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Hereditary motor neuron disease in a large Norwegian family with a "H46R" substitution in the superoxide dismutase 1 gene

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Abstract

Mutant genes associated with Charcot Marie Tooth type 2, distal hereditary motor neuropathy and familial amyotrophic lateral sclerosis may cause overlapping clinical phenotypes. We performed whole genome linkage analysis, haplotype analysis, sequencing and detailed clinical and neurophysiological investigations in a large Norwegian kindred with a condition that clinically had been classified as Charcot Marie Tooth type 2. The mutation c.140A>G, p.His47Arg (alias p.His46Arg or H46R) in the superoxide dismutase 1 gene (*SOD1*) segregated with the disease. The patients present a hereditary motor neuropathy-like clinical picture and long survival (mean 29 years). To our knowledge, this is the first extensive report describing a large non-Japanese kindred. The prognostic implications of the condition seen in this family have little in common with what is normally associated with sporadic amyotrophic lateral sclerosis and illustrates the complexity of the genetic etiology of lower motor neuron disease.

Keywords: H46R; SOD1; HMN; ALS; p.His47Arg

Introduction

Motor neuron diseases (MNDs) are a group of "neuronopathies" that affect the cell bodies of motor neurons. Their clinical heterogeneity as well as their considerable overlap of symptoms represent a challenge in diagnosing MND subtypes. Diseases such as hereditary spastic paraplegia and primary lateral sclerosis affect the upper motor neurons (UMNs) in relative isolation whereas progressive muscular atrophy and spinal muscular atrophy are caused by lower motor neuron (LMN) disease. The combination of UMN and LMN findings on clinical examination and post-mortem autopsy is the hallmark of amyotrophic lateral sclerosis (ALS).

Familial amyotrophic lateral sclerosis (FALS) has a wide phenotypic spectrum and may show considerable clinical overlap with other conditions, particularly when the symptoms are restricted to the LMN. The El Escorial criteria recognize this by defining isolated LMN signs as "suspected ALS" [2]. Although most ALS cases are sporadic, 1–13% of ALS cases show a familial pattern usually compatible with autosomal dominant inheritance. Mutations in the Cu/Zn superoxide dismutase (*SOD1*) gene have been documented in 12–23% of the FALS families [3]. Although

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the FALS group has a slightly earlier onset, the clinical phenotype of FALS caused by *SOD1* mutations and SALS is similar. Some SOD1 substitutions, however, are reported in association with pure LMN symptoms and long survival. Other substitutions like p.Ala90Val (alias p.Ala89-Val) and p.Asp91Ala (alias p.Asp90Ala) have been reported in association with sensory neuropathy, neuralgic pain or other atypical symptoms that would normally weigh against a diagnosis of ALS [4,5]. FALS may show considerable intra and inter familial variation in age at onset, severity and degree of bulbar involvement indicating an important influence of genetic or environmental modifying factors. The vascular endothelial growth factor gene (*VEGF*) [6], the ciliary neurothropic factor gene (*CNTF*) [7] and recently, a variant in the Chromogranin B gene (*CHGB*) causing the p.Pro413Leu substitution [8], have been identified as potential modifiers in some cases of FALS.

We have studied a large Norwegian family previously diagnosed with Charcot Marie Tooth type 2 (CMT2) in which the disease segregated in an autosomal dominant fashion, but with unknown genetic etiology. Genome wide linkage analysis and haplotype analysis of all genotyped family members pointed to a 1.9 Mb interval on chromosome 21 and subsequently the affected family members were found to carry a SOD1 substitution denoted "H46R" in the literature, but hereafter called p.His47Arg according to HGVS nomenclature. To our knowledge, this is the first extensive report on a large non-Japanese kindred with this substitution. The high number of affected family members, their age distribution and their cooperativeness has allowed us to further dissect the clinical phenotype associated with this *SOD1* mutation and, at least in part, explain the clinical complexity that has allowed the confusion of MND with CMT. The data presented here provide further knowledge to the spectrum of phenotypes associated with *SOD1* mutations and, most importantly, they point to the requirement for a diagnostic strategy that can meet the clinical and genetic heterogeneity associated with hereditary diseases of the motor neuron.

Materials and methods

Patients

Family history was obtained through interviews with patients and relatives. Three family members provided extensive genealogical data and their consistent recordings were important for the completion of the pedigree. Based on church records a local historian provided us with additional detailed records of the family. We evaluated 22 cases in total; 10 deceased and 12 living family members. Clinical records were available for the 10 deceased family members. Patient consents were obtained and procedures were in accord with the Helsinki Declaration of 1964. This study was approved by the Regional Committee for Medical Research Ethics, The Directorate of Health and the Norwegian Data Inspectorate.

Genetic analysis

DNA was extracted from peripheral blood cells using a Genovision M48 (Qiagen) or Biorobot EZ-1 (Qiagen) system.

Genome-wide genotyping was performed using HumanCNV370 chips (Illumina). We assumed an autosomal dominant model for the analysis. Penetrance was set at 95%. The frequency of the deleterious allele was set at 0.0001, the phenocopy rate at 0.001. Multipoint linkage analysis was performed using MERLIN [9]. Frequencies of marker alleles were estimated based on our database of genotyped individuals, which comprises approximately 700 individuals, mainly of Caucasian origin. Approximately 42,500 markers with a minor allele frequency of $\geqslant 0.15$ were selected for the analysis.

