected with alkaline phosphatase-conjugated second antibody (1:1000; Pierce, IL, USA) using an AP Conjugate Substrate kit (Bio-Rad Laboratories, Hercules, CA, USA).

Immunofluorescence staining

Transfected 293T or COS7 cells growing on glass slides were fixed with 4% paraformaldehyde. For intracellular staining, cells

were permeabilized by treatment with 0.5% Triton-X. Staining was performed using TI-35 mAb (1 µg/ml) or anti-FLAG M2 mAb (1 µg/ml). Bound antibody was detected with FITC-labeled anti-mouse IgG F(ab')$_2$ (Sigma-Aldrich) at a concentration of 20 µg/ml. Rhodamine-conjugated wheat germ agglutinin (WGA) (Vector Laboratories, Burlingame, CA, USA) was used for staining of the cell membrane. Nuclei were stained with DAPI (VECTASHIELD, Vector Laboratories). Slides were visualized using a fluorescence microscope (Biozero BZ-8000, Keyence, Osaka, Japan).

Flow cytometry analysis

The FACScan (Becton Dickinson, Mountain View, CA, USA) was used according to the manufacturer's instructions.

Immunohistochemistry

Tumor specimens were fixed with buffered formalin and embedded in paraffin. Five-micrometer sections were placed on glass slides, heated at 60°C overnight, and deparaffinized with xylene and ethanol. Glass slides were microwave-heated in antigen retrieval buffer (10 mmol/l citrate buffer, pH 6.0) using a pressure cooker for 20 min. After the inactivation of endogenous peroxidase with 0.3% H_2O_2 for 5 min, specimens were preincubated with serum-free blocking solution (DakoCytomation, Kyoto, Japan). TI-35 mAb was added at a concentration of 5 µg/ml and incubated for 2 h at room temperature. After washing, DAKO EnVision$^+$ horseradish peroxidase-conjugated goat anti-mouse IgG (Dako Cytomation) was applied. After incubation for 30 min at room temperature, the specimens were visualized with 3,3'-diaminobenzidine in H_2O_2 and counterstained with hematoxylin solution.

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Brief Report

Atypical Parkinsonism in Distal Myopathy With Rimmed Vacuoles

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Video (⌣)

Abstract: **A patient with distal myopathy with rimmed vacuoles (DMRV) exhibited Parkinsonism with a severe writing tremor that responded poorly to levodopa. Molecular genetic analysis revealed that the patient had the D176V/V572L compound heterozygous mutation in the UDP-*N*-acetylglucosamine 2-epimerase/*N*-acetylmannosamine kinase (GNE) gene. Histopathological examination of a biopsied muscle specimen yielded findings compatible with those of DMRV, which is characterized by the presence of rimmed vacuoles without inflammatory cell infiltration in muscle fibers. The finding of normal cardiac meta-iodobenzylguanide uptake makes the possibility of incidental Parkinson's disease in this patient unlikely. These observations raise the possibility that atypical Parkinsonism is a rare complication of DMRV associated with *GNE* mutation.** © 2008 Movement Disorder Society

Key words: writing tremor; dystonia; atypical Parkinsonism; rimmed vacuole; *GNE* mutation

Distal myopathy with rimmed vacuoles (DMRV),[1] also known as hereditary inclusion body myopathy,[2] is an autosomal recessive muscular disorder associated

with mutations in the gene encoding a bifunctional enzyme, UDP-*N*-acetylglucosamine 2-epimerase/*N*-acetylmannosamine kinase (GNE).[3] In patients with DMRV, mutations in *GNE* are responsible for decrease in sialic acid content in the skeletal muscle.[4] This suggests that the decrease in sialic acid underlies the disease process in DMRV, which is characterized pathologically by the presence of rimmed vacuoles without inflammatory changes in skeletal muscle. However, involvement of the central nervous system in this disease has not been examined.

CASE REPORT

A 58-year-old right-handed man first experienced difficulty in walking and climbing stairs at the age of 46 years. Prior to this, he noticed slowness in his movements and difficulty in writing due to hand tremor at the age of 42 years. These symptoms gradually worsened. His medical history included no psychotropic medications or head trauma. His parents were not consanguineous. Neither his parents nor siblings were affected by DMRV. He visited a department of neurology in a district hospital complaining of muscle weakness. He was suspected to have a degenerative muscular disorder; however, his clinical manifestations were not sufficiently prominent to make the diagnosis of a specific muscular disease. The accompanying hand tremor was also a confounding symptom in the diagnosis. Therefore, a biopsy was performed on his rectus femoris muscle under the provisional diagnosis of myopathy of uncertain cause. He therefore visited our hospital for further evaluations of his clinical symptoms as well as histopathological examinations of his biopsied muscle specimen.

