most common genotype groups in Japanese population. Each of the three age-matched and biopsy site-matched samples from c.1714G>C homozygous group and c.1714G>C/c.527A>T compound heterozygous group was compared. Muscle samples were taken from biceps brachii and frozen with isopentane cooled in liquid nitrogen. Serial frozen sections of 10 µm were stained using a set of histochemical methods including haematoxylin-eosin and modified Gomori trichrome.

Statistical analysis

Statistics were calculated using GraphPad Prism 5 software (GraphPad Software, La Jolla, California, USA). Between-group comparison for clinical data was performed using one-way analysis of variance with Dunnett's post-test. All values are expressed as means±SD. We performed two-sided tests with a $p<0.05$ level of significance.

RESULTS

Mutation profile

We identified homozygous or compound heterozygous *GNE* mutations in all 212 patients (see online supplement 1). In total, 63 different mutations were found including 50 missense mutations, 2 nonsense mutations, 1 insertion, 4 deletions, 5 intronic mutations and 1 single exon deletion (figure 1). Twenty-five novel mutations were identified including 17 missense mutations, 4 small deletions, 3 intronic mutations and 1 single exon deletion (figure 1, see online supplement).

Twenty-one mutations were found to be shared between two or more unrelated families. The three mutations occurring most frequently in the Japanese population were c.1714G>C (p.Val572Leu), c.527A>T (p.Asp176Val) and c.38G>C (p.Cys13Ser); these comprised 48.3%, 22.4% and 3.5%, respectively, of the total number of alleles examined (table 1).

Table 1 Allele frequency for GNE mutations in 212 Japanese GNE myopathy patients

	Allele frequency
Mutation type	
Missense	402 (94.8%)
Nonsense	3 (0.7%)
Insertion	1 (0.2%)
Small deletion	4 (0.9%)
Single exon deletion	2 (0.5%)
Intron	12 (2.8%)
Three most common mutations	
c.1765G>C (p.Val572Leu)	205 (48.3%)
c.578A>T (p.Asp176Val)	95 (22.4%)
c.38G>C (p.Cys13Ser)	15 (3.5%)
Total alleles	424

Genotype–phenotype correlations

The mean age of genetic analysis was 41.6±14.1 years (n=212), and the mean age of symptom onset based on the data available was 28.4±10.2 years (n=195). The earliest onset age was 10 and the latest was 61 years old in our cohort. Thirty-six among 154 patients (23.4%) were full-time wheelchair users at the point of genetic diagnosis with the average age at loss of ambulation being 36.8±11.3 years (n=36). The youngest wheelchair-bound age was 19, and the oldest ambulant age was 78. To investigate genotype–phenotype correlations in the major *GNE* mutations of Japanese population, we compared the age at symptom onset and loss of ambulation between the patients groups carrying either of the two most frequent mutations, c.1714G>C and c.527A>T (table 2). As with a previous report,[13] homozygous c.1714G>C mutations resulted in earlier symptom onset (23.9±7.1 years, $p<0.01$) and the majority of full-time wheelchair users were in this group. On the other hand, c.1714G>C/c.527A>T compound heterozygous patients first developed symptoms at a later age (37.6±12.6 years, $p<0.01$), and there were no wheelchair-bound patients at the time of genetic analysis in this group. Only three homozygous c.527A>T mutation patients were identified, and their average onset age (32.3±5.7 years) was also higher among total patients (28.4±10.2 years). All three patients were ambulant until the last follow-up visits (29, 40 and 44 years).

Among 212 cases, 80 patients underwent muscle biopsies. Overall pathological findings in our series were compatible with GNE myopathy. The characteristic rimmed vacuoles were observed in the majority (76/80, 95.0%) of the cases. Through the analysis of muscle biopsies from age-matched and biopsy site-matched samples, we found that the histopathological phenotypes were in line with these genotype–phenotype correlations (figure 2). Homozygous c.1714G>C mutations have led to much more advanced pathological changes with severe myofibre atrophy and increased numbers of rimmed vacuoles. Marked adipose tissue replacement was appreciated in a case with reflecting very advanced stage of muscle degeneration.

DISCUSSION

As shown in figure 1, mutations were located throughout the whole open reading frame of the *GNE* gene. The majority (94.8%, 402/424 alleles) of the mutations in our series were missense mutations (table 1), and there were no homozygous null mutations. These results are in accordance with previous reports[7 9] signifying that total loss of GNE function might be

