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McLeod myopathy revisited: more neurogenic and less benign

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The X-linked McLeod neuroacanthocytosis syndrome (MLS) has originally been denoted as 'benign' McLeod myopathy. We assessed the clinical findings and the muscle pathology in the eponymous index patient, Hugh McLeod, and in nine additional MLS patients. Only one patient had manifested with neuromuscular symptoms. During a mean follow-up of 15 years, however, eight patients including the initial index patient showed elevated skeletal muscle creatine kinase levels ranging from 300 to 3000 U/L, and had developed muscle weakness and atrophy. Two patients had disabling leg weakness. Muscle histology was abnormal in all 10 patients. Clear but unspecific myopathic changes were found in only four patients. All patients, however, had neurogenic changes of variable degree. Post-mortem motor and sensory nerve examinations support the view that muscle atrophy and weakness are predominantly due to an axonal motor neuropathy rather than to a primary myopathy. Multisystem manifestations developed in eight patients at a mean age of 39 years. Three patients manifested with psychiatric features comprising schizophrenia-like psychosis and personality disorder, two presented with generalized seizures and one with chorea. During follow-up, seven patients developed chorea, six had psychiatric disorders, five had cognitive decline and three had generalized seizures. Five patients died because of MLS-related complications including sudden cardiac death, chronic heart failure and pneumonia between 55 and 69 years. In conclusion, our findings confirm that MLS is not a benign condition but rather a progressive multisystem disorder sharing many features with Huntington's disease.

Keywords: McLeod syndrome; neuroacanthocytosis; chorea; myopathy; neuropathy

Abbrevations: CK = creatine kinase; CNS = central nervous system; EMG = electromyography; MLS = McLeod neuroacanthocytosis syndrome; MRI = magnetic resonance imaging; RBC = red blood cell

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Introduction

The X-linked McLeod neuroacanthocytosis syndrome (MLS) is a multisystem disorder with central nervous system (CNS) features resembling Huntington's disease (Walker *et al.*, 2007*b*). MLS is defined by absent expression of the Kx red blood cell (RBC) antigen, diminished expression of Kell glycoprotein RBC antigens, and RBC acanthocytosis (Allen *et al.*, 1961; Lee *et al.*, 2000). About 150 individuals carrying this so-called McLeod blood group phenotype are known to date, and virtually all of them have elevated serum creatine kinase (CK) levels (Danek *et al.*, 2001; Danek *et al.*, 2005). Since weakness in these patients may be subclinical or mild, the term 'benign X-linked myopathy with acanthocytes' was used in this journal when the syndrome first attracted the attention of neurologists (Swash *et al.*, 1983). Many patients, however, develop clinically relevant muscle weakness or atrophy during the disease course, which in some patients is disabling and may predispose to rhabdomyolysis (Danek *et al.*, 2001; Kawakami *et al.*, 1999; Jung *et al.*, 2001*a*; Jung and Brandner, 2002).

Most carriers of the McLeod blood group phenotype develop additional CNS abnormalities at a mean onset age of 40 years (Danek *et al.*, 2001; Jung *et al.*, 2001*a*; Danek *et al.*, 2005; Walker *et al.*, 2007*b*). CNS manifestations resemble Huntington's disease and comprise hyperkinetic movement disorders, psychiatric abnormalities, cognitive alterations, as well as epileptic seizures (Danek *et al.*, 2001; Jung *et al.*, 2001*a*; Danek *et al.*, 2005; Walker *et al.*, 2007*b*). These CNS manifestations may appear several decades after the immunohaematological diagnosis, progress relentlessly, and may lead to a considerable impairment of everyday activities (Danek *et al.*, 2001; Jung *et al.*, 2001*a*; Walker *et al.*, 2007*b*).

We describe the clinical features and the muscle biopsy findings of 10 McLeod patients from 8 different families, and delineate a clearly not benign neuromuscular and neurodegenerative disorder.

Methods

Patients

The patients originated from eight families from the United States, Germany, Switzerland and Chile (with German ancestors). Their clinical findings have previously been documented to a variable extent (Table 1). The series included the eponymous case of Hugh McLeod, whose clinical course, so far unreported, will be detailed below. Neurological and cognitive examination was performed in all patients and structured cognitive data were available in five patients (Table 1). Immunohaematological analysis of Kx and Kell expression, acanthocyte count and serum CK levels were available from all patients whereas sequence analysis of the XK gene was not performed in one (Table 2). Results of other technical examinations available were listed in the table (Table 2).

Muscle pathology

Tissue samples originated from quadriceps (cases 1, 2, 4, 5, 7–10) gastrocnemius (case 3) and deltoid muscles (case 6; Table 3), and were obtained by biopsy (cases 1–6, 9 and 10) or autopsy (cases 7 and 8). Findings were previously reported from cases 1 and 3 (Danek *et al.*, 2001), case 6 (Jung *et al.*, 2001*b*), case 9 (Jung *et al.*, 2003) and case 10 (Oechsner *et al.*, 1996). Frozen muscle samples stored at −80°C were available from all but the index patient (case 8), in whom formalin-fixed autopsy tissue and slides from frozen tissue of a muscle biopsy were available (Table 3). All muscle specimens were processed (or reprocessed) and analysed in a standardized way. Cryostat sections were cut at a thickness of 10 µm and stained according to standard protocols with haematoxylin-eosin (H&E), modified Gömöri trichrome, periodic acid-Schiff (PAS), Oil red O and Elastica-van Gieson (EvG). Fibre typing was performed using immunohistochemical stainings for myosin heavy chain-slow and myosin heavy chain-fast isotypes (clone WB-MHCs, diluted 1:50 and clone WB-MHCf, diluted 1:100, Novocastra, VisionBiosystems, Newcastle-upon-Tyne, UK), corresponding to type 1 and type 2 fibres, respectively. Additional immunohistochemical stains included dystrophin (Dys 1, clone Dy4/6D3, 1:10, Novocastra Newcastle-upon-Tyne, UK), spectrin (clone RBC/3D5, 1:100, Novocastra Newcastle-upon-Tyne, UK), and CD45 (leukocyte common antigen, LCA, clones 2b11 + PD7/26, 1:10, DAKO, Switzerland). Enzyme histochemistry was performed for cytochrome-c oxidase (COX), succinate dehydrogenase (SDH), and NADH dehydrogenase (NADHD). Formalin-fixed, paraffin-embedded tissue was available from four patients (cases 6–9). Sections were cut at a nominal thickness of 4 µm and stained with H&E, EvG, PAS and myosin heavy chain fast and slow isotypes.

