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BRIEF COMMUNICATION

Peripheral neuropathy in late-onset Krabbe disease: report of three cases

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Abstract Late-onset Krabbe disease may have variable misleading clinical manifestations and be a puzzling problem for physicians. We report clinical and peripheral nerve studies of three patients with adult-onset Krabbe disease. Two cases had a predominantly spastic paraparesis; in one case, the symptoms mimicked a cerebrovascular disorder. Predominantly, demyelinating neuropathy was observed in one case and axonal neuropathy in two cases. In all cases, no typical intracytoplasmic inclusions were found. These observations suggest that peripheral neuropathy in adult-onset Krabbe disease has variable clinical and pathological characteristics, different from those described in the classic form.

Keywords Krabbe's disease · Nerve biopsy · Intracytoplasmic inclusions · GALC gene

Introduction

Krabbe's disease (KD) or globoid cell leukodystrophy (GCL) is an autosomal recessive disorder caused by a deficiency of lysosomal galactocerebrosidase beta-galactosidase (GALC) activity, leading to widespread demyelination of central and peripheral axons. The disease is caused by mutations in the *GALC* gene encoding the GALC enzyme. The *GALC* gene is 57-kb long with 17 exons coding 669 amino acids and is mapped on chromosome 14q31 [1]. The disease can be diagnosed by detecting the deficiency of GALC activity in any available tissue sample and by molecular analysis of *GALC gene*. More than 80 mutations have been reported and they differ according to ethnicity. In individuals of European ancestry, a 30-kb deletion is the most frequent mutation with 40–50% allele frequency [2]. Patients with this homozygous mutation present with infantile-type disease, while mutations, such as G270D (809G>A) and L629R (1886T>G) are considered to contribute to the late-onset phenotype [3]. In the classic infantile form, demyelinating peripheral neuropathy, often overshadowed by massive central nervous system impairment, is characterized by fiber loss, segmental demyelination and thin myelin sheaths [4]. The pathological hallmark is typical crystalloid, tubular or prismatic inclusions in Schwann cells and endoneurial macrophages [4]. The rare late-onset form may have variable misleading clinical manifestations: they include spastic paraparesis with white matter abnormalities, visual deficit, ataxia. Mental deterioration may be absent. Peripheral neuropathy may have different characteristics from those of the infantile form. We describe the clinical manifestations and the pathology report of peripheral nerve biopsy in three patients with late-onset Krabbe disease.

Case reports

The peripheral nerve biopsies were performed with the written consent of the patients.

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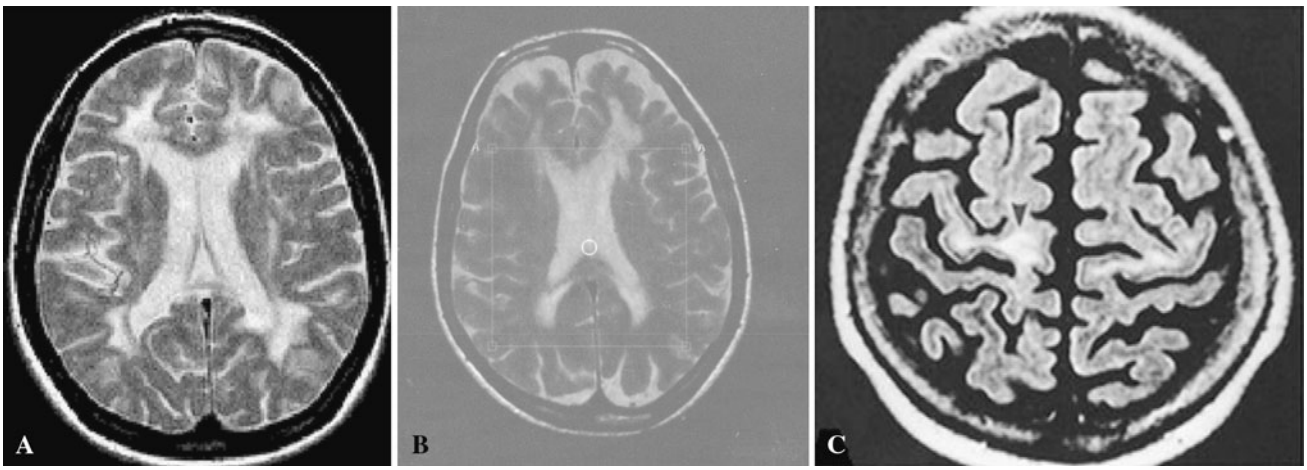