Mutation analysis was carried out by sequencing all coding exons of the *SOD1* gene (reference cDNA: NM_000454.4) in the index person.

Intronic primers used to PCR amplify the different exons were as follows:

exon 1 F:CCCAGTCATTCCCGGCCACT, R:GGGAGCGGCCTCGCAAACA,
exon 2 F:TTAAGCAGCTTGCTGGAGGT, R:CGACAGAGCAAGACCCTTTC,
exon 3 F:AGTCGTGATGCAGGTCAGCACT, R:TGGGGGAAACACGGAATTATCTTAGCA,
exon 4 F:GTGGCATCAGCCCTAATCCATCTG, R:AGAAACCGCGACTAACAATCAAAGTGA,
exon 5 F:GGAGGTAGTGATTACTTGACAGCCCA, R:AGTCTGGCAAAATACAGGTCATTGAA.

Partial PCR amplification and sequencing of exon 4 of the *CHGB* gene (reference cDNA: NM_001819) was carried out using the following exonic primers:

Exon 4 F:AACGTCAGCATGGCCAGTTTAG, R:GAGGGTCGTAGTATGGGTTGAACA.

Sequencing in both directions was performed with BigDye version 3.1 and an ABI 3130xl Genetic Analyzer. Sequences were analyzed using Sequencher 4.1 (Gene Codes).

Clinical analysis

All clinical records were evaluated by a specialist in neurology and a specialist in medical genetics. After the identification of the causative mutation, 12 living patients were re-examined neurologically including tests for neuropathic pain. Hypersensitivity to gentle touch (allodynia) was assessed by lightly stroking all four extremities with a brush (Somedic) in nine patients. In addition, signs of hyperalgesia to punctate stimuli were tested by an 83.7 mN von Frey filament.

EMG/neurography

Nerve conduction velocities (NCV), amplitudes and distal latencies of the median and ulnar (motor and sensory) nerves in one upper extremity were examined in nine patients. In the lower extremities the motor NCV, amplitudes and distal latencies of the peroneal and tibial nerves as well as the sensory sural and peroneal superficial nerves were examined in both legs. The results were regarded as pathological if they exceeded the normal values in use in our laboratory. Needle-EMG was performed in the following muscles in all patients: musculus opponens pollicis, extensor digitorum communis and deltoideus posterior in one arm, the anterior tibial and medial gastrocnemial in both legs and right lateral vastus.

Quantitative sensory testing (Thermotest)

Threshold temperatures for sensations of warmth, cold, heat-pain and cold-pain in nine patients were determined by standardized computerized equipment using the method of limits (Thermotest®, Somedic AB, Sweden) as described in detail elsewhere [10]. The results were compared with data from 38 healthy subjects (aged 22–66 years). The individual thresholds of the patients were considered pathological if they were greater than the 95th percentile (warmth, heat-pain) or less than the 5th percentile (cold) of values found in healthy subjects. Cold-pain detection <20 °C were considered normal. The thresholds were tested at the thenar eminence, lateral at the left thigh, bilaterally at the lower leg and at the dorsum of the foot. When the patient reported to have experienced complicated fractures or sciatica in one foot, the results from this extremity were disregarded.

Results

Genealogy

We studied affected individuals from a large Norwegian family with a disease history extending over seven generations (Fig. 1). The earliest obligate gene carrier identified was born in 1763 and lived in a village in South-Eastern Norway. In the second generation the family divided into two main branches. In the third generation the second branch split in two larger family groups. The inheritance pattern was autosomal dominant, as several cases of male to male transmission excluded X-linked and mitochondrial inheritance. Anticipation was not observed.

Genetic studies

We performed a genome-wide linkage analysis using SNP array genotyping. In order to use multipoint linkage analysis, we limited the analysis to the core family. We first performed an affected-only analysis using eight members of the core family. This analysis resulted in a multipoint LOD score of 3.0 between the markers rs1740165 and rs4816428 comprising an interval of 13 Mb on chromosome 21q21.1–q22.11. We did not detect any other region with a positive LOD score. The inclusion of three unaffected family members increased the LOD score to 3.8. To narrow down the interval, we performed a haplotype analysis of all genotyped family members. For this analysis, the family was divided into three overlapping parts. A recombination event in individual VI:4 placed the disease locus distal to marker rs2155475 and reduced the candidate region to 1.9 Mb. Besides a cluster of keratin-associated protein genes this region contained seven other genes, among them