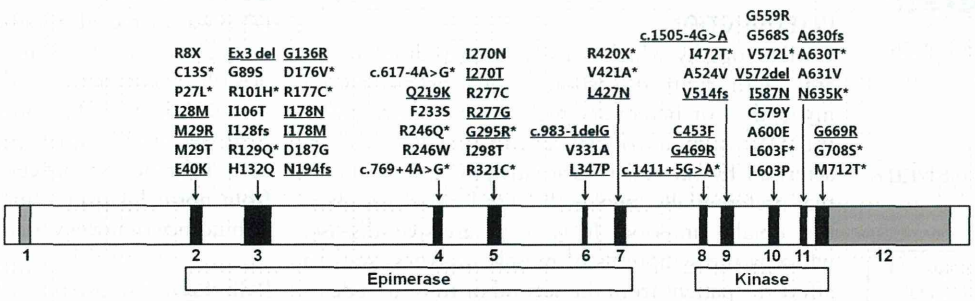

Figure 1 Mutation spectrum of *GNE* in the Japanese population. The mutations are located throughout the whole open reading frame. Twenty-five novel mutations are underlined, and 21 shared mutations are indicated with asterisks.

Table 2 Comparison of clinical course between two most frequent GNE mutations in Japanese population

Mutations	Age at exam (years)		Age at onset (years)		Age at WB (years)		Ambulant
c.1714G>C/c.1714G>C	38.6±13.4	(n=71)	23.9±7.1	(n=65)**	35.4±10.6	(n=28)	n=22
c.1714G>C/other	32.3±13.2	(n=25)	21.9±6.8	(n=22)*	37.0±8.6	(n=4)	n=16
c.1714G>C/c.527A>T	48.9±14.1	(n=38)	37.6±12.6	(n=35)**		(n=0)	n=29
c.527A>T/c.527A>T	37.7±7.7	(n=3)	32.3±5.7	(n=3)		(n=0)	n=3
c.527A>T/other	41.3±11.1	(n=51)	30.6±8.0	(n=46)		(n=2)	n=33
other/other	49.8±14.7	(n=24)	28.8±9.5	(n=24)		(n=2)	n=16
Total	41.6±14.1	(n=212)	28.4±10.2	(n=195)	36.8±11.3	(n=36)	n=118

Dunnett's multiple comparison test (control: total patients) *p<0.05, **p<0.01.
Other: a mutation other than c.1714G>C and c.527A>T; WB, wheelchair-bound.

lethal in human beings. The embryonic lethality of null mutation in *GNE* had also been proved in the mouse model.[14] Only three of total 212 patients carried a nonsense mutation; clinical data were available for two of them. Interestingly, one patient with compound heterozygous c.22C>T (p.Arg8X)/c.1714G>C (p.Val572Leu) mutations developed his first symptoms at the age of 15, while the other patient with c.1258C>T (p.Arg420X)/c.527A>T (p.Asp176Val) mutations developed her symptoms much later, at the age of 45. The similar difference was also observed in the phenotypes of patients with frame-shift mutations. A patient carrying c.383insT (p.I128fs) and c.1714G>C (p.Val572Leu) mutations developed his first symptom at the age of 13, whereas another two patients with c.1541-4del4 (p.Val514fs)/c.527A>T (p.Asp176Val) and c.581delA (p.N194fs)/c.527A>T (p.Asp176Val) mutations had later symptom onset, at the age of 30 and 32 years, respectively. This clinical variation can be explained as it reflects alternative missense mutations, because the two patients with very early onset shared the same missense mutation c.1714G>C, while the patients with the milder phenotype shared c.527A>T.

Among five intronic mutations identified in our series, c.617−4A>G and c.769+4A>G were previously reported as pathological mutations.[7 15] Three novel variants were located at splice junction of exon 6 (c.983−1delG), exon 8 (c.1411+5G>A) and exon 9 (c.1505−4G>A), raising the high possibility of relevant exons skipping. These variants were not detected in 200 alleles from normal Japanese individuals and also in the single nucleotide polymorphism (SNP) database.

