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ORIGINAL ARTICLE

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Molecular mechanism of rigid spine with muscular dystrophy type 1 caused by novel mutations of selenoprotein N gene

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Abstract Mutations of selenoprotein N, 1 gene (*SEPN1*) cause rigid spine with muscular dystrophy type 1 (RSMD1), multiminicore disease, and desmin-related myopathy. We found two novel *SEPN1* mutations in two Japanese patients with RSMD1. To clarify the pathomechanism of RSMD1, we performed immunohistochemical studies using a newly developed antibody for selenoprotein N. Selenoprotein N was diffusely distributed in the cytoplasm of the control muscle, but was reduced and irregularly expressed in the cytoplasm of a patient with RSMD1. The expression pattern was very similar to that of calnexin, a transmembrane protein of the endoplasmic reticulum. Selenoprotein N seems to be an endoplasmic reticulum glycoprotein, and loss of this protein leads to disturbance of muscular function. One of the families had the *SEPN1* homozygous mutation in the initiation codon 1_2 ins T in exon 1 and showed truncated protein expression. The other had a homozygous 20-base duplication mutation at 80 (80_99dup, frameshift at R27) which, in theory, should generate many nonsense mutations including TGA. These nonsense mutations are premature translation termination codons and they degrade immediately by the process of nonsense-mediated decay (NMD). However, truncated selenoprotein N was also expressed. A possible mechanism behind this observation is that *SEPN1* mRNAs may be resistant to NMD. We report on the possible molecular mechanism behind these mutations in *SEPN1*. Our study clarifies molecular mechanisms of this muscular disorder.

Keywords *SEPN1* · Rigid spine syndrome · Anti-selenoprotein N antibody · RSMD1 · SEPN-RM

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Introduction

The peculiar phenotype caused by the combination of muscle wasting and prominent spinal rigidity was first proposed by Dubowitz and termed “rigid spine syndrome (RSS)” [1, 2].

The spinal rigidity is a nonspecific feature; however, it is quite prominent in the X-linked, autosomal dominant, and autosomal recessive forms of Emery–Dreifuss muscular dystrophy [3, 4] and in some congenital myopathies [5, 6]. Several authors have pointed out distinctions between the spinal rigidity in other myopathies and that found in RSS [7, 8]. Although the syndrome has a good prognosis, some patients die of respiratory failure in the first or second decade. Nocturnal hypoventilation may be an early and characteristic symptom [9, 10]. In 1997, a subgroup of merosin-positive congenital muscular dystrophy characterized by slowly progressive weakness, rigid spine, and early respiratory failure was described [11]. Not long after, a gene locus on chromosome 1p35–36 was identified in consanguineous Moroccan, Iranian, and Turkish families affected by congenital muscular dystrophy with spinal rigidity and reduced vital capacity [12]. After linkage analysis, two frameshift, one nonsense, and three missense mutations in the selenoprotein N, 1 gene (*SEPN1,* OMIM 606210) were identified as the cause of rigid spine with muscular dystrophy type 1 (RSMD1) in the same families

[13]. *SEPN1* codes for selenoprotein N, a novel selenoprotein with an unknown function. Subsequent studies revealed that selenoprotein N is an endoplasmic reticulum glycoprotein, highly expressed in several human fetal tissues, but only slightly expressed in adult tissues including muscle based on Western blot analysis. However, immunostaining of selenoprotein N in human skeletal muscle has not yet been reported due to problems in sensitivity of reported antibodies [14].

In this study, we report two Japanese RSS patients with two novel *SEPN1* mutations. We examined their muscle pathology using a developed antibody directed against selenoprotein N. We detected selenoprotein N in biopsied human muscle tissues from these patients and from controls and describe its distribution. We also provide additional evidence to support the correlation between selenoprotein N localization and endoplasmic reticulum in muscle. One of our patients had the mutation in the initiation codon, which usually leads to absence of protein production. Our patient, however, showed truncated protein expression. We report on the possible molecular mechanism behind the mutation in the initiation codon and consequent protein expression. The other patient had a homozygous 20-base duplication mutation. In theory, this would generate many nonsense mutations. These nonsense mutations are premature translation termination codons that degrade immediately by the process of nonsense-mediated decay (NMD). However, truncated selenoprotein N was also expressed. A possible mechanism behind this observation is that *SEPN1* mRNAs may be resistant to NMD. Our studies not only shed light on a new diagnostic method for RSS caused by *SEPN 1* mutation, but also clarify molecular mechanisms of this muscular disorder.

Materials and methods

Patients

Patient 1 This 41-year-old man, whose parents were first cousins, was born after a normal pregnancy and uncomplicated delivery. His parents, one brother, and three sisters had no known muscle disease. An elder sister, who had delayed motor milestones, died at the age of 3 because of measles. It was, therefore, not possible to confirm whether she had the same disease as the patient.

