proposed that neuropathy can occur as a result of a direct toxic effect on peripheral nerves in patients with chronic alcoholism but no thiamine deficiency.[19]

In this study, we compared clinicopathological features of neuropathies associated with chronic alcoholism and thiamine deficiency in a series of consecutive patients who underwent careful determination of thiamine status and assessment of daily alcohol consumption to assess whether neuropathies caused by ethanol and thiamine deficiency were identical, and how thiamine deficiency was related to alcoholic neuropathy.

Patients and Methods

Patients

Consecutive patients with neuropathy who were referred to Nagoya University Hospital and its affiliated institutions between 1990 to 2002 and fulfilled the following criteria were included. Patients were assigned to one of three groups according to cause of neuropathy: pure alcoholic neuropathy without thiamine deficiency (ALN), alcoholic neuropathy with thiamine deficiency (ALN-TD), or pure, nonalcoholic thiamine-deficiency neuropathy (TDN). All patients with "alcoholic neuropathy" (ALN or ALN-TD) had chronic alcoholism, defined by regular intake of more than 100gm of ethanol daily for at least 10 years before onset of neuropathic symptoms. Individuals with lesser but daily consumption of alcohol were excluded from the TDN group. Four subjects not excluded had only occasional intake of alcohol, not exceeding 20gm per occasion. The remaining patients in the TDN group were total abstainers. The ALN group consisted of 36 men ranging from 31 to 70 years of age (mean ± standard deviation [SD], 50.7 ± 10.0); the ALN-TD group, 23 men and 5 women ranging from 27 to 68 years of age (mean ± SD, 51.1 ± 11.2); and the TDN group, 26 men and 6 women ranging from 18 to 81 years of age (mean ± SD, 54.5 ± 15.5; see Table 1). Causes of thiamine deficiency in patients with TDN were dietary imbalance in 12 patients and previous gastrointestinal surgery to treat ulcer or neoplasm in 20 patients.[20–22] Clinicopathological features of 17 patients in the TDN group who had undergone gastrectomy have been reported previously.[22] Of 28 patients in the ALN-TD group, 10 had a history of gastrectomy. Patients who had undergone operations to treat morbid obesity were excluded. A detailed history was obtained from each patient as well as their families concerning lifestyle, occupation, diet, and amount of daily consumption of alcohol. All patients underwent clinical and neurological assessment, routine blood and urine studies, blood thiamine determinations, cranial magnetic resonance imaging or computed tomography, and nerve conduction studies. Sural nerve biopsies were performed in 66 of 96 patients. A major manifestation of thiamine deficiency apart from peripheral neuropathy, Wernicke's encephalopathy, had occurred in 32 and 22% of patients in the ALN-TD and TDN groups, respectively. In the ALN group, no patients manifested this syndrome. Signs of heart failure possibly related to thiamine deficiency (cardiomegaly evident from chest radiographs, or pitting edema in the distal lower limbs) were observed in 50 and 69% of patients in the ALN-TD and TDN groups but no ALN patients. Patients with diabetic neuropathy, chronic inflammatory demyelinating polyneuropathy, Guillain–Barré syndrome, familial amyloid polyneuropathy, or other neuropathies unrelated to alcohol or thiamine deficiency were excluded. Patients' functional status was assessed at the peak phase according to modified Rankin score.[23]

Results of the various assessments in the three neuropathy groups defined above are described in the following order to facilitate comprehension: ALN, TDN, and ALN-TD.

Assessment of Thiamine Status

Thiamine status was determined at the time of the first referral to the hospital in all patients as previously described.[19,22] No patient had received thiamine at the time of

Table 1. Backgrounds of the Patients with Alcoholic Neuropathy and Nonalcoholic Thiamine-Deficiency Neuropathy

Characteristic	Alcoholic Neuropathy		Nonalcoholic Thiamine-Deficiency Neuropathy, n = 32 (%)
	Without Thiamine Deficiency, n = 36 (%)	With Thiamine Deficiency, n = 28 (%)	
Age (yr)	50.7 ± 10.0	51.1 ± 11.2	54.5 ± 15.5
Men/women	36/0	23/5	26/6
Duration of neuropathic symptoms (mo), mean ± SD	26.1 ± 39.3	16.5 ± 32.3	11.4 ± 23.5
Gastrointestinal tract operation			
Gastrectomy	0 (0)	10 (36)	18 (56)
Others	0 (0)	0 (0)	2 (6)
Associated symptoms			
Wernicke's encephalopathy	0 (0)	9 (32)	7 (22)
Heart failure	0 (0)	14 (50)	22 (69)
Total thiamine concentration (ng/ml), mean ± SD	38.5 ± 12.0	12.6 ± 4.0	11.0 ± 4.3

Normal values for whole-blood concentration of total thiamine were established in 100 normal volunteers (mean age ± SD, 29.7 ± 5.0 yr; male/female ratio, 50:50).
Normal thiamine concentration (mean ± 2 SD for normal control) is 20–50ng/ml.

SD = standard deviation.

this determination. Thiamine concentrations were measured by high-performance liquid chromatography as described previously.[13–15] Normal thiamine status was defined by a whole-blood concentration of total thiamine between 20 and 50ng/ml (the mean ± 2 SD for normal control subjects) and normal erythrocyte transketolase activity (between 123.8 and 206.2U/L, representing the mean ± 2 SD for normal controls). Thiamine deficiency was defined as total thiamine concentrations in whole blood below 20ng/ml and decreased erythrocyte transketolase activity less than 123.8U/L. Normal values for total thiamine concentration were established in 100 normal volunteers.[19,22] Normal values for erythrocyte transketolase activity were adopted from previous report.[24]

Electrophysiological Assessment

Motor and sensory conduction was measured in the median, ulnar, tibial, and sural nerves in all patients during their initial clinical assessment at the hospital, using a standard method with surface electrodes for stimulation and recording.[25,26]

Pathological Assessment of Sural Nerve Specimens

Sural nerve biopsy was performed in 29 patients with ALN, 18 patients with ALN-TD, and 19 patients with nonalcoholic thiamine-deficiency neuropathy, as described previously,[27–31] in most cases biopsy was performed before administration of thiamine. Informed consent was obtained beforehand. Specimens were divided into two portions. The first of these was fixed in 2.5% glutaraldehyde in 0.125M cacodylate buffer (pH 7.4) and embedded in epoxy resin for morphometric and ultrastructural study. Density of myelinated fibers was assessed in toluidine blue–stained semithin sections using a computer-assisted image analyzer (Luzex FS; Nikon, Tokyo, Japan), and densities of small and large myelinated fibers were calculated as described previously.[28–31] The extent of subperineurial edema was assessed by measuring total endoneurial area surrounded by perineurial cells and also determining endoneurial area after subtracting the increased subperineurial space representing edema. The latter then was subtracted from the former by the image analysis system.[22,32] Clusters of two or more small myelinated fibers enclosed by one basement membrane were designated as an instance of axonal sprouting.[19,33] Numbers of axonal sprouting were estimated as those of myelinated fibers. Cases with more than 5% of regenerating myelinated fibers (fibers which constitute axonal sprouting) in the total myelinated fibers were designated to be abundant with regenerating fibers.

For electron microscopic study, epoxy resin–embedded specimens were cut into ultrathin transverse sections and stained with uranyl acetate and lead citrate. To assess the density of unmyelinated fibers, we took electron microscopic photographs at a magnification of ×4,000 in a random fashion to cover the area of ultrathin sections as described previously.[28,30,31] Density of unmyelinated fibers was estimated from these electron micrographs. For determination of G-ratios (axon diameter/fiber diameter),[34] electron micrographs of up to 200 randomly selected fibers photographed at a magnification of ×8,000 were used. Numbers of neurofilaments were counted in systematically sampled squares of an overlying transparency placed upon the electron microscopic photographs at a final magnification of ×50,000. The first square was selected using a random table, and subsequent squares were sampled systematically. At least 30% of the axon area was counted, including subaxolemmal and central regions. At least 30 fibers were examined to calculate the mean density of neurofilaments in each case. Cases with abundant regenerating fibers were excluded for determination of the mean G-ratio and neurofilament density. Control values for G-ratio and neurofilament density were obtained from nine normal controls

A fraction of the glutaraldehyde-fixed sample was processed for teased-fiber study, in which at least 100 single fibers were isolated; their pathological condition was assessed microscopically according to criteria described previously.[28,35,36]

The second portion of the specimen was fixed in 10% formalin solution and embedded in paraffin. Sections were cut by routine methods and stained with hematoxylin and eosin as well as by the Klüver–Barrera and Masson trichrome methods.

Statistical Analyses

Quantitative data were presented as the mean ± SD and compared with previously described control values.[19,22,28,32] Statistical analyses were performed using the χ^2 test or the Mann–Whitney U test as appropriate. p values less than 0.05 were considered to indicate significance.

Results

Alcoholic Neuropathy without Thiamine Deficiency

All patients in this group, as well as in the other two groups, showed symmetric polyneuropathy with greater involvement of the lower than upper limbs. The initial symptom of neuropathy was pain or a painful burning sensation in the toes and/or ankle in all patients (see Table 2). This symptom gradually ascended to include the proximal part of the lower extremities, and occasionally to the lower trunk. In severely affected patients, the distal part of the upper limbs also were involved. Progression was mostly slow, occurring over months to years. Nineteen patients (53%) showed weakness in the lower extremities but with a sensory-dominant pattern; the remaining 17 patients (47%) showed a pure sensory pattern without any weakness in the limbs. Weakness in the upper extremities was seen in six patients (17%). Sensory disturbance was present in the lower limbs in all patients and also present in the upper limbs and trunk in 17 (47%) and 7 (19%) of patients, respectively. Almost all patients (97%) reported a painful sensation in the affected limbs and/or trunk. As for the modalities of sensation affected, loss of superficial sensation, particularly nociception, was predominant. Yet, involvement of all sensory modalities was seen in severely affected patients. Biceps, patellar, and Achilles tendon reflexes were reduced or absent in 10 patients (28%), 17 (47%), and 31 (86%),

Table 2. Neuropathic Symptoms of the Alcoholic Neuropathy and Nonalcoholic Thiamine-Deficiency Neuropathy

	Alcoholic Neuropathy			*p*		
	1. Without Thiamine Deficiency, n = 36 (%)	2. With Thiamine Deficiency, n = 28 (%)	3. Nonalcoholic Thiamine-Deficiency Neuropathy, n = 32 (%)	1 vs 2	2 vs 3	1 vs 3
Initial symptom						
Sensory disturbance	36 (100)	16 (57)	16 (50)	<0.0001	NS	<0.0001
Muscle weakness	0 (0)	12 (43)	16 (50)			
Progression						
<1 mo	2 (6)	11 (39)	18 (56)	0.0006	NS	0.001
1 mo to 1 yr	18 (50)	4 (14)	8 (25)			
>1 yr	16 (44)	13 (46)	6 (19)			
Type						
Motor-dominant	0 (0)	15 (54)	27 (84)	<0.0001	0.02	<0.0001
Sensory-dominant	19 (53)	11 (39)	3 (9)			
Pure sensory	17 (47)	2 (7)	2 (6)			
Presence of weakness						
Upper limbs	6 (17)	14 (50)	26 (81)	0.004	0.01	<0.0001
Lower limbs	19 (53)	26 (93)	30 (94)	0.0002	NS	<0.0001
Presence of sensory disturbance						
Upper limbs	17 (47)	19 (68)	25 (78)	NS	NS	0.03
Trunk	7 (19)	7 (25)	9 (28)	NS	NS	NS
Lower limbs	36 (100)	28 (100)	32 (100)	NS	NS	NS
Painful sensation	35 (97)	16 (57)	7 (22)	0.0001	0.005	<0.0001
Modality of sensory deficit						
Superficial sensation-dominant	22 (61)	7 (25)	3 (9)	0.005	NS	<0.0001
All modalities	13 (36)	15 (54)	20 (63)			
Deep sensation-dominant	1 (3)	6 (21)	9 (28)			
Deep tendon reflexes						
Biceps; reduced or absent	10 (28)	13 (46)	26 (81)	NS	0.005	<0.0001
Patellar; reduced or absent	17 (47)	23 (82)	29 (91)	0.004	NS	0.0001
Achilles; reduced or absent	31 (86)	28 (100)	32 (100)	0.04	NS	0.03
Functional status						
Unable to walk	9 (25)	15 (54)	27 (84)	0.02	0.009	<0.0001
Able to walk	27 (75)	13 (46)	5 (16)			
Modified Rankin score	2.1 ± 0.6	2.8 ± 0.9	3.6 ± 1.0	0.04	0.003	<0.0001

Patients' functional status was assessed at the peak phase according to modified Rankin score.[23] 0, asymptomatic; 1, nondisabling symptoms not interfering with lifestyle; 2, minor disability from symptoms leading to some restriction of lifestyle but not interfering with patients' capacity to look after themselves; 3, moderate disability from symptoms significantly interfering with lifestyle or preventing totally independent existence; 4, moderately severe disability from symptoms clearly precluding independent existence, though not requiring 24-hour attention from a caregiver; and 5, severe disability and total dependence, requiring constant attention day and night.

