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Novel SPEG mutations in congenital myopathies

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patients with previously performed screening of the *GLA* and/or the *TTR* genes or known mutations in either of the genes introduced a bias, with the risk of missing cases with either or both of the diseases.

Screening for hATTR amyloidosis and FD in patients with idiopathic SFN without any additional disease-specific symptoms or clinical characteristics in a Nordic population thus appears to be of little value in a clinical setting. Nevertheless, the divergent results in earlier genetic screening studies in patients with idiopathic SFN suggest that, in some populations, screening for these disorders may be worthwhile.[6,10]

Ethical Publication Statement: We (the authors) confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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NOVEL *SPEG* MUTATIONS IN CONGENITAL MYOPATHIES: GENOTYPE–PHENOTYPE CORRELATIONS

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ABSTRACT: *Introduction*: Centronuclear myopathies (CNMs) are a subtype of congenital myopathies (CMs) characterized by muscle weakness, predominant type 1 fibers, and increased central nuclei. *SPEG* (striated preferentially expressed protein kinase) mutations have recently been identified in 7 CM patients (6 with CNMs). We report 2 additional patients with *SPEG* mutations expanding the phenotype and evaluate genotype–phenotype correlations associated with *SPEG* mutations.
Methods: Using whole exome/genome sequencing in CM families, we identified novel recessive *SPEG* mutations in 2 patients.
Results: Patient 1, with severe muscle weakness requiring respiratory support, dilated cardiomyopathy, ophthalmoplegia, and findings of nonspecific CM on muscle biopsy carried a homozygous *SPEG* mutation (p.Val3062del). Patient 2, with milder muscle weakness, ophthalmoplegia, and CNM carried compound heterozygous mutations (p.Leu728Argfs*82) and (p.Val2997Glyfs*52).
Conclusions: The 2 patients add insight into genotype–phenotype correlations of *SPEG*-associated CMs. Clinicians should consider evaluating a CM patient for *SPEG* mutations even in the absence of CNM features.

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Congenital myopathies (CMs) are a group of muscle diseases that commonly present at birth or during infancy with muscle weakness and hypotonia. The clinical presentation ranges from mild hypotonia causing delays in achieving motor skills to severe muscle weakness causing death from respiratory involvement.[1] Centronuclear myopathies (CNMs) are a subtype characterized by increased central nuclei within myofibers, and often associated with disruption of excitation-contraction coupling.[1,2] Approximately 60–80% of CNMs are caused by dominant *DNM2* mutations, dominant and recessive *RYR1* and *CACNA1S* mutations, recessive *BIN1* mutations, and X-linked recessive *MTM1* mutations.[3–8] Recently, recessive *SPEG*

mutations have been identified in 6 CNM patients and 1 patient with non-CNM CM.[9–12] Here, we report 2 additional unrelated patients with CMs caused by recessive *SPEG* mutations, compare the clinical findings of all 9 patients, and discuss genotype–phenotype correlations thereby improving the understanding of *SPEG*-related CM.

METHODS

Patient Recruitment and Genetic Analysis. For patient 1, a CGH array was initially performed and then whole exome sequencing (WES) was performed in a diagnostic setting with a parent–offspring trio approach as previously described.[13] For Patient 2, the patient and her family were enrolled in an institutional review board-approved study (NINDS Protocol 12-N-0095). WES was initially performed through the National Institutes of Health (NIH) Intramural Sequencing Center using the Nimblegen SeqCap EZ Exome+UTR Library and Illumina HiSeq, and variants were analyzed using Varsifter.[14] Whole genome sequencing (WGS) was then performed by the Genomics Platform at the Broad Institute using Illumina HiSeq X Ten v2 chemistry, and variants were analyzed using Variant Effect Predictor.

Histopathology Studies. The muscle biopsy samples were frozen and processed using standard histological techniques.[15]