His intellectual milestones were normal, but he had delayed motor milestones and did not walk unsupported until the age of 3. At the age of 7, he had abnormal gait and he was never able to run or jump. There was an apparent rigidity of the spine and restricted forward flexion of the neck. At the age of 16, he began to have dyspnea during sleep. His dyspnea progressed to the point that a tracheotomy was performed at the age of 24. At the age of 27, he had limitation of spine flexion, scoliosis, and mild contracture of the ankle joints, but no limitation of elbow and wrist joint movement. Diffuse and mild muscle atrophy and weakness (MMT 4) were seen in the shoulder, sternocleidomastoideus, and axial muscles. Muscle strength of the upper and lower limbs were relatively preserved (MMT 4–5). He showed waddling gait and Gower's sign. Serum creatine kinase (CK) level was elevated at 590 IU/l, which is almost three times higher than the normal limit. Electromyographic findings showed myopathic change. Electrocardiogram showed negative T waves, but no conduction block. He has severe restrictive respiratory failure. Chest X-ray study showed an elevated diaphragm. He is on a respirator during sleep but is mostly off it during daytime. He was still able to walk albeit with support until the age of 34.

Patient 2 This 31-year-old woman is the child of healthy, unrelated parents, born after a normal pregnancy and uncomplicated delivery. Both parents and four siblings were normal. Her motor and intellectual milestones were normal during infancy. She began to walk at the age of 11 months and to run at the age of 7, although her running speed was relatively slow. At the age of 12, she felt nocturnal dyspnea and palpitations. X-ray examination revealed limitation of diaphragmatic movement. Serum CK level was elevated at 130 IU/l. At the age of 14, she had severe respiratory failure secondary to pneumonia and she underwent tracheotomy. Thereafter, she has been on a respirator during sleep but mostly off it during daytime. It was during this time that she visited our hospital for consultation. She had limitation of spine flexion, scoliosis, and restricted forward flexion of the neck. She showed a waddling gait and Gower's sign. Her face was myopathic. Diffuse and mild muscle atrophy and weakness (MMT 4) were seen in the shoulder and axial muscles. The muscle strength of the upper and lower limbs was relatively preserved (MMT 4–5). Neck flexion and extension, however, were particularly weak (MMT 3). She was diagnosed with muscular dystrophy based on muscle biopsy findings. Her muscle weakness progressed slowly, and she was still able to walk with support until the age of 30 years.

The patients, who were referred to us by their attending neurologists, and family members included in the study signed an informed consent approved by the Institutional Review Board of Kagoshima University. DNA was isolated from peripheral blood and skeletal muscle of all subjects.

Mutation screening

By aligning the human genomic sequence with the *SEPN1*cDNA, we were able to identify all coding exons. Using the Primer v3 program, we designed primers to amplify exons and intronic splice junctions and then screened the amplified PCR products from patient genomic DNA for mutations using ABI Prism 377 sequencer (Applied Biosystems, Foster City, CA, USA). Briefly, we amplified the coding region of *SEPN1* from 50 ng of patient genomic DNA using the primers and the hot start PCR method [15]. Using the presequencing kit (USB, Cleveland, OH, USA), we purified patient PCR products

from relatives and control chromosomes and sequenced them with dye-terminator chemistry using an ABI377 automated sequencer. We aligned the resulting sequences and evaluated mutations with the Sequencher sequence alignment program (Gene Codes, Ann Arbor, MI, USA). We then numbered the *SEPN1* cDNA sequence beginning with the adenine of the presumed initiating methionine and described mutations using standard nomenclature [16].

Real-time quantitative reverse transcriptase-polymerase chain reaction

Total RNA was isolated from whole blood using the QIAamp RNA Blood Mini Kit (QIAGEN, Valencia, CA, USA). The RNA comes in a 1-μg/μl concentration and the 260/280 absorbance ratio is between 1.7 and 2.1. Reverse transcriptase-polymerase chain reaction (RT-PCR) was carried out using SuperScript III First Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA) and hot Taq DNA polymerase (QIAGEN). We performed real-time quantitative RT-PCR using the Taqman system (Applied Biosystems). Primers for real-time RT-PCR and probes were obtained from TaqMan Gene Expression Assay catalog (ABI; SEPN1, Hs00898730_g1, NM_020451,2, Exon Boundary 5–6;SEPN1, Hs00898723_m1, NM_020451, 2, Exon Boundary 12–13) and TaqMan Rodent GAPDH Control Reagents VIC Probe (ABI). For real-time PCR, each target was amplified on the same plate as the reference, GAPDH, using the TaqMan Universal Master Mix and the ABI Prism 7700 Sequence Detection System (ABI), and the relative mRNA amounts and range were determined. Briefly, we normalized each set of samples using the difference in threshold cycles (C_T)