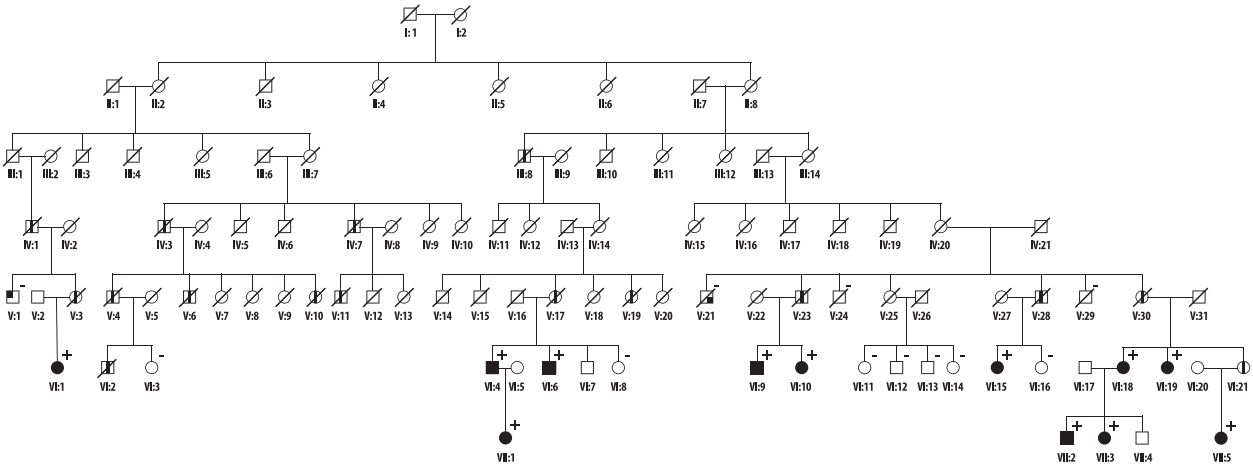

Fig. 1. Pedigree of the family. A symbol with a central vertical line indicates an affected individual based on family history and medical records. A filled symbol indicates an affected individual that has been examined by us. "+" indicates that the individual is a proven carrier of the p.His47Arg substitution, a "−" indicates that the individual is not a carrier of the p.His47Arg substitution. Squares indicate males, circles indicate females and oblique lines indicate deceased individuals. The patient with a quarter filled symbol on the upper left (V:1) turned out to be a phenocopy. The deceased male with a quarter filled symbol on the lower right (V:21) had an atypical clinical phenotype including axonal polyneuropathy with upper limb predominance. Case III: 8 was described in an old family book that was made available to us by the family. Case IV:3 and V:11 are mentioned as affected by independent sources but without enough precise details to include them in tables and calculations. Minor changes have been made to preserve anonymity of some of the family members.

Table 1
Clinical features at re-examination.

Patient	Gender	Age at disease onset (years)	Initial symptom	Disease duration (years)	Walking aid (years)	Wheelchair dependency (years)	Upper limb weakness (years)	Plantar response	Fasciculations	Fractures
VI:1	F	47	U l	0.5	–	–	–	↓	–	
VI:4	M	64	"WD"	6	–	–	–	↕	–	
VI:5	M	48	U r	19	Cane (16)	–	+(16)	↑	+	
VI:9	M	42	U r	21	Cane (6)	+(13)	+(17)	↑	+	
VI:10	F	51	U r	9	Crutches(NA)	+(8)	+(7)	–	–	Calf
VI:15	F	50	U	16	–	+(4)	+(7)	↕	–	Both legs
VI:18	F	55	U r	27	Crutches (3)	+(6)	+(NA)	–	+	Hip
VI:19	F	65	U l	15	NA	+(5)	+(NA)	–	–	
VII:1	F	36	U l	4	–	–	–	↓	–	
VII:2	M	50	U l	7	Crutches (4)	+(6)	+(4)	↓	+	
VII:3	F	40	U l	14	Walker (8)	+(10)	+(10)	↓	–	Foot
VII:5	F	22	U r	12	Crutches (12)	–	+(11)	↑	+	

Gender: F = female; M = male. Initial symptom: U = unilateral weakness in a distal lower limb; r = right; l = left; "WD" = "walking difficulties". Walking aid: – = no walking aid; NA = not available. Wheelchair dependency: – = not wheelchair dependent. Upper limb weakness: – = no upper limb weakness; NA = not available. Plantar response: ↓ = flexor plantar response uni- or bilaterally; ↑ = extensor plantar response uni- or bilaterally; ↕ = possible extensor plantar response uni- or bilaterally; – = no response bilaterally. Increased reflexes/muscle tone: – = no increased reflexes in the upper or lower extremities on clinical examination. Fasciculations: + = present at clinical examination; – = no fasciculations present at clinical examination. None of the patients had increased reflexes or increased muscle tone on examination.

Table 2
Disease history of deceased family members.

Patient	Gender	Age at disease onset (years)	Initial symptom	Disease duration (years)	Wheelchair dependency (years)	Upper limb weakness (years)	Deceased at age (years)	Additional information
III:8	M	40	"legs"	17	+	NA	57	Calf fracture after minimal trauma
IV:3	M	26	NA	11	+	+	37	
V:3	F	35	NA	33	+(20)	+	68	Died of obstructive hypertrophic cardiomyopathy
V:4	M	37	U l	26	+(10)	+	63	Died of post surgical complications /pneumonia
V:8	M	27	U l	20	+	+	47	Death due to pneumonia
V:10	F	35	U l	45	+(≈9)	+(1)	80	
V:17	F	42	U r	51	+(>20)	+	93	Fracture of hip, calf, wrist and femurs
V:19	F	37	"legs"	NA	NA	NA	NA	
V:28	M	43	U r	36	+	+(20)	79	Facial weakness
VI:2	M	42	U r	22	+(6)	+	63	Death due to post surgical complications

Gender: F = female; M = male. Initial symptom: U = unilateral weakness in a distal lower limb; r = right; l = left; "legs" = "weakness in the legs"; NA = information about site of onset not available. Wheelchair dependency: NA = information about wheelchair dependency not available. Upper limb weakness: NA = information about upper limb weakness not available.

