SIGMAR1 mutation associated with autosomal recessive Silver-like syndrome

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ABSTRACT

Objective: To describe the genetic and clinical features of a simplex patient with distal hereditary motor neuropathy (dHMN) and lower limb spasticity (Silver-like syndrome) due to a mutation in the sigma nonopioid intracellular receptor–1 gene (*SIGMAR1*) and review the phenotypic spectrum of mutations in this gene.

Methods: We used whole-exome sequencing to investigate the proband. The variants of interest were investigated for segregation in the family using Sanger sequencing. Subsequently, a larger cohort of 16 unrelated dHMN patients was specifically screened for *SIGMAR1* mutations.

Results: In the proband, we identified a homozygous missense variant (c.194T>A, p.Leu65Gln) in exon 2 of *SIGMAR1* as the probable causative mutation. Pathogenicity is supported by evolutionary conservation, in silico analyses, and the strong phenotypic similarities with previously reported cases carrying coding sequence mutations in *SIGMAR1*. No other mutations were identified in 16 additional patients with dHMN.

Conclusions: We suggest that coding sequence mutations in *SIGMAR1* present clinically with a combination of dHMN and pyramidal tract signs, with or without spasticity, in the lower limbs. Preferential involvement of extensor muscles of the upper limbs may be a distinctive feature of the disease. These observations should be confirmed in future studies. ***Neurology*®** **2016;87:1607–1612**

GLOSSARY

ALS = amyotrophic lateral sclerosis; **CMT** = Charcot-Marie-Tooth disease; **dHMN** = distal hereditary motor neuropathy; **dHMN-J** = distal hereditary motor neuropathy and pyramidal features identified in the Jerash region of Jordan; **ER** = endoplasmic reticulum; **ExAC** = Exome Aggregation Consortium database; **HSP** = hereditary spastic paraplegia; **MAF** = minor allele frequency; **MRC** = Medical Research Council; **σ1R** = sigma-1 receptor; **SIGMAR1** = sigma nonopioid intracellular receptor–1 gene; **UTR** = untranslated region; **WES** = whole-exome sequencing.

The distal hereditary motor neuropathies (dHMN) comprise a heterogeneous group of diseases that share the common feature of slowly progressive, symmetrical, and distal-predominant neurogenic weakness and amyotrophy.[1] There is phenotypic and genetic overlap between dHMN and other hereditary neuropathies and motor neuron disorders such as axonal forms of Charcot-Marie-Tooth disease (CMT2), juvenile amyotrophic lateral sclerosis (ALS), and hereditary spastic paraplegia (HSP). *BSCL2*- and *REEP1*-related disorders are an example of this overlap as autosomal dominant mutations in these genes can cause dHMN with upper limb predominance, pure HSP, or spastic paraplegia with amyotrophy of hands (Silver syndrome) or hands and feet (table 1).[2–6]

Homozygous mutations in the sigma nonopioid intracellular receptor-1 gene (*SIGMAR1*) have been reported as a cause of dHMN and juvenile ALS.[7,8] Here we describe a patient with a Silver-like syndrome carrying a homozygous mutation in *SIGMAR1* and review the

Supplemental data at Neurology.org

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Table 1 Genes and loci identified in distal hereditary motor neuropathies (dHMN) with upper motor neuron signs or with Silver syndrome

Gene/locus	Inheritance	Alternative name	MIM number
dHMN with upper motor neuron signs			
SETX	AD	ALS4	602433
BSCL2	AD	dHMN5	600794
GARS	AD	dHMN5	600794
4q34.3–q35.2	AD	HMSN5	600361
9p21.1–p12	AR	dHMN Jerash type	605726
Silver syndrome			
BSCL2	AD	SPG17	270685
REEP1	AD	SPG31	610250
SPAST	AD	SPG4	182601
4p16–p15	AD	SPG38	612335

AD = autosomal dominant; ALS = amyotrophic lateral sclerosis; AR = autosomal recessive; dHMN5 = distal hereditary motor neuropathy with upper limb predominance; HMSN = hereditary motor and sensory neuropathy; MIM number = catalogue assignment for the disease in the Mendelian Inheritance in Man system (omim.org/); SPG = spastic paraplegia.

phenotypic spectrum of mutations in this gene. We suggest that coding sequence mutations present clinically with a combination of dHMN and pyramidal tract signs, with or without spasticity, in the lower limbs, adding *SIGMAR1* to the list of genes that can cause overlapping motor neuron/nerve phenotypes.

