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Autosomal recessive adult-onset amyotrophic lateral sclerosis associated with homozygosity for Asp90Ala CuZn-superoxide dismutase mutation

A clinical and genealogical study of 36 patients

P. M. Andersen,[1] L. Forsgren,[1] M. Binzer,[1] P. Nilsson,[2] V. Ala-Hurula,[4] M.-L. Keränen,[5] L. Bergmark,[3] A. Saarinen,[6] T. Haltia,[7] I. Tarvainen,[8] E. Kinnunen,[9] B. Udd[10] and S. L. Marklund[2]

Departments of [1]Neurology, [2]Clinical Chemistry and [3]Clinical Neurophysiology, Umeå University Hospital, Umeå, Sweden, [4]Department of Neurology, Kemi Hospital, Kemi, [5]Department of Neurology, Lapin Keskussairaala, Rovaniemi, [6]Department of Neurology, Kainuun Keskussairaala, Kajaani, [7]Kauniala Hospital for injured Veterans, Kauniainen, [8]Department of Neurology Mikkeli Hospital, Mikkeli, [9]Department of Neurology, Hyvinkään Hospital, Hyvinkää, [10]Department of Neurology, Vaasa Hospital, Vaasa, Finland

Correspondence to: Dr Peter M. Andersen, Department of Neurology, Umeå University Hospital, S-901 85 Umeå, Sweden

Summary

We describe 36 patients (six were apparently sporadic cases and 30 were cases from nine families) with amyotrophic lateral sclerosis (ALS) characterized by a distinct phenotype associated with homozygosity for an Asp90Ala mutation in the CuZn-superoxide dismutase gene. The presenting motor manifestation in all patients was paresis in the legs, with slow progression to the upper extremities and finally to the bulbar muscles. The age of ALS onset varied from 20 to 94 years, with a mean of 44 years. Mean survival time was 13 years for the 11 deceased patients. However, this is probably biased and untypical (low) when compared with the disease duration in the surviving patients, and when considering other medical complications in the deceased patients. The rate of progression was highly variable, even within families. All patients showed signs of involvement of both upper and lower motor neurons. Other neurological features included painful muscle spasms and paraesthesiae in the lower extremities. Two-thirds of patients experienced difficulty with micturition. Electrophysiological studies confirmed the slow progression and spatial distribution of clinical symptoms in the peripheral motor system. Furthermore, EMG potentials evoked by transcranial magnetic stimulation (MEP) were compared with those evoked by cervical or lumbosacral electrical stimulation and often revealed marked slowing of transmission in central motor pathways. In Sweden and Finland ALS patients homozygous for the Asp90Ala mutation constitute a phenotypically characteristic subset of motor neuron disease.

Keywords: Asp90Ala CuZn-SOD mutation; autosomal recessive adult-onset amyotrophic lateral sclerosis; motor neuron disease

Abbreviations: ALS = amyotrophic lateral sclerosis; Asp90Ala = the aspartic acid to alanine mutation at codon 90 in the CuZn-SOD gene; CL = the shortest latency obtained with cortical stimulation; CML = central motor latency; CML-F = central motor latency corrected for F-response latencies; CuZn-SOD = copper- and zinc-containing superoxide dismutase; EMG = concentric needle electromyography; FALS = familial ALS; MEP = motor potentials evoked by transcranial magnetic stimulation; M% = the ratio of the highest amplitude from cortical magnetic stimulation to the highest amplitude of the distal motor response from supramaximal electrical stimulation expressed as a percentage; PCR-SSCP = polymerase chain reaction single stranded

conformational polymorphism; RL = the MEP latency following electical cervical or lumbosacral stimulation; SALS = sporadic ALS

Introduction

Amyotrophic lateral sclerosis is a type of motor neuron disease first described by Charcot in 1869. Although he reported only isolated cases, familial motor neuron disease had already been described by Aran in 1850. The possibility that ALS is a hereditary disease was long denied (Friedman and Freedman, 1950). Not until the study of Kurland and Mulder (1955) was it generally accepted that ALS may, in some cases, be familial and perhaps hereditary. Most authors now find that 5–10% of all ALS cases are hereditary or familial (FALS) (Hudson, 1981; Forsgren *et al.*, 1983; Mulder *et al.*, 1986; López-Vega *et al.*, 1988; Haverkamp *et al.*, 1995). The remaining ALS cases are considered sporadic (SALS) with unknown aetiology despite intense research. Several studies have suggested aetiological heterogeneity in ALS (Hirano *et al.*, 1967; Horton *et al.*, 1976; Siddique *et al.*, 1991). Rosen *et al.* (1993) reported the linkage between a subgroup of FALS and 11 different missense mutations in the gene encoding the CuZn-SOD enzyme in 13 North American FALS families. Since then, some 40 missense and deletion mutations in the CuZn-SOD gene have been reported worldwide. These mutations have all shown autosomal dominant traits, with varying degrees of penetrance and variable phenotypes, confirming earlier studies that there is a large variability in the phenotype within and between FALS families (Kurland and Mulder, 1955; Horton *et al.*, 1976; Chio *et al.*, 1987; Williams *et al.*, 1988).

We have previously reported the occurrence of FALS and SALS in Sweden and Finland associated with homozygosity for an Asp90Ala mutation in the CuZn-SOD gene (Andersen *et al.*, 1995). Here we describe the 36 ALS patients known to be homozygous for Asp90Ala, including five new families and two new sporadic cases not previously presented. The patients have a rather uniform, characteristic phenotype and constitute a distinct subtype of motor neuron disease which is the subject of this report.

Patients and methods

After informed consent, blood samples were collected from patients with motor neuron disease living in Canada, Denmark, Finland, Norway and Sweden. Samples were analysed for mutations in the CuZn-SOD gene using a polymerase chain reaction single stranded conformational polymorphism (PCR-SSCP) technique as described previously (Andersen *et al.*, 1995). All five exons of the CuZn-SOD gene were analysed. In samples with an abnormal SSCP band pattern, the existence of Asp90Ala was confirmed by direct nucleotide sequencing. After informed consent, samples were also collected from relatives of the patients with Asp90Ala. These samples were analysed either by PCR-SSCP or by a restriction enzyme test, as the Asp90Ala missense mutation introduces a new site for the restriction enzyme Fnu4H 1.

In patients with the Asp90Ala mutation, the family history and pedigree were documented by interviewing the patients and relatives and by inspection of case and church records. All patients were examined by neurologists, except one, who was examined by specialists in internal medicine. Twenty-seven of the 36 patients had been examined at least once in a neurological department of a university hospital. The case records of the patients were scrutinized (records from two patients were not available, but they both underwent a detailed interview), and 25 of the patients were re-examined by one of us (P.M.A.). One patient declined the examination, and one living patient was not accessible. Adult relatives were examined whenever possible.

To disclose environmental factors which may influence the age of onset of ALS or the disease progression rate, 27 of the patients underwent a detailed interview. Information on life habits prior to the disease presentation was collected according to a standardized protocol. In some cases, additional information was gathered from close relatives. All individuals were interviewed by the same investigator (PMA).

All but one of the 36 patients underwent an electrophysiological examination. Ten patients underwent sensory and motor nerve conduction studies and EMG recordings, and seven patients had MEP evaluated at the Department of Clinical Neurophysiology, Umeå University Hospital. The patients who underwent MEP for determination of the integrety of the pyramidal tracts, were stimulated (Magstim 200 with a 9 cm standard coil) transcranially and, in some, also peripherally at the cervical and lumbosacral levels. Recording was made from the abductor digiti minimi muscles in the hands and from the anterior tibial muscles in the legs. The central motor latency (CML) was calculated as the difference between the shortest latency obtained with cortical stimulation (CL) and the latency obtained by cervical or lumbosacral stimulation (RL). The CML was corrected for F-response latency (CML-F) as follows:

$$\text{CML-F} = (\text{minimal F-response latency} + M - 1)/2,$$

where M is the distal latency to muscular contraction at stimulation of the identical point (on a peripheral nerve) used for obtaining the F-response latency.

The ratio, M%, of the highest amplitude from cortical magnetic stimulation to the amplitude from the distal motor response from supramaximal electrical stimulation, expressed as a percentage, was calculated. Somatosensory evoked potentials were recorded in two patients by median and posterior tibial nerve stimulation.

The study was approved by the research ethical committee of Umeå University, Sweden.

