Review Article

An emerging phenotype of central nervous system involvement in Pompe disease: from bench to bedside and beyond

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Abstract: Pompe disease (PD) is a lysosomal storage disorder caused by deficiency of the lysosomal enzyme acid-alpha glucosidase (GAA). Pathogenic variants in the GAA gene lead to excessive accumulation of lysosomal glycogen primarily in the cardiac, skeletal, and smooth muscles. There is growing evidence of central nervous system (CNS) involvement in PD. Current research is focused on determining the true extent of CNS involvement, its effects on behavior and cognition, and effective therapies that would correct the disease in both muscle and the CNS. This review article summarizes the CNS findings in patients, highlights the importance of research on animal models, explores the probable success of gene therapy in reversing CNS pathologies as reported by some breakthrough preclinical studies, and emphasizes the need to follow patients and monitor for CNS involvement over time. Lessons learned from animal models (bench) and from the literature available to date on patients will guide future clinical trials in patients (bedside) with PD. Our preliminary studies in infantile PD show that some patients are susceptible to early and extensive CNS pathologies, as assessed by neuroimaging and developmental assessments. This article highlights the importance of neuroimaging which could serve as useful tools to diagnose and monitor certain CNS pathologies such as white matter hyperintense foci (WMF) in the brain. Longitudinal studies with large sample sizes are warranted at this time to better understand the emergence, progression and consequences of CNS involvement in patients with PD.

Keywords: Pompe disease (PD); central nervous system (CNS); white matter hyperintense foci (WMF); GAAKO mouse model; gene therapy

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Introduction

Pompe disease (PD) (OMIM: 232300), also known as glycogen storage disease type II (GSD II) or glycogenosis type II, is a lysosomal storage disorder caused by deficiency of the lysosomal enzyme acid-alpha glucosidase (GAA). Pathogenic variants in the *GAA* gene lead to deficient or absent GAA and excessive accumulation of lysosomal glycogen primarily in the heart, skeletal, smooth muscles, and the nervous system. PD encompasses a phenotypic continuum which is broadly classified into two main groups (I) infantile Pompe disease (IPD) at the severe end of the clinical spectrum and (II) late-onset Pompe disease (LOPD), which has less severe clinical outcomes (1). Children with IPD present with hypertrophic cardiomyopathy, hypotonia, and muscle weakness in the first few days to months of life. IPD also represents a continuum and is further classified into (I) classic IPD characterized by severe cardiomyopathy

and death typically within the first 1–2 years of life and (II) non-classic IPD, which presents in the first year of life with less severe cardiomyopathy and a more slowly progressive myopathy (1). LOPD is primarily differentiated from IPD by the absence of cardiomyopathy in the first year of life and the presence of a more slowly progressive myopathy (2). Based on the age at onset, LOPD is further classified into (I) childhood, juvenile, or muscular variant (second year to adolescence) and (II) adult-onset LOPD (second to sixth decade) (2,3). With the approval of enzyme replacement therapy (ERT) with recombinant human GAA (rhGAA, Alglucosidase alfa) by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) in 2006 and the subsequent success of immunomodulation protocols to prevent anti-drug antibody responses to ERT, along with newborn screening initiatives and a multidisciplinary management approach, patients with IPD are living longer and a new phenotype of survivors is emerging (4-6). This review article summarizes the CNS findings in patients, highlights CNS findings in animal models, explores gene therapy as a method of reversing CNS pathologies as reported by some breakthrough preclinical studies, and emphasizes the need to follow patients and monitor for CNS involvement over time.

CNS involvement in patients with PD

PD has long been considered a metabolic muscle disorder. While the clinical hallmarks of PD impact muscle function, it is recognized that the CNS is also involved. As early as 1965, brain autopsies demonstrated abnormal glycogen accumulation in the cerebral cortex, cerebellum, anterior horn cells, and spinal cord in patients with IPD and in the cerebral cortex and spinal cord in patients with LOPD (7-11) (see *Table 1*). However, to date, limited clinical data are available demonstrating the extent and clinical significance of CNS involvement in PD. As IPD survivors have aged, neurologic symptoms including sensorineural hearing loss, foot-slapping gait, bulbar weakness with dysarthria and dysphagia, and small fiber neuropathy have become apparent (6,20-22). Problems with processing speed, learning disabilities, and on rare occasions cognitive declines have also been described in some IPD patients (24,25). Patients with LOPD can also develop sensorineural hearing loss and there is some evidence of cognitive decline, mainly affecting executive functioning (26-29).