Fig. 1 Brain MR T2-weighted images showing bilateral areas of hyperintensity of white matter, accentuated in frontal and parietal regions (**a** case 1), periventricular region (**b** case 2) and enhanced signal in cortico-spinal tracts (**c** case 3)

Case 1

A 44-year-old woman was admitted to our hospital with a 7-year history of unsteady gait. Symptoms deteriorated progressively and the patient experienced multiple falls. Neurological examination revealed slight impairment of intellectual function, ataxic–spastic gait, mild dysphagia and dysarthria, brisk deep tendon reflexes, weakness of the left leg and bilateral Babinski sign. Brain MR showed bilateral areas of hyperintensity in the cerebral white matter, more accentuated in the parietal region; and slight cortical atrophy (Fig. 1a). Neurophysiological study showed a mild axonal neuropathy (see Table 1). Sural nerve biopsy showed a normal density of myelinated fibers (8,512/mm^2; NV 7,000–12,000/mm^2) and a relative reduction in those of large diameter. Some axons had thin myelin sheath (Fig. 2a). Occasional simple onion bulb formations were observed. Teased fibers were normal. There was ultrastructural evidence of membrane-bound vacuoles containing lamellar and tubular material in the cytoplasm of about 20% Schwann cells and in some macrophages (Fig. 3a). Occasional axons showing myelin disruption and axonal degeneration were also observed. Leukocyte lysosomal galactocerebrosidase activity was 0.8 nmol/h/mg protein (n.v. 2.0–6.0). Molecular analysis detected a homozygous mutation at cDNA position 121G>A (G41S) in the GALC gene associated with polymorphisms 1637T>C (I546T).

Case 2

A 57-year-old man presented with a 4-year history of progressive cognitive impairment, gait problems and unsteadiness. Family medical history was negative for neuromuscular disorders. Neurological examination showed dysphagia, dysarthria, right facio-brachio-crural weakness with accentuated deep tendon reflexes and right Babinski sign. Autonomous walking was difficult and cognitive function was slightly impaired. Brain MR showed diffuse cortico-subcortical atrophy, bilateral areas of hyperintensity in T2-weighted images of white matter, more accentuated in periventricular regions (Fig. 1b). Neurophysiological study revealed a severe axonal motor-sensory neuropathy (see Table 1). Nerve biopsy showed severe loss (543/mm^2) of myelinated fibers with complete disappearance of those of large diameter (Fig. 2b), rare axonal degenerations and rare fibers with thickened myelin sheaths. Ultrastructural examination showed profiles of curved lamellar inclusions in the cytoplasm of about 18% Schwann cells and in several endoneurial macrophages (Fig. 3b). On the basis of this finding, further biochemical analysis was performed. Leukocyte lysosomal galacto-cerebrosidase activity was 1.0 nmol/h/mg protein (n.v. 2.0–6.0). Molecular analysis detected a heterozygous mutation at cDNA position 809G>A (G270D) and 1637T>C (I546T) polymorphism in the GALC gene.

Case 3

A 54-year-old woman came to our observation at 44 years of age with a 2-year history of walking difficulty, leg pain and loss of weight. Family history was negative for neurological diseases. Neurological examination revealed ataxic–spastic gait, brisk deep tendon reflexes more accentuated in the upper limbs, loss of vibratory sensation in the distal lower limbs, bilateral Babinski sign. Psychic examination revealed anxious–depressive state; no clear cognitive impairment was detected. Brain MR showed

Table 1 The neurophysiological findings are listed

Case	1	2	3	M/SD
Median				
MCV	45	47.1	38.5	57.6 ± 3.7
DML	4.24	4.34	5.08	3.5 ± 0.5
CMAPa	13.6	5.4	11.5	14.6 ± 4.3
SCV	48	42.3	39.2	55.1 ± 4.9
SAPa	11.3	3.8	10.5	23.9 ± 6.6
Ulnar				
MCV	52	49	37.6	58.7 ± 3.9
DML	4.16	3.56	4.01	3.0 ± 0.4
CMAPa	11.7	4.7	10.8	15.9 ± 4.7
SCV	49.7	41.5	42.3	53.6 ± 5.2
SAPa	15.9	3.5	4.2	15.3 ± 3.5
Deep peroneal				
MCV	50	39.4	31.5	49.8 ± 4.7
DML	4.27	5.20	5.40	4.5 ± 0.4
CMAPa	8.9	1.1	0.870	5.7 ± 2.3
Superf. peroneal				
SCV	48.9	0	32.1	47.7 ± 4.3
SAPa	11.7	0	3.8	19.4 ± 7.3
Tibial				
MCV	45	37	33.5	46.7 ± 4.8
DML	5.20	6.20	6.34	5.5 ± 06
CMAPa	8.2	0.560	1.20	6.0 ± 4.4
Sural				
SCV	50.3	0	33.5	49.2 ± 4.5
SAPa	11.2	0	9.8	23.1 ± 6.5