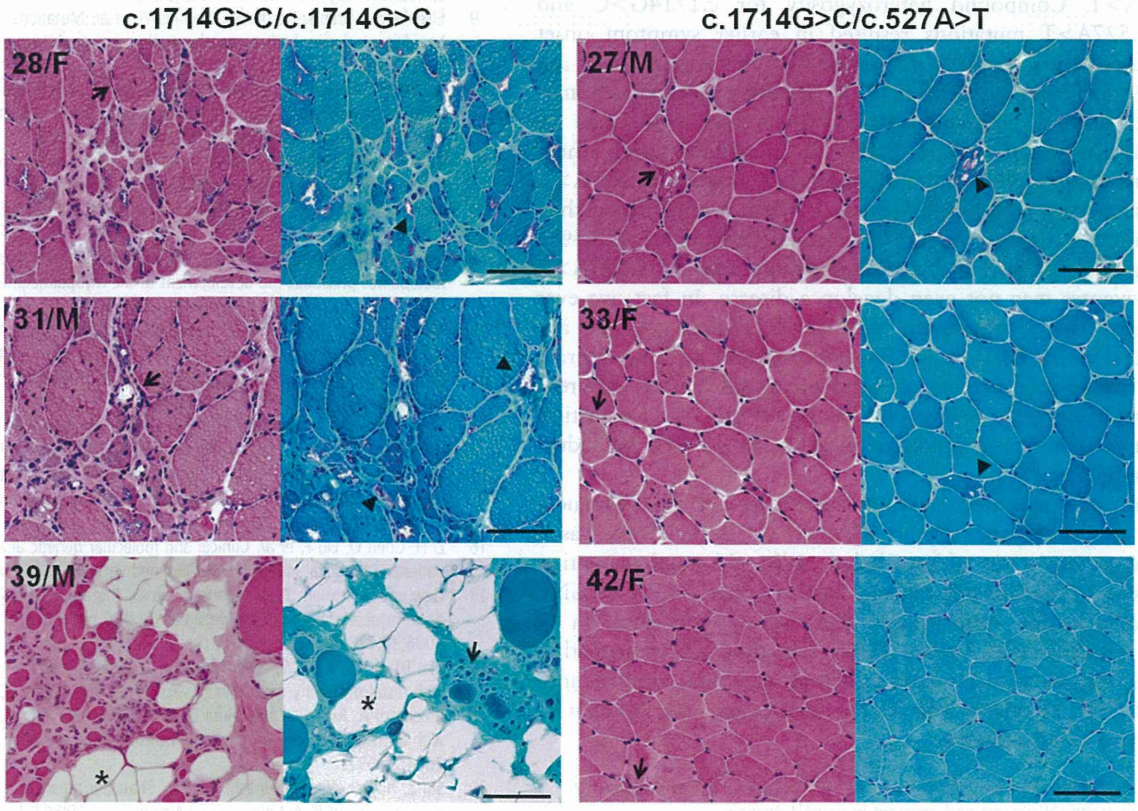

Figure 2 Comparison of muscle pathology between patients with homozygous c.1714G>C (p.Val572Leu) and with compound heterozygous c.1714G>C (p.Val572Leu)/c.527A>T (p.Asp176Val) mutations. Homozygous c.1714G>C (p.Val572Leu) mutations have led to much more advanced histopathological changes compared with compound heterozygous c.1714G>C (p.Val572Leu)/c.527A>T (p.Asp176Val) mutations. Haematoxylin-eosin (left) and modified Gomori trichrome (right) stains of muscle sections from age (c.1714G>C/c.1714G>C: 28, 31 and 39 years, c.1714G>C/c.527A>T: 27, 33 and 42 years) and biopsy site (biceps brachii muscles) matched samples. Bar=100μm; triangles: rimmed vacuoles; arrows: atrophic fibres; asterisks: adipose tissue.

As there are ethnic differences in *GNE* mutation frequencies,[9 16–19] establishing the mutation spectrum and defining predominant mutations in a certain population may be helpful for the diagnosis. Three most common mutations in the Japanese population and their allele frequencies (table 1) were in agreement with previous data.[7 13] The allele frequencies of top two mutations (c.1714G>C and c.527A>T) comprise more than two-third of the total number of alleles suggesting that founder effects are involved in the relatively higher incidence of GNE myopathy in Japan.

Although most of patients showed characteristic pathological features, the existence of exceptional cases with atypical biopsy findings implies that GNE myopathy cannot be totally excluded from the absence of rimmed vacuoles in muscle biopsies. On the other hand, we found 94 patients who were pathologically or clinically suspected but not had mutations in *GNE*. Several cases of VCP myopathy mutations in (*VCP*), myofibrillar myopathy mutations in (*DES*) and reducing body myopathy (*FHL1*) were later identified in this group, suggesting these diseases should be included as differential diagnosis of GNE myopathy.[20]

In terms of genotype–phenotype correlations, we confirmed that homozygosity for c.1714G>C (p.Val572Leu) mutation resulted in more severe phenotypes in clinical and histopathological aspects. In contrast, the second most common mutation, c.527A>T (p.Asp176Val), seems to be a mild mutation as the onset of the disease is much later in the compound heterozygotes with this mutation and c.1714G>C. Several evidences further strengthened the link between the more severe phenotype and c.1714G>C, and between the milder phenotype and c.527A>T. Compound heterozygosity for c.1714G>C and non-c.527A>T mutations resulted in earlier symptom onset (22.9±6.8 years, $p<0.05$) compared with the average onset age of the total group, whereas c.527A>T, both presented as homozygous and as compound heterozygous mutations, lead to slower disease progression (table 2). In addition, only three patients carrying this second most common mutation c.527A>T in homozygous mode were identified, which is much fewer than the number expected from high allele frequency (22.4%), raising a possibility that considerable number of c.527A>T homozygotes may not even develop a disease. In fact, we ever identified an asymptomatic c.527A>T homozygote at age 60 years.[7] Now he is at age 71 years and still healthy. Overall, these results indicate that different mutations lead to different spectra of severity. However, this is a result of a statistical summary that cannot predict clinical course of each individual patient.