Neuroimaging with magnetic resonance imaging (MRI) or angiograms (MRA), computed tomography, and ultrasounds have been used to analyze the structural anatomic areas of the brain in patients with PD (see *Table 2*). Collective data from serial MRIs on 13 patients with IPD (age range: 20 months–13 years) showed that all these patients with a normal baseline MRI in infancy later developed white matter hyperintense foci (WMF) at variable rates, from as early as 6 months to as late as 10 years (30,33,34,36,37,47). Periventricular, subcortical, and deep white matter were shown to be involved in reports by various authors (30,33,34,36,37,47). Additional research is required to determine whether PD has a certain pattern of WM involvement and its impact from a clinical perspective. In addition to WMF, other structural abnormalities seen in patients with IPD include ventricular enlargement, extra axial cerebrospinal fluid, or thinning of the corpus callosum during infancy with the resolution of these findings with increasing age (34). Studies using magnetic resonance spectroscopy show neuronal injury and loss of myelination, which may be suggestive of neurodegeneration in children with IPD (31,32). In LOPD, collective data from MRIs on 3 patients (age range: 6–45 years) showed WMF in the periventricular regions and the frontal lobe of cerebrum (14,27,28). It is known that cerebral blood vessel involvement in the form of intracranial aneurysms are one of the leading causes of death in LOPD (13). Intracranial aneurysm has also been described in a 7-year-old child with IPD (39).

Cognitive functioning in PD can be influenced by a number of factors such as genetic variants, rate of disease progression, other medical comorbidities that affect the wellbeing of an individual, and environmental exposures. Data on cognition in PD are limited. Developmental outcomes have been assessed in patients with IPD using different age-appropriate neuropsychological tests (see *Table 2*). The overall profile of skills shown by those participants with below average academic skills was consistent with a learning disability diagnosis rather than an intellectual disability (24). Relative weakness in the processing speed has been reported in the survivors of IPD (35,45). Problems in certain domains of cognition such as sustained attention (n=5), visual-spatial integration (n=3), and working memory (n=5) have been reported (35) for patients with IPD. In LOPD, developmental delays in childhood have been documented in a single case report (28), and significant impairments in executive functions, visual-constructive abilities, and short-term memory have been observed in adults (n=1 to 21) (29,43,44,46). Further research is needed to better understand the extent of

Table 1 Autopsy findings of the central nervous system in patients with Pompe disease

