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Journal of Human Genetics (2013) 58, 259–266

www.nature.com/jhg

ORIGINAL ARTICLE

Exome sequencing identifies a novel *TTN* mutation in a family with hereditary myopathy with early respiratory failure

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Myofibrillar myopathy (MFM) is a group of chronic muscular disorders that show the focal dissolution of myofibrils and accumulation of degradation products. The major genetic basis of MFMs is unknown. In 1993, our group reported a Japanese family with dominantly inherited cytoplasmic body myopathy, which is now included in MFM, characterized by late-onset chronic progressive distal muscle weakness and early respiratory failure. In this study, we performed linkage analysis and exome sequencing on these patients and identified a novel c.90263G>T mutation in the *TTN* gene (NM_001256850). During the course of our study, another groups reported three mutations in *TTN* in patients with hereditary myopathy with early respiratory failure (HMERF, MIM #603689), which is characterized by overlapping pathologic findings with MFMs. Our patients were clinically compatible with HMERF. The mutation identified in this study and the three mutations in patients with HMERF were located on the A-band domain of titin, suggesting a strong relationship between mutations in the A-band domain of titin and HMERF. Mutation screening of *TTN* has been rarely carried out because of its huge size, consisting of 363 exons. It is possible that focused analysis of *TTN* may detect more mutations in patients with MFMs, especially in those with early respiratory failure.
Journal of Human Genetics (2013) **58,** 259–266; doi:10.1038/jhg.2013.9; published online 28 February 2013

Keywords: A-band; cytoplasmic body; Fn3 domain; hereditary myopathy with early respiratory failure; HMERF; myofibrillar myopathy; titin; *TTN*

INTRODUCTION

Myofibrillar myopathies (MFMs) were proposed in 1996 as a group of chronic muscular disorders characterized by common morphologic features observed on muscle histology, which showed the focal dissolution of myofibrils followed by the accumulation of products of the degradative process.[1] The clinical phenotype of MFM is characterized by slowly progressive muscle weakness that can involve proximal or distal muscles, with onset in adulthood in most cases. However, other phenotypes are highly variable. Although 20% of patients with MFMs have been revealed to have mutations in *DES*, *CRYAB*, *MYOT*, *LDB* (*ZASP*), *FLNC* or *BAG3*, the major genetic basis of MFMs remains to be elucidated.

Respiratory weakness is one of the symptoms of MFMs. The early or initial presentation of respiratory failure is not a common manifestation of MFMs as a whole, and there are limited reports regarding a fraction of patients with *DES*,[2] *MYOT*[3] or *CRYAB*[4] mutation. In 1993, our group reported a Japanese family with dominantly inherited cytoplasmic body (CB) myopathy,[5] which is now included in MFM. Currently, this family includes 20 patients in five successive generations who show almost homogeneous clinical features characterized by chronic progressive distal muscle weakness and early respiratory failure. However, the underlying genetic etiology in this family was unknown. The aim of this study was to determine the genetic cause in this family. To identify the responsible genetic mutation, we performed linkage analysis and whole-exome sequencing.

MATERIALS AND METHODS

This study was approved by the Ethics Committee of the Tohoku University School of Medicine, and all individuals gave their informed consent before their inclusion in the study.

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Received 23 October 2012; revised 9 January 2013; accepted 10 January 2013; published online 28 February 2013

Clinical information on the family

This family includes 20 patients (13 males and 7 females) in five successive generations (Figure 1). The family is of Japanese ancestry, and no consanguineous or international mating was found. Of all patients, seven underwent a muscle biopsy, and two were autopsied. All of the histological findings were compatible with MFM (see clinical data).

The age of onset ranged from 27–45 years. The most common presenting symptom was foot drop. At the initial evaluations, muscle weakness was primarily distributed in the ankle dorsiflexors and finger extensors. The patients were generally built and showed no other extramuscular abnormalities. In addition to this chronic progressive distal muscle weakness, respiratory distress occurred between 0 and 7 years from the initial onset (average 3.8 years) in seven patients (IV-9, V-2, A, B, E, H, and J) with adequate clinical information. Two patients who had not had any respiratory care died of respiratory failure approximately a decade from the initial onset. The other patients have been alive for more than 10 years (maximum 18 years) but require nocturnal non-invasive positive pressure ventilation. They were 37–58 years of age as of 2012 and able to walk independently with or without a simple walking aid. Although the time at which patients recognized dysphagia or dysarthria varied between 1 to more than 10 years from the initial onset, decreased bulbar functions had been noted at the initial evaluation in most cases. Cardiac function was normally maintained in all patients of the family.

Clinical data

The level of serum creatine kinase was normal or mildly elevated. Electromyography of affected muscles showed a chronic myogenic pattern, and the nerve conduction study did not suggest any neuropathic involvement. Muscle imaging showed focal atrophy in the tibialis anterior, tibialis posterior, extensor hallucis and digitorum longus, peroneal and semitendinosus muscle on initial assessment (Figure 2A), and atrophy became clear in cervical muscles, shoulder girdles, intercostals and proximal limb muscles in the following several years. Upon muscle biopsy, the most common finding was numerous cytoplasmic bodies (CBs), which were found on 7.3% of myofibers in the tibialis anterior of individual E (Figure 2B (a–c)) and 50–80% of intercostals in other cases.[5] Other nonspecific findings were increased variability in the size of myofibers, central nuclei and rimmed vacuoles observed on a few fibers. No strong immunoreaction of desmin was seen in the CBs (Figure 2B (d, e)). An electron microscope examination showed that the regular sarcoplasmic pattern was replaced by abnormal fine filamentous structures, which seemed to attach to the Z-band. CBs were also found in almost all skeletal muscles and some smooth muscles in autopsied cases.[5] Cardiac myofibers also contained numerous CBs in one of the autopsied cases (V-2),[5] although the patient did not present any cardiac complication. The sequence analysis of the coding regions and flanking introns of *DES* and *MYOT* showed no pathogenic mutation in individual E. An array comparative genomic hybridization performed with the Agilent SurePrint G3 Human CGH 1M microarray format in individual A did not reveal any aberrations of genomic copy number.

Linkage analysis

DNA was extracted by standard methods. Linkage analysis was performed on nine family members (A–I in Figure 1; four of them were affected, and the others were unaffected) through genotyping using an Illumina Human Omni 2.5 BeadChip (Illumina, San Diego, CA, USA). We chose single-nucleotide polymorphisms (SNPs) that satisfied all of the following criteria: (1) autosomal SNPs whose allele frequencies were available from the HapMap project (http://hapmap.ncbi.nlm.nih.gov/), (2) SNPs that were not monomorphic among members and (3) SNPs that were not in strong linkage disequilibrium with neighboring SNPs (r2 values <0.9). Then, we selected the first five SNPs from each position of integer genetic distance from SNPs that met the above criteria for the initial analysis. The details were as follows; we chose a SNP closest to 0 cM and the neighboring four SNPs. If the genetic distance of a SNP was the same as that of the next SNP, we considered the genomic position to determine their order. We repeated this process at 1 cM, 2 cM and so on.

We performed a multipoint linkage analysis of the data set (17 613 SNPs) using MERLIN[6] 1.1.2 under the autosomal dominant mode with the following parameters: 0.0001 for disease allele frequency, 1.00 for individuals heterozygous and homozygous for the disease allele and 0.00 for individuals

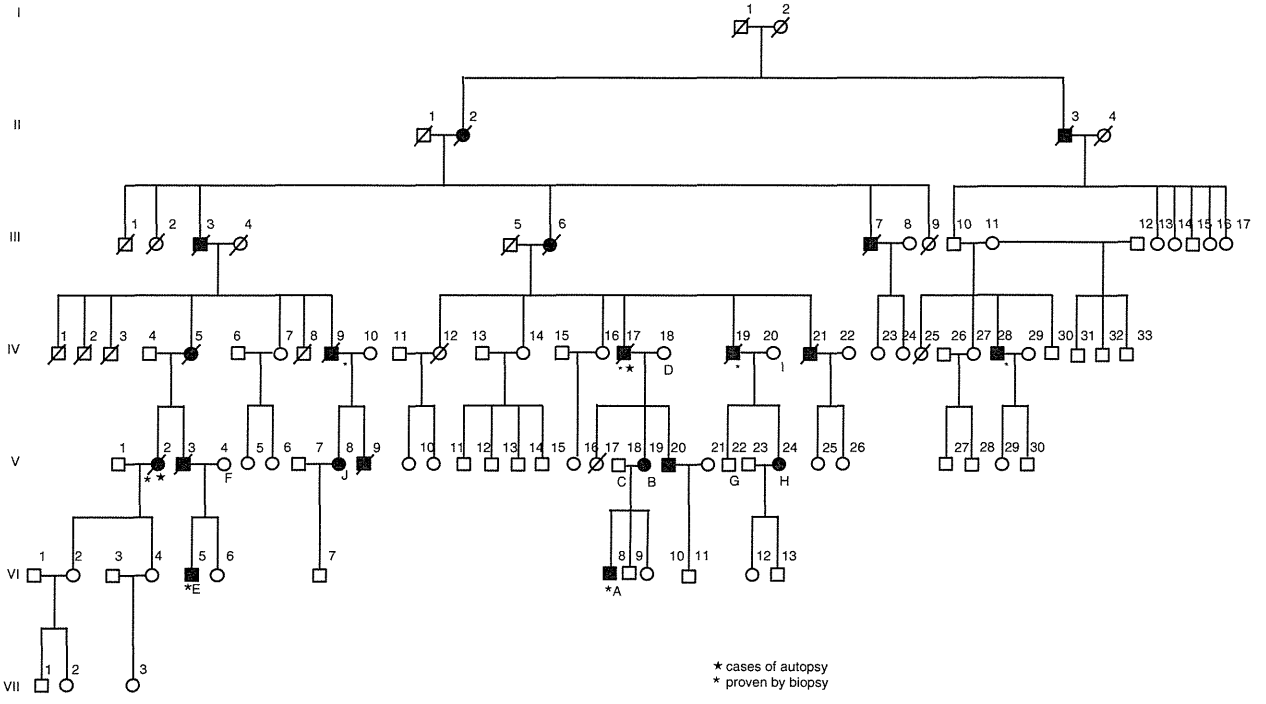


Figure 1 Family pedigree. Filled-in symbols indicate individuals with MFM. Empty symbols indicate unaffected individuals. A star and asterisk indicate autopsy-proven and muscle biopsy-proven cases, respectively. (A–J) indicates individuals whose DNA was used for this study.