We performed fibre size measurement using the analySIS™ morphometry software package (Olympus, Switzerland). Representative muscle regions were selected, and for type 1 and type 2 fibres were measured on the basis of their 'lesser diameter', defined as the maximum distance at a right angle to a fibre's longest diameter. Frequency of internalized nuclei was defined by the number of internalized nuclei per 100 fibres in the selected region. Variability, hypertrophy and atrophy coefficients were calculated according to commonly used definitions (Brooke and Engel, 1969). At least 150 fibres per sample were measured. Mean fibre diameter, variability, atrophy and hypertrophy coefficients were calculated for type 1 and type 2 fibres, respectively. Although no formal cut-off values are available, variability coefficients above 250, and atrophy and hypertrophy coefficients above 250–350 are commonly considered as clearly pathological (Brooke and Engel, 1969).

Nerve pathology

For conventional nerve histology, paraffin-embedded nerve samples obtained at autopsy were available from cases 7 (femoral and sural nerves) and 8 (peroneal and sural nerves). Sections were cut at a nominal thickness of 4 µm and stained with H&E and EvG, and immunohistochemically reacted for neurofilament and myelin basic protein.

Semithin sections and electron microscopy

Resin-embedded tissue was available from case 6 (deltoid muscle), case 7 (quadriceps muscle, femoral and sural nerves) and case 9 (quadriceps muscle). Formalin-fixed tissue from case 8 (quadriceps muscle, peroneal and sural nerves) was post-fixed with glutaraldehyde and subsequently embedded in epon resin. Semithin and ultrathin sections were prepared according to standard protocols.

Results

Case report of Hugh McLeod

The initial index patient (case 8) had been identified during screening for allogenic antibodies while he was a Harvard dental student at the age of 25 years. His RBC's had shown an unexplained weak reactivity to Kell antisera and thus defined the Kell blood group phenotype that came to be known by his name (Allen *et al.*, 1961; Danek, 2004). General areflexia was noted at about that time. Subsequently, Dr McLeod episodically donated blood for patients with chronic granulomatous disease and the McLeod phenotype (Giblett *et al.*, 1971), an association later shown to be due to a contiguous gene syndrome (Francke *et al.*, 1985). At age 35 years, acanthocytosis was first detected (Wimer *et al.*, 1977) and soon after, high serum CK levels were noted (Marsh *et al.*, 1981).

At age 51 years, physical examination revealed moderately increased weight (body mass index-BMI-of 29.7),

Table 1 Clinical findings

Patient[a]	Earlier report of case	Age at onset (years)	Initial Presentation	Age at last examination	Muscle weakness	Muscle atrophy	Clinical signs of cardiopathy	PNS	Chorea; age of onset (years)	Other movement disorders	Last UHDRS	Psychiatric Symptoms; age of onset (years)	Cognitive alterations; age of onset (years)
1	Case 16 of Danek *et al.* (2001*a*)	43	Seizure	62[b]	UL no LL M4	LL slight	No	UL and LL areflexia PNP	UL, LL; 51	FD, IV Dysarthria	n.d.	Paranoid delusions; 43	Moderate; 43
2	Case 2 of Walker *et al.* (2007*c*)	n.a.	None; McLeod blood group phenotype	57	No	No	No	No	No	No	n.d.	No	No
3	Case 1 of Danek *et al.* (2001*a*)	26	Exercise intolerance	34	UL no LL M4	No	No	LL areflexia	No	No	10	No	No
4 Brother of case 5	Case 1 of Miranda *et al.* (2007)	23	Schizophrenia	57[b]	UL M4 LL MI-2	UL moderate LL severe	No	UL and LL areflexia PNP	Trunk, UL; 41	FD IV Dysarthria	n.d.	Schizophrenia; 28	Moderate; 46 MMS 23
5 Brother of case 4	Case 2 of Miranda *et al.* (2007)	48	Chorea	56	UL M4 LL M4	UL slight LL slight	No	UL and LL areflexia PNP	Trunk, UL; 40	FD, IV Dysarthria	n.d.	Obsessive-compulsive disorder; 50	No
6 Cousin of case 7	Case IV-13 of Jung *et al.* (2001)	25	Personality disorder	55[b]	UL M4-5 LL M3-4	UL slight LL moderate	No	UL and LL areflexia	Trunk, UL, LL; 30	FD, Dystonia	61	Personality disorder; 25	Moderate; 45 ERFC 39.5/50
7 Cousin of case 6	Case IV-5 of Jung *et al.* (2001)	39	Schizophrenia	55[b]	UL no LL M4-5	UL slight LL moderate	No	UL and LL areflexia	Trunk, UL. LL; 50	Dysarthria	38	Schizophrenia; 39	Mild; 48 MMS 25/30
8 (Index)	Case 21 of Danek *et al.* (2001*a*)	n.a.	None; McLeod blood group phenotype	69[b]	UL no LL M3-4	UL no LL slight	Dyspnoea	UL and LL areflexia decreased vibration sense LL	UL, 64	No	n.d.	No	No
9	Case II-2 of Jung *et al.* (2003)	n.a.	None; McLeod blood group phenotype	52	No	No	No	No	No	No	0	No	No ERFC 50/50
10	Case 4 of Oechsner *et al.* (1996)	58	Generalized Seizure	61	ULM4 LL MI-3	UL moderate LL severe	No	UL and LL areflexia PNP	Trunk, UL, LL; 48	FD, IV Dystonia TLB	n.d.	Schizophrenia, compulsion, personality disorder; 48	Moderate; 58 MMS 20/30

ERFC = évaluation rapide des fonctions cognitives; a population-based validated method for short and comprehensive cognitive testing (Gil *et al.*, 1986); FD = facial dyskinesia; IV = involuntary vocalizations; LL = lower limbs; MMS = mini mental state examination; n.a. = not applicable; n.d. = not determined; PNP = polyneuropathy; PNS = peripheral nervous system; TLB = tongue and lip biting; UHDRS = Unified Huntington's Disease Rating Scale; UL = upper limbs.
[a]Patients are listed according to the position of the mutation in the XK gene. [b]Age at death.