Tissue type	Pathologic findings		Clinical correlation
	Infantile Pompe disease	Late onset Pompe disease	
Brain and spinal cord	• Marked ballooning and glycogen accumulation in medulla (12); • Glycogen accumulation in the neurons of cortex, midbrain, pons, medulla, and cranial nerve nuclei. Ballooning of neurons in substantia nigra, dorsal raphae nuclei, pontine nuclei, inferior olivary nuclei, dentate nucleus. Glycogen accumulation in Schwann cells of nerve sheaths in cortex, in glial cells and astrocytes of white matter, throughout nuclei of brainstem and cerebellum, with relative sparing of Purkinje cells (13); • Sporadic glycogen accumulation in Purkinje cells in cerebellum (11); • Neuronal loss with areas of gliosis (7)	• Numerous vacuoles and glycogen in smooth muscle cells in the media of anterior cerebral artery. Glycogen in smooth muscles in intima of large arteries that comprise the Circle of Willis (14); • Glycogen in capillary pericytes (vascular smooth muscle cells) in cerebellum (10,14). Ballooned pericytes in the cerebral cortex (14); • Cerebral artery aneurysms- fusiform aneurysms of the basilar artery (15), small aneurysms of small cerebral and cerebellar arteries (14); • Broadened gyri. Moderately increased number of astrocytes in cerebrum and cerebellum. Prominent subependymal glial proliferation was present. Myelin changes ranging from focal areas of demyelination to necrosis. Lipofuscin deposits in neurons and astrocytes (14); • Glycogen in Schwann cells of the nerve sheaths in the cerebral cortex and spinal cord. Neuronal cell bodies with lysosomal glycogen. Multiple structures resembling corpora amylacea or spheroid-in spinal cord (10)	• Extensive vacuolar degeneration of the media may lead to weakness of arterial wall and eventually formation of aneurysms (14); • In the brain, along with endothelium and astrocytes, pericytes form the protective blood brain barrier and also provide immunologic defenses and act as scavenger cells (16). In mice, vascular damage in pericyte-deficient mice precedes neurodegeneration, learning and memory impairment and the neuroinflammatory response making pericytes key controllers of neurovascular functioning (17). In humans this mechanism is still unclear; • Cerebral aneurysms are one of the major causes of death in LOPD (18); • Astrogliosis can cause a range of abnormalities from reversible cell hypertrophy to long-lasting scar formation (19); still unclear for Pompe disease; Patients with IPD have sensorineural hearing loss, gait abnormalities, bulbar weakness with dysarthria and dysphagia, hyporeflexia or absent reflexes, motor deficits in limbs, abnormal nerve conduction studies, and cognitive declines (6,20-22)
Anterior horn cells	• Marked ballooning and glycogen accumulation (7,11-13,18); • Fibrillary gliosis; no glycogen increase (18)		Patients with IPD have gait abnormalities, hyporeflexia or absent reflexes, motor deficits in limbs, and abnormal nerve conduction studies
Ocular tissue	Retinal ganglion cells, photoreceptors, and optic nerve glial cells (13)	Minor atrophy of bilateral optic nerves (10)	Strabismus, myopia, astigmatism seen in IPD (23)

nervous system involvement in PD across the disease spectrum and to explore possible correlations between abnormalities detected through neuroimaging and developmental outcomes.

CNS histopathology in PD

Based on published literature, pathogenesis of muscle damage in PD has been attributed to dysregulated autophagy, lysosomal glycogen accumulation, impaired calcium homeostasis, increased reactive oxygen species, and mitochondrial defects (48). However, CNS pathogenesis is still unclear. To better understand CNS disease progression and the underlying mechanism in PD, it is important to integrate the knowledge obtained from autopsy data from patients and preclinical animal studies.

Histopathology findings in patients with PD

The major histopathological changes seen in patients with PD are due to extensive glycogen accumulation in the brain, spinal cord, and anterior horn cells (see *Table 1*). Autopsy reports of patients with IPD have shown abnormal glycogen accumulation in the neurons of the cerebral cortex,

midbrain, pons, medulla, cranial nerve nuclei, Schwann cells, glial cells, astrocytes throughout the brainstem and cerebellum (13). Vacuolization was seen in the medulla, certain nuclei in the brain, and anterior horn cells (12,13). Gliosis has also been seen in patients with IPD (12). The most prominent histological finding in patients with LOPD was the involvement of the cerebral vasculature showing vacuolization, scant glycogen in cerebellar pericytes, and aneurysms in the large, medium, and small arteries of the brain (10,14,15). In addition, astrogliosis was seen in the cerebrum and cerebellum on autopsy of a 40-year-old male patient with LOPD (14). The patient had myelin abnormalities, ranging from focal areas of demyelination to necrosis in the brain (14) (*Table 2* for clinical details). These histopathological findings, seen in both IPD and LOPD, point to what could be the cause for the development of WMF, namely vasculopathies causing ischemia and small cerebral artery damages, gliosis, and myelin changes (49) (*Tables 1*,*2*).

Normal myelination requires a close association between glial cells (oligodendrocytes, astrocytes, and Schwann cells) and neurons. In IPD, glycogen accumulation was seen in oligodendrocytes in the second trimester of gestation (9). As described above, autopsy reports of IPD show that astrocytes, Schwann cells, and neurons in the anterior horn cells and cortex had glycogen accumulation, along with neuronal losses in the brain and spinal cord (7,10,13). Therefore, abnormalities in these cells could be the cause of problems in the white matter integrity (such as de-/dys-myelination) in patients with IPD (50). In children with IPD, studies using magnetic resonance spectroscopy have described neuronal injury and loss of myelination, which may be suggestive of neurodegeneration (31,32).