RESULTS

Clinical Description. Patient 1 was the first child of healthy consanguineous parents, with normal intellect and no family history of neuromuscular disease. He has been reported in a large series of cardiomyopathy patients with minimal clinical information.[13] The pregnancy was reportedly uncomplicated, and he was delivered by vacuum extraction at 37 weeks gestation. At birth, he presented with severe hypotonia and left-sided inguinal hernia. At age 4, he developed progressive proximal muscle weakness and was noted to have marked atrophy of his lower leg muscles, pes planovalgus, and a high-arched palate. His history was significant for recurrent abdominal pain and diarrhea, recurrent otitis media, frequent upper airway infections, recurrent pneumonias, and multiple bone fractures (distal ulna, medial condyle, distal tibia, all after trauma). His serum creatine kinase level ranged from 9 to 60 U/L (normal < 171 U/L).[16] At age 6, an electrocardiogram (EKG) showed biventricular hypertrophy, and an echocardiogram demonstrated severe left ventricular dilation with poor muscle contractility. He was started on digoxin, captopril, and diuretics; tube feeding; and nocturnal noninvasive ventilation. At age 7, a gastrostomy tube was inserted. At age 12, ophthalmoplegia and mild lumbar torsion-scoliosis was diagnosed. His dilated cardiomyopathy was progressive; his shortening fraction decreased from 20% at age 10 to 9% at age 16 with severe mitral valve
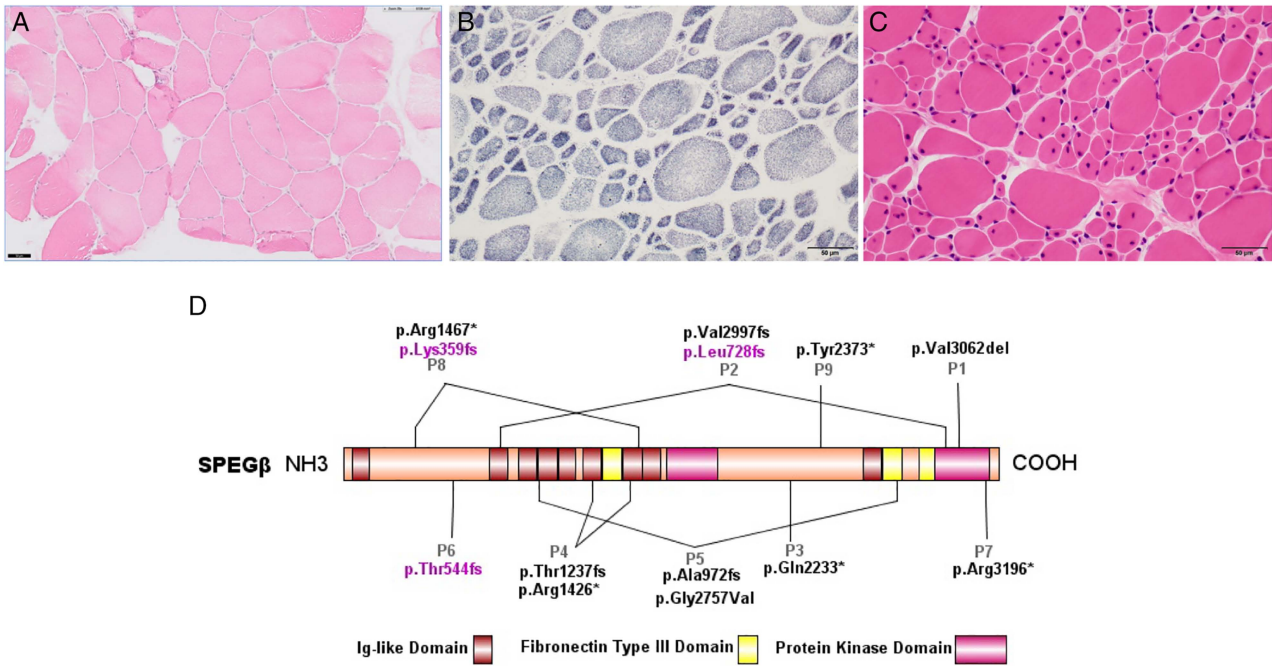
FIGURE 1. Histological examination of patients' muscle biopsies and *SPEG* schematic. **(A)** Hematoxylin and eosin (H&E) staining of Patient 1's muscle biopsy specimen, performed at 9 years of age. The muscle biopsy shows a mild increase in fiber size variability, several atrophic fibers, and only a few internal/central nuclei, consistent with non-CNM CM. Succinate dehydrogenase **(B)** and H&E **(C)** staining of Patient 2's muscle biopsy, performed at 3 years of age. The muscle biopsy reveals marked variability in fiber size with hypotrophic type 1 fibers and hypertrophic type II fibers with many central nuclei, consistent with CNM. Scale bar = 50 µm for all images. **(D)** Schematic of *SPEG*β domain organization with positions of identified mutations generated by Illustrator for Biological Sequences. Mutations affecting both *SPEG*α and *SPEG*β are in black, while mutations affecting only *SPEG*β are in pink. [Color figure can be viewed at wileyonlinelibrary.com]

insufficiency. Despite maximum support, he died at age 17 due to cardiopulmonary insufficiency.