METHODS The proband and 16 additional patients screened for *SIGMAR1* mutations were identified from those attending neuropathy clinics at the National Hospital for Neurology and Neurosurgery, Queen Square, London, UK. The proband was investigated as part of a larger study to determine the genetic etiology in patients with inherited neuropathies using whole-exome sequencing (WES). All patients had undergone clinical and instrumental assessments during the routine diagnostic process and mutations in common neuropathy-related genes had been excluded in all cases. Nerve conduction studies, EMG, laboratory tests, and MRI scans were performed using standard methods. WES, Sanger sequencing, and in silico analysis methods are detailed in the supplementary material at Neurology.org.

Standard protocol approvals, registration, and patient consents. The study had ethical approval from the National Hospital for Neurology & Neurosurgery/Institute of Neurology Joint Research Ethics Committee. All patients gave written informed consent for genetic testing.

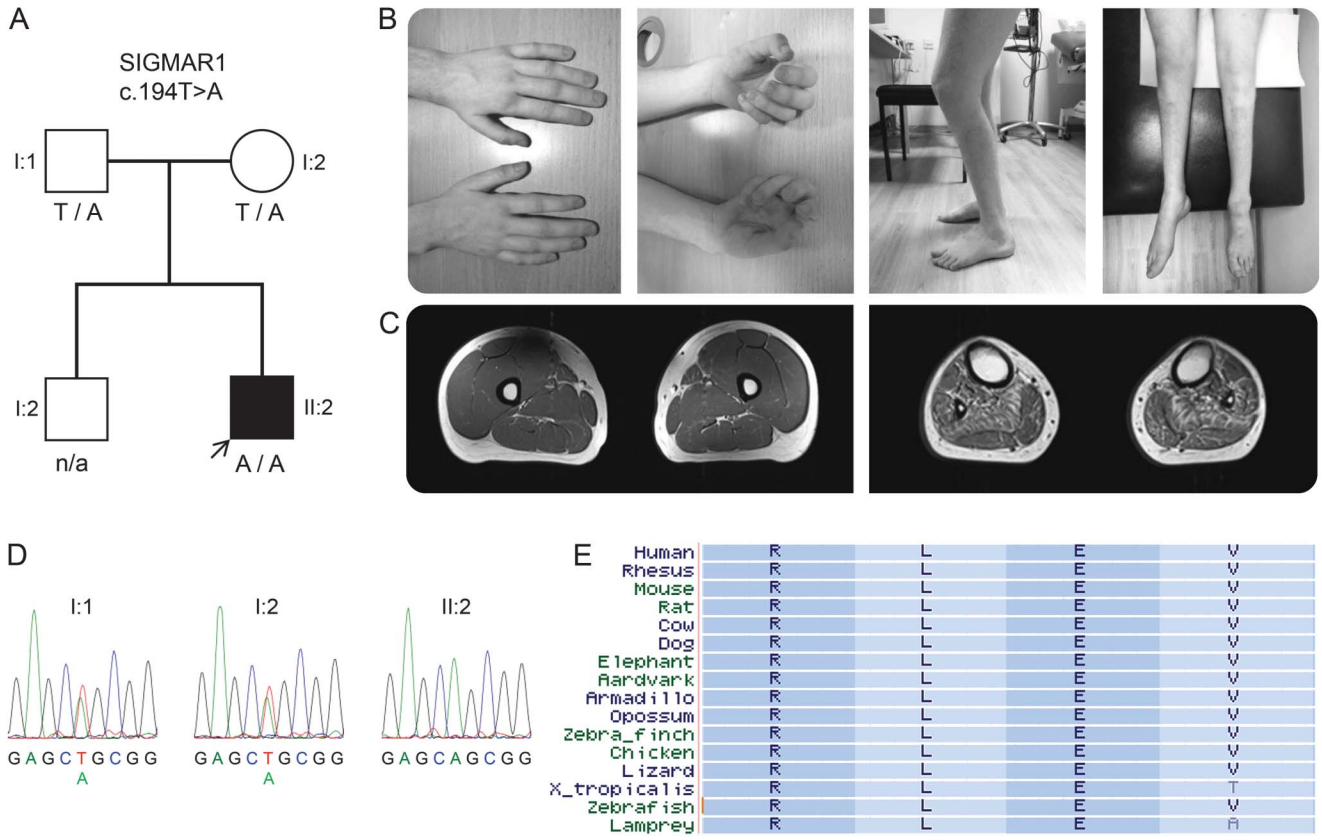
RESULTS **Clinical features.** The proband (figure, A) was the second child of healthy, nonconsanguineous parents of British and French descent. He walked independently at age 12 months. At age 3 years, he was noted to have bilateral foot drop and frequent falls and, over the following years, he developed progressive muscle weakness and atrophy in the lower limbs. Hand weakness and lower limb stiffness developed during the second decade of life. He underwent orthopedic surgery on his feet at ages 13–14 years and tendon-transfer surgery on both thumbs at ages 16–17 years with good functional outcomes. He has normal intellect, no sensory symptoms, and no sphincter problems. His family history was unremarkable and clinical and electrophysiologic examination of the parents was normal.

