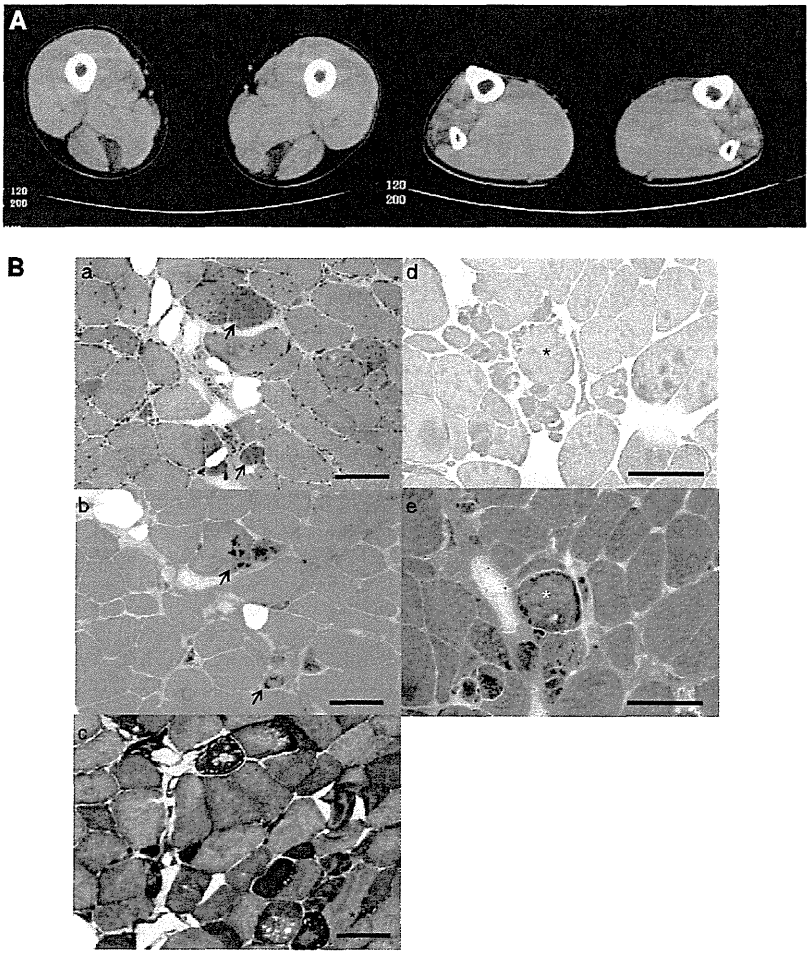



**Figure 2** Family clinical data. (**A**) Muscle computed tomography of affected lower extremity. The imaging in the initial assessment of individual A showed symmetrical atrophy and fatty replacement of the semitendinosus in the proximal lower extremities (left) and the tibialis anterior, tibialis 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.

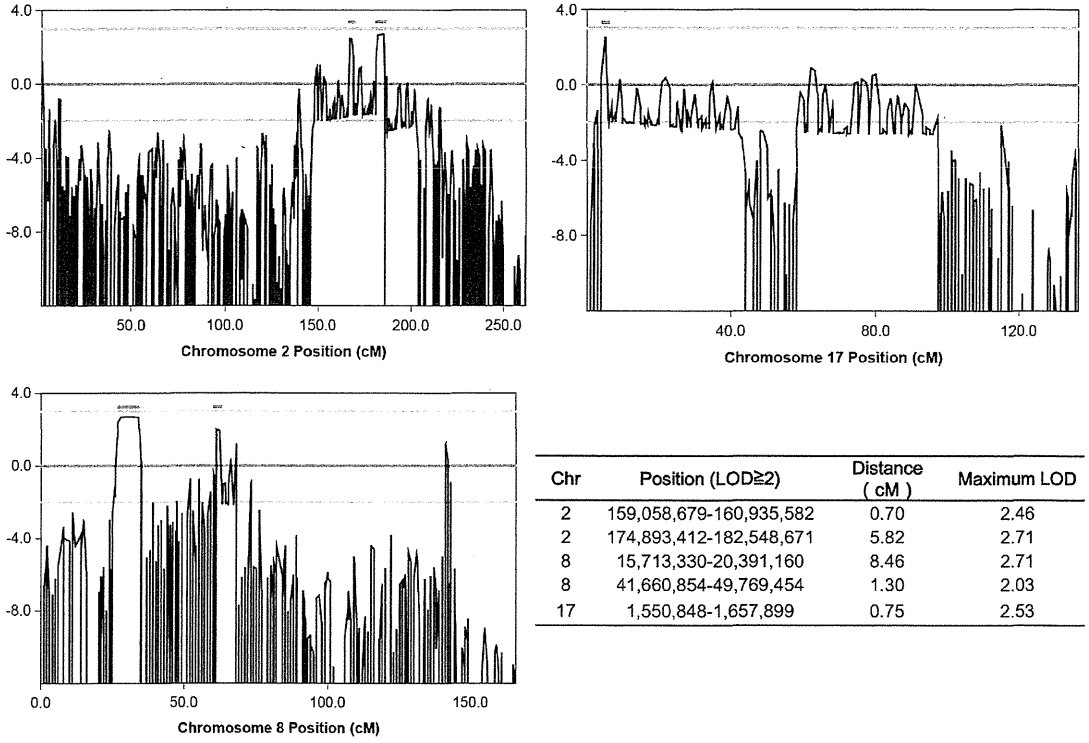
### Sanger sequencing

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*<br>*Morbidity* | *A*<br>*Affected* | *B*<br>*Affected* | *C*<br>*Unaffected* | *D*<br>*Unaffected* | *E*<br>*Affected* | *H*<br>*Affected* | *I*<br>*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**

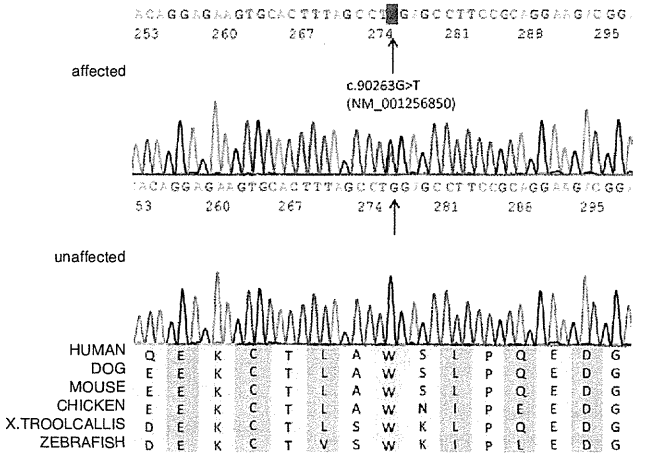



**Figure 4** Identified mutations by exome sequencing. (**a**) We performed segregation analysis of two candidates. (**b**) The identified *TTN* mutation and its conservation among species. Sanger sequencing confirmed the heterozygous G to T substitution (indicated by the arrow) at the position chr2;179 410 777, 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 | French | Swedish English | Japanese | Portuguese | Swedish | French | Finnish | Finnish | Italian | Belgian | French | Spanish | French | Sudanese | Moroccan |
| Inheritance | AR | AD | AD | AD | AD | AD | AD | AR | AD | AD | AD | AD | AD | Consanguineous siblings | 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.

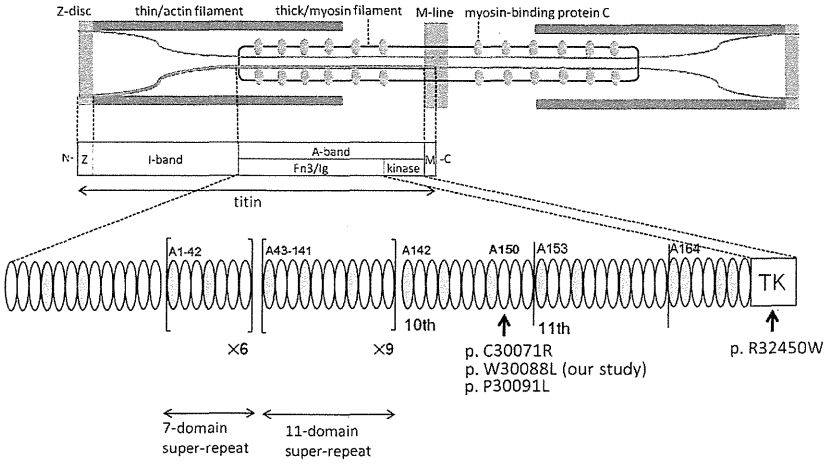



**Figure 5** Structure of titin and mutation distribution in the A-band domain. Human *TTN* was mapped to 2q31.2. *TTN* is 294 kb and is composed of 363 exons that code for a maximum of 38 138 amino-acid residues and a 4.20-MDa protein[32] called titin. Titin is expressed in the cardiac and skeletal 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.