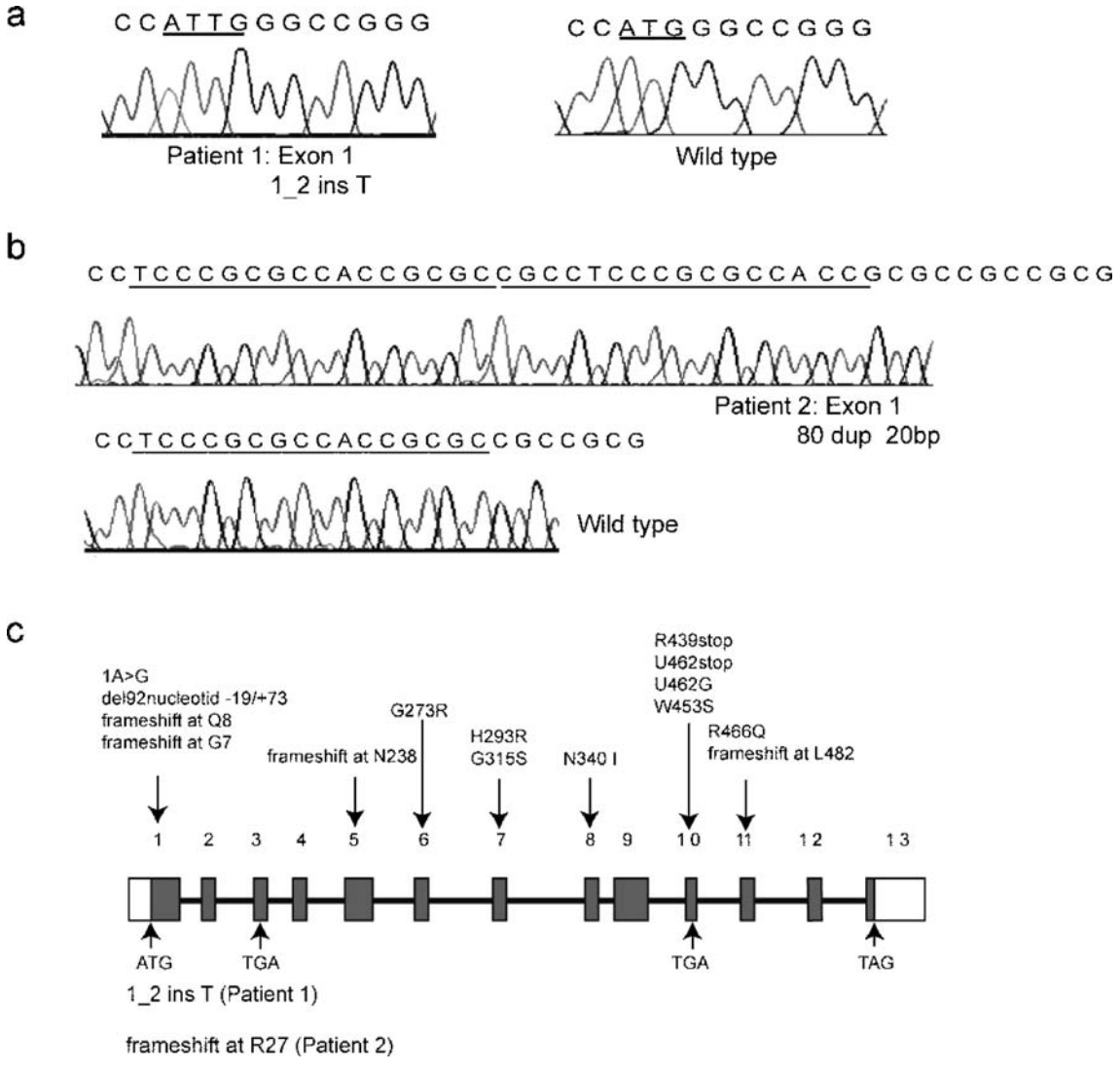

Fig. 1 Identification of the novel *SEPN1* mutations in Japanese patients with RSMD1. Direct sequence analysis of the *SEPN1* gene. **a** Electropherogram shows the homozygous 1_2 ins T mutation in exon1 of *SEPN1* in patient 1. This change is absent in normal controls. **b** Electropherogram shows the homozygous 80 dup 20-bp mutation in exon 1 of *SEPN1*; this causes a frameshift at R27. This change is absent in normal controls. **c** Schematic representation of the *SEPN1* gene, showing the localization of mutations in SEPN1-related myopathy

between the sample gene and housekeeping gene (GAPDH): $\Delta C_{\mathrm{T}} = \left(\Delta C_{\mathrm{T\ sample}} - \Delta C_{\mathrm{T\ GAPDH}}\right)$. The calibrator sample ($\Delta C_{\mathrm{T\ calibration}}$) was assigned as the sample with the highest ΔC_{T} in each set. Relative mRNA levels were calculated by the expression $2^{-\Delta\Delta CT}$ where $\Delta\Delta C_{\mathrm{T}} = \Delta C_{\mathrm{T\ sample(n)}} - \Delta C_{\mathrm{T\ calibration(n)}}$. Each reaction was done in triplicate, at the least.

Immunohistochemistry

Frozen biopsied biceps brachii muscle specimens from the patients (biopsies performed at ages 24 and 14 for patients 1 and 2, respectively) were obtained. Specimens were cut into 8-μm sections and picked up on aminosilane-coated slides. Immunohistochemical studies were performed on the components of the cell membrane and extracellular matrix. A rabbit polyclonal antibody, directed against a synthetic peptide of the C-terminal region (566–583 NH2–GCEDPSTATYMQFLKEGLRR–OH) of selenoprotein N, was generated and used (Bio-synthesis, Lewisville, TX, USA; 1:200 dilution). Monoclonal antibodies comprised a 1:400 dilution of calnexin, which is known to be a transmembrane protein of the endoplasmic reticulum (Sigma, St Louis, MO, USA); a 1:1,000 dilution of merosin (Gibco BRL, Gaithersburg, MD, USA); a 1:2000 dilution of laminin beta 1 (Gibco); a 1:50 dilution of dystrophin C terminus (Novocastra, Newcastle, UK); and a 1:50 dilution of alpha-sarcoglycan (Novocastra). Biotinylated anti-mouse IgG was used as a secondary antibody, and the ABC method [17] was used for signal detection (ABC kit; Vector, Burlingame, CA, USA). All immunohistochemical procedures were performed as reported previously [18].