Histopathology findings in spontaneous animal models of PD

Studies of naturally occurring animal models of PD have been reported in cats, sheep, cattle, dogs, and quail (*Table 3*). GAA deficiency was not tested in the cat and sheep models but the affected animals exhibit pathological phenotypes that closely resemble human PD. Electron microscopy analysis showed the presence of large amounts of rounded glycogen-loaded bodies that are limited by a single membrane in both the nerve and glial cells in the spinal medulla of the affected cats (51). In the diseased sheep, neurons in the midbrain, brain stem, cerebellum, and dorsal root ganglia were heavily affected with PAS-positive granules. Electron microscopy image showed that the affected neurons contained both

Table 2 Neurologic findings reported in the literature in patients with Pompe disease

Assessments	IPD/LOPD	n	Age/gender	Neurologic involvement	Clinical correlates	Ref
Neuroimaging studies	IPD	10	Range: newborns-90 months	• Out of the 10 CRIM-positive patients, 5 patients had WMF, 2 had hypomyelination, and 2 had normal brain MRI. Follow-up MRIs in 5 patients showed increase in the hyperintensity with time in the deep white matter of brain, but the areas of involvement did not enlarge; • delayed milestones in infancy. Gross motor development slowed and lagged further behind, when compared to their age-matched typical peers; • independent ambulation and respiration (without mechanical ventilators). Pompe-based functional skills assessment showed that normative scores declined after age 2 years, although their scaled scores increased until age 5–6 years; • cognitive tests: most patients had normal scores at 1 year age, slightly impaired at 2 years of age and then improvement thereafter-with ERT	• Neurogenic involvement may contribute to the symptoms despite early-treatment with ERT	(30)

Table 2 (*continued*)

Table 2 (*continued*)

Assessments	IPD/ LOPD	n	Age/gender	Neurologic involvement	Clinical correlates	Ref
		1	2 years/female	• MRI: Generalized hypomyelination and parenchymal volume loss; • Magnetic resonance spectroscopy (MRS) suggested neuronal injury and hypomyelination injury; • Electromyography and nerve conduction studies were consistent with a severe chronic motor axonal peripheral polyneuropathy, with no evidence of reinnervation; • Antibody titers peaked at 102,400 at 20 months (no details of the CRIM status provided). At age 23 months, severe muscular weakness, worsening respiratory distress and hypercapnia requiring intubation > died after extubation	• Slowly progressive neurodegenerative process in IPD, affecting the motor neurons	(31)
		5	Median age at baseline MRI 6 months, median age at follow-up 13 months (3 males, 2 females)	• Delayed myelination in 5/5 patents; normal myelination in 4/5 on ERT; slow myelination in 1/5 on ERT (both neuron and myelination loss, suggested by follow-up MRI and MRS)	• Myelination defects seen in IPD patients	(32)
		4	54 months/male	• Out of 4 CRIM-negative patients, 1 had an extensive WMF in periventricular areas, centrum semiovale, internal and external capsules (5th and 54th month scans); • Motor and speech deficits, continues to improve (this was attributed to the immunomodulation therapy given)	• Progression of WMF over time in a CRIM-negative IPD case	(33)
		8	Range: 3–15 months. 2/8 patients had follow-up MRIs after 10 years	• 7/8 had mild structural abnormalities such as ventricular enlargement, extra axial cerebrospinal fluid, or thinning of corpus callosum. These mild abnormalities disappeared with time, for all patients. 2/8 patients had an emergence of white matter hyperintense foci (WMF) in the periventricular and deep frontoparietal areas with subtle signal abnormalities in the basal ganglia (age 11.5 and 13.2 years). 1/8 had delayed myelination at 9 months and again seen at 4.1 years	• New emerging WMF in the second decade of life; the role of neuroimaging as part of the clinical evaluation of IPD to be considered to assess for white matter abnormalities and cerebral aneurysms	(34)
		11	8–17 years	• 11/11 had periventricular WMF and 6/11 had deep WMF in the MRI; • IQ scores lay in the range of intellectual disability; • Working memory (5 patients) and memory impairment (2 patients) seen. Five patients had problems in sustained attention, not in selective attention. Visual-spatial integration problems were found in 3 patients; • Brain involvement appeared to be independent of motor functioning of the extremities	• Suggestive of cognitive declines in certain domains in patients with IPD	(35)