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Short communication

# Comparison of REM sleep behaviour disorder variables between patients with progressive supranuclear palsy and those with Parkinson's disease

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ABSTRACT

*Purpose:* Rapid eye movement (REM) sleep behaviour disorder (RBD) is an important indicator of underlying synucleinopathies. However, the frequency of RBD in tauopathies such as progressive supranuclear palsy (PSP) remains unclear. In this study, we compared RBD-related symptoms and polysomnographic (PSG) findings between patients with PSP and those with Parkinson's disease (PD).
*Methods:* We conducted clinical interviews of 20 patients with PSP, 93 patients with PD and their caregivers regarding RBD-related symptoms, and conducted PSG recordings on all the subject patients. We then compared the clinical backgrounds, PSG parameters, and frequency of RBD-related symptoms between the two groups.
*Results:* PSP patients had more severe symptoms of Parkinsonism and cognitive impairment, and took lower doses of dopaminergic agents compared with PD patients. The PSP group had lower values for both estimated total sleep time and sleep efficiency on PSG compared with the PD group ($p = 0.002$, $p = 0.021$, respectively). The PSP group also included a significantly smaller number of patients having REM sleep without atonia (RWA) compared with the PD group ($n = 5$, 20.0% vs. $n = 56$, 60.2%, $p = 0.003$). None of the PSP patients were experiencing RBD-related symptoms at the time of the investigation, while 30 PD patients (32.3%) had RBD-related symptoms.
*Discussion:* The existence of RWA as well as RBD-related symptoms was less frequent in patients with PSP versus patients with PD. Differences in brain stem pathology and/or disease course between the two disorders might influence this difference.



## 1. Introduction

Recently, several reports have revealed that rapid eye movement (REM) sleep behaviour disorder (RBD) precedes or follows the onset of synucleinopathies such as Parkinson's disease (PD) and dementia with Lewy bodies (DLB), but only a few reports have investigated the prevalence of RBD among patients with tauopathies [1]. It is believed that RBD is rarely co-morbid with tauopathies such as progressive supranuclear palsy (PSP), cortico-basal degeneration or Alzheimer's disease. This belief comes from the idea that RBD is a specific preclinical symptom of synucleinopathies. However, a recent report showed a similar rate of REM sleep without atonia (RWA), an important physiological basis for RBD occurrence [1], in patients with PSP and those with PD [2,3]. Most patients with PSP have degeneration of cholinergic neurons at the level of the pedunculopontine tegmentum (PPT), which plays a primary role in the regulation of REM sleep [4]. Given this, it is possible that patients with PSP are susceptible to RBD. However, there have been no studies on this issue in Japanese patients with PSP. Therefore, we compared the results of polysomnographic (PSG) examination and clinical interviews regarding RBD symptoms between patients with PSP and those with PD.

## 2. Subjects and methods

This study was approved by the ethics committees of Tottori University, and all subjects gave informed consent to take part in the study. We examined patients with PSP or PD who were consecutively hospitalised at the Department of Neurology in Tottori University Hospital from December 2004 to March 2011. Twenty patients (15 male and five female) with PSP (mean age of 75 ± 7 years) and 93 patients (39 male and 54 female) with PD (mean age 73.4 ± 7.9 years old) were targeted. The diagnoses of the disorders were made according to the standard criteria for PD[5] and PSP [6]. The PSP group consisted of 13 patients with probable PSP and seven patients with possible PSP based on information about the period between the onset of vertical supranuclear palsy and prominent postural instability accompanying falls [6]. Moreover, we divided patients with PSP into six sub-types according to the clinical

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features reported by Barsottini et al. [7], that is, Richardson's syndrome (RS), PSP-parkinsonism (PSP-P), pure akinesia with gait freezing (PAGF), PSP-progressive non-fluent aphasia, PSP-cerebellar syndrome and PSP-corticobasal syndrome.

We compared descriptive variables including age at the time of investigation, gender, dose of dopaminergic agents as levodopa dose equivalents (LDEs) [5], length of morbidity, Hohen and Yahr grade and scores on the Mini-Mental State Examination (MMSE) between the patients with PD and those with PSP. We also recorded details related to medications, especially central nervous system-acting drugs that may have influenced PSG measures (including RBD-related variables) in patients with both disorders [8].

Overnight PSG recordings together with video monitoring were performed by standardised methods [5]. During REM stage sleep, submental phasic electromyographic (EMG) activity (defined as 3-s mini-epochs containing phasic twitches that are at least four times higher than the background EMG activity) or submental tonic EMG activity with durations of more than half of a 30-s epoch, were scored as RWA [5].

Systematic interviews were made on the subjects and their caregivers regarding sleep problems, with special focus on dream enactment sleep talking and abnormal motor behaviours while dreaming that occurred within 1 month prior to the investigation. The interviews were conducted by physicians specialising in sleep disorders. Based on the PSG findings and the results of the interviews, we evaluated the existence of RWA on PSG as well as its relationship to nocturnal RBD-related symptoms. We diagnosed a patient as having violent RBD if there were both RWA on PSG and violent dream-enactment behaviour according to the 2nd edition of the International Classification of Sleep Disorders [1]. We also diagnosed non-violent RBD when a patient had RWA and sleep talking without having clear violent behaviour while dreaming, and defined both violent RBD and non-violent RBD as RBD-related symptoms [5].

For statistical analyses between the two groups, we used chi-square tests for categorical variables and Mann–Whitney U tests for continuous variables. Data were expressed as mean ± standard deviation (SD). A $p$-value <0.05 was considered to be statistically significant. The statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS, ver. 15.0J, 2006; SPSS, Tokyo, Japan).

## 3. Results

At the time of investigation, there was no significant difference in age between the patients with PD and those with PSP. However, there was a significantly larger ratio of male patients in the PSP group (M:F ratio = 15:5) compared with the PD group (M:F ratio = 39:54) ($p = 0.007$). Demographic variables and disease-related information on all the patients, including the dose of dopaminergic agents taken expressed as LDEs [5], were examined. The length of PD morbidity was 7.3 ± 6.9 years, their LDEs at the time of the investigation were 314 ± 219 mg day$^{-1}$ and their Hohen and Yahr grade was 2.8 ± 0.8. The MMSE score in patients with PD was 25.9 ± 3.7. The numbers of patients with PD taking clonazepam, antipsychotics and donepezil were 7, 2 and 2 (respectively). The number of patients taking clonazepam and antipsychotics, donepezil and antipsychotics, donepezil and clonazepam, antipsychotics and Yi Gan San and antipsychotics and benzodiazepines other than clonazepam were 2, 1, 1, 1 and 1 (respectively). In comparison, the PSP patients were taking dopaminergic drugs with LDEs of 202 ± 168 mg day$^{-1}$, which was significantly smaller than the doses used in the PD patients ($p = 0.026$). The length of PSP morbidity was 5.4 ± 3.3 years, which was roughly similar to the length of morbidity of PD patients. The Hohen and Yahr grade in patients with PSP (3.9 ± 0.9, $p = 0.001$) was higher than that observed in PD patients, and the MMSE score in patients with PSP was lower than that seen in the PD group (PSP: 20.3 ± 8.1, $p = 0.001$) (Table 1). The number of patients with PSP taking benzodiazepines other than clonazepam, clonazepam and both clonazepam and other benzodiazepines were 2, 1 and 1 (respectively). Sixteen patients with PSP did not take any of these kinds of drugs. There were no patients taking antidepressants in either group at the investigation.