Table 2 Technical findings

Patient	Kell blood group phenotype	*XK* mutation	RBC Acantho-cytes (%)	CK min-max (U/l)	ECG	Echo	EEG	EMG	ENG	Cerebral MRI/CT	Muscle MRI	Cerebral SPECT/PET
1	K2 (+), K4+, K5 0, K7 +, K9 0, K11 0, K17 0	50 000 bp major deletion	3	100–1200	AF	LVSD EF 38%	n.d.	Normal	Sensory-motor axonal	Caudate atrophy	n.d.	IBZM-SPECT: decreased striatal D2- binding; PET: decreased striatal FDG-uptake
2	Kx 0, K1 0, K2 +, K3 0, K4 +, K5 +, K6 0, K7 +, K17 0 K21 0	IVS2 + 5G>A	<1	20	Normal	Normal	n.d.	n.d.	n.d.	Mild caudate atrophy	n.d.	n.d.
3	Kx 0, K20 0	768–769delTT	5	400–1190	Normal	n.d.	Normal	Normal	Sensory axonal	Normal	n.d.	IBZM-SPECT: normal; PET: decreased striatal FDG-uptake left
4	Kx 0, K1 0, K2 +, K3 0, K4 0	938–942delCTCTA	3 (26[a])	300	Normal	n.d.	Normal	Myopathic No SA	Normal	Caudate atrophy	n.d.	ECD-SPECT: caudate hypoperfusion
5	Kx 0, K1 0, K2 +, K3 0, K4 0	938–942delCTCTA	2 (35[a])	300	Normal	n.d.	Normal	Myopathic No SA	Normal	Caudate atrophy	n.d.	ECD-SPECT: caudate hypoperfusion
6	Kx 0, K1 0, K2 (+), K3 0, K4 (+), K5 (+), K7 (+)	977C > T	20	3000	Normal	Normal	n.d.	n.d.	n.d.	Caudate and putamen atrophy	n.d.	PET: decreased striatal FDG-uptake
7	Kx 0, K1 0, K2 (+), K3 0, K4 (+), K5 (+), K7 (+)	977C > T	18	1500–160 820	Normal	Excentric LVH EF 43%	n.d.	n.d.	n.d.	Mild caudate atrophy	n.d.	PET: decreased striatal FDG-uptake
8 (Index)	Kx 0, K1 0, K2 (+), K3 0, K4 (+), K5 (+), K6 0, K7 (+), K11 +, K12 +, K13 (+), K14 (+), K 18 (+), K17 0	1020-1033del	8–85	1000	AF LAFB	EF reduced; enlargement LA and RV	n.d.	Neurogenic SA	Sensory-motor axonal	Mild caudate and general cerebral atrophy	Fatty degeneration	n.d.
9	Kx 0, K1 0, K2 +, K3 0, K4 +, K7 +	106IG>A	0	298	Normal	Normal	n.d.	n.d.	n.d.	Normal	n.d.	FDG-PET: normal
10	Kx 0, K20 0	n.d.	5	120–1600	Normal	n.d.	Left temporal theta focus, no epileptic activity	Myopathic Neurogenic SA	Sensory-motor axonal	Caudate atrophy	n.d.	n.d.

AF = Atrial fibrillation; CT = computed tomography; Echo = echocardiography; ECG = Electrocardiogram; EEG = electoencephalography; EF = ejection fraction; EMG = electromyography; ENG = electroneurography; LA = left atrium; LAFB = left anterior fascicular block; LVH = left ventricular hypertrophy; LVSD = left ventricular systolic dysfunction; MRI = magnetic resonance imaging; n.d. = not determined; RV = right ventricle; PET = positron emission tomography; SA = pathological spontaneous activity; SPECT = single photon emission computed tomography.
[a]1:1 dilution with saline.

Table 3 Muscle histology

Patient	Muscle	Myopathic alterations				Neurogenic alterations			
		Increased fibre size variation	Necrosis	Internalized nuclei per fibre population	Endomysial fibrosis	Fibre group atrophies	Angulated fibres	Fibre-type grouping	Fibre-type predominance (type)
1	Quadriceps	–	–	6	–	–	–	++	–
2	Quadriceps	–	–	4	–	–	–	(+)	–
3	Gastrocnemius	(+)	–	3	–	(+)	(+)	++	–
4	Quadriceps	–	–	5	–	–	–	++	2: ++
5	Quadriceps	–	–	2	–	–	–	+	1: +
6	Deltoid	+	–	24	–	++	+	+	1: ++
7	Quadriceps	+	–	5	–	+	(+)	+	–
8 (Index)	Quadriceps	+	–	21	+	+	+	+	–
9	Quadriceps	–	–	1	–	–	–	–	2: ++
10	Quadriceps	–	–	16	–	–	–	++	–

– indicates absent; (+) indicates subtle/rare; + indicates moderate/occasional; ++ indicates pronounced/frequent.

normal blood pressure, and slightly irregular cardiac rhythm with marked splitting yet otherwise normal cardio-pulmonary findings. The spleen was palpated 11 cm below the costal margin. Neurological examination demonstrated moderately reduced strength of hamstrings and toe extensors (MRC grade 4), areflexia and decreased vibration sense at the ankles yet otherwise normal sensation. Electrophysiology was compatible with a sensory and motor axonal neuropathy. Nerve conduction studies demonstrated an absence of sural nerve action potentials as well as absent or prolonged F-waves in the legs, with motor velocities at the lower range of normal. Electromyography disclosed high-amplitude interference patterns in distal arm and leg muscles. In addition, rare fibrillations and fasciculations were found. At age 54 years, electrocardiography (ECG) showed left axis deviation, sinus bradycardia and left anterior fascicular block. Echocardiography showed normal but somewhat globular left ventricular size, mildly reduced systolic function, and a mild enlargement of the left atrium as well as the right heart chambers. At age 60 years, leg weakness had slightly progressed, with particular difficulty walking on toes, and there were a positive Trendelenburg sign and atrophy of thigh and foot muscles. A movement disorder was not observed and no cognitive alterations were reported. In the following years, while still working as a dentist, he developed progressive proximal weakness, with difficulty walking upstairs and inability to take more than 10 steps in succession. In addition, atrial fibrillation was noted and oral anticoagulation was started. At age 64 years, a movement disorder was noted for the first time, described as repetitive right arm shoulder shrugs. His condition deteriorated further, with increasing immobility because of weakness, obesity (BMI 40) and shortness of breath. Hugh McLeod died at the age of 69 years from heart failure.