Table 2 (*continued*)

Table 2 (*continued*)

Assessments	IPD/ LOPD	n	Age/gender	Neurologic involvement	Clinical correlates	Ref
		1	44 months/female	• Fast progression of deep WMF (20–44 months) in a CRIM-negative patient; • Markedly reduced attention span, behavioral problems, and speech-language delays, along with a lag in the overall developmental age when compared to her typically developing peers	• Neuroimaging and developmental outcomes were studied together (cognition, speech and language problems observed)	(36)
		1	4 years/female	• Rapid progression of WMF in six months (frontoparietal predominance involving the periventricular, deep, and subcortical white matter); • MRS showed myelin and neuronal loss; • Gait changes, hyperreflexia of the ankles, lower limb spasticity; Wechsler scale for IQ showed global severe impairment	• Rapidly progressive CNS involvement	(37)
		8		• Before the availability of ERT, this study showed that 2 had ventricular dilation and 1 patient had brain parenchymal atrophy		(38)
		1	7 years/female	• Basilar artery aneurysm	• Only case of IPD in the literature with a brain (vascular) aneurysm	(39)
		1	5 months/female	• Before the availability of ERT: multifocal dural thickening, underdeveloped neocortex (bilateral perisylvian pachygyria and open opercula) on MRI	• Underdeveloped neocortex could be due to excessive glycogen deposition during embryologic stages of development	(40)
		1	8 months/male	• Severe hydrocephalus	• Hydrocephalus may result from oversecretion, obstruction, or impaired absorption of CSF	(41)
	LOPD	31	3rd-6th decade of life	• Significant abnormalities in the cerebral vasculature (12/21 patients), signs of lacunar encephalopathy (13/21 patients), dilatation of cerebral arteries in the posterior circulation (3/10 patients) in the form of dolichoectasia of the vertebrobasilar system, and intracranial aneurysms. Significantly decreased brain connectivity in the salience network with a more relevant reduction in the bilateral middle and superior frontal gyri on functional resting MRI. Gray matter atrophy; • Mild impairment in executive functions and visual-constructive abilities	• Small and large cerebral artery are involved, based on these studies; cerebral aneurysm is one of the leading causes of death in LOPD. Middle and frontal gyri affect executive functions and primary motor control	(13,42,43)

Table 2 (*continued*)

Table 2 (*continued*)

Assessments	IPD/ LOPD	n	Age/gender	Neurologic involvement	Clinical correlates	Ref
		1	6 years/male	• WMF in the right middle frontal gyrus/frontal cortex; • Psychodevelopmental delay (including delayed language development)	• Middle and frontal gyri mostly affect the executive functions and primary motor control	(28)
		1	39 years/male	• WMF in periventricular areas. Hyperintense anterior pole of right thalamus bulging forward into adjacent horn of lateral ventricle; • Coexisting neurogenic and myopathic lesions in EMG; • Severe muscle weakness at age 7 with progressive muscle wasting and weakness thereafter; suffered from episodes of dizziness, tachycardia, right bundle branch block, very high blood pressure, transient left homonymous hemianopia, and progressive dyspnea in the last year of his life; evaluated at age 40 for occipital headaches, light sensitivity, blurring of vision, severe problems in visual perception	• Problems in vision, headaches, dizziness, and visual perception-suggestive of CNS pathology; • Vasculopathies in posterior cerebral arteries or branches can cause transient homonymous hemianopia	(14)
		1	40 years/male	• WMF in the corona radiata, centrum semiovale and periventricular areas > progressed in 10 years; • Mesial temporal sclerosis	• Progressive WMF in LOPD	(27)
		1	45 years/male	• MRS showed disturbances in the salience network, particularly in the cingulate gyrus and medial frontal cortex	• Results are indicative of significant abnormalities in the functional connectivity of the brain	(29)
		1	65 years/male	• Normal pressure hydrocephalus. Midbrain atrophy. Short-term memory impairment. Extra-pyramidal signs-postural instability	• Atrophy of the midbrain is suggestive of neurodegeneration	(44)
Neuropsychological testing	IPD	20	<18 years	• Relative weakness in the processing speed has been reported in the survivors	• The overall level of adaptive functioning was lower than their IQ scores on cognitive testing	(35,45)
	LOPD	21	Mean age 49±18.4 years	• Mild cognitive declines in half the patients, with an impairment in the visual constructive subtests in all patients	• Certain domains in cognitive declines seen in these patients with LOPD	(43)
		22		• Significant impairments in executive functions		(29,43)
		1	17 years/male	• Wechsler Adult Intelligence Scale revealed lower than average verbal score and full-scale score, compared to peers	• Reportedly the first study to show declines in cognitive skills measured by a neuropsychiatry test	(46)