Comparison of the PSG parameters between the two groups showed that the patients with PSP had lower values for estimated total sleep time and sleep efficiency compared with those with PD ($p = 0.002$, $p = 0.021$, respectively, Table 2). There were no significant differences in the percentages of stage 1, stage 2, stage 3/4 or REM stage sleep between the two disorder groups. However, the number of patients having RWA in the PSP group was significantly smaller compared with the PD group (5/20 vs. 56/93, $p = 0.004$). There was also a significant difference in the proportion of RWA/(RWA + REM) between the two patient groups ($p = 0.021$).

**Table 1**
Comparison of descriptive variables between patients with PSP and PD.

| | PSP ($n = 20$) | PD ($n = 93$) | $p$-value |
|---|---|---|---|
| Age (years) | 75 ± 7 | 73.4 ± 7.9 | n.s. |
| Gender (Male/Female) | 15/5 | 39/54 | 0.007 |
| Length of morbidity (years) | 5.4 ± 3.3 | 7.3 ± 6.9 | n.s. |
| Hohen &Yahr grade | 3.9 ± 0.9 | 2.8 ± 0.8 | 0.001 |
| Levodopa dose equivalents (mg/day) | 202 ± 168 | 314 ± 219 | 0.026 |
| Mini Mental State Examination (scores) | 20.3 ± 8.1 | 25.9 ± 3.7 | 0.001 |

Levodopa dose equivalents = Levodopa + Selegiline × 0.75 + Prampexole × 67 + Pergolide × 0.1 + Bromocriptine × 10 + Talipexole × 37.5.
The values are expressed as mean ± SD except for gender.
n.s. = not significant, Mann–Whitney U test.

With regard to PSP sub-types, nine patients were categorised as RS, eight patients as PSP-P and three patients as PAGF. Among these, one patient with RS, two patients with PSP-P and two patients with PAGF had RWA on PSG. There were no significant differences in the proportion of patients having RWA among the three sub-types.

Thirty patients with PD (32.3%) had RBD-related symptoms. Among these, seven patients had violent RBD symptoms and the other remaining 23 patients had non-violent RBD symptoms. Out of 93 PD cases, 26 PD patients with RWA (28.0%) had no experiences of possible RBD-related symptoms. As for the relationship between the clinical course of PD and RBD-related symptoms, 15 patients (15.6%) out of 93 PD cases reported having symptoms from the period before the onset of PD to the time of the investigation. Out of these 15 patients, two patients reported that the RBD symptoms changed from violent symptoms before the onset of PD to non-violent symptoms after the onset of PD. One patient (1.1%) having RBD-related symptoms before the onset of PD reported that the symptoms completely disappeared after the onset of PD. In comparison, 14 patients (14.6%) reported that the symptoms appeared after the onset of PD.

By contrast, none of the PSP patients had symptoms of sleep talking or violent nocturnal behaviour while dreaming. As a result, there was a significant difference between the two disorder groups in terms of the number of patients with RBD-related symptoms ($p = 0.012$).

## 4. Discussion

Previously, Montplaisir et al. reported that patients with PSP had a significantly shorter total sleep time and reduced sleep efficiency compared with controls [9]. Sixel-Doring et al. also reported that

**Table 2**
Comparison of PSG findings between patients with PSP and PD.

| | PSP ($n = 20$) | PD ($n = 93$) | $P$ value |
|---|---|---|---|
| Total sleep time (min) | 244 ± 84 | 312 ± 92 | 0.002 |
| Sleep efficiency (%) | 46.6 ± 16.0 | 56.3 ± 17.4 | 0.021 |
| Stage 1 (%) | 17.7 ± 7.9 | 18.2 ± 7.6 | n.s. |
| Stage 2 (%) | 27.2 ± 13.9 | 33.6 ± 14.7 | n.s. |
| Stage 3 + 4 (%) | 1.4 ± 2.8 | 2.9 ± 5.7 | n.s. |
| Total stage REM (RWA + REM) (%) | 8.9 ± 4.9 | 11.0 ± 8.9 | n.s. |
| Proportion of patients having RWA (%) | 20 (5/20) | 60.2 (56/93) | 0.003 |
| RWA/(RWA + REM) (%) | 10.5 ± 28.2 | 13.1 ± 22.1 | 0.021 |

RWA:REM sleep without atonia. Sleep stage variables are expressed as absolute values and the proportions. A $p$-value <0.05 was considered statistically significant.
n.s. = not significant. Mann–Whitney U test.

polysomnographically recorded sleep was more severely impaired in patients with PSP versus patients with PD [3]. In the present study, patients with PSP had lower values of estimated total sleep time and sleep efficiency versus those with PD. Taking these findings together, PSP is accompanied by disturbances in the initiation and/or maintenance of nocturnal sleep.

When diagnosing RBD, it is necessary to confirm both the existence of RWA on PSG and violent dream-enactment behaviour [1]. In this study, we conducted thorough clinical interviews on all the subject patients and their caregivers regarding RBD-related symptoms. In this regard, only two previous studies examined RWA together with RBD-related symptoms in patients with PSP [2,3], and this study was the third to systematically investigate both PSG findings and clinical RBD symptoms among patients with PSP. The number of our PSP patients was the same as that in the report by Sixel-Doring et al [3].

The percentage of PSP patients with RWA in this study (5/20, 25%) was quite similar to the rate reported by Arnulf et al. (4/15, 27%). In that report, they stated that the percentage of patients with RWA was not different between the PD group and the PSP group. However, the percentage of PD patients with RWA in Arnulf's report (4/15, 27%) [2] was clearly lower than that observed in our study (56/93, 60.2%), which is quite consistent with reported rates of RWA positivity in PD [10]. Comparatively, not only the number of PSP patients with RWA (17/20, 85%) but also the number of PD patients with RWA (19/20, 95%) in Sixel-Doring's reports [3] were strikingly higher than both the present results and Arnulf's reports [2]. The reason for the difference among the studies is unclear. However, in contrast to the study by Montplasir et al., in which none of the PSP patients had RWA on PSG [8], recent studies including the present study demonstrate that a significant number of PSP patients have RWA. We speculate that while a certain number of patients with PSP could have RWA, the rate of PSP patients having RWA is lower relative to RWA occurrence in PD. In addition, our results raise the possibility that even in RWA-positive PSP patients the amount of RWA is lower than that in RWA-positive PD patients.

It has been hypothesised that RWA occurs based on the dysfunction of the locus coeruleus (LC) and the pedunculopontine nucleus (PPN), both of which regulate REM sleep. Warren et al. indicated that cholinergic nerves in PPN are degenerated even in the early stages of PSP [4]. Also, Mann et al. pointed out that neuronal loss in LC is clearly more severe in cases with PD than in cases with PSP [10]. Given these studies, we speculate that a combined degeneration of PPN and LC is important for the formation of RWA, and that the difference in the frequency of RBD between PD and PSP comes from differences in the severity of LC pathology.

Generally, the percentage of PD patients having RBD symptoms ranges from 15% to 34% [11]. Lavault et al. showed that the length of PD morbidity does not correlate with the occurrence of RBD [12]. Of note, none of the patients with PSP in our study had experienced RBD-related symptoms, while the rate of PD patients having symptoms was 32.3%, which is quite consistent with previously reported rates [11]. In addition, no clear relationship between the clinical course of PD and the occurrence of RBD was observed, consistent with the aforementioned report by Lavault et al. [12] In Sixel-Doring's report, the percentage of PSP patients having clinical RBD symptoms (35.0%) was clearly lower than the percentage of PD patients with these symptoms (68.4%) [3]. Thus, it is possible that RBD-related symptoms are less likely to occur in PSP compared with PD even in RWA-positive cases. The reason for this phenomenon is unclear. However, one possible cause is that a higher severity of motor symptoms and/or cognitive impairment, both of which are known to progress more rapidly in PSP versus PD [6], might act to mask the RBD symptoms. As a second possible cause, PSP patients are less likely to have terrifying and uncomfortable dreams, the rate of which is considerably higher in PD populations [13].

The present study has several limitations. First, we confirmed RBD-related symptoms by retrospective interviews with patients and their caregivers. For this reason, it is possible that we might have overlooked the existence of mild RBD-related symptoms. Second, some patients were medicated with drugs that can impact RBD-related symptoms and PSG findings. Third, our study sample included patients from only one institute, and it is possible that a sampling bias existed.

