CASE OF THE MONTH

ABSTRACT: Adult polyglucosan body disease (APBD) is characterized by the accumulation of insoluble glucose polymers within the central and peripheral nervous systems. A common missense mutation in the glycogen branching enzyme (GBE1) gene has been identified in Ashkenazi patients with APBD. We report on a non-Jewish patient with APBD on whom we performed proton magnetic resonance spectroscopic imaging of the brain. GBE activity in fibroblasts was markedly reduced, and a novel heterozygous mutation was identified in the GBE1 gene. Our findings widen the spectrum of APBD genotypes, underline the importance of performing GBE analysis in all APBD patients, and suggest that brain white matter degeneration in APBD may result from tissue damage involving axons and myelin.

Adult Polyglucosan Body Disease: Proton Magnetic Resonance Spectroscopy of the Brain and Novel Mutation in the GBE1 Gene

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Adult polyglucosan body disease (APBD) is a rare disorder involving both the central and peripheral nervous systems. Patients with APBD usually present between the fourth and the sixth decade of life with a variable combination of symptoms and signs commonly including corticospinal involvement, peripheral neuropathy, bladder dysfunction, and, in about half of the patients, cognitive impairment.10,21,23,33 A few magnetic resonance imaging (MRI) studies have shown nonspecific, leukodystrophy-like changes in cerebral white matter (WM), along with brain and spinal cord atrophy.5,22,23,33 Most cases are apparently sporadic but a few familial clusterings involving siblings have been reported.6,12,23,30

The diagnosis of APBD is generally made by exclusion of other clinical-pathological entities, and most cases have been defined by examination of autopsy material. However, since a peripheral neuropathy is invariably present, a diagnosis in vivo is possible by means of nerve biopsy studies. The pathological hallmark of this condition is the rather diffuse accumulation of polyglucosan bodies (PBs) in the brain and peripheral nerves. PBs consist of diastase-resistant glucose polymers that stain with periodic acid–Schiff (PAS) and, ultrastructurally, they are composed of filamentous and finely granular material.30 Axonal damage, induced by PB deposition, is generally considered the pathogenic mechanism of peripheral neuropathy.

The accumulation of this amylopectin-like polyglucosan is usually ascribed to the deficiency of glycogen branching enzyme (GBE), although in the initial reports the activity of this enzyme was either not assayed or, in a few cases, found to be normal.10,12,21,30 However, APBD is presently considered a clinical variant of GBE deficiency, which is charac-
terized, in its classic form, by liver or neuromuscular involvement with onset in infancy.\textsuperscript{11,27}

PB deposition is not peculiar of APBD, since it can be found in other disorders, such as phosphofructokinase deficiency and Lafora’s disease, or in normal aging.\textsuperscript{1,13,25} A recognized mechanism leading to PB formation is an increase in the normal ratio between glycogen synthase and branching enzyme.\textsuperscript{1,28}

In APBD patients from Ashkenazi Jewish families, genetic analysis of the \textit{GBE1} gene has identified a homozygous missense mutation (Tyr329Ser).\textsuperscript{24} Only one patient with reduced GBE activity and \textit{GBE1} mutations has been reported so far among the non-Ashkenazi population.\textsuperscript{35} Here we report on an Italian non-Jewish woman with APBD and a likely familial presentation. In order to detect possible disease markers in vivo we performed proton magnetic resonance spectroscopic imaging (\textit{\textsuperscript{1}H-MRSI}) of the brain. Biochemical and genetic studies revealed GBE deficiency produced by a novel mutation in the \textit{GBE1} gene.

**CASE REPORT**

A 44-year-old woman, the fourth and youngest child of unrelated parents, was admitted to the hospital with complaints of urinary urgency for 2 years, dysphagia for 4 months, and slurred speech and dizziness for several weeks. On examination she had dysarthria and dysphagia for liquids. Gait was ataxic; dysmetria and dysdiadochokinesia were present in the upper extremities, especially the left. Strength and sensation were normal and deep tendon reflexes were brisk but symmetrical. Plantar responses were flexor.

Needle electromyography showed diffuse neurogenic changes, with motor unit potentials of increased amplitude and duration, and a reduced interference pattern during maximal voluntary contraction. Nerve conduction studies revealed an axonal sensory-motor neuropathy in the lower limbs, with markedly decreased amplitude of compound muscle action potentials in both tibial nerves and absent sensory action potentials in both sural nerves. Neuropsychological testing failed to detect any cognitive deficit and cerebrospinal fluid examination was normal (lactate values were not measured). A brain MRI showed diffuse hyperintensity of hemispheric WM in T2-weighted images. Cardiac function, as assessed by electrocardiography and echocardiography, was normal.