On examination, marked atrophy and weakness were observed in his anterior tibial muscle, whereas his quadriceps muscles were well preserved. His neck and shoulder girdle muscles were mildly atrophic. His tendon reflexes were diminished in the extremities. Muscle computed tomography and magnetic resonance imaging (MRI) demonstrated that his anterior tibial muscles were atrophic. Routine blood and urine tests, including determination of serum creatine kinase level, revealed no abnormalities. Needle electromyography (EMG) of the anterior tibial muscle revealed mildly high-amplitude and polyphasic units.
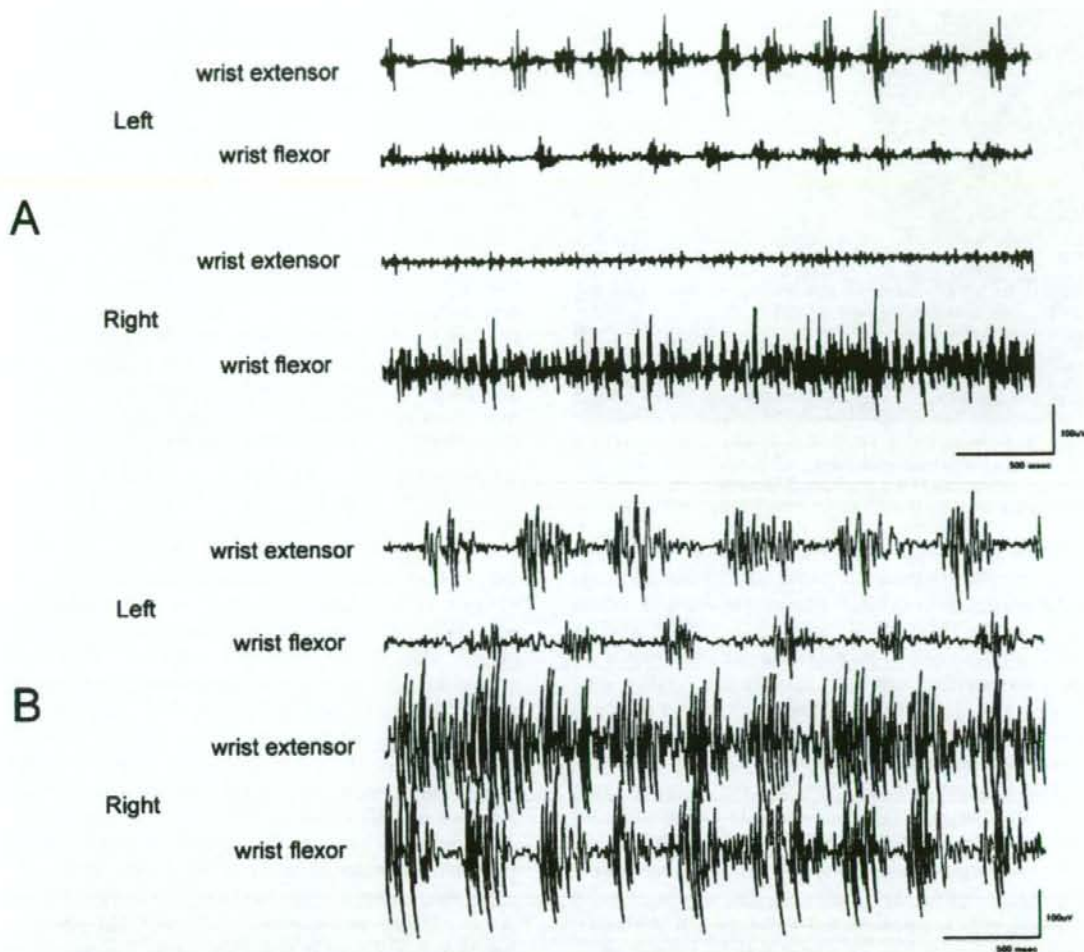
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FIG. 1. Surface EMG of both arms during periods of rest and task of writing. (A) At rest, EMG reveals a 3- or 4-Hz tremor in the left arm and a continuous dystonic discharge in the right arm. (B) During writing, bursts of EMG activity are observed simultaneously in the right wrist extensor and flexor muscles due to their co-contraction.

Bradykinesia and mild rigidity of the neck and bilateral extremities were observed as Parkinsonian features. A resting tremor was observed in his left hand. Poor arm swing and instability were observed in his gait and station, respectively. There was no general intellectual deterioration, cerebellar ataxia, or autonomic failure. There was no limitation in ocular movements. His writing was severely affected; when he began to write using his right hand, muscle spasm and frequent jerks appeared immediately, making correct writing very difficult (video). When he wrote with his left hand, severe jerks of the same type forced him to stop

writing. Surface EMG of his arms was performed. At rest, EMG revealed a 3- or 4-Hz tremor in the left arm, and a continuous dystonic discharge in the right arm (Fig. 1A). During the task of writing, bursts of EMG activity were observed simultaneously in the right wrist extensor and flexor muscles due to their co-contraction (Fig. 1B). Brain MRI demonstrated no abnormality in the putamen, midbrain, or pons, except for a small ischemic spot in the left medial part of the thalamus. Magnetic resonance angiography revealed normal flow signals in the major vessels. Single-photon-emission computed tomography (SPECT) using
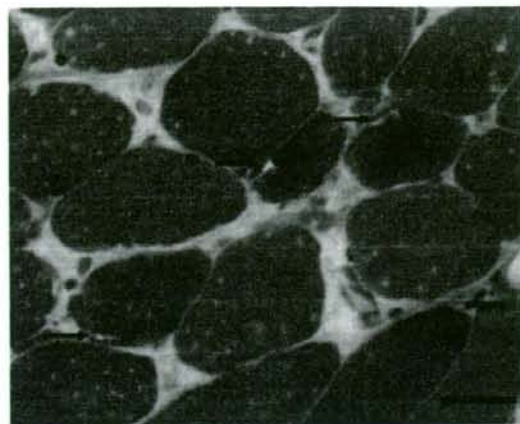
FIG. 2. Histopathological examination of muscle reveals rimmed vacuoles (arrows) without inflammatory cell infiltration in muscle fibers. Gomori trichrome stain. Bar = 50 μm. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Tc-99m ECD suggested a reduced blood flow in the basal ganglia bilaterally. Myocardial 123-labeled meta-iodobenzylguanide (MIBG) scintigraphy showed normal tracer uptake (heart-mediastinum ratio: 2.92). Levodopa therapy (300 mg/day) partly ameliorated the symptoms of Parkinsonism such as bradykinesia and resting tremor; however, his writing tremor responded poorly to this treatment. Finally, clonazepam (1.5 mg/day) was found to be moderately effective for his writing tremor.

The biopsied muscle specimen was obtained from the district hospital in which a muscle biopsy had been performed on his rectus femoris muscle under the provisional diagnosis of myopathy of uncertain cause. Histopathological examination of the muscle revealed rimmed vacuoles without inflammatory cell infiltration in muscle fibers (Fig. 2). There was no other specific feature suggestive of the presence of other myopathies. High-molecular-weight genomic DNA was extracted from the peripheral white blood cells of the patient. Mutation analysis of *GNE* was performed as described previously.[5] Results showed that the patient had a D176V/V572L compound heterozygous mutation in *GNE*. Informed consent was obtained from the patient before the above evaluations were performed.

