REVIEW

McLeod syndrome: a neurohaematological disorder

H. H. Jung,[1] A. Danek[2] & B. M. Frey[3]

[1]*Department of Neurology, University Hospital Zürich, Zürich, Switzerland*
[2]*Department of Neurology, Hospital of the University of Munich Großhadern, Ludwig-Maximilians-Universität, Munich, Germany*
[3]*Regional Blood Transfusion Service, Swiss Red Cross, Zürich, Switzerland*

Vox Sanguinis

The X-linked McLeod syndrome is defined by absent Kx red blood cell antigen and weak expression of Kell antigens, and this constellation may be accidentally detected in routine screening of apparently healthy blood donors. Most carriers of this McLeod blood group phenotype have acanthocytosis and elevated serum creatine kinase levels and are prone to develop a severe neurological disorder resembling Huntington's disease. Onset of neurological symptoms ranges between 25 and 60 years, and the penetrance of the disorder appears to be high. Additional symptoms of the McLeod neuroacanthocytosis syndrome that warrant therapeutic and diagnostic considerations include generalized seizures, neuromuscular symptoms leading to weakness and atrophy, and cardiopathy mainly manifesting with atrial fibrillation, malignant arrhythmias and dilated cardiomyopathy. Therefore, asymptomatic carriers of the McLeod blood group phenotype should have a careful genetic counseling, neurological examination and a cardiologic evaluation for the presence of a treatable cardiomyopathy.

Key words: cardiopathy, chorea, McLeod blood group phenotype, myopathy, neuroacanthocytosis syndrome, neurodegeneration.

Introduction

McLeod syndrome (MLS) is an X-linked multisystem disorder that is assigned to the neuroacanthocytosis syndromes (see Table 1 [1,2] and http://www.geneclinics.org/profiles/mcleod). Haematologically, MLS is characterized by the absence of Kx red blood cell (RBC) antigen, weak expression of Kell RBC antigens, acanthocytosis and compensated haemolysis [3]. Asymptomatic carriers of the McLeod blood group phenotype may be accidentally recognized by blood bank testing [3,4]. All McLeod carriers reported up to date have elevated serum creatine kinase (CK) levels. They may develop neurological signs and symptoms with a mean onset age from 30 to 40 years, and high penetrance of the disorder [4–6]. Neuromuscular manifestations include myopathy, sensory-motor axonal neuropathy and cardiomyopathy [1]. Central nervous system (CNS) manifestations are similar to that of Huntington's disease, and consist of a choreatic movement disorder,

'subcortical' neurobehavioural deficits, psychiatric abnormalities and generalized seizures [1,2]. McLeod syndrome is caused by mutations of the *XK* gene encoding the XK protein, which carries the Kx RBC antigen [7]. Although the exact function of the human XK protein is not yet known, available data suggest an important role for apoptosis regulation. Thus, MLS might be a model disorder to study principal mechanisms that are not only involved in RBC physiology but also in neurodegeneration.

The Kell blood group system

The Kell system was first identified in 1946 by Coombs with the help of his newly developed antiglobulin test [8]. Rhesus incompatibility with the mother, Mrs Kell, had been ruled out as the cause of haemolytic disease in her child. Subsequently, Kell became the third most important blood group system after the ABO and rhesus systems.

The McLeod phenotype of the Kell system was detected by routine screening for allogenic antibodies at Harvard University blood bank in 1960 [3]. The RBCs of the propositus, Hugh McLeod, showed an unexplainably weak reactivity to Kell antisera. Immunohaematologically, the McLeod blood group

Correspondence: Hans H. Jung, Department of Neurology, University Hospital Zürich, Frauenklinikstrasse 26, 8091 Zürich, Switzerland
E-mail: hans.jung@usz.ch

Core neuroacanthocytosis syndromes

    Chorea-acanthocytosis (ChAc)

    McLeod syndrome (MLS)

    Huntington's disease-like 2 (HDL2)

    Pantothenate kinase associated neurodegeneration (PKAN; including

    HARP subtype)

Neuroacanthocytosis with lipoprotein disorders

    Abetalipoproteinemia (Bassen–Kornzweig syndrome)

    Familial hypobetalipoproteinemia

    Anderson disease

    Atypical Wolman disease

Acanthocytosis in systemic diseases where neurological findings may also be present

    Severe malnutrition (e.g. anorexia nervosa)

    Cancers, sarcoma

    Thyroid disorders, myxoedema

    Splenectomy

    Liver cirrhosis, hepatic encephalopathy

    Psoriasis

    Eales' disease

    MELAS

HARP, hypoprebetalipoproteinemia, acanthocytosis, retinitis pigmentosa, pallidal degeneration; MELAS, mitochondrial encephalopathy with lactic acidosis and stroke-like episodes.

phenotype was later characterized by absent expression of Kx RBC antigen, weak expression of Kell glycoprotein RBC antigens and X-linked inheritance [9]. Subsequently, the McLeod blood group phenotype was also observed in boys with X-linked chronic granulomatous disease (CGD) as part of a contiguous gene syndrome [10].

Molecular basis

The Kell protein is a 93-kDa glycoprotein that is encoded by the *KEL* gene on chromosome 7q34. *KEL* contains 19 exons and shares a consensus sequence with a large family of zinc-dependent endopeptidases [11]. The Kell glycoprotein is a type II red cell membrane protein with a short intracellular amino terminal, a single transmembrane and a large extracellular domain [12]. It shows substantial homology with the M13 subfamily of mammalian neutral endopeptidases, including endothelin converting enzyme-1 (ECE1; 600423). ECE-1 converts big endothelin-3 into endothelin-3, the bioactive peptide [11]. Its extracellular conformation is stabilized by seven intramolecular disulphide bonds preserving endopeptidase activity of KEL protein as well as most of the KEL antigens.

The *XK* gene contains three exons and is located on Xp21·1. It shares important homologies with the ced-8 protein of the nematode *Caenorhabditis elegans* where it

controls the timing of programmed cell death [13]. The XK protein is predicted to have 10 transmembrane domains and shows structural characteristics of procaryotic and eucaryotic membrane transport proteins [7]. The XK protein carries the Kx RBC antigen [14] and is linked to the Kell glycoprotein by a single disulphide bond (XKcys347-KellCys72) [15].