SOD1, which we regarded as a good candidate in light of its expression pattern and involvement in motor neuron diseases. Sequencing of the coding region in a single affected and a single unaffected family member revealed a mutation, c.140A>G, resulting in p.His47Arg (formerly known as H46R), which has previously been reported in Japanese families [11–15]. Mutation testing of the extended family confirmed the segregation with the disease as shown in the pedigree (Fig. 1). Investigation of 19 family members of whom 11 were affected did not identify any carriers of the p.Pro413Leu substitution in CHGB.

Clinical phenotype

We evaluated 22 cases in total; 10 deceased and 12 living family members with a male to female ratio of 1:1.2. Table 1 shows the clinical features of the re-examined patients, Table 2 shows clinical details from the disease history of deceased family members and Table 3 summarizes the clinical details of all affected family members.

Age at disease onset

First sign of muscular weakness was documented in 20 cases. Weakness distally in one of the lower limbs was the initial symptom in 17 cases. In the remaining three, first symptoms were "walking difficulties" or "weakness in the legs". Muscle cramps in the calves in association with, or prior to, onset of weakness was documented in six cases; probably a common finding. Mean age at onset was 42.5 years ranging from 22 to 65 years (Figs. 2–4 and Tables 1–3). One case of non-penetrance was plausible in the obligate carrier IV:20, who was remembered by multiple family members as physically strong without any signs of disability prior to her death at the age of 79 years.

Table 3
Summary of clinical features.

Feature	*N*	Mean	Median	Range
Age at disease onset (years)	22/22	42.5	42.0	22–65
Leg cramps before onset	6/12			
Onset with weakness distally in one lower limb	17/22			
Interval before wheelchair dependency (years)	12/22	9.7	8.5	4–20
Interval before upper limb weakness (years)	9/22	10.3	10.0	1–20
Fasciculations	5/12			
Tongue atrophy	0/12			
Bulbar symptoms	0/12			
Respiratory problems	0/12			
Dementia	0/22			
Disease duration; deceased patients (years)	9/10	29.0	26.0	11–51
Age at death (years)	9/10	65.2	63.0	37–93

The data represents a summary of 22 patients in total of whom 10 were deceased family members and 12 were re-examined patients.

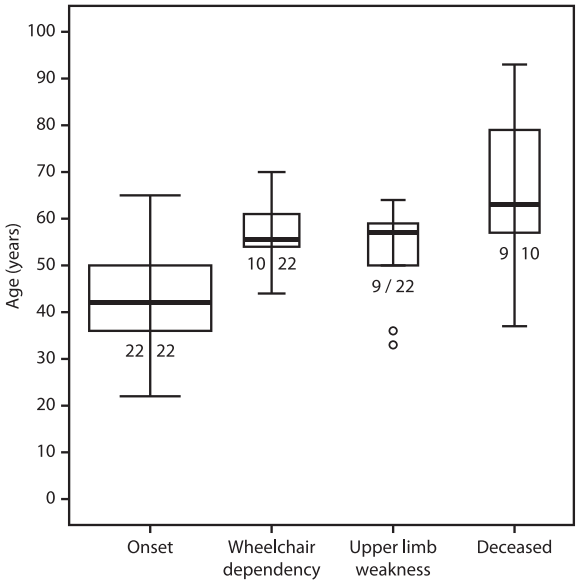

Fig. 2. Age distribution of onsets of the most important clinical milestones. The number of patients included in the calculations is indicated by the width of the boxplots and is also listed below each of the boxes. Deceased = age at death.

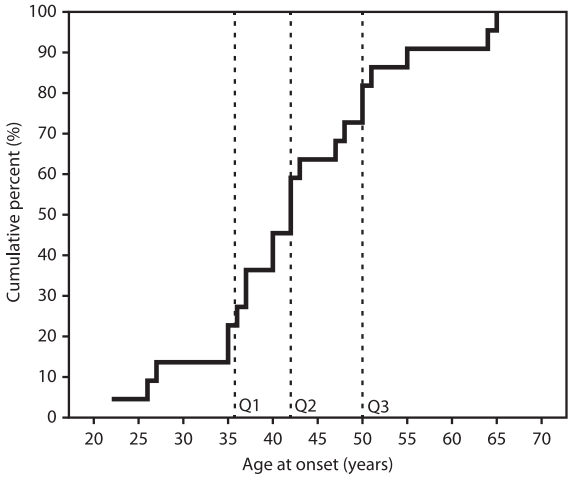

Fig. 3. Cumulative distribution (percent) of age at onset in the family members ($n = 22$). The median age at onset was 42 years and is marked by the line denoted Q2. The cut off value between the first and second quartile was 35.75 years and is marked by the line denoted Q1. The cut off value between the third and the fourth quartile was 50 years and is marked by the line denoted Q3. The youngest age at onset was 22 years; the oldest 65 years. One asymptomatic obligate mutation carrier died at an age of 79 years.

Disease duration

Deceased family members died at a mean age of 65.2 years with a range of 37–93 years (Figs. 2 and 4 and Table 2). Average disease duration before death was 29 years with a range of 11–51 years. The exceptional case of V:17 is well documented. She had onset at 42 with limping, weakness in the right foot and muscle cramps in the calves. She could walk independently for at least 20 years after onset. She died at the age of 93. The last year of her life she had severe weakness in all four limbs, absent tendon reflexes and indifferent plantar responses. Her medical record made 1 year before death specified that she had no respiratory difficulties and that cognitive functions were very well preserved. Five patients died before the age of 65. The cause of death was surgical complications to a colorectal cancer operation in one case, intestinal hemorrhage and pneumonia in a second, and pneumonia in a third case. The cause of death is not known in the remaining two patients (Table 2).