NS = not significant.

respectively. Plantar responses were flexor in all patients. Autonomic symptoms including urinary retention, constipation, impaired sweating, and orthostatic hypotension were not prominent in patients of this group. In all patients, sensory disturbance compromised activities of daily living significantly, but 27 patients (75%) could walk unaided at the time of first referral to the hospital. Functional status determined by the modified Rankin score was 2.1 ± 0.6.

Nerve conduction studies showed more profound abnormalities in the lower than upper limbs (see Table 3). Moderate reduction of compound muscle action potentials (CMAPs) in the tibial nerves and severe reduction of sensory nerve action potentials in the sural nerves were seen. In contrast, CMAPs in the median nerves were relatively preserved, and sensory nerve action potentials in the median nerves were only moderately decreased. Mild to moderate slowing of motor nerve conduction velocity in the median and tibial nerves and of sensory nerve conduction velocities in the median and sural nerves also was observed. Distal latencies in the median and tibial nerves also were mildly prolonged.

Myelinated fiber density in the sural nerve was significantly reduced (see Table 4). Densities of large myelinated fibers were 1307 ± 864 fibers/mm^2 (43% of normal control); those of small myelinated fibers were 1381 ± 1278 fibers/mm^2 (27% of normal control). In

Table 3. Nerve Conduction Studies, Mean ± SD

	Alcoholic Neuropathy			*p*			Controls
	1. Without Thiamine Deficiency, n = 36	2. With Thiamine Deficiency, n = 28	3. Nonalcoholic Thiamine-Deficiency Neuropathy, n = 32	1 vs 2	2 vs 3	1 vs 3	(n = 121 ~ 191)
Median nerve							
MCV (m/sec)	51.2 ± 4.7[a]	50.4 ± 5.2[a]	52.3 ± 5.9[a]	NS	NS	NS	57.8 ± 3.7
DL (msec)	4.2 ± 0.7[a]	4.0 ± 0.6[a]	3.6 ± 0.6	NS	0.02	0.005	3.4 ± 0.4
CMAP (mV)	8.4 ± 4.2[b]	8.7 ± 5.0[a]	6.7 ± 4.5[a]	NS	NS	NS	10.7 ± 3.5
Not elicited	None	None	None				
SCV (m/sec)	48.0 ± 7.2[a]	46.9 ± 8.4[a]	47.9 ± 9.3[a]	NS	NS	NS	57.8 ± 4.7
SNAP (μV)	8.7 ± 6.4[a]	6.8 ± 4.9[a]	7.8 ± 8.4[a]	NS	NS	NS	23.5 ± 8.4
Not elicited	2 cases (6%)	1 case (4%)	8 cases (25%)				
Tibial nerve							
MCV (m/sec)	40.6 ± 4.2[a]	40.8 ± 5.6[a]	42.8 ± 4.5[a]	NS	NS	NS	46.9 ± 3.5
DL (msec)	5.3 ± 0.8[a]	5.3 ± 1.8[c]	4.9 ± 1.0[c]	NS	NS	NS	4.5 ± 0.8
CMAPs (mV)	4.6 ± 3.5[a]	5.4 ± 5.1[a]	2.6 ± 2.4[a]	NS	0.045	0.002	10.9 ± 3.8
Not elicited	None	3 cases (11%)	3 cases (10%)				
Sural nerve							
SCV (m/sec)	38.8 ± 6.9[a]	40.5 ± 6.3[a]	41.0 ± 11.8[c]	NS	NS	NS	51.0 ± 5.1
SNAP (μV)	2.4 ± 3.3[a]	1.5 ± 2.3[a]	2.0 ± 3.5[a]	NS	NS	NS	11.5 ± 4.7
Not elicited	14 cases (39%)	17 cases (61%)	21 cases (66%)				

[a] $p < 0.001$, [b] $p < 0.005$, and [c] $p < 0.05$ (Mann–Whitney *U* test) for the control values.

SD = standard deviation; MCV = motor nerve conduction velocity; DL = distal latency; CMAP = compound muscle action potential; SCV = sensory nerve conduction velocity; SNAP = sensory nerve action potential; NS = not significant.
Control values are based on previously published reports.[19,22]

Table 4. Pathology of the Sural Nerves, Mean ± SD

	Alcoholic Neuropathy			*p*			Controls
	1. Without Thiamine Deficiency, n = 29	2. With Thiamine Deficiency, n = 18	3. Nonalcoholic Thiamine-Deficiency Neuropathy, n = 19	1 vs 2	2 vs 3	1 vs 3	(n = 9)
Total MFD (no./mm^2)	2,687 ± 1,875[a]	2,727 ± 1,649[a]	2,367 ± 1,868[a]	NS	NS	NS	8,190 ± 511
Large MFD (no./mm^2)	1,307 ± 864[a]	928 ± 764[a]	663 ± 693[a]	NS	NS	0.005	3,068 ± 294
Small MFD (no./mm^2)	1,381 ± 1,278[a]	1,800 ± 1,245[a]	1,704 ± 1,310[a]	NS	NS	NS	5,122 ± 438
Axonal sprouting (no./mm^2)	137.6 ± 264.8	157.3 ± 271.4	2.8 ± 9.5	NS	0.01	0.046	
Small/large							
All cases	1.8 ± 2.9 (n = 29)	4.4 ± 8.0 (n = 18)	13.6 ± 27.0 (n = 19)	NS	0.02	<0.0001	1.7 ± 0.2
Cases with abundant RMFs are excluded	0.7 ± 0.3[a] (n = 20)	4.7 ± 9.6 (n = 12)	13.6 ± 27.0 (n = 19)	0.03	0.02	<0.0001	1.7 ± 0.2
G-ratio	0.58 ± 0.07[a]	0.54 ± 0.06[a]	0.56 ± 0.06[a]	NS	NS	NS	0.73 ± 0.03
Neurofilamant density (no./μm^2)	187.0 ± 44.1[a]	188.2 ± 41.2[a]	193.5 ± 43.8[a]	NS	NS	NS	108.3 ± 25.9
UMFD (no./mm^2)	7,029 ± 4,153[a]	11,194 ± 4,386[a]	10,585 ± 4,867[a]	0.004	NS	0.006	29,913 ± 3,457
Subperineurial edema (%)	6.6 ± 2.9[b]	7.3 ± 4.2[c]	10.3 ± 4.5[a]	NS	0.02	0.002	4.6 ± 1.0
Teased fiber study							
Myelin irregularity (%)	17.3 ± 11.3	9.2 ± 7.7	5.7 ± 5.2	0.01	NS	0.0003	
De/remyelination (%)	9.0 ± 5.2	10.3 ± 8.2	3.4 ± 4.8	NS	0.003	0.001	9.5 ± 8.8
Axonal degeneration (%)	30.9 ± 19.7[a]	45.3 ± 30.7[a]	57.8 ± 25.3[a]	NS	NS	0.001	1.7 ± 1.4

[a] $p < 0.001$, [b] $p < 0.005$, and [c] $p < 0.05$ (Mann–Whitney *U* test) for the control values.

SD = standard deviation; MFD = myelinated fiber density; small/large = ratio of small myelinated fibers to large myelinated fibers; UMFD = unmyelinated fiber density; RMFs = regenerating myelinated fibers, NS = not significant.
Cases with abundant regenerating fibers were excluded for determination of the mean *G*-ratio and neurofilament density.
Control values are based on previously published reports,[28,32] and control values for *G*-ratio and neurofilament density are obtained from nine normal volunteers.

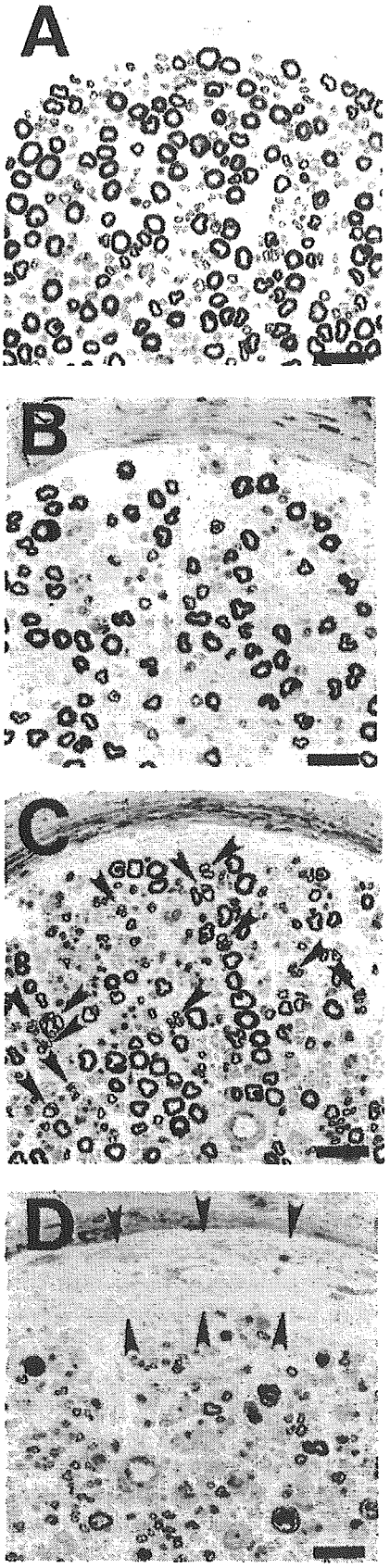
nine cases (31%) axonal sprouting was abundant (more than 5% of regenerating fibers in the total myelinated fibers; see Fig 1C). The duration of neuropathic symptoms of these nine cases was extremely long (70.5 ± 49.5 months), and the regenerating myelinated fibers increased the proportion of small myelinated fibers (small/large, 4.3 ± 4.4; control, 1.7 ± 0.2). In the remaining 20 cases (69%), duration of neuropathic symptoms was much shorter (9.7 ± 11.6 months). Reduction of small myelinated fibers in these 20 cases was more profound than reduction of large myelinated fibers (small/large, 0.7 ± 0.3), hence small-fiber-predominant loss was clearly evident (see Figs 1B and 2). Axonal shrinkage with increased neurofilament density accompanied by a redundant loop of myelin was observed in some myelinated fibers. Decreased G-ratio (0.58 ± 0.07; control, 0.73 ± 0.03) and increased neurofilament density (187.0 ± 44.1 filaments/μm^2; control, 108.3 ± 25.9) indicated axonal atrophy. Reduction of unmyelinated fiber density also was profound (7029 ± 4153 fibers/mm^2). Clusters of small unmyelinated fibers, suggestive of regenerating fibers, were seen in cases with abundant axonal sprouting of myelinated fibers. Varying degrees of subperineurial edema was seen. In teased-fiber preparations, the frequency of axonal degeneration was prominent (30.9 ± 19.7%), and myelin irregularity was conspicuous in the remaining fibers (17.3 ± 11.3%). The proportion of segmental de/remyelination was 9.0 ± 5.2%. This demyelination consisted of widening of consecutive nodes of Ranvier resulting from attenuation of the internodes of the myelin sheath (see Fig 3).

Nonalcoholic Thiamine Deficiency Neuropathy

All patients manifested symmetric polyneuropathy with more involvement in the lower than upper limbs, showing a centripetal pattern of progression. The initial symptom of neuropathy was variable, in contrast with alcoholic neuropathy without thiamine deficiency (ALN); this was weakness in the lower extremities in 16 patients (50%) and numbness in the distal lower limbs in 16 patients (50%; see Table 2). Progression rate also varied; acute progression within 1 month was seen in 18 patients (56%), whereas slow progression over more than 1 year was seen in 6 patients (19%). On average, progression was more rapid than in ALN ($p = 0.001$). Impairment usually was motor dominant, as in 27 patients (84%), which contrasted to the sensory-dominant pattern characteristic of ALN. Some patients whose motor weakness progressed over days initially were thought to have Guillain–Barré syndrome. Motor symptoms were more predominant in the lower than upper extremities; even so, 26 patients (81%) showed weakness in the upper limbs. Sensory disturbance was present in the lower limbs in all patients and also was present in the upper limbs and trunk in 25 patients (78%) and 9 patients (28%), respectively. Varying degrees of numbness with or without painful sensations were noted in all patients, but painful sensations were reported by only 7 patients (22%). Involvement of all sensory modalities was a common feature in TDN, in contrast with predominant affection of nociception in ALN; superficial sensation was most affected in only 3 patients (9%), deep sensation was most involved in 9 patients (28%), and both modalities were equally affected in 20 patients (63%). Biceps, patellar, and Achilles tendon reflexes were reduced or absent in most patients. Plantar responses were flexor in all patients. Autonomic symptoms were absent or only mildly present in most patients, but six patients who had severe thiamine deficiency manifested flaccid bladder requiring urethral catheterization or severe intestinal gas retention that mimicked ileus. Activities of daily living were significantly more impaired than in ALN ($p < 0.0001$) mainly because of rapid progression of muscle weakness. Only five patients (16%) could walk unaided at the time of initial examination. Functional status determined by modified Rankin score was 3.6 ± 1.0.

Findings of nerve conduction studies were similar to those in ALN (see Table 3). Only distal latency of the median nerve was significantly prolonged ($p = 0.005$), whereas CMAPs in the tibial nerves were significantly smaller ($p = 0.002$) in ALN than in TDN.