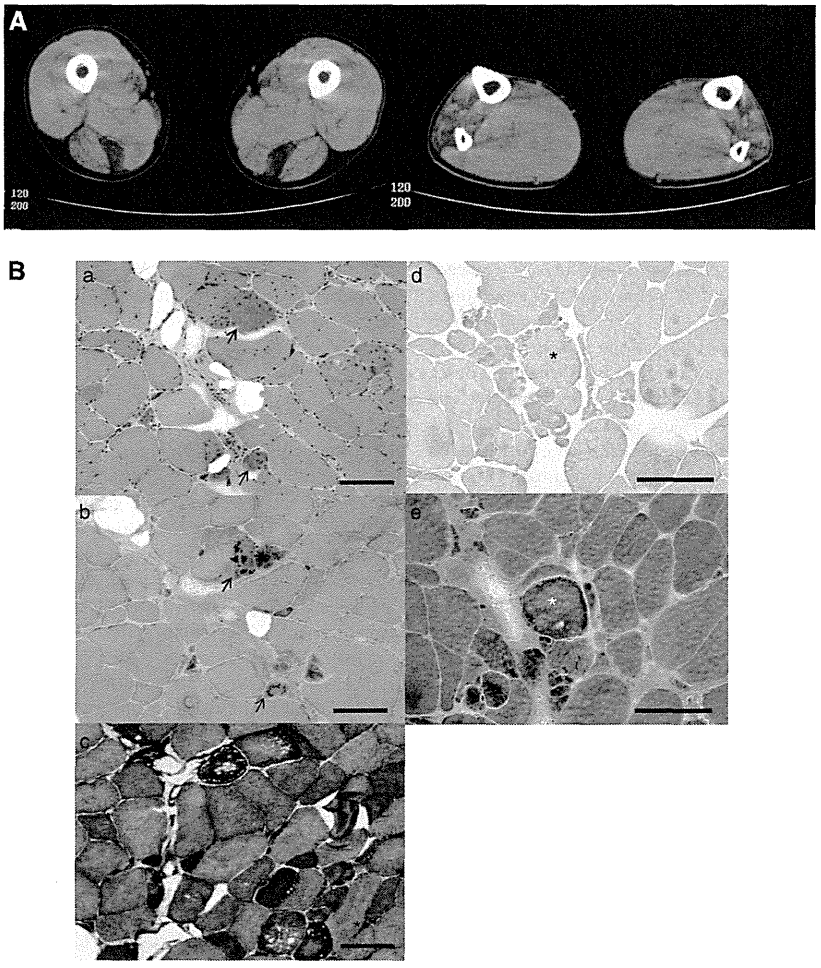


Figure 2 Family clinical data. (**A**) Muscle computed tomography of affected lower extremity. The imaging in the initial assessment of individual A showed symmetrical atrophy and fatty replacement of the semitendinosus in the proximal lower extremities (left) and the tibialis anterior, tibialis posterior, extensor hallucis and digitorum longus, and peroneal muscle in the distal (right) lower extremities. (**B**) Pathology of muscle biopsy. Hematoxylin-eosin (a), Gomori-trichrome (b) and NADH (nicotinamide adenine dinucleotide)-tetrazolium reductase (c) staining of the muscle biopsy sample from the tibialis anterior of individual E are shown. CBs are indicated by arrows. CBs were round or oval, 5–10 μm in diameter and predominantly located in the periphery of type 1 fibers, which stained eosinophilic with hematoxylin-eosin and blue–purple with Gomori-trichrome. NADH-tetrazolium reductase staining showed disorganization of the myofibrillar network. Immunostaining for desmin (d) and Gomori-trichrome staining (e) are serial sections of the muscle biopsy from individual E. Stars indicate corresponding fibers. No strong immunoreaction of desmin was seen in the CBs. Scale bars = 100 μm

homozygous for the alternative allele. After this first analysis, a second analysis was performed with all SNPs fulfilling the above criteria around the peaks identified in the first analysis.

Exome sequencing

Exome sequencing was performed on seven family members in three generations (A–E, H and I in Figure 1), four of whom were affected. Exon capture was performed with the SureSelect Human All Exon kit v2 (individuals E, H and I) or v4 (A–D) (Agilent Technologies, Santa Clara, CA, USA). Exon libraries were sequenced with the Illumina Hiseq 2000 platform according to the manufacturer's instructions (Illumina). Paired 101-base pair reads were aligned to the reference human genome (UCSChg19) using the Burrows-Wheeler Alignment tool.[7] Likely PCR duplicates were removed with the Picard program (http://picard.sourceforge.net/). Single-nucleotide variants and indels were identified using the Genome Analysis Tool Kit (GATK) v1.5 software.[8] SNVs and indels were annotated against the RefSeq database and dbSNP135 with the ANNOVAR program.[9] We used the PolyPhen2 polymorphism phenotyping software tool[10] to predict the functional effects of mutations.

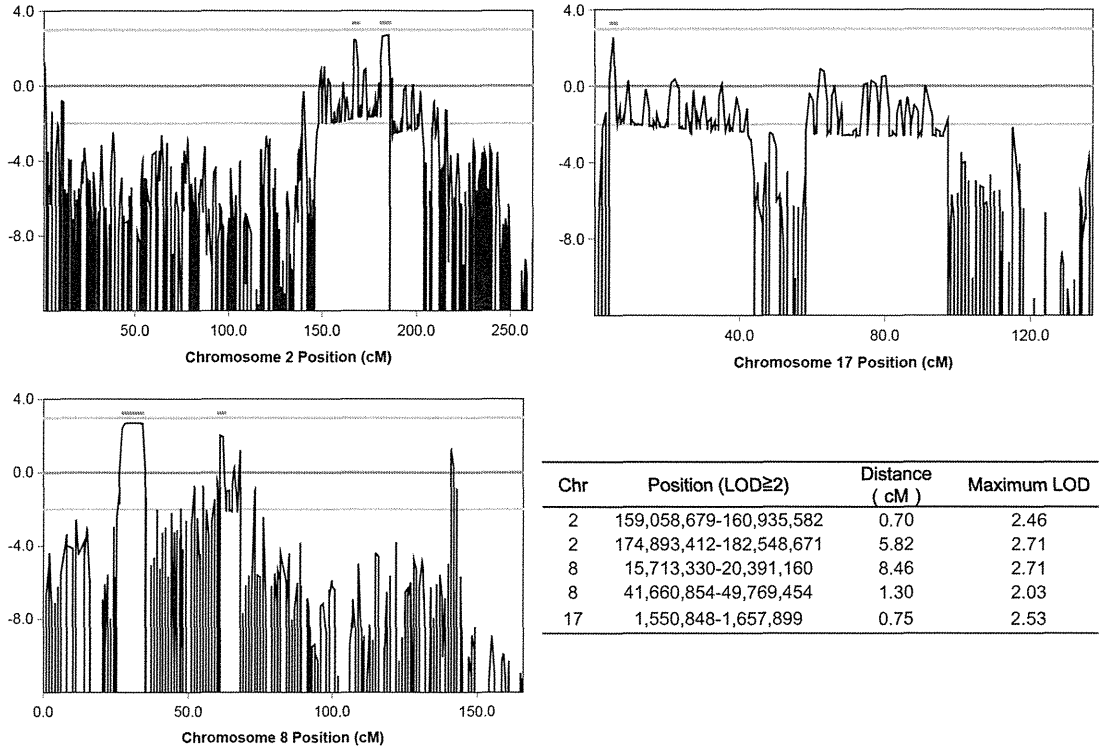
Sanger sequencing

To confirm that mutations identified by exome sequencing segregated with the disease, we performed direct sequencing. PCR was performed with the primers shown in Supplementary Table 1. PCR products were purified with a MultiScreen PCR plate (Millipore, Billerica, MA, USA) and sequenced using BigDye terminator v1.1 and a 3500xL genetic analyzer (Applied Biosystems, Carlsbad, CA, USA).

RESULTS

Linkage analysis

The first linkage analysis identified five regions across autosomes with a logarithm of odds (LOD) score greater than 2 (Figure 3). Of the five regions, two were on chromosome 2 (from 167 cM to 168 cM, with a maximum LOD score of 2.46 and from 182 cM to 185 cM, with a maximum LOD score of 2.71), the other two were on chromosome 8 (from 27 cM to 34 cM, with a maximum LOD score of 2.71 and at 61 cM, with a maximum LOD score of 2.03), and one was on



Chr	Position (LOD≧2)	Distance (cM)	Maximum LOD
2	159,058,679-160,935,582	0.70	2.46
2	174,893,412-182,548,671	5.82	2.71
8	15,713,330-20,391,160	8.46	2.71
8	41,660,854-49,769,454	1.30	2.03
17	1,550,848-1,657,899	0.75	2.53

Figure 3 Linkage analysis. Linkage analysis was performed on nine family members (four of them were affected, the others were unaffected) using an Illumina Human Omni 2.5 BeadChip. Five regions with an LOD score greater than 2 (indicated by bar) were identified. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

Table 1 Summary of detected variants by exome sequencing

Individual *Morbidity*	*A* *Affected*	*B* *Affected*	*C* *Unaffected*	*D* *Unaffected*	*E* *Affected*	*H* *Affected*	*I* *Unaffected*	*Segregated in seven family members*
Exonic, splicing	10089	10064	10079	10065	10230	10194	10216	64
Nonsynonymous, splicing, indel, nonsense	4987	5020	5055	5038	5143	5234	5200	32
Allele frequency not available	577	600	536	555	671	794	786	2

chromosome 17 (at 5 cM, with a maximum LOD score of 2.53). In the second detailed linkage analysis, these peaks were determined to range from 167.49 cM at rs4233674 at position 159 058 679 to 168.19 cM at rs7598162 at position 160 935 582, and from 181.23 cM at rs4402725 at position 174 893 412 to 187.05 cM at rs7420169 at position 182 548 671 on chromosome 2; from 26.42 cM at rs2736043 at position 15 713 330 to 34.88 cM at rs9325871 at position 20 391 160, and from 61.02 cM at rs6999814 at position 41 660 854 to 62.32 cM at rs10957281 at position 49 769 454 on chromosome 8; and from 4.7 cM at rs11078552 at position 1 550 848 to 5.45 cM at rs1057355 at position 1 657 899 on chromosome 17. Haplotypes shared by affected individuals in these regions were confirmed by visual inspection. There were a few incompatible SNPs in these regions, presumably due to genotyping error.