At age 17 years, on neurologic examination, he had clawed hands with no fixed contractures, bilateral finger and foot drop, knee bobbing, marked muscle atrophy from mid-forearms and knees down (figure, B), and weakness of wrist extension (Medical Research Council [MRC] grade 2/5 right, 4/5 left), finger extension (1/5), intrinsic hand muscles (0/5), ankle dorsiflexion (0/5 right, 1/5 left), ankle plantar flexion (1/5), toe extension (1/5), and toe flexion (0/5). Muscle strength in proximal muscles and finger flexors was normal. There was marked spasticity of the lower limbs and normal muscle tone in the upper limbs. Deep tendon reflexes were present in the upper limbs, increased at the knees, and absent at the ankles. Babinski sign was present bilaterally. Sensory and cranial nerve examinations were unremarkable. He walked with a combination of spastic and steppage gait. There was no scoliosis. Nerve conduction studies were consistent with a motor axonal neuropathy that was more severe in the lower limbs (table e-1). The superficial peroneal sensory action potential was normal and although the sural sensory nerve action potential amplitude was mildly reduced this may have been due to previous surgery rather than a sensory neuropathy. EMG of the biceps brachii showed features of chronic denervation. Brain and spinal cord MRIs were normal. Charcot-Marie-Tooth Neuropathy Score v.2 was 13.[9]

At age 20, neurologic examination confirmed progression of wrist extension weakness (MRC grade 1/5) causing hand drop and weakness of finger flexion (4/5) (video). The remainder of the examination was unchanged. MRI of the lower limbs demonstrated normal appearance of the thigh muscles and atrophy of all lower leg muscles with mild fatty replacement (figure, C). Serum creatine kinase level was normal at 184 IU/L.

Genetic studies. Molecular genetic analysis of the 17p11.2 chromosome region and direct sequencing of *MFN2*, *HSPB1*, *HSPB8*, *HSPB3*, *BSCL2,* and *GARS* genes revealed no pathogenic variants. WES on the proband revealed a total of 25,073 exonic variants (table e-2). The GEM.app software was used to focus on nonsynonymous, splice-site, and coding indel variants that were present in <5 families in the GEM.app database and that had a minor allele

Figure Family segregation, conservation of the *SIGMAR1* variant, and clinical images of the proband



(A) Segregation of the *SIGMAR1* variant c.194T>A in the family; genotypes are indicated below tested individuals. (B) Photographs of the proband show atrophy of intrinsic hand muscles, clawed hands, flexed knee posture (characteristic of knee bobbing), and atrophy of leg and foot muscles. (C) Axial T1-weighted MRI of the mid-thighs (left) and mid-legs (right) demonstrate normal appearance of the thigh muscles and atrophy of all lower leg muscles with mild fatty replacement especially of tibialis anterior, tibialis posterior, soleus, and peroneal muscles. (D) Sanger sequencing electropherograms demonstrate sequence variants in the proband and his parents. (E) Conservation of leucine (L) at amino acid position 65 of the σ-1 receptor encoded by *SIGMAR1*; a subset of 16 species were chosen, representing the 100 species available at the USCS browser.

frequency (MAF) <0.5% in the Exome Variant Server database (http://evs.gs.washington.edu/EVS/). From a total of 198 variants that met these filtering criteria, 9 of them involved genes associated with pure and complex inherited neuropathies/neuronopathies. Six were heterozygous variants in genes associated with autosomal recessive disorders (*ERCC6*, *LAMA2*, *MTPAP*, *MTTP*, *NTRK1*, and *PRX*). Two heterozygous variants were detected in genes associated with autosomal dominant spinal muscular atrophy and CMT2 (*BICD2* and *TRPV4*) and one homozygous variant was detected in *SIGMAR1*, a gene associated with autosomal recessive dHMN and juvenile ALS. The variants detected in *BICD2*, *TRPV4*, and *SIGMAR1* were validated by direct sequencing.