Myelinated fiber density was significantly reduced (see Table 4). Reduction of large meylinated fibers was greater than in ALN ($p = 0.005$). Densities of large myelinated fibers were 663 ± 693 fibers/mm^2 (22% of normal control), whereas those of small myelinated fibers were 1704 ± 1310 fibers/mm^2 (33% of normal control). The mean ratio of small to large myelinated fibers was 13.6 ± 27.0 (control, 1.7 ± 0.2), significantly higher than in ALN ($p < 0.0001$). Axonal sprouting was scarce in all cases. In contrast with ALN, all cases showed more loss of large myelinated fibers than loss of small mylinated fibers except one (see Figs 1D and 2). The mean G-ratio was 0.56 ± 0.06 (control, 0.73 ± 0.03) and the density of neurofilaments was 193.5 ± 43.8 filaments/μm^2 (control, 108.3 ± 25.9), not differing significantly from those in ALN. Reduction of unmyelinated fibers also was seen but was less profound than in ALN ($p = 0.006$). Regeneration of unmyelinated fibers was scarce. Subperineurial edema was more severe than in ALN ($p = 0.002$). In teased-fiber preparations, significantly more axonal degeneration was seen than in ALN ($p = 0.001$). The proportion of fibers showing segmental de/remyelination was small compared to ALN ($p = 0.001$). Myelin irregularity was observed in 5.7 ± 5.2% of fibers, significantly less often than in ALN ($p = 0.0003$).



Alcoholic Neuropathy with Thiamine Deficiency

Neuropathic symptoms in alcoholic neuropathy with thiamine deficiency (ALN-TD) were variable, showing characteristics of both ALN and TDN (see Table 2). The initial symptom was numbness or painful paresthesias in the lower limbs in 16 patients (57%) but was weakness in 12 others (43%). Progression varied from acute (within 1 month) in 11 patients (39%) to chronic (occurring over 1 year) in 13 (46%). Relative degrees of motor and sensory deficits also were variable; 15 patients (54%) showed a motor-dominant pattern, whereas the remaining 13 (46%) showed a sensory-dominant or purely sensory pattern. Muscle weakness was present in the lower limbs in 26 patients (93%) and in the upper limbs in 14 (50%). Sensory disturbance was present in the lower extremities in all patients extending to the trunk and distal portion of the upper extremities in 7 patients (25%) and 19 patients (68%), respectively. Painful paresthesias were reported by 16 patients (57%), significantly less often than in ALN ($p = 0.005$). Modaliy of sensory deficit also was variable; superficial and deep sensations were affected equally in 15 patients (54%), deep sensation predominated slightly in 6 (21%), and predominant involvement of nociception associated with painful paresthesias as in ALN was seen in 7 (25%). Deep tendon reflexes were reduced in the biceps, patellar, and Achilles tendons in 13 patients (46%), 23 (82%), and 28 (100%), respectively. Plantar responses were flexor in all patients. The modified Rankin score was 2.8 ± 0.9, intermediate between ALN and TDN scores.

Findings of nerve conduction studies were similar to those in ALN and TDN (see Table 3).

Sural nerve biopsy specimen findings also were variable occupying a range between ALN and TDN (see Table 4). Densities of large myelinated fibers were 928 ± 764 fibers/mm^2 (30% of normal control) and those of small myelinated fibers were 1,800 ± 1,245 fibers/mm^2 (35% of normal control). The mean ratio of small to large myelinated fibers was 4.4 ± 8.0,

Fig 1. Light microscopic observations of the sural nerve. (A) Transverse section of a sural nerve specimen from a control case. (B) A specimen from a patient with a 4-month history of alcoholic neuropathy without thiamine deficiency. Small myelinated fibers show more loss than large myelinated fibers. Subperineurial edema is slight. (C) A specimen from a patient with a 5-year history of alcoholic neuropathy without thiamine deficiency does not show small-fiber-predominant axon loss as in B, because of the presence of abundant axonal sprouting (arrowheads), *which indicates regeneration of axons. (D) A specimen from a patient with nonalcoholic thiamine-deficiency neuropathy. In contrast with B, large myelinated fibers show more loss than small myelinated fibers, although both types of myelinated fibers are significantly reduced. Subperineurial edema is marked (between* arrowheads). *Bar = 30μm.*

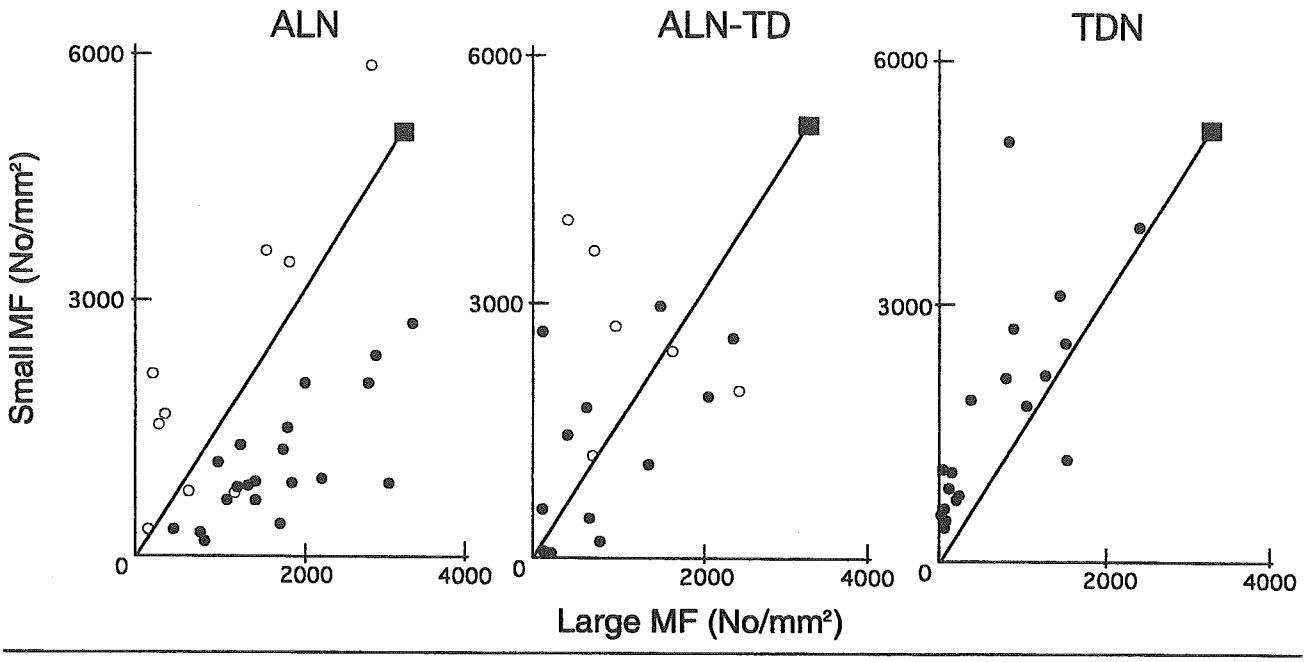


Fig 2. Relationships between large and small myelinated fibers. Boldface lines represent the normal ratio of small to large myelinated fibers (small/large = 1.7). Black boxes indicate the mean value in control cases. Circles located below the boldface lines indicate predominance of large myelinated fibers, whereas those above the boldface lines indicate predominance of small myelinated fibers. Black circles represent cases with few regenerating myelinated fibers (<5% of total myelinated fibers), whereas white circles represent cases with abundant regenerating myelinated fibers (> 5% of total myelinated fibers). Abundant regeneration of myelinated fibers increase the number of small myelinated fibers. In the pure alcoholic neuropathy (ALN) group, all cases with few regenerating fibers showed predominance of large myelinated fibers, reflecting predominantly small-fiber loss; all except one nonalcoholic thiamine-deficiency neuropathy (TDN) cases showed predominance of small myelinated fibers, reflecting predominantly large-fiber loss.

which was intermediate between ratios in ALN and TDN. Axonal sprouting was abundant in six cases (33%). The proportions of large and small myelinated fibers were highly variable between cases (see Fig 2). The mean G-ratio was 0.54 ± 0.06 and the density of neurofilaments was 188.2 ± 41.2 filaments/μm^2, not differing significantly from those in ALN or TDN. In teased-fiber preparations, the frequency of axonal degeneration was 45.3 ± 30.7%. Myelin irregularity was conspicuous in the remaining fibers (9.2 ± 7.7%). The proportion of segmental de/re-myelination was 10.3 ± 8.2%. Values for the teased-fiber preparations were intermediate between the range of ALN and TDN.

Discussion

The pathogenesis of alcoholic neuropathy, especially its relationship to thiamine deficiency, has remained unclear. Recent studies indicated a direct neurotoxic effect of ethanol or its metabolites, involving ethanol-induced glutamate neruotoxicity,[18,37] decreased production of neurofilament protein or its phosphorylated form,[38,39] or impairment of fast axonal transport.[40] Axonal degeneration has been documented in animals receiving ethanol while maintaining normal thiamine status.[41] Human studies also have suggested a direct toxic effect, because a dose-dependent relationship has been observed between severity of neuropathy and amount of ethanol consumed.[17] In addition to this direct toxic effect, thiamine deficiency is closely related to chronic alcoholism[4] and also can induce neuropathy in alcoholic patients. Ethanol diminishes thiamine absorption in the intestine and reduces hepatic stores of thiamine.[42,43] Ethanol also decreases phosphorylation of thiamine, reducing availability of the active form of the vitamin.[44–46] In addition, patients with chronic alcoholism tend to have dietary imbalance. These relationships make chronic alcoholism a risk factor for thiamine-deficiency neuropathy.

Clinicopathological features of alcoholic neuropathy have remained obscure despite its wide prevalence, in large part because of incomplete differentiation from beriberi neuropathy. Although sometimes attributed to inadequate nutritional assessment in reported cases, technical limitations of thiamine status assessment contributed greatly to the problem. Only in the 1960s were assays of erythrocyte transketolase activity intro-

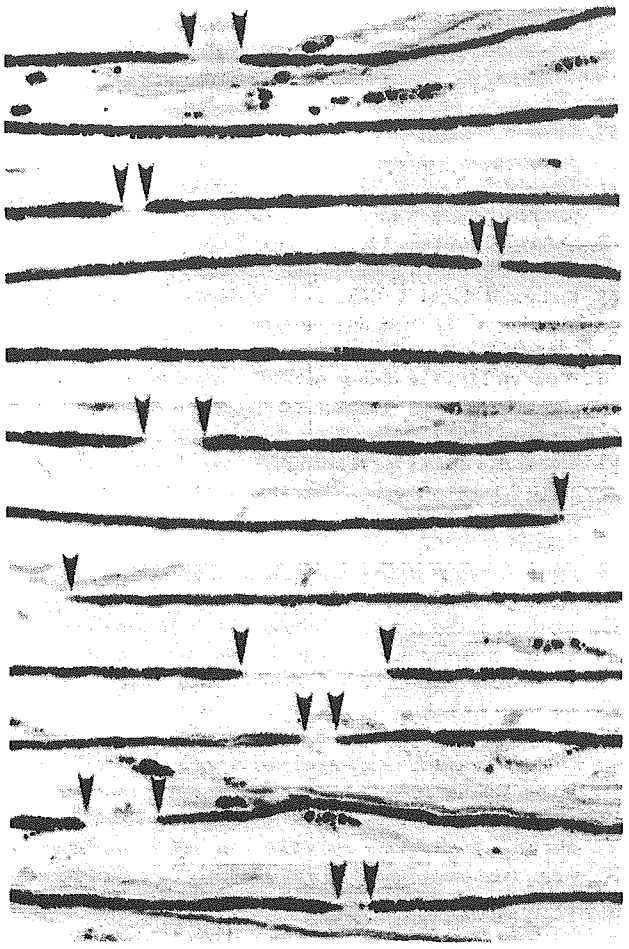


Fig 3. Consecutive portions along the length of a single teased fiber from a patient with alcoholic neuropathy. Note marked irregularity of myelin. Segmental demyelination has resulted from widening of consecutive nodes of Ranvier (arrowheads).

duced,[47] and this method assesses thiamine only indirectly. In the 1980s, a direct, highly sensitive, and convenient high-performance liquid chromatographic assay for thiamine became widely available.[13–15] When we assess clinicopathological features of "alcoholic neuropathy," both direct toxicity of ethanol or its metabolites and concomitant effects of thiamine deficiency need to be considered. In addition, clinicopathological features of pure thiamine-deficiency neuropathy need to be studied in nondrinkers.

In this study, we divided consecutively recruited patients with "alcoholic neuropathy" into two groups based on thiamine status. Alcoholic neuropathy without thiamine deficiency (ALN) was considered to be attributable solely to direct toxicity of ethanol or ethanol metabolites. Characteristics of ALN-TD were considered to reflect combined cause of ethanol toxicity and thiamine deficiency. On the other hand, patients with nonalcoholic (ie, "pure") thiamine-deficiency neuropathy (TDN), identical to previously reported "beriberi neuropathy," were assessed for comparison. We thus were able to differentiate the distinct clinicopathological features of the individual neuropathies and confirm that effects of thiamine deficiency can modify the features of ALN.