Results

Genealogy

We studied 30 FALS patients from Sweden, Finland and Canada and their nine families. Analysis of available blood

Table 1 *Clinical features of 36 patients with autosomal recessive ALS*

No.	Genotype	Family	Born	Sex	Age (years) at onset of ALS	Occupation	Bladder problem	Disease duration (years)
1*	mm‡	A, III-1	1934	f	46	Farmer's wife	+	15.4
2*	mm	B, IV19	1896	m	94(?)	Mine work	na	† 4.6
3*	mm	B, IV28	1914	m	77	Mine work	na	† 4.3
4*	mx	B, V8	1932	f	45.9	Office work	na	† 7.0
5*	mm	B, V25	1930	m	48.5	Technician	+	17.0
6*	mx	B, V27	1936	f	37.2	Secretary	+	† 8.0
7*	mm	B, V32	1945	m	42.3	Office work	+	8.3
8	xx	C, III-3	1894	f	64	Waitress	+	†16.0
9	mx	C, III-9	1907	f	36	Housewife	+	†24.5
10	mm	C, III-13	1918	f	45.7	Farmer's wife	+	†21.9
11	xx	C, III-20	1892	m	44(?)	Cabinet maker	+	†22.1
12	mx	C, IV-26	1925	m	41.7	Carpenter	+	†12.8
13	mm	C, IV-28	1945	f	49.3	Office work	-	1.4
14	mm	D, III-11	1923	m	62	Sailor/factory work	na	10.4
15	mm	D, III-16	1932	f	42	Office work	+	21.3
16	mm	D, IV-1	1933	f	44.7	Shop assistant	na	17.7
17	mm	D, IV-2	1936	f	51.6	Shop assistant	+	7.3
18*	mm	E, III-4	1938	m	40	Teacher	+	17.5
19*	mm	E, III-6	1946	f	40	Nurse	-	9.2
20*	mm‡	F, III-4	1954	f	34.3	Nurse assistant	-	7.0
21	mx	G, III-4	1936	m	44	Forestry/factory work	+	†12.3
22	mm	G, III-6	1938	m	44.0	Factory worker	+	†11.0
23	mm	G, III-11	1948	m	31	Forestry/factory work	+	16.5
24*	mm	H, III-1	1952	m	20	Forestry worker	-	23.5
25*	mm	H, III-3	1954	m	32	Forestry worker	-	9.4
26*	mm	H, III-4	1956	f	20	Farmer	+	19.6
27	mm	J, II-1	1953	f	31	Bank clerk	+	11.5
28	mm	J, II-5	1961	f	20	Hairdresser	+	14.5
29	mm	L, III-2	1939	m	48.1	Carpenter	+	8.5
30*	mm	L, III-6	1947	f	31	Shop assistant	na	17.4
31	mm	T, V-2	1939	m	50	Forestry worker	+	6.5
32	mm	T, V-4	1943	f	37.5	Post worker	-	14.6
33	mm‡	V, III-3‡	1955	f	37.6	Business woman	+	2.7
34*	mm‡	–	1924	m	61.7	Managing director	+	10.2
35	mm‡	–	1939	f	45	Bank clerk	+	11.4
36*	mm‡	–	1941	m	37.0	Factory worker	+	17.3

† = deceased. *Previously reported (Andersen *et al.*, 1995); mm = individual homozygous for the Asp90Ala CuZn-SOD mutation; mx = no biological material available but offspring tested heterozygous for the mutation, and the spouses of the deceased patient tested homozygous for the wild type allele. xx = patient who could not be tested and had no children but who had a younger sibling (FALS patient) shown to be homozygous for Asp90Ala; ‡SALS.

samples (from the 23 surviving patients) had revealed that they were all homozygous for Asp90Ala (Table 1). The offspring of five deceased patients tested were heterozygous for Asp90Ala, and the spouses of four of them were homozygous for the wild-type allele. The spouse of the fifth ALS patient was deceased and no biological material was available for testing, but six of their seven children were heterozygous for Asp90Ala, while the last child declined to be tested. The results from these relatives are compatible with, indeed suggestive of, the deceased patients having been homozygous for Asp90Ala. No biological material was available from the remaining two deceased patients in Family C and they had no children. However, the phenotype of these two patients is similar to the other four cases in the Family C and one of their younger siblings was a FALS patient homozygous for Asp90Ala, suggesting that they also carried the same Asp90Ala allele.

Pedigrees of two of the FALS families (B and E) have previously been published (Andersen *et al.*, 1995) and are not reproduced here. The pedigrees of the other seven families are presented in the Appendix. The two patients in Family H, originally described by Myllylä *et al.* (1979) are still alive, and a third family member has also recently developed ALS. In addition to the 30 patients in the nine FALS families, we also found six apparently sporadic ALS (SALS) patients homozygous for Asp90Ala. Pedigrees of three of these six cases are shown in the Appendix as Families A, F and V.

The patient group included 19 females and 17 males. All 36 patients were born either in the Torne valley in northern Sweden

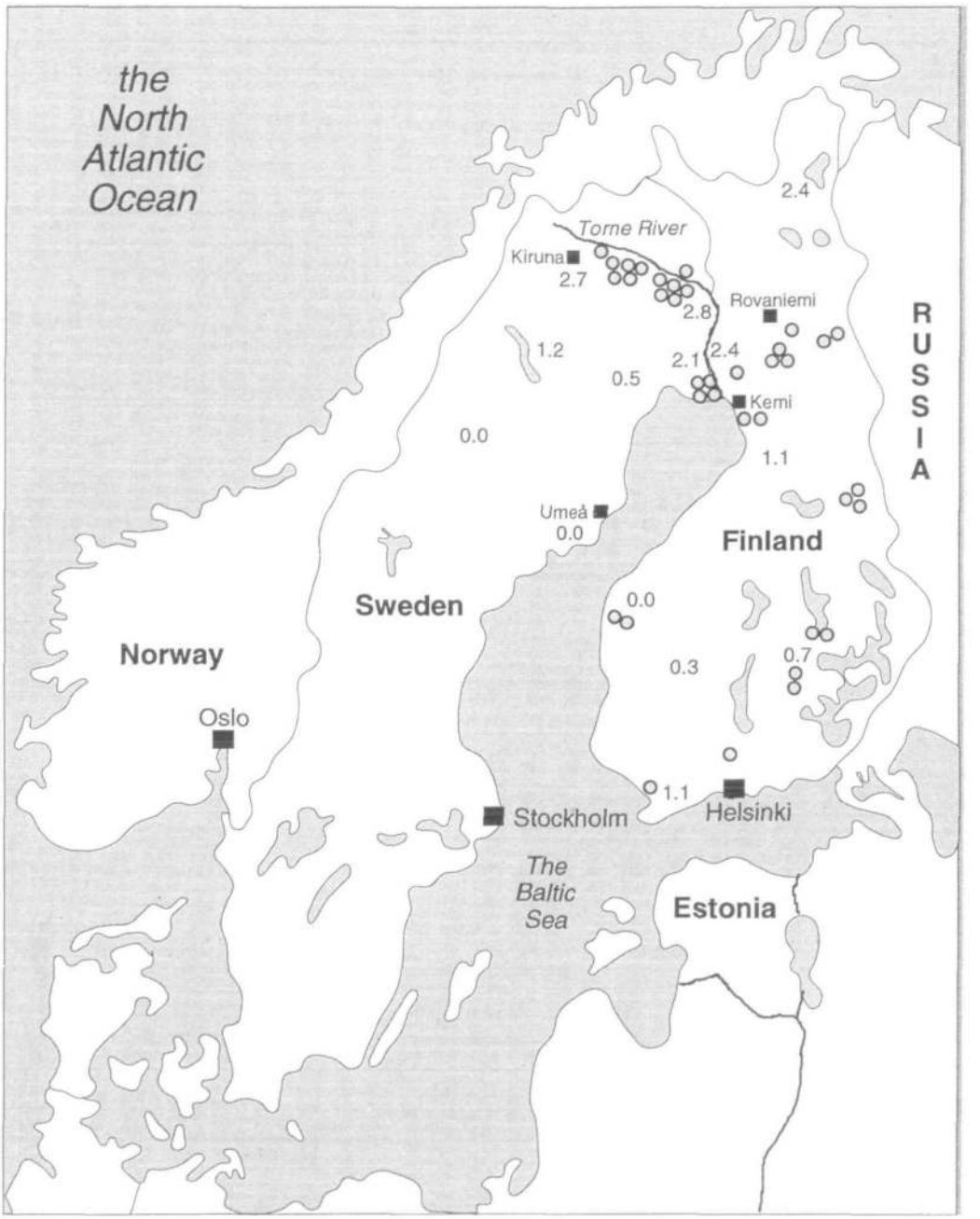

Fig. 1 Map showing the birth places (circles) of all 36 ALS patients, and the overall homozygous allele frequency (%) of Asp90Ala in local populations. Nine of the 36 patients moved in early adulthood from northern Sweden and northern Finland to central or southern Sweden, and one Finnish patient moved to Canada. In both Sweden and Finland the frequency of the Asp90Ala allele is highest in the scarcely populated north; this may be due to the founder effect. In the region of the city of Kiruna (population 22 000 inhabitants) the homozygous allele frequency was 2.7% implying that ~ 5% of the population should be heterozygous for Asp90Ala. Allele frequency data were compiled from Beckman (1973), Beckman and Pakarinen (1973) and Eriksson (1973).

or in Finland (Fig. 1). Ten of the patients moved in their early adulthood to central or southern Sweden, or to Canada (one patient).

Among the relatives of the 36 ALS patients tested, we found 99 individuals who were heterozygous for Asp90Ala. At the time of blood sampling, they had a mean age of 56.1 years (SD = 13.9 years), 20 of them being over the age of 70 years. None of the 99 individuals showed signs of motor neuron disease. An additional seven relatives were found to be homozygous for Asp90Ala (*see* separate section below).