Exome sequencing and segregation analysis

In exome sequencing, an average of 215 million reads enriched by SureSelect v4 (SSv4) and 319 million reads enriched by SureSelect v2 (SSv2) were generated, and 99% of reads were mapped to the reference genome by Burrows-Wheeler Alignment tool. An average of 57% (SSv4) and 61% (SSv2) of those reads were duplicated and removed, and an average of 80% (SSv4) and 66% (SSv2) of mapped reads without duplicates were in target regions. The average coverage of each exome was 163-fold (SSv4) and 130-fold (SSv2). An average of 85% (SSv4) and 69% (SSv2) of target regions were covered at least 50-fold (Supplementary Table 2). On average, 10 133 SNVs or indels, which are located within coding exons or splice sites, were identified per individual (Table 1). A total of 64 variants were common among patients and not present in unaffected individuals, and 32 of those were left after excluding synonymous SNVs. In these variants, only the heterozygous mutation c.90263G>T (NM_001256850) at position 179 410 777 of chromosome 2, which was predicted to p.W30088L in *TTN*, was novel (that is, not present in dbSNP v135 or 1000 genomes). Polyphen2 predicted this mutation as probably damaging. This mutation was located in a candidate region suggested by the linkage analysis in the present study. The other variants were registered with dbSNP135, and the allele frequencies, except for one SNV, rs138183879, in *IKBKB*, ranged from 0.0023 to 0.62.

a

Gene	Chr	Position	Variant type	Reference	Variant	dbSNP v135	Coding change	Prediction	Segregaion analysis
TTN	2	179 410777	Missense	G	T	Not present	W30088L	Probably damaging	Segregating
IKBKB	8	42 188 457	Missense	C	T	rs138183879	T742M	Benign	Non-segregating

b

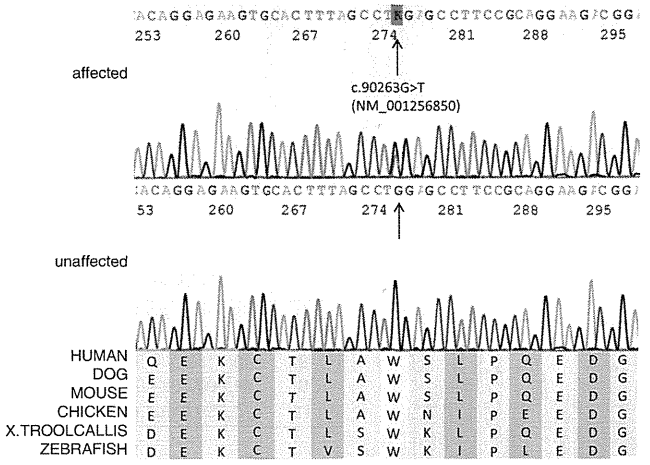


Figure 4 Identified mutations by exome sequencing. (**a**) We performed segregation analysis of two candidates. (**b**) The identified *TTN* mutation and its conservation among species. Sanger sequencing confirmed the heterozygous G to T substitution (indicated by the arrow) at the position chr2;179 410 777, which corresponds to c.90263G>T in exon 293 (NM_001256850.1). The substitution leads to p.W30088L (NP_001243779.1), and this amino acid is conserved among species.

These values were not compatible with the assumption that MFM was a rare disease and showed complete penetrance in this family. The allele frequency of rs138183879 was not available in dbSNP135, and this SNV was in the candidate region on chromosome 8 based on linkage analysis.

We then performed a segregation analysis on the two candidates, the novel mutation c.90263G>T in *TTN* and rs138183879 in *IKBKB*, through Sanger sequencing in 10 family members (A–J in Figure 1; Figure 4a). The rs138183879 SNP was not found in individual J, that is, it was not segregated with the disease in this family. In contrast, the novel mutation c.90263G>T in *TTN* was detected in all patients ($n=5$) and not detected in any of the unaffected family members ($n=5$) or 191 ethnically matched control subjects (382 chromosomes). These results suggested that this rare mutation in *TTN* segregated with the disease in this family.

DISCUSSION

In this study, we found that a novel missense mutation in *TTN* segregated with MFM in a large Japanese family. The identified c.90263G>T mutation in *TTN* (NM_001256850) was considered to be the genetic cause of MFM in our family, because (1) exome sequencing revealed that this was the best candidate mutation after filtering SNPs and indels, (2) this mutation is located in a region on chromosome 2 shared by affected family members, (3) the segregation with MFM was confirmed by Sanger sequencing, (4) this mutation was not detected in 191 control individuals, (5) this mutation was predicted to alter highly conserved amino acids (Figure 4b) and (6) *TTN* encodes a Z-disc-binding molecule called titin, which is similar to all of the previously identified causative genes for MFMs, which also encode Z-disc-associated molecules.

Recently, three mutations in *TTN* have been reported as the causes of hereditary myopathy with early respiratory failure (HMERF, MIM #603689),[11–16] which has similar muscle pathology to MFMs. The identified novel missense mutation c.90263G>T in our study was located on the same exon as recently reported HMERF mutations: c.90272C>T in a Portuguese family[16] and c.90315T>C in Swedish and English families[14,15] (Table 2). This finding suggests the possibility that our family can be recognized as having HMERF from a clinical aspect.

Compared with symptoms described in the past three reports on HMERF (also see Table 2), our patients have common features, such as autosomal dominant inheritance, early respiratory failure, the absence of clinically apparent cardiomyopathy, normal to mild elevation of serum CK and histological findings compatible with MFM. Early involvement of the tibialis anterior is also common, except for the Portuguese family, who reported isolated respiratory insufficiency and a milder presentation of HMERF. Thus, our family shares major clinical manifestations with patients with HMERF, suggesting that the identified mutation is novel for MFM and HMERF.

To date, mutations in *TTN* have been identified in skeletal myopathy and cardiomyopathy.[17,18] The relationship between the variant positions on *TTN* and phenotypes accompanied by skeletal or respiratory muscle involvement is summarized in Table 2. Titin is a large protein (4.20 MDa) that extends from the Z-disk to the M-line within the sarcomere, and it is composed of four major domains: Z-disc, I-band, A-band and M-line (Figure 5). All four HMERF mutations detected by other groups and our study were consistently located in the A-band domain, while mutations in tibial muscular dystrophy (TMD) (MIM #600334),[19–24] limb-girdle muscular dystrophy type 2J (LGMD2J) (#608807)[19,25] and early-onset myopathy with fatal cardiomyopathy (#611705)[26] were located in the M-line domain. HMERF and TMD have some common clinical characteristics, such as autosomal dominant inheritance with onset in adulthood and strong involvement of the tibialis anterior muscle.

Table 2 Previously reported TTN mutations with skeletal and/or respiratory muscle involvement

Phenotype	*LGMD*	*HMERF*	*Our family*	*HMERF*	*HMERF*	*TMD*	*TMD*	*LGMD2J*	*TMD*	*TMD*	*TMD*	*TMD*	*TMD*	*Early-onset myopathy with fatal cardiomyopathy*	*Early-onset myopathy with fatal cardiomyopathy*
Reported by	Vasli *et al.*[16]	Ohlsson *et al.*,[14] Pfeffer *et al.*[15]	Abe *et al.*[5]	Vasli *et al.*[16]	Edstrom *et al.*,[12] Nicolao, *et al.*[11] Lang e *et al.*[13]	Hackman *et al.*[23]	Udd *et al.*,[20] Hackman *et al.*[19]	Udd *et al.*,[25] Hackman *et al.*[19]	Pollazzon *et al.*[24]	Van den Bergh *et al.*[22]	Seze *et al.*,[21] Hackman *et al.*[19]	Hackman *et al.*[23]	Hackman *et al.*[23]	Carmignac *et al.*[26]	Carmignac *et al.*[26]
Mutation identified in Nucleotide (NM_001256850.1)	2012 c.3100G>A, c.52024G>A	2012 c.90315T>C	2012 c.90263G>T	2012 c.90272C>T	2005 c.97348C>T	2008 c.102724delT	2002 102857_ 102867 del11ins11	2002 102857_ 102867 del11ins11	2010 c.102914A>C	2003 c.102917T>A	2002 c.102944T>C	2008 c.102966delA	2008 c.102967C>T	2007 g.289385del ACCAAGTG	2007 g.291297delA
Protein (NP_001243779.1)	p.V1034M, p.A17342T	p.C30071R	p.W30088L	p.P30091L	p.R32450W				p.H34305P	p.I34306N	p.L34315P		p.Q34323X		
Domain	I-band, A-band	A-band (Fn3)	A-band (Fn3)	A-band (Fn3)	A-band (kinase)	M-line	M-line	M-line	M-line	M-line	M-line	M-line	M-line	M-line	M-line
Population Inheritance	French AR	Swedish English AD	Japanese AD	Portuguese AD	Swedish AD	French AD	Finnish AD	Finnish AR	Italian AD	Belgian AD	French AD	Spanish AD	French AD	Sudanese Consanguineous siblings	Moroccan Consanguineous siblings
Onset	35	33–71	27–45	46	20–50s	20–30s	35–55	20–30s	50–60s	47	45	40–50s	30s	Neonatal	Infant-early childhood
Skeletal muscles															
Major	Proximal UL and LL	TA, PL, EDL, ST	TA, ST	No	TA, neck flexor, proximals	TA, GA,HAM, pelvic	TA	All proximals	TA	TA	TA	TA	TA, HAM, pelvic	General muscle weakness and hypotonia	Psoas, TA, GA, peroneus
Minor		Neck flexor	Cervical, shoulder girdles, intercostals, proximal limb			QF				EDL, peroneal, TP	GA, femoral, scapular	HAM, GA	GA, distal UL		QF, proximal UL, neck, facial, trunk flexor
Spared			Facial			Proximal UL	Facial, UL, proximals	Facial		UL, proximal LL	Facial	UL	Proximal UL, QF		
Cardiac muscles	ND	No	No	ND	ND	ND	No	No	ND	ND	ND	ND	ND	DCM, onset; in the first decade	DCM, onset; 5–12 years old
Respiratory failure	ND	Yes, within 5–8 years	Yes, within 7 years	Isolated respiratory failure	Yes, as first presentation	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Muscle pathologic features	ND	Inclusion bodies (major) and RVs (minor)	Cytoplasmic bodies (major) and RVs (minor)	Cytoplasmic bodies	Cytoplasmic bodies, positive for rhodamine-conjugated phalloidin	Dystrophic pattern without vacuoles	Nonspecific dystrophic change	Nonspecific dystrophic change, loss of calpain-3	Dystrophic pattern with RVs	Nonspecific, RV	Nonspecific	Dystrophic pattern with RVs	Nonspecific	Minicore-like lesions and abundant central nuclei	Minicore-like lesions and abundant central nuclei

Abbreviations: AD, autosomal dominant; AR, autosomal recessive; DCM, dilated cardiomyopathy; EDL, extensor digitorum longus; GA, gastrocnemius; HAM, hamstrings; LL, lower limb; ND, not described; no, no involvement; PL, peroneus longus; QF, quadriceps femoris; RV, rimmed vacuole;
ST, semitendinosus; TA, tibialis anterior; TMD, tibial muscular dystrophy; TP, tibialis posterior; UL, upper limb.