However, conclusively, RBD was commonly observed in PD patients, but the rate of PSP patients having RWA was lower and none of the PSP patients had RBD symptoms. To draw more definitive conclusions about the existence of RWA and RBD-related symptoms in patients with PSP and its relation to PSP sub-types, it would be necessary to conduct a prospective study including neuropathological examination on a large number of patients with the disorder.

## Conflict of interest

The authors have no conflicts of interest pertaining to this study.

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**Original Research Article**

# Epidemiological Survey of Frontotemporal Lobar Degeneration in Tottori Prefecture, Japan

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**Key Words**
Prevalence · Frontotemporal dementia · Progressive nonfluent aphasia · Semantic dementia · Tau gene

**Abstract**
***Background:*** The prevalence of frontotemporal lobar degeneration (FTLD) in Japan is unknown. An epidemiological survey study of FTLD was undertaken in Tottori Prefecture, a district in the western region of Japan. ***Methods:*** Hospitals in Tottori Prefecture were surveyed by a two-step questionnaire in 2010, and the prevalence of FTLD per 100,000 inhabitants was calculated using the actual number of patients and inhabitants in Tottori Prefecture on the prevalence day of October 1, 2010. ***Results:*** In this survey, 66 patients were diagnosed with FTLD. The subtypes of FTLD were as follows: 62 cases of frontotemporal dementia (FTD), 3 cases of progressive nonfluent aphasia, and 1 case of semantic dementia. Among the FTD cases, 5 cases were FTD with motor neuron disease and 1 case was FTD with parkinsonism linked to chromosome 17. The prevalence of FTD in the total population of Tottori Prefecture was 11.2 per 100,000 inhabitants. Based on these results, the prevalence of FTLD in Japan in 2008 was estimated to be 9.5 per 100,000 individuals. ***Conclusions:*** Our epidemiological survey results suggest that there are at least 12,000 FTLD patients in Japan, indicating that FTLD is not a rare disease.



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## Introduction

Frontotemporal lobar degeneration (FTLD) is a neurodegenerative disorder predominantly affecting the frontal and temporal lobes. Two major clinical types are recognized in FTLD: behavioral variant frontotemporal dementia (FTD) and progressive aphasia. The latter is divided into progressive nonfluent aphasia (PNFA) and semantic dementia (SD) [1]. Few epidemiological surveys have been conducted concerning FTLD in Japan, and no epidemiological study focusing particularly on FTLD has been reported. In small community-based studies on the prevalence of dementia in individuals 65 years of age or older, a small percentage of the dementia cases were attributed to FTLD [2, 3]. In clinic-based survey studies, FTLD was the most third frequent cause of early-onset dementia in patients less than 65 years of age [4, 5]. The prevalence of FTLD in Japan is unknown. Here, we report a survey study of FTLD in Tottori Prefecture, the least populated district in Japan.

## Methods

We used a questionnaire to perform a retrospective surveillance study of cases of FTLD in Tottori Prefecture. Tottori Prefecture is located in a rural area of western Japan (fig. 1); it had a population of 587,772 (280,602 males and 307,170 females) on the prevalence day of October 1, 2010.

In 2010, we sent inquiries with registration criteria for each category of FTLD to the departments of neurology and psychiatry in the 47 hospitals in Tottori Prefecture where patients with dementia were treated, asking if they had admitted or examined any cases of FTLD during the past year. We then sent a second questionnaire to the departments who responded affirmatively enquiring about the type of FTLD, sex and age of patients, age of onset, symptoms, neuroimaging results, and treatment. If permission was obtained, board-certificated neurologists (K.W.-I., S.I., S.N., and M.Y.) visited the hospitals to examine the patients.

The diagnosis of FTLD was based on the consensus criteria by Neary et al. [1] and the criteria of the International Behavioural Variant FTD Criteria Consortium [6]. Structural neuroimaging [cerebral computed tomography (CT) or magnetic resonance imaging (MRI)] was performed to support the clinical diagnosis. Functional imaging data [cerebral blood flow evaluated by single-photon emission computed tomography (SPECT)] were obtained, if available. Patients were diagnosed as having probable or possible amyotrophic lateral sclerosis using the El Escorial criteria [7, 8].

The prevalence of FTLD per 100,000 inhabitants and 95% confidence interval (CI) were calculated using the actual number of patients and inhabitants in Tottori Prefecture on the prevalence day, October 1, 2010.

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.

## Results

*Survey Results*

Sixty-six patients were diagnosed with FTLD in Tottori Prefecture on the prevalence day (fig. 2). The subtypes of FTLD were as follows: 62 cases of FTD, 3 cases of PNFA, and 1 case of SD. Among the FTD cases, 5 were FTD with motor neuron disease, and 1 was FTD with parkinsonism linked to chromosome 17 (FTDP-17). Overall, the mean age of patients with

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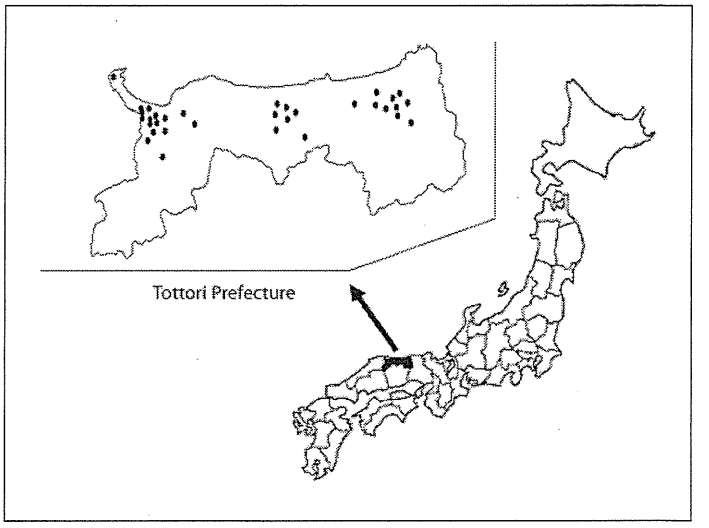


**Fig. 1.** Location of the surveyed hospitals in Tottori Prefecture. Tottori Prefecture is located in western Japan. The marked hospitals and clinics (circles) were included in our survey.

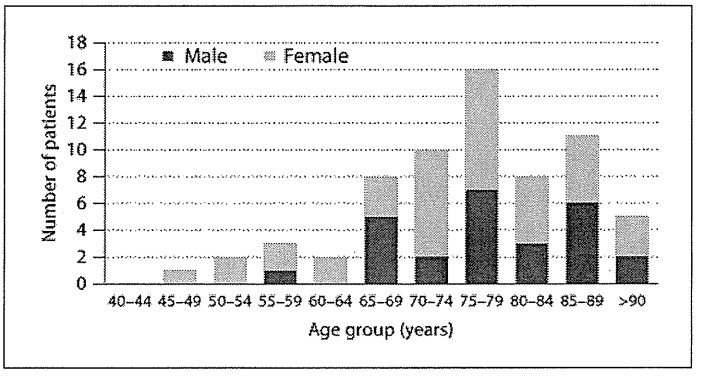


**Fig. 2.** Age-specific number of patients with FTLD.

FTD was 76.5 ± 11.0 years. The mean age of FTD patients with motor neuron disease was 65.8 ± 14.3 years, less than the mean age of FTD patients without motor neuron disease. The mean age of the 3 patients with PNFA was 71.7 ± 8.1 years, and the age of the patient with SD was 72 years. Three patients with FTLD had a family history of the disease. Genetic analysis revealed that the patient with FTDP-17 had an intronic mutation IVS10 C>T in the microtubule-associated protein tau (MAPT) gene.

*Prevalence*

Table 1 shows the age-specific prevalence of FTLD per 100,000 inhabitants of Tottori Prefecture in 2010. The survey results indicate that the overall prevalence of FTLD in Tottori Prefecture was at least 11.2 per 100,000 inhabitants. Based on the demographics of Japan in 2008, the data suggest an estimated prevalence of FTLD in Japan of 9.5 per 100,000 inhabitants, or an overall prevalence of at least 12,000 individuals.