Databank analysis demonstrated that the human XK gene belongs to a gene family with several different members [16]. Sequence similarity searches identified a family of nine full-length human genes related to ced-8, as well as a series of eight Y chromosome-linked partial sequences. Sequence comparisons confirmed that previously identified highly conserved motifs in ced-8 and XK are shared with the related genes. Several residues in these motifs were identical between ced-8 and other family members, but not conserved with XK [17]. Recently, two cDNAs, XPLAC and XTES, have been cloned. XPLAC, like XK, has three exons and is located on X chromosome at q22·1, while XTES has four exons and is located at 22q11·1. While XK is ubiquitously expressed, XPLAC predominantly in placenta and adrenal gland, and XTES is exclusively expressed in primate testis [16].

Several studies demonstrated that XK and Kell are co-expressed in erythroid tissue [7,16,18,19]. In the erythroid tissue, Kell and XK most probably formed a functional complex, as Kell antigen expression, as well as Kell protein density on the RBC membrane, was dependent on the expression of XK protein [20,21]. In other tissues, however, the Kell and XK protein had a differential expression pattern. In skeletal muscle, there was no colocalization of Kell and XK [22]. In rodent and human brain, XK was expressed in intracellular compartments of neurons, whereas Kell expression was restricted to RBCs in cerebral vessels [19]. Another study showed that XK, but not Kell, was significantly expressed in brain, spinal cord, muscle, heart, small intestine, stomach, bladder and kidney [23]. In brain, XK was predominantly expressed in neuronal cells [23]. Co-expression of Kell and XK in erythroid tissues and the different expressions in non-erythroid tissues suggests that XK may have a complementary haematological function with Kell and a separate role in other tissues.

Immunohaematology

At present, 35 mutations of the *KEL* locus are characterized [24]. At the moment, the International Society of Blood Transfusion (ISBT) recognizes 31 distinct antigens of the Kell system, including four pairs of antithetical antigens and one triplet, 18 antigens with high frequency and 10 of low frequency (Table 2). According to the ISBT, the Kell antigens are numbered from K1 to K31, including two obsolete antigens [25]. Most KEL blood group polymorphisms are located in the extracellular, amino-terminal half of the protein (exon 6–10) while the carboxy-terminal half of the protein (exon 11–19)

Table 2 Antigens of the Kell blood group system as recognized by the ISBT (October 2006)

Number	ISBT nomenclature	Symbol Generic name	Mutation	Exon	AA	Prevalence	Antithetic
001	KEL1	K, Kell, K1	T698C	6	Thr193Met	Low	1
002	KEL2	k, Cellano, K2	C698T	6	Met193Thr	High	1
003	KEL3	Kp^a, K3	T961C	8	Arg281Trp	Low	2
004	KEL4	Kp^b, K4	C961T	8	Trp281Arg	High	2
005	KEL5	Ku, K5	complex	n.d.	n.d.	High	
006	KEL6	Js^a, Sutter a, K6	C1910T	17	Leu597Pro	Low	3
007	KEL7	Js^b, Sutter b, K7	T1910C	17	Pro597Leu	High	3
008	obsolete	Kw	n.a.	n.a.	n.a.	n.a.	
009	obsolete	KL	n.a.	n.a.	n.a.	n.a.	
010	KEL10	Ul^a, K10	T1601A	13	Glu494Val	Low	
011	KEL11	Côté, K11	T1025C	8	Val302Ala	High	4
012	KEL12	Boc, K12	A1763G	8	His548Arg	High	
013	KEL13	SGRO, K13	T1106C	9	Leu329Pro	High	
			G1716A	15	Trp532Stop		
014	KEL14	Scan, K14	G659C	6	Arg180Pro	High	5
015	XK019001	Kx	n.a.	n.a.	n.a.	High	
016	KEL16	k-like, K16	n.d.	n.d.	n.d.	High	
017	KEL17	Wk^a, K17	C1025T	8	Val302Ala	Low	4
018	KEL18	K18	C508T	4	Arg130Trp	High	
			G509A		Arg130Gln		
019	KEL19	K19	G1595A	13	Arg492Gln	High	
020	KEL20	Km, K20	n.d.	n.d.	n.d.	High	
021	KEL21	Kp^c, Levay, K21	A962G	8	Arg281Gln	Low	2
022	KEL22	N.I., Ikar, K22	C1085T	9	Ala322Val	High	
023	KEL23	Centauro, K23	G1265A	10	Arg382Gln	Low	
024	KEL24	CL, Callais, Cls, K24	C659G	6	Pro180Arg	Low	5
025	KEL25	VLAN	A863G	8	Gln248Arg	Low	
026	KEL26	TOU	G1337A	11	Arg406Gln	High	
027	KEL27	RAZ	G865A	8	Glu249Lys	High	
028	KEL28	VONG	n.d.	n.d.	n.d.	n.d.	
029	KEL29	KALT	G1988A	17	Arg623Lys	High	
030	KEL30	KTIM	G1033A	8	Asp305Asn	High	
031	KEL31	KYO	n.d.	n.d.	n.d.	n.d.	

n.d., not defined; n.a., not applicable.

is phylogenetically conserved preserving the endopepitase activity [12]. Some of the polymorphism are located in the active part of the protein, among those the antithetical pair K6/K7 (Js^a/Js^b), which is characterized by C1910T (Leu597Pro) in exon 17 [12]. However, a functionally active Kell glycoprotein for humans might not be essential as there are numerous K_0 phenotypes that do not exhibit clinical deficiencies.

Table 2 gives an overview of the currently recognized Kell protein antigens and their underlying molecular mechanisms and shows the systematic nomenclature of Kell antigens as suggested by the ISBT in perspective to the historical and generic nomenclature of the Kell antigens [25]. Besides the Kell protein antigen polymorphisms there are numerous mutations at the *KEL* locus that create a K_{Null} (K_0) phenotype. Most of these mutations are point mutations, frameshift

mutations or splice site mutations that lead to protein truncation or accelerated protein degradation [25–28]. In addition, several mutations in the coding sequence of *KEL* locus are associated with weak expression of the Kell epitopes. These phenotypes are assigned to K_{mod} phenotypes [29]. K_0 and K_{mod} phenotypes are characterized by strongly expressed Kx antigen. In contrast, McLeod phenotypes lack the Kx antigen and the RBCs present weak or missing expression of all Kell epitopes.