MCV motor conduction velocity, *DML* distal motor latency, *CMPa* compound muscle action potential amplitude, *SCV* sensory nerve conduction velocity, *SAPa* sensory action potential amplitudes, *NP* not performed, *0 inelicitable M/SD* (laboratory reference values) mean, standard deviation

bilateral areas of hyperintensity along cortico-spinal tracts in T2-weighted images (Fig. 1c). Neurophysiological study showed prevalently demyelinating motor-sensory polyneuropathy (see Table 1). Sural nerve biopsy showed slightly reduced density of myelinated fibers (5,922/mm^2; n.v. 7,000–12,000/mm^2). Several axons had relatively thin myelin sheaths (Fig. 2c); no onion bulb formations were observed. Teasing showed signs of de-remyelination in 6% fibers. Ultrastructural examination showed membrane-bound inclusions containing lamellar zebra-body-like material in the cytoplasm of about 14% Schwann cells (Fig. 3c) and in some endoneurial macrophages. Leukocyte lysosomal galactocerebrosidase activity was 1.1 nmol/h/mg protein (n.v. 2.0–6.0). Molecular study: detected a mutation at cDNA position 809G>A (G270D) and a deletion in the exon 10 at nucleotide 1026 (1026del10, Gly362fs) in the GALC gene.

Discussion

The clinical picture of patients here reported was not readily indicative for Krabbe disease. In case 2, the clinical picture recalled a cerebrovascular disorder, but the presence of bilateral brain white matter lesions and peripheral neuropathy, although without the typical biopsy findings, prompted us to investigate for Krabbe disease. In the other cases, the association of progressive spastic paraparesis, brain white matter abnormalities and peripheral neuropathy was more clearly suggestive for a diagnosis of Krabbe disease.

All patients showed deficiency of galactocerebrosidase activity, whose association with known pathogenetic mutations made the diagnosis reliable. In fact, in case 1 molecular analysis detected a homozygous mutation at cDNA position 121G>A (G41S) in the GALC gene associated with polymorphisms 1637T>C (I546T); and in case 3 a mutation at cDNA position 809G>A (G270D) associated with a deletion in the exon 10 at nucleotide 1026 (1026del10, Gly362fs) in the GALC gene were found. In case 2, molecular analysis detected a heterozygous mutation at cDNA position 809G>A (G270D) and 1637T>C (I546T) polymorphism in the GALC gene; the detection of at least one pathogenetic mutation in association with reduced level of galactocerebrosidase in subjects with central and peripheral myelin pathology constitutes an important diagnostic achieve. In addition, in this patient, a T1637C polymorphism was found. Polymorphisms play a significant role in producing the wide range of GALC values measured in the "normal" population, in the family members of affected individuals and among obligate heterozygotes. There are reports of healthy people with very low GALC activities. This makes almost unreliable testing carrier only by measurement of GALC enzyme activity. Polymorphisms may also play a role in the development of clinical disease; in fact several studies demonstrated that some disease-causing mutations may be more deleterious if associated with one ore more polymorphisms [5, 6].

In Krabbe disease, demyelinating peripheral neuropathy is caused by progressive damage to myelin sheaths through failure of Schwann cell in maintaining metabolic support [4]. This type of neuropathy was classically described in the infantile form. Crystalloid, tubular and prismatic inclusions in Schwann cells and histiocytes are the ultrastructural hallmark of the disease [4]. These inclusions are usually long and straight with clear contents, but may occasionally be curvilinear and lamellar [4]. Before the advent of diagnosis by molecular analysis, nerve biopsy study was regarded as a valid diagnostic tool in addition to biochemical tests. In the last few decades, several adult-onset forms were reported and it was noticed that peripheral neuropathy was no longer a constant finding and that