Clinical and paraclinical findings

The clinical and paraclinical findings are summarized in the tables (Tables 1 and 2). By definition, all patients had absent Kx RBC antigen and weak Kell antigens. In three individuals, including the initial index patient, this McLeod blood group phenotype had been incidentally detected in blood banks (cases 2, 8 and 9). In standard blood smears, significant RBC acanthocytosis of more than 5% was found in three patients (cases 6–8). Five additional patients also showed acanthocytosis but less than 5% (cases 1, 3–5 and 10). In two individuals, no acanthocytes were detected (cases 2 and 9). In two patients (cases 4 and 5), a standardized method was applied to increase the sensitivity of acanthocyte determination by using a dilution with 1:1 isotonic saline and phase contrast microscopy, thus considerably increasing acanthocyte numbers (Storch *et al*., 2005). Serum CK levels were elevated in eight patients, ranging from 300 to 3000 U/L. Case 7 experienced an episode of massive rhabdomyolysis possibly related to neuroleptic medication, with CK levels up to 160820 U/l and consequent renal failure (Jung and Brandner, 2002). Case 9 had serum CK levels at the upper limit of the normal range (298 U/L; normal for the laboratory < 300) and case 2 had entirely normal levels.

These latter two individuals did not show CNS or neuromuscular symptoms at the last follow-up examination at the age of 57 years (case 2) and 52 years (case 9), respectively. Age of onset of CNS or neuromuscular symptoms in the remaining patients ranged between 23 and 58 years (mean 39 years). Only one patient presented with a choreatic movement disorder (case 5). During follow-up, however, seven patients developed chorea that mainly affected trunk and arms (cases 1, 4–8 and 10). Additional movement disorders included facial dyskinesia (cases 1, 4–6 and 10), involuntary vocalizations (cases 1, 4, 5 and 10), dysarthria (1, 4, 5 and 7), dystonia (6 and 10) and involuntary tongue and lip biting (case 10). Three patients presented with psychiatric manifestations (schizophrenia in cases 4 and 7; personality disorder in case 6), and three further patients developed psychiatric disorders during the disease course (paranoid delusion in case 1;

obsessive-compulsive disorder in case 5; schizophrenia, compulsion and personality disorder in case 10). There was no clinical evidence for tardive dyskinesia in any patient: In one patient (case 5), the choreatic movement disorder was present before the onset of the psychiatric disorders. Neuroleptic treatment was only initiated after onset of the movement disorder (case 4) or was not used at all (cases 1, 8 and 10). In a further case (case 7), typical chorea without features of tardive dyskinesia appeared with a longer delay of several months after the use of clozapine with gradual progression.

Whereas no patient had cognitive impairment at the initial examination, in five patients (cases 1, 4, 6, 7 and 10) it subsequently developed to a variable degree, with a mean onset age of 48 years. Generalized seizures were the presentation of two patients (cases 1 and 10), and occurred during follow-up in a third patient at the age of 56 years (case 4).

EEG was abnormal in only one of five patients examined (case 10). Cerebral imaging was normal in two patients (cases 3 and 10) but caudate atrophy of variable degree with consequent enlargement of the lateral ventricles was found in the remaining eight cases studied. One patient each had additional atrophy of putamen (case 6) or mild generalized cerebral atrophy (case 8). IBZM–SPECT demonstrated decreased striatal D2-binding in one patient (case 1) and was normal in another patient (case 3). ECD–SPECT and FDG–PET showed reduced striatal tracer uptake in six patients (cases 1 and 3–7). One individual had a normal FDG–PET (case 9) (Jung *et al.*, 2003).

Only one patient had a neuromuscular presentation (case 3), but neuromuscular features developed in altogether eight cases during the disease course. Weakness and atrophy affected mainly the legs and were distally pronounced. Two patients had considerable walking problems due to pronounced leg weakness (cases 4 and 10) and two additional patients (cases 6 and 8) had moderate weakness and atrophy of their lower limbs. Four patients had mild lower limb weakness and atrophy (cases 3, 5 and 7). Generalized areflexia was noted in eight patients, and lower limb areflexia was found in an additional patient (Table 1). Sensory findings were present in 4 patients (cases 1, 4, 5 and 10).

EMG demonstrated myopathic alterations in three patients (cases 4, 5 and 10), neurogenic alterations in 2 patients (case 8 and 10), and was normal in two patients (cases 1 and 3). NCV studies demonstrated axonal changes in 4 patients (cases 1, 3, 8 and 10). The two patients with myopathic EMG alterations had normal NCV studies (cases 4 and 5). Lower leg MRI was reported with muscle atrophy and fatty degeneration in the index patient (case 8).

The index patient had clinical signs and symptoms suggesting chronic heart failure. ECG demonstrated atrial fibrillation in two patients (cases 1 and 8) and was normal in the remaining eight patients. Echocardiography was normal in two of five patients examined (Table 2). Three patients (cases 1, 7 and 8) had left ventricular systolic dysfunction of variable degree, and one of them (case 7) also had left ventricular hypertrophy. The index patient (case 8) had asymmetric dilatation of the left atrium and the right ventricle (Table 2).

Five patients died because of MLS-related complications including sudden cardiac death (cases 1, 6 and 7), chronic heart failure (case 8), and pneumonia (case 4) between 55 and 69 years (mean age at death 59.6 years; mean disease duration 23.4 years).

Molecular genetic analysis

Sequence analysis of the *XK* gene was performed in all but one patient (case 10; Table 2). A major deletion including the entire *XK* gene was found in one patient (case 1), and minor deletions in four other patients (cases 3–5, 8). One patient had a splice-site mutation (case 2). Two brothers of the Chilean family that originated from Germany had a 4 bp deletion leading to a frame shift and a premature stop codon (cases 4 and 5). One patient had a 2 bp deletion leading to a frame shift and a premature stop codon (case 3). Two Swiss cousins had a nonsense mutation leading to a premature stop codon (cases 6 and 7). All these mutations predicted an absent or truncated XK protein devoid of the Kell-binding site. One patient with absent CNS and neuromuscular symptoms had a missense mutation (case 9) (Jung *et al.*, 2003). Except for the mild phenotype in the patient with the missense mutation, there was no clear correlation between clinical symptoms and the position of the mutations in the gene.

Muscle histology

Sufficient preservation of muscle tissues was demonstrated by normal spectrin immunohistochemistry in all muscles. No structural abnormalities within muscle fibres were observed. In particular, no vacuoles or inclusions were present.