DISCUSSION

Specific vacuolar degeneration of muscle fibers characterized by the presence of rimmed vacuoles is a pathological hallmark of DMRV or inclusion body myositis (IBM). Vacuolated muscle fibers of patients with DMRV or IBM contain ubiquitin, β-amyloid precursor protein, phosphorylated tau,[6] and alpha synuclein.[7]

These molecules also play crucial roles in the pathogenesis of neurodegenerative diseases of the central nervous system. Transgenic mouse models overexpressing the β-amyloid precursor protein exhibit IBM-like histopathology.[8] These observations raise the question whether the pathogenic mechanism underlying the rimmed vacuole formation also affects the central nervous system, which is vulnerable to the accumulation of such proteins. There is an interesting report of a case of L-dopa-responsive hand tremor occurring in a patient with IBM.[9] This observation suggests that the pathogenic mechanism underlying the rimmed vacuole formation coexists with dopaminergic disruption of the central nervous system. However, in patients with IBM, it is difficult to identify a common mechanism underlying rimmed vacuole formation and dopaminergic disruption, since IBM is a sporadic disease the pathogenesis of which has not been determined at the molecular level.

Detailed evaluations of the Parkinsonian features in patients with DMRV, which is a genetically distinctive disease entity, may yield findings useful for determining whether there is a common etiology for the rimmed vacuole formation and the vulnerability of neurons in the dopaminergic system. Interestingly, our patient with DMRV exhibited Parkinsonism with a severe writing tremor, which responded poorly to L-dopa. This severe writing tremor may correspond to hand dystonia, which is atypical compared with the common form of action tremor in Parkinson's disease.[10] Furthermore, the normal tracer uptake found on myocardial MIBG scintigraphy makes the possibility of incidental Parkinson's disease in this patient unlikely. There are phenotypes that predominantly exhibit hand dystonia in hereditary neurodegenerative diseases such as autosomal recessive Parkinsonism linked to a mutation in the Parkin gene[11] or autosomal dominant early-onset dystonia linked to a mutation in the DYT1 gene.[12] However, the coincidental existence of such a mutation in a DMRV patient would probably be extremely rare. The atypical Parkinsonian features in this patient raise the possibility of coincidental occurrence of a rare neurodegenerative disease such as progressive supranuclear palsy or multiple system atrophy; however, the patient's clinical findings do not meet the diagnostic criteria for these diseases. Of note, in this patient, the onset of the atypical Parkinsonian features was earlier than that of myopathic symptoms, whose onset was quite late; most DMRV patients manifest the onset of myopathic symptoms during the second or third decade of life. Our patient thus has an unusual phenotypic expression of DMRV characterized by late-onset myopathy and atypical Parkinsonian fea-

tures. We hypothesize that a genetic factor responsible for a common etiology of the late-onset myopathy and vulnerability of neurons in the dopaminergic system triggered this unusual phenotypic expression of DMRV.

Our patient with DMRV carries a D176V/V572L compound heterozygous mutation in *GNE*, one of the mutations causing DMRV. GNE is a ubiquitous protein that is also expressed also in the central nervous system.[13] GNE is the key enzyme in the biosynthesis of sialic acid.[14] This biosynthesis involves the intracellular concentration of sialic acid, which regulates the polysialylation of the neural cell adhesion molecule (NCAM).[15] The polysialylated form of NCAM has been reported to play an important role in the development of striatal neurons.[16] Furthermore, experimental findings suggest the molecule may be involved in the control of migration and synthesis of tyrosine hydroxylase in dopaminergic cells of the developing mesencephalon.[17] Taken together, these observations suggest the hypothesis that the *GNE* mutation also affects the polysialylated form of NCAM in the central nervous system, which is essential for the development of the dopaminergic system in the nigrostriatum. Strikingly, our patient with DMRV who carries the *GNE* mutation exhibited atypical Parkinsonian features with severe hand dystonia that poorly responded to L-dopa, suggesting involvement of the nigrostriatum and the surrounding area. This is supported by the SPECT finding suggestive of decreased perfusion of the basal ganglia. Further investigation is needed to determine whether the *GNE* mutation leads to vulnerability of neurons functionally related to the dopaminergic system.

There is no prior report of atypical Parkinsonism in patients with DMRV. The D176V/V572L compound heterozygous mutation was found to be relatively rare in a DMRV cohort,[18] and its clinical phenotype has not been fully characterized. Although other genetic modifiers might affect its phenotypic expression in this patient, our observations raise the possibility that the D176V/V572L compound heterozygous mutation in *GNE* is at least partly responsible for the development of the atypical Parkinsonian features in this patient. Further studies are needed to elucidate the effects of *GNE* mutations on the central nervous system.

LEGEND TO THE VIDEO

Segment 1. Writing tremor in the patient. Muscle spasm and jerks severely affected the fluency of his writing, making correct writing very difficult.

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Executive Dysfunction Using Behavioral Assessment of the Dysexecutive Syndrome in Parkinson's Disease

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Abstract: The objective of this study was to evaluate the executive dysfunction (ExD) in Parkinson's disease (PD) using the Behavioral Assessment of the Dysexecutive Syndrome (BADS), which provides a wide-range assessment of ExD. The BADS and the Unified Parkinson's Disease Rating Scale (UPDRS) were investigated in 63 nondemented PD patients who revealed scores of ≥24 points on the Mini-Mental State Examination based on the DSM-IV. Multiple logistic regression analysis was performed to evaluate the predisposing factors to ExD, which was defined as <70 points on the age-controlled standardized score. The total score on the UPDRS was a significant independent predisposing factor to ExD. Among the various parts of the UPDRS, part II was the significant factor for ExD. The profile scores of all subtests on the BADS in patients with ExD were significantly lower than those of patients without ExD. All profile scores decreased with severity of PD, but the changes among these scores differed. ExD in nondemented PD predisposed to a greater severity of PD, particularly as regards the activity of daily living impairment. Nondemented PD revealed wide-range components of ExD. All components of ExD were impaired with severity of PD, but the patterns of each component exhibited variety. © 2007 Movement Disorder Society

Key words: executive function; Parkinson's disease; predisposing factor; behavioral assessment of the dysexecutive syndrome (BADS); Unified Parkinson's Disease Rating Scale (UPDRS).