Mobility

Wheelchair dependency was documented in 9 of the 10 deceased patients; in the remaining case information was not available (Fig. 2 and Table 2). Average disease duration in the re-examined patients was 12.5 years with a range of 0.5–27 years (Table 1). Seven of them were wheelchair dependent, but patient VI:4 still walked with a cane 19 years after onset. Duration from onset of symptoms to wheelchair dependency was known in a total of 12 cases and was 9.7 years on average with a range of 4–27 years. One patient lost ambulation 4 years, another 8 years and a third 10 years after onset in succession to leg fractures. Walking aid was needed on average 8 years after onset with a range of 3–16 years.

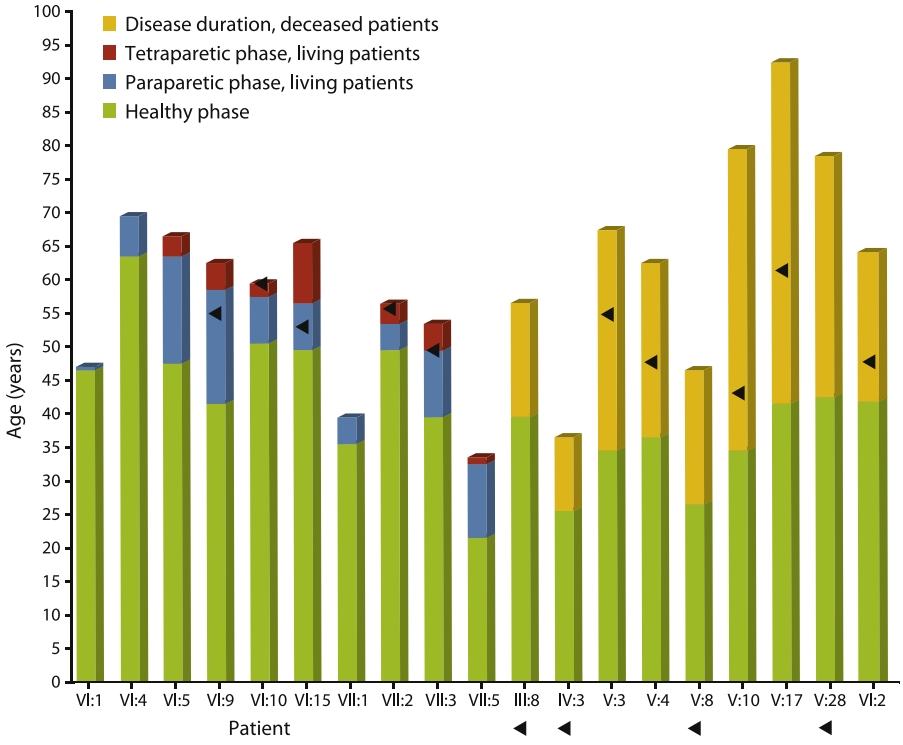

Fig. 4. Disease history of living (left) and deceased (right) patients. Period before disease onset is shown in green. The progression of symptoms from the lower to the upper extremities in individual re-examined patients is shown in brown and purple. Disease duration in deceased patients is shown in yellow. The upper limit of the bars symbolize their age at last examination (left) or at death (right). Age at onset of wheelchair dependency is indicated by ◄. In the cases where the exact age at onset of wheelchair dependency is not known the ◄ is placed below the column. Case V:19 (deceased), VI:18 and VI:19 (living) were omitted due to lack of exact data for one or more parameters.

Clinical features; limbs

Symptoms always started asymmetrically and distally in the lower limbs, mostly with weakness and atrophy of calf muscles. Some of the family members reported a preparetic phase with muscle cramps and pain in the lower limbs. Usually the other leg was affected before the symptoms spread further proximally. The disease course proceeded with atrophy and weakness of the whole limb but a certain degree of asymmetry was sometimes preserved even in later stages. Several years later symptoms appeared in the hands (Table 3), usually presenting with unilateral or bilateral weakness of the thenar muscles and the small hand muscles. Eventually the patients showed a ubiquitous weakness and atrophy of both upper limbs, but particularly of the opponens and with relative preservation of finger- and wrist extensors. Seven of the re-examined patients had upper limb weakness (Table 1). The mean interval between first symptoms and upper limb weakness was 10.3 years with a range of 4–17 years. The medical record of one of the deceased patients; V:28, specified an onset of upper limb weakness after 20 years disease duration whereas the records of another deceased patient; V:10, stated upper limb weakness after 1 year of disease duration (Table 2).

Fasciculations were documented on clinical examination in 5/12 re-examined patients. One of the re-examined patients; VI:6, showed increased vibratory threshold below the knees, however, sensory examinations were otherwise normal. None of the re-examined patients had *pes cavus*.

Pain

Most patients experienced cold extremities; especially cold feet. One patient, VI:15, reported burning pain in the feet during the last 4–5 years. The burning pain was most prominent during the evenings. None of the patients had mechanical allodynia or hyperalgesia.

Bulbar symptoms

One of the re-examined patients; VI:4, with disease duration of 19 years, reported occasional swallowing difficulties. Examination by an ear, nose and throat specialist showed normal results. Another patient, VI:19, had a slight dysarthria, probably in relation to a prior stroke. Clinical examination showed that tongue and throat was normal in all patients and there were no other reports of bulbar symptoms.