A sural nerve biopsy was performed. Light microscopy of cryostat sections revealed a conspicuous loss of myelinated fibers, together with the presence of fiber enlargements, corresponding to PBs. These were intra-axonal, PAS-positive, and diastase-resistant spherical or ovoidal bodies (Fig. 1A). In resin semithin sections, PBs appeared as concentrically laminated structures with a targetoid aspect determining an enlargement of the axon that reached a maximum diameter of 40 \( \mu \text{m} \) (Fig. 1B,C). In some cases, PBs were located at paranodal sites, whereas others were distributed in short chains, with a beadwork appearance. Degenerating axons were rare, but numerous clusters of axonal regeneration were observed. Some remyelinated axons were present, as well as a few irregular and thickened myelin sheaths resembling tomacula. There was no inflammation but mast cells were widespread in all nerve compartments and sometimes appeared to be close to nerve fibers. Teased-fiber preparations showed sporadic demyelinated and remyelinated internodes. Interestingly, only a few (6 of 100) myelinated fibers showed focal thickenings produced by PBs but in these fibers PBs were disseminated along the fiber length (Fig. 1D). Electron microscopy showed the presence of PBs also in unmyleinated axons and in perineurial and endoneurial cells, the latter being fibroblasts or, less commonly, Schwann cells. PBs appeared as cytoplasmic, non–membrane-bound, granulo-filamentous structures displacing normal axoplasm to the margins of the axon. Other frequent alterations were demyelination and remyelination, with sporadic clusters of supernumerary Schwann cells forming onion bulbs. Mitochondria had a consistently normal appearance. In view of these findings a diagnosis of APBD was made.

The patient was clinically reexamined 1 year later and her status was unchanged. After 3 years, however, dysarthria, dysphagia, and ataxia had worsened. Dysmetria and dysdiadochokinesia of upper and lower extremities and urinary incontinence were also evident. Extensor plantar reflexes and palomomental reflexes were present bilaterally. At this time neuropsychological tests showed a severe cognitive impairment affecting both cortical and subcortical functions. A new brain and spinal MRI showed an extension of the WM changes, which affected most of the cerebral hemispheres, together with moderate cortical atrophy and severe atrophy of the corpus callosum, cerebellum, brainstem, and spinal cord. In order to investigate changes in cerebral metabolism we performed \textit{\textsuperscript{1}H-MRSI} of the brain. In addition, GBE activity was measured in cultured fibroblasts obtained from a skin biopsy and genetic studies of the \textit{GBE1} gene were performed. Nine
months later the patient died of pneumonia at home. Autopsy was not performed.

**Family History.** The elder brother of the patient had complained 10 years earlier, at the age of 36 years, of urinary urgency and sporadic incontinence. Since the age of 40 he had complained of slurred speech, “dizziness,” and weakness in all limbs. His clinical record reported dysarthria, dystadiakinesia, ataxic gait, and generalized hypotonia. Computed tomography of the brain showed cerebral atrophy with dilation of the ventricular system and bilateral periventricular confluent hypodensities. He was diagnosed with normal pressure hydrocephalus and underwent ventriculo-peritoneal shunting. On follow-up, no clinical improvement was reported. A few months later he died of a septic complication related to his shunt. No material was available for morphological and genetic analysis.

The family history was otherwise unremarkable. The surviving brother and sister of the patient, both in their 50s, and the patient’s three children (two sons, one daughter), all in their 20s, were in good health.

**\(^1\text{H-MRSI of the Brain.}\)** The \(^1\text{H-MRSI} \) examination was performed by using a Philips Gyroscan NT operating at 1.5 T (Philips Medical Systems, Best, The Netherlands). Multislice spin-echo and fluid-attenuated inversion recovery (FLAIR) images were obtained in a transverse plane parallel to the anteroposterior commissure line. The MR images were used to position a large intracranial volume of interest (VOI, 100 mm anteroposterior × 20 mm craniocaudal × 100 mm left–right) for spectroscopy, which was centered on the corpus callosum to include mostly WM and some mesial cortex of both hemispheres (Fig. 2). We used this specific VOI to obtain metabolic information from a VOI that is particularly important in a WM disorder, as it includes a portion of the brain where much of the visible MR pathology occurs and where axonal projections converge.