Western blotting analysis

To determine the amounts of selenoprotein N and calnexin, 15 slices of 4-μm frozen muscle sections from patients and healthy volunteers were collected and lysed on ice for 20 min in 1 ml of lysis buffer containing 150 mM NaCl, 10 mM Tris pH 7.2, 0.1% sodium dodecyl sulfate (SDS), 1.0% Triton X-100, 1% deoxycholate, 5 mM EDTA, and a cocktail of protease inhibitors (Roche, Indianapolis, IN, USA). The lysates were spun, and the 20-μl supernatants were collected. The same volume (20 μl) of double-

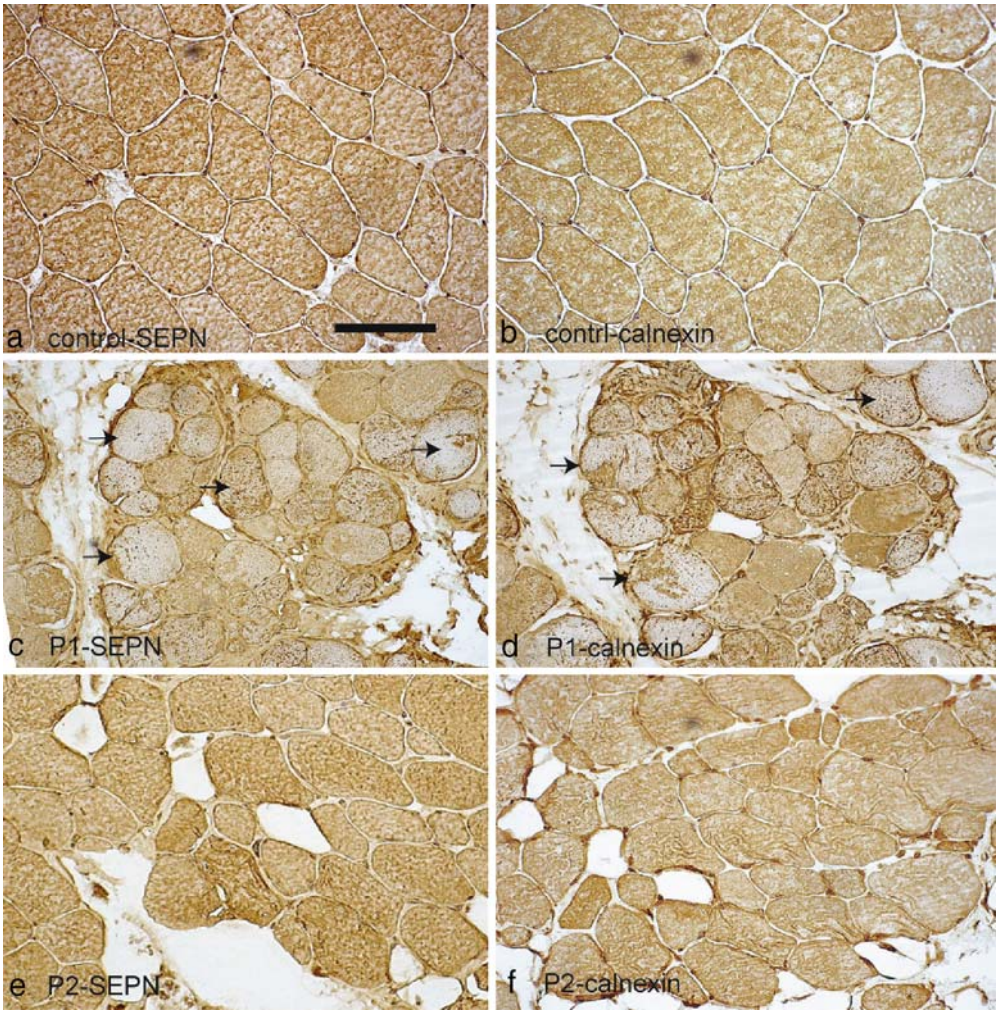

Fig. 2 Immunohistochemical analysis of selenoprotein N (**a**, **c**, **e**) and calnexin (**b**, **d**, **f**) in biopsied muscle specimens from control and two patients with RSMD1 (control: **a**, **b**; patient 1: **c**, **d**; patient 2: **e**, **f**). In the control specimen, selenoprotein N and calnexin were both found to be diffusely expressed in muscle cytoplasm, but not in the nucleus, as compared with the muscle membrane and connective tissue elements. In patient 1, selenoprotein N and calnexin were either reduced or absent in the cytoplasm of several muscle fibers (see *arrow*). In patient 2, however, the expression patterns of selenoprotein N and calnexin were not reduced, and were similar to the control samples. **a**–**f** Serial sections; bar = 100 μm

strength sample buffer (20% glycerol, 6% SDS, 10% 2-mercaptoethanol) was added, and the samples were then boiled for 10 min. Proteins were analyzed on 10% polyacrylamide gels by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred electrophoretically to nitrocellulose membranes at 150 mA for 1 h using a semidry system. The membranes were incubated with rabbit polyclonal anti-human selenoprotein N antibody and mouse anti-human calnexin monoclonal antibody (Sigma) followed by sheep anti-rabbit or mouse IgG coupled with horseradish peroxidase (Amersham Pharmacia Biotech, Piscataway, NJ, USA). Peroxidase activity was visualized by the Enhanced Chemiluminescence Detection System (Amersham).