Executive dysfunction (ExD) in Parkinson's disease (PD) has been described in many previous studies, but the reported tasks for evaluation of the ExD were able to detect one or several components of ExD. The ExD in PD has been widely assessed by the Wisconsin Card Sorting test (WCST).[1–5] The WCST was designed mainly to detect the ability for set shifting.[6] In previous studies employing the WCST,[1–5] the frequency of impairment of shifting conceptual sets of ExD at the early stage of PD was found to be higher than that in age-matched normal control subjects. However, the early stage of PD in these previously reported subjects consisted of Hohen & Yahr (H&Y) stage I and II, or stage I to III. There was only one reported comparative study on ExD estimated by the WCST among different H&Y stages in PD.[7] Although the number of subjects in this previous study[7] was small, the ExD did not present at H&Y stage I but was evident at more than stage II. The impairment of set shifting, as estimated by the Trail-Making test or Stroop Color-Word test, had also been reported to present at the early stage of PD[8,9] and to be related to the UPDRS score for the severity of PD.[10] Moreover, Tower tasks such as the Tower of London task (TOL) mainly detect the ability for set elaboration and planning.[6] In previous studies[2,11,12] that employed the TOL, the initial thinking time was impaired at the

stage of mild PD and the minimum move solution was revealed at the severe stage of PD. Impairment of the motor set had also been found to be evident at the mild to moderate stage of PD, but providing an external cue at the start of the examination could improve the performance.[13] On the other hand, patients with advanced PD have been described as exhibiting the disturbance of every cognitive measure.[14,15]

Wilson et al. developed a new test battery providing a wide-range assessment of ExD, the Behavioral Assessment of the Dysexecutive Syndrome (BADS).[16] Lezak et al. recently indicated that the BADS was appropriate as a wide-range assessment for ExD.[17] Bennett et al. also reported that the BADS was more sensitive to ExD than many traditional tests including the WCST, Trail Making Test, Porteus Maze Test, Controlled Oral Word Association Test, and Tinker Toy Test.[18] However, the important issue is not whether the BADS is more sensitive or specific than other well-established batteries or not, but that the BADS has a notable ability to examine with only one scale all aspects of the dysexecutive syndrome: it is this which gives special relevance to the BADS. In the present study, we therefore employed the BADS for the first time to estimate the ExD.

The BADS consists of 6 different subtests as follows: the Rule Shift Cards Test, Action Program Test, Key Search Test, Temporal Judgement Test, Zoo Map Test, and Modified Six Elements Test. The BADS offers a more comprehensive assessment of ExD, in that it involves not only set shifting and inhibition control (e.g., the Rule Shift Cards Test), but also the planning of behavior under concept formation to novel situations (e.g., the Action Program Test), problem solving (e.g., the Key Search Test and Zoo Map Test), even the reasoning (the Temporal Judgement Test), and problem solving under planning in the face of several competing tasks (e.g., the Modified Six Elements Test).[16,19] The BADS is thus considered to provide a more integrated battery with "ecological validity" based on the simulation of problem-solving situations that patients may confront in actual daily life.[20–22]

Performance on each of the 6 subtests in the BADS is scored according to the BADS manual. These scores are summed to derive a composite total profile score, providing an overall measure of ExD. The total profile score is then converted into a standardized score with a mean (M) of 100 and a standard deviation (SD) of 15 based on data obtained from normal control subjects in the same manner as for IQ scores on the Wechsler Adult Intelligence Scale, Revised.[16] In 2003, Kashima et al. translated and revised the Japanese version of the BADS, which was adapted for validity and normalization in

Japanese people, under the supervision of Wilson.[23] The total profile score and profile scores of each subtest in this Japanese version were confirmed to have an identical potential against the original version of the BADS.[23] Moreover, Ihara et al. reported that the Japanese version was consistent with the original version of the BADS based on comparative data for Japanese and English ExD patients.[24] It is therefore possible to classify the standardized score on the BADS into five grades: impaired, borderline, low average, average, high average, and superior, in the same manner as in the traditional IQ test. Since executive function decreases even in normal, aged subjects, a controlled standardized score is also provided for the BADS based on data obtained from different age groups of normal control subjects.[16,23] In the present assessments, we thus employed the age-controlled standardized score of the BADS to estimate the ExD of nondemented PD patients.

Most previous studies on ExD in PD have, however, utilized single variable analysis. Such analysis tests the significance of each variable separately, whereas multiple logistic regression analysis tests the independent effect of each variable after considering the associations among the variables. A predisposing factor for ExD in PD using multiple logistic regression analysis has been reported in only one study: Muslimovic et al. found that patient's age was the only significant predisposing factor employing such analysis.[25] Although many earlier studies using two-paired comparisons indicated that the ExD in PD was associated with the severity or motor disability of PD,[5,10,12,14] axial impairment and speech disorder as motor symptoms were only enrolled in this previous multiple logistic regression analysis.[25]

The present study therefore represents the first evaluation of predisposing factors to ExD based on multiple logistic regression analysis, which included the total score and score of part III on the Unified Parkinson's Disease Rating Scale (UPDRS) as measures of the severity and motor disability of PD.

METHODS

Patient Definitions

The number of consecutive patients in this study after giving informed written consent was 106 out of 146, who were diagnosed as having sporadic form PD at the Neurology Clinic of our hospital during the period between December 2004 and May 2006. The diagnosis of sporadic PD was made according to the UK Parkinson's Disease Brain Bank criteria.[26] Based on the clinical features and neuroradiological findings, we excluded other forms of Parkinsonism that included (1) dementia with

Lewy bodies (DLB),[27,28] (2) drug-induced Parkinsonism, (3) vascular Parkinsonism, and (4) atypical Parkinsonism with absent or minimal responses to anti-parkinsonian drugs. Thus, the enrolled patients with PD in the present study did not become demented within 1 year of onset of motor manifestations and also lacked any history of visual hallucinations or fluctuating cognitive ability suggestive of a clinical diagnosis of DLB.

One of us (S.K.) diagnosed 96 out of the 106 patients as having sporadic PD, and excluded the remaining 10 patients from the present study.