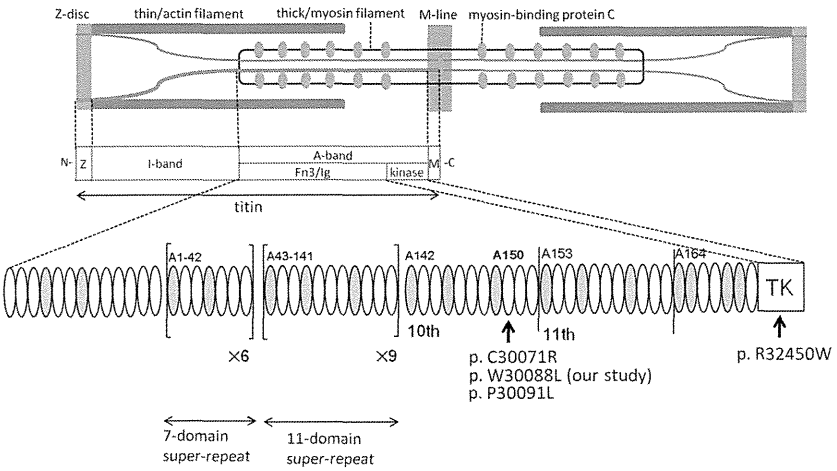


Figure 5 Structure of titin and mutation distribution in the A-band domain. Human *TTN* was mapped to 2q31.2. *TTN* is 294 kb and is composed of 363 exons that code for a maximum of 38 138 amino-acid residues and a 4.20-MDa protein[32] called titin. Titin is expressed in the cardiac and skeletal muscles and spans half the sarcomere, with its N-terminal at the Z-disc and the C-terminal at the M-line.[33] Titin is composed of four major domains: Z-disc, I-band, A-band and M-line. I-band regions of titin are thought to make elastic connections between the thick filament (that is, myosin filament) and the Z-disc within the sarcomere, whereas the A-band domain of titin seems to be bound to the thick filament, where it may regulate filament length and assembly.[34] The gray and white ellipses indicate an Ig-like domain and fibronectin type 3 domain, respectively. Our mutation (p.W30088L) and the neighboring two mutations (that is, p.C30071R and p.P30091L) were all located in the 6th Fn3 domain in the 10th domain of large super-repeats. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

In contrast, one of the distinctive features of TMD is that early respiratory failure has not been observed in patients with TMD. Histological findings of TMD usually do not include CBs but show nonspecific dystrophic change. The underlying pathogenic processes explaining why mutations on these neighboring domains share some similarities but also some differences are unknown.

Three of four HMERF mutations in the A-band domain are located in the fibronectin type 3 and Ig-like (Fn3/Ig) domain, and one of four HMERF mutations is located in the kinase domain (Table 2, also see Figure 5). The missense mutation c.97348C>T in the kinase domain was the first reported HMERF mutation. It has been shown that the kinase domain has an important role in controlling muscle gene expression and protein turnover via the neighbor of BRCA1 gene-1-muscle-specific RING finger protein-serum response transcription factor pathway.[13] Moreover, the Fn3/Ig domain is composed of two types of super-repeats: six consecutive copies of 7-domain super-repeat at the N-terminus and 11 consecutive copies of 11-domain super-repeat at the C-terminus.[27–29] These super-repeats are highly conserved among species and muscles. Our identified mutation (c.90263G>T) and the neighboring two mutations (that is, c.90272C>T and c.90315T>C shown in Table 2) were all located on the 6th Fn3 domain in the 10th copy of 11-domain super-repeat (that is, A150 domain[30]) (Figure 5). Although some Fn3 domains are proposed to be the putative binding site for myosin,[31] the role with the majority of Fn3 domains, how it supports the structure of each repeat architecture, and the identity of its binding partner have not been fully elucidated. Our findings suggested that the Fn3 domain, in which mutations clustered, has critical roles in the pathogenesis of HMERF, although detailed mechanisms of pathogenesis remain unknown.

In conclusion, we have identified a novel disease-causing mutation in *TTN* in a family with MFM that was clinically compatible with HMERF. Because of its large size, global mutation screening of *TTN* has been difficult. Mutations in *TTN* may be detected by massively parallel sequencing in more patients with MFMs, especially in patients with early respiratory failure. Further studies are needed to understand the genotype–phenotype correlations in patients with mutations in *TTN* and the molecular function of titin.

ACKNOWLEDGEMENTS

We thank the patients and their family. We are grateful to Yoko Tateda, Kumi Kato, Naoko Shimakura, Risa Ando, Riyo Takahashi, Miyuki Tsuda, Nozomi Koshita, Mami Kikuchi and Kiyotaka Kuroda for their technical assistance. We also acknowledge the support of the Biomedical Research Core of Tohoku University Graduate School of Medicine. This work was supported by a grant of Research on Applying Health Technology provided by the Ministry of Health, Labor and Welfare to YM, an Intramural Research Grant (23-5) for Neurological and Psychiatric Disorders of NCNP and JSPS KAKENHI Grant number 24659421.

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Supplementary Information accompanies the paper on Journal of Human Genetics website (http://www.nature.com/jhg)

Clinical Neurology and Neurosurgery 115 (2013) 1555–1557

Contents lists available at SciVerse ScienceDirect

Clinical Neurology and Neurosurgery

journal homepage: www.elsevier.com/locate/clineuro

Case report

Autopsy-confirmed progressive supranuclear palsy with decreased uptake of metaiodobenzylguanidine

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ARTICLE INFO

Article history:
Received 4 September 2011
Received in revised form 5 January 2013
Accepted 12 January 2013
Available online 5 February 2013

Keywords:
Progressive supranuclear palsy
MIBG
Pathology

1. Introduction

Progressive supranuclear palsy (PSP) is a clinical syndrome comprising supranuclear gaze palsy, postural instability, and dementia. In clinical practice, it is sometimes difficult to distinguish between PSP, early-stage Parkinson's disease (PD), or multiple system atrophy. In the context of these difficulties, decreased cardiac uptake of metaiodobenzylguanidine (MIBG), a physiological analog of norepinephrine, has been reported in patients with PD and dementia with Lewy bodies [1,2]. Some patients with PSP also show slightly reduced MIBG uptake in comparison to healthy controls [3]; however, pathological analyses of these cases have not been conducted. Here, we investigated a case of autopsy-confirmed PSP with decreased MIBG uptake.

2. Case report

This male patient was age 76 at time of death. He was healthy while employed at an office and there was no family history of neurological disorders. At age 69, he had difficulty in walking and looking downward when he was putting on shoes. At the same time, his family noticed him becoming forgetful. He was initially diagnosed with PD and he received L-DOPA/decarboxylase inhibitor (300 mg/day) without effect. Gradually, his gait became unstable and he fell often. At age 72, he was admitted to our hospital. He showed supranuclear vertical gaze palsy, rigidity of neck and extremities without asymmetry, akinesia, postural instability, and dementia (Mini-mental state examination score: 19/30). He showed no apraxia, agnosia, aphasia, or cerebellar signs. He showed no orthostatic hypotension, but suffered from neurogenic bladder. Brain MRI showed atrophy of the midbrain tegmentum (Fig. 1A). Single-photon emission tomography showed hypoperfusion in the frontal lobe. He was diagnosed with possible PSP according to the National Institute of Neurological Disorders and the Society for PSP criteria [4]. Two years after this diagnosis, the patient could not walk and speak. Hemoglobin A1c level, brain natriuretic peptide level, and cardiothoracic ratio on chest radiography were all within normal limits. Cerebrospinal fluid, electrocardiography, coefficient of variation of the R–R interval, and echocardiography all showed no abnormalities. To confirm whether he had concomitant PD pathology or not, we performed MIBG scintigraphy. MIBG scintigraphy performed one month before his death showed decreased cardiac uptake; the heart-to-mediastinum ratio was 1.71 (mean ratio of control patients in our institute ± SD: 2.23 ± 0.31 [5]) in the early image and 1.32 (2.16 ± 0.41) in the delayed image; washout rate was 45.9% (32.4 ± 7.9) (Fig. 1B and C). Drugs that may affect MIBG uptake, such as tricyclic and tetracyclic antidepressants, serotonin reuptake inhibitors, sympathomimetics, sympatholytics, and calcium channel antagonists, were not administered. Monoamine oxidase inhibitor was administered for one year until the patient was age 75. He died of pneumonia at age 76.

An autopsy was performed 12 h after death. The general autopsy revealed severe pneumonia. There was no myocardial infarction. The brain weighed 1200 g after fixation. Gross examination confirmed mild frontal atrophy. The midbrain tegmentum showed

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0303-8467/$ – see front matter
http://dx.doi.org/10.1016/j.clineuro.2013.01.004