Several members of the families who have not reportedly

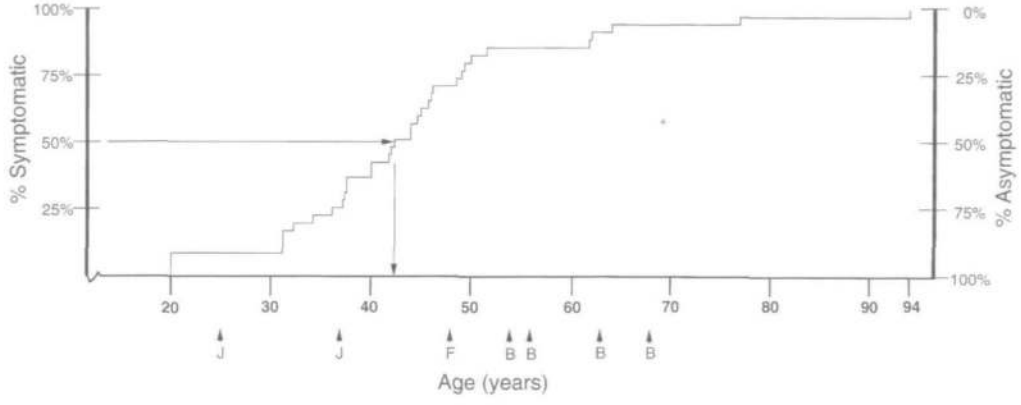

Fig 2. Cumulative distribution of age of onset of first symptoms (n = 35). One patient was excluded here since exact data were not available. The median age of onset was 42.3 years. The ages of the seven relatives of patients homozygous for Asp90Ala who have not developed ALS are indicated by arrows with a letter indicating the family of the individual.

Table 2 *Initial symptom(s) of 35 ALS patients with adult-onset recessive ALS*

Symptom	Right side	Left side	Bilateral	Side unknown	Other	Total (%)
Stiffness in the legs			9			9 (26)
Muscular cramps		2	5	1		8 (23)
Severe lower back pain					6	6 (17)
Pain in the leg(s)	1	3	6			9 (26)
Paresthesies			7			7 (20)
Paresis of lower limb	5	5				10 (29)
Fatigue					4	4 (11)
Clumsiness in the leg(s)	1	5	1			7 (20)
Muscle twitches in the legs			1			1 (3)

Table of first symptoms as recorded in case records or as remembered by the patient and relatives. Some patients had more than one symptom initially. The most common combination was painful muscular cramps and stiffness in the lower limbs.

developed ALS, have migrated to other parts of Europe, Canada, the USA and Australia. Thus, the Asp90Ala mutation should be expected in some of their offspring.

Age of onset

The mean and median age of onset of first symptoms were 44 and 42 years, respectively, with a wide range of 20– 94 years (Fig. 2). The age of onset showed large inter- and intrafamilial variability, e.g. ranging from 37–94 years in Family B (Table 1).

Clinical characteristics

The symptomatology of ALS patients homozygous for Asp90Ala can be divided into two phases: a preparetic phase followed by a phase of slowly progressive ascending paresis.

The preparetic phase

The onset of paresis was preceded by an insidious sense of stiffness and muscular cramps in the legs, unsteadiness or clumsiness and general fatigue in 25 patients (Table 2). Twelve of the 25 patients initially had a burning and aching pain in the lumbar area, buttocks, hips and/or, in the legs. Several patients obtained little relief from the pain with ordinary analgesics. The duration of this initial phase of the disease was highly variable ranging from a few months to seven years with large variations in the duration and intensity of symptoms even among patients of the same family (Fig. 3). According to available records, in this phase of the disease the clinical examination was essentially normal, with no reports of muscle atrophy, fasciculations, abnormal deep tendon reflexes or paresis. This was also the case in the seven patients who complained of clumsiness or unsteadiness in the legs. Two of these patients were referred to neurologists because of pain and unsteadiness in the legs, but the initial neurological examinations were normal. Three patients underwent EMG examination during this initial phase. They had had symptoms for 18 months, 26 months and 3 years when the examinations was performed; the EMG was considered normal in all three.

The paretic phase

In six patients, paresis was one of the initial symptoms. In another four patients paresis was an initial symptom but since only scarce information was available they may have

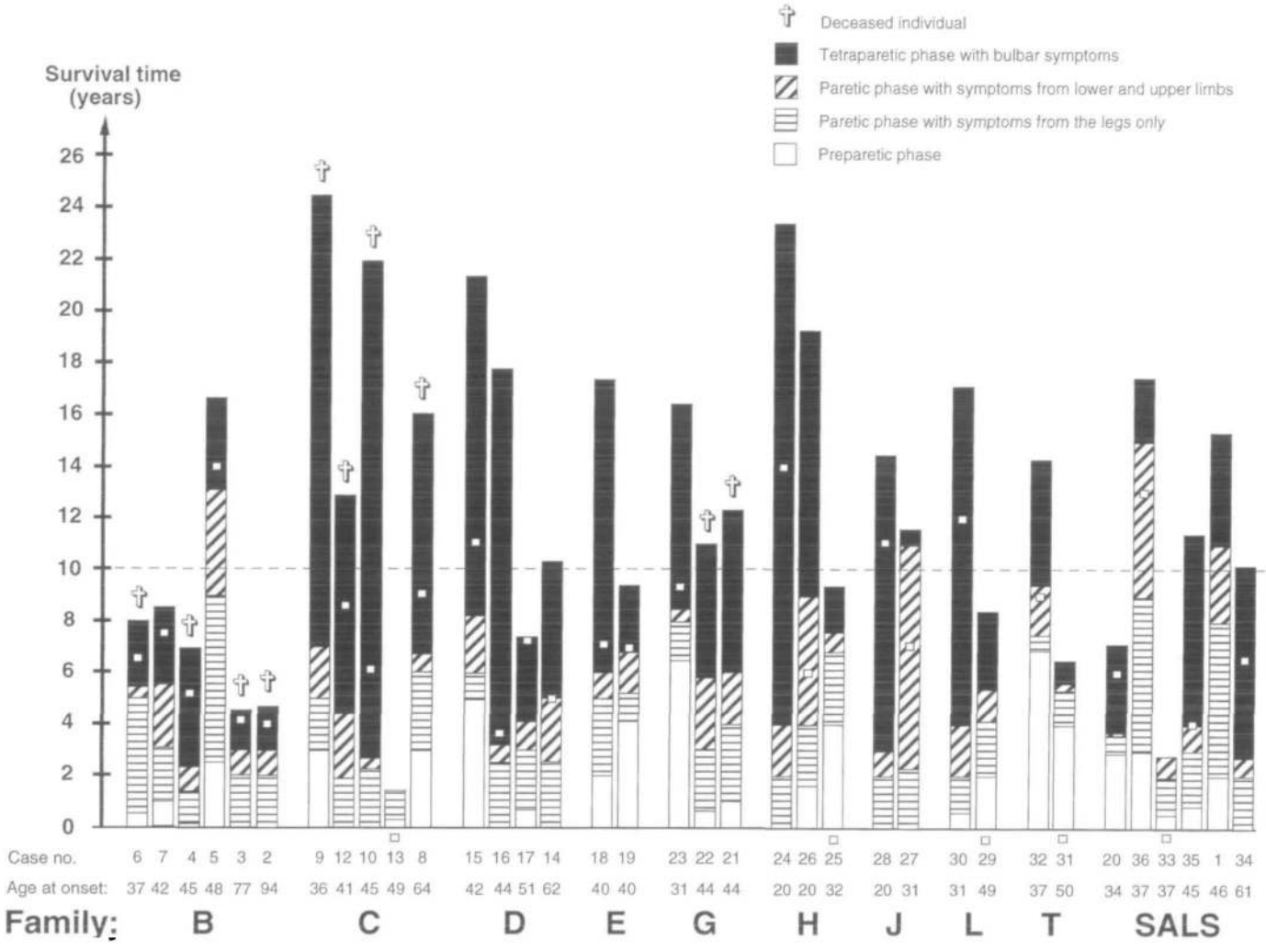

Fig. 3 Histogram showing the progression of symptoms from the lower limbs upwards in individual patients. The combined height of the bars for each individual patient indicates the total survival time from onset of first symptom. An unfilled (white) square in the bar indicates the approximate time the patient first used a wheelchair. Five patients are not using a wheelchair; for these cases the squares have been placed below the bar. The time the patient first used a wheelchair is not known for four patients (1, 9, 21 and 22). Within each family, the patients are arranged according to their age at onset of first symptoms. The approximate age at onset for each patient is written below with the number of the patient corresponding to Table 1. One patient in Family C was omitted since exact data were not available. Also, there is some uncertainty regarding the duration of symptoms in the extremities of Patient 27.

had additional symptoms preceding muscular paresis. Only one patient (E; III-4) clearly presented with paresis as the single initial symptom. This patient later developed leg and low back pain. In all patients the paresis appeared first in the legs, asymmetrically in all but three cases, and predominantly on the left side. In all cases paresis first appeared in the same limb in which the patient had experienced the most pronounced symptoms during the initial phase. Upon the appearance of paresis, most patients were referred to a neurologist. The initial neurological examinations generally revealed muscle wasting (often asymmetrical), fasciculations, absence of the deep ankle reflexes, normal to brisk patellar reflexes and, in 24 patients, hyperactive biceps reflexes, although these patients had no symptoms or other signs of involvement of the upper limbs at this stage of the disease. Slight dysmetria was found in the arms in two patients.

Symptoms appeared in the upper limbs, on average, 4.1 years after onset of first symptoms in the lower limbs (Table 3). One patient (J;II-5) with onset in the left leg had fasciculations in the left arm before the onset of symptoms in the right leg. All other patients developed a paraparesis before symptoms appeared in the arms. The first symptoms in the upper limbs were fasciculations in four patients and distal paresis or clumsiness in 21 patients. In contrast to the frequent occurrence of painful cramp as the initial symtom in the lower limbs, painful cramp was the initial symptom in the upper limbs in only two patients. One patient had no symptoms in the upper extremities, but they were early in the disease. The first symptom in the upper limbs in the remaining eight patients is unknown. In all cases wasting first appeared asymmetrically and distally in the upper extremity first involved.

