Case Report

Adult polyglucosan body disease with reduced glycogen branching enzyme activity and heterozygous GBE1 mutation mimicking a low-grade glioma

Maria A Franco-Palacios¹, Michael D Martin², Klaas J Wierenga³, Jack R Lake⁴, Ashley C Davis⁵, Eric P Wartchow⁷, Gary W Mierau⁷, Kar-Ming Fung¹,6

Departments of ¹Pathology, ²Neurosurgery, ³Pediatrics (Genetics), ⁴Radiology, University of Oklahoma Health Sciences Center, Oklahoma, OK, USA; ⁵DNAXPRT Consulting, LLC, Overland Park, KS; ⁶Department of Pathology, Oklahoma City Veteran Affairs Medical Center, Oklahoma, OK, USA; ⁷Department of Pathology, Children’s Hospital Colorado, Aurora, CO, USA

Received December 16, 2015; Accepted February 26, 2016; Epub March 1, 2016; Published March 15, 2016

Abstract: Adult polyglucosan body disease (APBD) is a rare neurologic disease characterized clinically by progressive upper and lower motor neuron dysfunction, neurogenic bladder, distal sensory loss, cerebellar dysfunction and dementia, while histologically featured by diffuse accumulation of polyglucosan bodies throughout the nervous system and in other organs. In some cases, this entity has been proved to be associated with reduced activity of glycogen branching enzyme and mutations of GBE1. We are reporting here a case of adult polyglucosan body disease with an atypical clinical presentation and imaging features that mimic a low-grade glioma. On enzymatic studies, there was reduced activity of glycogen branching enzyme in muscle but normal glycogen content. We also identified a heterozygous consensus splice site variant in intron 5 (c.691+2T>C) and a homozygous variant of unknown significance (c.-35dupC). Our case illustrates the possible clinical, imaging, pathologic, and genetic diversity of APBD.

Keywords: Corpora amylacea, adult polyglucosan body disease, glycogen storage disease, Lafora body, Bielschowsky body, glioma, GBE1, glycogen branching enzyme

Introduction

Adult polyglucosan body disease (APBD) is a rare neurologic disease characterized clinically by progressive upper and lower motor neuron dysfunction, neurogenic bladder, distal sensory loss, cerebellar dysfunction and dementia associated with large numbers of polyglucosan bodies throughout the nervous system as well as in other organs [1-7]. In common with Lafora body disease and Bielschowsky body disease, APBD is also characterized by numerous amorphous inclusions that involve the central nervous system (CNS).

APBD can occur sporadically or in familial clusters, and many of the cases reported are of Ashkenazi Jewish origin [3, 4, 6, 8, 9]. APBD has been described in association with reduced glycogen branching enzyme (GBE) activity in leukocytes, peripheral nerves, and skeletal muscle [8, 10, 11] but cases with normal GBE enzyme activity have also been described [2, 3, 8]. Deficient GBE activity is also seen in glycogen storage disease (GSD) type IV also known as Andersen's disease [12-14]. Many different mutations have been described in the different clinical forms of GSD type IV and APBD [1, 4, 6, 12-17]. Interestingly, some of these genetic aberrations are described in both diseases suggesting that these two entities, even though clinically and pathologically different, may in fact be the manifestation of dysfunction allelic variants of the same gene [1, 4, 6, 12-17].

We are reporting here a non-Ashkenazi Caucasian woman with APBD with atypical clinical and imaging features that mimicked a low grade glioma. Her condition was approached in an integrated fashion with imaging, light and
APBD with reduced glycogen branching enzyme activity and heterozygous GBE1 mutation
APBD with reduced glycogen branching enzyme activity and heterozygous GBE1 mutation

**Figure 1.** A, B. The lesion presented as a poorly defined T1 hypointense and FLAIR hyperintense, non-enhancing (2.2 × 3.3 × 3.1 cm) lesion with subtle mass effect on the adjacent gyri in the left frontal lobe in 2007. C, D. Two years later, the lesion has expanded in size and evolved into a vaguely symmetrical lesion involving the frontal area. The abnormal FLAIR signal seems to have crossed the midline to the right frontal region. E, F. Four years after the initial presentation, the lesion has expanded in size and appears rather symmetrical on FLAIR. The lesion is non-enhancing over the course.