Here, we presented the molecular bases of 212 Japanese GNE myopathy patients with 25 novel *GNE* mutations. Based on the current status of knowledge, sialic acid supplementation may lead to considerable changes in the natural course of GNE myopathy within near future. The ongoing identification of *GNE* mutations and further studies regarding the clinicopathological features of each mutation will provide better understanding of GNE myopathy and lead to accelerated development of treatment for this disease.

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Contributors AC had full access to all of the data in the study and wrote the manuscript; YKH supervised all aspects of this study including study design, data interpretation and manuscript preparation; KM and YO participated in collecting and analysing all the clinical and genetic data; SN, I Nonaka and I Nishino were involved in data analysis and interpretation and also supervised manuscript preparation.

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Competing interests None.

Ethics approval This study was approved by the ethics committee of National Center of Neurology and Psychiatry.

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Case report

Deep sequencing detects very-low-grade somatic mosaicism in the unaffected mother of siblings with nemaline myopathy

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Abstract

When an expected mutation in a particular disease-causing gene is not identified in a suspected carrier, it is usually assumed to be due to germline mosaicism. We report here very-low-grade somatic mosaicism in *ACTA1* in an unaffected mother of two siblings affected with a neonatal form of nemaline myopathy. The mosaicism was detected by deep resequencing using a next-generation sequencer. We identified a novel heterozygous mutation in *ACTA1*, c.448A>G (p.Thr150Ala), in the affected siblings. Three-dimensional structural modeling suggested that this mutation may affect polymerization and/or actin's interactions with other proteins. In this family, we expected autosomal dominant inheritance with either parent demonstrating germline or somatic mosaicism. Sanger sequencing identified no mutation. However, further deep resequencing of this mutation on a next-generation sequencer identified very-low-grade somatic mosaicism in the mother: 0.4%, 1.1%, and 8.3% in the saliva, blood leukocytes, and nails, respectively. Our study demonstrates the possibility of very-low-grade somatic mosaicism in suspected carriers, rather than germline mosaicism.

Keywords: Nemaline myopathy; *ACTA1*; Deep resequencing; Next-generation sequencer; Low-grade somatic mosaicism

1. Introduction

Nemaline myopathy is a common form of congenital myopathy characterized clinically by general hypotonia and muscle weakness, and pathologically by the presence of nemaline bodies within the myofibers [1,2]. *ACTA1* is one of the nine known genes associated with nemaline myopathy [3].

Sometimes in the clinic, an expected mutation is not identified in a suspected carrier. This is usually assumed to be because of germline mosaicism, in which mosaicism occurs in the carrier's germline only. Here we report, for the first time, very-low-grade somatic mosaicism detected by deep resequencing using a next-generation sequencer (NGS) in an unaffected mother of two affected siblings.

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0960-8966/

2. Case report

2.1. The proband

The proband was a boy who was 6 years old at the time of the study. He was the first child of healthy nonconsanguineous Japanese parents. He had an affected sister (Fig. 1a). He was born by cesarean section at 40 weeks of gestation after an uneventful pregnancy. He was admitted to a neonatal intensive care unit immediately after birth because of asphyxia with loss of spontaneous respiration and general hypotonia. His birth weight was 2640 g, height 48 cm, and occipitofrontal head circumference 33 cm. His Apgar scores were 1 and 4 at 1 and 5 min, respectively. He quickly recovered with ventilatory support. Laboratory tests showed normal findings except mildly elevated creatine kinase (653 IU/L). When he was discharged at age 2 months, general hypotonia remained with absent deep tendon reflexes. Antigravity movements were not observed. Arthrogryposis and mild cardiomegaly were noted. Ultrasound cardiography revealed mild dilatation and dyskinesia of the left ventricle. At 7 months, he was given a tracheostomy, and home ventilation therapy was introduced because of aspiration pneumonia (Supplementary Fig. 1a). Tube-feeding was also started because of poor swallowing. At 9 months, a muscle biopsy was performed. On a modified Gomori trichrome stain, nearly all the muscle fibers contained nemaline rods. Intranuclear rods were also scattered (Fig. 1b). On staining for ATPases, type 1 fiber atrophy and predominance were seen. Immunolabeling of a muscle biopsy from the patient with α-actinin showed intensely stained rod bodies (Fig. 1c). Co-staining with α-actinin, lamin A (as a marker of the nuclear envelope), and DAPI clearly revealed intranuclear rods (Fig. 1c). His condition was diagnosed as nemaline myopathy. He could sit unassisted at age 2 years, move on his hip at age 3, and walk with assistance at age 5. He showed normal intellectual ability. At 5 years, his cardiac function was re-evaluated by ultrasound cardiography. Mild left ventricular dilatation and dyskinesia remained.