As for clinical features, ALN in our series uniformly showed slowly progressive, sensory-dominant symptoms. Painful paresthesias were major complaints and that often limited daily activities in these patients. In pure TDN, in contrast, many patients manifested an acutely progressive, motor-dominant pattern leading to loss of ambulation, although variation including slow progression or sensory-dominant pattern was apparent in some patients. Clinical features of ALN-TD were particularly variable, constituting a spectrum raging from a picture of ALN to that of TDN among individual patients.

Major electrophysiological and histopathological findings in our three groups of patients indicated axonal neuropathy, in agreement with previous descriptions of both alcoholic neuropathy and beriberi neuropathy.[6–11] The electrophysiological features that we observed were similar in ALN and TDN. Like the clinical findings, these studies showed predominant lower limb involvement in both neuropathies. Lower amplitude of CMAPs in TDN than in ALN were reflected by more severe muscle weakness in TDN. Electrophysiological findings commonly associated with myelin damage (slowing of MCV and SCV as well as prolongation of DL) also were observed in patients with ALN and those with TDN, even though axonal damage was dominant histopathologically.

Sural nerve specimens showed more clear-cut differences between these neuropathies than did electrophysiological studies. ALN showed loss of mainly small fibers, less subperineurial edema, and more frequent myelin irregularity and segmental de/remyelination. In contrast, TDN showed predominantly large-fiber loss, more subperineurial edema, and less myelin irregularity and segmental de/remyelination. In ALN, small-fiber-predominant axonal loss (small-fiber axonopathy) was most evident in cases with recent onset. In long-standing cases, abundant regenerating fibers obscured some small-fiber loss. The finding of small-fiber-predominant loss was in accord with previous descriptions of painful alcoholic neuropathy.[19] Relative preservation of deep tendon reflexes in ALN reflected relative sparing of the large fibers that mediate them. ALN-TD showed an extensive range of pathology from small-fiber-predominant loss to large-fiber-predominant loss with features of both ALN and TDN. Axonal sprouting in long-standing cases and large-fiber-predominant loss in some ALN-TD cases may have

obscured the characteristic feature of small-fiber-predominant loss in ALN in some previous reports describing loss of nerve fibers throughout the entire range of fiber diameter.[7,8,10] Axonal atrophy as determined by the G-ratio and the density of neurofilaments did not differ between the three groups, but irregularity of myelin, which also indicates axonal atrophy,[48] was significantly more frequent in ALN than in TDN. Segmental de/remyelination in teased-fiber preparations also was more frequent in ALN than in TDN. This change is consisted of the widening of consecutive nodes of Ranvier. Irregularity of the myelin sheath also was seen in these fibers; so these findings are supposed to reflect axonal atrophy.[48,49] Differences in relative frequency of these changes are supposedly caused by different mechanism of axonal atrophy in ALN from TDN.

Another important characteristic of "alcoholic neuropathy" is a presence of postgastrectomy patients; 36% of ALN-TD patients but no ALN patients. This finding suggests that gastrectomy is a risk factor for thiamine deficiency in patients with chronic alcoholism and establishes thiamine deficiency as a cause of postgastrectomy polyneuropathy.[19]

In conclusion, the nature of "alcoholic neuropathy" has been unclear because of an often undetected or overestimated influence of thiamine deficiency, whereas the clinical picture of thiamine-deficiency neuropathy (ie, beriberi neuropathy) can be distorted by concomitant effects of ethanol. We compared these two neuropathies with careful consideration of interactions, confirming that the two neuropathies are clinically and pathologically distinct. Not only thiamine deficiency but also direct toxic effects of ethanol or its metabolites can cause alcoholic neuropathy. Although clinicopathological features of pure alcoholic neuropathy are remarkably uniform, extensive variation results when thiamine deficiency is present.

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References

1. Shattuck GC. Relation of beriberi to polyneuritis from other causes. Am J Trop Med 1928;8:539–543.
2. Victor M, Adams RD. On the etiology of the alcoholic neurologic diseases. Am J Clin Nutr 1961;9:379–397.
3. Novak DJ, Victor M. The vagus and sympathetic nerves in alcoholic polyneuropathy. Arch Neurol 1974;30:273–284.
4. Darnton-Hill I, Truswell AS. Thiamine status of a sample of homeless clinic attenders in Sydney. Med J Aust 1990;152:5–9.
5. Pekelharing CA, Wrinkler C. Mittheilung über die Beri-Beri. Dtsch Med Wochenschr 1887;13:845–848.
6. Takahashi K. Thiamine deficiency neuropathy, a reappraisal. Int J Neurol 1981;15:245–253.
7. Walsh JC, McLeod JG. Alcoholic neuropathy; an electrophysiological and histopathylogical study. J Neurol Sci 1970;10: 457–469.
8. Tredici G, Minazzi M. Alcoholic neuropathy; an electronmicroscopic study. J Neurol Sci 1975;25:333–346.
9. Takahashi K, Nakamura H. Axonal degeneration in beriberi neuropathy. Arch Neurol 1976;33:836–841.
10. Behse F, Buchthal F. Alcoholic neuropathy: clinical, electrophysiological, and biopsy findings. Ann Neurol 1977;2:95–110.
11. Ohnishi A, Tsuji S, Igisu H, et al. Beriberi neuropathy: morphometric study of sural nerve. J Neurol Sci 1980;45:177–190.
12. Denny-Brown DE. The neurological aspects of thiamine deficiency. Fed Proc 1958;17(suppl 2):35–39.
13. Kimura M, Fujita T, Itokawa Y. Liquid-chromatographic determination of the total thiamine content of blood. Clin Chem 1982;28:29–31.
14. Warnoch LG. The measurement of erythrocyte thiamine pyrophosphate by high- performance liquid chromatography. Anal Biochem 1982;126:394–397.
15. Froridi A, Pupita M, Palmerini CA, et al. Thiamine pyrophosphate determination in whole blood and erythrocyts by high performance liquid chromatography. Int J Vitam Nutr Res 1984;54:165–167.
16. Claus D, Eggers R, Engelhardt A, et al. Ethanol and polyneuropathy. Acta Neurol Scand 1985;72:312–316.
17. Monforte R, Estruch R, Valls-Solé J, et al. Autonomic and peripheral neuropathies in patients with chronic alcoholism; a dose-related toxic effect of alcohol. Arch Neurol 1995;52: 45–51.
18. Ikonomidou C, Bittigau P, Ishimaru MJ, et al. Ethanol-induced apoptotic neurodegeneration and fetal alcohol syndrome. Science 2000;287:1056–1060.
19. Koike H, Mori K, Misu K, et al. Painful alcoholic polyneuropathy with predominant small-fiber loss and normal thiamine status. Neurology 2001;56:1727–1732.
20. Markkanen T. Metabolic disturbance after gastro-oesophageal resection. Int J Vitam Nutr Res 1973;43:549–554.
21. Shimomura T, Mori E, Hirono N, et al. Development of Wernicke-Korsakoff syndrome after long intervals following gastrectomy. Arch Neurol 1998;55:1242–1245.
22. Koike H, Misu K, Hattori N, et al. Postgastrectomy polyneuropathy with thiamine deficiency. J Neurol Neurosurg Psychiatry 2001;71:357–362.
23. van Swieten JC, Koudstaal PJ, Visser MC, et al. Interobserver agreement for the assessment of handicap in stroke patients. Stroke 1988;19:604–607.
24. Herve C, Beyne P, Lettéron P, et al. Comparison of erythrocyte transketolase activity with thiamine and thiamine phosphate ester levels in chronic alcoholic patients. Clin Chim Acta 1995; 234:91–100.
25. Kimura J. Principles of nerve conduction studies. In: Kimura J, ed. Electrodiagnosis in diseases of nerve and muscle: principles and practice. 2nd ed. Philadelphia: F.A, Davis, 1989:78–102.
26. Kimura J. Assessment of individual nerves. In: Kimura J, ed. Electrodiagnosis in diseases of nerve and muscle: principles and practice. 2nd ed. Philadelphia: F.A, Davis, 1989:103–138.
27. Sobue G, Yasuda T, Mitsuma T, et al. Expression of nerve growth factor receptor in human peripheral neuropathies. Ann Neurol 1988;24:64–72.
28. Sobue G, Hashizume Y, Mukai E, et al. X-linked recessive bulbospinal neuronopathy: a clinicopathological study. Brain 1989; 112:209–232.
29. Sobue G, Nakao N, Murakami K, et al. Type I familial amyloid polyneuropathy. A pathological study of the peripheral nervous system. Brain 1990;113:903–919.

30. Hattori N, Ichimura M, Nagamatsu M, et al. Clinicopathological features of Churg- Strauss syndrome-associated neuropathy. Brain 1999;122:427–439.
31. Misu K, Hattori N, Nagamatsu M, et al. Late-onset familial amyloid polyneuropathy type I (transthyretin Met 30-associated familial amyloid polyneuropathy) unrelated to endemic focus in Japan; clinicopathological and genetic features. Brain 1999;122: 1951–1962.
32. Nagamatsu M, Terao S, Misu K, et al. Axonal and perikaryal involvement in chronic inflammatory demyelinating polyneuropathy. J Neurol Neurosurg Psychiatry 1999;66:727–733.
33. Vital A, Ferrer X, Lagueny A, et al. Histopathological features of X-linked Charcot-Marie-Tooth disease in 8 patients form 6 families with different connexin32 mutations. J Peripher Nerv Syst 2001;6:79–84.
34. Llewelyn JG, Gilbey SG, Thomas PK, et al. Sural nerve morphometry in diabetic autonomic and painful sensory neuropathy. Brain 1991;114:867–892.
35. Sobue G, Li M, Terao S, et al. Axonal pathology in Japanese Guillain-Barré syndrome; a study of 15 autopsied cases. Neurology 1997;48:1694–1700.
36. Dyck PJ, Giannini C, Lais A. Pathologic alterations of nerves. In: Dyck PJ, Thomas PK, Griffin JW, Low PA, Poduslo JF, eds. Peripheral neuropathy. 3rd ed. Philadelphia: Saunders.: 1993:514–595.
37. Ikonomidou C, Bosch F, Miksa M, et al. Blockade of NMDA receptors and apoptotic neurodegeneration in the developing brain. Science 1999;283:70–74.
38. Guru SC, Shetty KT, Shankar SK. Effect of chronic ethanol ingestion on phosphate content of neurofilament proteins and neurofilament associated protein phosphatase in rat spinal cord. Neurochem Res 1991;16:1193–1197.
39. Saunders DE, DiCerbo JA, Williams JR, et al. Alcohol reduces neurofilament protein levels in primary cultured hippacampal neurons. Alcohol 1997;14:519–526.
40. McLane JA. Decreased axonal transport in rat nerve following acute and chronic ethanol exposure. Alcohol 1987;4:385–389.
41. Bosch EP, Pelham RW, Rasool CG, et al. Animal models of alcoholic neuropathy: morphologic, electrophysiologic, and biochemical findings. Muscle Nerve 1979;2:133–144.
42. Hoyumpa AM Jr, Breen KJ, Schenker S, et al. Thiamine transport across the rat intestine. II. Effect of ethanol. J Lab Clin Med 1975:803–816.
43. Dancy M, Evans G, Gaitonde MK, et al. Blood thiamine and thaimine phosphate ester concentrations in alcoholic and non-alcoholic liver diseases. Brit Med J 1984;289:79–82.
44. Hoyumpa AM Jr. Mechanism of thiamine deficiency in chronic alcoholism. Am J Clin Nutr 1980;33:2750–2761.
45. Paladin F, Russo Perez G. The haepatic thiamine level in the course of alcoholic neuropathy. Eur Neurol 1987;26:129–133.
46. Poupon RE, Gervaise G, Riant P, et al. Blood thiamine and thiamine phosphate concentrations in excessive drinkers with or without peripheral neuropathy. Alcohol Alcoholism 1990;25: 605–611.
47. Dreyfus PM. Clinical application of blood transketolase determinations. N Eng J Med 1962;267:596–598.
48. Dyck PJ, Lais AC, Karnes JL, et al. Permanent axonotomy, a model of axonal atrophy and secondary segmental demyelination and remyelination. Ann Neurol 1981;9:575–583.
49. Ohi T, Kyle RA, Dyck PJ. Axonal attenuation and secondary segmental demyelination in myeloma neuropathies. Ann Neurol 1985;17:255–261.

LETTERS

An unusual phenotype of McLeod syndrome with late onset axonal neuropathy

McLeod syndrome is a rare multisystem disorder defined by weak expression of the Kell glycoprotein antigens and the absence of a red blood cell surface antigen, Kx.[1 2] The gene responsible for McLeod syndrome, *XK*, was cloned in 1994.[1] The XK protein contains the Kx antigen missing in patients with McLeod syndrome. Mutation analysis of the *XK* gene has shown different deletions or point mutations in families with this condition.[2 3]

Clinical features of McLeod syndrome are reported to be heterogeneous.[2 4 5] Clinical manifestations include acanthocytosis, an increased level of serum creatine kinase (CK), progressive muscular atrophy, seizures, and involuntary movement. As the symptoms and signs of this syndrome seem to be variable even among siblings, it is sometimes difficult to distinguish the condition from other neuromuscular disorders by clinical features and conventional examination.