**Figure 2.** (A) Numerous corpora amylacea are present in the squashed cytological preparation (arrow). While most of them are round, occasional ones are elongated (arrow). (B) Some of these corpora amylacea are rimmed by brightly eosinophilic cytoplasm reminiscent of reactive astrocytes (arrow). (C) On frozen section, the background is edematous with numerous corpora amylacea present. (D, E) Numerous corpora amylacea are demonstrated and many of them tend to arrange in small clusters or around blood vessels. Note that the blood vessels are sclerotic. (F) Immunohistochemistry for glial fibrillary acidic protein demonstrated a gliotic background. Note that the corpora amylacea has fallen out during immunohistochemistry and left numerous round vacuoles (arrow). (G) The extreme increase in corpora amylacea is best appreciated with PAS stain. Note the great variation of diameter of the corpora amylacea and also the dust like positive material consistent with minute corpora amylacea (inset, PAS stain with diastase pretreatment). (H) Similar PAS positive round bodies are identified in the sweat glands. (I) Similar bodies are demonstrated in muscle biopsy using PAS stain with diastase pretreatment. Original magnification is 20× for (D, G), 40× for (A, C, F), 60× for (B, E, H), inset in (H and I).

electron microscopy, genetic and biochemical studies. Of particular interest is the finding of a heterozygous, GBE1 mutation associated with reduced branching enzyme activity but normal glycogen content in skeletal muscle. This mutation has only been previously described once in a patient with a severe, non-lethal neonatal neuromuscular form of Glycogenosis type IV and recently by Bigio et al in a case of APBD with frontotemporal lobar degeneration [1, 17].

**Clinical presentation and imaging studies**

The patient was a 57 year-old, Caucasian, non-Ashkenazi woman with no family history of neurological disorders. She presented with “auras”,...
consisting of lightheadedness with near fainting episodes, cacosmia (sensation of foul smell) and tingling in the left upper extremity. Physical examination was remarkable for truncal ataxia and mild cognitive impairment. Imaging studies showed a poorly-defined (2.2 × 3.3 × 3.1 cm) non-enhancing lesion (Figure 1A) with hyperintense T2-weighted signal and fluid attenuated inversion recovery (FLAIR) signals (Figure 1B) in the medial aspect of the left frontal lobe with subtle mass effect on the adjacent and contralateral gyri. A diagnosis of low-grade glioma was made as per imaging findings and a diagnostic stereotactic biopsy was offered. The patient declined the biopsy but agreed to follow-up. The lesion remained largely stable with very slow progression. Two years after the initial presentation, the lesion started to appear vaguely symmetrical and the abnormal signals were suspicious of involving the contralateral gyri on FLAIR images (Figure 1C, 1D).

Approximately 4 years after the initial presentation, she started to develop word-finding difficulties and the lesion became more extensive and symmetrical on imaging studies. A stereotactic biopsy of the left frontal lobe was performed followed by muscle and axillary sweat gland biopsies.

Pathology and special studies

Brain biopsy, cytological preparation and frozen section: A stereotactic core biopsy was submitted for intraoperative consultation. The specimen was confirmed with the neurosurgeon to be obtained from deep white matter in the left frontal lobe. On cytological (squash) preparation there were reactive astrocytes and...
many purple round bodies consistent with corpora amylacea (Figure 2A, 2B). What was unusual was that the number of corpora amylacea was very high and that many of these bodies were elongated ovals (arrow in Figure 2A) rather than round. A few of these bodies appeared to be wrapped within the cytoplasm of reactive astrocytes (arrow in Figure 2B). Frozen section showed an edematous background and substantial increase in the number of corpora amylacea. No atypical cells to suggest neoplastic proliferation was noted (Figure 2C). APBD was the major differential diagnosis. A separate specimen for electron microscopy was obtained. Additional biopsy cores were also procured and a request for sweat gland biopsy was also made at the time of intraoperative consultation.

**Brain biopsy, permanent sections:** Hematoxylin and eosin stained sections of the non-frozen cores demonstrated findings very similar, if not identical observations to that of the frozen section. In essence, there was a substantial increase in the density of corpora amylacea particularly in the white matter. The density was variable in different areas of the cores. In some areas, the corpora amylacea had a perivascular distribution around sclerotic blood vessels (Figure 2D, 2E). Some of the corpora amylacea were significantly larger than normal ones and some of them were elongated in shape.