Bulbar symptoms appeared after an average of 5.4 years

Table 3 *Disease time course in adult-onset recessive ALS (in years)*

	No. of patients*	Mean	±SD	Range
Age at onset of first symptom				
All patients	36	43.8	14.7	20–94
Males only	17	48.1	17.6	20–94
Females only	19	39.9	10.5	20–64
Age at onset of paresis (in the lower limbs)	36	45.3	14.4	20–94
Time from onset of first symptom in the lower limbs to:				
first symptom in the upper limbs	34	4.1	2.3	1–9
bulbar symptoms	28	5.4	2.4	1–11
use of a walking stick	25	4.8	2.9	1–13
use of wheelchair	24†	7.8	3.3	3–13
Survival time to death	11	13.1	8.1	4.3–24.5

*Patients from whom reliable data were not available have been omitted. †Five patients are in early stages of the disease and are not using a wheelchair; six other patients are known to have used a wheelchair but the time when they began using it is unknown.

from first symptoms in the lower limbs. The most common first bulbar symptom was dysarthria. Dysphagia occurred later than dysarthria and has been a less prominent symptom. Two patients have not yet developed bulbar symptoms. The bulbar symptoms in most cases have progressed slowly, resulting in anarthria late in the disease. Atrophy of the neck, face and masticatory muscles was slight even after several years disease duration. None of the patients showed signs of involvement of the external ocular muscles. Gustatory and olfactory function was reported to be normal in all but one patient who had impaired olfactory function. The cranial symptoms and signs were mainly of a lower motor neuron type though several of the patients also had a pseudobulbar component in their dysarthria. The jaw jerk was brisk in eight patients but not in another three who had other signs of bulbar involvement. Patients with very long disease duration showed mild inappropriate laughing and crying but none have shown signs of cognitive impairment.

The Babinski sign was found bilaterally in all patients and was found early in the disease in some patients, initially unilaterally. An increase in muscle tone was found in 31 patients but not in two who were in the early stages of the disease. In the three remaining patients the records gave no information on muscle tone.

Paraesthesia (including hypaeresthesia) was one of the initial symptoms in seven patients, and appeared later in another nine patients. Sensory examination for pain, touch or temperature in all these patients was essentially normal except in Patient G;III-6 who also suffered from type I diabetes mellitus complicated by polyneuropathy. Eleven patients had periods of weeks to months during the first 5 years of the disease when they experienced a sensation of intense heat in the legs, most pronounced in the feet. In two patients, the sensation of heat in the feet preceded all other symptoms. All eleven patients with heat sensations later developed an intense chilling sensation in the lower part of the legs. This was also reported by another five patients who had no initial sense of intense heat. The chilling sensation developed before severe wasting and persisted throughout the duration of the disease. Vibratory sensation in the feet was slightly reduced in 14 patients.

The six SALS cases were clinically indistinguishable from the 30 FALS cases.

Micturition

Urgency of micturition and/or difficulty initiating urination was reported by 24 patients (Table 1). In a few patients, the urgency of micturition became so pronounced that a permanent catheter needed to be inserted. Hypertrophy of the prostate was excluded as the cause of difficulties in voiding. Cystometrograms revealed uninhibited contractions of the detrusor muscle. The onset of bladder disturbance was difficult to estimate but two patients reported urgency before the onset of paresis in the legs. In most other patients the bladder symptoms appeared after >6 years disease duration. Six patients did not complain of difficulties in urination. They had an average disease duration of 10.5 years (range 1.2–23 years). None of the 36 patients developed vesico-rectal incontinence.

Progression and survival

The disease progressed slowly but relentlessly without signs of a plateau phase or remission in any patient. The mean interval from onset of first symptoms to walking with the aid of a stick was 4.8 years and to the use of a wheelchair 7.8 years with large variations both within and between the families (Fig. 3 and Table 3). Eight patients have fallen and suffered fractures (the tibia in six and the collum femoris in two) forcing them to use a walking aid or wheelchair earlier than would have been anticipated had they not had fractures.

The patients were followed to death or to the beginning of 1996. Eleven of the patients have died with a mean survival time of 13 years. Seven (64%) of the deceased patients survived for >10 years from onset, and three for >20 years. Two patients are still alive after >21 and >23 years disease duration. None of the patients who died had respiratory support. At the time of death, the patients had generalized wasting with severe tetraplegia. Most died of respiratory failure, which was aggravated by pneumonia in three cases. Patient G;III-4 died of acute myocardial infarction confirmed by autopsy. The exact cause of death of Patient B;IV-28, who died aged 81 years after the shortest survival time of 4.3 years, is unknown. He also suffered from ischaemic heart disease.

Possible risk factors

Infections

None of the patients had had poliomyelitis or had had known contact with patients with poliomyelitis. All patients had been vaccinated against polio (no information available from three patients). No patient reported having had serious infectious symptoms in the 2 years preceding the onset of ALS.

Intoxication

Three of the patients had major exposure to heavy metals (B;IV-19, B;IV-28, D;III-11). Heavy metal urine screening was normal in the eight patients examined (Cd, Cu, Hg, Mn, Pb, Se, Zn; not all metals were examined in all eight patients). Only two patients had been occupationally exposed to organic solvents (T;V-2 to petrol fumes while working a chainsaw and D;III-11 to different chemicals at a plastic factory).

Trauma

Four patients had experienced major traumas, but none within 5 years before the onset of ALS. Two patients (B;IV-19 and B;IV-28) had suffered repeated traumata in their jobs as mine workers. Both developed ALS many years after retirement. One patient (L;III-2) had multiple electrical traumata, all minor and without serious injury.

Occupation

The occupations of the patients are listed in Table 1. Four patients had lived on farms as children and in early adulthood but had not been exposed to pesticides or had much contact with animals.

Athletic activity

Some patients had been athletes. In most cases the kind of activity involved demanded long endurance as in marathon running or cross-country skiing. Some of the athletically active patients developed ALS before older siblings who were homozygous for Asp90Ala. On the other hand some of the younger patients had never participated in any athletic activities apart from the ordinary sports at school.

Homozygous individuals without a diagnosis of ALS

Out of all the relatives tested we have found seven individuals in three families who were homozygous for the Asp90Ala mutation but who have not been diagnosed with ALS (Fig. 2). In Family B, four siblings of the present living patients V-25 and V-32 are homozygous for Asp90Ala. The four are aged between 54 and 68 years. Of the four, a 59-year-old woman has shown progressive difficulties in walking for the past 3 years and is now walking with the aid of a stick. She declines examination. Another of the four siblings (B;V-31, 54 years old) who we earlier reported as being healthy (Andersen *et al.*, 1995), has over the past year, developed increasing pain in the lower back and legs similar to the initial symptoms of other family members who later developed ALS. A recent EMG disclosed slight neuropathic changes in the L5-S1 myotomes bilaterally. The two other Asp90Ala homozygous siblings in Family B are both reported to be healthy. One of them, a 68-year-old man, lives next door to his younger brother (65 years old), who has had ALS for the past 17 years. The two brothers experienced the same upbringing and shared the same environment with similar work and leisure activities.

Patient F;III-1 (aged 48 years), the sister of the SALS Patient F;III-4 (aged 41 years), is homozygous for Asp90Ala and without symptoms of ALS. Her recent neurological examination and EMG (like that of her brother and parents, all heterozygous for Asp90Ala) was normal. In Family J, two siblings aged 37 and 25 years are both homozygous for Asp90Ala and are reported to be without symptoms.

Laboratory investigations

Muscle morphology

Ten patients had muscle biopsies and they all showed atrophy, fibre type grouping and small angulated fibres consistent with a peripheral neuropathy.

Clinical neurophysiology

Thirty-five of the patients underwent EMG and nerve conduction studies. All showed results compatible with the diagnosis of ALS. In the 10 patients examined at Umeå University Hospital, the EMG suggested collateral reinnervation. Distal lower limb muscles were first affected, followed by distal upper limb and proximal lower limb. Eventually all limbs were affected severely, both distally and proximally. Sensory conduction studies in the hands were

Table 4 *The MEP latencies (in ms) and relative amplitudes in the patients that underwent transcranial magnetic stimulation and nerve conduction studies*

Patient	History length (years)	MEP recorded in:									
		abductor digiti minimi in the hand (right/left)					anterior tibial muscle (right/left)				
		CL	RL	CML	CML-F	M%	CL	RL	CML	CML-F	M%
B;V-25	15	NR/34.8	12.9/12.9	NR/21.9	F/15.2	NR/11	47.5/50.2	15.8/16.0	31.7/34.2	27.2/30.1	110/110
B;V-32	5	20.2/19.7	11.9/11.3	8.3/8.4	5.3/3.9	22/5.4	NR/NR			F/F	NR/NR
C;IV-28	1	18.2/18.9		5.2/6.0	34/15	38.8/37.2				23.2/22.0	30/21
D;IV-2	8	37.5/33.1	14.1/14.4	23.1/18.7	21.6/19.2	52/28	44.7/51.3	14.6/NR	30.1/NR	29.0/35.4	
E;III-4	17	37.5/36.3	12.9/15.8	24.6/20.5	20.2/19.5	4.4/25	NR/NR			*/*	NR/NR
E;III-6	9	37.9/40.7	15.0/15.9	22.9/24.8	NDF/20.8	47/28	46.4/46.4	16.0/15.2	30.4/31.2	28.7/26.2	48/56
G;III-11	16	26.7/NR	16.6/16.9	10.1/NR	7.9/F	20/NR	55.6/NR	15.3/17.8	40.3/NR	37.8/F	47/NR
Normal	–	≤25.5	≤18.0	≤10.0	≤9.0	≥10	≤35.0	≤18.5	≤20.0	≤17.5	≥10