The formerly KEL15 (Kx) antigen has been regrouped into the new antigen system 019 (XK019001), as it was discovered that the XK protein is a product of the *XK* locus on chromosome Xp21·1. The XK protein carries only one single antigen, Kx. The absence of Kx reflects loss of XK protein on the RBC membrane, which might be due to mutational, transcriptional

or translational deficiency of the XK gene [30,31]. *XK* is located close to the locus of CGD, Duchenne muscle dystrophia and retinitis pigmentosa on the X chromosome Xp21·1. Its alteration is necessary but not predictive of the neurological manifestation of MLS [4,6,32–34]. Kx negativity of RBC reflects the serologically specific finding of the McLeod phenotype. The associated abortive expression of Kell protein, reflected by low or missing reactivity of all Kell antigens, serves as a major surrogate for the presence of dysfunctional *XK* mutations.

Transfusion hazards

Boys with X-linked chronic granulomatous disease (CGD) (see below) are at risk of developing anti-Kx antibodies, which may lead to serious transfusion hazards when receiving multiple transfusions [35]. Rarely, carriers of the McLeod phenotype without CGD may develop anti-Kx antibodies [36]. Therefore, transfusion hazards must be considered in all carriers of the McLeod phenotype due to allogenic antibody production, which occurs exclusively in male carriers of the McLeod mutation and requires previous exposure to Kx antigens as a result of homologous blood transfusion. Autologous blood banking for males with the McLeod blood group phenotype should be considered before carrying out elective surgical procedures that may possibly require transfusions. Female carriers of McLeod mutations are always hemizygous mutation carriers and therefore not prone to anti-Kx generation.

Laboratory findings

Most carriers of the McLeod blood group phenotype have significant RBC acanthocytosis (Fig. 1). However, examination for RBC acanthocytosis has to be done according to a standardized protocol with 1 : 1 dilution of the blood in heparinized saline and phase contrast microscopy [37]. Additional laboratory findings in most McLeod patients include compensated haemolysis and elevated serum CK levels [4–6,9,33].

Hepatosplenomegaly was found in about a third of McLeod patients and MLS may rarely present as hepatic disease [4,38]. More likely, hepatosplenomegaly is due to an MLS-associated extravascular haemolytic state. Absence of the XK protein in the RBC membrane leads to a lipid imbalance between the inner and outer leaflet of the RBC membrane involving phosphatidylserine (PS), which is normally enriched in the inner leaflet. Diminished PS in the inner leaflet causes shrinkage and acanthocytic deformation of the RBC [39]. Although McLeod RBC have normal adenosine triphosphate (ATP) levels, normal range of glycolytic and non-glycolytic enzymes, and normal osmotic fragility [39], they are eliminated from circulation more rapidly than normal RBC by the reticuloendothelial system (RES) due to
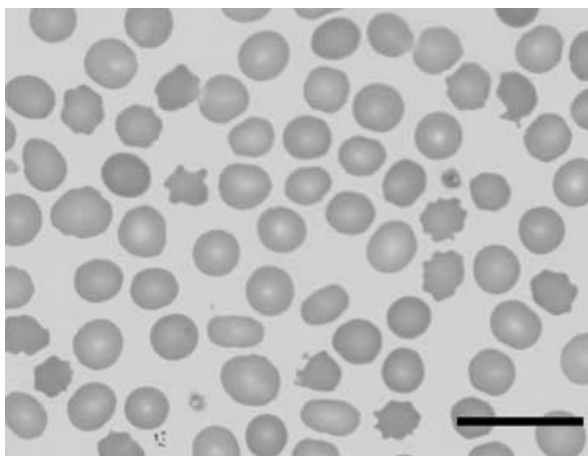
Fig. 1 Acanthocytes. Peripheral blood smear of the index patient showing significant acanthocytosis (May Gruenwald-Giemsa; ×100; scale bar = 25 μm). This patient manifested with a recurrent schizoid psychosis after the age of 39. A first neurological examination at the age of 48 revealed absent tendon reflexes and moderate cognitive deficits [6,57]. During the following years, a progressive choreatic movement disorder as well as leg and distally prominent weakness and atrophy developed. He died at the age of 55 because of sudden cardiac death.

overexpression of PS on the outer membrane leaflet [40–42]. Clinically, this leads to MLS-associated non-immune mediated, extravascular haemolysis. Maintenance of lipid bilayer asymmetry and normal RBC shape are dependent on ATP and Ca^{2+} consuming processes [43,44]. Therefore, the abnormalities of McLeod RBC might be explained by the absence of XK protein that may interfere with transmembrane transport of essential metabolites.

McLeod syndrome and chronic granulomatous disease

CGD is an inherited disorder of phagocyte function, characterized by recurrent bacterial and fungal infections and by granuloma [45]. Neutrophils, monocytes/macrophages and eosinophils of CGD patients cannot generate microbicidal oxygen metabolites due to a defect in any of the four components of the phagocyte nicotinamide adenine dinucleotide phosphate oxidase, namely, gp91-, p22-, p47- and p67-phox [46]. Three of these subtypes are autosomal recessive disorders, namely, cytochrome b negative CGD caused by mutations in the gene encoding p22-phox (CYBA; MIM-Nr. 233690), cytochrome b positive CGD type I caused by mutation in the NCF1 gene that encodes the p47-phox (phagocyte oxidase) protein (MIM-Nr. 233700), cytochrome b positive CGD type II caused by mutation in the NCF2 gene that encodes the p67-phox (phagocyte oxidase) protein (MIM-Nr. 233710). X-linked CGD is caused by mutations in the gene encoding p91-phox (CYBB; MIM-Nr. 306400). A vast number of different CYBB

mutations including deletions, insertions, missense, non-sense and splice site mutations have been identified to date [47]. Rarely, large deletions may cause a contiguous gene syndrome including the loci for Duchenne muscular dystrophy (MIM-Nr. 310200), X-linked retinitis pigmentosa (MIM-Nr. 300389), and the McLeod blood group phenotype [10,48,49]. Only a minority of boys with X-linked CGD carry the McLeod blood group phenotype. There are no reports demonstrating that these boys have CNS or neuromuscular manifestations of MLS, most probably because these manifestations have a late adult onset. However, these boys are at risk to develop anti-Kx antibodies, which may lead to serious transfusion hazards when receiving multiple transfusions as indicated above [35].