UMN symptoms/central involvement

The patients investigated had normal or decreased muscle tone and there were no cases with brisk tendon reflexes although some had asymmetric reflexes, particularly in early stages. Three of the 12 re-examined patients showed a convincing extensor plantar response, two had a possible

Babinski sign with subtle or ambiguous extension of the great toe.

Additional findings

Bone fractures occurring after disease onset were reported in two of the deceased and four of the re-examined patients. In one case, V:17, the fractures were multiple and involved both lower and one upper limb. One patient, V:28, showed facial weakness, particularly in the lower parts of the face and he was unable to whistle. We did not perform formal testing for dementia, but none of the re-examined patients were suspected of having cognitive deficits and there were no reports on dementia among the deceased family members. Muscle strength with regards to neck flection was normal in all patients and none of them had respiratory problems, even 27 years after onset. Independent breathing with normal oxygen saturation 50 years after onset was documented in the case of V:17. Plasma creatine kinase (P-CK) levels were available in 3 male and 5 female patients. Two men had elevated P-CK levels at 403 U/L and 510 U/L (Reference intervals in serum for men >50 years 40–280 U/L [16]). Three women had elevated P-CK levels at 258 U/L, 278 U/L and 387 U/L (Reference intervals in serum for women >18 years 35–210 [16]).

Patient V:1 was first examined by us at the age of 67. He first experienced weakness in an ankle at the age of 40 with subsequent slow progression. At examination 27 years later he was still mobile and, with some effort, he could tiptoe and stand on his heels. He had atrophy of the calves; more pronounced, however, on the right side. Achilles tendon reflexes were absent; patellar reflexes were weak and

Table 4
Motor and sensory neurography from nine patients with a proven p.His47Arg substitution.

Nerve/Patient	VI:6	VII:2	VI:9	VII:5	VI:10	VII:3	VI:15	VI:4	V:1
Median (m)									
Amplitude (mV)	3	0.5*	0.3*	3.5	0.7*	0.7*	1.0*	4.5	11.6
CV (m/s)	56.9	51.1	41.2*	52	51.3	53	51.4	51.2	54.8
Median (s) 2.finger									
Amplitude (μV)	6.9	4.2	7	12	4.3	6.7	14	2.1	5.9
CV (m/s)	59.2	51.2	48.4	58	56	48.9	54.8	48.4	51.9
Ulnar (m)									
Amplitude (mV)	6.1	4.2	1.9*	5	8.3	2.8*	1.3*	7.8	6.7
CV (m/s)	57.3	46.8*	52.9	63.8	54.7	47.8*	47.7*	55.4	61.4
Ulnar (s) 5.finger									
Amplitude (μV)	8.1	1.9	6.4	7.7	10	7.2	11	3.9	5.3
CV (m/s)	63.6	48.6	52.5	54.7	55	47.9	57.5	46.2	60.9
Right peroneal (m)		NM*	NM*			NM*	NM*		
Amplitude (mV)	0.9*			1.4	1.5			0.5*	4.7
CV (m/s)	38			40.9	49.2			41.9	49.2
Left peroneal (m)		NM*	NM*				NM*		
Amplitude (mV)	1.2			1.0*	1.0*	0.3*		0.1*	5.7
CV (m/s	37			43.8	41.9	37*		40.2	50
Right tibial (m)		NM*					NM*		
Amplitude (mV)	1.2		0.1*	0.7*	1.4*	0.7*		0.2*	5.9
CV (m/s)	42		NM*	39.2*	39.9	NM*		37.6	–
Left tibial (m)			NM*				NM*		
Amplitude (mV)	0.8	0.1*		0.4*	0.7*	0.3*		1.0	6.0
CV (m/s)	38.1	NM*		43.3	40	NM*		42.5	44
Right sural (s)									
Amplitude (μV)	11	1.1*	2.4	20	14	3.2*	8.7	2.4	4.2
CV (m/s)	43.8	35.4	54.3	45.2	45.2	46.4	46.7	41.6	62.6
Left sural (s)			NM*						
Amplitude (μV)	9.4	1.5*		8.6	18	3.3*	3.9	2.1	9.7
CV (m/s)	42.9	42.2		50	42.4	41.2	45.2	42.4	51.9
Right superf. peroneal		NM*	NM*			NM*		NM*	
Amplitude (μV)	5.4			7.3	5.5		4.6		7.6
CV (m/s)	49			43.8	51.9		46.7		59.6
Left superf. peroneal		NM*	NM*					NM*	ND
Amplitude (μV)	6.5			7.1	5.7	4.6	1.9		
CV (m/s)	38.9			44.2	48	46.6	50		

m = motor nerve, s = sensory nerve, CV = conduction velocity, NM = not measurable, ND = not done, * = pathological.

Table 5
EMG findings from nine patients with a proven p.His47Arg substitution (the same patients as in Table 4).

EMG findings	Normal	Slightly pathological	Pathological	Severely pathological
Motor unit potentials	0/9	0/9	6/9	3/9
Fasciculation potentials	4/9	5/9	0/0	0/0
Fibrillations and/or positive sharp waves	0/0	0/0	4/9	5/9
Complex repetitive discharges	2/9	3/9	4/9	0/9

Slightly pathological = pathology in 1–2 of 8 investigated muscles. Pathological = pathology in 3–6 muscles. Severely pathological = pathology in 7–8 muscles. The pathological motor unit potentials showed chronic neurogenic changes with increased amplitude, polyphacy and increased duration.