Assessments

All 96 registered patients underwent the same assessments, including a detailed PD history, family history of neurological diseases, assessment of educational background, drug history, H&Y stage, and UPDRS evaluations for the severity of PD, and a Mini-Mental State Examination (MMSE) of their mental state. The UPDRS, H&Y stage, and MMSE in all patients were evaluated at the Neurology Clinic by S.K. Nondemented PD was defined as patients with a score of ≥24 points on the MMSE based on the *Diagnostic and Statistical Manual of Mental Disorders, 4th ed.* (DSM-IV) criteria for dementia according to a previously reported study.[29] For the estimation of ExD, the BADS (Japanese version) was performed in 71 nondemented PD patients by 3 neuropsychologists (M.I., R.K., and Y.T.) at the Clinic of Psychosomatic Medicine. Since 7 patients demonstrated a wearing-off phenomenon in their parkinsonism, the assessments including the UPDRS, H&Y stage, MMSE, and BADS were undertaken at the time of the on-period of PD. The assessments of the BADS were performed within one week after those of the UPDRS, H&Y stage, and MMSE. The BADS in all patients was rated according to the BADS manual[16] by M.I., R.K. or Y.T.

Statistical Analysis

SPSS software version 12.0 (SPSS, Chicago, Illinois) was used. At the end of May 2006, the statistical analyst (K.H.) at another independent institute collected the data for the clinical parameters from S.K. and those for the neuropsychological parameters from M.M.. ExD was defined as "impaired" when it showed a value of below 70 on the age-controlled standardized score in accordance with the BADS manual.[16,23] Since the SD for that standardized score was 15, this criterion represented two SD below when compared to the age-controlled normal subjects. The level of statistical significance in this study was defined as 0.05.

Estimation of the Inter-Rater Reliability of BADS.

Wilson et al. stated that the inter-rater reliability of the BADS was high based on results for the correlation coefficient of two different raters.[16] We also tested the inter-rater reliability in the present study by estimating statistically the intraclass correlation coefficient using a two-way mixed effects model among the 3 raters. At the beginning of the present study, the above 3 raters assessed the first 15 registered patients independently in order to evaluate the inter-rater reliability of the BADS. The total profile scores, i.e., the sums for the 6 component tasks of the BADS, were compared among these raters.

Assessment of Differences in Each Variable Between Patients With and Without ExD.

Fisher's exact probability test was employed for estimating the difference of sex between patients with and without ExD, for comparing the frequency of ExD patients at different H&Y stages, and for assessing the differences in frequency of drug administration. The Mann-Whitney U test was also performed for estimating the differences of M-values between 2 groups in terms of the age at assessment, duration from onset, educational level, and UPDRS scores.

Multiple Logistic Regression Analysis for Predisposing Factors to ExD in Nondemented PD.

Multiple logistic regression analysis was employed to assess the predisposing factors to ExD in PD. A dichotomous dependent variable of ExD was assigned a value of 1 when the age-controlled standardized BADS score was <70, and 0 when the score was ≥70. The independent variables were assigned as (1) sex (male = 0, female = 1), (2) patient's age at this assessment (years; real number), (3) duration from onset (months; real number), (4) educational level (years; real number), and (6) total score or score on each part of the UPDRS (real number). Since the number of patients at H&Y stage I, which was a reference categorical variable, was too small (only 6 patients), the H&Y stage [variable (5)] was deleted from the present analysis. The UPDRS [variable (6)] was thus utilized for assessment of the severity of PD in the present analysis. Moreover, since 33 out of the 63 enrolled patients with nondemented PD were also examined for their Zung Self-Rating Depression Scale (SDS), multiple logistic regression analysis including the score on the SDS as an independent variable was also undertaken.

Assessment of Differences in Each Profile Score of the 6 BADS Subtests Between Patients With and Without ExD, and Changes in Each Profile Score With Progression of H&Y Stage.

The Mann-Whitney U test was performed to estimate the differences in M-values of the 6 profile scores on the BADS between patients with and without ExD. Moreover, the change in each profile score of the 6 subtests on the BADS according to the progression of the H&Y stage was evaluated from the difference the M-value in the percentage of the maximum profile score among different H&Y stages (Mann-Whitney U test).

RESULTS

Six out of the 10 excluded patients in the present study had possible vascular parkinsonism (4 patients) or DLB (2 patients). In the other 4 patients, the severity of PD could not be evaluated clinically because of disability in the postoperative state following transcervical fracture of the femur (2 patients), and pain due to lumbar compression fracture (1 patient) and osteoporosis (1 patient). Ninety-six patients were thus confirmed with a diagnosis of sporadic PD. Among these 96 patients, 71 patients were diagnosed as having nondemented PD based on the above-mentioned MMSE and DSM-IV criteria. Among the 71 serial patients, 8 were unable to complete the BADS: 5 patients became too fatigued to continue the examination, 2 patients failed to complete because of marked tremor of their right hands, and 1 patient entered the off-period during the examination. The remaining 63 consecutive patients were thus enrolled for the present study. According to the BADS manual, it takes about 30 minutes to complete the examination. However, since the PD patients had motor disability, approximately 1 hour was required.

ExD, defined as less than 70 points on the aged-controlled standardized score in accordance with the BADS manual, was revealed in 13 out of the 63 patients studied (20.6%). The demographic and clinical features of the groups of patients which were separated by scores of <70 and ≥70 on the age-controlled standardized BADS are summarized in Table 1. The 63 nondemented PD patients included 40 males and 23 females [mean age at this assessment, 65.8 ± 9.8 years (range, 46–87), with disease duration 67.1 ± 51.1 months (range, 6–228), and 13.0 ± 2.9 years (range, 6–16) of education].

Inter-Rater Reliability of BADS

The intraclass correlation coefficient for the total profile scores in the first 15 registered patients among the 3 raters was 0.994 (95% CI: 0.985 to 0.998). The clinical

profile of these 15 patients was included: 10 males and 5 females (mean age at this assessment, 64.0 ± 11.1 years (range, 46–87), with disease durations 68.5 ± 50.9 months (range, 12–228) and 13.5 ± 2.6 years (range 9–16) of education].

Differences in Each Variable Between Patients With and Without ExD

The differences in each variable between the patients with and without ExD are summarized in Table 1. The age at assessment with ExD was greater than that without ExD. The frequency of ExD at H&Y stage III was higher than that at stage II. Moreover, the total scores and scores on part II (Activities of daily living; ADL) and part III (Motor examination) of the UPDRS were also higher in the patients with ExD than in those without ExD.