2.2. The affected sister

The proband's only sibling, a sister 3 years younger, was also affected (Fig. 1a). She was born by cesarean section at 38 weeks of gestation after an uneventful pregnancy. She was similarly admitted to a neonatal intensive care unit immediately after birth because of hypoventilation and hypotonia. Her birth weight was 2620 g, height 48.5 cm, and occipitofrontal head circumference 36.5 cm. Her Apgar scores were 4 and 6 at 1 and 5 min, respectively. Serum creatine kinase was 190 IU/L, which was within the normal range. She recovered with ventilatory support, but needed continuous oxygen therapy. She had difficulty thriving and tube-feeding was introduced. Her cardiac function was normal without cardiomegaly. General hypotonia remained. After she was discharged at age 2 months, frequent aspiration pneumonia occurred. She started to use nocturnal noninvasive positive pressure ventilation at the age of 22 months. At age 2 years, she was given a tracheostomy and was controlled under nocturnal ventilation after respiratory syncytial virus infection following respiratory failure (Supplementary Fig. 1a). Because of her clinical presentation, she was also suggested to have congenital (nemaline) myopathy. At present, she shows antigravity movement of the extremities although she has not acquired head control and cannot roll over.

2.3. Genetic and three-dimensional structural analysis

First we checked for *ACTA1* (NM_001100.3) mutation in the proband's DNA, considering the presence of intranuclear rods. We identified a novel heterozygous missense mutation, c.448A>G (p.Thr150Ala), by Sanger sequencing. Because autosomal recessive inheritance was possible from the family tree, we performed whole-exome sequencing of the proband, affected sister, and their parents to identify a further genetic cause. Genomic DNA obtained from blood leukocytes was captured using a SureSelect^{XT} Human All Exon 50 Mb Kit (Agilent Technologies, Santa Clara, CA) and sequenced on a HiSeq2000 (Illumina, San Diego, CA) with 101-bp paired-end reads, as previously described [4]. The mean depth of coverage was 123× to 143×. We selected rare protein-altering and splice-site variants after filtering against dbSNP135 and 408 in-house control exomes. Among the rare variant calls, we first screened for genes known to cause nemaline myopathies, namely *ACTA1* (MIM 102610) [3], *TPM3* (MIM 191030) [5], *NEB* (MIM 161650) [6], *TPM2* (MIM 190990) [7], *TNNT1* (MIM 191041) [8], *CFL2* (MIM 601443) [9], *KBTBD13* (MIM 613727) [10], *KLHL40* (MIM 615340) [11], and *KLHL41* (MIM 607701) [12]. We identified only the novel heterozygous missense mutation c.448A>G (p.Thr150Ala) in *ACTA1* in both the affected brother and sister, which we confirmed by Sanger sequencing. Copy number analysis by eXome Hidden Markov Model (XHMM) [13] using next-generation sequencing (NGS) data revealed that there were no copy number changes within the *ACTA1* locus (Supplementary Fig. 1b). This mutation was not identified in either of the parents, after testing DNA obtained from their saliva, hair, nails, and blood by Sanger sequencing (Fig. 2a). This mutation, which alters the evolutionarily well-conserved Thr150 to Ala (Fig. 2b), was not present in the NHLBI Exome Sequencing Project (ESP6500). Two of three web-based prediction programs suggested that this mutation is pathogenic (PolyPhen-2: benign; SIFT: deleterious; MutationTaster: disease-causing). We also searched for any rare variants that were compatible with an autosomal recessive inheritance model, such as a homozygous mutation or compound heterozygous