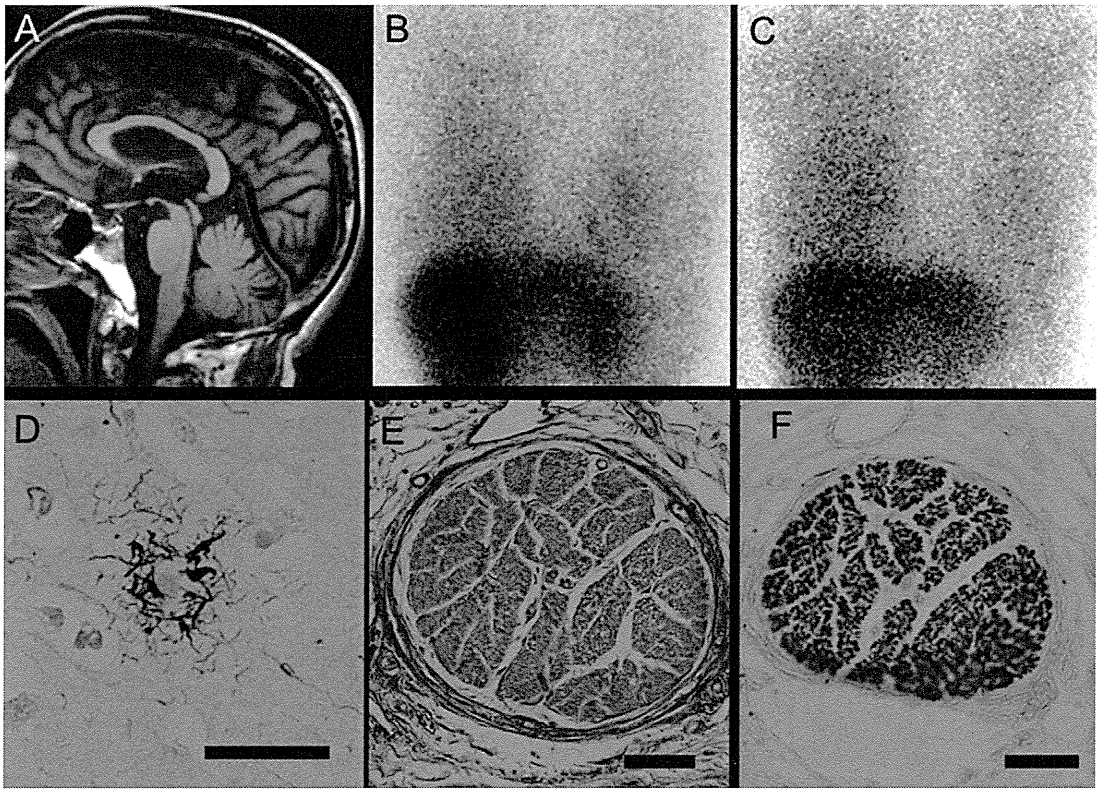

Fig. 1. Clinical images and pathologic findings. (A) Sagittal T2-weighted brain MRI shows severe atrophy of the midbrain tegmentum. The heart-to-mediastinum ratio for the post-injection metaiodobenzylguanidine scan was 1.71 in the early image (B) and 1.32 in the delayed image (C); washout rate was 45.9%. (D) Gallyas–Braak-positive, tuft-shaped astrocytes were present in the putamen; scale bar = 30 μm. Nerve bundles in the left ventricular wall were well preserved as shown by Azan staining (E) and by immunohistochemistry for phosphorylated neurofilament (F); scale bars = 60 μm.

marked atrophy, and the substantia nigra and locus coeruleus showed pigmentation loss. The subthalamic and red nuclei showed atrophy and grayish discoloration. Representative areas of formalin-fixed, paraffin-embedded brain tissue were sectioned and stained with hematoxylin and eosin (HE) and Klüver–Barrera staining. Selected sections were subjected to Gallyas–Braak silver staining. Cardiac left ventricle tissues were sectioned and stained with HE and Azan staining. For immunohistochemistry, we used antibodies to phosphorylated tau (AT8; Innogenetics, Temse, Belgium; 1:1000), β-amyloid 11–28 (12B2; IBL, Maebashi, Japan; 1:1000), phosphorylated α-synuclein (#64; Wako, Osaka, Japan; 1:5000), tyrosine hydroxylase (TH16; Sigma–Aldrich, MO, USA; 1:3000), and phosphorylated neurofilament (SMI-31; Sternberger, Baltimore, USA; 1:10,000). Peroxidase labeling was visualized with diaminobenzidine.

Microscopy showed neuronal loss and gliosis were severe in the globus pallidus, subthalamic nucleus, substantia nigra, dentate nucleus, and inferior olivary nucleus. Tuft-shaped astrocytes were scattered in the putamen, globus pallidus, red nucleus, and superior colliculus (Fig. 1D). Globoid type neurofibrillary tangles (NFT) were prominent in the midbrain, pons, medulla, and dentate nucleus. The neuropathologic diagnosis was definite PSP. Alzheimer-type pathology was minimal (Braak amyloid stage A and NFT stage I). There was no Lewy body–related α-synucleinopathy in the amygdala, dorsal nucleus of vagus, locus coeruleus, substantia nigra, transentorhinal cortex, hippocampus, or anterior cingulate gyrus. Sympathetic ganglia were not collected at autopsy. Nerve bundles of the epicardium and myocardium were well preserved as shown by azan staining and immunostaining for phosphorylated neurofilament and tyrosine hydroxylase (Fig. 1E and F).

3. Discussion

We present a case of autopsy-confirmed PSP with a clinically identified decrease in cardiac MIBG uptake. In Lewy body disease, cardiac sympathetic denervation precedes neuronal loss in the sympathetic ganglia [2] and decreased MIBG uptake has been established as a biomarker of Lewy body pathology *in vivo*. Decreased MIBG uptake has also been reported in some cases of PSP; concomitant PD pathology, involvement of the autonomic nervous system, and cardiovascular events have been postulated as causes [3]. These causes are unrelated to the present case and pathologically the cardiac nerves were well preserved. Functional abnormalities of the cardiac sympathetic nerve terminal may be responsible for the decreased MIBG uptake. For example, in the early scintigraphy image, dysfunction of noradrenergic transporters or monoamine transporters, or antagonism between MIBG and norepinephrine could be responsible for decreased MIBG uptake. In the delayed image, increased exocytosis due to hyperactivity of sympathetic function, reduced reuptake due to dysfunction of monoamine transporters could be responsible for decreased MIBG uptake. Proving the presence of these conditions with the neuropathological methods currently available is difficult; however, it is possible to evaluate cardiac nerve denervation. These conditions may be present in PSP or in only the akinetic mutism state like in the present case. We should examine many more cases of autopsy-proven PSP cases that include MIBG scintigraphy in clinical practice.

4. Conclusion

This case provides important information about the interpretation of MIBG scintigraphy in clinical practice.

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Journal of the Neurological Sciences 331 (2013) 76–80

Contents lists available at SciVerse ScienceDirect

Journal of the Neurological Sciences

journal homepage: www.elsevier.com/locate/jns

Impulsive compulsive behaviors in Japanese Parkinson's disease patients and utility of the Japanese version of the Questionnaire for Impulsive–Compulsive Disorders in Parkinson's disease

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ARTICLE INFO

Article history:
Received 26 January 2013
Received in revised form 4 May 2013
Accepted 10 May 2013
Available online 2 June 2013

Keywords:
Pathological gambling
Compulsive sexual behavior
Compulsive buying
Compulsive eating
Punding
Dopamine dysregulation syndrome

ABSTRACT

Background: In order to evaluate impulsive compulsive behaviors (ICBs), such as pathological gambling, compulsive sexual behavior, compulsive buying, compulsive eating, punding, and dopamine dysregulation syndrome (DDS) in Japanese Parkinson's disease (PD) patients, we constructed a Japanese version of the Questionnaire for Impulsive–Compulsive Disorders in Parkinson's disease (J-QUIP) and evaluated the utility of the J-QUIP in Japanese PD patients.

Methods: J-QUIP was administered to 121 PD patients. Diagnoses of ICBs were made via interview of patients or their caregivers. Subsequently, in order to evaluate risk factors related to these conditions, we evaluated demographic and clinical characteristics, clinical features, and medications utilized.

Results: We were able to administer the J-QUIP to 118 of 121 PD patients (97.5%). Sensitivity and specificity of J-QUIP were similar to that reported for the original version of QUIP. In our study, the actual prevalence of each disorder diagnosed via interview was as follows: pathological gambling (6.5%), compulsive sexual behavior (3.2%), compulsive buying (3.2%), compulsive eating (3.2%), punding (6.5%), and DDS (2.2%). Significantly risk factors for these conditions were younger age ($p = 0.047$), earlier age of disease onset ($p = 0.015$), longer PD duration ($p = 0.001$), total levodopa equivalent dose ($p = 0.006$), and dosage of levodopa ($p = 0.019$).

Conclusions: We evaluated the prevalence of ICBs in Japanese PD patients along with factors associated with these behaviors via J-QUIP.

1. Introduction

Besides classical motor symptoms, patients with Parkinson's disease (PD) also experience non-motor symptoms. These non-motor symptoms affect quality-of-life, institutionalization, and healthcare costs. Certain behaviors associated with PD are produced by chronic treatment with dopaminergic medications. These behaviors are linked by their incentive- or reward-based and repetitive natures [1], and are termed impulsive compulsive behaviors (ICBs). These behaviors include pathological gambling, compulsive sexual behavior, compulsive buying, compulsive eating, punding, and dopamine dysregulation syndrome (DDS). DDS means compulsive medication use. Especially, pathological gambling, compulsive sexual behavior, compulsive buying, and compulsive eating are often called impulsive–compulsive disorders (ICDs) [2]. Although ICBs represent one of the non-motor symptoms of PD, they are not very well identified by patients or family caregivers and are frequently overlooked in the clinical setting. In addition, whereas the prevalence and the risk factors of these behaviors were estimated in recent reports, it has not fully considered. Recently, Weintraub et al. have reported the utility of the Questionnaire for Impulsive–Compulsive Disorders in Parkinson's disease (QUIP), a self-administered questionnaire for impulsive–compulsive disorders [2]. QUIP can also screen punding and DDS. Of note, QUIP was used in Malaysia [3]. In this study, we developed a Japanese version of QUIP (J-QUIP) and evaluated the usefulness of J-QUIP in Japanese PD patients. Furthermore, we estimated the prevalence and the risk factors of ICBs in Japanese PD patients.

2. Material and methods

2.1. Development of J-QUIP

We developed a Japanese version of QUIP, employing procedures accepted internationally [4]. After obtaining permission from the authors of the original version of QUIP to produce the J-QUIP, we translated the original version of QUIP into the Japanese language. Next, the Japanese-translated QUIP was re-translated into English

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http://dx.doi.org/10.1016/j.jns.2013.05.013

by a person unassociated with the first translation. Finally, we asked the original authors whether this back-translated version preserved the same meanings as the original, and this ultimately resulted in the J-QUIP. Although the original version of QUIP included both a full version questionnaire and a short version questionnaire, we employed only the short version questionnaire since no differences exist in the test sensitivity between these two versions [2].