We report here two cases of McLeod syndrome in brothers and emphasise the variable features of the disease. Phenotypic variability was obvious in the two patients, and one case was unusual because the clinical features greatly resembled an axonal form of Charcot-Marie-Tooth disease.

Case reports

Case 1

A 50 year old man had been complaining of weakness and paraesthesiae in both legs. He first noted weakness in the right leg at the age of 37. Subsequently, the symptom extended to both legs, and he began to be unsteady on his feet. At age 47, he noticed muscular atrophy in his legs. There was no consanguinity in the family. A neurological examination in August 2000 revealed sensorimotor neuropathy with severe weakness and atrophy in both calves and shins (fig 1A). Deep tendon reflexes were diminished in the lower limbs. The ability to sense pinprick and light touch was mildly impaired in the distal parts of the lower extremities. Vibration sense was impaired in both feet. Abnormal involuntary movement was not seen.

Laboratory investigations were unremarkable except for a raised serum CK concentration (1510 IU/l, normal <255). Serum levels of thyroid hormones, vitamin B-12, vitamin E, antinuclear antibody, anti-DNA antibody, and anti-SS-A/SS-B antibodies were normal. In nerve conduction studies, neither compound motor action potentials (CMAP) nor sensory nerve action potentials (SNAP) were elicited in the patient's lower extremities.

Histopathological features of a sural nerve biopsy specimen showed moderate myelinated fibre loss and abundant axonal sprouting in residual myelinated fibres (fig 1B), while onion bulb formation was absent. No apparent amyloid deposits or inflammatory cell infiltrates were seen in the epineurial and endoneurial tissues. An axonal form of Charcot-Marie-Tooth disease was strongly suspected from the clinical features and pathological findings. Although mutation analysis available for the peripheral myelin protein zero and connexin-32 was done, no mutation was detectable in these genes.

Case 2

A 62 year old man, an elder brother of case 1, was admitted for evaluation of a progressive movement disorder in December 2001. On neurological examination, he had choreic involuntary movement of the extremities, mild weakness in the thighs, and hyporeflexia in all limbs. Pathological reflexes were not elicited, and he showed no sensory disturbance. No personality change or cognitive impairment was seen.

A peripheral blood smear showed acanthocytes in 4% of the red blood cells by May-Giemsa staining. Serum CK was raised to 1710 U/l, with predominant MM isozyme. Brain magnetic resonance imaging showed mild atrophy of the bilateral frontal lobes and caudate nuclei (fig 1C). Nerve conduction studies of the lower limbs suggested mild sensory neuropathy, showing reduced SNAP in the sural nerves (left 2.3 μV, right 3.6 μV).

A muscle biopsy specimen taken from the left biceps brachii showed increased variability in fibre diameter. The most striking findings were some scattered necrotic fibres, several basophilic fibres, and an increased number of central nuclei (fig 1D).

An evaluation of Kell antigen expression was subsequently undertaken. Expression of Kell antigens (K2, K4, and K7) on red blood cells was reduced, a result consistent with McLeod syndrome.

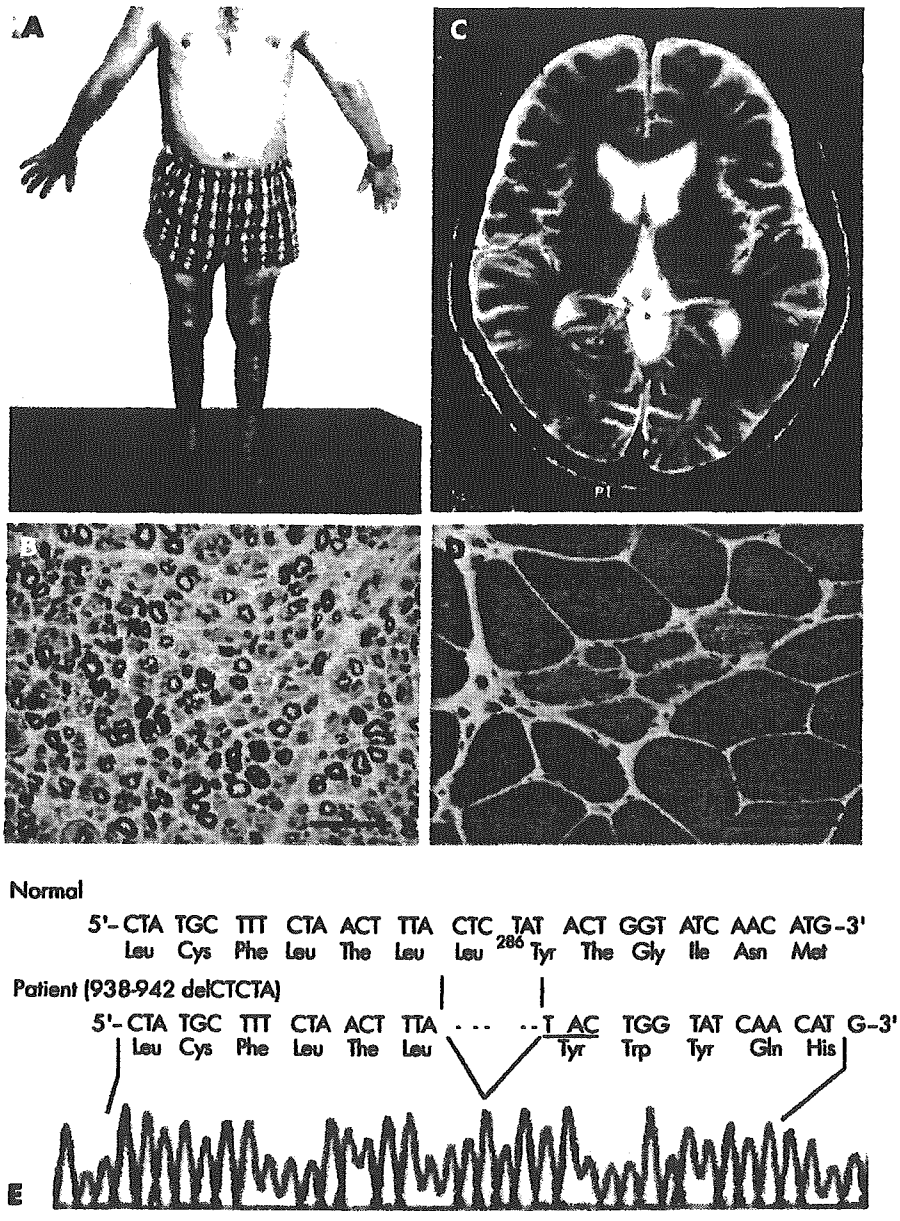

Figure 1 (A) Marked amyotrophy of both legs in case 1. (B) Sural nerve specimen obtained from case 1 showing moderate loss of myelinated fibres. Onion bulb formation is not observed. Toluidine blue stained transverse semithin section. Bar = 50 μ. (C) Axial T2 weighted magnetic resonance image of case 2, showing atrophy of the heads of the caudate nuclei. (D) Muscle biopsy specimen obtained from the left biceps brachii of case 2. The specimen shows increased variability of fibre diameter, and a group of basophilic fibres. Bar = 100 μ. (E) Direct DNA sequencing of the *XK* gene. A five base deletion in exon 3 is present at nt position 938 to 942 from the 5′ end of the cDNA.

Molecular analysis

After informed consent had been obtained from the brothers, genomic DNA was extracted from peripheral blood by standard procedures. Exons of the *XK* gene were subsequently amplified by polymerase chain reaction as described by Ho *et al*.[1] The analysis showed a five base deletion in exon 3 at nt positions 938 to 942 from the 5′ end of the cDNA. This mutation results in a frame shift at codon 286 and the premature stopping of translation at codon 301, as reported previously.[2] This mutation was found in both cases 1 and 2, whose clinical phenotypes were extremely different.

After mutation analysis of the *XK* gene, we confirmed the presence of acanthocytes in a peripheral blood smear of case 1.

Comment

To date, the clinical features of McLeod syndrome have been reported to be heterogeneous.[2 5] The clinical features and conventional pathological findings in this condition are sometimes difficult to distinguish from other neuromuscular disorders because the expression of symptoms and signs seems to be variable, even among siblings.[2 5] In many cases, chorea, seizures, or muscular atrophy are the most frequently presented symptoms. Danek *et al* recently reported clinical features of 22 affected patients with mutation analysis of the *XK* gene.[2] In their investigations, limb chorea—which reflects CNS involvement in McLeod syndrome—was described in all patients. It is extremely difficult to make a diagnosis of this disease where the symptoms and signs are restricted to the peripheral nervous system.

In the present investigation, case 2 was characterised clinically by choreic movement and mild muscular atrophy, frequently seen in the reported cases of McLeod syndrome. In contrast, the symptoms in case 1 were extremely rare. Case 1 showed late onset of symptoms, slowly progressive weakness and amyotrophy of the lower extremities, areflexia, glove and stocking type sensory impairment, an increased level of serum CK, and pathological features with axonal degeneration of the nerve biopsy specimen. He showed no apparent central nervous system involvement 14 years from onset.

Our case 1 was clinically and pathologically indistinguishable from an axonal form of Charcot-Marie-Tooth disease without McLeod serology.

McLeod syndrome should be considered in patients with axonal sensorimotor neuropathy and high CK activity. Abnormal red cell morphology may be a clue to the diagnosis.

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References

1 Ho M, Chelly J, Carter N, *et al*. Isolation of the gene for McLeod syndrome that encodes a novel membrane transport protein. *Cell* 1994;77:869–80.
2 Danek A, Rubio JP, Rampoldi L, *et al*. McLeod neuroacanthocytosis: genotype and phenotype. *Ann Neurol* 2001;50:755–64.
3 Ueyama H, Kumamoto T, Nagao S, *et al*. A novel mutation of the McLeod syndrome gene in a Japanese family. *J Neurol Sci* 2000;176:151–4.
4 Witt TN, Danek A, Reiter M, *et al*. McLeod syndrome: A distinct form of neuroacanthocytosis. Report of two cases and literature review with emphasis on neuromuscular manifestations. *J Neurol* 1992;239:302–6.
5 Hardie RJ, Pullon HWH, Harding AE, *et al*. Neuroacanthocytosis. A clinical, haematological and pathological study of 19 cases. *Brain* 1991;114:13–49.

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Cytogenetic and Genome Research

Transgenic mouse models of spinal and bulbar muscular atrophy (SBMA)

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Abstract. Spinal and bulbar muscular atrophy (SBMA) is a late-onset motor neuron disease characterized by proximal muscle atrophy, weakness, contraction fasciculations, and bulbar involvement. Only males develop symptoms, while female carriers usually are asymptomatic. A specific treatment for SBMA has not been established. The molecular basis of SBMA is the expansion of a trinucleotide CAG repeat, which encodes the polyglutamine (polyQ) tract, in the first exon of the androgen receptor (AR) gene. The pathologic hallmark is nuclear inclusions (NIs) containing the mutant and truncated AR with expanded polyQ in the residual motor neurons in the brainstem and spinal cord as well as in some other visceral organs. Several transgenic (Tg) mouse models have been created for studying the pathogenesis of SBMA. The Tg mouse model carrying pure 239 CAGs under human AR promoter and another model carrying truncated AR with expanded CAGs show motor impairment and nuclear NIs in spinal motor neurons. Interestingly, Tg mice carrying full-length human AR with expanded polyQ demonstrate progressive motor impairment and neurogenic pathology as well as sexual difference of phenotypes. These models recapitulate the phenotypic expression observed in SBMA. The ligand-dependent nuclear localization of the mutant AR is found to be involved in the disease mechanism, and hormonal therapy is suggested to be a therapeutic approach applicable to SBMA.

Numerous animal models of neurodegenerative diseases have been created to elucidate the molecular pathogenesis and develop therapeutic approaches. Some transgenic mouse models of SBMA, the first polyglutamine (polyQ) disease to be discovered, recapitulate the symptoms and pathologic features of the disease, and provide insight into its pathogenesis. Moreover, with our latest model, we developed an effective treatment for SBMA. Here we review transgenic mouse models of SBMA, and discuss the therapeutic strategies against SBMA as well as other polyQ diseases.

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Spinal and bulbar muscular atrophy (SBMA)

SBMA, also known as Kennedy's disease, is a late-onset motor neuron disease characterized by proximal muscle atrophy, weakness, contraction fasciculations, and bulbar involvement (Kennedy et al., 1968; Sobue et al., 1989). Lower motor neurons are markedly depleted through all spinal segments and in brainstem motor nuclei except for the third, fourth and sixth cranial nerves (Sobue et al., 1989). Sensory neurons in the dorsal root ganglia are less severely affected, and there is also a distally accentuated sensory axonopathy in the peripheral nervous system. This disease affects males, and male patients often show signs of androgen insensitivity such as gynecomastia, testicular atrophy, and decreased fertility (Fig. 1). Female carriers are usually asymptomatic, although some express subclinical phenotypes, including high amplitude motor unit potentials on electromyography (Sobue et al., 1993; Mariotti et al., 2000; Schmidt et al., 2002). No specific treatment for SBMA has been established. Testosterone may improve motor function in some patients, although it has no effect on the progression of SBMA (Danek et al., 1994; Goldenberg et al., 1996; Neuschmid-Kaspar et al., 1996).