Table 6
Previous reports on clinical features associated with the p.His47Arg substitution in SOD1.

Ethnicity	Age at onset (years)	Initial symptom	Unilateral onset	UMN symptoms	Bulbar symptoms	Respiratory muscle weakness	Duration (years)	*N*	Reference
Japanese	Mean 49.6 SD +/−10.9 (*n* = 10)	LL	+	+	+	–	DD: Mean: 17.3 SD: +/− 10.7 (*n* = 4)	13	[12]
Japanese	Mean 48.0 SD +/− 9.5 (*n* = 14)	LL	+	+	–	–	DD: Mean: 16.8 SD: +/− 6.8 (*n* = 9)	15	[12]
Japanese	Mean 39.7 SD +/−10.5 (*n* = 9)	LL	+	–	+ (1:8)	+ (1:15)	DD: Mean: 18.1 SD: +/− 13.2 (*n*=?)	15	[13]
Japanese	Mean 42.9 SD +/− 4.7 (*n* = 7)	LL	+	–	–	–	DD: Mean: 17.2 SD: +/− 8.1 (*n*=?)	15	[14]
Japanese	Mean 44.3 SD +/− 8.7 (n = 17)	LL	+	+ (1:17)	+ (1:12)	+ (7:17)	DOR: Mean: 17 SD: +/− 7.3 (*n* = 7)	17	[15]
Japanese	46, 56, 49	LL	+	–	–	–	All tree alive, in one case 38 years after onset	3	[18]
Pakistani	55	LL	+	+	–	–	S: Range: 17–21	12	[32]
Norwegian	Mean 42.5 SD +/−10.9 Range 22–65 (n = 22)	LL	+	+ (3:12)	–	–	S: Mean 29.0 SD +/− 13.3 Range 11–51 (n = 9)	22	This study

Initial symptom: LL = lower limb(s); Unilateral onset: + = one or more patients with unilateral symptoms at onset are described; no patients with symmetric symptoms at onset are described. UMN/Bulbar symptoms: + = present; − = absent; Respiratory muscle weakness: + = one or more patients with respiratory weakness during their disease course; − = no patients with respiratory weakness are reported. Duration: DD = disease duration from the onset of symptoms in years, DOR = disease duration from onset of symptoms to respiratory failure; S = survival (duration from disease onset to death). *N* = number of affected patients in the family/families.

symmetrical. Reflexes in the upper limbs were well preserved. The left thenar eminence was slightly atrophic, and he had a discrete weakness in the hands. Sensation of pain and proprioception in the feet was reduced. After genetic testing he turned out not to be a carrier of the p.His47Arg causing mutation and we assume that he is a phenocopy with a neuropathy of different genesis.

Electro-diagnostic studies and thermo-test

The most prominent finding was an axonal motor involvement with a reduction in amplitude of motor nerves, measured by neurography in the lower extremities, and neurogenic changes as measured with EMG. In four patients at least one motor nerve was not measurable because of atrophy, and in all but two patients; VI:6 and V:1, more than one motor nerve in the lower extremities were low in amplitude or not measurable (Table 4). As shown in Table 5, most patients showed massive EMG findings implying neurogenic involvement with pathological motor unit potentials, denervation activity and complex repetitive discharges in several muscles both in the upper and the lower extremities. However, relatively few muscles had fasciculation potentials. Sensory findings were sparse, with no sensory involvement judged by neurography in the upper extremity, except carpal tunnel syndrome. In some patients there were findings implying axonal involvement of the sensory nerves in the lower extremity with low or not measurable amplitudes.

Six of the patients had increased thresholds for cold detection (CDT) at the dorsum of their feet indicating a possible small fiber involvement.

Discussion

The substitution p.His47Arg was first described in association with mild FALS in two Japanese families in 1993 [11]. In subsequent reports a homogenous clinical phenotype with complete penetrance, but variable age at onset was delineated [12–15]. The family reported here represents, to our knowledge, the first extensive presentation of a large non-Japanese kindred.

Mean age at onset was 42.5 years which is slightly earlier than reported in the Japanese patients with the p.His47Arg substitution (Table 6). Average age at onset associated with *SOD1* mutations as a group has been reported to be 46.9 years [17], and 56 years in sporadic ALS which indicates an overall lack of correlation between age at onset and disease severity.

Unilateral weakness in a lower limb was the presenting symptom in all known cases, usually in the form of weakness in a calf muscle, which is consistent with a previous report [18]. An additional feature of the family presented here is the (relative) preservation of the extensor muscles of the fingers and wrists in late stages of the disease. Some of the family members reported a preparetic phase with muscle cramps and pain in the lower limbs resembling aspects of the preparetic phase described in association with the p.Asp91Ala substitution [5]. The disease course in the family presented here was slowly progressing with wheelchair dependency occurring after 9.7 years (range 4–20), and upper limb weakness after 10.3 years (range 1–20), but with great intra familial variation. Surprisingly, six patients experienced fractures, several after minor trauma. Osteoporosis and an increased susceptibility to fractures have not previously been reported to be associated with *SOD1* mutations and may be related to the long lasting disuse of the limbs. One patient had facial weakness, but in contrast to previous reports none of the patients reported here had bulbar findings [12,13] nor the need of assisted ventilation, although some had decreased coughing force and an increased susceptibility to pneumonia.