Enzyme histochemistry (COX, NADHD, SDH) showed normal intensity as well as regular patterns of staining. There was no evidence for accumulation of either glycogen (PAS) or neutral lipids (Oil red O). Patterns and intensities of dystrophin immunohistochemistry were normal in all muscles analyzed.

Alterations suggesting a myopathic process were not frequent and-if present-not pronounced (Fig. 1). The most common finding was an increase in the number of internalized nuclei. Three muscles (cases 6, 8 and 10) demonstrated a clearly increased number of internalized nuclei above 15%. One muscle (case 1) yielded borderline abnormalities, with a fraction of 6% internalized nuclei. The remaining muscles showed values of 5% or less (Table 3). The three muscles with clearly increased numbers (cases 6, 8 and 10) also showed increased fibre size variation, and mild endomysial fibrosis was present in one muscle (case 8). Only one muscle (case 6) had few

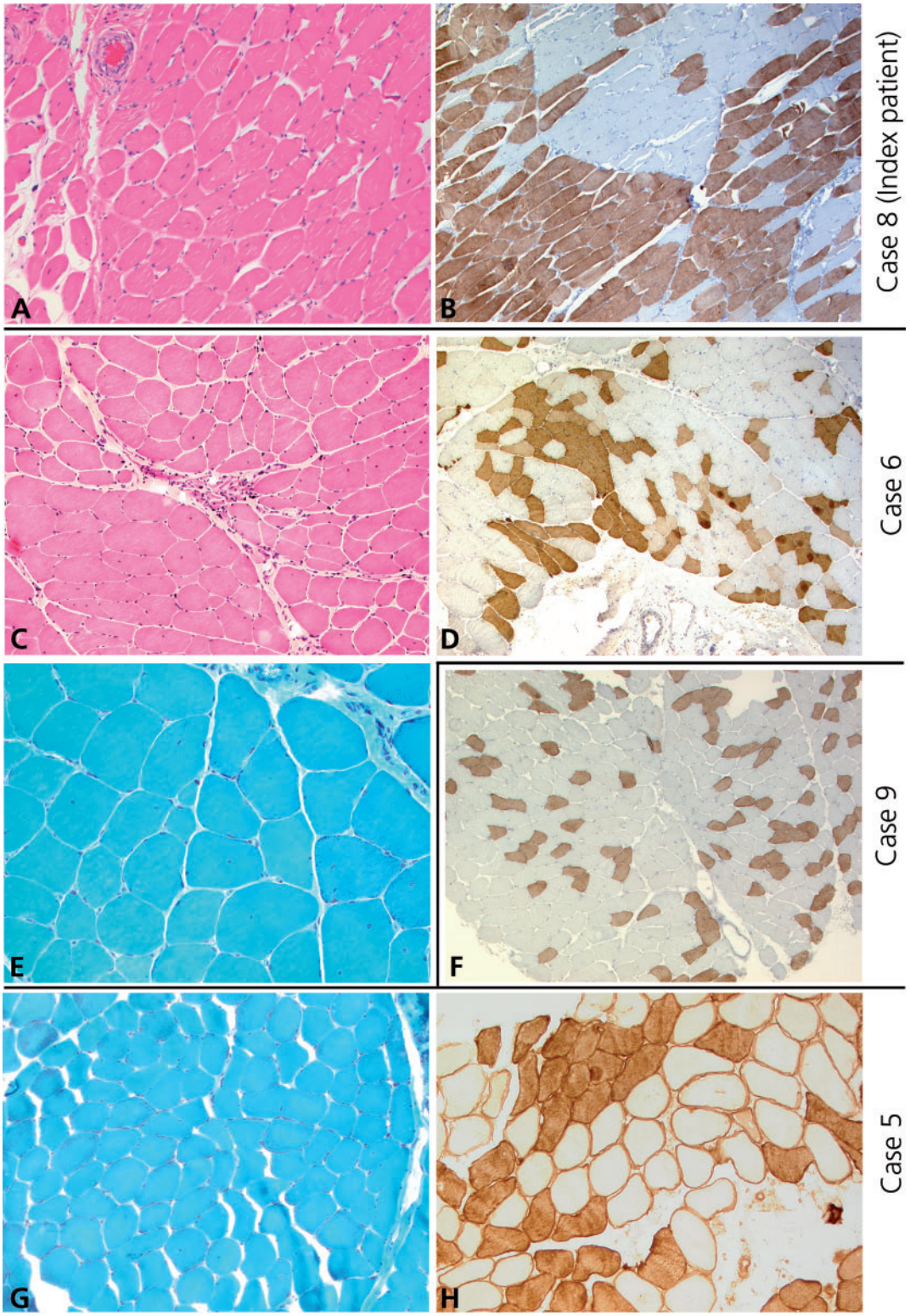

Fig. 1 Muscle histology (**A**, **B**) The muscle autopsy of the index patient (case 8) demonstrates fibre-type grouping and numerous internalized nuclei, while atrophic fibres were not frequently seen. (**C**–**E**) The muscle biopsy of case 6 shows the most pronounced pathology of the present series with groups of atrophic and angulated fibres, some fibre-type grouping and frequent internalized nuclei. However, the morphology of the individual muscle fibres was normal. E, F: The muscle biopsy of case 9 shows a predominance of type 2 fibres, without additional abnormal findings. (**G**, **H**) The muscle biopsy of case 5 shows moderate pathology: While conventional morphology was unremarkable and in particular atrophic fibres were absent, there was clear fibre-type grouping (Stainings: A, C–H&E; B, D, F–immunohistochemistry for type I fibres-myosin heavy chain slow isoform-MHCs; E, G: trichrome; double immunohistochemistry for MHCs and Dystrophin).

CD45-positive leukocytes without evidence of polymyositis. Signs of necrosis were not seen in any of the samples.