Immunohistochemistry for glial fibrillary acidic protein (GFAP) also demonstrated a severely gliotic background (Figure 2F) but all of the corpora amylacea fell off from the slides (arrow in Figure 2F) leaving an empty space. Similar falling off was also noted during immunohistochemistry for neurofilament. Therefore, the intracytoplasmic location of these bodies could not be determined by immunohistochemistry. Periodic acid Schiff (PAS) stain showed a very high density of corpora amylacea with increase in perivascular distribution (Figure 2G). PAS stain also highlighted numerous minute, dust-like, positive deposits. These deposits could not be recognized on hematoxylin and eosin stained sections and were resistant to diastase pretreatment indicating that these were not glycogen granules (inset in Figure 2H) and were best interpreted as minute corpora amylacea.

Immunohistochemistry for GFAP (Clone EP67-2Y, Cell Marque, Hot springs, AR) and neurofilament (Clone FNP7, Invitrogen, Camarillo, CA) were performed with a Benchmark automated stainer (Ventana, Tucson, AZ) with antigen retrieval and dilution recommended by the vendor.

**Sweat gland and skeletal muscle:** The corpora amylacea were also identified in the sweat gland biopsy (Arrow in Figure 2H) and they were also resistant to diastase pretreatment. A biopsy of the left quadriceps was subsequently performed for histology, histochemistry, and biochemical studies. Scant corpora amylacea were identified in the muscle biopsy and were best highlighted on PAS stain with diastase pretreatment (Arrow in Figure 2I).

**Electron microscopy:** Semithin resin-embedded sections of the brain revealed numerous non-membrane bound bodies of variable diameter, with a concentric appearance, and a dense core (Inset in Figure 3A). Electron microscopy showed that the hyaline bodies identified on semithin sections corresponded to round fibrillary depositions of variable size. These rare bodies were partially rimmed by some intermediate filaments (Arrow in Figure 3A). The center of the bodies tended to be more electron dense (Figure 3B). Although they were often found in perivascular locations, they did not appear to be associated with or arising from the vessel wall (Figure 3B). No bodies were found inside myelinated axons (Figure 3C). Minute bodies were also found and corresponded to the dust like PAS positive material identified on light microscopy (white arrow in Figure 3C). Fused and elongated bodies were also identified (black arrow in Figure 3C). On high magnification, these bodies were composed of filamentous material about 8.3 nm in diameter (Figure 3D). An exhaustive search revealed only a single intracellular perinuclear body in what was likely to represent a glial cell and the filamentous morphology of this body was identical to the rest in this case.

**Biochemical studies:** The glycogen branching enzyme activity and glycogen content were measured in the muscle in an outside reference laboratory. The glycogen content was found to be normal (0.71% with the control being 0.94 ± 0.55%) and the glycogen branching activity was found to be reduced to 13.5 micro mol/min/gram tissue with a control of 32.
± 10 micro mol/min/gram tissue representing 42% of the control, consistent with haploinsufficiency.

**Genetic studies:** genetic studies were done after DNA extraction of blood leukocytes. Polymerase chain reaction was used to amplify the indicated exons plus additional flanking intronic or other non-coding sequence. Sequencing was performed separately in both the forward and reverse directions. A heterozygous c.691+2T>C mutation of the GBE1 was found along with a homozygous variant of unknown significance (c.-35dupC) in the 5'UTR. No deletion or amplification mutations within the genomic region encompassing the GBE1 were detected by comparative genomic hybridization.

**Follow-up**

Approximately 8 months later after diagnosis, the patient presented again to the hospital with a 6 week history of gait disturbance, falling episodes, weakness, word-finding difficulty and deterioration of mental status. No bladder or bowel disturbances were present. Magnetic resonance image (MRI) studies revealed an asymmetric dilation of the lateral ventricles, left greater than right with periventricular hyperintensity on T2-weighted images suggesting transependymal flow of cerebral spinal fluid. There was also a rightward midline shift, effacement of the quadrigeminal plate cistern and cisterns around the foramen magnum. The patient’s course progressed with worsening of the cerebral edema that progressed until tonsillar herniation and death. An autopsy could not be obtained.

**Discussion**

Adult polyglucosan body disease (APBD) is a rare and poorly understood neurodegenerative disorder. Diagnosis is often made at autopsy as most cases are not suspected antemortem. Histologically, APBD is characterized by numerous corpora amylacea-like polyglucosan bodies diffusely involving both gray and white matter.