CL = shortest latency obtained with cortical stimulation.; RL = latency obtained with cervical or lumbrosacral stimulation; CML = central motor pathway delay, CML-F = central delay corrected for F-response latencies; M% = ratio of highest amplitude response to cortical stimulation to that following supramaximal electrical stimulation. Normal: upper reference limits for normal latencies based on mean plus 3 SDs and the lower limits for relative amplitudes; NR = no responses could be seen or difference/ratio could not be calculated due to lack of cortical or peripheral response; F = F-responses could be elicited, indicating that NR to cortical stimulation was due to upper motor neuron involvement. NDF-no definite F-responses could be elicited.*Small distal MEP amplitude and no F-responses indicating that NR by cortical stimulation was associated with lower motor neuron involvement

abnormal in two of the 10 patients studied; one patient had a probable carpal tunnel syndrome, and the other patient was aged 98 years. Studies of the sural nerve gave abnormal results in the three oldest patients examined. Motor conduction was more frequently affected, especially in the lower limbs and in the arms late in the course of the disease in four out of the 10 patients.

The MEP examination was pathological in all seven patients studied with prolonged latencies (CML and/or CML-F) following cortical stimulation or inability to evoke any responses with cortical stimulation (*see* CML and CML-F in Table 4). Six of the seven patients had pathological MEP values in the legs. The seventh had a severe peripheral neuropathy in the legs which probably accounted for the absence of responses to cortical stimulation. This patient also had slow conduction to the hands as well as a diminished M% ratio in one hand (reduced relative to the response to more distal stimulation; *see* the 'M%' ratio in Table 4). In addition, three of the patients had latency prolongations to the hands and one patient had borderline latencies. In two patients the M% ratio was normal in the legs but showed decreased values in one hand. One of these patients had normal latencies to that hand. Sensory evoked potential tests done on two patients both gave normal results.

Blood and chemistry

Routine blood analyses were essentially normal in all patients. *S*-Creatine phosphokinase was slightly elevated in 22 of 28 patients. Electrophoresis of plasma proteins and total plasma IgA, IgG and IgM were normal (n = 21). Analysis for serum antibodies against a panel of gangliosides (including GM1) was normal in the four patients examined. Mean protein concentration in the cerebrospinal fluid was 440 mg l^{-1} (n = 25). In Patients B;V-25 and G;III-11, the protein concentration was slightly elevated to 668 mg l^{-1} and 900 mg l^{-1}, respectively. In both, the examination was later repeated and similar elevations were obtained. Evidence for intrathecal synthesis of immunoglobulins or oligoclonal bands was not found in any patient.

Imaging examinations

Sixteen patients underwent myelograms and/or MRI of the spine and almost all patients underwent X-ray examination of the spine (whole or part). No clinically significant abnormalities were found in any of the patients. Nine patients underwent cranial CT or MRI early in the disease; no significant lesions were found.

Other diseases among the patients

Most of the patients claimed to have been healthy until they developed ALS. Only one patient had developed a cancer: Patient C;III-3 developed breast cancer in 1949 with metastasis to the axillary lymph nodes. Surgical treatment and radiotherapy was successful, and the last control in 1965 revealed no signs of cancer. In 1964, this patient developed ALS with a phenotype similar to the Asp90Ala homozygous patients. Patient G;III-6 developed diabetes mellitus at the age of 46 years (2 years after the onset of ALS) requiring treatment with insulin. The diabetes was complicated by axonal motor and sensory polyneuropathy.

Ischaemic heart disease was found in three patients and hypertension in another three. In one of these (G;III-11),

the development of ALS symptoms coincided with the development of severe hypertension.

Patient H;III-3 developed streptococcus-induced glomerulonephritis with complete recovery some 20 years before the onset of ALS. Patient C;III-11 suffered from what was most probably viral meningitis in 1954. Thirty-one years later he developed ALS with the same phenotype as the three other members of Family C. None of the patients have suffered from polyradiculitis.

Diseases among the relatives

Dementia. The mother of the two patients in Family T was diagnosed with familial Alzheimer's disease, her first symptom being insidious loss of memory function. In Family B, as well as in Family F, a maternal aunt developed vascular dementia. Analysis showed the aunt in Family B was heterozygous for Asp90Ala and that the aunt in Family F was homozygous for the wild-type allele. No other forms of dementia were found in the families.

Parkinson's disease. In Family F, the paternal aunt F;II-11 and grandmother F;I-4 both developed Parkinson's disease. The aunt is homozygous for the wild-type allele, no material is available from the deceased grandmother. A neurological examination (including EMG) of the father (F;II-10, aged 69 years) of the SALS patient F;III-4 was normal. In Family B, the two cousins B;V-49 and B;V-59 (different parents) have developed an L-dopa-responsive Parkinson's disease. Analysis showed them to be heterozygous and homozygous for the wild-type allele, respectively. In Family H, the mother of the three ALS cases (H;II-8) has developed a mild L-dopa responsive form of Parkinson's disease. She is heterozygous for Asp90Ala. In Family C, a brother (C;III-18) and a cousin (C;III-24) are reported to have developed Parkinson's disease in old age. No further details are available. In the other branch of Family C, no cases of Parkinson's disease have been found. In none of the families have the children of the ALS patients developed Parkinson's disease.

Thyroid disease. Two siblings of the ALS patients have been treated for cancer of the thyroid: F;III-1 is homozygous for Asp90Ala and at the age 48 years she developed a papillary carcinoma of the thyroid gland. A recent neurological examination and EMG revealed no signs of ALS. H;III-5 is heterozygous for Asp90Ala and was diagnosed with a medullary carcinoma of the thyroid gland at the age of 26 years. He shows no signs of ALS. Two heterozygous men, sons of deceased ALS patients, have been treated for thyrotoxicosis.

Multiple sclerosis. Relative T;V-3, homozygous for the wild-type allele, emigrated from Finland to Canada in 1961 and developed multiple sclerosis of the relapsing–remitting type in 1972. The diagnosis was supported by MRI of the brain and the finding of oligoclonal bands in the CSF.

Other. Relative F;III-2, the sister of two Asp90Ala homozygous individuals, died in 1949 at the age of 8 months of 'pneumonia and muscular weakness'. She is reported to have had an uncomplicated gestation and delivery, but never learned to sit or walk 'since she suffered from congenital muscular weakness'. Her diagnosis was 'mongolism', but it is not known on what criteria the diagnosis was based.

Discussion

Age of onset

We have studied 36 ALS patients, 29 of which have been shown to be homozygous for Asp90Ala, and the remaining seven, although not tested were, according to the family pedigree very likely to be homozygous too. The mean age of onset of first symptoms and the onset of muscular weakness are in accordance with earlier studies which show that the mean age of onset in FALS cases is ~10 years earlier than the mean age of onset of SALS (Kurland and Mulder, 1955; Emery and Holloway, 1982).

The 36 patients show a rather uniform phenotype and have in common (i) disease onset in the lower limbs and (ii) similar order of appearance of new symptoms. The heterogeneity between the patients lies in (i) age of onset of disease and (ii) disease progression rate, emphasizing the involvement of other factors in the ALS phenotype of individuals homozygous for Asp90Ala.

The age of onset varied from 20 years in the three youngest patients to 77 and 94 years in the oldest patients. Even within individual families the range of the age of onset is wide. This suggests that the onset of ALS in individuals homozygous for Asp90Ala is influenced by factor(s) other than the mutated CuZn-SOD molecule. Roe (1964) described a FALS family with only small differences in the age of onset between the five cases, but most authors find wide differences in the age of onset of ALS within FALS families (Strong *et al.*, 1991).

Several of the exogenous factors considered as possible risk factors for ALS can also be considered as factors influencing the age of onset. (i) Viral infections, especially poliomyelitis, have been causally related to ALS (Mulder *et al.*, 1972; Woodall *et al.*, 1994). (ii) Intoxication has frequently been suggested as the cause of, or a contributing factor to, ALS (Aran, 1850; Campbell *et al.*, 1970; Felmus *et al.*, 1976; Deapen, 1986; Gunnarsson *et al.*, 1992). The intoxicants include heavy metals such as manganese, lead, mercury and selenium. (iii) Antecedent mechanical traumata have been related to the cause of motor neuron disease (Gallagher, 1987; Granieri *et al.*, 1988). Electrical trauma have been correlated with sporadic motor neuron disease (Deapen *et al.*, 1986; Gallagher, 1987). (iv) The patient's occupation may be a factor: farming and/or heavy labour as well as athletics have been associated with motor neuron

disease in some studies (Felmus *et al.*, 1976; Granieri *et al.*, 1988; Gunnarsson *et al.*, 1991*a*). Although a formal epidemiological study has not been performed in our study, a detailed questionnaire concerning occupational exposure, medical history, life-style factors and examination of case records did not reveal any obvious differences between patients with early onset and patients with late onset ALS.