From haematology to neurology

Marsh and colleagues demonstrated that male carriers of the McLeod blood group phenotype have elevated serum levels of CK reflecting muscle cell pathology [50]. Subsequently, it was recognized that McLeod carriers had a 'neurological disorder characterized by involuntary dystonic or choreiform movements, areflexia, wasting of limb muscles, elevated CK, and congestive cardiomyopathy' [51]. This first short description of 1981 as well as the subsequent clinical observations defines the MLS as a multisystem disorder with haematological, neuromuscular and CNS involvement [4–6,52].

Central nervous system involvement

Central nervous system manifestations of MLS closely resemble Huntington's disease. Symptoms and signs comprise the prototypic triad of a progressive neurodegenerative basal ganglia disease including:
- choreatic movement disorder,
- 'subcortical' cognitive impairment, and
- psychiatric symptoms.

Movement disorder

Choreatic movements are the presenting sign in about 30% of McLeod patients [4–6,53]. Abnormal motor findings may be subtle such as a slight generalized restlessness with frequent changes of posture or with tic-like movements [4,6,53]. During the course of the disorder, however, the majority of patients develop chorea [4–6]. Additional involuntary movements include facial dyskinesia and dysarthria as well as involuntary vocalization [4–6,53]. There is considerable inter- and intrafamilial variability with respect to the type and the severity of the movement disorder [6]. In contrast to autosomal recessive chorea-acanthocytosis (ChAc; MIM 200150; Levine–Critchley syndrome), only very few McLeod patients have habitual lip or tongue biting, dysphagia, dystonia or extrapyramidal signs [2,4,5].

Cognitive impairment

Cognitive impairment is not a major presenting finding in MLS. During the course of the disease, however, about 50% of the patients show cognitive decline [4–6,54,55]. The data available suggest a 'subcortical' pattern of cognitive deficits similar to Huntington's disease [56]. Severity of cognitive alterations shows a remarkable intrafamilial variability and ranges from slight memory impairment to frank dementia [6].

Psychiatric abnormalities

About 20% of McLeod patients manifest with psychiatric abnormalities including personality disorder, anxiety, depression, obsessive-compulsive disorder, bipolar disorder, or schizoaffective disorder [4–6,57]. A psychiatric presentation may predominate in certain families [6] and develop in a majority of McLeod patients during the course of disease [4–6]. The spectrum of psychiatric abnormalities is comparable to other neurodegenerative basal ganglia disorders such as Huntington's disease [56,58].

Seizures

About 20% of McLeod patients may present with generalized seizures and up to 40% experience them during the course of disease, most probably with secondary generalization [4,5].

Neuroradiology

Computed tomography and magnetic resonance imaging studies demonstrate atrophy of caudate nucleus and putamen, particularly with advanced disease [4–6]. Exceptionally, there may also be white matter changes [59]. 18F-fluorodeoxyglucose (FDG)-positron emission tomography (PET) demonstrates impaired striatal glucose metabolism (Fig. 2) [6,60], but no impairment of glucose metabolism was found in the cerebral cortex by quantified FDG-PET [6]. Magnetic resonance spectroscopy demonstrates subtle metabolic abnormalities in different extrastriatal brain regions related to the psychiatric and cognitive findings [61].

Pathology

Neuropathological examination of one McLeod patient revealed marked neuronal loss and astrocytic gliosis in caudate nucleus and putamen. In contrast, no alterations were found in cortex, thalamus, subthalamic nucleus, brainstem and cerebellum [5,62]. Similar findings were observed in the exceptional case of a female *XK* mutation carrier who clinically manifested MLS (Case 5 of [5] and [63]). Although the prominent psychiatric and cognitive manifestations in McLeod patients indicate a significant and widespread

Fig. 2 Magnetic resonance imaging (MRI) and fluorodeoxyglucose-positron emission tomography (FDG-PET). (a) T1-weighted cerebral MRI was performed on a 1·5 Tesla MRI system (GE Medical Systems, Milwaukee, WI, USA) using routine parameters demonstrates only subtle atrophy of the head of the caudate nucleus. (b) FDG-PET of the same patient was performed on an Advance PET scanner (GE Medical Systems) after injection of 200 MBq [18F]-FDG (2-fluoro-2-deoxy-glucose, manufactured in-house). Emission scans demonstrated severe impairment of the FDG-uptake of the head of the caudate nucleus (CN) and less pronounced in the putamen (Pu). FDG-uptake in the thalamus (Th) and the cerebral cortex appears to be normal.
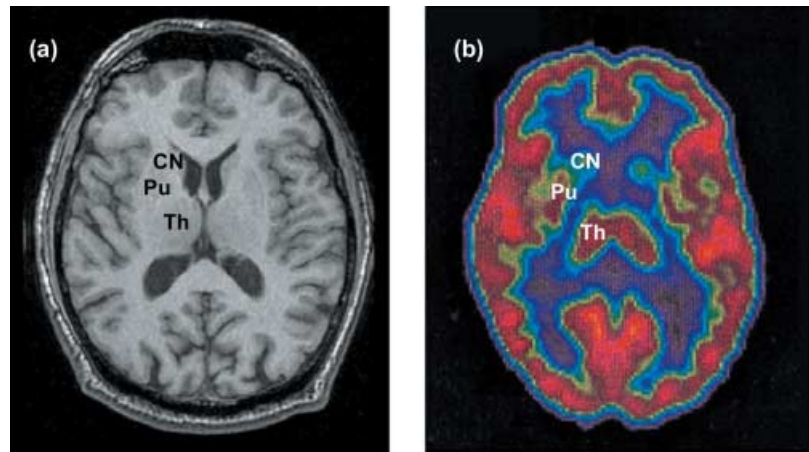
cortical, rather than purely subcortical dysfunction, the cerebral pathological alterations were restricted to the striatum. Therefore, the clinical symptoms may be explained by a neuronal dysfunction due to impaired basal ganglia – cortical circuits [57].

Neuromuscular involvement

Axonopathy

All McLeod patients reported up to date lack deep tendon reflexes, at least at the ankles [4–6]. About a third of McLeod patients have reduced vibration sense in the feet, but only a minority of patients have sensory symptoms [4–6]. Neurography demonstrates a sensory-motor axonal neuropathy, and electromyography may reveal myopathic as well as neurogenic changes [4,5].

Myopathy

Elevated serum CK levels as a sign of subclinical or manifest myopathy are present in virtually all carriers of the McLeod blood group [4–6,64]. CK elevation is usually mild and is usually < 4000 U/l. About 50% of McLeod patients have a clinically significant muscle weakness or atrophy. The deterioration rate of the myopathy is slow, and only a minority of McLeod patients develop severe weakness or rhabdomyolysis [65,66]. In one patient, the findings resembled inflammatory changes [67].