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**Table 1.** Age-specific prevalence estimates of FTLD in Tottori Prefecture

| | Patients, n | Prevalence (95% CI)[1] |
|---|---|---|
| Age group | | |
| 45–54 years | 3 | 4.0 (–5.3 to 8.6) |
| 55–64 years | 5 | 5.6 (0.7–10.5) |
| 65–74 years | 18 | 25.8 (13.9–37.7) |
| 75–84 years | 24 | 40.5 (24.3–56.7) |
| >85 years | 16 | 64.0 (32.6–95.0) |
| Population ≥45 years | 66 | 20.7 (15.7–26.0) |
| Total Population | 66 | 11.2 (8.5–14.0) |

[1] Per 100,000 inhabitants.

## Discussion

We conducted an epidemiological survey focusing on the prevalence of FTLD in Tottori Prefecture of Japan. Our previous epidemiological study in Ama-cho [3], a small island town, revealed that only 1 patient with FTLD was diagnosed among 943 subjects with dementia aged 65 years or older, suggesting that more subjects would be needed to examine the true prevalence of FTLD. Therefore, we decided to carry out an epidemiological survey in Tottori Prefecture, which has a total population of 587,772.

The prevalence of FTLD has also been reported in areas outside of Japan. In population-based studies, the prevalence of FTLD between 45 and 64 years of age has varied from 4.0 per 100,000 individuals in the Zuid-Holland district in the Netherlands to 22 per 100,000 individuals in Brescia, Italy [9–12]. A nationwide hospital-based clinicoepidemiological study in Germany showed a high estimated prevalence of FTLD of 43.1 per 100,000 individuals between 45 and 64 years of age [13]. In contrast, Ikejima et al. [14] reported the prevalence of restricted FTD patient in Ibaraki Prefecture, Japan, to be 2.0 per 100,000 individuals between 45 and 64 years of age. Taken together with our results, the prevalence of FTLD in individuals under 65 years of age in Japan might be less than that in Europe.

Although FTLD is generally considered to be a presenile dementia, a review of demographic characteristics of 353 FTLD patients by Johnson et al. [15] indicated that approximately one quarter of the patients diagnosed as having FTD and semantic dementia and half of PNFA patients had a disease onset after age 65 years. The prevalence of FTLD was 3.8 per 100,000 individuals between 70 and 79 years of age in the Zuid-Holland district in the Netherlands [9]. A nationwide study in Germany estimated the prevalence of FTLD to be 49.3 per 100,000 individuals between 70 and 79 years of age [11]. Gislason et al. [16] reported a much higher prevalence of FTD of 3% in a cohort of 85-year-old individuals in Gothenburg, Sweden. In the current study, the prevalence of FTLD was 37.6 per 100,000 inhabitants 65 years of age or older. These epidemiological data indicate that the prevalence of FTLD among elderly subjects might be higher than previously described. One challenging aspect of FTLD is that the clinicopathological features of FTLD in elderly patients may differ from those in patients with presenile-onset FTLD. Baborie et al. [17] proposed that FTLD in elderly patients might exist as a separate entity from presenile-onset FTLD in that the characteristics of clinically frequent memory loss and behavioral changes predominate over language and semantic dysfunction. It was suggested that FTLD in elderly patients is under-recognized, and FTLD should be considered in elderly subjects presenting with an 'atypical Alzheimer's disease' phenotype.

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Although epidemiological studies in Europe have reported that a large percentage of FTLD patients have a family history (29% in the UK, 43% in the Netherlands), only 4.5% of FTLD patients in our current study had a family history. Only 1 patient (1.5%) of 66 FTLD patients in Tottori Prefecture had a mutation of MAPT, compared with 32 (13.8%) of 245 FTD patients in the study in the Zuid-Holland district in the Netherlands. These results suggest that genetic factors for the development of FTLD may have a less important role in the Japanese population.

There are several limitations in our estimates of the prevalence of FTLD in Tottori Prefecture of Japan. First, the diagnosis of FTLD was dependent on clinical symptoms only, due to the absence of a definitive biomarker. We could not confirm the diagnosis neuropathologically in any case in this survey. Careful clinical examinations are needed because of the similarities in symptoms between syndromes such as corticobasal degeneration, progressive supranuclear palsy, Alzheimer's disease, vascular dementia, and FTLD. In this study, board-certificated neurologists and psychiatrists reported the diagnosis of FTLD patients based on their clinical assessment and results of neuroimaging such as cerebral CT, MRI, or cerebral blood flow by SPECT. Further, board-certificated neurologists who have scientific interest in dementia or neurodegenerative disorders visited the clinic or hospital for assessment of the patients when required. For the diagnosis of FTD, we applied the criteria by Neary et al. [5] as well as the criteria of the International Behavioural Variant FTD Criteria Consortium [6]. The former criteria are thought to be relatively insensitive and difficult to apply in the early stage of FTD, whereas the sensitivity of the latter criteria is reported to be better [6].

Further, only patients diagnosed with FTLD who had a medical consultation with the department of neurology or psychiatry were included in the survey. A clinical survey in an academic hospital indicated that the prevalence of PNFA or SD was similar to that of FTD [4]. In the current survey, the proportion of patients with PNFA or SD was much less than that reported in the previous survey, suggesting that the prevalence of PNFA and SD may have been underestimated.

In conclusion, the results of this study suggest that there are as many as 12,000 patients with FTLD in Japan, indicating that FTLD is not a rare disease at all.

## Acknowledgment

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## Disclosure Statement

We certify that there is no conflict of interest.

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# Cerebrospinal fluid amyloid β and tau in *LRRK2* mutation carriers

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## ABSTRACT

**Objective:** The goal of the current investigation was to examine a cohort of symptomatic and asymptomatic *LRRK2* mutation carriers, in order to address whether the reported alterations in amyloid β (Aβ) and tau species in the CSF of patients with sporadic Parkinson disease (PD) are a part of PD pathogenesis, the aging process, or a comorbid disease in patients with PD, and to explore the possibility of Aβ and tau as markers of early or presymptomatic PD.

**Methods:** CSF Aβ42, total tau, and phosphorylated tau were measured with Luminex assays in 26 *LRRK2* mutation carriers, who were either asymptomatic (n = 18) or had a phenotype resembling sporadic PD (n = 8). All patients also underwent PET scans with $^{18}$F-6-fluoro-L-dopa (FD), $^{11}$C-(±)-α-dihydrotetrabenazine (DTBZ), and $^{11}$C-d-*threo*-methylphenidate (MP) to measure dopaminergic function in the striatum. The levels of CSF markers were then compared to each PET measurement.

**Results:** Reduced CSF Aβ42 and tau levels correlated with lower striatal dopaminergic function as determined by all 3 PET tracers, with a significant association between Aβ42 and FD uptake. When cases were restricted to carriers of the G2019S mutation, the most common *LRRK2* variant in our cohort, significant correlations were also observed for tau.

**Conclusions:** The disposition of Aβ and tau is likely important in both *LRRK2*-related and sporadic PD, even during early phases of the disease. A better understanding of their production, aggregation, and degradation, including changes in their CSF levels, may provide insights into the pathogenesis of PD and the potential utility of these proteins as biomarkers. ***Neurology***® **2012;78:55–61**

## GLOSSARY

**Aβ** = amyloid β; **AD** = Alzheimer disease; **DTBZ** = $^{11}$C-(±)-α-dihydrotetrabenazine; **FD** = $^{18}$F-6-fluoro-L-dopa; **MP** = $^{11}$C-d-*threo*-methylphenidate; **p-tau** = phosphorylated tau; **PD** = Parkinson disease; **t-tau** = total tau.

Recent studies, particularly those with large cohorts of subjects, have reported altered CSF levels of amyloid β (Aβ) and tau species in patients with sporadic Parkinson disease (PD).[1–6] However, it is unclear whether these alterations are mechanistically important to PD pathogenesis, or represent a comorbidity or aging process. To address this question, Aβ and tau species need to be measured in patients with early (ideally preclinical) PD. Moreover, unique protein alterations in early/preclinical cases, alongside with other early (nonmotor) symptoms,[7] could assist in identifying PD at premotor stages.

Studying sporadic PD prior to the onset of clinical symptoms has been impractical, making early intervention exceedingly challenging. Autosomal dominant mutations in the leucine-rich repeat kinase (*LRRK2*) gene, the most common known genetic cause of parkinsonism, result in a

Supplemental data at www.neurology.org

Supplemental Data

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clinical phenotype similar to sporadic PD.[8,9] *LRRK2* mutation carriers have been extensively characterized by PET studies, demonstrating neurochemical changes similar to sporadic PD cases, as well as detectable dopaminergic dysfunction in asymptomatic carriers.[10,11] Thus, subjects with *LRRK2* mutations constitute an excellent cohort for studying preclinical and early PD.