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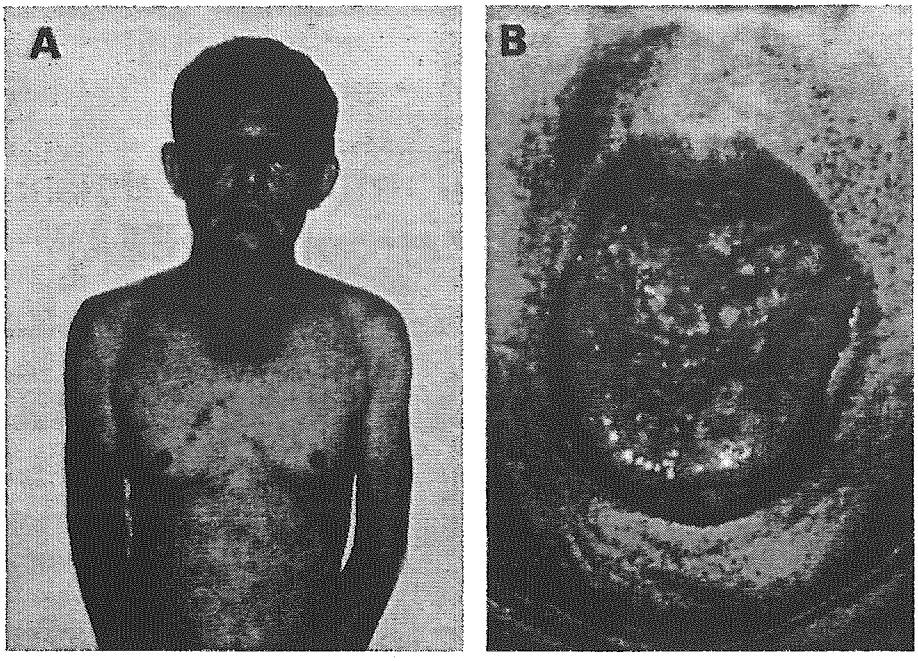

Fig. 1. SBMA patient. SBMA patients show muscle atrophy of the upper arms and gynecomastia (**A**) as well as tongue atrophy (**B**).

The molecular basis of SBMA is the expansion of a trinucleotide CAG repeat, which encodes the polyQ tract, in the first exon of the androgen receptor (AR) gene (La Spada et al., 1991). The CAG repeat within AR ranges in size from 5 to 33 repeats in normal subjects, but from 40 to 62 in SBMA patients (Tanaka et al., 1996, Merry, 2001). Expanded polyQ tracts have been found to cause several neurodegenerative diseases, including SBMA, Huntington's disease (HD), several forms of spinocerebellar ataxia, and dentatorubral and pallidoluysian atrophy (DRPLA) (Zoghbi and Orr, 2000). These disorders, known as polyQ diseases, share salient clinical features such as anticipation, somatic mosaicism (Tanaka et al., 1999), and selective neuronal and non-neuronal involvement despite widespread expression of the mutant gene (Doyu et al., 1994). There is also an inverse correlation between the CAG repeat size and the age at onset, or the disease severity adjusted by the age at examination in SBMA (Doyu et al., 1992; La Spada et al., 1992) as well as other polyQ diseases (Zoghbi and Orr, 2000).

Previously we reported nuclear inclusions (NIs) containing the mutant and truncated AR with expanded polyQ in the residual motor neurons in the brain stem and spinal cord (Li et al., 1998a) as well as in the skin, testis and some other visceral organs of SBMA patients (Li et al., 1998b). These inclusions had similar epitope features detectable by antibodies that recognize a small portion of the N-terminus of the AR protein only, and they were ubiquitinated. The presence of NIs is a pathologic hallmark of most other polyQ diseases, and is likely to be related to pathogenesis (Zoghbi and Orr, 2000). Although considerable controversy surrounds the importance of NIs in the pathogenesis of the polyQ diseases (Tobin and Signer, 2000), several studies have implied that nuclear localization of mutant protein is essential for inducing neuronal cell dysfunction and degeneration in the majority of polyQ diseases (Ross 2002). Although molecular mechanisms still remain to be elucidated, several studies have revealed that nuclear accumulation of the mutant protein resulted in sequestration of transcriptional regulatory proteins in polyQ diseases (Steffan et al., 2000; Nucifora et al., 2001).

SBMA is unique among polyQ diseases in that the mutant protein, AR, has a specific ligand, testosterone, which alters the subcellular localization of the protein by favoring its nuclear uptake. No ligands of causative protein have been found in other polyQ diseases. AR is normally confined to a multi-heteromeric inactive complex in the cell cytoplasm, and translocates into the nucleus in a ligand-dependent manner (Zhou et al., 1994; Zhou et al., 1995). This intracellular trafficking of AR may play an important role in the pathogenesis of SBMA.

Early mouse models of SBMA failed to show phenotypes

All of the available animal models of SBMA are transgenic mice with truncated or full-length human AR (Table 1). Transgenic mice were first created using the full-length AR containing 45 CAGs, which is equivalent to the repeat length observed in SBMA patients, driven by the interferon-inducible Mx promoter or the neuron-specific enolase (NSE) promoter (Bingham et al., 1995). Expression of mutant AR was found in mice with the inducible Mx promoter, but at a lower level than normal endogenous expression. The mice demonstrated neither phenotype nor repeat length instability.

Another transgenic mouse model was created with yeast artificial chromosomes (YACs) carrying the AR gene in the context of flanking non-coding sequences (La Spada et al., 1998). Studies on independent lines of AR YAC transgenic mice carrying 45 CAGs revealed intergenerational instability, which was greater with maternal transmission and with age of the transmitting mother. However, this model failed to show

Table 1. Comparison between transgenic mouse lines and SBMA patients

Reference	Transgene construct	CAG repeat instability	Motor impairment		Neuropathology		Muscle pathology
			Symptoms	Gender effect	Nuclear inclusions	Cell loss	
Bingham et al., 1995	Mx or NSE promoter, full-length human AR with 45 CAGs	(–)	(–)	(–)	(–)	(–)	(–)
LaSpada et al., 1998	YAC transgenic full-length human AR, with 45 CAGs	mild	(–)	(–)	(–)	(–)	(–)
Merry et al., 1996	NSE or NFL promoter, full-length human AR with 66 CAGs	(–)	(–)	(–)	(–)	(–)	(–)
Adachi et al., 2001	human AR promoter, sole 239 CAGs	mild	weakness, amyotrophy, incoordination	(–)	spinal cord, cerebrum cerebellum	(–)	(–)
Abel et al., 2001	NFL promoter, truncated human AR with 112 CAGs	(–)	weakness, foot clasping	(–)	spinal cord, cerebrum brainstem	(–)	(–)
Abel et al., 2001	PrP promoter, truncated human AR with 112 CAGs	(–)	hypoactivity, foot clasping, tremor, seizure	(–)	all neurons	(–)	(–)
Katsuno et al., 2002	chicken ß-actin promoter, full-length human AR with 97 CAGs	(–)	weakness, amyotrophy	significant	spinal cord, cerebrum brainstem	(–)	grouped atrophy
McManamny et al., 2002	cytomegalovirus promoter, full-length human AR with 120 CAGs	(–)	weakness, amyotrophy, foot clasping	mild	(–)	(+)	grouped atrophy fiber-type grouping hypertrophic fiber
Kennedy et al., 1968 Sobue et al., 1989	SBMA patients	mild	weakness, amyotrophy, fasciculation	significant	spinal cord, brainstem	(+)	grouped atrophy fiber-type grouping hypertrophic fiber

the expression of mutant AR in RT-PCR or Western blot analysis.

In order to enhance the toxicity of mutant AR, a transgenic mouse model was created with human AR containing 66 CAGs, which was longer than the longest repeat observed in SBMA patients, driven by the NSE promoter or the neurofilament light chain (NFL) promoter (Merry et al., 1996). Although expression levels of mutant AR were 2–5 times the endogenous AR levels, these mice showed no neurologic symptoms, presumably because the CAG repeat was not long enough.

Transgenic mouse models with truncated AR recapitulate neurologic symptoms

Since mouse models of SBMA could not be created using full-length AR, truncated AR with an expanded CAG repeat was used in later models. This strategy was based on the results reported on transgenic mice of Huntington disease and Machado-Joseph disease, where truncated protein had a particularly pronounced effect (Ikeda et al., 1996; Mangiarini et al., 1996). In SBMA and other polyQ diseases, NIs are detected by antibodies against an N-terminal epitope, but not by antibodies against a C-terminal epitope (Li et al., 1998a, b; DiFiglia et al., 1997). These findings suggest that truncated polyQ-containing proteins confer the toxicity in polyQ diseases. It should be noted that truncated mutant AR is more toxic than the full-length AR in an SBMA cell model (Merry et al., 1998; Kobayashi et al., 2000). Additionally, in vitro translated full-length AR protein with an expanded polyQ tract is cleaved by caspase-3, liberating a polyQ-containing fragment, and the susceptibility to cleavage is polyQ repeat length-dependent (Kobayashi et al., 1998). Thus, cleavage of polyQ-containing proteins is likely to contribute the toxicity of polyQ tracts.

Not like previous models, a transgenic mouse model carrying 239 CAGs driven by the human AR promoter demonstrated motor impairment and revealed that the polyQ tract is sufficient to induce the pathogenic process of SBMA (Adachi et al., 2001). Symptomatic lines exhibited small body size, weakness, truncal and limb incoordination, reduced activity and short lifespan. These phenotypes apparently developed within 4–8 weeks of birth in both lines, and gradually became severe at 8–16 weeks. Impairment of the rotarod task appeared within 8 weeks of birth, and the subsequent progression was monophasic. The most striking pathologic observation was widespread occurrence of NIs, which were distributed in the neuronal cell nuclei in the cerebrum, cerebellum, brainstem and spinal cord, but to a lesser extent or not at all in the basal ganglia. This pathologic distribution was limited by the promoter used, and was more widely spread than that of human SBMA. In the regions with their marked occurrence, the NIs were also prominently observed in the nuclei of the glial cells. The electron microscopic structure of NIs was very similar to that observed in SBMA patients; granular materials densely aggregate without a limiting membrane or filamentous materials. These NIs were positive for ubiquitin and colocalized with proteasome components (20s, PA28α, and PA28γ). Despite abundant NIs, there was no evidence of active neuronal degeneration or reactive astrogliosis. Thus neuronal dysfunction, rather than neuronal degeneration, is likely to be the pathogenesis of this mouse model. Expression of the transgene assessed by RT-PCR was revealed in the cerebrum, cerebellum, spinal cord, pituitary, lung, eye and skin; its distribution was compatible to that of NIs as well as mouse AR distribution. The mice showed subtle meiotic instability of the CAG repeat, in agreement with SBMA.

Simultaneously, another SBMA mouse model was created with truncated human AR containing 112 CAGs, driven by the

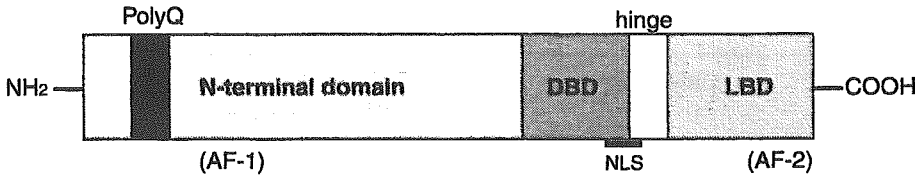

Fig. 2. Structure of AR protein. The AR protein consists of three major domains: N-terminal transactivating, DNA-binding, and ligand-binding domains. The polyglutamine tract is located in an N-terminal domain, which possesses major transactivating function (AF-1). The ligand binding domain (LBD) in the C-terminus also contains weak transactivating function (AF-2). The nuclear localization signal (NLS) in the DNA-binding domain (DBD) and hinge lesion is unmasked upon ligand-binding.

NFL or prion protein promoter (Abel et al., 2001). The mice with the prion protein promoter showed non-specific features such as tremor, seizure and loss of body weight, whereas those with the NFL promoter demonstrated motor impairment similar to SBMA patients, accompanied by upper motor deficits. Widely expressed mutant AR may account for these neurological phenotypes, because the distribution of histopathological involvement, which depended on the expression level of the transgene, was more extensive than that of SBMA. Immunohistochemical analysis of AR expression revealed transgenic AR-positive nuclear inclusions in isolated neurons in several restricted regions of the central nervous system including the brainstem and the cortex and at lower frequency in spinal cord motor neurons. NIs were ubiquitinated and contained several molecular chaperones, including HDJ-2 and Hsc70. About half of the NIs were positive for CREB-binding protein, a transcriptional coactivator (McCampbell et al., 2000). In spite of neurologic symptoms and NIs, neither neuronal loss nor neurogenic muscle atrophy was demonstrated in this model.