Cardiomyopathy

Cardiac manifestations of MLS include congestive cardiomyopathy, dilated cardiomyopathy, atrial fibrillation and tachyarrhythmia [68–70]. Up to 60% of McLeod patients develop cardiac manifestations during the course of the disease [4]. Cardiac problems such as malignant arrhythmia or heart failure might be an important cause of death [4]. Due to the possibility of a curative intervention, McLeod patient should be carefully monitored for cardiac disease.

Histopathology

Sural nerve biopsies display an unspecific sensory-motor axonal neuropathy [5]. Histological findings of skeletal muscle in McLeod patients reveal fibre type grouping, type 1-fibre predominance, type 2-fibre atrophy, increased variability in fibre size, and increased central nucleation [22,64]. In normal skeletal muscle, XK immunohistochemistry reveals a type 2 fibre-specific intracellular staining possibly confined to the sarcoplasmic reticulum. XK staining is absent in McLeod myopathy. This finding correlates to the observed type 2-fibre atrophy, and suggests that the XK protein is crucial for the maintenance of normal structure and function [22]. Cardiac muscle needle biopsy in one patient showed non-specific findings of hypertrophy with some dilatations [69].

Manifesting female McLeod carriers

Two female heterozygotes were reported to develop the typical McLeod phenotype [5,65]. In one McLeod family, female McLeod mutation carriers had slight cognitive deficits [6]. In addition, reduction of striatal glucose uptake was seen with quantified cerebral FDG-PET indicating subtle CNS involvement in heterozygotes without manifest movement disorder [6]. The most probable reason for these findings is an unfavourable inactivation of the chromosome carrying the normal XK gene (Lyon effect), as was demonstrated in one heterozygote [5,63]. Female heterozygotes with or without clinical manifestation of the MLS may demonstrate a Kell blood group chimerism detectable by Kell antigen flow cytometry (Fig. 3).
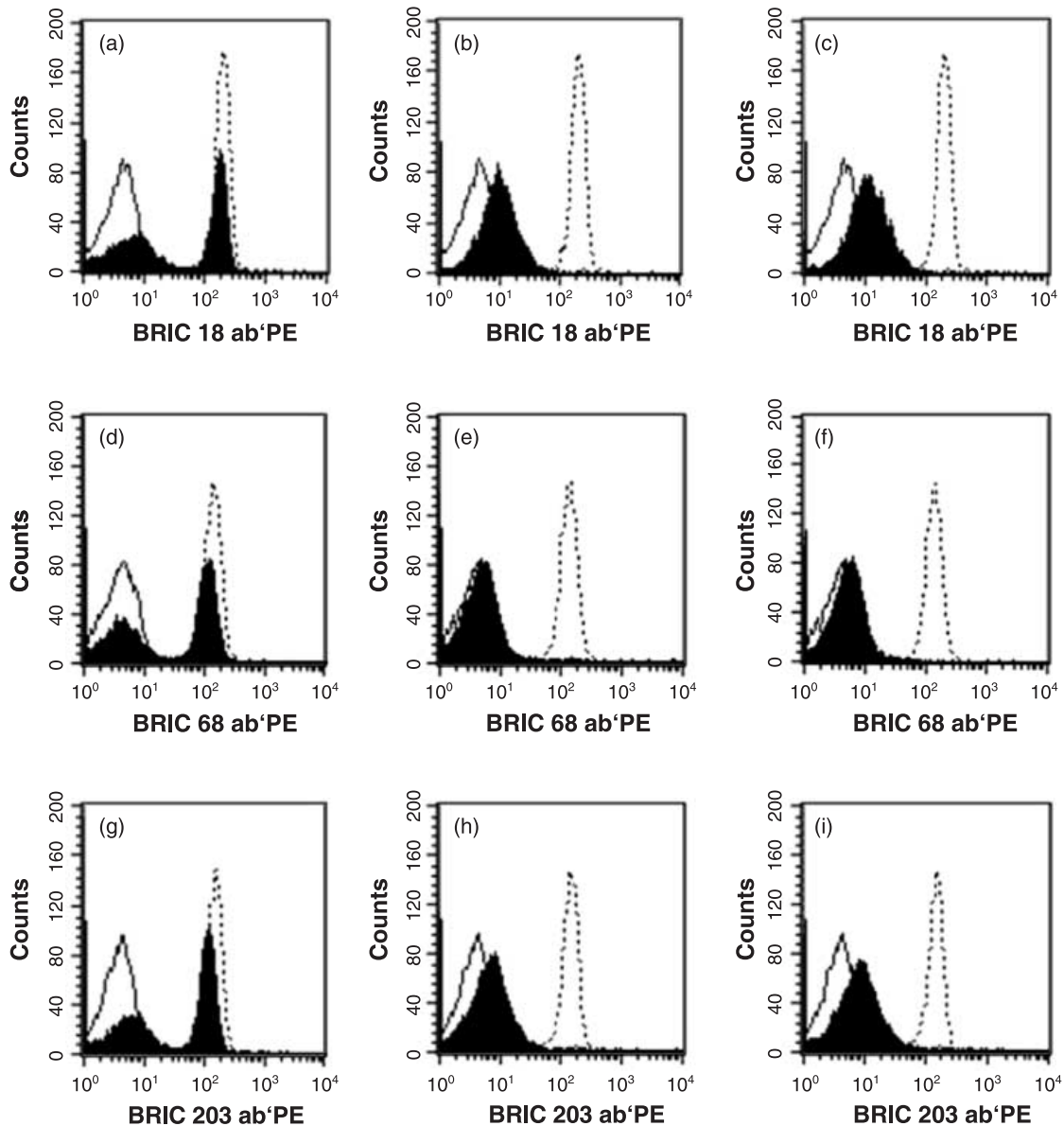
Fig. 3 Flow cytometry. Kell glycoprotein expression on red blood cell (RBC) was assessed by flow cytometry using a panel of Kell-specific murine monoclonal antibodies (BRIC 18; BRIC 68; BRIC 203; kindly provided by G. Daniels, Bristol Institute for Transfusion Sciences, Bristol, UK). RBC were suspended in FACSFlow™ (Becton Dickinson, Franklin Lakes, NJ, USA) with 0·8% BSA (Immucor GmbH, Rödermark, Germany), and incubated with the primary Kell antibodies for 60 min at 37 °C. After washing, RBCs were incubated with phycoerythrin-conjugated rabbit-antimouse Fab-fragment (Dako, Glostrup, Denmark) for 30 min at 4 °C. Analysis of at least 50 000 events was performed on a FACSCalibur™ flow-cytometer, using the Cellquest™ software (Becton Dickinson). RBC of a mother (a, d, g) and her two sons (b, e, h and c, f, i) with molecular genetically determined McLeod syndrome (mutation 938–942delCTCTA) as described were assessed by flow cytometry [72]. With all three antibodies, the clinically normal mother reveals two populations of RBC. One population shows normal Kell protein staining, while the other population has only very weak expression of Kell glycoproteins. The two sons show only a single RBC population with very low expression of Kell glycoprotein as surrogate for the identified *XK* mutation.