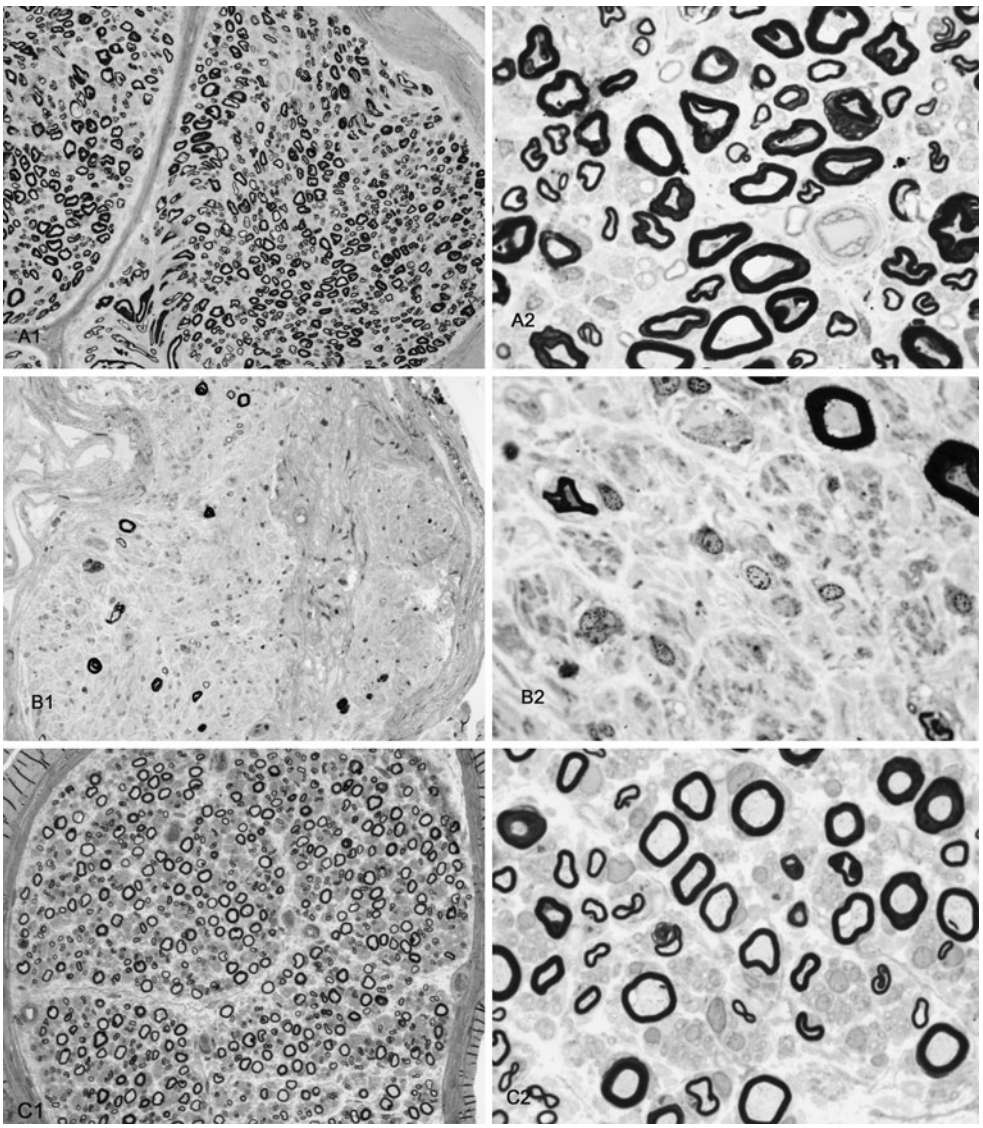

Fig. 2 Sural nerve biopsy (semithin sections; toluidine blue stain: **a** case 1, **b** case 2, **c** case 3. *Left column* ×200 magnification, *right column* ×1,000 magnification. **a** Some axons have a thin myelin sheath. **b** Severe fiber loss with disappearance of large diameter fibers. **c** Several axons with thin myelin sheath