Table 3 Neurological involvement in animal models of Pompe disease

Animal model categories	Animal	Neurological phenotypes	Ref
Spontaneous animal models	Cat†	• Glycogen accumulation in both neuron and glial cells in the brain and spinal cord	(51)
	Sheep†	• Glycogen accumulation in neuron in the brain stem and cord • Swollen neurons in the mid and posterior brain stem, cord, and cerebellum • Vacuolated neurons in spinal cord	(52)
	Cattle	• PAS-positive glycogen staining in the brain and spinal cord:	(53-57)
		• Almost all neurons in the thalamic, midbrain, medulla oblongata, cerebellar roof nuclei, basal ganglia, cerebral cortex, hippocampus, and cerebellar granular layer in the brain • All neurons of the spinal grey matter	
		• Swollen and vacuolated neurons in the central and autonomic nervous system	
	Dog	• Granulovesicular neurons in all layers of the cerebral and cerebellar cortex, hippocampus, brain stem ganglia, retinal ganglion layer, and spinal cord ventral horns • PAS-positive granulation	(58)
	Quail	• Reduced GAA activity in the brain • Swollen and vacuolated nerve cells in the brain and spinal cord • PAS-positive materials in the brain and spinal cord	(59,60)
Genetically modified rodent models	Δ13/Δ13 mice	• Glycogen accumulation in different subpopulations of neurons and non-neuronal cells in CNS	(61)
	$\Delta 14^{neo}/\Delta 14^{neo}$ mice	• PAS-positive glycogen staining in the brain	(62)
	$6^{neo}/6^{neo}$ mice	• Glycogen accumulation in the spinal cord and entire brain:	(62-71)
		• Cerebral cortex, olfactory bulb, striatum, thalamus, hippocampus, hindbrain, cerebellum, and medulla	
		• Behavioral abnormalities:	
		• Rota-rod, wire hanging, foot fault test, footprint test, cylinder test, beam walking, novel object recognition, and von Frey test	

†, GAA deficiency was not confirmed in this model.

empty vacuoles and lysosome-like structures filled with granular material resembling glycogen (52).

Several reports have described CNS involvement in cattle (*Table 3*). Glycogen content was increased and GAA activity reduced in both the brain and spinal cord of affected cattle (56). Swollen neurons containing PAS-positive vacuoles were seen throughout the CNS (53-56). In particular, many neurons in the cerebral cortex, mid-brain thalamus, hippocampus, brain stem, roof nuclei, molecular and granular layers of the cerebellum, and medulla were found to be affected (53,56). Glial cells in the white matter of the cerebrum and cerebellum were enlarged and contained PAS-positive glycogen (56). Purkinje cells also contained PAS-positive granules but were rarely swollen or vacuolated (53,56).

Similar to the cattle model, a dog model of PD revealed the presence of PAS-positive granulated neurons in the cerebral and cerebellar cortex, hippocampus, brain stem ganglia, retinal ganglion layer, and spinal cord ventral horns (58). Japanese quail, once used in the preclinical studies before the development of mouse models, showed decreased GAA enzyme activity in the brain and spinal cord on necropsy (60). Histological results showed that nerve cells were swollen and vacuolated and the accumulation of PAS-positive glycogen was observed in the brain and spinal cord (59).

Collective data from these naturally occurring animal models seem consistent with CNS findings in patients with PD.