2.2. Subjects

The subjects for this study comprised 121 consecutive patients diagnosed with idiopathic PD in the Department of Neurology at Tottori University Hospital between April 2011 and December 2011. The clinical diagnosis of PD was based on the UK PD Society Brain Bank criteria [5]. Demographic and clinical characteristics are as follows: Age (70.5 ± 9.7 years), Sex (male: female = 56: 65), PD duration (9.9 ± 7.3 years), and Hoehn–Yahr scale (2.6 ± 0.9). We evaluated demographic and clinical characteristics of each group, such as sex, age, age of disease onset, PD duration, degree of severity of motor symptoms, non-motor symptoms (depression, apathy, sleep disturbance, excessive daytime sleepiness, REM sleep behavior disorder, restless leg syndrome, fatigue, orthostatic hypotension, constipation, visual hallucination, and olfactory dysfunction), medications (total levodopa equivalent dose (LEDs) [6], dopamine agonist-only LEDs, levodopa, pramipexole, ropinirole, selegiline, and amantadine), heart-mediastinum (H/M) ratio of ^{123}I-meta-iodobezylguanidine (MIBG) myocardial scintigraphy [7], and amount of activity in actigraphy [8]. We assessed motor symptoms by using the Hoehn–Yahr scale, and certain non-motor symptoms via questionnaires. The employed questionnaires included the Geriatric Depression Scale-15 (GDS-15) [9], the Apathy Scale (AS) [10], the Pittsburgh Sleep Quality Index (PSQI) [11], the Japanese version of the Epworth Sleepiness Scale (JESS) [12], the REM Sleep Behavior Disorder Screening Questionnaire (RBDSQ) [13], and the Parkinson Fatigue Scale (PFS) [14]. GDS-15 has been validated for the diagnosis of depression, with the cut-off point being 5/6. AS has also been validated for apathy, with the cutoff point being 15/16. The cutoff value for the PSQI for poor sleep was 5/6 points, for the JESS to assess excessive daytime sleepiness was 9/10 points, and for the RBDSQ to detect REM sleep behavior disorder was 5/6 points. In addition, PFS was used to evaluate fatigue, and the cutoff point was 3.3. Regarding restless leg syndrome, fatigue, orthostatic hypotension, constipation, visual hallucination, and olfactory dysfunction, we assessed it positive or negative.

2.3. Diagnosis of ICBs

After administration of J-QUIP, we interviewed the patients directly or by telephone to confirm whether the patient experienced abnormal behaviors related to ICBs. In case we were unable to gather sufficient information from the patient, we also interviewed their caregiver. We diagnosed pathological gambling, compulsive sexual behavior, compulsive buying, compulsive eating, punding and DDS according to various diagnostic criteria as listed in the review by Voon and Fox [1].

2.4. Data analysis

Data analysis was conducted with SPSS for Windows version 18 (Chicago, IL). The results are presented as mean ± standard deviation. Intergroup differences were analyzed using a Mann–Whitney *U* test. Categorical variances were examined using a χ^2 test. We used a level of 95% ($P < 0.05$) as the criterion for statistical significance.

This study was planned and conducted in accordance with the Declaration of Helsinki. The Ethics Committee of the Tottori University Faculty of Medicine approved the study prior to its implementation.

3. Results

3.1. Validation of J-QUIP and prevalence of ICBs

We were able to administer the J-QUIP to 118 of 121 PD patients (97.5%), almost all within 5 min. Three patients rejected the survey due to their severe cognitive impairment. Of the 118 PD patients, 93 patients were able to confirm whether they experienced symptoms of ICBs via interviewing directly or by telephone. 25 patients were not able to confirm because we couldn't contact them. So, we decided to turn these 93 patients into this study (Fig. 1).

The prevalence of QUIP positivity in our patients was as follows: pathological gambling (14.0%), compulsive sexual behavior (14.0%), compulsive buying (10.8%), compulsive eating (10.8%), punding (16.1%), and DDS (18.3%) (Fig. 1).

The actual prevalence of ICBs which is determined by interview for patients was indicated in below: pathological gambling (6.5%), compulsive sexual behavior (3.2%), compulsive buying (3.2%), compulsive eating (3.2%), punding (6.5%), and DDS (2.2%) (Fig. 1 and Table 1). Regarding DDS, DDS was highest QUIP positive, but only 2 of 17 PD patients actually experienced compulsive medication use. Overall, 21.5% of PD patients had a history of at least one ICB and 12.9% of PD patients had a history of at least one ICD. In addition, we detected 2 patients (2.2%) who concealed their ICBs (one is pathological gambling, the other is punding) from their caregiver's interview.

Based on these results, we validated the utility of J-QUIP. We calculated sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for each behavior via diagnostic concordance rate between actual diagnosis and result of J-QUIP. These are shown in Table 2.

3.2. Risk factors for ICBs

We evaluated risk factors for actual ICBs diagnosed via interview.

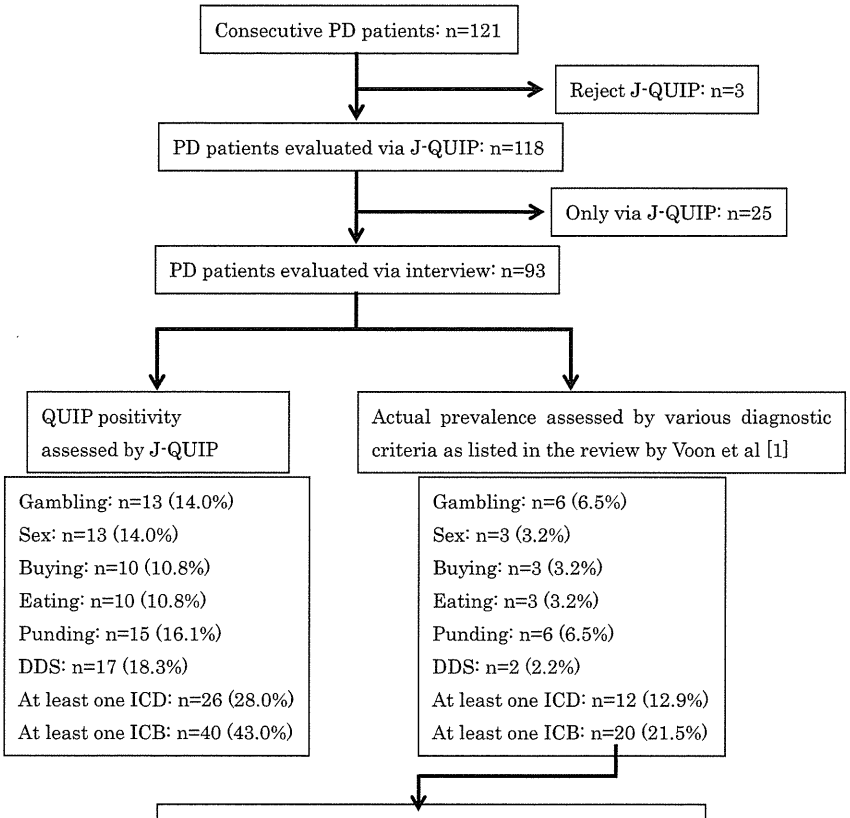
Regarding demographic features, younger age ($p = 0.047$), earlier age of disease onset ($p = 0.015$), and longer PD duration ($p = 0.001$) were all related to ICBs. Gender was unrelated to these conditions (Table 3).

Regarding motor symptoms, Hoehn–Yahr scale scores were not related to ICBs. Regarding non-motor symptoms, none of the symptoms studied, including depression, apathy, sleep disturbances, excessive daytime sleepiness, REM sleep behavior disorder, restless leg syndrome, fatigue, orthostatic hypotension, constipation, visual hallucination, or olfactory dysfunction, were related to ICBs (Table 3).

Regarding dopamine replacement therapy as medications, LEDs ($p = 0.006$) and dosage of levodopa ($p = 0.019$) were related to the prevalence of ICBs. Mean dose of dopamine agonist-only LEDs in the PD patients with ICBs was higher than that in the PD patients without ICBs, but the difference did not reach statistical significance. Other medications, such as dosage of pramipexole, dosage of ropinirole, dosage of selegiline, or dosage of amantadine, were not related. The use of dopamine agonists (any one of pramipexole, ropinirole, cabergoline, bromocriptine, or pergolide) was not also statistically related to the presence of ICBs (Table 3).

4. Discussion

In this study, we created a Japanese version of QUIP, which represents a screening questionnaire for ICBs. We also evaluated the usefulness of this test since the sensitivity and specificity of J-QUIP share similar detection rates as the original version of QUIP (Table 2). Previously, the Japanese versions of BIS-11 (Barratt Impulsive Scale-11) [15], SOGS (South Oaks Pathological gambling Screen) [16], and MOCI (Maudsley Obsessional–Compulsive Inventory) [17] have been used for the screening of impulsivity in Japan. In addition, the MIDI (Minnesota Impulsive Disorders Interview) has been used in foreign countries although it has



ICBs of each patient (* Patients who concealed their ICBs)

No.	ICBs	7	Gambling	14	DDS
1	Gambling, Buying	8	Punding	15*	Gambling
2	Gambling, Eating	9	Eating	16	Punding
3	Eating	10	Punding	17	Punding
4	Gambling, Buying	11	DDS	18*	Punding
5	Punding	12	Sex	19	Gambling
6	Sex	13	Sex	20	Buying

Gambling=pathological gambling, Sex=compulsive sexual behavior
Buying=compulsive buying, Eating=compulsive eating

Fig. 1. Diagnostic flow chart for this study.

not been employed in Japan to our knowledge. However, these questionnaires assess limited symptoms and do not assess all types of ICBs, and these questionnaires were not developed specifically for PD. In contrast, QUIP can evaluate a comprehensive range of ICBs, such as pathological gambling, compulsive sexual behavior, compulsive buying, compulsive eating, punding, and DDS. Furthermore, QUIP was designed for PD patients. Therefore, it is likely that QUIP represents the most suitable questionnaire for PD patients in evaluating their ICBs.

Table 1
Prevalence of symptoms.

Report	Area	Gambling	Sex	Buying	Eating	ICDs	Punding	DDS	ICBs
Several authors [18]	Western	1.7~ 6.1%	2.0~ 10.0%	0.4~ 5.7%	2.4~ 4.3%	6.1~ 13.6%	1.4~ 14.0%	3.4~ 4.3%	–
Weintraub D et al. [19]	Western	5.0%	3.5%	5.7%	4.3%	13.6%	–	–	–
Lee JY et al. [20]	Korea	1.3%	2.8%	2.5%	3.4%	10.1%	4.2%	–	–
Chiang HL et al. [18]	Taiwan	1.49%	2.99%	0.00%	0.37%	4.48%	0.37%	1.12%	–
Fan W et al. [21]	China	0.32%	1.92%	0.00%	0.32%	3.21%	–	0.64%	–
[a]Lim SY et al. [3]	Malaysia	2.6%	8.2%	3.6%	8.7%	15.4%	13.8%	2.0%	24.6%
Present study	Japan	6.5%	3.2%	3.2%	3.2%	12.9%	6.5%	2.2%	21.5%

Gambling = pathological gambling, Sex = compulsive sexual behavior.
Buying = compulsive buying, Eating = compulsive eating.
[a] QUIP positivity (results from assessment only by QUIP).