Cosegregation analyses allowed exclusion of the *BICD2* and *TRPV4* variants as pathogenic (e-Results) and confirmed that the proband's parents were heterozygous carriers of the *SIGMAR1* variant c.194T>A located in exon 2 (Ensembl ID ENST00000277010) (figure, D). This variant was present in 4 out of 97,910 alleles (MAF 0.004%) in the Exome Aggregation Consortium database (ExAC http://exac.broadinstitute.org/), with none of the carriers being homozygous, and was absent in the GEM.app database and in an in-house exome database of 138 clinically and neuropathologically normal controls. c.194T>A leads to the substitution of nonpolar leucine for polar glutamine at amino acid position 65 (p.Leu65Gln), affects a highly conserved nucleotide and amino acid (figure, E), and is predicted as being deleterious or disease-causing by pathogenicity prediction tools (table e-3).

The 4 coding exons, intron/exon boundaries, and the 3′-untranslated region (3′-UTR) of *SIGMAR1* were Sanger sequenced in the proband and 16 additional unrelated dHMN patients. No other exonic or intronic changes were identified (table e-4).

DISCUSSION Using WES in a simplex patient with distal weakness and amyotrophy and lower limb spasticity and electrophysiologic features consistent with

a motor neuropathy, we have identified *SIGMAR1* as the probable causative gene. The phenotype of the patient mimics classical Silver syndrome except for the absence of upper limb predominance and the autosomal recessive pattern of inheritance; we termed this novel phenotype Silver-like syndrome. Pathogenicity of the *SIGMAR1* variant is supported by the in silico analysis described above and previous reports of individuals carrying homozygous mutations in the same gene, which are summarized in table 2.[7,8]

Like the present case, affected individuals from previously reported families developed a combination of distal weakness in the upper ± lower limbs and pyramidal features in the lower limbs. Both our case and those with the mutation c.304G>C, located in exon 2, presented within the first 3 years of life and had severe involvement of forearm extensors and lower limb spasticity.[7] Although the authors designated the cases with c.304G>C as juvenile ALS, they had no bulbar involvement and EMG evidence of

Table 2 Genetic and clinical features of patients with *SIGMAR1* mutations

	Present study, 2016	Li et al., 2015[8]	Al-Saif et al., 2011[7]
No. cases/kindred	1/1	3/1	6/1
Ethnicity	French-British	Chinese	Saudi Arabian
Type of mutation	Homozygous, missense	Homozygous, splice-site	Homozygous, missense
Exon/intron	Exon 2	Intron 1	Exon 2
cDNA change	c.194T>A	c.151+1G>T	c.304G>C
Protein change	p.Leu65Gln	p.Gly31_Ala50del[a]	p.Glu102Gln
Protein domain[14]	Cytoplasmic	Cytoplasmic	Transmembrane
Disease onset, y	3	9-12	1-2
Motor delay	No	No	NA
Initial symptoms	Foot drop, frequent falls	Foot drop, pes varus	Lower limb spasticity and weakness
Hand involvement, y	12-13	11-15	9-10
Upper limb weakness	Yes; finger and wrist extensors > flexors	Yes; difficulty to straighten fingers at age 13 in one patient	Yes; paralysis of forearm extensors and triceps
Lower limb weakness	Yes	Yes	Yes
Distal predominant wasting/weakness	Yes	Yes	Upper limbs
Clinical fasciculations	No	No	NA
Upper limb spasticity	No	No	NA
Lower limb spasticity	Yes	No	Yes
Upper limb DTRs	Normal	NA	NA
Knee DTRs	Increased	Increased	Increased[b]
Ankle DTRs	Absent	Absent	Increased[b]
Babinski sign	Yes	Yes	NA
Sensory deficit	No	No	No
Bulbar involvement	No	No	No
Respiratory involvement	No	No	No
Cognitive impairment	No	No	No
Brain MRI	Normal	Normal	Normal
Spinal cord MRI	Normal	Normal	NA
Creatine kinase	Normal	Normal	NA
Phenotype description	dHMN with UMN signs/ spasticity in lower limbs	dHMN with UMN signs in lower limbs	ALS; UMN signs/spasticity in lower limbs

Abbreviations: ALS = amyotrophic lateral sclerosis; dHMN = distal hereditary motor neuropathy; DTRs = deep tendon reflexes; *SIGMAR1* = sigma nonopioid intracellular receptor-1 gene; UMN = upper motor neuron.
Esembl reference sequences for *SIGMAR1*: ENSG00000147955; ENST00000277010.
[a] c.151+1G>T causes an alternative splicing event that generates an in-frame deletion of 60 bp in exon 1 (c.92_151del, p. Gly31_Ala50del).
[b] Reported originally as lower limb spasticity and weakness accompanied by exaggerated tendon reflexes.

active denervation is not mentioned in the report. Thus, it appears that all these patients with exon 2 mutations share the same phenotype.