Disease course and therapy

Patients with MLS usually show a slow progression of disease with a mean onset between 30 and 40 years of age [4–6].

Disease duration ranged from 7 to 51 years, and mean age at death was 53 years, ranging from 31 to 69 years [4–6,70]. Hugh McLeod, the original propositus, recently died at the age of 69 after he had developed all major MLS manifestations

(personal communications by Dr Ruth Walker, New York). Cardiovascular events, epileptic seizures and aspiration pneumonia might be the major causes of death in older McLeod patients [4–6]. At present, no causal or preventive therapy is available to alter the progression of the syndrome. Recognition of treatable MLS complications, for example cardiac problems and seizures, is presently the most important issue. Because of possible rhabdomyolysis, serum CK levels should be carefully monitored, in particular if excessive movement disorder or neuroleptic medication are present [66]. Psychiatric problems should be treated according to the clinical presentation. Dopamine antagonists such as tiapride, clozapine or quetiapine may be useful to ameliorate choreatic movements. And last but not least, an extended and continuous multidisciplinary psychosocial support should be provided for patients and their families.

Challenging diagnosis of McLeod syndrome

The diagnosis of MLS is based on the immunohaematological findings of absent Kx antigen and weak Kell antigens. Carriers of the McLeod blood group phenotype may therefore be recognized in blood banks. In most carriers, neurological data are not available, and cognitive, neuromuscular, cardiac and neuroimaging examinations are rarely performed [3,71]. Long-term follow-up of McLeod phenotype carriers, however, including the eponymous index case, demonstrate development of neurological manifestations during the course of disease [4]. There is converging evidence that the clinical penetrance of MLS is high, in particular after the age of 50 [4–6]. Yet, because of the gradual onset and wide diversity of signs and symptoms, the diagnosis is frequently delayed and often mistaken for familial haemolytic anaemia, hepatopathy [38], Huntington's disease, Tourette's syndrome, myopathy or spinal muscle atrophy.

Conclusions

Carriers of the McLeod blood group phenotype are prone to develop a severe neurological disorder that resembles Huntington's disease but shows additional neuromuscular and cardiac manifestations. If such carriers are recognized in blood donation programmes, genetic counseling, neurological and cardiologic examination as well as follow-up examinations in a specialized neurological centre are warranted.

References

1 Danek A (ed.): *Neuroacanthocytosis Syndromes*. Dordrecht, The Netherlands, Springer, 2005

2 Walker RH, Jung HH, Dobson-Stone C, Rampoldi L, Sano A, Tison F, Danek A: Neurologic phenotypes associated with acanthocytosis. *Neurology* 2007; **68**:92–98

3 Allen FH, Krabbe SMR, Corcoran PA: A new phenotype (McLeod) in the Kell blood-group system. *Vox Sang* 1961; **6**:555–560

4 Danek A, Rubio JP, Rampoldi L, Ho M, Dobson-Stone C, Tison F, Symmans WA, Oechsner M, Kalckreuth W, Watt JM, Corbett AJ, Hamdalla HHM, Marshall AG, Sutton I, Dotti MT, Malandrini A, Walker RH, Daniels G, Monaco AP: McLeod neuroacanthocytosis: genotype and phenotype. *Ann Neurol* 2001; **50**:755–764

5 Hardie RJ, Pullon HW, Harding AE, Owen JS, Pires M, Daniels GL, Imai Y, Misra VP, King RH, Jacobs JM: Neuroacanthocytosis. A clinical, haematological and pathological study of 19 cases. *Brain* 1991; **114**:13–49

6 Jung HH, Hergersberg M, Kneifel S, Alkadhi H, Schiess R, Weigell-Weber M, Daniels G, Kollias S, Hess K: McLeod syndrome: a novel mutation, predominant psychiatric manifestations, and distinct striatal imaging findings. *Ann Neurol* 2001; **49**:384–392

7 Ho M, Chelly J, Carter N, Danek A, Crocker P, Monaco AP: Isolation of the gene for McLeod syndrome that encodes a novel membrane transport protein. *Cell* 1994; **77**:869–880

8 Coombs RRA, Mourant AE, Race RR: *In vivo* isosensitisation of red cells in babies with haemolytic disease. *Lancet* 1946; **i**: 244–266

9 Wimer BM, Marsh WL, Taswell HF, Galey WR: Haematological changes associated with the McLeod phenotype of the Kell blood group system. *Br J Haematol* 1977; **36**:219–224

10 Francke U, Ochs HD, de Martinville B, Giacalone J, Lindgren V, Disteche C, Pagon RA, Hofker MH, van Ommen GJ, Pearson PL: Minor Xp21 chromosome deletion in a male associated with expression of Duchenne muscular dystrophy, chronic granulomatous disease, retinitis pigmentosa, and McLeod syndrome. *Am J Hum Genet* 1985; **37**:250–267

11 Lee S, Lin M, Mele A, Cao Y, Farmar J, Russo D, Redman C: Proteolytic processing of big endothelin-3 by the Kell blood group protein. *Blood* 1999; **94**:1440–1450

12 Lee S: Molecular basis of Kell blood group phenotypes. *Vox Sang* 1997; **73**:1–11

13 Khamlichi S, Bailly P, Blanchard D, Goossens D, Cartron JP, Bertrand O: Purification and partial characterization of the erythrocyte Kx protein deficient in McLeod patients. *Eur J Biochem* 1995; **228**:931–934

14 Stanfield GM, Horvitz HR: The ced-8 gene controls the timing of programmed cell deaths in *C. elegans. Mol Cell* 2000; **5**:423–433