Results

By DNA sequencing, we screened each coding exon of *SEPN1* for mutations in the two patients. Patient 1 was homozygous for the transition mutation 1_2 ins T in exon1 of *SEPN1*; this causes a loss of the translation starting codon ATG (Fig. 1a). The unaffected parents and sisters were heterozygous for these mutations. Patient 2 was homozygous for the transition mutation 80 dup 20 bp in exon 1 of *SEPN1*; this mutation leads to a frameshift mutation at R27 (Fig. 1b). The unaffected parents and sisters were heterozygous for these mutations.

We did not find the same mutations in 100 Japanese control chromosomes.

On histochemical examination in patient 1, the muscle fibers ranged from 10 to 120 μm in diameter. Atrophic and hypertrophic fibers were admixed with each other. Internal nuclei were moderately increased, and fatty and fibrous connective tissues were likewise increased. In NADH dehydrogenase reacted sections, there was focal decrease of oxidative enzyme activities in a fair number of fibers; however, there were no multiminicore lesions found. Other cell membrane and extracellular matrix components examined were all normal.

On histochemical examination in patient 2, the muscle fibers ranged from 5 to 80 μm in diameter. Occasional fibers were regenerating and rare fibers were necrotic. Many fibers were replaced by adipose tissues. In NADH dehydrogenase reacted sections, there was focal decrease of oxidative enzyme activities in a fair number of muscle fibers. Occasional lobulated fibers were observed; however, multiminicore lesions were not observed. Other cell membrane and extracellular matrix components examined were all normal.

On immunohistochemical analysis using the anti-selenoprotein N antibody, selenoprotein N was found to be diffusely expressed in control muscle cytoplasm. Calnexin also showed a very similar expression pattern in control muscle. In patient 1, 40–50% of muscle fibers showed an extremely low expression of selenoprotein N with spotted aggregation (Fig. 2c). Calnexin staining also showed an expression pattern very similar to selenoprotein N (Fig. 2d). On the other hand, expression patterns of both selenoprotein N and calnexin were not reduced in patient 2 (Fig. 2e,f).

We carried out additional Western blotting analysis of selenoprotein N and calnexin in patients 1 and 2 to confirm the decreased expression in muscle biopsy specimens in the former. The normal 70-kDa size of selenoprotein N was not detected in patient 1 but calnexin was normally expressed. Although the 60-kDa bands were seen in the control and in patient 1, the 62–63 kDa band was only seen in patient 1 (Fig. 3). The size of the expressed selenoprotein N in patient 2 appears to be a few kilodaltons smaller than the normal control. The expression of calnexin was normal in patient 2 (Fig. 4).

Expressions of mRNA were measured by real-time quantitative RT-PCR. The relative *SEPN1* mRNA of two targets (Exon boundary 5–6 and 12–13) showed similar results. The expressions were decreased by 27–71% in

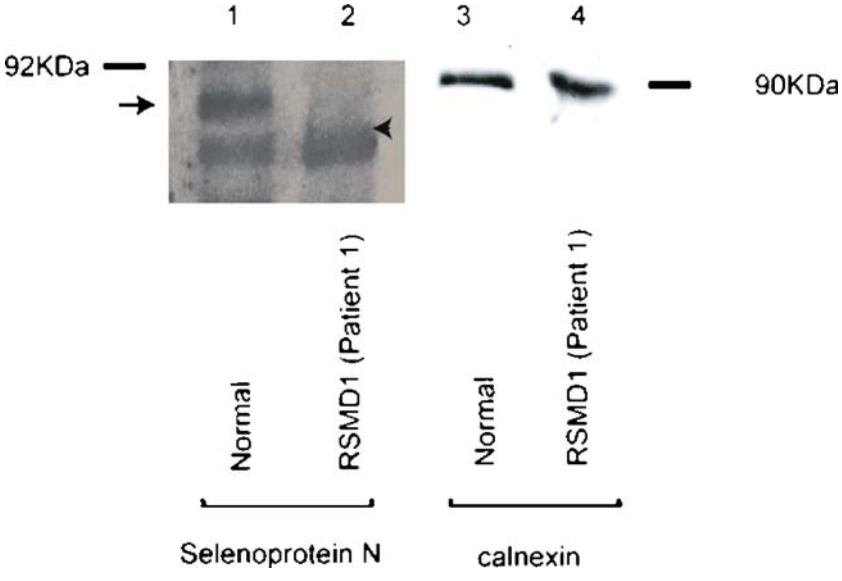

Fig. 3 Detection of selenoprotein N (lanes *1* and *2*) and calnexin (lanes *3* and *4*) by Western blot (using polyclonal antibody anti-selenoprotein N and monoclonal antibody anti-calnexin, respectively). Lanes *1* and *3*, control; lanes *2* and *4*, patient 1; 10% SDS-PAGE. The expression of selenoprotein N was reduced in patient 1 compared to the control (see *arrow*). The 62–63 kDa band of selenoprotein N was seen in patient 1 (see *arrowhead*)