The cases with dHMN due to the splice-site mutation c.151+1G>T, resulting in deletion of the last 20 amino acids encoded by exon 1, had a later age at onset and lacked spasticity.[8] As suggested by the authors, this phenotype is similar to that described in patients with dHMN and pyramidal features identified in the Jerash region of Jordan (dHMN-J) and mapped to 9p21.1-p12, which encompasses *SIGMAR1*.[10] It is also significant that, as observed in our patient and those with the mutation c.304G>C, patients with dHMN-J had preferential involvement of forearm extensors over flexors.

The homozygous variant rs4879809 (9:34635598T>C, c.*31A>G), located in the 3′-UTR of *SIGMAR1*, has been detected in a consanguineous family with adult-onset ALS.[11] The authors suggested that c.*31A>G could contribute to the pathogenesis of ALS in this family. The allele C in 9:34635598, however, has a frequency of 99.5% in the ExAC database (99% homozygotes) and was present in all patients from our cohort and the proband's unaffected parents so we regarded it as nonpathogenic. Heterozygous 3′-UTR variants in *SIGMAR1* have been also identified in individuals with frontotemporal lobar degeneration–motor neuron disease but their pathogenic role is still unclear.[12,13]

SIGMAR1 encodes for the sigma-1 receptor (σ_1R), an endoplasmic reticulum (ER) chaperone that resides at the specialized mitochondrial-associated ER membrane.[14] σ_1R is involved in a wide array of cellular processes, including ER-mitochondrial Ca^{2+} signaling and cell survival, and is highly expressed in motor neurons of the brainstem and spinal cord.[14,15] Recent studies suggest that loss of σ_1R function causes ER–mitochondria disconnection and ER stress activation and disrupts mitochondrial function and axonal transport, leading to motor neuron axonal degeneration followed by cell death.[16] This dying-back degeneration process would be consistent with the distal predominant pattern of motor involvement seen in patients with *SIGMAR1* mutations.

Based on the present study and previous reports, we propose that coding sequence mutations in *SIGMAR1* manifest clinically with a combination of dHMN and pyramidal tract signs, with or without spasticity, in the lower limbs, and that preferential involvement of forearm extensors may be a distinctive feature of the disease. These observations should be confirmed in future studies.

AUTHOR CONTRIBUTIONS

A. Horga, P.J. Tomaselli: study design; acquisition, analysis, and interpretation of data; drafting and revising the manuscript. M.A. Gonzalez: acquisition, analysis, and interpretation of data; revising the manuscript. M. Laurà, F. Muntoni, A.Y. Manzur, M.G. Hanna, J.C. Blake, H. Houlden: interpretation of data; revising the manuscript. S. Züchner: study concept and design; acquisition, analysis, and interpretation of data; revising the manuscript. M.M. Reilly: study supervision; study concept and design; acquisition, analysis, and interpretation of data; drafting and revising the manuscript.

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DISCLOSURE

The authors report no disclosures relevant to the manuscript. Go to Neurology.org for full disclosures.

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This podcast begins and closes with Dr. Robert Gross, Editor-in-Chief, briefly discussing highlighted articles from the October 11, 2016, issue of *Neurology*. In the second segment, Dr. Mark Keegan talks with Dr. Ilya Kister about his paper on the various approaches neurologists may contribute to cost containment while maintaining and enhancing the quality of MS care. Dr. Andy Southerland interviews Dr. Lyell Jones about the topic of the Axon Registry and how it interfaces with practice and reimbursement for our "What's Trending" feature of the week. This is part 2, with part 1 appearing last month. In the next part of the podcast, Dr. Bryan Eckerle focuses his interview with Dr. Steven Messé on a *Neurology Today* story about the AAN Guideline published in *Neurology* on the topic of recurrent stroke with patent foramen ovale.

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