Predisposing Factors to ExD in Nondemented PD

The results of the multiple logistic regression analysis in relation to ExD indicated that the only significant independent variable related to the presence of ExD in PD was the total score on the UPDRS [odds ratio = 1.117 (95%CI: 1.040 to 1.200), $P = 0.0025$]. The other factors including sex, age at assessment, duration from onset, and educational level were not significant. The results for each part of the UPDRS employing multiple logistic regression analysis against the presence of ExD in PD revealed that the only significant independent variable was the score on part II (ADL) of the UPDRS [odds ratio = 1.115 (95%CI: 1.183–3.100), $P = 0.0082$]. The other factors including the UPDRS part I, part III, and part IV were not significant.

Furthermore, the results of the multiple logistic regression analysis including the SDS score in 33 patients also indicated that the only significant independent variable related to the presence of ExD in PD was the total score on the UPDRS [odds ratio = 1.117 (95%CI: 1.036 to 1.209), $P = 0.0145$]. The correlation between the SDS score and aged-controlled standardized score of the BADS in these 33 patients was not significant ($r = 0.263$, $P = 0.197$).

Differences in Each Profile Score of the 6 BADS Subtests Between Patients With and Without ExD, and Changes in Each Profile Score With Progression of H&Y Stage

The results for the differences in each profile score of the 6 subtests on the BADS between patients with and without ExD are summarized in Table 2. Every profile score of the 6 subtests on the BADS in the ExD patients was significantly lower than that in those without ExD. Since the clinical severity of PD, which was indicated by

TABLE 1. *Demographic and clinical characteristics of the nondemented PD patients studied*

Parameter	Age-controlled standardized score of BADS		Statistical methods and results	P value
	<70 (ExD) (number of patients: 13)	≥70 (number of patients: 50)		
(1) Male (%)	8 patients (61.5)	32 patients (64.0)	Frequency of males between the 2 groups[a]	NS
(2) Age at assessment (years) (minimum, mean, median, and maximum)	58, 73.6, 74, 87	46, 63.7, 67, 81	Difference of mean values[b]: patients with ExD were older than patients without ExD	0.0033[c]
(3) Months after onset (minimum, mean, median, and maximum)	6, 61.2, 52, 156	7, 68.6, 60, 228	Difference of mean values[b]	NS
(4) Educational level (years) (minimum, mean, median, and maximum)	9, 12.9, 12, 16	6, 13.0, 12, 16	Difference of mean values[b]	NS
(5) Hohen & Yahr stage (%)	I: 0 patients (0)	I: 6 patient (12.0)	Frequency of patients with ExD at different stages[a]:	
	II: 1 patient (7.7)	II: 29 patients (58.0)	Stage III was higher than stage II	0.0021[c]
	III: 8 patients (61.5)	III: 13 patients (26.0)	Comparisons between other pairs of stages	NS
	IV: 3 patients (23.1)	IV: 2 patients (4.0)		
	V: 1 patient (7.7)	V: 0 patients (0)		
(6) UPDRS (minimum, mean, median, and maximum)			Difference of mean values[b]:	
Total score	35, 51.4, 48, 80	8, 30.4, 28, 62	Patients with ExD exhibited higher scores than patients without ExD	<0.0001[c]
Part I (Mentation, behavior, mood)	0, 3.0, 3, 7	0, 2.0, 2, 7		NS
Part II (Activities of daily living)	9, 17.5, 17, 25	1, 8.7, 8, 20	Patients with ExD exhibited higher scores than patients without ExD	<0.0001[c]
Part III (Motor examination)	16, 28.8, 27, 50	6, 18.6, 16, 38	Patients with ExD exhibited higher scores than patients without ExD	0.0011[c]
Part IV (Complications of therapy)	1, 2.0, 1, 7	0, 1.2, 1, 5		NS
(8) Treatments				
(a) Dopaminergic drugs (%)	11 patients (84.6)	45 patients (90.0)	Frequency of administration between the 2 groups[a]	NS
(b) Anticholinergic drug (%)	1 patient (7.7)	12 patients (24.0)	Frequency of administration between the 2 groups[a]	NS

[a]Fisher's exact probability test.
[b]Mann-Whitney U test.
[c]Statistically significant ($P < 0.01$).
[d]Statistically significant ($0.01 \leq P < 0.05$).
UPDRS, Unified Parkinson's Disease Rating Scale; BADS, behavioral assessment of the dysexecutive syndrome; NS, not significant.

the total score on the UPDRS, was identified as a significant predictor for ExD in the present study, we also assessed the changes in each profile score of the 6 subtests with the progression of H&Y stage. The results for the changes in each profile score among the different H&Y stages are summarized in Figure 1. The profile scores of all 6 subtests decreased with progression of the H&Y stages. However, the patterns of these changes were not uniform among the subcomponents of the executive function. The profile scores for the Rule Shift Cards Test and Action Program Test were relatively preserved at H&Y stage I, whereas those for the other 4 subtests which consisted of the Key Search Test, Temporal Judgement Test, Zoo Map Test, and Modified Six Elements Test tended to be impaired even at H&Y stage I. Another notable finding was that the profile scores for

the Rule Shift Cards Test decreased markedly from H&Y stage I to IV plus V stage.

DISCUSSION

Patient's age, severity of PD, and scores on part III (Motor examination) of the UPDRS have been reported as predisposing factors to ExD in PD based on two-pair comparisons.[5,10,12,14] Our results for the correlation of variables against ExD demonstrated that patient's age, H&Y stage, total score, and scores on parts II and III of the UPDRS represented significant factors. Our findings were thus consistent with these earlier results using two-pair comparisons.[5,10,12,14]

However, PD is a slowly progressive disease that advances with increase in the patient's age. Moreover, since the majority of traditional neuropsychological tests

TABLE 2. *Results for differences in profile score on the BADS between patients with and without executive dysfunction (ExD)*

	Age-controlled standardized score of BADS		Statistical methods and results (Difference of mean values[a])	P value
	<70 (ExD)	≥70		
Profile score on BADS (minimum, mean, median, and maximum)				
Total profile score	7, 9.1, 9, 11	11, 16.5, 17, 23	Patients with ExD exhibited lower scores than patients without ExD in both their total profile score and profile scores for each subtest	<0.0001[b]
Rule Shift Cards test	0, 1.6, 1, 4	0, 3.2, 3, 4		0.0004[b]
Action Program test	0, 2.5, 3, 4	2, 3.6, 4, 5		<0.0001[b]
Key Search test	0, 0.9, 1, 2	0, 2.0, 2, 4		0.0110[c]
Temporal Judgement test	0, 1.5, 2, 3	0, 2.5, 2, 4		0.0028[b]
Zoo Map test	−1, 0.5, 0, 2	0, 2.2, 2, 4		<0.0001[b]
Modified Six Elements test	0, 2.0, 2, 3	0, 3.0, 3, 4		0.0011[b]