Clinical features

The symptomatology during the preparetic phase described by most of the homozygous Asp90Ala patients is so consistent that we consider it to be characteristic of the symptomatology of ALS itself in the patients homozygous for the Asp90Ala mutation.

Subjective disturbances of sensation are frequent in ALS patients, but objective sensory findings are less common (Friedman and Freedman, 1950). Almost all of our patients experienced sensory symptoms at some time during the disease. Most characteristic was a burning and aching pain in the lower back and/or leg muscles. In some patients this was the initial symptom, in others it appeared during the first years of the disease. As the wasting progressed, the patients were relieved of the burning and aching pain. However, articular pain, especially in the back and neck were common in cases with advanced amyotrophy, probably because of loss of muscular support for the joints. Pain has been reported, albeit rarely, in ALS patients (Swank and Putman, 1943; Friedman and Freedman, 1950; Murray *et al.*, 1974; Drake, 1983; Gubbay *et al.*, 1985). Two cases of autopsy-confirmed ALS beginning with severe pain in the lower extremities (described as 'neuritis' and 'coldness') have been reported (Weschler *et al.*, 1929).

A reduced sense of vibration was noted in 14 patients, confirming findings by others that sensory pathways may be involved in familial cases (Friedman and Freedman, 1950; Hirano *et al.*, 1967; Li *et al.*, 1988).

The rarity of bedsores in patients with ALS is well established (Forrester, 1976). Two of our patients developed small bedsores in the buttock after very long immobilization.

Muscular cramps were reported by most patients and were especially severe at the onset of disease, often, but not always, associated with pain. The cramps tended to disappear as wasting became profound. Cramps are often overlooked in ALS (Gubbay *et al.*, 1985), though in his original description of progressive muscular atrophy (1850), Aran described a patient with insidious onset of cramps and muscle quiverings. Cramps have also been described as an initial symptom in patients with spino-bulbar muscular atrophy (Kennedy *et al.*, 1968).

The sequential order of appearance of symptoms and the way they slowly ascended from the limbs to bulbar innervated muscles has been identical in all patients studied, but large inter- and intrafamilial variability in the rate of disease progression was found, even among siblings living under nearly identical conditions (Fig. 3). Younger individuals tend to have a slower rate of disease progression and longer disease duration than individuals who are older at the age of onset, as has previously been found among FALS cases (Giménez-Roldán *et al.*, 1977; Strong *et al.*, 1991) and SALS cases (Müller, 1952; Eisen *et al.*, 1993). In contrast, Mulder *et al.* (1986) found that survival time in FALS was not associated with the age of onset. Eleven of our patients have died with a mean survival time of 13 years. This is probably an underestimation of the real survival time of patients homozygous for Asp90Ala, since it includes the two very old patients who also suffered from cerebrovascular and cardiac diseases. Also, of the 25 patients presently alive, 16 (64%) have been ill for >10 years already and this percentage should rise considerably for the whole group, whereas only 64% of the deceased group had been ill for >10 years.

In most epidemiological studies performed, a small percentage of ALS patients are long survivors, challenging the dogma that ALS is invariably a rapidly progressing fatal disease (Müller, 1952; Osuntokun *et al.*, 1974; Mulder and Howard, 1976; Forsgren *et al.*, 1983; Gubbay *et al.*, 1985; Ben Hamida *et al.*, 1990; Eisen *et al.*, 1993). Our results indicate that ALS patients homozygous for the Asp90Ala mutation constitute a distinct, long surviving subgroup of ALS patients in Sweden and Finland.

All patients showed signs of involvement of the upper motor neuron system early in the disease with lower limb stiffness as a frequent first symptom. A bilateral Babinski sign was found in all patients and in some of these it appeared very early in the course of the disease. The first signs from the upper limbs were brisk deep tendon reflexes which, in several patients, were found before the appearance of paresis in the upper limbs. This suggests early involvement of the descending tracts of the upper motor neuron system.

Urgency of micturition has been suggested to reflect rapid advancement of involvement of the pyramidal tract (Swank and Putnam, 1943). Vesico-rectal symptoms are seldom reported in ALS patients (Friedman and Freedman, 1950; Müller, 1952; Metcalf and Hirano, 1971; Hattori *et al.*, 1983; Schröder and Reske-Nielsen, 1984; Gubbay *et al.*, 1985; López-Vega *et al.*, 1988), and are usually considered uncommon and an exclusion criterion for ALS (Charcot and Joffroy, 1869). Eleven of Friedman and Freedman's (1950) patients were autopsied. No specific pathology was found. Loss of neurons in the nucleus of Onufrowicz has been reported in long-surviving patients on ventilatory support (Hayashi *et al.*, 1989). Twenty-four of our patients complained of urgency of micturition and/or difficulty in initiating micturation. Since it has not been possible to find any specific cause of bladder dysfunction, we are left to conclude that difficulties in the neural control of voiding occur in the majority of ALS patients homozygous for Asp90Ala. No histological material is available from autopsy of a patient homozygous Asp90Ala, and it remains to be seen if there are pathological changes in the nucleus of Onufrowicz or the sacral autonomic nuclei. Bladder dysfunction has been reported in patients with primary lateral sclerosis (Russo,

1982), although autopsy in one case did not find any neuropathological correlate in the sacral autonomic and Onufrowicz nuclei (Pringle *et al.*, 1992).

Clinical neurophysiology

The MEP examination was pathological in all seven of the patients tested, even early in the disease. Prolonged latencies were the main finding and were often very marked, while decreased amplitudes were a much less prominent feature. In contrast to our findings, most authors have seen low amplitudes with normal or slightly prolonged latencies or normal results in patients with ALS (Barker *et al.*, 1986; Berardelli *et al.*, 1987; Ingram and Swash 1987; Schriefer *et al.*, 1989; Eisen and Shytbel, 1990; Eisen *et al.*, 1990; Berardelli *et al.*, 1991; Caramia *et al.*, 1991). In the studies where individual latencies were presented, only a few patients had markedly prolonged latencies. Latency values corresponding with our findings have been reported in patients with primary lateral sclerosis (Brown *et al.*, 1992); the cause of these latency aberrations is not clear. Different mechanisms have been proposed (Ingram and Swash, 1987; Schriefer *et al.*, 1989; Eisen *et al.*, 1990; Mills, 1995).

Other diseases in the families

We found Parkinson's disease in four, and dementia in three, of the relatives in the Asp90Ala families. The phenotype of our Asp90Ala homozygous ALS patients appears to be unaffected by the familial concurrence of Parkinson's disease or dementia. It has been suggested that dementia and parkinsonism are associated with ALS (Appel, 1981; Calne *et al.*, 1986; Deapen and Henderson, 1986) and may share aetiopathogenic factors (Hudson, 1981; Eisen and Calne, 1992; Majoor-Krakaur *et al.*, 1994). Numerous reports of familial concurrence of ALS and Parkinson's disease and/or dementia (Brait *et al.*, 1973; Alter and Schaumann, 1976; Schmitt *et al.*, 1984; Frecker *et al.*, 1990; Gunnarsson *et al.*, 1991*b*) suggest a shared genetic susceptibility to these disorders or subtypes of them. Burrow and Blumbergs (1992) found significant loss of neurons in the substantia nigra in 14 cases of motor neuron disease without signs of parkinsonism. Mild neuronal loss and neurofibrillary tangles were found in the substantia nigra, globus pallidus, locus coeruleus and inferior olivary nuclei upon autopsy of a long surviving FALS patient with the Ile113Thr, CuZn-SOD mutation (Orrell *et al.*, 1995*a*). The patient did not show signs of extrapyramidal disease. Bandmann *et al.* (1995) sequenced the CuZn-SOD gene in 23 patients with familial Parkinson's disease and found no mutations.

Appel *et al.* (1986) found either past or present thyroid disease (one tumor, two hyperthyroid, eight hypothyroid) in 11 out of 58 patients with ALS, and a family history of thyroid disease was found in 19% of the 58 ALS patients. One of our patients had been treated for hypothyreosis earlier in life. Among the relatives of the Asp90Ala patients, two individuals developed a malignant neoplasm of the thyroid and another two had been treated for thyrotoxicosis. In 16 Swedish and five Finnish FALS families without CuZn-SOD mutations, we have found no relatives of ALS patients with either cancer of the thyroid gland or thyrotoxicosis, but they have previously been reported by others (Williams *et al.*, 1988).

Sporadic ALS cases

Six of our patients homozygous for the Asp90Ala mutation were referred to us as sporadic cases. Cases of SALS heterozygous for CuZn-SOD mutations (Ile113Thr, Glu21Lys) have previously been reported but in these few cases the parents of the patients had died relatively early and it was not possible to exclude illegitimacy in the families (Jones *et al.*, 1995). Decreased penetrance has been suggested in a SALS case heterozygous for the Ile113Thr mutation (Suthers *et al.*, 1994).

CuZn-SOD polymorphism

In the three Swedish families (B, C and D), we found ALS patients homozygous for Asp90Ala in multiple generations, illustrating the occurrence of the polymorphism of the Asp90Ala CuZn-SOD mutation which exists in northern Sweden. Without the knowledge of the patients being homozygous for Asp90Ala, the families would most likely be considered as having autosomal dominant inheritance with incomplete penetrance.