15 Lee S, Russo D, Redman CM: The Kell blood group system: Kell and XK membrane proteins. *Semin Hematol* 2000; **37**:113–121

16 Calenda G, Peng J, Redman CM, Sha Q, Wu X, Lee S: Identification of two new members, XPLAC and XTES, of the XK family. *Gene* 2006; **370**:6–16

17 Phelan JK, Wong K, Jung HH, Hengartner MO: Sequence analysis identifies a family of human genes related to ced-8. *15th Biennial International C. elegans Conference*, June 25–29. University of California, Los Angeles, California, USA, 2005

18 Russo D, Wu X, Redman CM, Lee S: Expression of Kell blood group protein in nonerythroid tissues. *Blood* 2000; **96**:340–346

19 Claperon A, Hattab C, Armand V, Trottier S, Bertrand O, Ouimet T: The Kell and XK proteins of the Kell blood group are not co-expressed in the central nervous system. *Brain Res* 2007; **1147**:12–24

20 Russo D, Redman C, Lee S: Association of XK and Kell blood group proteins. *J Biol Chem* 1998; **273**:13950–13956

21 Russo D, Lee S, Redman C: Intracellular assembly of Kell and XK blood group proteins. *Biochim Biophys Acta* 1999; **1461**:10–18

22 Jung HH, Russo D, Redman C, Brandner S: Kell and XK immunohistochemistry in McLeod myopathy. *Muscle Nerve* 2001; **24**:1346–1351

23 Lee S, Sha Q, Wu X, Calenda G, Peng J: Expression Profiles of Mouse Kell, XK, and XPLAC mRNA. *J Histochem Cytochem* 2007; **55**:365–374

24 Cooper RA: Kell blood group. The human gene mutation database at the Institute of Medical Genetics in Cardiff (BIOBASE), 2007, cited 25 February 2007; available from: http://www.hgmd.cf.ac.uk/ac/gene.php?gene=KEL

25 Daniels G, Flegel WA, Fletcher A, Garratty G, Levene C, Lomas-Francis C, Moulds JM, Moulds JJ, Olsson ML, Overbeeke MA, Poole J, Reid ME, Rouger P, van der Schoot CE, Scott M, Sistonen P, Smart E, Storry JR, Tani Y, Yu LC, Wendel S, Westhoff CM, Zelinski T: International Society of Blood Transfusion Committee on terminology for red cell surface antigens: Cape Town report. *Vox Sang* 2007; **92**:250–253

26 Wester ES, Storry JR, Schneider K, Nilsson SB, Poole J, Olsson ML: Genetic basis of the K(0) phenotype in the Swedish population. *Transfusion* 2005; **45**:545–549

27 Yu LC, Twu YC, Chang CY, Lin M: Molecular basis of the Kell-null phenotype: a mutation at the splice site of human KEL gene abolishes the expression of Kell blood group antigens. *J Biol Chem* 2001; **276**:10247–10252

28 Lee S, Russo DC, Reiner AP, Lee JH, Sy MY, Telen MJ, Judd WJ, Simon P, Rodrigues MJ, Chabert T, Poole J, Jovanovic-Srzentic S, Levene C, Yahalom V, Redman CM: Molecular defects underlying the Kell null phenotype. *J Biol Chem* 2001; **276**:27281–27289

29 Lee S, Russo DC, Reid ME, Redman CM: Mutations that diminish expression of Kell surface protein and lead to the Kmod RBC phenotype. *Transfusion* 2003; **43**:1121–1125

30 Daniels GL, Weinauer F, Stone C, Ho M, Green CA, Jahn-Jochem H, Offner R, Monaco AP: A combination of the effects of rare genotypes at the XK and KEL blood group loci results in absence of Kell system antigens from the red blood cells. *Blood* 1996; **88**:4045–4050

31 Kell and XK Blood Group System: *dbRBC – Blood Group Antigen Gene Mutation* database, 2006, cited 15 January 2007

32 Jung HH, Hergersberg M, Vogt M, Pahnke J, Treyer V, Röthlisberger B, Kollias SS, Russo D, Frey BM: McLeod phenotype associated with a *XK* missense mutation without hematological, neuromuscular, or cerebral involvement. *Transfusion* 2003; **43**:928–938

33 Jung HH: McLeod syndrome: a clinical review; in Danek A (ed.): *Neuroacanthocytosis Syndromes*. Dordrecht, Springer, 2004:45–53

34 Walker RH, Danek A, Uttner I, Offner R, Reid M, Lee S: McLeod phenotype without the McLeod syndrome. *Transfusion* 2007; **47**:299–305

35 Holland SM: Chronic granulomatous disease and MLS. *Mov Disord* 2005; **20**:1677

36 Russo DC, Oyen R, Powell VI, Perry S, Hitchcock J, Redman CM, Reid ME: First example of anti-Kx in a person with the McLeod phenotype and without chronic granulomatous disease. *Transfusion* 2000; **40**:1371–1375

37 Storch A, Kornhass M, Schwarz J: Testing for acanthocytosis A prospective reader-blinded study in movement disorder patients. *J Neurol* 2005; **252**:84–90

38 Walker RH, Danek A, Jung HH, Davey R, Reid M: McLeod syndrome presenting with hepatic disease. *Mov Disord* 2005; **20** (Suppl. 10):S6–S7

39 Redman CM, Huima T, Robbins E, Lee S, Marsh WL: Effect of phosphatidylserine on the shape of McLeod red cell acanthocytes. *Blood* 1989; **74**:1826–1835

40 Lang KS, Lang PA, Bauer C, Duranton C, Wieder T, Huber SM, Lang F: Mechanisms of suicidal erythrocyte death. *Cell Physiol Biochem* 2005; **15**:195–202

41 Mandal D, Mazumder A, Das P, Kundu M, Basu J: Fas-, caspase 8-, and caspase 3-dependent signaling regulates the activity of the aminophospholipid translocase and phosphatidylserine externalization in human erythrocytes. *J Biol Chem* 2005; **280**:39460–39467

42 Schroit AJ, Tanaka Y, Madsen J, Fidler IJ: The recognition of red blood cells by macrophages: role of phosphatidylserine and possible implications of membrane phospholipid asymmetry. *Biol Cell* 1984; **51**:227–238

43 Smriti, Nemergut EC, Daleke DL: ATP-dependent transport of phosphatidylserine analogues in human erythrocytes. *Biochemistry* 2007; **46**:2249–2259