In the present study, we examined CSF Aβ1–42 ($A\beta_{42}$) peptide, total tau (t-tau), and phosphorylated tau (p-tau) in symptomatic and asymptomatic *LRRK2* mutation carriers. CSF protein levels were correlated with dopamine denervation (assessed by PET measurements), to determine whether $A\beta_{42}$ and tau species are altered during early PD processes, and to explore the utility of these proteins as early or presymptomatic PD diagnostic or progression markers.

**METHODS Subjects.** This study focused on preclinical and asymptomatic *LRRK2* mutation carriers: 18 of the 26 carriers included in the study were asymptomatic at the time of evaluation. The remaining 8 subjects had clinically confirmed PD; most of them were at early disease stages that permitted long-range travel and limited confounding due to complicated CNS changes associated with advanced PD. Details on subject recruitment can be found in the e-Methods on the *Neurology*® Web site at www.neurology.org. Demographic and clinical information is listed in table 1 for all subjects.

Table 1 **Summary of demographics and PET values of subjects**

| Subject | Source | Age, y | Sex | Mutation status | Clinical status | UPDRS III[a] | FD ($k_{occ}$)[b] | DTBZ ($BP_{ND}$)[b] | MP ($BP_{ND}$)[b] |
|---|---|---|---|---|---|---|---|---|---|
| 1,451 | Norway | 74 | F | G2019S | PD | 41 | 0.3831 | 0.1662 | 0.2051 |
| 1,454 | Norway | 69 | F | G2019S | U | 6 | 1.0824 | 0.8420 | 0.7905 |
| 1,457 | Norway | 60 | M | G2019S | U | 8 | — | 0.9504 | 0.9653 |
| 1,464 | Norway | 60 | M | G2019S | U | 7 | 0.9491 | 1.0489 | 0.9913 |
| 1,465 | Norway | 57 | M | G2019S | U | 4 | 0.9725 | 0.8173 | 0.7843 |
| 1,474 | Norway | 51 | F | G2019S | U | 5 | 0.8061 | 0.6640 | 0.6422 |
| 1,515 | Norway | 40 | M | G2019S | U | 7 | 1.0561 | 0.9743 | 0.7584 |
| 1,521 | Norway | 29 | M | N1437H | U | 2 | 1.0585 | 1.1693 | 0.8132 |
| 1,522 | Norway | 51 | F | N1437H | PD | 31 | 0.3854 | 0.2746 | 0.2264 |
| 1,523 | Norway | 50 | F | N1437H | U | 11 | 0.5273 | 0.3620 | 0.3035 |
| 1,526 | Norway | 26 | M | N1437H | U | 2 | 1.0094 | 0.9345 | 0.6876 |
| 1,529 | Norway | 30 | M | N1437H | U | 2 | 0.7339 | 1.0166 | 0.7537 |
| 1,539 | US | 80 | M | G2019S[c] | PD | 13 | 0.4759 | 0.4088 | 0.5153 |
| 1,540 | US | 77 | M | G2019S[c] | PD | 25 | 0.4113 | 0.2216 | 0.2442 |
| 1,541 | US | 56 | F | R1441H | U | 0 | 1.0138 | 1.0873 | 0.9621 |
| 1,542 | US | 55 | M | R1441C | U | 0 | 0.7791 | 0.8508 | 0.6589 |
| 1,543 | US | 61 | F | R1441C | U | 11 | 0.8858 | 0.8479 | 0.7901 |
| 1,544 | US | 57 | M | R1441C | PD | 7 | 0.9704 | 0.8878 | 0.9806 |
| 1,546 | US | 57 | F | R1441C | U | 0 | 0.8215 | 0.8584 | 0.7140 |
| 1,547 | US | 67 | F | R1441C | PD | 10 | 0.3771 | 0.3159 | 0.3188 |
| 2,241 | Norway | 55 | F | G2019S | U | 8 | 0.7326 | 0.6026 | 0.4629 |
| 2,246 | Norway | 47 | F | G2019S | U | 7 | 0.9997 | 1.0639 | 0.8189 |
| 2,250[d] | Japan | 65 | M | I2020T | PD | 9 | 0.2328 | 0.2005 | 0.1914 |
| 2,251[d] | Japan | 66 | F | I2020T | PD | 4 | 0.2579 | 0.1829 | 0.1536 |
| 2,252 | Japan | 62 | M | I2020T | U | NA | 0.9490 | 0.8032 | 0.6567 |
| 2,253[d] | Japan | 59 | M | I2020T | U | NA | 0.9741 | 0.9950 | 0.8013 |

Abbreviations: DTBZ = $^{11}$C-(±)-α-dihydrotetrabenazine; FD = $^{18}$F-6-fluoro-L-dopa; MP = $^{11}$C-d-*threo*-methylphenidate; PD = Parkinson disease; U = unaffected/asymptomatic; UPDRS = Unified Parkinson's Disease Rating Scale.

[a] Subject 1,523 had mainly mild left-sided bradykinesia and rigidity, as well as mild axial symptoms, but no tremor. She had no other signs or complaints consistent with Parkinson disease during the follow-up assessments. Subject 1,543's high UPDRS score might be due to a locomotion problem caused by other previous disease, rather than reflecting the parkinsonian phenotype. Some symptomatic subjects had low UPDRS scores, likely due to the medication (levodopa).

[b] Values shown are averages of left and right putamen expressed as a fraction of age-matched control values.

[c] Homozygote.

[d] Excluded from $A\beta_{42}$ analyses due to high blood contamination in the CSF sample.

**Standard protocol approvals, registrations, and patient consents.** This study was approved by the institutional review boards of all participating institutions. All individuals provided written informed consent to participate.

**PET, CSF samples, and Luminex assays.** Within 1 year of CSF sample collection, each subject was scanned with 3 tracers, $^{18}$F-6-fluoro-L-dopa (FD, a marker for the uptake and decarboxylation of levodopa as well as the trapping of dopamine in synaptic vesicles), $^{11}$C-(±)-α-dihydrotetrabenazine (DTBZ, a vesicular monoamine transporter 2 [VMAT2] ligand), and $^{11}$C-d-*threo*-methylphenidate (MP, a dopamine transporter [DAT] ligand). CSF $A\beta_{42}$, t-tau, and p-tau levels of all cases were measured as previously described.[2] To minimize any age effects, PET data were normalized to age-matched control values. Details can be found in the e-Methods.

**Statistical analysis.** All analyses were performed with PASW Statistics 18.0 (SPSS Inc., Chicago, IL). Nonparametric correlation methods (Kendall rank correlation) were used to assess the relationship between the skewed PET measurements and the CSF biomarkers and to minimize the effects of outliers. See more details in the e-Methods.

RESULTS **PET measurements.** Subjects were scanned for putaminal tracer binding/uptake, and the average values of left and right putamen, expressed as a fraction of age-matched control values, are shown in table 1. Representative PET images from *LRRK2* PD patients, asymptomatic mutation carriers, and healthy controls are shown in figure e-1.

Except for subject 1,544, all *LRRK2* PD subjects had significantly reduced PET values for all 3 tracers (figure 1 and table 1). Among asymptomatic mutation carriers, 4 subjects had significantly reduced values for all 3 tracers; in subject 1,523, PET abnormalities demonstrated asymmetry, typical of sporadic PD. Subject 1,542 had significantly reduced MP (DAT) binding and FD uptake values only. In subject 1,526, DAT binding was significantly reduced; reduced DTBZ (VMAT2) binding was observed only in the right putamen. Subjects 1,454 and 2,246 showed borderline decrease in right putaminal FD uptake and DAT binding, respectively. All other asymptomatic mutation carriers demonstrated normal uptake/binding of all 3 tracers in the putamen. Subjects 1,465 and 1,543 showed abnormal VMAT2 and DAT binding in the caudate, but not the putamen. Subject 1,457 could not complete the FD scan.