Two-dimensional spectroscopic imaging was obtained using a point-resolved spectroscopy (PRESS)
sequence for volume selection (repetition time, 2000 ms; echo time, 272 ms; 250 × 250 mm field of view; 32 × 32 phase-encoding steps; one signal average per step) as previously described. Magnetic field homogeneity was optimized to a line-width of about 5 Hz over the VOI, using the proton signal from water. Water suppression was achieved by placing frequency-selective excitation pulses at the beginning of the MRSI sequence. Prior to the water-suppressed acquisition another MRSI was acquired without water suppression (repetition time, 850 ms; echo time, 272 ms; 250 × 250 mm field of view; and 16 × 16 phase-encoding steps) to allow for B₀ homogeneity correction.

Postprocessing of the raw ¹H-MRSI data was performed as previously described. The nominal voxel size of raw ¹H-MRSI data was 8 × 8 × 20 mm, giving a resolution of about 12 × 12 × 20 mm after k-space filtering. In these cerebral volumes, resonance intensity values of N-acetyl groups (mainly NAA), choline (Cho, choline-containing compounds arising mainly from tetramethylamines), creatine and phosphocreatine (Cr), and lactate (Lac) were determined. In particular, Lac resonance intensities were from the methyl doublet resonance centered at 1.33 ppm (with 7-Hz splitting). Using this method the Lac resonance intensity can be detected only under pathological conditions, as it is below detection limits in the normal brain. Resonance intensity values of metabolites were assessed using a combination of Xunspec1 software (Philips Medical Systems, Andover, Massachusetts) and free software developed at the Montreal Neurological Institute (AVIS; Samson Antel, PhD, Magnetic Resonance Spectroscopy Unit, MNI, Montreal, Canada). Using this software, Gaussian-fitted peak areas were determined relative to a baseline computed from a moving average of the noise regions of each spectrum. No attempt was made to provide an absolute quantitation of our ¹H-MRSI data for the known complexity and inaccuracies of deriving absolute (mmol/L) concentrations from in vivo ¹H-MRSI, which would require knowledge about individual metabolite relaxation and correction for spatial variations of the excitation pulse and B₀ inhomogeneities. Mean values of NAA/Cr, Cho/Cr of the whole brain region were obtained by averaging each value for all the voxels in the spectroscopic VOI. Values of Lac/Cr were calcu-
lated by averaging only voxels with detectable Lac resonance intensities. As chemical shift artifacts associated with selective excitation might affect spectra at the edges of the VOI, they were deleted before averaging. $^1$H-MRSI values of the patient were compared to those of a group of 12 age-matched normal controls obtained by using the same $^1$H-MRSI procedure.

The results of the $^1$H-MRSI examination showed decreases of the NAA/Cr ratio in the whole VOI (2.7, normal controls = 3.05 ± 0.15) and normal values of Cho/Cr (1.27, normal controls = 1.3 ± 0.20). Resonance intensities at 1.33 ppm, deriving from the methyl doublet of Lac, were found in each voxel of the periventricular WM of both hemispheres, with a mean resonance intensity of Lac/Cr = 1.2 (Fig. 2).

**Biochemical and Genetic Studies.** GBE activity was assayed in cultured fibroblasts as described. Total cellular RNA and genomic DNA were extracted from cultured fibroblasts according to standard procedures. Six overlapping fragments encompassing the entire coding region of $\text{GBE1}$ were directly amplified from the patient’s total RNA with the Superscript Pre-Amplification System kit (Life Technology, Gaithersburg, Maryland), as previously described. The promoter region, the entire coding region sequence, and exon–intron boundaries of the $\text{GBE1}$ gene were amplified by polymerase chain reaction (PCR) using a set of intronic primers. The PCR products were purified and directly sequenced using the ABI Prism Big-Dye terminator cycle sequencing kit and an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, California). To confirm the identified mutation, specific primer extension techniques were employed using minisequencing analyses (ABI Prism SNaPshot multiplex kit; Applied Biosystems).

GBE residual activity in cultured fibroblasts was less than 5% of the normal value. By sequencing the $\text{GBE1}$ gene a heterozygous A-to-G transition in exon 12, changing a tyrosine with a cysteine (Tyr535Cys), was identified (Fig. 3). This mutation was absent in a panel of 200 human control chromosomes. Sequencing of the entire coding region and of the promoter region failed to detect any other variant.

**DISCUSSION**

Although a “typical” combination of symptoms and signs may be recognized in most patients with APBD, several clinical variants, mimicking spinocerebellar ataxia, extrapyramidal disorders, or motor neuron disease, have been described. In our patient, clinical presentation at onset lacked some of the “typical” features, such as cognitive impairment and corticospinal signs, and was characterized by unusual signs, such as cerebellar dysfunction, dysthria, and dysphagia that correlated with MRI evidence of cerebellar and brainstem atrophy. However, follow-up disclosed the full “typical” clinical setting during the course of the disease.