[a]Mann-Whitney U test.
[b]Statistically significant ($P < 0.01$).
[c]Statistically significant ($0.01 \leq P < 0.05$).
BADS, behavioral assessment of the dysexecutive syndrome; NS, not significant.

concentrate on building blocks or card sorting, ExD in PD cannot always be readily assessed because of the confounding motor disability.[30] Nevertheless, in view of the fact that ExD constitutes a nonmotor symptom, since part III (Motor examination) of the UPDRS had been reported previously to be a predisposing factor,[5,10,12,14] doubt existed as to whether or not ExD in PD reflected only the difficulty in tasks for executive function due to the motor disability in PD. We therefore evaluated the predisposing factors for ExD by multiple logistic analysis including patient's age and motor disability (part III on the UPDRS) as independent variables. Since other parts of the UPDRS except for part III had not been

estimated in the previous studies, the total score and each part of the UPDRS were examined in the present study. The results obtained revealed first that the total score of the UPDRS was a significant predisposing factor for the presence of ExD. Moreover, they also demonstrated that while part II (ADL) was a significant predisposing factor, part III (Motor examination) was not a significant predisposing factor. These findings suggested that ExD in nondemented PD patients did not predispose to motor disability of PD, but affected the impairment of actual daily life based on wide-range disturbances of ExD.

Since the BADS provides an integrated assessment of ExD, it has the ability to detect many different aspects of
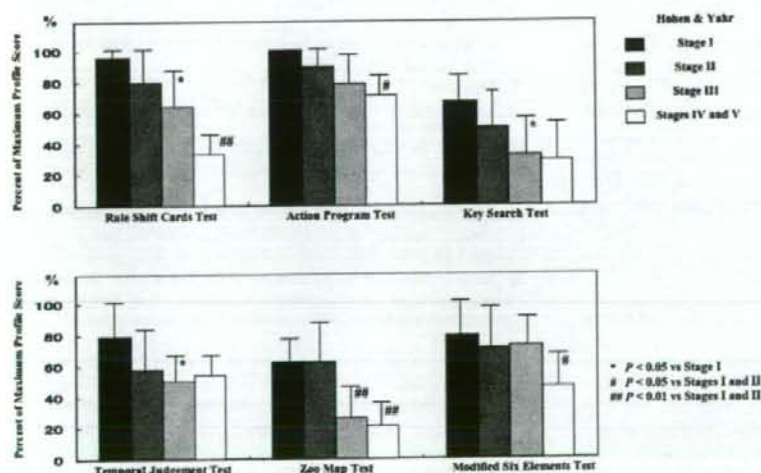

FIG. 1. Mean (and standard deviation) percentage of the maximum possible score on each profile of the BADS for patients with different Hohen & Yahr stages of nondemented PD.

ExD at the same time. The present results for the subtests on the BADS in nondemented PD patients revealed that every profile score of the 6 subtests in the patients with ExD was significantly lower than that in those without ExD. The data for the subtests indicated that ExD in nondemented PD was seen for set shifting, inhibition control, reasoning, planning, and problem solving. Moreover, the impairments of ExD occurred on the test with a time constraint (Modified Six Elements Test) and the test that involved a reaction time measure (Rule Shift Cards Test). These observations support the previous finding that PD patients exhibited a prolonged thinking time.[2,15] Nondemented PD patients thus suffer from many different aspects of ExD.

Although there have been many reported investigations of ExD in relation to the severity of PD, the detailed changes in different subcomponents of ExD in nondemented PD according to the progression of PD are far from being established. One notable result of the present study was that ExD in nondemented PD presented non-uniform findings with different patterns among the subcomponents of the executive function according to progression of the H&Y stage. This might reflect certain differences in the affected lesions and/or disturbance of neurotransmitters among the subcomponents of ExD. In summary, the present study on nondemented PD revealed a wide-range ExD which was related to the severity of PD, although some different patters of progression were evident among the subcomponents of the executive function.

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Impaired Proliferation and Th1 Differentiation of CD4⁺ T Cells of SHPS-1 Mutant Mice

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Background & Aims : SHPS-1 is a transmembrane protein that binds the protein tyrosine phosphatases SHP-1 and SHP-2 through its cytoplasmic region. It is highly expressed on the surface of CD11c⁺ dendritic cells (DCs) and macrophages. We have recently shown that priming of CD4⁺ T cells by DCs is markedly impaired in mice that express a mutant form of SHPS-1 lacking most of the cytoplasmic region. We have now evaluated further the functions of CD4⁺ T cells derived from SHPS-1 mutant mice. **Methods** : The expression of cell surface molecules on CD4⁺ T cells was examined by flow cytometry. The proliferation of CD4⁺ T cells was measured by [³H] thymidine incorporation. Cytokine production by CD4⁺ T cells was measured by ELISA. **Results** : SHPS-1 is expressed at low level on CD4⁺ T cells of wild-type mice. The T cell receptor (TCR)-stimulated proliferation of CD4⁺ T cells from SHPS-1 mutant mice was markedly decreased, whereas the TCR-stimulated production of IL-2 and IFN-γ by these cells was markedly increased, compared with those apparent with wild-type cells. Differentiation of CD4⁺ T cells from SHPS-1 mutant mice into Th1 cells was also impaired. **Conclusions** : Present results suggest that SHPS-1 is essential for proper regulation of CD4⁺ T cell functions.
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Key Words : Th1/Th2 cells, autoimmunity, cell surface molecules, transgenic/knockout mice