44 Lin S, Yang E, Huestis WH: Relationship of phospholipid distribution to shape change in Ca(2+)-crenated and recovered human erythrocytes. *Biochemistry* 1994; **33**:7337–7344

45 Johnston RB, Jr: Clinical aspects of chronic granulomatous disease. *Curr Opin Hematol* 2001; **8**:17–22

46 Roos D: The genetic basis of chronic granulomatous disease. *Immunol Rev* 1994; **138**:121–157

47 Hayflick SJ, Westaway SK, Levinson B, Zhou B, Johnson MA, Ching KH, Gitschier J: Genetic, clinical, and radiographic delineation of Hallervorden–Spatz syndrome. *N Engl J Med* 2003; **348**:33–40

48 Bertelson CJ, Pogo AO, Chaudhuri A, Marsh WL, Redman CM, Banerjee D, Symmans WA, Simon T, Frey D, Kunkel LM: Localization of the McLeod locus (XK) within Xp21 by deletion analysis. *Am J Hum Genet* 1988; **42**:703–711

49 Frey D, Machler M, Seger R, Schmid W, Orkin SH: Gene deletion in a patient with chronic granulomatous disease and McLeod syndrome: fine mapping of the Xk gene locus. *Blood* 1988; **71**:252–255

50 Marsh WL, Marsh NJ, Moore A, Symmans WA, Johnson CL, Redman CM: Elevated serum creatine phosphokinase in subjects with McLeod syndrome. *Vox Sang* 1981; **40**:403–411

51 Schwartz SA, Marsh WL, Symmans A, Johnson CL, Mueller KA: 'New' clinical features of McLeod syndrome. *Transfusion* 1982; **22**:404

52 Danek A: Neuroacanthocytosis syndromes: what links red blood cells and neurons? in Danek A (ed.): *Neuroacanthocytosis Syndromes*. Dordrecht, The Netherlands, Springer, 2004:1–14

53 Danek A, Tison F, Rubio J, Oechsner M, Kalckreuth W, Monaco AP: The chorea of McLeod syndrome. *Mov Disord* 2001; **16**:882–889

54 Danek A, Uttner I, Vogl T, Tatsch K, Witt TN: Cerebral involvement in McLeod syndrome. *Neurology* 1994; **44**:117–120

55 Kartsounis LD, Hardie RJ: The pattern of cognitive impairments in neuroacanthocytosis. A frontosubcortical dementia. *Arch Neurol* 1996; **53**:77–80

56 Danek A, Sheesley L, Tierney M, Uttner I, Grafman J: Cognitive and neuropsychiatric findings in McLeod syndrome and in chorea-acanthocytosis; in Danek A (ed.): *Neuroacanthocytosis Syndromes*. Dordrecht, The Netherlands, Springer, 2004:95–115

57 Jung HH, Haker H: Schizophrenia as a manifestation of X-Linked McLeod – neuroacanthocytosis syndrome. *J Clin Psychiatry* 2004; **65**:722–723

58 Ring HA, Serra-Mestres J: Neuropsychiatry of the basal ganglia. *J Neurol Neurosurg Psychiatr* 2002; **72**:12–21

59 Nicholl DJ, Sutton I, Dotti MT, Supple SG, Danek A, Lawden M: White matter abnormalities on MRI in neuroacanthocytosis. *J Neurol Neurosurg Psychiatr* 2004; **75**:1200–1201

60 Oechsner M, Buchert R, Beyer W, Danek A: Reduction of striatal glucose metabolism in McLeod choreoacanthocytosis. *J Neurol Neurosurg Psychiatr* 2001; **70**:517–520

61 Dydak U, Mueller S, Sandor PS, Meier D, Boesiger P, Jung HH: Cerebral metabolic alterations in McLeod syndrome. *Eur Neurol* 2006; **56**:17–23

62 Brin MF, Hays A, Symmans WA, Marsh WL, Rowland LP: Neuropathology of McLeod phenotype is like choreaacanthocytosis (CA). *Can J Neurol Sci* 1993; **20** (Suppl):S234

63 Ho MF, Chalmers RM, Davis MB, Harding AE, Monaco AP: A novel point mutation in the McLeod syndrome gene in neuroacanthocytosis. *Ann Neurol* 1996; **39**:672–675

64 Swash M, Schwartz MS, Carter ND, Heath R, Leak M, Rogers KL: Benign X-linked myopathy with acanthocytes (McLeod syndrome). Its relationship to X-linked muscular dystrophy. *Brain* 1983; **106**:717–733

65 Kawakami T, Takiyama Y, Sakoe K, Ogawa T, Yoshioka T, Nishizawa M, Reid ME, Kobayashi O, Nonaka I, Nakano I: A case of McLeod syndrome with unusually severe myopathy. *J Neurol Sci* 1999; **166**:36–39

66 Jung HH, Brandner S: Malignant McLeod myopathy. *Muscle Nerve* 2002; **26**:424–427

67 Barnett MH, Yang F, Iland H, Pollard JD: Unusual muscle pathology in McLeod syndrome. *J Neurol Neurosurg Psychiatr* 2000; **69**:655–657

68 Faillace RT, Kingston WJ, Nanda NC, Griggs RC: Cardiomyopathy associated with the syndrome of amyotrophic chorea and acanthocytosis. *Ann Intern Med* 1982; **96**:616–617

69 Witt TN, Danek A, Reiter M, Heim MU, Dirschinger J, Olsen EG: McLeod syndrome: a distinct form of neuroacanthocytosis. Report of two cases and literature review with emphasis on neuromuscular manifestations. *J Neurol* 1992; **239**:302–306

70 Malandrini A, Fabrizi GM, Truschi F, Di Pietro G, Moschini F, Bartalucci P, Berti G, Salvadori C, Bucalossi A, Guazzi G: Atypical McLeod syndrome manifested as X-linked chorea-acanthocytosis, neuromyopathy and dilated cardiomyopathy: report of a family. *J Neurol Sci* 1994; **124**:89–94

71 Russo DC, Lee S, Reid ME, Redman CM: Point mutations causing the McLeod phenotype. *Transfusion* 2002; **42**:287–293

72 Miranda M, Castiglioni C, Frey BM, Hergersberg M, Danek A, Jung HH: Phenotypic variability of a distinct deletion in McLeod syndrome. *Mov Disord* 2007 [Epub ahead of print]