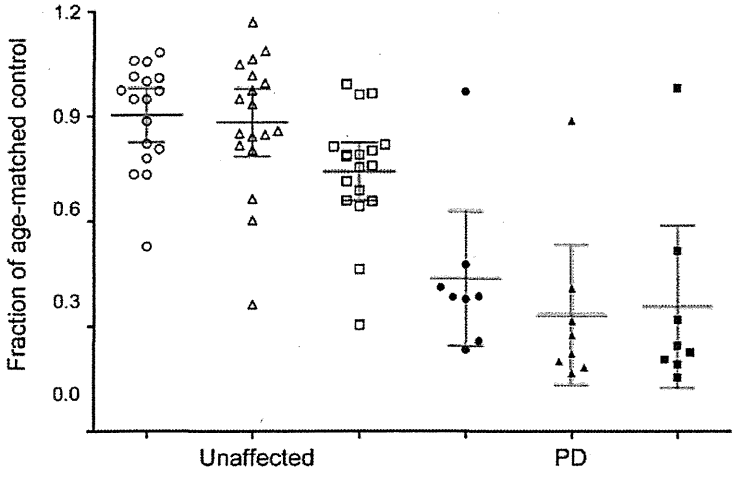
**Correlation of CSF biomarkers with PET data.** Blood contamination of CSF, which occasionally occurs during lumbar puncture collection, can significantly affect CSF levels of $A\beta_{42}$ (but not t-tau or p-tau).[2] To control for this variable, 3 subjects (1 *LRRK2* PD and 2 asymptomatic) whose CSF hemoglobin levels were >250 ng/mL were excluded from further analysis of $A\beta_{42}$.

Similar to our previous report in patients with sporadic PD,[2] CSF levels of all analytes were lower in symptomatic *LRRK2* mutation carriers compared to asymptomatic carriers (symptomatic [mean ± SD]: $A\beta_{42}$, 504.1 ± 128.5 pg/mL, t-tau, 24.4 ± 22.6 pg/mL, p-tau, 16.7 ± 3.8 pg/mL; asymptomatic: $A\beta_{42}$, 601.5 ± 234.1 pg/mL, t-tau, 36.4 ± 32.0 pg/mL, p-tau, 21.1 ± 9.0 pg/mL; individual values can be found in table e-1); but this trend did not reach significance, likely due to the limited number of subjects included. Additionally, because symptomatic subjects were older than asymptomatic carriers (mean 67.1 ± 9.8 vs 51.3 ± 12.3 years), the extent of decrease in CSF tau levels in *LRRK2* PD may have also been reduced by the known age-dependent increase in CSF tau levels ($A\beta_{42}$ is stable as a function of age in healthy controls).[2] Nonetheless, the correlations between CSF analyte levels and PET measurements are unlikely to be substantially affected by age because all the PET data were standardized to age-matched control values.

CSF levels of $A\beta_{42}$ in the entire *LRRK2* cohort decreased with decreasing uptake of FD (Kendall tau = 0.316, $p$ = 0.040), indicating that decreasing protein levels correspond with progressive loss of dopamine function (figure 2A). Decreased levels of $A\beta_{42}$ also corresponded to lower values of the other PET tracers, as did decreased levels of t-tau or p-tau with all PET tracers, but these relationships did not reach significance (figure 2).

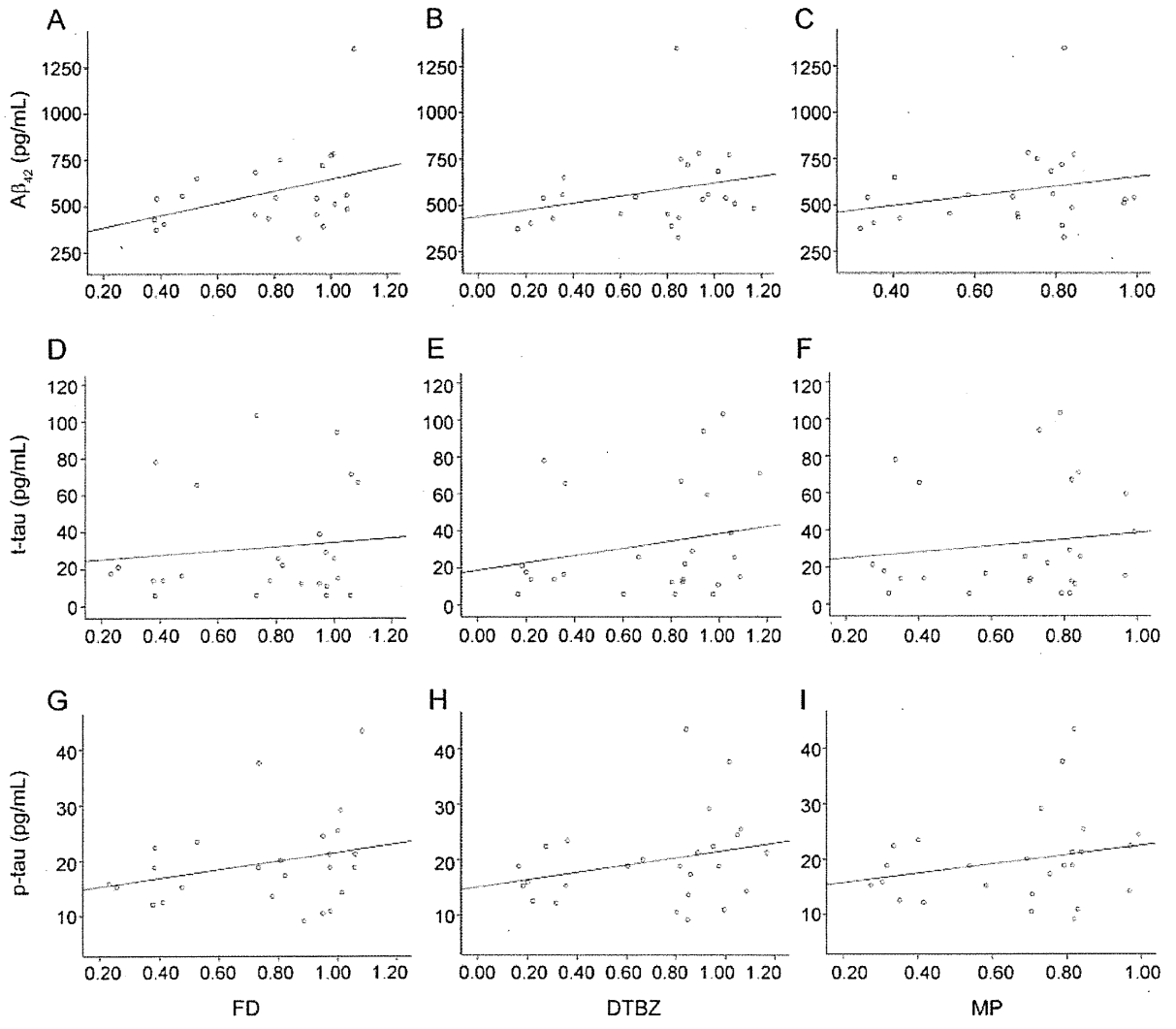
Because *LRRK2* mutations are heterogeneous, this analysis was repeated using the subset of patients

Figure 1 **Putaminal tracer binding/uptake in *LRRK2* mutation carriers**




The average left and right putaminal tracer binding/uptake values in *LRRK2* mutation carriers are given as a fraction of age-matched healthy control values. The means with 95% confidence intervals for asymptomatic (unaffected) subjects and subjects with clinically confirmed *LRRK2*-Parkinson disease (PD) are also shown. Circles indicate $^{18}$F-fluoro-L-dopa (FD) uptake, triangles indicate $^{11}$C-dihydrotetrabenazine (DTBZ) binding, and squares indicate $^{11}$C-d-threo-methylphenidate (MP) binding.