By contrast, all muscles displayed signs of an acute or chronic neurogenic process with fibre type grouping of variable degree that ranged from a few groups of both types of fibres to an almost complete loss of the normal checkerboard pattern in others (Table 3). In four muscles, groups of atrophic fibres of variable extent as well as occasional angulated fibres were seen (cases 3, 6–8). In two muscles (cases 7 and 8), atrophic fibres belonged to both fibre types. One sample each showed predominant atrophy of type 1 (case 7) or type 2 fibres (case 3), respectively. Although earlier reports suggested a predominance of type 1 fibres as a feature of neuromuscular pathology in the McLeod syndrome, six muscles in our series (cases 1–3, 7, 8 and 10) showed an equal representation of both fibre types. Two samples each showed predominance of type 1 fibres (cases 5 and 7), and type 2 fibres (cases 4 and 9), respectively (Table 3). The results of the morphometric analysis are listed in Table 4, demonstrating normal mean fibre diameters in all muscles, increased variability of fibres diameters of type 1 and type 2 fibres in 6 muscles (cases 1, 3, 4, 6, 7 and 10), pathological atrophy coefficients in 2 muscles only for type 2 fibres (cases 6 and 7), and pathological hypertrophy coefficients of type 1 and type 2 fibres in five muscles (cases 2–4, 7 and 8; Table 4).

The samples of the two patients without evidence of CNS or neuromuscular involvement (cases 2 and 9) showed only minimal alterations (fibre type grouping in case 2; fibre type 2 predominance in case 9). Remarkably, the patient with the deletion of the entire *XK* gene (case 1) showed only moderate neurogenic alterations, and there were no myopathic changes. The two patients with pronounced walking difficulties (cases 4 and 10) had only moderate neurogenic alterations in the muscle biopsy. However, this might be misleading since the weakness was distally pronounced, and biopsies were performed in the quadriceps muscles in both patients.

Ultrastructural muscle pathology

None of the three samples available (cases 6, 7 and 9) showed alterations of the sarcomeres or any of the other muscle fibre compartments. In two muscles (cases 6 and 9), however, occasional defects of the sarcolemma were seen. Since the same observation could be made after rotation of the section, this could not been simply attributed to tangential cutting (Fig. 2). In the third sample (case 7), obtained at autopsy, preservation of the sarcolemma was insufficient to arrive at unequivocal conclusions.

Nerve pathology

Cross sections of sural and femoral nerves (case 7) or sural and peroneal nerves (case 8) showed a pronounced loss of myelinated fibres and both endoneural and perineural fibrosis (Fig. 3). Digestion chambers that may indicate recent axonal degeneration were not frequent. Onion bulbs, a sign of primary demyelination, were not seen. Electron microscopy (case 7, sural nerve) occasionally showed sheets of Schwann cell processes devoid of axons, indicating additional loss of unmyelinated axons, but ultrastructural evidence of a primary demyelination was absent.

Table 4 Morphometry

	Mean fibre diameter (μm)		Variability coefficient		Atrophy coefficient		Hypertrophy coefficient	
Patient	Type 1	Type 2	Type 1	Type 2	Type 1	Type 2	Type 1	Type 2
1	56.4	59.8	**264**	**289**	115	188	115	188
2	69.9	77.3	237	179	52	0	**397**	**667**
3	84.7	69.8	245	**303**	30	103	**1403**	**641**
4	86.8	66.9	**260**	216	38	24	**1654**	229
5	64.2	51.1	185	181	27	160	125	0
6	53.4	45.2	**281**	**370**	254	**644**	48	68
7	60.0	59.7	**265**	**463**	132	**414**	150	**672**
8 (Index)	69.1	62.6	232	192	32	34	**372**	86
9	54.2	61.2	161	192	0	22	0	65
10	50.8	53.4	**263**	220	297	137	31	0

Mean fibre diameter, variability, atrophy and hypertrophy coefficients were calculated for type 1 and type 2 fibres, respectively. Pathological values are marked in bold.

Discussion

Out of the 10 McLeod patients from eight families, only two presented with neuromuscular symptoms. Initial serum CK levels, by contrast, were clearly elevated in eight patients, and all these successively developed weakness and atrophy with distal and leg predominance and areflexia was found at least of the lower limbs. Proximal muscles as well as distal arm muscles were much less severely affected. The remaining two patients showed a 'McLeod phenotype without the McLeod syndrome' (Jung *et al.*, 2003; Walker *et al.*, 2007*a*), and were free of neuromuscular symptoms until their sixth decade. In our cohort of McLeod patients, the proportion of patients who developed muscle weakness and atrophy was 80% which exceeds the 59–68% reported earlier (Danek *et al.*, 2001). The proportion of 40% patients with sensory signs or symptoms in the present study is within the range of the previously describe rate of 27–59% (Danek *et al.*, 2001). Thus, subtle neuromuscular features might have been overlooked in the past.

Predominant neurogenic alterations were found in the muscle samples of all our patients. Myopathic changes, were present in 4 out of 10 patients, but were less prominent than neurogenic alterations. Although the myopathic alterations might be secondary to the neurogenic process, highly elevated CK levels in some patients and rhabdomyolysis in one patient point towards an additional myopathy. Based on the pathological findings in our cohort, however, the myopathy is far less prominent. The significance of the ultrastructural alterations of the sarcolemma remains unclear but suggests a defective