Patient 2 is a 6.5-year-old female. She was born at term by means of Cesarean section and presented with a weak cry, respiratory distress, hypotonia, and reduced deep tendon reflexes. She had bilateral vocal cord paralysis, and a gastrostomy tube was placed at age 4 weeks due to swallowing concerns. She attained head control at 4 months, rolled over at 6–9 months, got into a sitting position at 9–12 months, crawled at 18 months, pulled to stand at 18–20 months, and walked at 2 years. EKG at 3 years and 10 months revealed sinus tachycardia; an echocardiogram was normal. Her serum creatine kinase level was within normal limits at 89 IU/L. At age 4, she had mild lower facial weakness, axial hypotonia, and proximal muscle weakness (MRC 3–4/5 range) with subgravity neck flexion. She has nearly complete ophthalmoplegia, bilateral ptosis, and intermittent strabismus. She has a high-arched palate and nasal speech. She has a weak cough and has had recurrent respiratory infections. She has difficulty feeding by mouth and receives all nutrition by means of a gastrostomy tube. Although she has a history of delayed motor milestones, she continues to demonstrate improvements. At age 4.5, she was still unable to run and jump. A nerve conduction study at age 5 showed a reduced compound muscle

action potential (CMAP) amplitude of 2.3 mV (normal > 3.0 mV) of the ulnar motor nerve recorded at the abductor digiti minimi muscle.

Muscle Biopsy Findings. Patient 1 had a quadriceps muscle biopsy at age 9, which is consistent with non-CNM CM and shows a mild increase in fiber size variability, several atrophic fibers, and only a few internal/central nuclei (<20% of fibers) (Fig. 1A). No clear fiber size hypertrophy is noted. Patient 2 had a quadriceps muscle biopsy at age 3, which is consistent with CNM and shows good fiber type differentiation without clear fiber type predominance, hypotrophic Type 1 fibers and hypertrophic Type 2 fibers, and many central nuclei (~50% of fibers and 60% of Type 1 fibers) (Fig. 1B,C). Electron microscopy for Patient 2 shows a few myofibers with unstructured cores.

Genetic Results. Copy number variant analysis for Patient 1 using array-CGH identified a deletion of chromosome 4q35.2 (190,462,807-191,041,681; 579 kb), a deletion of chromosome 7q11.22 (66,692,376-68,103,955; 1,412 Mb), and copy neutral homozygosity of 6 regions >10 Mb, confirming consanguinity. Both deletions did not correlate to a phenotype and were identified in his father; the first includes *BC087857*, and the second does not include any genes. Sanger sequencing of *FKRP*, *SEPN1*, and *RYR1* was unrevealing. Trio WES

Table 1. Clinical and molecular findings in individuals carrying *SPEG* mutations.