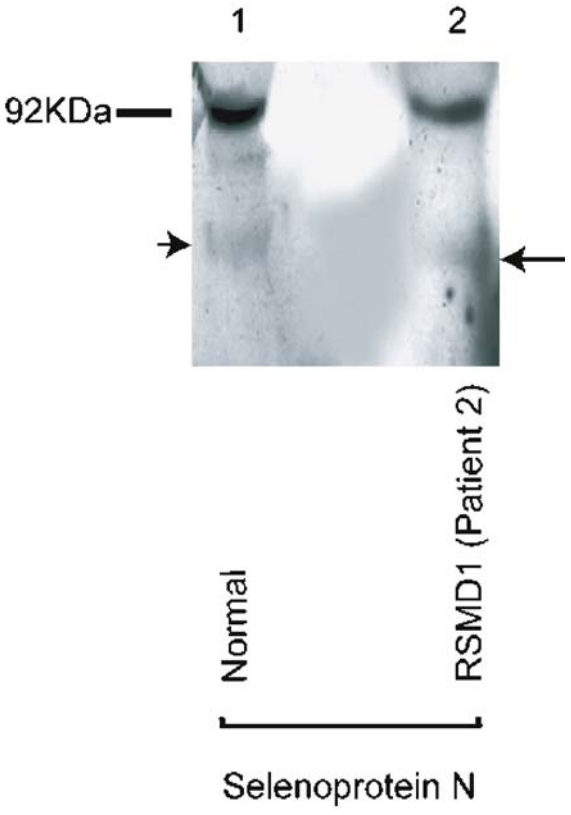

Fig. 4 Detection of selenoprotein N (lanes *1* and *2*) by Western blot (using polyclonal antibody anti-selenoprotein N). Lane *1*, control; lane *2*, patient 2; 10% SDS-PAGE. The size of the expressed selenoprotein N in patient 2 appears to be smaller than normal control (see *arrow*)

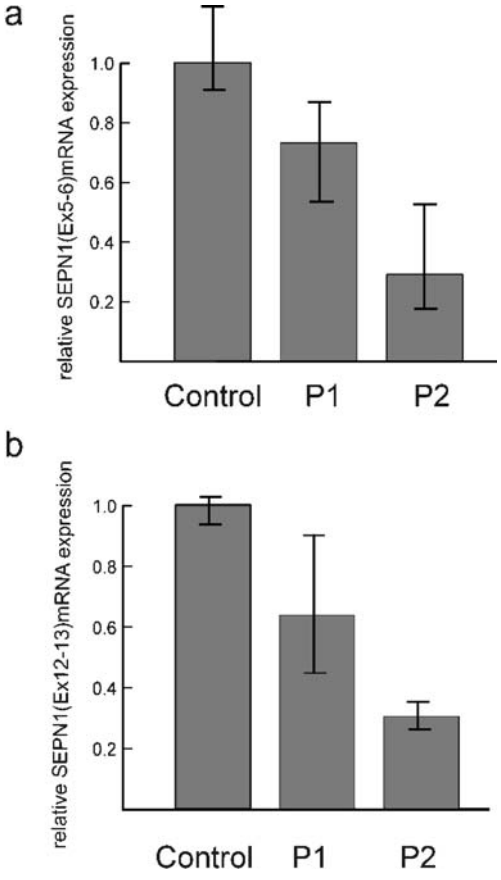

Fig. 5 Relative *SEPN1* mRNA expression in quantitative real-time PCR. Relative *SEPN1* mRNA expression of Exon boundary 5–6 (**a**) and Exon boundary 12–13 (**b**) showed lower levels in patients 1 and 2 compared to control. In both targets, *SEPN1* mRNA levels of patient 2 were less than that of patient 1

patients 1 and 2 compare with control. *SEPN1* mRNA expression in patient 2 was lower than in patient 1 (Fig. 5).

Discussion

We found two novel *SEPN1* mutations in two patients with rigid spine syndrome and confirmed abnormal selenoprotein N expression in human muscle by Western blotting and immunostaining using anti-selenoprotein N antibody. We were able to localize selenoprotein N in muscle from normal control and RSMD1 patients.

Rigid spine syndrome is a condition found in a subset of patients affected by myopathy with early contractures as a prominent feature [1, 2]. Studies of informative families in recent years have succeeded in assigning the locus for congenital muscular dystrophy with spinal rigidity (RSMD1) to chromosome 1 p35–36. Further studies identified the selenoprotein N gene, 1, *SEPN1*, as the cause of RSMD1. Another study revealed that mutations in *SEPN1* were also responsible for multiminicore disease and desmin-related myopathy with Mallory body-like inclusions, for which the authors proposed the joint term of *SEPN*-related myopathy (SEPN-RM). Selenoprotein N is the first selenoprotein shown to be responsible for certain genetic disorders [13, 19–21]. This protein contains a selenium atom, in the form of a selenocysteine residue, which is characteristic of the selenoprotein family. Selenocysteine (Sec) is encoded by an in-frame UGA codon, implying the existence of a mechanism capable of distinguishing the UGA selenocysteine codon from a translational stop. The selenocysteine insertion requires a *cis*-acting selenocysteine insertion sequence (SECIS) usually located in the 3′ UTR of selenoprotein mRNAs [22].