In the presence of such clinical findings the results of sural nerve biopsy led to the unequivocal diagnosis of APBD. We observed PBs not only in myelinated and unmyelinated fibers, but also within endoneurial fibroblasts and perineurial cells, as previously reported in a juvenile case of polyglucosan body disease. Interestingly, our teased-fiber findings suggest that deposition of PBs, rather than occurring randomly, affects single nerve fibers throughout their length, possibly favored by local metabolic stress. Besides, we detected several examples of segmental demyelination and remyelination,
Schwann cell vacuolation and hyperplasia, and endoneurial mast cell infiltration. These data support the hypothesis that accumulation of PBs, in addition to its deleterious effects on axonal integrity, may also induce direct or indirect myelin damage. Indeed, demyelination accompanies axonal loss in the brain, according to the few available postmortem studies.21,30,32

Brain MRI findings showed severe alterations of the hemispheric WM that, although described in APBD, are not distinctive compared to those seen in the leukodystrophies.5,22,23,33 In considering our 1H-MRSI findings, a reduction of NAA/Cr should be interpreted, as in other neurological disorders, as an index of neuro-axonal damage or loss due to the decrease of NAA.7,17 The presence of widespread neuro-axonal damage in postmortem APBD brains seems to give further support to this hypothesis.20,30,32 However, increases in cerebral Cr levels, resulting from gliosis occurring in the cerebral WM, cannot be categorically excluded.

The diffuse presence of a large Lac signal in the altered brain WM was the most interesting 1H-MRSI feature in our patient. Lac is the endproduct of glycolysis and accumulates when oxidative metabolism cannot meet energy requirements. Lac resonance intensity detected by in vivo spectroscopy can come from both intra- and extracellular compartments and its increases are usually seen in patients with either focal brain injuries or mitochondrial abnormalities.18,19 In conditions associated with focal inflammation, Lac accumulation reflects the metabolism of inflammatory cells.18 In contrast, increases in brain Lac resonance intensities not localized to visible MRI lesions are usually due to signals coming from the brain parenchyma as a consequence of mitochondrial disturbance.19 Thus, the large and diffuse presence of Lac signal in the abnormal WM of our patient could be related to infiltrating macrophages recruited by an intense demyelination, as described in postmortem studies.21,30,32 Interestingly, this mechanism has been proposed in recent 1H-MRSI studies to explain WM accumulation of Lac in different forms of leukodystrophies.8,34 However, an impaired mitochondrial metabolism of the brain parenchyma cannot be definitely ruled out, although we did not observe morphological mitochondrial alterations in the peripheral nerve. Therefore, our 1H-MRSI data, in agreement with pathological findings, suggest that brain WM degeneration in APBD may result from tissue damage involving both axons and myelin.

In our patient we identified a novel missense mutation in the GBE1 gene in a heterozygous condition. We consider this single amino acid substitution to be pathogenic because: (1) it is associated with a deficiency of GBE activity; (2) it replaces an amino acid that is evolutionarily conserved with a residue of different conformation; and (3) the mutation was absent in a panel of 200 control chromosomes. To our knowledge, this is the first Italian case of APBD to be reported, and the first non-Ashkenazi patient with a likely familial presentation. Indeed, examination of the proband’s brother clinical records allowed us to challenge his diagnosis of normal pressure hydrocephalus, suggesting instead that he had APBD. Age at onset, the rich combination of symptoms and signs, very similar to those presented by his sister, and the lack of clinical improvement after ventricular shunting support this hypothesis. Moreover, his brain CT scan findings were comparable to those in other cases of APBD.5

To date, GBE deficiency and mutations in the GBE1 gene have been found almost exclusively in APBD patients of Ashkenazi descent.24 The only non-Jewish patient reported so far carried two previously unreported heterozygous mutations (Arg515His and Arg524Gln).35 Recently, two cases of typical APBD with “atypical” GBE insufficiency have been described. The first case was a non-Jewish patient with complete GBE deficiency and no mutation in the GBE1 gene.22 The second case was a patient of Ashkenazi descent with partial GBE deficiency (in the heterozygous range) and the Tyr329Ser mutation in heterozygosity. At variance with other carriers, this patient displayed the full-blown disease.39 In order to explain PB deposition in these two cases the occurrence of posttranscriptional or posttranslational defects and the influence of environmental factors have been suggested.22,53 This hypothesis may apply to other cases with “atypical” GBE insufficiency.

In conclusion, our findings widen the spectrum of APBD genotypes and underline the importance to perform GBE enzymatic assay and GBE1 gene analysis in all APBD patients, independent of their ethnic background. Finally, brain 1H-MRSI data may provide a better understanding of the pathogenesis of APBD.

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