Patient/sex	P1/M (this study)	P2/F (this study)	P3/F[9]	P4/F[9]	P5/M[9]	P6/M[10]	P7/M[10]	P8/M[12]	P9/M[11]
Age (when reported)	Died at 17 years	6.5 years	Died at 3 weeks	6 years	1.5 years	3 years	7 years	10 years	Died at 19 weeks
SPEG exons Allele 1 (maternal)	Exon 38 c.9185_9187delTGG; p.Val3062del	Exon 10 and 38 c.2183delT; p.Leu728fs	Exon 30 c.6697C>T; p.Gln2233*	Exons 18 and 13 c.4276C>T; p.Arg1426*	Exons 10 and 35 c.2915_2916delCCinsA; p.Ala972fs	Exon 4 c.1627-1628insA; p.Thr544fs	Exon 40 c.9586C>T; p.Arg3196*	Exon 4 and 20 c.1071_1074dup; p.Lys359fs	Exon 30 c.7119C>A p.Tyr2373*
Allele 2 (paternal)	same as above	c.8962_8963ins25; p.Val2997fs	same as above	c.3709_3715 + 29del36; p.Thr1237fs	c.8270G>T; p.Gly2757Val	same as above	same as above	c.4399C>T; p.Arg1467*	same as above
Family history	Consanguineous parents, one healthy sister	No known consanguinity	Consanguineous parents, two sisters died early	No known consanguinity	No known consanguinity, sibling died early	parents from village in Turkey	Likely consanguineous	Non-consanguineous	Consanguineous parents
Birth history	Full term, severely hypotonic	Full-term, hypotonic	Full-term, breech delivery, severely hypotonic	Severely hypotonic	Born at 36 weeks of gestation, severely hypotonic	Full-term, hypotonic	Full-term, poor fetal movements	Uneventful pregnancy, hypotonic	Uneventful pregnancy, severely hypotonic
Neurological findings	symmetric atrophy of lower extremities, wheel chair bound at 17 years	normal early motor milestones, walked at 2 years, unable to run or jump	Died of severe muscle weakness	Sit unsupported at 2.5 years, unable to walk unsupported	Head control at 16 months, sit unsupported at 1 8 months	Head control at 6 months, sit unsupported - 12 months, unable to walk	Head control at 18 months, sitting at 30 months, walking- 4 years	sit -11 months, walk - 30 months, short distances	Contracture of right ankle and lacked deep tendon reflex, antigravity movement at 1 week
Eye findings	Ophthalmoplegia	Ophthalmoplegia, bilateral ptosis	No known evaluation	Ophthalmoplegia	None	ophthalmoplegia, mild ptosis	None	None	None
Respiratory issues	non-invasive ventilation during night, recurrent pneumonia	Weak cough	Insufficient respiratory efforts	Tracheostomy, mechanical ventilation dependent	brief NICU stay for respiratory issues, no assisted ventilation	NICU for apnea, no intubations, recurrent lung infections	non-invasive ventilation during first 48 hours of life	None	Intubation required immediately after birth, weaned at 10 weeks for palliative care
Feeding issues	Gastrostomy tube from age 6	gastrostomy tube	Gastrostomy tube early in life	Gastrostomy tube early in life	NG feeding	None	NG feeding until day 13	Gastrostomy tube from age 9	Gastrostomy tube
Cardiac issues	Dilated cardiomyopathy at age 7, severe mitral valve insufficiency	No cardiomyopathy at 3 years 10 months, sinus tachycardia	No cardiac evaluation	Dilated cardiomyopathy	Dilated cardiomyopathy, mitral valve insufficiency	None	Dilated cardiomyopathy mild mitral insufficiency	Reduced myocardial contraction, no ventricular dilation at 5 year	Enlarged atria, abnormal trabeculation of left ventricle
Skeletal issues	Torsion scoliosis	Ulnar fracture at age 4, condyle fracture at age 5, tibia fracture at age 11 (all after trauma)	Not applicable	None	None	Pectus excavatum and mild scoliosis	None	Scoliosis developed at age 4	Not applicable

identified a homozygous mutation in exon 38 of *SPEG*, c.9185_9187delTGG (p.(Val3062del)). The amino acid at this position is highly conserved and located in the protein kinase domain, which is critical for *SPEG* function. This variant was heterozygous in the parents and unaffected sister.

WES analysis for Patient 2 initially identified a maternally inherited c.2183delT (p.(Leu728Argfs*82)) mutation in exon 10 of *SPEG*. Due to regions of low coverage, WGS was then performed, and identified the same maternally inherited mutation in compound heterozygosity with a paternally inherited 25 base pair insertion in exon 38, c.8962_8963insCGGGGCGAACGTTCGTGGCCAAGAT (p.(Val2997Glyfs*52)). These variants result in frameshifts and thus are classified as loss-of-function. The variants identified in both patients were predicted deleterious by MutationTaster and absent from ExAC, gnomAD, and 1000 Genomes databases.

DISCUSSION

We report 2 additional patients with *SPEG*-associated CMs: Patient 1 with muscle pathology consistent with nonspecific CM, and Patient 2 with muscle pathology consistent with CNM (P1 and P2 in Fig. 1D). The clinical and molecular findings of all 9 patients reported so far including ours are summarized in Table 1, and pathological findings are described in Supplementary Table S1, which is available online.

SPEG is alternatively spliced into 4 tissue-specific isoforms: APEG (aortic preferentially expressed gene), BPEG (brain preferentially expressed gene), and *SPEGα* and *SPEGβ* (expressed in skeletal and cardiac muscle).[17] *SPEGβ* is the longer isoform with *SPEGα* missing amino acids 1–854.[9] Clinical data from Patients 6 and 8 suggests that *SPEGα* may partially rescue mutations affecting only *SPEGβ*, possibly preserving cardiac function.[10,12] This appears to be the case for Patient 2, who carries 1 variant sparing *SPEGα*, and has not yet developed signs of cardiac dysfunction. In contrast, Patient 1 carried a homozygous mutation affecting *SPEGα* and *SPEGβ*, and developed dilated cardiomyopathy, also seen in Patients 3, 4, 5, and 7 carrying mutations affecting both isoforms.[9,10] Of interest, Patient 9, who also carries a mutation affecting both isoforms, developed noncompaction cardiomyopathy.[11]