The classic clinical presentation of APBD is that of a late middle aged adult presenting with progressive upper and lower motor neuron dysfunction, sensory loss, bladder dysfunction and dementia [3, 5, 7]. However, many of the patients described lacked some of the “classical” symptoms and others showed rarer clinical phenomena including extrapyramidal syndrome, spinocerebellar ataxia or amyotrophic lateral sclerosis like syndrome [18, 19]. In our case, the patient initially presented with simple partial seizures that progressed several years later to the more typical or characteristic clinical presentation including dysarthria, ataxia and memory loss. As in many of the cases, the diagnosis was clinically unsuspected. Moreover, the initial MRI findings were interpreted as being consistent with a low-grade glioma. It was not until the patient progressed with dysarthria (five years after the initial presentation), that the patient gave consent to a stereotactic biopsy. There are three unusual clinical and radiologic features in this case that go against a diagnosis of low-grade glioma. First, the patient was over 50 years old and gliomas in this age group tend to be high grade. Second, the lesion was poorly defined and did not form a distinct mass. Third, the lesion started to appear vaguely symmetrical and involved the bilateral mid frontal lobes. This feature is extremely uncommon in low-grade gliomas.

The intraoperative pathologic examination showed numerous corpora amylacea-like bodies, some with unusual shape that initiated the diagnostic query for APBD. These inclusions or “polyglucosan bodies” constitute the hallmark of the disease. These are round to oval, PAS positive and diastase-resistant bodies. Histologically, they are identical to corpora amylacea. Although corpora amylacea are common in the CNS, their distribution is typically heavier in subpial areas particularly those from the base of the brain. The key leading to high degree of suspicion of APBD at the time of intraoperative consultation in this case was the substantial increase in the number of corpora amylacea, some with atypical shape and size, in a location (deep white matter) that was not expected to have such a high density of corpora amylacea.

Corpora amylacea must also be distinguished from dense microspheres. These are round, eosinophilic, PAS negative, membrane bound bodies that are found in younger subjects [20, 21]. Gertz at al. have also described non-membrane bound granules that are densely packed α- and β-glycogen granules. These are found in
the cortex of subjects over 60 years of age. Although scant, they may reach a diameter of 50 μm [22].

Polyglucosan bodies were originally described within astrocytic or neuronal processes and are distinct from Lafora bodies and Bielschowsky bodies. Lafora bodies refer to polyglycosan bodies in Lafora body disease [5, 7]. The polyglucosan bodies are found in intraperikaryal location in association with progressive epilepsy, myoclonus and dementia, and occasionally with other chronic neurological conditions. Bielschowsky bodies are found in perikarya and neurites also and can be considered an incomplete form of Lafora body disease.

Electron microscopy performed in our case, showed the inclusion bodies to be located mostly in the neuropil in extracellular locations. There were a few inclusions partially rimmed by small traces of intermediate filaments. Only one intracellular perinuclear body in a possible glial cell was identified after exhaustive searching. These findings are congruent with studies done on the ultrastructure of the corpora amylacea that revealed these bodies start forming in the astrocytic fiber and thus are commonly seen in the vicinity of the astrocytic nuclei. As the corpora amylacea increases in size, the normal fiber pattern of the astrocyte gradually disappears or remains only at the edge, appearing extracellular in location [23, 24]. As there was a time lag of 4 years between the initial presentation and the biopsy, the area where the biopsy was obtained might represent an area of advanced change which correlates with the predominantly extracellular location of the polyglucosan bodies.

The pathogenesis of APBD is still enigmatic, with deficient GBE activity reported in some patients [8, 10, 11]. This enzyme catalyzes the transfer of alpha-1,4-linked glucosyl units from the outer end of a glycogen chain to an alpha-1,6 position on the same or a neighboring glycogen chain making a soluble spherical molecule. The activity can be measured in muscle, leukocytes, nerve tissue and skin fibroblasts [8, 10, 13, 14]. Deficiency of GBE is best known to cause type IV glycogen storage disease (GSD), which in its classic presentation manifests in childhood with progressive liver cirrhosis. One hypothesis is that absent or very low enzymatic levels are characteristic of GSD-IV and that reduced or normal levels are seen in APBD [4, 12-14]. In our patient, we observed reduced GBE activity as measured in muscle (42% of the control). Most cases report lower levels of activity and moreover, cases with similar levels of activity can be found on unaffected offspring of individuals with the disease [4]. However, on most of the reported cases, the activity is usually measured in leukocytes or skin fibroblasts [3, 4, 15, 16]. Bruno et al, in one of the earlier articles on GBE deficiency on APBD, found normal enzyme activity in muscle but decreased activity in leukocytes and skin fibroblasts in a subgroup of patients with adult polyglucosan body disease suggesting that the defect might be tissue specific [8].