In a previous study, it was revealed that selenoprotein N is an endoplasmic reticulum glycoprotein and is present in high levels in several human fetal tissues and in lower levels in adult fibroblasts from control and RSMD1 patients. However, polyclonal antibodies that were used were not sensitive enough to detect selenoprotein N in adult human tissues. Thus, ours is the first report to describe the distribution of human selenoprotein N in biopsied human muscles. We were able to demonstrate that selenoprotein N is expressed and activated in adult tissues of normal and RSMD1 patients. The expression pattern of selenoprotein N and calnexin, a transmembrane protein of the endoplasmic reticulum, showed a faint and aggregated staining in the sarcoplasm in patient 1. This anti-selenoprotein N antibody has the possibility of cross-

reactivity for a wide variety of proteins because it is a polyclonal antibody. However, by immunohistochemical analysis using the anti-selenoprotein N antibody, selenoprotein N was found to be diffusely expressed in muscle cytoplasm, but not in the nucleus, as compared with the muscle membrane and connective tissue elements. The expression pattern of selenoprotein N resembles that of calnexin, which suggests that selenoprotein N is an endoplasmic reticulum glycoprotein. A previous study reported that selenoprotein N is present in lower levels in adult fibroblasts relative to fetal tissues. The expression level of selenoprotein N might differ between fibroblasts and adult human muscles. Normal calnexin, but not normal selenoprotein N, content was detected by Western blotting in patient 1. These findings suggest that the loss of normal selenoprotein N could lead to disruption of the endoplasmic reticulum of muscle fibers. On the other hand, the expression patterns of selenoprotein N and calnexin were similar to those of normal controls in patient 2, and selenoprotein N was detected by immunohistochemical staining. However, a normal selenoprotein N content was not detected by Western blotting.

In contrast to the morphological and genotypic differences, the two patients have remarkably similar clinical phenotypes. This might depend on the mechanism of action of selenoprotein N translation. Reported *SEPN1* mutations are distributed throughout the entire gene. It is interesting to note that patient 1, as well as three previously reported unrelated families, had a homozygous mutation of the initiation codon (Fig. 1c) [19]. These mutations, including those of patient 1, most likely result in a null mutation, since the initiator ATG is lost. Although we confirmed the loss of regular-sized selenoprotein N by Western blotting, selenoprotein N was found to be expressed in the muscle as detected by immunohistochemical study using the C-terminal antibody. These findings suggest the possible expression of a truncated selenoprotein. To explain the molecular mechanisms of patient 1, we know that, although the mutation is located in the initiation codon, the next ATG plays the role of a putative translation start. In mutations of the *SMPD1* gene, the cause of Niemann–Pick type B, a homozygous 2T>G mutation inactivates the first in-frame translation start site leading to a mild form of the disease, with 20–25% residual enzyme activity with respect to the normal value. The authors of the study concluded that when the first ATG is not functional, the second initiation codon could act as a substitute [23]. The optimal context for initiation of translation in mammals is GCCRCCaugG. In experimental tests, the biggest reduction in efficiency was seen when the purine (R) in position-3 or the G in position +4 was mutated. Thus, initiation sites are usually designated “strong”or “weak”based on those two positions. In our study, because these sequences were observed at four points, these ATG codons can be potential initiation codons (Fig. 6b) [24–26]. Patient 1 did not present with the severe form of *RSMD1*; thus, another initiation codon could have produced partially functional proteins (Fig. 6b). In the Western blotting study of this patient, although extra bands were found, the pattern did not significantly differ from control (data not shown). The expression of a 62- to 63-kDa band compared to control, however, shows the possible expression of the truncated selenoprotein N on this nonspecific extra band.

Patient 2 was homozygous for the transition mutation 80 dup 20 bp in exon 1 of *SEPN1*; this mutation leads to a frameshift mutation at R27. In theory, this would generate many nonsense mutations including TGA (Fig. 6c). Since frameshift or nonsense mutations are premature translation termination codons, they degrade immediately by nonsense-mediated decay process (NMD) and are a major