A CuZn-SOD polymorphism has previously been described in Sweden and Finland (Kirjarinta *et al.*, 1969; Beckman, 1973; Beckman and Pakarinen, 1973; Eriksson, 1973). Recently, the polymorphic allele in northern Sweden was found to be identical to Asp90Ala (Själander *et al.*, 1995). In these studies both heterozygous and a few homozygous individuals were found, but the polymorphism could not be associated with any disease, most probably since only a few, young homozygous individuals were examined (Beckman *et al.*, 1975).

The highest allele frequency, of 2 –2.8% (Beckman, 1973), was found in the northern part of the province of Norrbotten in northern Sweden, in particular in the valley of the River Torne. In the southern half of the province of Norrbotten, the frequency was found to be lower, and very low in central and southern Sweden. In Finland the highest allele frequency reported was 2.4%, in northern Finland (Eriksson, 1973). In central and southern Finland, the allele frequency varied from 0.35% to 1.1% (Kirjarinta *et al.*, 1969; Eriksson, 1973). However, it must be recognized that samples were collected from only a few locations and it is likely that higher allele frequencies can be found locally due to the founder effect.

Clinical impact of Asp90Ala

Since the Asp90Ala allele is associated with ALS, it might be expected that the disease is more prevalent in the areas

Table 5 *Prevalence of ALS in northern Sweden and Northern Finland on January 1, 1995*

	Province of Norrbotten Sweden	Rovaniemi health district Finland	Kemi health district Finland
Population	267 648	130 282	72 043
No. of ALS cases 1.1.1995	19	6	5
No. of ALS cases homozygous for Asp90Ala	5	3	2
%ALS cases homozygous for Asp90Ala	26	50	40
Prevalence of ALS with the Asp90Ala mutation	1.9/100 000	2.3/100 000	2.8/100 000
Prevalence of ALS on 1.1.1995	7.1/100 000	4.6/100 000	6.95/100 000

These 30 cases were all tested for Asp90Ala mutations; the 10 cases which were homozygous for Asp90Ala were all included in our group of 36 patients in this study.

with high Asp90Ala allele frequency. The prevalence of ALS in northern Sweden and northern Finland on January 1, 1995, are shown in Table 5. On that day there were 19 known cases of definitive ALS (El Escorial criteria) in the province of Norrbotten in northern Sweden, of which five were homozygous for Asp90Ala (the two individuals in our Family B who are showing symptoms of suspected early ALS are not included). We found the prevalence of ALS in Norrbotten was 7.1 patients per 100 000 inhabitants. If the homozygous Asp90Ala patients are omitted, the prevalence is 5.2. In the combined health districts of Kemi and Rovaniemi in northern Finland, on the same day, there were 11 patients with definitive ALS of which five were homozygous for Asp90Ala. Four of the five patients were apparently SALS cases, the fifth (T;V-2; the sister T;V-4) lives in Canada. We conclude, that Asp90Ala has a major impact on the prevalence of ALS in northern Sweden and northern Finland.

Estimation of the incidence of ALS patients homozygous for Asp90Ala in Finland is difficult since samples could only be collected from 10 neurological departments, some of them geographically distant from the others. However, samples were collected from all parts of Finland. Of 74 samples from motor neuron disease patients born and living in Finland (patients born in Finland who have emigrated to Sweden and Canada have been excluded), 14 (19%) were homozygous for Asp90Ala. Jokelainen (1977) found the mean survival time for all ALS patients in Finland to be 2.6 years which is about one-fifth the mean survival time of our 11 deceased Asp90Ala patients. With reservation for irregular sampling bias and small numbers, our results indicate that as many as 3% of all new cases of ALS in Finland should be homozygous for the Asp90Ala mutation.

Since we have found no individual heterozygous for Asp90Ala with ALS in any of the families, the children of patients homozygous for Asp90Ala are probably only at risk of developing ALS if the other parent is heterozygous for Asp90Ala (as in Family B). However, it cannot be excluded that heterozygosity for the Asp90Ala mutation is a low risk factor for ALS. With a very stable population of about five million people and assuming an Asp90Ala allele frequency for the whole of Finland of 1%, there should be ~500 Asp90Ala homozygous and 99 000 Asp90Ala heterozygous individuals in Finland (assuming the conditions of the Hardy-Weinberg theorem are fulfilled, which is probably the case in Finland). Of 72 blood samples from sporadic cases of motor neuron disease born in Finland (including some patients who lived as adults in Sweden and the USA), six proved to be homozygous for Asp90Ala (the six SALS cases in this article) and two were heterozygous for Asp90Ala. The two heterozygous cases had a phenotype very different from the homozygous Asp90Ala, with rapidly progressing bulbar paresis and death within 2–3 years (Andersen *et al.*, 1995) suggesting that other aetiologic factors were involved (different from those in the patients homozygous for Asp90Ala).

Disease penetrance

The question of disease penetrance is closely linked to the question of the age of onset of disease. Onset of motor neuron disease in very old individuals has been reported previously (Friedman and Freedman, 1950). Williams *et al.* (1988) reported onset of familial motor neuron disease in two individuals in their ninth decade, while a 90-year-old obligate gene carrier was clinically unaffected. In our Family B, three of Patient B;IV-19's children had developed ALS and one had been dead for 9 years (B;V-27) by the time B; IV-19 himself first showed symptoms of ALS. We found seven individuals homozygous for Asp90Ala without a diagnosis of ALS, although one, and maybe two, of the seven are showing signs of incipient ALS. Five of these seven are older than the median age at onset of ALS for the 35 patients (Fig. 2), suggesting that homozygosity for Asp90Ala may be associated with decreased penetrance as has been found for the Ala4Thr (Nakano *et al.*, 1994) and Ile113Thr CuZn-SOD mutations (Suthers *et al.*, 1994). A bias in estimating the penetrance for ALS of individuals homozygous for Asp90Ala is introduced by our sampling procedure of selective sampling from Ala90Asp homozygous individuals with ALS. A large-scale epidemiological study is needed to estimate the disease penetrance for individuals homo- and heterozygous for the Asp90Ala mutation.

Genetic counselling

Caution is recommended when delivering genetic counselling to relatives of ALS patients homozygous for Asp90Ala. Since we have found a very large range of age of onset of ALS (74 years) and since the penetrance for ALS in Asp90Ala homozygous individuals is currently unknown, predictive testing must be considered premature.

CuZn-SOD mutations

At present some 40 different mutations in the CuZn-SOD gene have been reported in FALS families from different parts of the world. The Asp90Ala mutation is the only mutation which has been associated with autosomal recessive inheritance. Another distinguishing feature of Asp90Ala is the essentially normal CuZn-SOD activity in erythocytes (Andersen *et al.*, 1995). The mechanism by which the mutations in CuZn-SOD participate in the degeneration of neurons in ALS is not understood, but appears to be related to the gain of an adverse property rather than to the loss of enzymic activity (Gurney *et al.*, 1994; Rosen *et al.*, 1994; Andersen *et al.*, 1995). That homozygosity is needed suggests that the Asp90Ala has a less powerful adverse function than other CuZn-SOD mutations. The long survival time of our homozygous Asp90Ala patients also supports this notion.

Little clinical data has been published on most CuZn-SOD mutations and, for most mutations, the number of patients has been small. The most common point mutation reported previously is Ala4Val, which is associated with rapidly progressive ALS with a mean survival time of only 1.2± 0.8 years, but with a surprisingly high mean age of onset (51.5±11.2 years) for the 24 patients of Rosen *et al.* (1994). Patients with the autosomal dominantly inherited His46Arg mutation show long survival (n = 6; Aoki *et al.*, 1993) and a phenotype (Aoki *et al.*, 1994), which resembles that of the Asp90Ala patients. A very different course of disease has been found in individuals with Gly93Arg (Orrell *et al.*, 1995*b*) and Ile113Thr mutations (Suthers *et al.*, 1994; Orrell *et al.*, 1995*a*). Judging from the available reports on the different CuZn-SOD mutations, lower motor neuron symptoms appears to be a dominating feature of patients with a CuZn-SOD mutation. In this context the development of difficulty of micturition and the pathological MEPs in patients homozygous for Asp90Ala is noteworthy. Variability in the age of onset, disease progression rate and onset of disease in different parts of the body both within and between families, have been found for several mutations (reviewed by de Belleroche *et al.*, 1995). Patients homozygous for Asp90Ala seem to show less phenotypic variability than patients with other CuZn-SOD mutations, with the exception of the variability of the age of onset of first symptoms, where the Asp90Ala patients show the widest span (74 years). Thus, considerable phenotypic heterogeneity of ALS patients with mutations in the CuZn-SOD gene emphasizes the involvement of other factors in the pathogenesis of ALS.

Addendum

Since the submission of this paper, we have identfied a seventh SALS patient homozygous for the Asp90Ala CuZn-SOD mutation. The patient is a 57-year-old man from the Torne Valley in northern Sweden. He has, for the past 2 years developed a paraparesis with a phenotype similar to the patients described in this paper.