The GBE enzyme of our case is reduced to 42% of control but the glycogen content was within normal limit. At least one reported case of APBD with GBE deficiency assayed in muscle with reduced GBE activity (48% of control) was reported [6]. Possible explanations might be that lower levels of activity can be found on leukocytes and skin fibroblasts in contrast to activity assayed in muscle or it may indicate possible interactions of reduced GBE levels with other enzymes involved in glycogen metabolism or other environmental factors [6].

Since the determination of the genomic structure of the human GBE1 in 1993 [25], several mutations have been described. APBD associated with GBE1 mutation is inherited in an autosomal recessive pattern [4, 26]. However, manifesting heterozygous individuals have been described, raising the possibility of post-transcriptional and post-translational defects and environmental factors playing a role in the pathogenesis [1, 6, 15, 16]. In our patient we identified a heterozygous mutation affecting a consensus splice site on intron 5 (c.691+2T>C) and a homozygous variant of unknown significance (c.-35dupC). To date, all the mutations described on APBD were also reported on different variants of GSD type IV strongly suggesting that these 2 changes represent allelic disorders encoded by mutations in the same GBE1 in spite of the very different clinical presentations [1, 12-16]. Thus GSD type IV demonstrates extensive clinical heterogeneity with the most common and classic form presenting in childhood with progressive liver cirrhosis and on the other end of the spectrum, with a later-
onset phenotype with central and peripheral nerve dysfunction in the form of APBD [4, 12-15].

Most of the reported mutations in APBD consist of missense mutations that were characteristically associated with higher retained GBE activity and therefore, compatible with “milder” forms of disease [4, 6, 15, 16]. Our patient however, had a mutation predicted to be severe as it affects a consensus splice site. Recently, Bit-Ivan et al described the same heterozygous mutation in a 35 year-old patient with APBD and concomitant frontotemporal degeneration associated with a GBE activity of approximately 58% measured on homogenized brain tissue [1]. The c.691+2T>C was also described in the non-progressive hepatic form of GSD IV and on a case of non-lethal neuromuscular variant of GSD type IV associated with a missense mutation (c.563A>C) [14, 17]. Reviewing the ExAC browser data, the c.691+2T>C SNP (rs1920-44702) is rare, with allele frequency of 0.0011, from 98,680 studied alleles. However, given the 50% reduced GBE activity we interpret this SNP as associated with loss-of-function, and as consistent with a wild-type second GBE1 allele. We also felt that no further testing of the other allele was indicated, as the presence of a hypomorphic or amorphic ‘trans’ allele, as identified recently, would result in GBE activity well below 50% [27].

Our case represents an atypical example of this enigmatic disease that defied the “typical or classic” findings on this disease. From the histopathologic diagnostic perspective, it is important to emphasize that even though the density of corpora amylacea is variable in different parts of the brain, an unusually high density of corpora amylacea, particularly when being found in an unusual location should raise a concern of APBD. Demonstration of PAS positive dust-like depositions and polyglucosan bodies in sweat glands, peripheral nerve, and muscle are useful adjuncts for confirmation of the diagnosis. We also expand on the neuroimaging findings in this disease showing that a more localized pattern that may mimic a low-grade glioma is possible. We also report a severe, heterozygous mutation (c.691+2T>C) recently described on a case of APBD with frontotemporal dementia and previously reported on a case of non-lethal neonatal neuromuscular variant of GSD-IV confirming that these 2 diseases, are in fact allelic disorders. Our case illustrates the value of integrating clinical, imaging, pathologic, metabolic and genetic findings in reaching a complex and challenging diagnosis.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Kar-Ming Fung, Department of Pathology, University of Oklahoma Health Sciences Center, BMSB451, 940 Stanton Young Blvd, Oklahoma 73104, OK, USA. Tel: 405-271-5653; Fax: 405-271-2524; E-mail: Karming-fung@ouhsc.edu

References

APBD with reduced glycogen branching enzyme activity and heterozygous GBE1 mutation