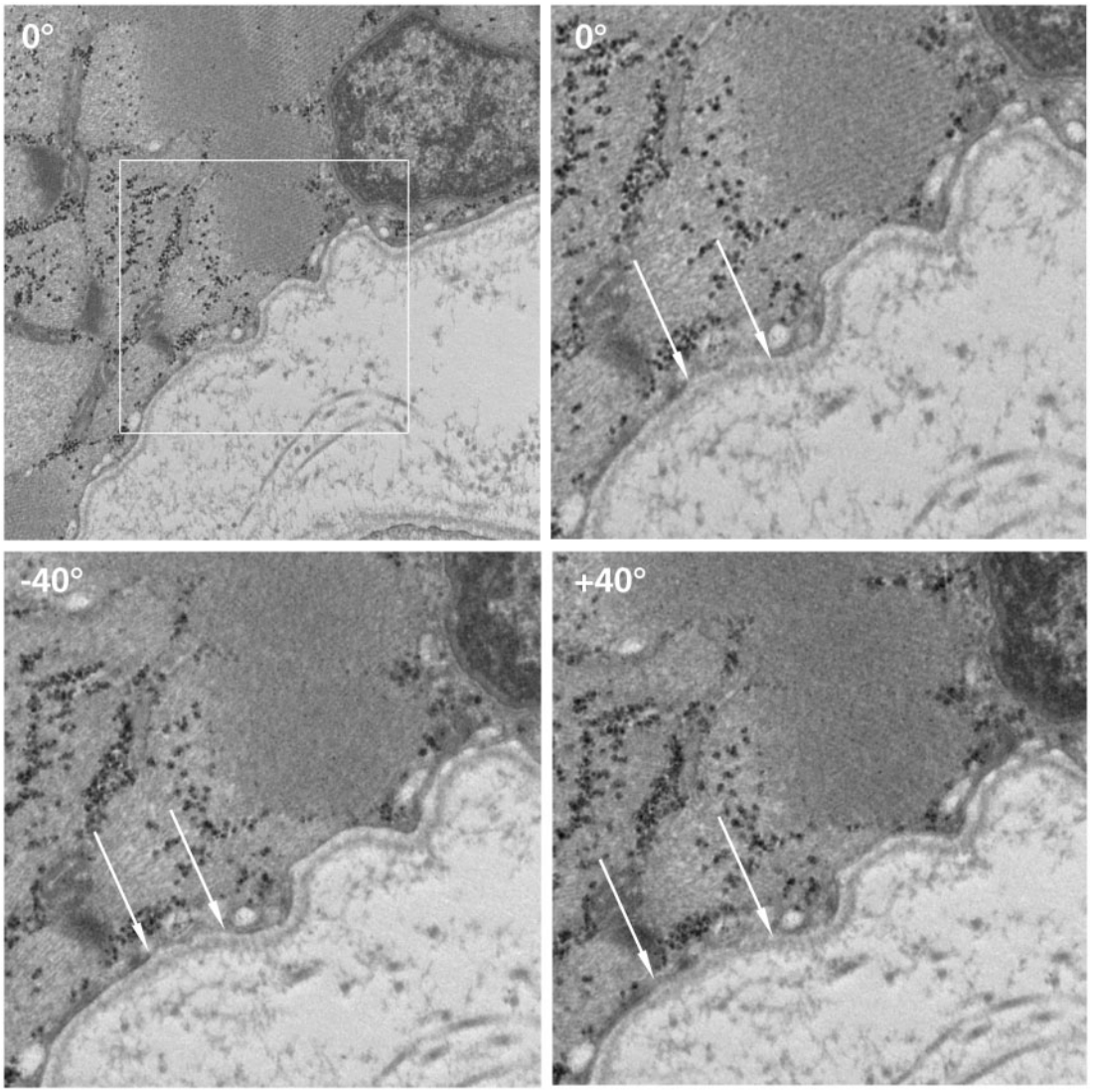

Fig. 2 Muscle ultrastructure. Muscle ultrastructure was normal except for occasional sarcolemma defects, which remained unchanged after goniometric analysis (i.e. rotation of the section by various angles; −40° −0° −+40°). The arrows indicate borders between normal sarcolemma and defects.

plasma membrane. None of our patients had relevant inflammatory changes as reported previously in a single case (Barnett *et al.*, 2000). Myopathic EMG alterations did not predict myopathic findings upon muscle biopsy. Presentation with neuromuscular symptoms and pronounced atrophy, on the other hand, was not clearly associated with more pronounced neurogenic alterations. The two patients with pronounced walking difficulties had only moderate neurogenic alterations in the muscles. This might be explained by the fact that the weakness in these patients was prominent in distal muscles but biopsy tissues were taken from the less severely affected, proximal muscles.

Previous reports of MLS muscle histology documented a variety of unspecific changes such as type 1 fibre predominance and type 2 fibre atrophy, but also clearly myopathic changes such as increased fibre size variability and increased numbers of centralized nuclei as well as fibre type grouping that is usually considered as a reinnervation phenomenon due to axonal neuropathy (Swash *et al.*, 1983; Witt *et al.*, 1992; Malandrini *et al.*, 1994; Jung *et al.*, 2001*b*; Dotti *et al.*, 2004). The latter had been previously interpreted as a myopathic finding in resemblance to findings in female carriers of Duchenne muscular dystrophy (Witt *et al.*, 1992).

In line with previous observations, EMG demonstrated myopathic as well as neurogenic changes in the majority of patients (Danek *et al.*, 2001). NCV studies were normal in the two patients with purely myopathic EMG alterations, but indicated sensory and motor axonopathy in all patients with neurogenic EMG as well as in two patients with normal EMG. In accordance with these findings, NCV studies from the literature regularly suggested a mixed axonal-demyelinating neuropathy (Malandrini *et al.*, 1994;