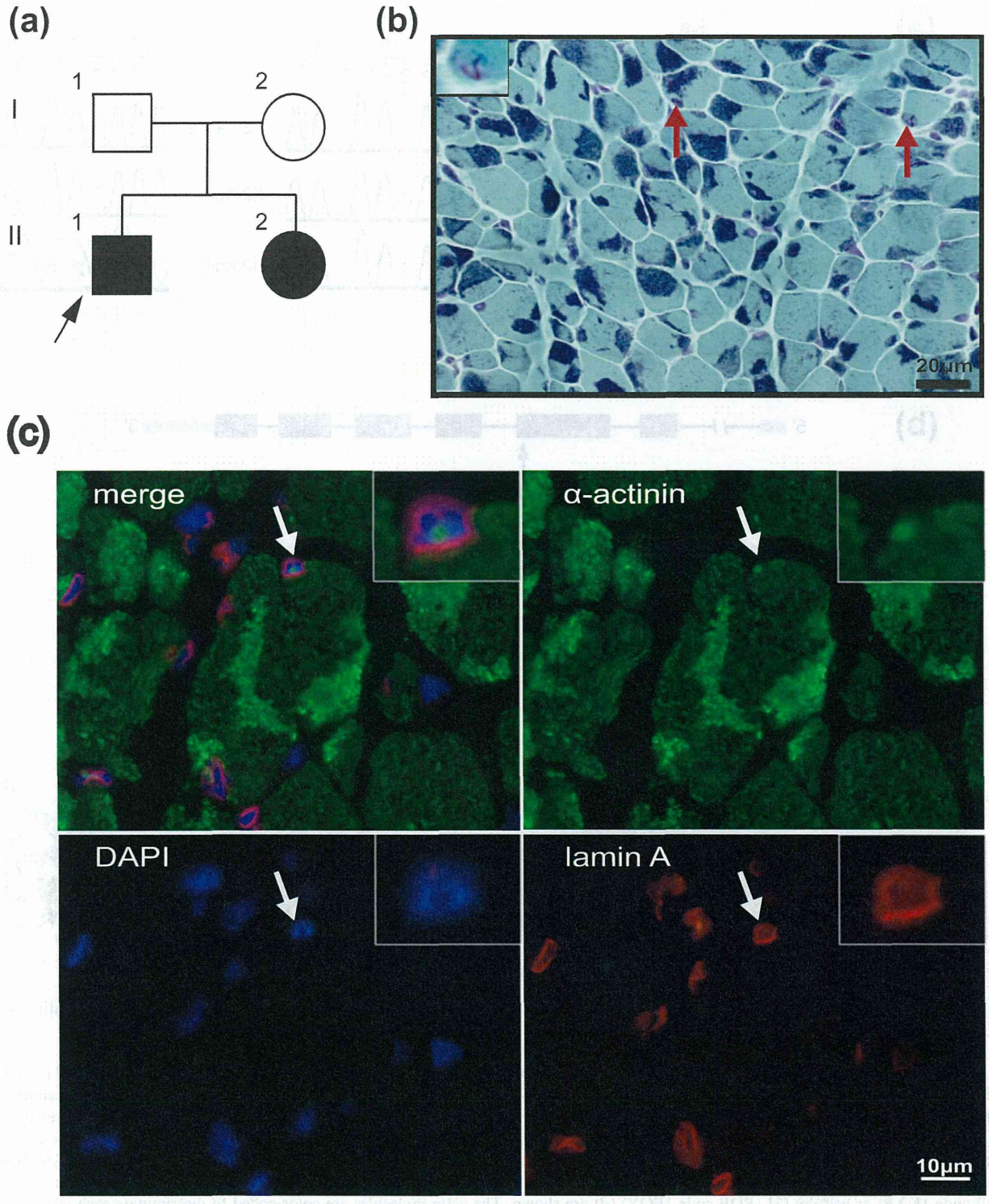

Fig. 1. (a) Family pedigree. (b) Light microscopic images of the muscle of the proband at age 9 months. With a modified Gomori trichrome stain, nearly all the muscle fibers can be seen to contain nemaline rods. Intranuclear rods (arrows, and upper-left window) are also scattered in some nuclei. Bar = 20 μm. (c) Immunohistochemical analysis of a muscle biopsy using anti-α-actinin (EA-53; Sigma, St. Louis, MO) (green) and anti-lamin A (red) antibodies [19]. Nuclei were stained with DAPI (blue). Nemaline rods were strongly stained by anti-α-actinin. An intranuclear rod was also seen (arrows, and higher-magnification inset boxes). Scale bar = 10 μm.

mutation, but no candidate mutations were identified (data not shown).

To explore the effect of the *ACTA1* p.Thr150Ala mutation, we mapped the mutation onto reported crystal structures. Thr150 is located near the polymerization/interaction interfaces between actin monomers (Fig. 2c) and between actin and its interacting proteins (Fig. 2d, Supplementary Fig. 1c). Thus, p.Thr150Ala may