Table 2
Validation of the J-QUIP.

		Gambling	Sex	Buying	Eating	Punding	DDS
Weintraub et al. study [2]	Sensitivity	91%	95%	80%	86%	63%	–
	Specificity	95%	90%	91%	85%	93%	–
	PPV	59%	48%	38%	21%	50%	–
	NPV	99%	100%	99%	99%	96%	–
Present study	Sensitivity	83.3%	100%	100%	100%	83.3%	100%
	Specificity	90.6%	100%	92.0%	92.0%	88.2%	81.7%
	PPV	38.5%	100%	30.0%	30.0%	33.3%	11.8%
	NPV	98.7%	100%	100%	100%	98.7%	100%

Gambling = pathological gambling, Sex = compulsive sexual behavior.
Buying = compulsive buying, Eating = compulsive eating.

The sensitivity and specificity for each ICB for J-QUIP were all over 80%, while NPVs for each ICB were close to 100%, demonstrating the utility of J-QUIP as a screening questionnaire in daily practice. J-QUIP is also a rapid screening questionnaire as it takes only 5 min to complete. However, it is necessary to interview patients who are screened as positive (in order to confirm the diagnosis of ICBs) because of the low PPVs and high false-positive rates for ICBs. If patients are screened as being negative, it strongly suggests that they do not have a history of ICBs. Furthermore, we need to note that some patients are screened as negative because they may conceal their symptoms. If symptoms are concealed, they are not detectable by the questionnaire. Indeed, at least 2 patients (2.2%) concealed their symptoms in our study.

The prevalence of ICBs in PD patients has also been reported in areas outside of Japan (Table 1). The prevalence of ICBs in our Japanese PD patients is similar to those reported in Western countries [18], although the prevalence was greater than that reported for other Asian countries, particularly Taiwan and China [18,21]. Although the reasons for these differences in prevalence are unclear, the frequency of using dopamine agonist therapy might account for the differences. Several studies have revealed that treatment with dopamine agonists is associated with the development of ICBs in PD patients [22,23,24,25]. The frequency of dopamine agonist utilization is much less in Taiwan and China than in Japan, Korea, or Western countries [21]. The prevalence of ICBs in Korean PD patients was almost the same as that in our Japanese patients, while only the prevalence of pathological gambling in Korean patients was much less compared to our Japanese patients [20]. In addition, the prevalence of pathological gambling in Japanese patients was somewhat higher than observed in Western patients. Pachinko (pinball) was reported to be the most prevalent avenue for pathological gambling in Japan [26]. In this study, almost all patients who experienced pathological gambling also played Pachinko. Thus, one possible reason why the prevalence of pathological gambling is high in Japanese PD patients is that it is influenced not only by medication, but also by environmental factors including easy access to Pachinko.

Previous studies have indicated that numerous factors including young age, early age of disease onset, longer duration of disease, dopamine agonist medications, pramipexole use, amantadine use, LEDs, and dosage of levodopa are related to ICBs in PD [22,23,24,25,27,28]. In our study, younger age, earlier age of disease onset and longer PD duration were related to ICBs. In addition, LEDs and dosage of levodopa were related to ICBs. Regarding dopamine agonist therapies, there were no significant differences in the frequency of dopamine agonist use between PD patients with ICBs and those without, but dopamine agonist-only LEDs ($p = 0.057$) might have some part of relation with ICBs. Regarding non-motor symptoms, depression was found to be associated with ICBs in PD [24,27,28], although relationships with non-motor symptoms are less reported than relationships with demographic factors and medications. In our study, no non-motor symptoms were related to control and repetitive behaviors. So, it seems likely that non-motor symptoms have little to no association with ICBs.

There are a few limitations of the current study to note. First, since we diagnosed ICBs based on interviews of patients and their caregivers, we might have overlooked symptoms when the patients failed to disclose

Table 3
Factors related to ICBs.

	Overall	ICBs group	Non-ICBs group	Statistical value
Demographic factors	n = 93	n = 20	n = 73	
Male:Female	46:47	12:8	34:39	p = 0.322*
Age (years)	69.3 ± 9.8	66.3 ± 7.6	70.2 ± 10.1	p = 0.047
Age of disease onset (years)	59.7 ± 11.9	53.0 ± 9.2	61.5 ± 11.9	p = 0.015
PD duration (year)	9.6 ± 6.6	12.9 ± 8.3	8.7 ± 5.9	p = 0.001
Motor, non-motor symptoms				
Hoehn–Yahr scale	2.6 ± 0.9	2.6 ± 0.8	2.6 ± 0.9	p = 0.851
MIBG (early image)	1.67 ± 0.39	1.61 ± 0.32	1.69 ± 0.40	p = 0.393
MIBG (late image)	1.50 ± 0.42	1.47 ± 0.40	1.51 ± 0.42	p = 0.615
Actigraphy	263 ± 190	216 ± 167	284 ± 200	p = 0.164
Depression(n)	60(65%)	11(55%)	49(67%)	p = 0.802*
Apathy(n)	58(62%)	9(45%)	49(67%)	p = 0.116*
Sleep disturbance(n)	49(53%)	14(70%)	35(48%)	p = 0.128*
Excessive daytime sleepiness(n)	30(32%)	9(45%)	21(29%)	p = 0.186*
REM sleep behavior disorder(n)	36(39%)	11(55%)	25(34%)	p = 0.121*
Restless leg syndrome(n)	14(15%)	4(20%)	10(14%)	p = 0.491*
Fatigue(n)	49(53%)	14(70%)	35(48%)	p = 0.130*
Orthostatic hypotension(n)	42(45%)	11(55%)	31(42%)	p = 0.447*
Constipation(n)	70(75%)	15(75%)	55(75%)	p = 1.000*
Visual hallucination(n)	25(27%)	6(30%)	19(26%)	p = 0.778*
Olfactory dysfunction(n)	34(37%)	9(45%)	25(34%)	p = 0.436*
Medication				
LEDs	554 ± 470	676 ± 325	520 ± 499	p = 0.006
Dosage of levodopa (mg)	374 ± 379	412 ± 145	366 ± 421	p = 0.019
Dosage of pramipexole (mg)	0.57 ± 1.13	1.10 ± 1.56	0.43 ± 0.94	p = 0.121
Dosage of ropinirole (mg)	1.40 ± 3.18	1.45 ± 3.14	1.39 ± 3.22	p = 0.860
Dosage of selegiline (mg)	1.48 ± 2.13	2.00 ± 2.76	1.33 ± 1.91	p = 0.402
Dosage of amantadine (mg)	18.8 ± 56.6	20.0 ± 54.8	18.5 ± 57.4	p = 0.865
Dopamine agonist-only LEDs	86.4 ± 119.2	139 ± 147	71.6 ± 106.8	p = 0.057
Taking dopamine agonist(n)	52(56%)	13(65%)	39(53%)	p = 0.449*

Mann–Whitney *U* test.
* χ^2 test

them and/or if their caregivers took no notice of them. Therefore, the prevalence in our study may represent a minimum, and it is possible that the actual prevalence was higher than that reported. Second, we could not conduct a multivariate analysis with respect to each ICB due to the small number of patients. Finally, since our study population was only PD patients who were able to visit our hospital, we did not study a true random sample of PD patients.

In conclusion, we verified the usefulness of the J-QUIP as a screening questionnaire for ICBs, and report that the prevalence of ICBs in Japanese PD patients appears to be similar to that reported in patients in Western countries. In addition, some demographic factors as well as medications were associated with the prevalence of ICBs in our study.

Conflict of interest

We have no conflict of interest.

Acknowledgments

We thank Weindraub D for the permission of use QUIP and the staff at the Department of Neurology at Tottori University for their help in recruiting the patients. This work was supported by Grants-in-Aid from the Research Committee of CNS Degenerative Disease, the Ministry of Health, Labour and Welfare of Japan.

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RESEARCH ARTICLE

Pathology and Sensitivity of Current Clinical Criteria in Corticobasal Syndrome

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ABSTRACT: The aim of this study was to investigate corticobasal syndrome with respect to underlying pathologies, the ability of current clinical criteria to detect early stages of disease, and symptoms and signs predicting background pathologies. We retrospectively analyzed the clinicopathological findings from patients with corticobasal syndrome. We also analyzed whether those findings fulfilled the diagnostic criteria for corticobasal degeneration (CBD). Finally, we investigated characteristic clinical features that are specific to each background pathology. Of 10 consecutive autopsied patients who had corticobasal syndrome (mean age ± standard deviation, 67.9 ± 9.3 years; male:female ratio, 6:4), three had corticobasal degeneration pathology, three had progressive supranuclear palsy, three had Alzheimer's disease, and one had atypical four-repeat tauopathy. Nine patients fulfilled Mayo criteria, and all 10 patients fulfilled modified Cambridge criteria at the later stage, but only two patients fulfilled either clinical criteria within 2 years of disease onset. Five patients fulfilled the clinical criteria for possible CBD (p-CBD), and one patient fulfilled the clinical research criteria for probable sporadic CBD (cr-CBD) at the later stage. Only two patients fulfilled the criteria for either p-CBD or cr-CBD within 2 years of disease onset. Although we could not find any predictive characteristic clinical features that were specific to CBD pathology, only patients with progressive supranuclear palsy developed apraxia of eyelid opening and cerebellar ataxia. Myoclonus and memory impairment, especially if they appear at an early stage of the disease, may predict Alzheimer's disease pathology. Sensitivity of the available clinical criteria for corticobasal syndrome was poor within 2 years of disease onset. © 2013 International Parkinson and Movement Disorder Society

Key Words: corticobasal syndrome; corticobasal degeneration; diagnostic criteria

The terminology related to corticobasal degeneration (CBD) is confusing because a constellation of clinical features may be seen in patients with pathologies other than CBD. For example, the clinical features of CBD are observed in other neurodegenerative disorders, such as progressive supranuclear palsy (PSP),[1,2] Alzheimer's disease (AD),[3-5] Pick's disease,[6,7] and frontotemporal lobar degeneration with TAR DNA binding protein 43 (TDP-43)-immunoreactive inclusions (FTLD-TDP)[8]. Several proteinopathies, including tauopathy, amyloidopathy, and TDPopathy, can underlie the same clinical phenotype. Additionally, the topographic distribution of neurodegeneration may dictate the clinical phenotype.[9] Therefore, the term *corticobasal syndrome* (CBS) was proposed to characterize the constellation of clinical features that were initially considered the defining characteristics of CBD, and the use of the term CBD was reserved for the pathological disorder.[10]

Additional Supporting Information may be found in the online version of this article.