Figure 2 **Correlation of CSF biomarkers with PET measurements in the entire *LRRK2* cohort**



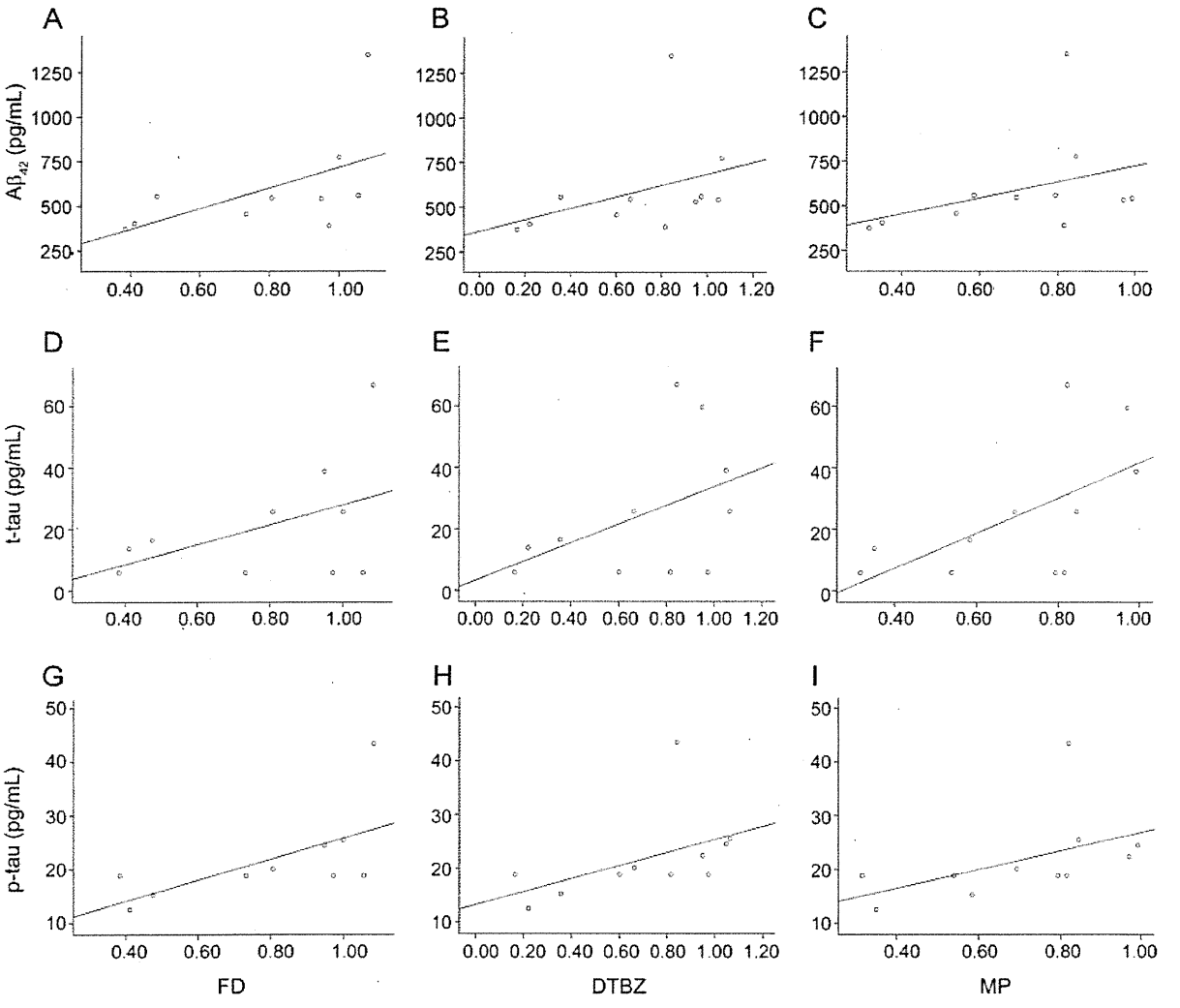

The correlations of protein levels in CSF with putaminal PET values expressed as fractions of age-matched healthy control values are shown. For $A\beta_{42}$ (A–C), FD: Kendall tau = 0.316, $p$ = 0.040; DTBZ: Kendall tau = 0.223, $p$ = 0.119; MP: Kendall tau = 0.217, $p$ = 0.146. For total tau (t-tau) (D–F), FD: Kendall tau = 0.065, $p$ = 0.656; DTBZ: Kendall tau = 0.182, $p$ = 0.200; MP: Kendall tau = 0.119, $p$ = 0.401. For phosphorylated tau (p-tau) (G–I), FD: Kendall tau = 0.226, $p$ = 0.117; DTBZ: Kendall tau = 0.203, $p$ = 0.151; MP: Kendall tau = 0.190, $p$ = 0.178. $A\beta_{42}$ values restricted to 23 out of 26 cases due to high blood contamination in CSF, which does not influence t-tau or p-tau values. Note that regression lines were generated from linear regression for visualization only. DTBZ = $^{11}$C-dihydrotetrabenazine; FD = $^{18}$F-fluoro-L-dopa; MP = $^{11}$C-d-threo-methylphenidate.

with the G2019S mutation (n = 11), the most common variant in our cohort. Remarkably, there was a clear reduction in the levels of all proteins with decreasing PET measurements in the striatum, regardless of the tracer used (figure 3). CSF levels of $A\beta_{42}$ and p-tau were both reduced with greater degrees of reduced FD uptake (i.e., diminished dopamine function; $A\beta_{42}$: Kendall tau = 0.556, $p$ = 0.025; p-tau: Kendall tau = 0.597, $p$ = 0.020). In addition, reduced CSF p-tau significantly correlated with reduced DTBZ binding (Kendall tau = 0.597, $p$ = 0.013) and MP binding (Kendall tau = 0.559, $p$ = 0.020), but t-tau only significantly correlated with MP binding (Kendall tau = 0.506, $p$ = 0.037).

One symptomatic *LRRK2* PD subject (1,544) with normal PET measurements demonstrated relatively lowered t-tau and p-tau levels, i.e., behaved more like a patient with PD than a normal control, suggesting that CSF tau might be a more sensitive indicator than the PET measurements.

DISCUSSION Several independent investigations reported that CSF Aβ levels decreased (but not as substantially as in Alzheimer disease [AD]) in pa-

Figure 3 **Correlation of CSF biomarkers with PET measurements in *LRRK2* G2019S mutation carriers**




The correlations of protein levels in CSF with putaminal PET values expressed as fractions of age-matched healthy control values are shown ($\hat{n}$ = 11). For $A\beta_{42}$ (A–C), FD: Kendall tau = 0.556, $p$ = 0.025; DTBZ: Kendall tau = 0.455, $p$ = 0.052; MP: Kendall tau = 0.382, $p$ = 0.102. For total tau (t-tau) (D–F), FD: Kendall tau = 0.339, $p$ = 0.192; DTBZ: Kendall tau = 0.331, $p$ = 0.199; MP: Kendall tau = 0.506, $p$ = 0.037. For phosphorylated tau (p-tau) (G–I), FD: Kendall tau = 0.597, $p$ = 0.020; DTBZ: Kendall tau = 0.597, $p$ = 0.013; MP: Kendall tau = 0.559, $p$ = 0.020. Note that the regression lines were generated from linear regression for visualization only. DTBZ = $^{11}$C-dihydrotetrabenazine; FD = $^{18}$F-fluoro-L-dopa; MP = $^{11}$C-d-threo-methylphenidate.

tients with sporadic PD, particularly in those with cognitive impairment.[2,5,6,12,13] Additionally, studies with large cohorts (≥50 subjects in either sporadic PD or control group) suggest that, unlike what is typically described for AD, tau levels tend to decrease in PD compared to controls.[2,4,6] The obvious trend of decreasing CSF levels of $A\beta_{42}$ and tau in *LRRK2* PD and asymptomatic carriers (figure 2 and 3, table e-1) is in line with these observations. This raises the question of whether decreased concentrations of CSF tau and Aβ might be mechanistically involved in PD pathology. Tau pathology and amyloid plaques, though increasingly reported,[14,15] are not typically seen in the brains of patients with PD. Therefore, it is possible that the decrease in CSF tau and Aβ levels occurs as soluble tau/p-tau and Aβ oligomers, often considered more toxic,[16] deposit in the brains of patients with PD without formation of amyloid plaques and neurofibrillary (tau) tangles.

Regardless of the cause of decreased CSF $A\beta_{42}$, t-tau, and p-tau in asymptomatic and symptomatic mutation carriers, our data suggest that metabolism of Aβ and tau in brain is likely dysfunctional in *LRRK2*-related PD, even before motor symptoms appear. If the same is true of sporadic PD, then tau and amyloid pathology in patients with PD, rather than just indicating comorbidity or general aging pro-