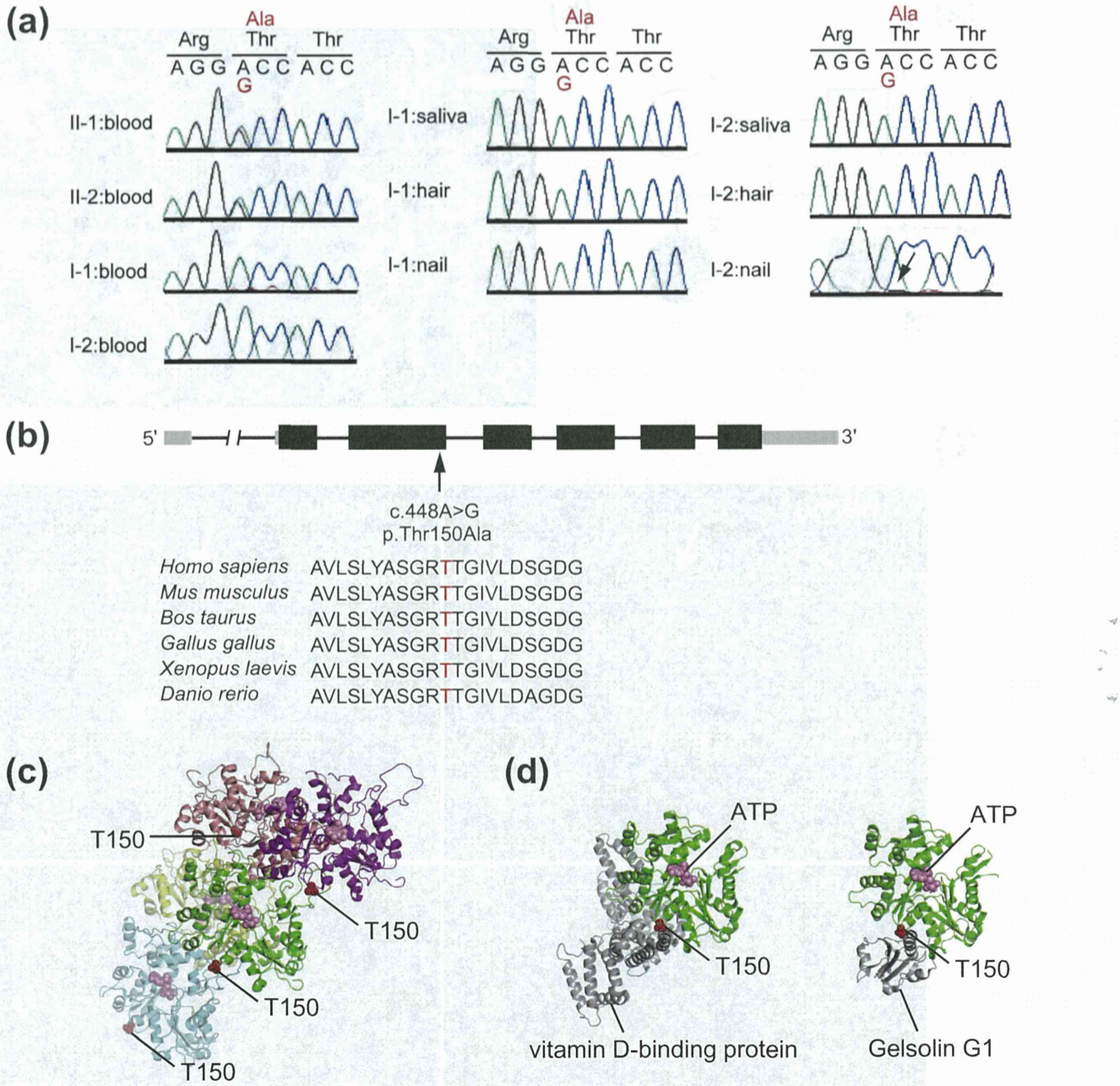

Fig. 2. (a) Sanger sequencing of the c.448A>G mutation using DNA from the affected siblings and the parents obtained from blood (left), and DNA from the father (middle) and mother (right) obtained from saliva, hair, and nails. The heterozygous mutation was identified in the affected siblings but not in any of the parental samples. No clear peak for the G allele was observed in the mother's nail DNA sample (arrow). (b) Schematic representation of *ACTA1*. The light gray bars represent untranslated regions and the black bars represent coding exons. Exon 1 is a non-coding exon. The c.448A>G mutation changes the well-conserved amino acid Thr150 (red) into Ala. (c, d) Structural implications of the p.Thr150Ala mutation in *ACTA1*. Structures of bare F-actin filaments determined by electron microscopy (Protein Data Bank (PDB) code 4A7N) (c) and G-actins in complex with vitamin D-binding protein (PDB code 1KXP) or gelsolin G1 (PDB code 1EQY) (d) are shown. The actin molecules are color-coded to discriminate each monomer in the F-actin filaments or are colored green in the G-actin complexes. Thr150 and ATP molecules are shown as red and pink space-filling spheres, respectively.

affect polymerization and/or the interactions of actin with other proteins.

We expected autosomal dominant inheritance from either parent. To test this, we performed deep resequencing for this mutation using an Illumina MiSeq platform. We used DNAs from the blood of the affected siblings, and from the saliva, nails, hair, and blood of their parents. The total read depth at c.448A in *ACTA1* was 131495× to 425933×. Very-low-grade somatic mosaicism was confirmed in the mother: 0.4%, 1.1%, and 8.3% in saliva, blood, and nails, respectively (all beyond 0.1% of the background level) (Table 1).

We used allele-specific PCR to confirm the presence of the mutation in the mother. The primer sequences and

Table 1
Deep resequencing of c.448A>G in *ACTA1* in various samples from each individual.