The fact that neuronal loss is not evident despite abundant NIs has been demonstrated in various mouse models of other polyQ diseases (Rubinsztein, 2002). Formation of NIs preceded cerebellar ataxia in a transgenic mouse model of SCA1 (Burright et al., 1995). Loss of cerebellar Purkinje cells, however, was not revealed until the symptoms progressed in this model. The life span of these model mice may not be long enough to demonstrate neuronal cell loss, although some other models have shown neuronal degeneration (Reddy et al., 1998; Hodgson et al., 1999; McManamny et al., 2002). These findings suggest that the pathogenesis of human polyQ disease could be neuronal dysfunction rather than neuronal loss in early stages.

Symptomatic full-length model provides therapeutic approach to SBMA

Unlike the profound gender difference of phenotypes in SBMA patients, neither a Tg mouse model of SBMA expressing expanded pure 239 CAGs (Adachi et al., 2001) nor another model carrying truncated AR with 112 CAGs (Abel et al., 2001) showed any remarkable phenotypic difference with gender, because the transgenes of these Tg mice did not contain the ligand-binding domain located in the C-terminus of AR (Fig. 2).

Recently, we generated Tg mice expressing the full-length human AR containing 24 or 97 CAGs under the control of the cytomegalovirus enhancer and the chicken β-actin promoter (Katsuno et al., 2002). This model recapitulated not only the neurologic disorder, but also the phenotypic difference with gender which is a specific feature of SBMA. Three out of five lines with 97 CAGs (AR-97Q) exhibited progressive motor impairment, although no lines with 24 CAGs showed any manifested phenotypes. All symptomatic lines showed small body size, muscle atrophy, weakness, reduced activity and short lifespan; all of which were markedly pronounced and accelerated in the male AR-97Q mice, but either not observed or far less severe in the female AR-97Q mice regardless of the line. Early mortality of male mice, which is not common in SBMA, appears to be caused by cachexia due to progressive amyotrophy. Western blot analysis revealed the transgenic protein retained in the stacking gel in all symptomatic lines. We detected these proteins in the spinal cord, cerebrum, heart, muscle and pancreas. Although the male AR-97Q mice had more protein within the stacking gel than their female counterparts, the female AR-97Q mice had more monomeric AR protein. The nuclear fraction contained most of the mutant AR within the stacking gel. Despite the profound sexual difference of the mutant AR protein expression, there was no significant difference in the expression of the transgene mRNA between the male and female AR-97Q mice. These observations indicate that the testosterone level plays important roles in the sexual difference of phenotypes, especially in the post-transcriptional stage of the mutant AR.

In the AR-97Q mice, we detected diffuse nuclear staining and less frequent NIs with 1C2, an antibody specifically recognizing the expanded polyQ, in the neurons of the spinal cord, cerebrum, cerebellum, brainstem and dorsal root ganglia as well as non-neuronal tissue such as the heart, muscle and pancreas. The regions with diffuse nuclear staining and NIs also showed immunoreactivity to an antibody to AR (N-20). Neither 1C2 nor N-20 revealed immunoreactivity in the cytoplasm. The male AR-97Q mice showed markedly more abundant diffuse nuclear staining and NIs than females, in agreement with the symptomatic and Western blot profile differences with gender. Muscle histology revealed significant grouped atrophy and small angulated fibers in the male AR-97Q mice as well as mild myopathic change. Although neuronal cell loss was not evident in the spinal cord, morphologic studies

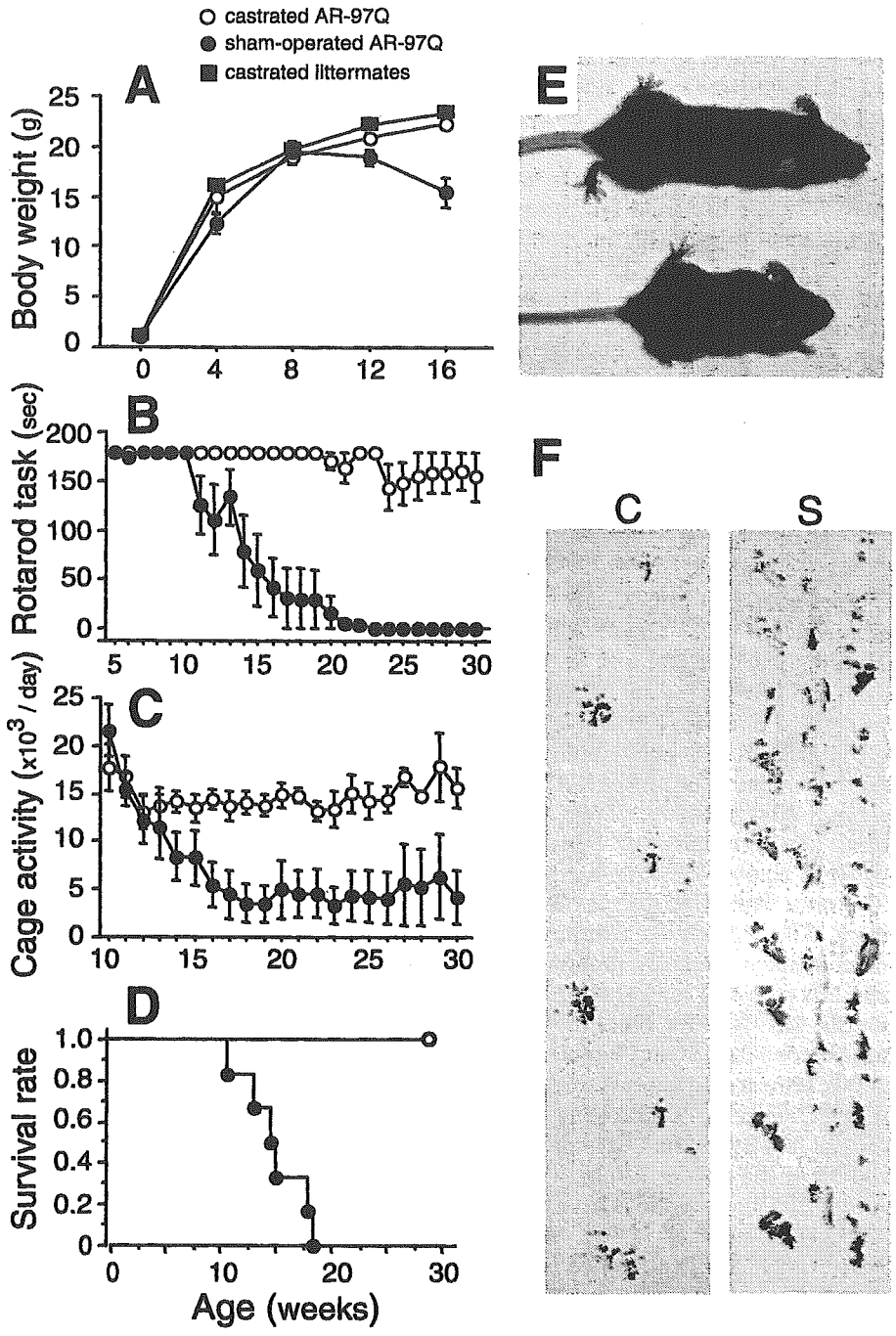

Fig. 3. Effects of castration on symptomatic phenotypes of male AR-97Q mice. (**A**, **B**, **C** and **D**). Body weight (**A**), rotarod task (**B**), cage activity (**C**), and survival rate (**D**) of castrated (ι, $n = 6$) and sham-operated (λ, $n = 6$) male AR-97Q mice. All parameters are significantly different between sham-operated male AR-97Q mice and castrated male AR-97Q mice or castrated male littermates (■, $n = 2$) ($P = 0.0001$, $P < 0.0001$, $P = 0.006$, and $P = 0.0006$, respectively). (**E**) A castrated AR-97Q mouse (top) shows no muscular atrophy, which is striking in a sham-operated male AR-97Q mouse (bottom) (#2-6, 12-week-old). (**F**) Footprints of 12-week-old castrated (C) and sham-operated (S) male AR-97Q mice. Front paws are in red, and hind paws in blue.

revealed axonal atrophy of the ventral nerve root as well as shrinkage of the spinal motor neurons. The female AR-97Q mice showed no neurogenic changes. The neuronal cell populations in the cerebrum, cerebellum and dorsal root ganglia were also fairly well preserved despite the abundant diffuse nuclear staining and NIs. These results indicate that dysfunction of the lower motor neurons contributes to the motor impairment in this model, and this dysfunction is far more severe in the male Tg mice than in the females.

Because of such dramatic sexual difference of phenotype, we examined the effects of hormonal interventions in this mouse model. Castrated male AR-97Q mice, which had a decreased testosterone level, showed significant improvement of symptoms, pathologic findings, and nuclear localization of the mutant AR compared with the sham-operated male AR-97Q mice. The castrated male AR-97Q mice weighed the same as their castrated male littermates, whereas the sham-operated male AR-97Q mice showed progressive emaciation (Fig. 3A). Motor impairment assessed by rotarod and cage activity was significantly less or virtually absent in the castrated male AR-97Q mice as compared with the sham-operated male AR-97Q mice (Fig. 3B and C). The castrated male AR-97Q mice showed

motor impairment similar to that of the female AR-97Q mice. The life span was also significantly prolonged in the castrated male AR-97Q mice (Fig. 3D). Castration ameliorated muscle atrophy and body size reduction (Fig. 3E). In a foot print analysis, the sham-operated male AR-97Q mice exhibited motor weakness with dragging of their legs, while the castrated male AR-97Q mice had mild symptoms (Fig. 3F). In the Western blot analysis using N-20, the mutant AR appearing within the stacking gel was markedly diminished in the castrated male AR-97Q mice compared with the sham-operated male AR-97Q mice (Fig. 4A). The mutant AR in the nuclear fraction also significantly decreased in the castrated male AR-97Q mice (Fig. 4B). Castration markedly diminished diffuse nuclear staining and NIs (Fig. 4C). These observations suggested that castration markedly prevented nuclear localization of the mutant AR protein. Leuprorelin, an LHRH agonist that reduces testosterone release from the testis, also rescued motor dysfunction and nuclear accumulation of mutant AR in this model (Katsuno et al., 2003).

In contrast to castration of the male mice, testosterone administered to the female AR-97Q mice caused significant exacerbation of symptoms, pathologic features, and nuclear localization of the mutant AR. The motor impairment assessed by rotarod and cage activity was significantly exacerbated in the female AR-97Q mice administered testosterone, similar to the case of untreated male AR-97Q mice. Western blot analysis using N-20 revealed the mutant AR in the stacking gel in whole tissue homogenates as well as in the nuclear fraction, which was larger in amount in the testosterone-administered female AR-97Q mice than in the sesame oil-administered female AR-97Q mice. The testosterone-administered female AR-97Q mice demonstrated markedly pronounced diffuse nuclear staining and NIs with 1C2 compared with the sesame oil-administered female AR-97Q mice. Since the nuclear translocation of AR is testosterone-dependent (Stenoien et al., 1999; Simeoni et al., 2000), testosterone appears to show toxic effects in the female AR-97Q mice by accelerating nuclear translocation of the mutant AR. By contrast, castration prevented the nuclear localization of the mutant AR by reducing the testosterone level. Although a few exceptions have been reported (Hodgson et al., 1999; Hackam et al., 1999), the nuclear localization of the mutant protein with expanded polyQ is important in inducing neuronal cell dysfunction and degeneration in the majority of polyQ diseases. Addition of a nuclear export signal to the mutant huntingtin eliminated aggregate formation and cell death in cell models of HD (Saudou et al., 1998; Peters et al., 1999), whereas a nuclear localization signal had the opposite effect (Peters et al., 1999). In Tg mice of SCA1 having a mutated nuclear localization signal, ataxin-1 was distributed in the cytoplasm, and the mice did not show any neurologic disorders (Klement et al., 1998). These findings suggest that reduction of testosterone ameliolates phenotypic expression by preventing nuclear localization of the mutant AR, while castration may enhance the protective effects of heat shock proteins (HSPs), which are normally associated with AR and dissociate upon ligand binding.

The castrated AR-97Q mice showed phenotypes similar to those of the female AR-97Q mice, implying that motor impairment in SBMA patients can be reduced to the level in female carriers. SBMA has been considered to be an X-linked disease, whereas other polyQ diseases show autosomal dominant inheritance. In fact, SBMA female carriers hardly manifest clinical phenotypes, although they possess similar numbers of a CAG repeat in the disease allele as their siblings with SBMA (Sobue et al., 1993; Mariotti et al., 2000). Indeed the lower level of mutant AR expression in female carriers due to X inactivation may cause the escape from the manifestation, but our present study also strongly suggests that the low level of testosterone prevents the nuclear localization of the expressed mutant AR, resulting in a lack of phenotypic manifestations in the female carriers. This hypothesis is supported by the finding that manifestation of symptoms is minimal even in homozygous females of SBMA (Schmidt et al., 2002). The testosterone-dependent neurodegenaration was also recently revealed in a fly model of SBMA (Takeyama et al., 2002). Thus, hormonal intervention to diminish testosterone level appears to be applicable to human therapy.

Another Tg mouse model carrying the full-length AR with 120 CAGs driven by the cytomegalovirus promoter showed slowly progressive motor impairment and neurogenic muscle atrophy (McManamny et al., 2002). The affected mice also displayed a progressive reduction in sperm production consistent with androgen insensitivities in SBMA patients. Although this model showed no neuronal inclusions throughout the nervous system, loss of motor neurons was demonstrated in the spinal cord. Notably, as expected, mild but evident sexual difference of phenotypes was observed. This finding supports the hypothesis that testosterone level is implicated in the phenotypic expression of SBMA. The above study and ours indicate that the C-terminus of mutant AR is necessary to recapitulate the testosterone-dependent pathogenesis of SBMA in mouse models.