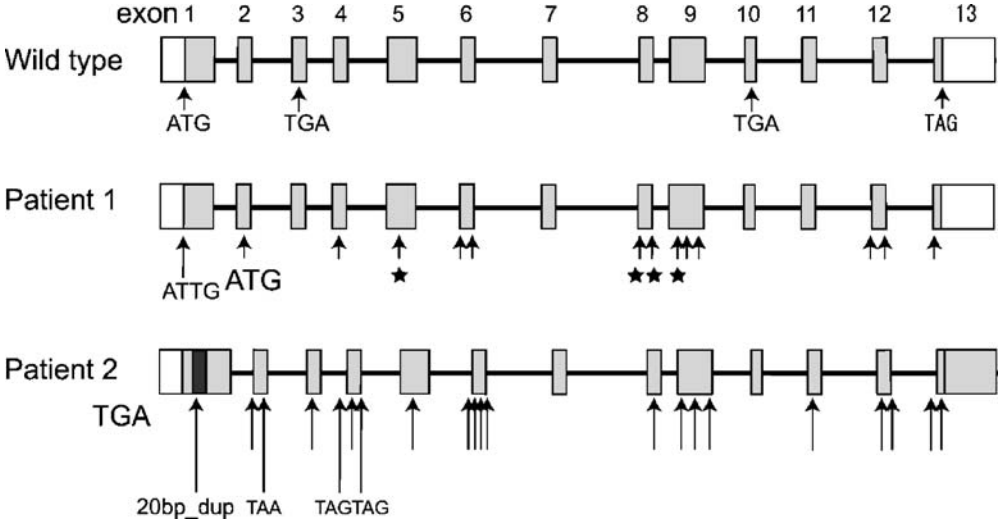

Fig. 6 Schematic representation of the *SEPN1* gene. Wild type, patients 1 and 2. The mutation in patient 1 (1_2 insT) most likely resulted in a null mutation, since the initiator ATG was lost; however, succeeding ATGs that may act as putative translation starts to exist in many numbers. The locations of hypothetical ATGs are indicated by *arrowheads*. *Stars* indicate “Kozak sequence.” Patient 2 was homozygous for the transition mutation 80 dup 20 bp in exon 1 of *SEPN1*; this leads to a frameshift at R27 and, in theory, may have generated many nonsense mutations including TGA. The locations of hypothetical TGAs are indicated by *vertical arrows*. Three *longer arrows* indicate TAA or TAG

cause of inherited genetic disorder [27]. NMD is a process by which most mRNAs having premature stop codons located >50–55 nucleotides upstream of most 3′-exon-exon junctions are rapidly degraded. However, in selenoproteins, translational termination is also linked to another process that has a bearing on the success or failure of Sec incorporation. Under conditions of limited selenium, differential mRNA stability appears to be the primary means of regulation for selenoprotein expression. However, some selenoprotein mRNAs are resistant to NMD even under conditions of limited selenium [28]. Indeed in our study, the phenotype of patient 2 corresponded to that of RSMD1, and the size of expressed selenoprotein N was about a few kilodaltons smaller than normal control as detected by Western blotting. Immunopathological findings in patients 2 were indistinguishable from control. A possible mechanism behind this observation is that *SEPN1* mRNAs may be resistant to NMD despite the existence of numerous TGA codons and, by means of the selenocysteine translation system, truncated selenoprotein N are then expressed. Another possibility is that the SECIS element may have changed the reading frame or start codon of mRNA. A recent study revealed that each stop codon (UGA, UAG, UAA) in the *SEPN1* gene might become a readthrough codon to various degrees in the presence of the 3′UTR SECIS element [29]. We confirmed that the relative *SEPN1* mRNA expression of two targets (Exon boundary 5–6 and 12–13) was reduced in patients 1 and 2 by quantitative real-time PCR. Expression levels of mRNA in patient 1 were 27–37% reduced, although this change was not found to be significant. On the other hand, the expression levels of mRNA in patient 2 were found to be significantly reduced by 70–71% compared to control. It is uncertain how the degree of resistance to NMD occurred under the influence of the SECIS element. The 1_2 ins T mutation did not turn out TAA codons through the entire coding sequence; however, 80 dup 20 bp produced a TAA codon in exon 2 (Fig. 6). Since the SECIS element basically works on the TGA codon, it could not prevent NMD by the TAA codon. The correlation between the SECIS element and NMD has not yet been discovered and our studies could provide some insights regarding this. We could not provide the exact mechanism behind the discrepancies in the results of the mutation, mRNA studies, and protein studies. Further examinations of more patients with *SEPN1* mutation are necessary to determine the exact relationship between the gene abnormality, the expression level of selenoprotein N, and clinical features of RSMD1.

In summary, we reported the clinical features of RSMD1 with novel mutations. We also described the expression pattern of selenoprotein N in biopsied muscle of control and RSMD1. We confirmed the localization of selenoprotein N in the endoplasmic reticulum pathologically. This analysis of the correlation between *SEPN1* mutations and protein expression could be the first report to describe the molecular mechanism between NMD and Sec incorporation in humans. These observations add to a growing body of evidence implicating specific genes/proteins in skeletal muscle function and delineating the pathological consequences of their dysfunction.

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Electronic database information

Accession numbers and URLs for data in this article are as follows:

1. Online Mendelian Inheritance in Man (OMIM): http://www.ncbi.nlm.nih.gov/entrez/query.fcgi? db=OMIM (for SEPN1 [MIM *606210], *LMNA* [MIM *150330],)
2. GenBank: http://www.ncbi.nih.gov/Genbank (for human *SEPN1* mRNA sequence [NM_020451], *LMNA* mRNA sequence [NM_170707])
3. BLAST: http://www.ncbi.nlm.nih.gov/BLAST/
4. Primer v3 program, Whitehead Institute for Biomedical Research: http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi

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