Introduction

Src homology 2 domain-containing protein tyrosine phosphatase substrate-1 (SHPS-1),[1] also known as signal-regulatory protein α[2,3] or BIT,[4] is a transmembrane protein whose extracellular region comprises three immunoglobulin (Ig)-like domains and whose cytoplasmic region contains four tyrosine phosphorylation sites that mediate the binding of the protein tyrosine phosphatases SHP-1 and SHP-2. Tyrosine phosphorylation of SHPS-1 is regulated by various growth factors and cytokines as well as by integrin-mediated cell adhesion to extracellular matrix proteins.[5,6] SHPS-1 thus functions as a docking protein to recruit and activate SHP-1 or SHP-2 at the cell membrane in response to extracellular stimuli. CD47

is a ligand for the extracellular region of SHPS-1.[7,8] This protein, which was originally identified in association with $\alpha v\beta3$ integrin, is also a member of the Ig superfamily, possessing an Ig-V-like extracellular domain, five putative membrane-spanning segments, and a short cytoplasmic tail.[9]

Among hematopoietic cells, SHPS-1 is especially abundant in dendritic cells (DCs), macrophages, and neutrophils, being barely detectable in T or B lymphocytes.[8,10-13] In contrast, CD47 is expressed in a variety of hematopoietic cells including red blood cells (RBCs) and T cells.[9] Indeed, the interaction of CD47 on RBCs with SHPS-1 on macrophages is thought to prevent phagocytosis of the former cells by the latter through activation of SHP-1, which forms a complex with SHPS-1.[14-16] Similarly, SHPS-1, through its
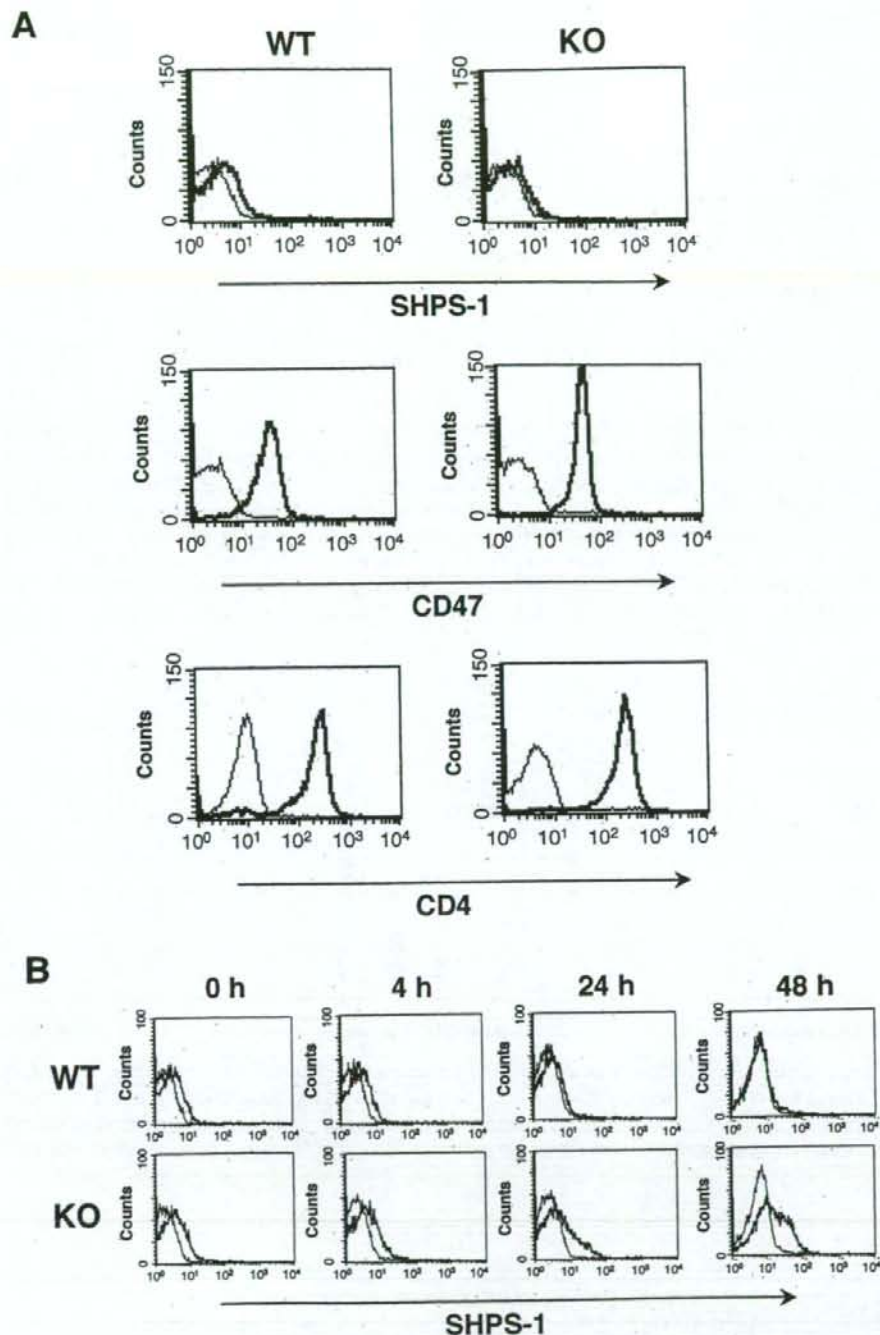
Fig. 1 Flow cytometric analysis of the surface expression of SHPS-1 and CD47 in CD4+ T cells. (A) Freshly purified CD4+ T cells from WT or SHPS-1 mutant (KO) mice were stained with a biotin-conjugated mAb to SHPS-1 (thick trace), a biotin-conjugated mAb to mouse CD47 (thick trace), or a biotin-conjugated control rat IgG (thin trace), as indicated. The cells were then incubated with PE-conjugated streptavidin. Cells were also labeled with an FITC-conjugated mAb to mouse CD4 (thick trace) or an FITC-conjugated control rat IgG (thin trace). All stained cells were analyzed by flow cytometry. (B) Freshly purified CD4+ T cells from WT or SHPS-1 mutant mice were cultured for the indicated times on plates coated with a mAb to CD3 (10μg/ml), after which the cells were stained first with a biotin-conjugated mAb to SHPS-1 (thick trace) or a biotin-conjugated control rat IgG (thin trace) and then with PE-conjugated streptavidin. The stained cells were analyzed by flow cytometry. All results are representative of three separate experiments.