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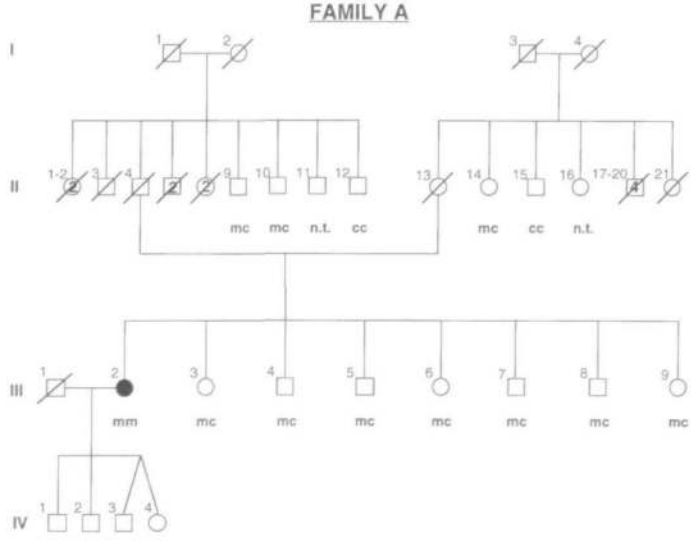
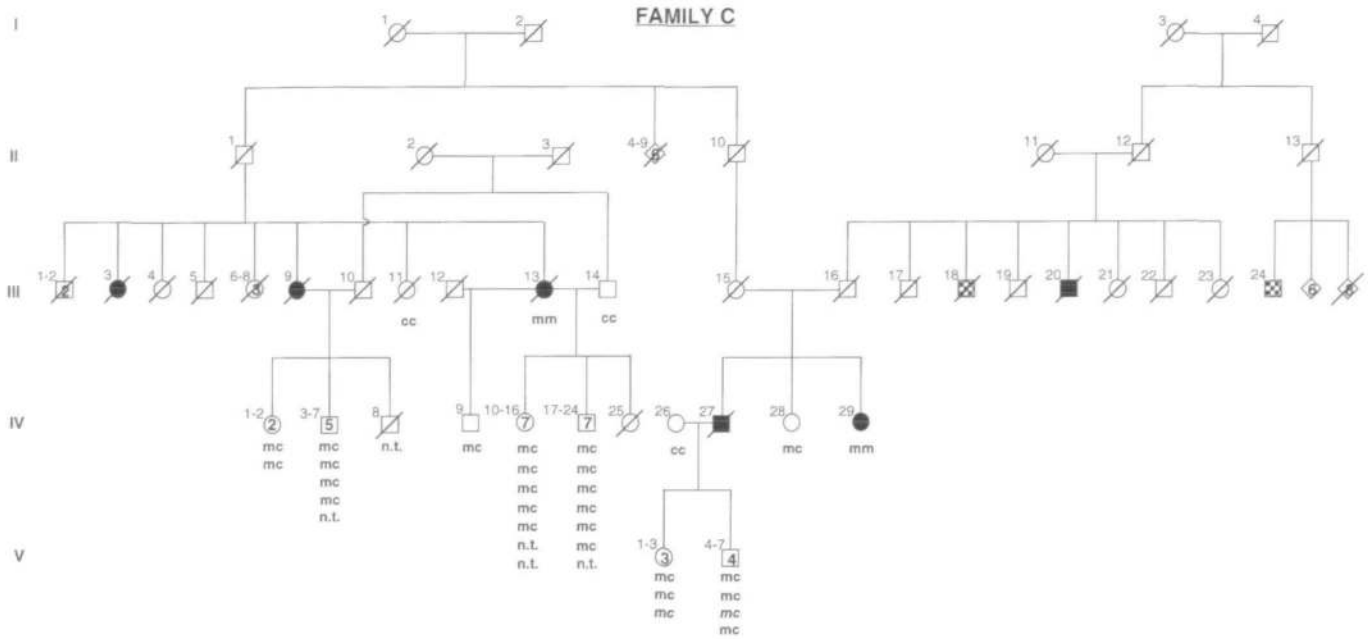
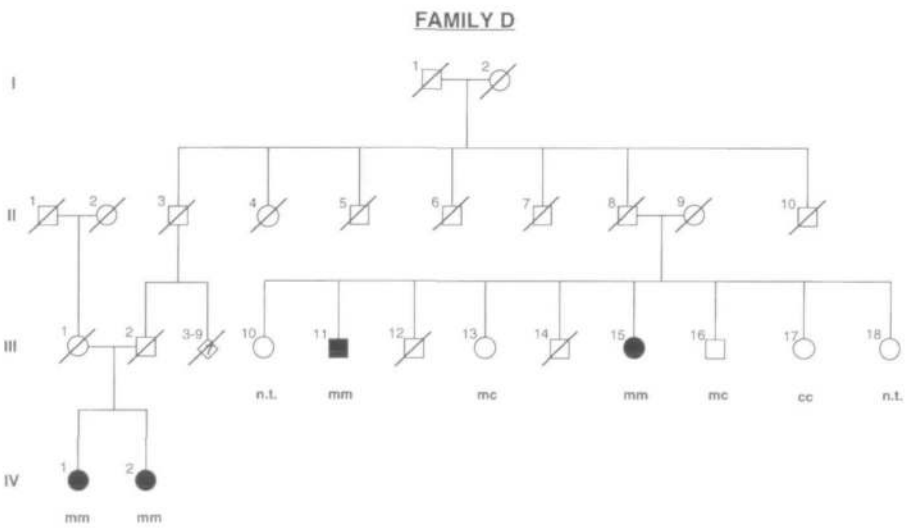
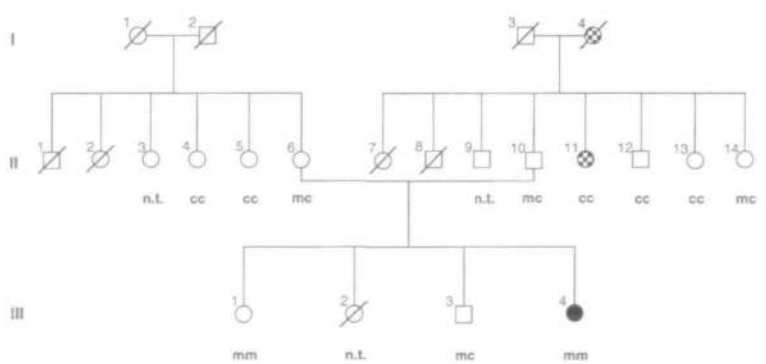
Appendix

Pedigrees of 10 of the families studied. The families of three of the SALS cases are included (A, F and V). The pedigrees of Family B and Family E have already been published (Andersen *et al.*, 1995). Individuals are indicated by generation and pedigree number. Circle = female; square = male; diamond = unspecified sex; filled symbols = affected with ALS; symbols with horizontal lines = Alzheimer's disease; symbols with check pattern = Parkinson's disease; oblique line = deceased individual. Numbers in circles or squares indicate numbers of individuals, the genotypes of some or all of them are listed below the symbol: mm = individual homozygous for Asp90Ala (the m indicates a CuZn-SOD allele with the Asp90Ala mutation); mc = heterozygous for Asp90Ala; cc = homozygous for the common allele (wild-type allele); n.t. = individual not tested.









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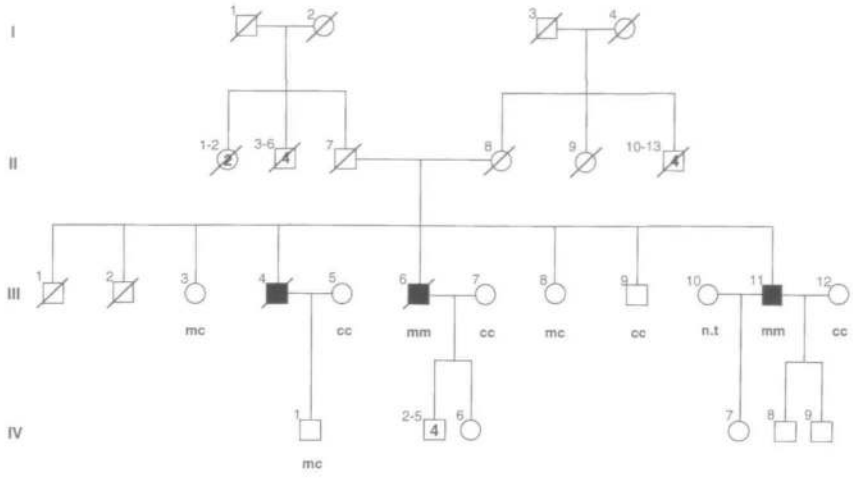

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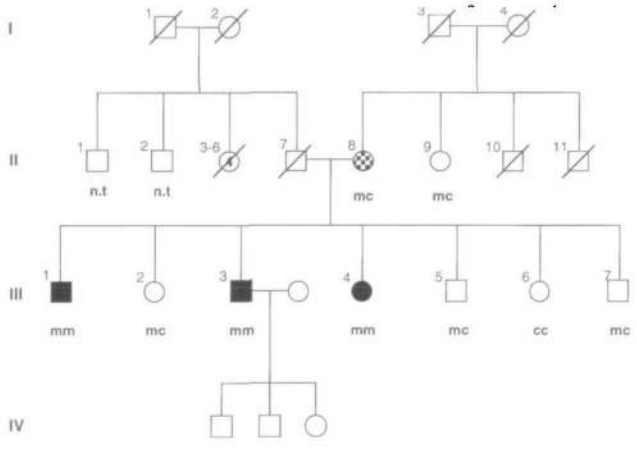

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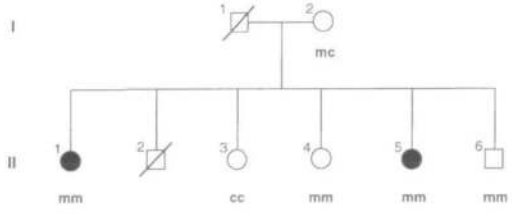

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