Individual	Tissue	Total depth (×)	Disease status	Wild-type allele (×)	Mutant allele (×)	% of wild-type allele	% of mutant allele
II-1 (Proband)	Blood	380016	Affected	192510	186808	50.7	49.2
II-2 (Sister)	Blood	425933	Affected	215496	209612	50.6	49.2
I-1 (Father)	Blood	261948	Unaffected	260897	355	99.6	0.1
I-2 (Mother)	Blood	364850	Unaffected	360030	4103	98.7	1.1
I-1 (Father)	Saliva	245895	Unaffected	245130	299	99.7	0.1
I-1 (Father)	Hair	190636	Unaffected	189929	253	99.6	0.1
I-1 (Father)	Nail	325282	Unaffected	324198	381	99.7	0.1
I-2 (Mother)	Saliva	239339	Unaffected	237872	999	99.4	0.4
I-2 (Mother)	Hair	324289	Unaffected	323120	400	99.6	0.1
I-2 (Mother)	Nail	131495	Unaffected	120210	10956	91.4	8.3

PCR conditions are available upon request. Both the wild-type and mutant alleles were amplified in the proband and the affected sister at a similar level. Both alleles could also be amplified in the mother, but the wild-type allele was amplified at a much greater level than the mutant allele. The wild-type allele only was amplified in the father (Supplementary Fig. 2a). Sanger sequencing of these amplicons confirmed the mutation in the proband, sister, and mother (Supplementary Fig. 2b).

There are various conventional methods to detect somatic mosaicism: Sanger sequencing to detect a small variant peak compared with the wild-type peak, high-resolution melting (HRM) analysis to detect an aberrant melting pattern, allele-specific PCR to amplify only the mutant allele, and pyrosequencing and SNaPshot analysis for quantitative variant detection [14]. We explored whether our very-low-grade somatic mosaicism could be detected by HRM, because this has been suggested to be one of the more sensitive methods [15]. We performed HRM analysis as previously described [16] using DNAs from normal controls, the affected siblings, the father (all DNA derived from blood), and the mother (DNA derived from the nails, which showed the highest rate of mosaicism (8.3%). The melting curves of both affected siblings were aberrant and were called mutant, but those of the father and mother were called normal (Supplementary Fig. 2c). In other words, this technique could not detect the 8.3% mosaicism.

3. Discussion

Here, we report very-low-grade somatic mosaicism in the unaffected mother of siblings with nemaline myopathy, identified by deep resequencing using NGS. Our study is significant in two ways. First, we demonstrate the possibility of very-low-grade somatic mosaicism in a suspected carrier, rather than germline mosaicism; this is likely to be a very rare event. Second, we present another example of the clinical application of NGS.

The novel heterozygous mutation c.448A>G (p.Thr150Ala) in *ACTA1* is likely to be responsible for the nemaline myopathy in this pedigree based on four lines of evidence: the mutation is not registered in the ESP6500 database, the substituted amino acid is well conserved, two previously reported mutations also involve residue 150 (Thr150Asn, Thr150Ser) [17,18], and three-dimensional structural modeling suggests an impact on polymerization and/or the interactions of actin with other proteins. Interestingly, the mutation exists within a region where most of the mutations identified in patients with intranuclear rod myopathy, a variant of nemaline myopathy associated with *ACTA1*, are located [18].

The majority of reported *ACTA1* mutations are *de novo* heterozygous mutations in sporadic cases. This is likely to be due to the severity of nemaline myopathy with *ACTA1* mutation. However, autosomal dominant inheritance has been observed in a few situations: a pedigree with a relatively mild phenotype or incomplete penetrance, or parental somatic/germline mosaicism [2,18]. To date, there have been three reported cases of somatic mosaicism of *ACTA1* in one parent of a severely affected patient [18].

In our study, allele-specific PCR, despite being non-quantitative, was sufficiently sensitive to detect mosaicism in blood leukocytes from the mother (mosaic rate 1.1%). Thus, this method is worth trying to confirm a suspected low-grade mosaicism. In contrast, as we were unable to detect 8.3% mosaicism using HRM, NGS should be the first choice for detecting very-low-grade somatic mosaicism that other methods might miss. A recent paper has described using NGS to detect somatic *BRAF* mutations down to 2% allele frequency, demonstrating the increased sensitivity of this method compared with HRM (limit 6.6% allele frequency), pyrosequencing (limit 5% allele frequency), and Sanger sequencing (limit 6.6% allele frequency) [15].

In our family, the mother does not seem to have any neurological problems in her daily activities, although she has not been clinically examined and no muscle imaging studies or biopsies have been undertaken.

The proband had mild left ventricular dilatation with dyskinesia without a hypertrophic phenotype. In the literature, cardiomegaly appears to be a rare complication. Patients with *ACTA1* mutation usually have hypertrophic cardiomegaly [18,19].

In conclusion, we used NGS to confirm very-low-grade somatic mosaicism in the mother. Using conventional methods, the mother might have been judged to have germline mosaicism. Clinically, our data on the rate of

somatic mosaicism could be used to estimate the recurrence risk, although prenatal diagnosis would be required to provide certainty.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.nmd.2014.04.002.

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