"Loss of function" model of SBMA

As in other autosomal dominant polyQ diseases, a toxic gain of the mutant AR function has been considered the mainstream of SBMA pathogenesis. Although the expansion of polyQ tract in AR deteriorates the transcriptional activities of AR (Mhatre et al., 1993; Brooks et al., 1997), motor impairment has not been observed in severe testicular feminization (Tfm) patients lacking AR function (Gottlieb et al., 1999). Thus, the neurologic impairment in SBMA cannot be attributed to the loss of AR function (Maclean et al., 1995), a reason why testosterone shows insufficient and transient effects when used as a therapeutic agent for SBMA (Danek et al., 1994; Goldenberg et al., 1996; Neuschmid-Kaspar et al., 1996). Animal models of Tfm demonstrate a decreased number of motor neurons in the spinal nucleus of the bulbocavernosus (Sengelaub et al., 1989) without any other motor neuron involvement.

However, re-examination of earlier studies and recent data has revealed that loss of normal protein function also plays a role in the neuronal involvement in polyQ diseases. In a huntingtin knockout mouse model, the heterozygotes demonstrated motor impairment and basal ganglia degeneration (Na-

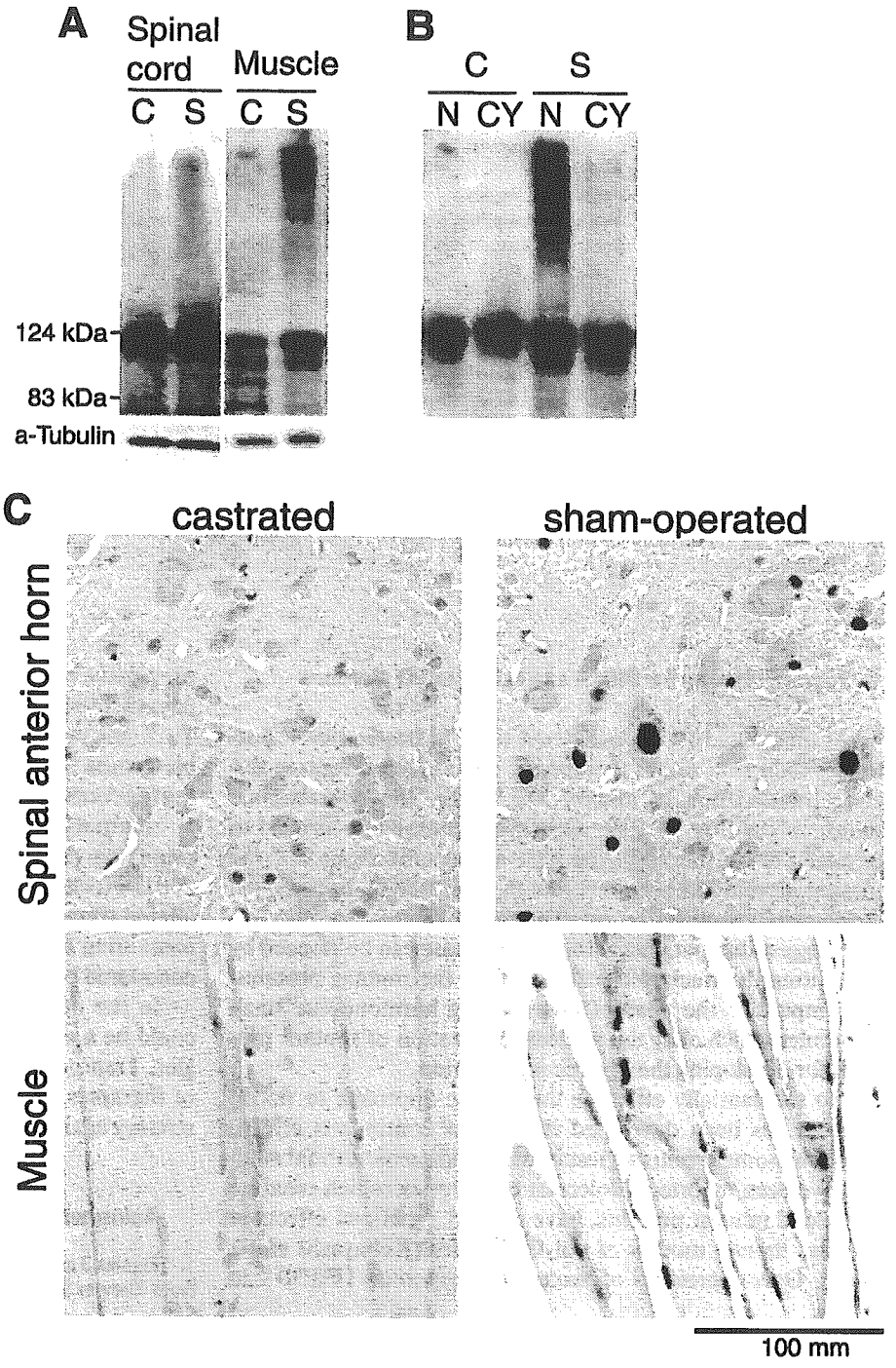

Fig. 4. Effects of castration on transgene expression and neuropathology of male AR-97Q mice. (**A**) Western blot analysis of total homogenates from the spinal cord and muscle of castrated (C) and sham-operated (S) male AR-97Q mice, that were immunolabeled by N-20. (**B**) Western blot analysis of nuclear (N) and cytoplasmic (CY) fraction from muscle of castrated (C) and sham-operated (S) male AR-97Q mice, immunolabeled by N-20. (**C**) Immunohistochemical study using 1C2 showed marked differences of diffuse nuclear staining and nuclear inclusions between castrated and sham-operated AR-97Q mice in the spinal anterior horn and muscle.

sir et al., 1995). More recently, conditional inactivation of wild-type huntingtin selectively in forebrain and testis resulted in a progressive degenerative neuronal phenotype and sterility (Dragatsis et al., 2000). Additionally, an anti-apoptotic effect of wild-type huntingtin was revealed in a cell model (Rigamonti et al., 2000). These results imply that loss of normal huntingtin function may contribute to the neurodegeneration in HD.

Androgens have been found to have neuroprotective effects. Administration of testosterone immediately after nerve injury impacts positively on functional recovery through actions mediated by the androgen receptor (Jones at al., 2001). In a cell culture model, AR with 24 CAGs showed trophic effects, whereas AR with 65 CAGs did not demonstrate any neuroprotection (Lieberman et al., 2002). The role of normal AR function in the pathogenesis of SBMA should be further studied in cell and animal models.

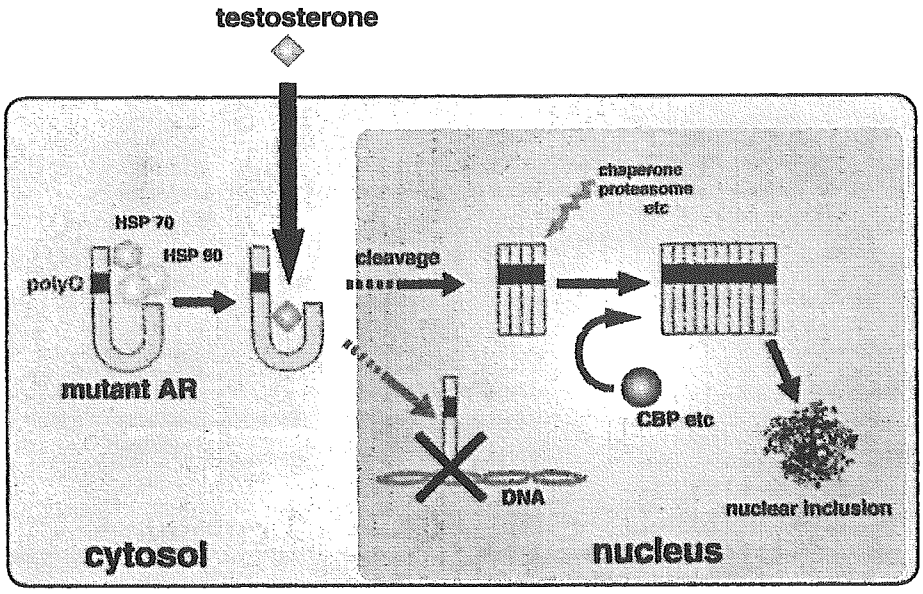

Fig. 5. Hypothetical dynamics of mutant AR in SBMA. In the absence of ligand, mutant AR is confined to a multi-heteromeric inactive complex with heat shock proteins (HSPs) and immunophilins in the cell cytoplasm. Upon testosterone-binding, the conformational change of mutant AR facilitates its dissociation from the complex and translocation into the nucleus. Mutant AR is cleaved and aggregates in the nucleus, whereas cellular mechanisms such as molecular chaperone and ubquitin-proteasome system attempt to mitigate its toxicity. Aggregation sequestrates critical cellular proteins such as CREB-binding protein (CBP) resulting in aberrant transcription, and finally forms nuclear inclusions. On the other hand, decreased transactivating function of mutant AR may contribute to the neurodegeneration and androgen insensitivity in SBMA.

Toward therapy for SBMA and other polyQ diseases

As mentioned above, our recent study indicated that testosterone reduction exerts therapeutic effects by preventing nuclear translocation of mutant AR in the SBMA transgenic mouse model (Fig. 5). This approach can easily be applied to human SBMA therapy. Although no specific ligand of the mutant protein has been revealed in other polyQ diseases, the striking therapeutic effects of castration in our SBMA mice further suggest that patients with polyQ disease can be rescued by preventing the nuclear translocation of the mutant proteins. We emphasize the need of investigating hormone-like small molecules which alter the nuclear localization of mutant proteins for developing therapeutic intervention.

No substantially effective therapeutic approach to polyQ diseases has been developed in spite of continuous efforts. However, some promising results using transgenic animal models have been reported. Molecular chaperones, which renature misfolded mutant proteins, have exerted beneficial effects in cell and animal models of polyQ diseases (Kobayashi et al., 2001). Over-expression of molecular chaperone HSP70 had preventive effects in cell and Tg mouse models of SBMA (Kobayashi et al., 2000; Adachi et al., 2003) as well as in an SCA1 Tg mouse model (Cummings et al., 2001). It may also be possible to treat SBMA by increasing the expression level or enhancing the function of molecular chaperones.

Alternatively, histone deacetylase inhibitors could be a promising candidate therapy for polyQ diseases (Steffan et al., 2001; Hockly et al., 2003). These drugs potentially correct altered transcription due to toxic effects of the mutant protein containing expanded polyQ, although their toxicity should be conquered before clinical application.

In the near future, an ideal treatment for polyQ diseases could be a combination of these and other therapeutic strategies. Transgenic mice will be useful in testing the effectiveness of therapies, and further contribute to the development of a strategy against polyQ diseases including SBMA.

Acknowledgements

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References

Abel A, et al: Expression of expanded repeat androgen receptor produces neurologic disease in transgenic mice. Hum molec Genet 10:107–116 (2001).

Adachi H, et al: Transgenic mice with an expanded CAG repeat controlled by the human AR promoter show polyglutamine nuclear inclusions and neuronal dysfunction without neuronal cell death. Hum molec Genet 10:1039–1048 (2001).

Adachi H, et al: HSP70 chaperone over-expression ameliorates phenotypes of the SBMA transgenic mouse model by reducing nuclear-localized mutant AR protein. J Neurosci 23:2203–2211 (2003).

Bingham PM, et al: Stability of an expanded trinucleotide repeat in the androgen receptor gene in transgenic mice. Nature Genet 9:191–196 (1995).

Brooks BP, et al: Characterization of an expanded glutamine repeat androgen receptor in a neuronal cell culture system. Neurobiol Dis 3:313–323 (1997).

Burright EN, et al: SCA1 transgenic mice: a model for neurodegeneration caused by an expanded CAG trinucleotide repeat. Cell 82:937–948 (1995).

Cummings CJ, et al: Over-expression of inducible HSP70 chaperone suppresses neuropathology and improves motor function in SCA1 mice. Hum molec Genet 10:1511–1518 (2001).

Danek A, et al: Decrease in androgen binding and effect of androgen treatment in a case of X-linked bulbospinal neuronopathy. Clin Investig 72:892–897 (1994).

DiFiglia M, et al: Aggregation of huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain. Science 277:1990–1993 (1997).

Doyu M, et al: Severity of X-linked recessive bulbospinal neuronopathy correlates with size of the tandem CAG repeat in androgen receptor gene. Ann Neurol 32:707–10 (1992).

Doyu M, et al: Androgen receptor mRNA with increased size of tandem CAG repeat is widely expressed in the neural and nonneural tissues of X-linked recessive bulbospinal neuronopathy. J Neurol Sci 127:43–47 (1994).

Dragatsis I, et al: Inactivation of Hdh in the brain and testis results in progressive neurodegeneration and sterility in mice. Nature Genet 26:300–306 (2000).