This article was published online on 20 NOV 2013. An error in the title was subsequently identified. *Corticobasal* was misspelled. The article has since been corrected.

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Relevant conflicts of interest/financial disclosures: Nothing to report. Full financial disclosures and author roles may be found in the online version of this article.

Received: 27 July 2013; **Revised:** 8 October 2013; **Accepted:** 21 October 2013

Published online 20 November 2013 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mds.25746

However, several issues need to be resolved. First, all of the aforementioned observations were based on reports from patients with CBS from Western populations, and the clinicopathological characteristics of other ethnicities remain to be elucidated. Second, although several clinical diagnostic criteria have been proposed, including those published by Boeve et al. (Mayo Clinic criteria)[10] and by Mathew et al. (modified Cambridge criteria),[11] as well as the diagnostic criteria for CBD,[12] the proportion of patients who satisfy early stage criteria remains unknown. Third, although accurate antemortem diagnoses will become increasingly important for designing future pharmacological trials, the characteristic symptoms or signs that could predict the pathological background of patients with CBS also remain unknown. Here, we analyzed Japanese patients with CBS who satisfied the current clinical criteria at their later disease stages to investigate the background of their pathologies, the sensitivity of these criteria for detection at early stages, and the symptoms and signs that were indicative of their pathologies.

Patients and Methods

Patients

We retrospectively reviewed our institutional database between October 1996 and February 2011 and identified the records of patients who satisfied the clinical criteria for CBS whose bodies were donated to our institute. The diagnosis of CBS was made when the patient met either set of clinical criteria proposed by Boeve et al. (Mayo Clinic criteria)[10] or Mathew et al. (modified Cambridge criteria).[11] We also analyzed whether these patients fulfilled the diagnostic criteria for CBD.[12] All procedures were carried out with the ethical approval of the Ethics Committee of the Niigata University School of Medicine.

Pathological Examination

Brains were fixed with formalin, and multiple tissue blocks were embedded in paraffin. Histological examinations were performed on 4-μm-thick sections with several stains, including hematoxylin and eosin (H&E), Klüver-Barrera, and Gallyas-Braak. These sections also were immunostained with a mouse monoclonal antibody against hyperphosphorylated tau (AT8; Innogenetics, Ghent, Belgium; 1:200 dilution). We assessed neuronal loss with gliosis and the severity of tau pathology in several selected areas, including the cerebral cortices (prefrontal cortex, supplementary motor area [SMA], primary motor cortex, postcentral cortex, and insular cortex), the basal ganglia (globus pallidus, putamen, and caudate), the substantia nigra, and the cerebellum (Purkinje cells and the dentate nucleus). The selected cortical regions were determined by reference to previous studies of CBS.[13] Neuronal loss with gliosis was assessed semiquantitatively with H&E-stained sections and was recorded using a 4-point scale (0, absent; 1, mild; 2, moderate; 3, severe). The numbers of AT8-positive neurofibrillary tangles (NFTs), which included pretangles/tangles, neuropil threads, and glial fibrillary tangles, were assessed using a 4-point rating scale (−, absent or nearly absent; +, sparse; ++, moderate; and +++, numerous). The pathological diagnoses were based on the established consensus criteria for CBD,[14] PSP,[15] and AD.[16] A diagnosis of *atypical tauopathy* was made when pathological findings did not satisfy the above-mentioned criteria despite the presence of neurodegeneration with tau-positive neuronal and glial cytoplasmic inclusions.

Clinical Data Collection

We reviewed the patients' medical records and determined their clinical features. A feature was regarded as present if it appeared at any stage during the clinical course. We defined *sign absent* as cases in which the sign was described as absent in the medical record. We defined *not examined* as cases in which the sign was either not examined or was not described in the medical chart. The clinical features extracted were defined according to a previous report.[11] With respect to levodopa (L-dopa) resistance, the patient and clinician's interpretations of subjective improvement were assessed from the medical records. Patients were also examined for the presence of asymmetric atrophy of the cerebral cortex using magnetic resonance imaging (MRI) and for asymmetric cerebral blood flow using single-photon emission computed tomography (SPECT) in both the left and right hemispheres if they were examined with those neuroimaging tools. We also investigated the proportions of patients who satisfied the Mayo Clinic or modified Cambridge criteria[10,11] as well as the diagnostic criteria for CBD[12] at the early stage (within 2 years of onset) and at the later stage in their illness.

Results

Variety of Background Pathology

We identified 11 patients (seven men and four women) who had a clinical diagnosis of CBS. We excluded one patient because he did not satisfy either the Mayo Clinic criteria or the modified Cambridge criteria.[10,11] None of the patients had a family history of similar symptoms. The male:female ratio was 6:4, the mean ± standard deviation age at onset was 67.9 ± 9.3 years (see Supporting Table 1), and the mean ± standard deviation duration of illness was 6.9 ± 3.3 years. Pathological analyses revealed that, of the 10 patients who had CBS, three had CBD pathology (CBS-CBD), three had PSP (CBS-PSP), and three had AD (CBS-AD) (see Supporting Table 1).

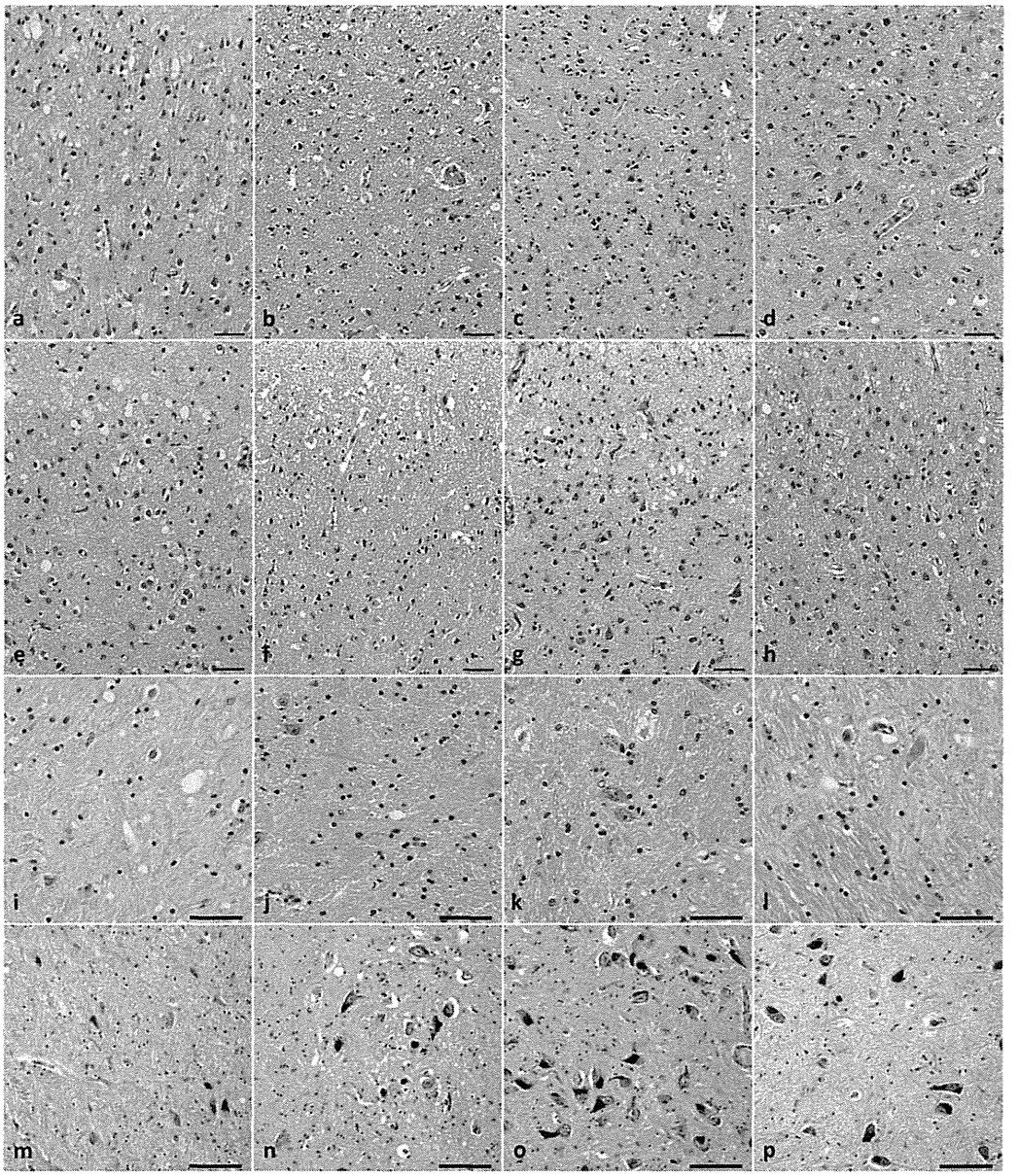

FIG. 1. Hematoxylin and eosin-stained sections show histopathological findings in patients who had corticobasal syndrome with different background pathologies, including neuronal loss with gliosis of (**a-d**) the primary motor cortex, (**e-h**) the supplementary motor area, (**i-l**) the globus pallidus, and (**m-p**) the substantia nigra. Patient 3 (**a,e,i,m**) had corticobasal degeneration, patient 5 (**b,f,j,n**) had progressive supranuclear palsy, patient 8 (**c,g,k,o**) had Alzheimer's disease, and patient 10 (**d,h,l,p**) had atypical tauopathy. Scale bars = 50 μm in **a-l**, 100 μm in **m-p**.

The pathological diagnosis of patient 10 (Supporting Table 1) was an atypical tauopathy that had been reported previously by our institute.[17,18] Her initial symptoms were asymmetrical parkinsonism, muscle weakness, and apraxia, which appeared 2 years after the initial symptoms. The patient exhibited neurodegeneration with widespread neuronal and glial four-repeat tau lesions in the central nervous system, including the upper and lower motor neuron systems. Neuronal loss with gliosis was evident in the primary motor and premotor cortices, including the SMA (Fig. 1d,h), and was less severe in the basal ganglia (Fig. 1l) and substantia nigra (Fig. 1p). AT8-positive and Gallyas-Braak-negative neuronal cytoplasmic inclusions resembling NFTs and atypical astrocytic tau lesions, which were distinct from astrocytic plaques in CBD or tufted astrocytes in PSP, were observed.[17,18]

Common Topographic Distribution of Patients With CBS

We examined the severity of neuronal loss with gliosis in all 10 patients and compared the patients according to pathology subgroup (Supporting Table 1). We