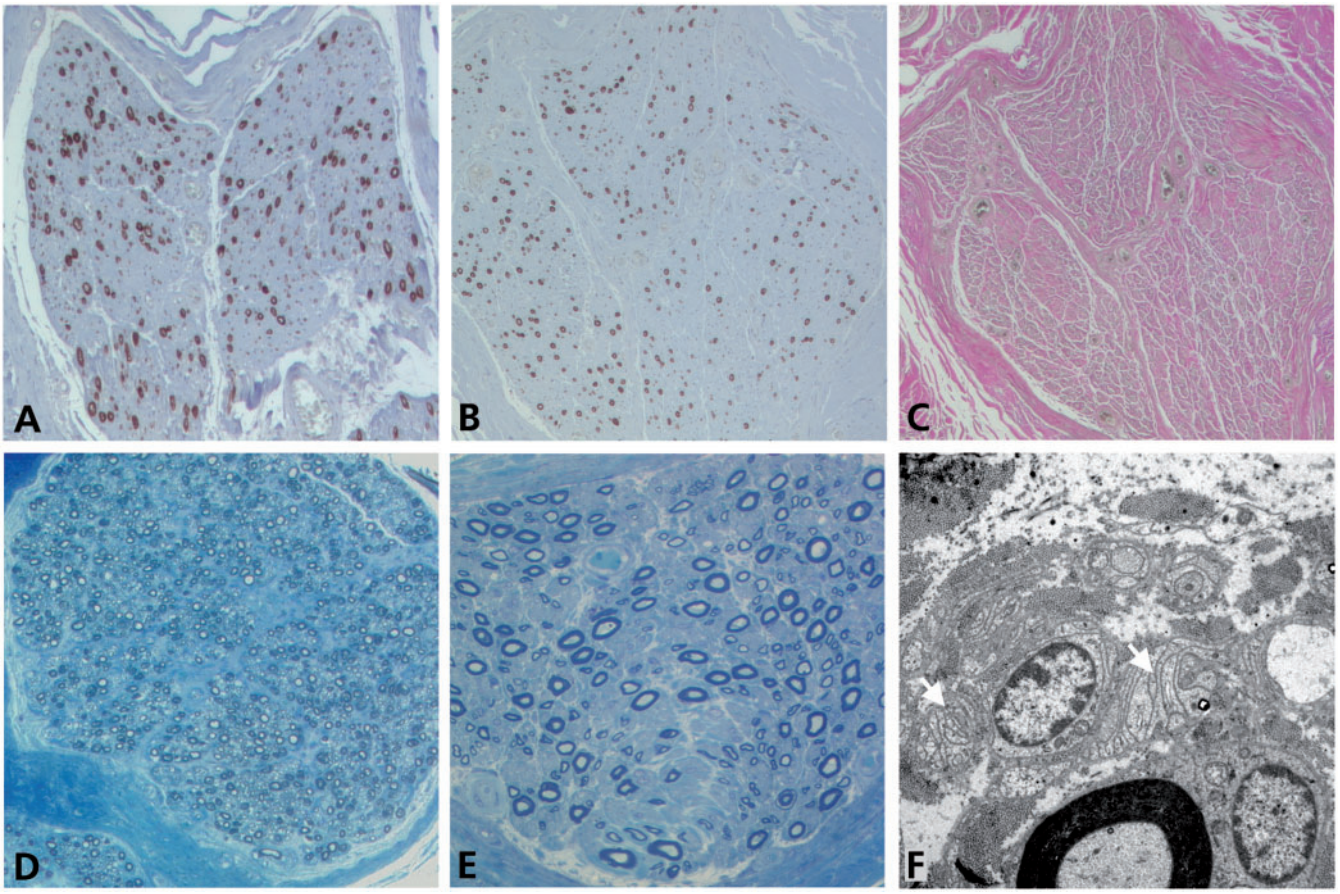

Fig. 3 Nerve pathology. The peroneal (**A**) and sural (**B**) nerves of case 8 show a marked loss of myelinated axons (MBP). In addition, EvG staining demonstrates a pronounced endo- and perineural fibrosis (**C**). Semithin sections of the femoral nerve of case 7 show a moderate loss of myelinated axons (**D**), which was more pronounced in the sural nerve (**E**). Ultrastructurally, no overt abnormalities of the remaining myelinated axons were observed in the sural nerve (**F**). However, there were sheets of empty Schwann cell processes, indicating additional loss of unmyelinated axons (F, arrow).

Danek *et al.*, 2001; Dotti *et al.*, 2004). In contrast to the unspecific sural nerve findings from single cases in the literature, however, the samples available here give clear structural evidence of a sensory-motor axonopathy in MLS. Since spinal cord tissue was not available, we cannot decide about an underlying anterior horn cell pathology. In summary, electrophysiological as well as muscle and nerve pathology findings in McLeod syndrome demonstrate predominant neurogenic alterations underlying the clinically observed areflexia, weakness and atrophy, yet lacking disease-specific structural features. These findings strongly support a motor neuropathy as the predominant cause of weakness and atrophy in MLS, rather than a myopathy.

The majority of our patients developed a syndrome similar to Huntington's disease with chorea, psychiatric manifestations and cognitive decline (Danek *et al.*, 2001; Jung *et al.*, 2001*a*). Involuntary tongue and lip biting, a hallmark of autosomal recessive chorea-acanthocytosis (Walker *et al.*, 2007*b*), was observed in only one patient. Psychiatric disorders represented the initial manifestation in three of our patients, and developed in more than half of the patients during the disease course. Cognitive abnormalities, not evident initially, developed during the disease course in almost half the patients and were regularly associated with psychiatric manifestations. Three patients (30%) of our cohort had epilepsy, a proportion at the lower range of the previously estimated prevalence of 27–73% (Danek *et al.*, 2001) The clinical characteristics of the two pairs of patients harbouring the same mutation (cases 4 and 5 and 6 and 7, respectively) demonstrated a considerable intrafamilial phenotypic variability. The follow-up of the present cohort covered a mean of 15 years. It clearly indicates a slow progression of the disease as documented in the typical case of the eponymous index patient. Also based on our previous observations, we propose that there might be a complete clinical penetrance of MLS by the age of 60 years (Danek *et al.*, 2001; Walker *et al.*, 2007*c*). Five patients died because of MLS-related complications at a mean age of 60 years and mean disease duration of over 20 years because of probable sudden cardiac death, chronic heart failure and pneumonia indicating a clearly reduced life-expectancy.

The majority of our patients had low acanthocyte counts. Late appearance or even absence of acanthocytosis in

neuroacanthocytosis syndromes has been reported previously (Sorrentino *et al*., 1999) Acanthocyte determination after dilution of the blood with 1:1 heparinized saline, however, raised the sensitivity considerably, also in two of our patients available for this examination (Storch *et al*., 2005). This underlines the importance to use such a validated method in the evaluation of chorea patients. Molecular genetic analysis of the *XK* gene revealed a causative mutation in all cases available. As already noted in the past, the vast majority of McLeod patients have loss of function mutations in the *XK* gene (Ho *et al*., 1994; Walker *et al*., 2007*a*). In such patients of our cohort, no clear phenotype-genotype correlation was possible. In fact, the patient with a large deletion of the entire *XK* gene lacked pronounced neuromuscular symptoms and CNS symptoms were not worse than in other patients. On the other hand, missense or splice-site mutations in the *XK* gene can be associated with only a mild phenotype and even absence of symptoms for decades. One oligosymptomatic patient carrying the R222G missense mutation that allows XK protein expression in cell culture but leads to its absence on RBC's (Walker *et al*., 2007*a*), had clearly elevated CK levels suggesting subclinical neuromuscular involvement. Another individual with the E327K missense mutation and serum CK levels at the upper limit of normal did not show any cerebral pathology based on MRI and FDG–PET (Jung *et al*., 2003). Few other McLeod patients with *XK* missense mutations are known. One was associated with the prototypic neuroacanthocytosis phenotype including chorea, cognitive decline, and generalized seizures after the age of 44 years (Danek *et al*., 2001), another showed only moderate suppression of Kell antigens compared to XK nonsense mutations (Russo *et al*., 2002).

Our data delineate MLS as a deleterious basal ganglia disorder that resembles Huntington's disease. Weakness and atrophy are common and may seriously interfere with mobility. Although myopathic alterations may occur, the predominant neurogenic changes on muscle biopsy as well as the neurophysiological findings suggest that muscle weakness and atrophy are mostly due to motor neuropathy. The euphemistic designation as 'benign X-linked myopathy with acanthocytes' is misleading. MLS clearly is not a pure myopathy nor is it benign and it should be recognized as a potentially life-threatening neurodegenerative condition.

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