The disease penetrance is high; however, according to the wide range of age at onset (22–65 years) and the fact that one obligate carrier, IV:20, remained unaffected until her death at the age of 79, it is not complete. To our knowledge, this is the first documented case showing non-penetrance.

The deceased family members showed disease duration of 29 years in average (range 11–51 years). This is longer than what has been reported in the Japanese patients with the p.His47Arg substitution (range 12.1–17.3 years). The etiology of this discrepancy remains unknown; however, it might be related to environmental or population-specific genetic modifying factors. *Cis*-acting, modifying factors have been suspected among carriers of the p.Asp91Ala substitution of Finno-Scandinavian decent [19]. Patient V:17 died at the age of 93, 51 years after the initial symptoms. To our knowledge this is the longest disease duration published in association with a pathogenic *SOD1* mutation. Two of the men that died at a young age (37 and 47 years) also had the youngest age at onset (26 and 27 years). The causes of death in these cases are not known to us, and in the material as a whole we could not document a relationship between age at onset and age at death. We found no significant correlation between gender and age at onset or between gender and time span between disease onset and wheelchair dependency.

Neurophysiology showed mainly axonal motor involvement, but also axonal involvement of the sensory nerves in the lower extremities with low or not measurable amplitudes in some patients. The lack of or decreased sensory amplitudes could be due to technical problems since many of the patients had considerable peripheral oedema. The EMG showed widespread subacute and chronic neurogenic changes (Table 5) as can be seen in motor neuron diseases [20]. However fasciculation potentials were scarce as opposed to what is usually seen in ALS [21]. Thermotest indicated some sensory involvement also including small fibers (mainly cold). Some sensory involvement has been reported in up to one third of SALS cases [22] and may be more pronounced in patients with *SOD1* mutations [4].

In spite of careful examination we did not find spasticity or increased reflexes in any of the re-examined patients. A minority (3/12) did present extensor plantar responses as evidence of a certain degree of corticospinal tract involvement, but not more than what have been reported in association with dHMN [23]. Clinical findings of UMN symptoms in the Japanese families were mostly reported as lacking, transient or modest, even after long disease duration (Table 6). Minor UMN symptoms and findings are not uncommon in association with *SOD1* mutations. However, mild or absent UMN symptoms do not necessarily correlate with a favorable prognosis since this feature was reported in association with the p.Ala5Val ("p.Ala4Val") substitution which is associated with a very rapid disease progression [24].

As to the nosology it is not clear how to classify the disease in this family. The distal onset, with gradual proximal involvement and the absence of bulbar symptoms mimics an axonal length dependent degeneration and, correspondingly, the initial diagnosis in this family was CMT2. Clinically the patients have more in common with previously reported families with dHMN than with CMT2, due to scarcity of sensory symptoms and findings. However, the clinical and neurophysiologic findings over time, with prominent proximal motor involvement, is more in line with LMN findings in ALS. In particular, the asymmetric onset with weakness of the flexor muscles of the distal lower limb separates the phenotype in this family from what to be expected in dHMN/dSMA as well as CMT2. A variable clinical expression has also been documented for mutations in genes associated with non-*SOD1* FALS.

The phenotypic effect of the p.Pro56Ser substitution in *VAPB*, for example, ranges from ALS, atypical ALS to late onset SMA [25,26]. Likewise, mutations in *SETX* have been associated with the benign autosomal dominant juvenile ALS (ALS4), dHMN type II [27] and even with sporadic ALS [28].

To date over 150 *SOD1* mutations have been reported, although many are private and of uncertain pathogenicity [29]. Some mutations are associated with a rapidly progressive form (survival <2 years), a classic form or a relatively benign form (survival >5 years). To our knowledge the family presented here has the longest mean disease duration reported in association with any *SOD1* mutation. FALS may also show considerable intra and inter familial variation in age at onset indicating an important influence of genetic or environmental modifying factors. The Chromogranin B (*CHGB*) variant leading to the substitution p.Pro413Leu has been associated with earlier age of disease onset in a French, French–Canadian and Swedish cohort of SALS cases [8]. However, the association could not be replicated in subsequent studies of a French–Caucasian SALS cohort [30] and a Dutch SALS and FALS cohort [31]. Due to the wide range of age at onset in this family we investigated 11 affected family members with regards to the p.Pro413Leu substitution. We did not identify any carriers; hence it remains to be determined whether other potential causative or modifier genes influence the age of onset.

It has been proposed that p.His47Arg is relatively unique to the Japanese population [13]. To our knowledge there is only one detailed description, a Norwegian man of Pakistani decent [32], and a report of a few single patients from Germany [33] and North America [34]. The c.140A>G allele frequency among patients with a HMN/SMA like phenotype may be underestimated. The number of descendants in the seven generation family presented here, and the fact that we have recently identified this mutation in unrelated Norwegian families, makes p.His47Arg a common pathogenic SOD1 substitution and likely the most frequent genetic cause of HMN/dominant SMA in Norway. We therefore, suggest that patients with this distinct phenotype, consistent of adult onset of unilateral weakness in a calf muscle progressing with a distal hereditary motor neuropathy-like picture, should be tested for the c.140A>G mutation (p.His47Arg).

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