neurophysiological and pathological characteristics often differed from those of the classical form [7–13]. The latter is described as demyelinating neuropathy characterized by thin myelin sheaths, onion bulb formations and signs of de-remyelination in teased fibers. However, these findings are also found in different acquired and degenerative demyelinating polyneuropathies, the true diagnostic marker has always been considered ultrastructural evidence of typical inclusions. In adult-onset Krabbe disease, peripheral neuropathy occurs in about 60% of patients. It may be the prominent symptom associated with pyramidal features [7–10, 13] in which cases differential diagnosis with respect to complicated forms of CMT or HSP is necessary. In the present series, the presence of brisk tendon reflexes was due to the hyperexcitability of the motorneurons caused by the cortico-spinal tract involvement and by the fact that peripheral neuropathy was not so severe to interrupt the reflex arc. Paradoxically; when clinical presentation is atypical, inclusions in Schwann cells and macrophages might be the only diagnostic marker of the adult-onset form. In the present series, case 1 showed initial axonal neuropathy with mild biopsy findings; while in case 2, there was a severe loss of myelinated fibers. Case 3 showed neurophysiological evidence for a predominantly demyelinating neuropathy with thin myelin sheaths and without significant onion bulb formations at nerve biopsy.

In the present series, many inclusions in Schwann cells and macrophages resembled normal Reich bodies. However, the inclusions detected in case 1 might also be compatible with adult-onset Krabbe disease; those

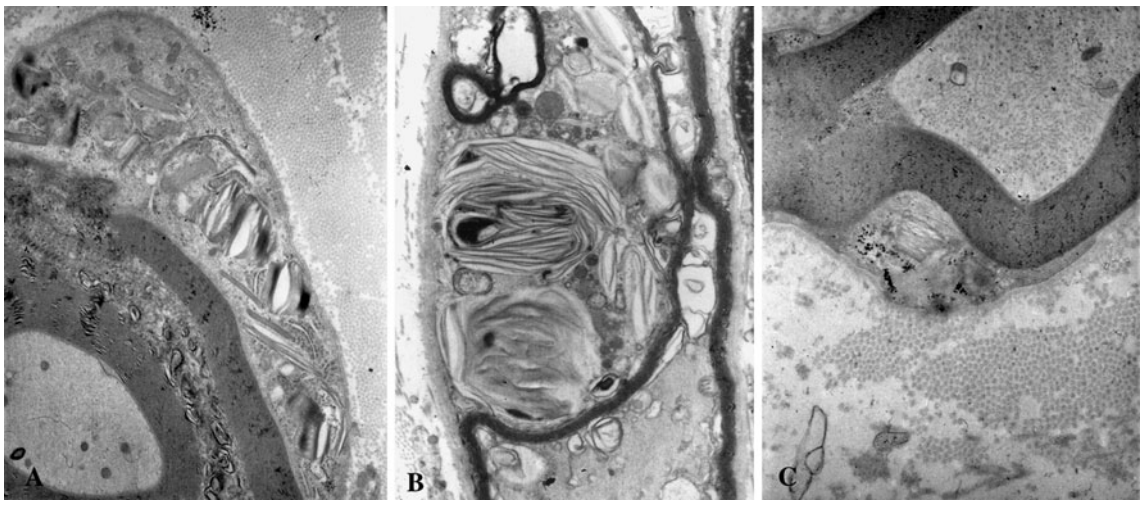

Fig. 3 Sural nerve (TEM) **a** (case 1): membrane-bound vacuoles containing lamellar and tubular material in Schwann cell cytoplasm, **b** (case 2): profiles of curved lamellar inclusions; **c** (case 3) membrane-bound inclusions containing lamellar zebra-body-like material in cytoplasm of a Schwann cell

observed in case 2 might suggest further molecular analysis. Similar findings have already been reported in adult-onset Krabbe disease.

Different factors may explain the multiform findings of peripheral neuropathy in adult-onset Krabbe disease: (1) the pathology findings may depend on the stage the peripheral nerve is investigated. In longstanding neuropathy, there may be severe loss of large diameter fibers and what was initially demyelinating neuropathy may be perceived as axonopathy in the final stage. Alternatively, the metabolic deficit may be particularly severe in peripheral nerves, leading to hypomyelinating neuropathy "ab initio", as previously reported [8, 12]. (2) The morphological aspects of deposits of galactocerebroside and possibly psychosine may vary in time, so instead of the typical prismatic and tubular inclusions, lamellar zebra-body-like inclusions are observed. Furthermore, additional degradation products may determine variable morphological aspect of the inclusions in longstanding neuropathy. Supporting our observation, various inclusions have been reported in late-onset Krabbe disease, but they have no specific diagnostic connotation beyond the clinical and laboratory setting [8–10, 13].

In cases with progressive white matter abnormalities associated with peripheral nerve involvement, even if features are different from those described in the classic form, late-onset Krabbe disease should be considered in differential diagnosis.

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