Skeletal muscle dysfunction seems more severe in patients with mutations affecting both isoforms, as seen in Patients 1, 3, and 9 dying early, and Patient 4 needing constant mechanical ventilation.[9,11] The other 2 patients with both isoforms affected are Patients 5 and 7.[9,10] In Patient 5, the disease was relatively mild, likely due to 1 variant being missense while all *SPEG* variants described so far have been loss-of-function, suggesting haploinsufficiency.[9] In Patient 7, the milder phenotype may be due to the

mutation being very close to the C-terminus, thereby escaping nonsense mediated decay and potentially having less effect on protein function.[10] Overall, these findings suggest *SPEGα* has a critical role in skeletal and cardiac function and the disease is more severe when both isoforms are affected. Future studies should investigate the role of *SPEGα* in compensating for mutant *SPEGβ*. The clinical features of all patients have phenotypic similarities, most notably the presence of respiratory problems (Patients 1–7, and 9), eye involvement (Patients 1, 2, 4, and 6), and scoliosis (Patients 1, 6, and 8).[9–12]

In summary, this study expands the genetic heterogeneity of *SPEG*-associated CMs and further elucidates genotype–phenotype correlations to help guide appropriate clinical screening and management. The phenotype of *SPEG*-associated CM is varied and expanding, including ophthalmoplegia, and diagnostic markers that were initially considered, such as the presence of centralized nuclei on muscle biopsy and dilated cardiomyopathy, do not capture all cases. Thus, it is important for clinicians to consider evaluating a patient with congenital myopathy for *SPEG* mutations using WES even in the absence of type 1 fiber predominance, central nuclei, or cardiomyopathy.

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INCREASED RISK OF MELANOMA IN *C9ORF72* REPEAT EXPANSION CARRIERS: A CASE–CONTROL STUDY

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ABSTRACT: *Introduction:* Amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD) are considered part of the same pathological spectrum. There is an increased risk of ALS in patients who have had melanoma. The risk of FTLD in melanoma (or cancer) patients is unknown. We aimed to study if *C9ORF72* expansion is linked to a higher prevalence of melanoma. *Methods:* We selected patients with a diagnosis in the ALS-FTLD spectrum who were tested for pathogenic mutations. Medical history was reviewed, to identify those with pathologically documented melanomas. *Results:* We included 189 patients. Sixty-two had identified pathogenic mutations (39 *C9ORF72*). *C9ORF72* carriers had a significantly higher risk of melanoma (odds ratio = 24.709; *P* < 0.007). There was no association with phenotype. *Conclusions:* These findings suggest that patients with a history of melanoma may have an increased probability of carrying a *C9ORF72* repeat expansion. ALS or FTLD carriers of *C9ORF72* should undergo surveillance for skin changes.

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Several studies have examined the risk of cancer in neurodegenerative diseases, including Parkinson and Alzheimer diseases, which have been reported to have a protective effect against the overall development of cancer.[1–5] The same overall protective effect has been reported recently in amyotrophic lateral sclerosis (ALS),[6] but some types of cancer seem to have increased prevalence in this population. There is mounting evidence of an increased risk of ALS in patients who have had melanoma.[7,8] ALS and frontotemporal lobar degeneration (FTLD) are considered part of the same pathological spectrum and share common pathogenic genetic mutations, with the chromosome 9 open reading frame 72 (*C9ORF72*) expansion being the most common in both.[9] A small study in FTLD reports overall lower risk of cancer.[10]

Several genes have been implicated in the association between cancer and neurodegenerative diseases, including progranulin (GRN),[11] fused-in-sarcoma (FUS),[12] and SQSTM1.[13] However, we still lack the understanding of the underlying mechanism that would explain such association.[14] Exploring the relationship between cancer and neurodegenerative diseases could provide new clues relevant to the etiologies and potential therapies for both sets of conditions.

Of interest, analysis of skin biopsies of *C9ORF72* repeat expansion ALS patients revealed early TDP-43 deposition and abnormal extracellular matrix findings,[15] suggesting a possible link between this expansion and skin disease.

We aimed to study the hypothesis that *C9ORF72* expansion is linked to a higher prevalence of melanoma.

PATIENTS AND METHODS

Patients. We selected ALS-FTLD spectrum patients, followed at the dementia and neuromuscular clinics of our