Histopathology findings in rodent models of PD

Mice share many common genetic features with humans

and mouse models are extremely useful for studying human disease and testing new therapies. To date, four genetically modified knockout mouse (GAAKO) models of PD have been reported. The first mouse model (Δ13/Δ13) was generated by disrupting exon13 in the *Gaa* gene (72); the second is $6^{neo}/6^{neo}$, in which the *Gaa* gene reading frame is disrupted by inserting a neomycin-resistance gene (73); the third is Δ6/Δ6, in which exon 6 was deleted from the *Gaa* gene by Cre/lox-mediated recombination (73); and the fourth model, $\Delta14^{neo}/\Delta14^{neo}$, was created by a targeted deletion of exon 14 (62). Unlike in humans where disease severity and age of onset are closely associated with the level of residual GAA activity, all these knockout mouse models had undetectable GAA enzyme activity but variable disease severity. All four mouse models accumulated glycogen in the heart, skeletal muscles, and the brain, however, only the $6^{neo}/6^{neo}$ and $\Delta14^{neo}/\Delta14^{neo}$ mice showed a severe phenotype by developing early functional impairments of mobility and muscle strength (62,73). The $6^{neo}/6^{neo}$ model resembles critical features of both the infantile and adult forms of the human disease and has been the most used animal model for pre-clinical therapy development over the past 20 years (62,73). CNS involvement in GAAKO mice have been reported in the Δ13/Δ13, $6^{neo}/6^{neo}$, and $\Delta14^{neo}/\Delta14^{neo}$ mouse models (*Table 3*).

Recent studies have shown progressive glycogen accumulation in the CNS of GAAKO mice by PAS-staining of tissue sections. Noticeable glycogen accumulation was observed in the brain and spinal cord of 2-week-old GAAKO mice and PAS-positive glycogen was even detected in some motor neurons in the brainstem on the first day after birth (70,71). At 1 month of age, still asymptomatic, GAAKO mice showed a moderate accumulation of glycogen in the entire brain and spinal cord, which continued to increase over time (64,70,71). By 3 months of age, there was a striking increase in the amount of accumulated glycogen, and by 12 months the pathology could be characterized as extensive (71).

In the brain, glycogen accumulation occurs in both neurons and glial cells in the cerebral cortex, corpus callosum, and hippocampus (64,71). A dramatic increase in glycogen accumulation was seen in the granule cell neurons in the glomerular layer of the olfactory bulb (64,71). In the cerebellum, massive glycogen accumulation was observed in the Golgi epithelial cells in the cortex, in the granule cells, and the cytoplasm of most glial cells in the cerebellar cortical white matter (63,64,70,71). In brainstem (mainly medulla) and spinal cord, PAS staining revealed extensive glycogen accumulation in the large neurons, especially in the motoneurons (also known as motor neurons) (64,68-71). Swollen degenerating axons and motoneurons containing numerous vacuoles were also found in these regions (64,68).

In the GAAKO mice, neurodegeneration and neuroinflammation were detected in the brain by immunohistochemical markers, but showed no evidence of a correlation with the glycogen accumulation (68). Transcriptome analysis of the spinal cord revealed that both the cell death and proinflammatory signaling pathways were dramatically upregulated. TUNEL (transferase dUTP nick end labeling) assay confirmed the progressive cell death in the spinal cord of GAAKO mice (69). Astrogliosis was observed in some old GAAKO mice and asymptomatic young mice particularly in the white matter of spinal cord (64,68). Microglial activation was noted in the spinal cord of GAAKO mice even at a young age (at 6 weeks) (68).

The age-wise, progressive glycogen accumulation in the CNS observed in the GAAKO mouse models may be helpful to better understand the underlying disease progression and identify novel endpoints in humans since there are very few longitudinal studies in patients with PD that explore the extent of CNS lesions and its impact on clinical outcomes. Neurodegeneration described here also requires to be translated to clinical studies in patients with PD.

Defects of neurological functions in GAAKO mice

Functional tests including grip strength, wire hang, treadmill, and rotarod tests that have been used in most preclinical studies with GAAKO mice were mainly used to measure skeletal muscle strength, and did not define the role of a neurological component, if any, in the overall neuromuscular dysfunction. Respiratory dysfunction was previously considered a consequence of muscle weakness, but recent studies have suggested that respiratory dysfunction may be a measure of neurological impairment, which can also contribute to the respiratory problems in PD (65). The muscle-specific transgenic GAAKO mice expressing hGAA only in skeletal muscle showed a normal functioning diaphragm muscle. Even with normal diaphragm function, the transgenic mice still showed a similar abnormal ventilation pattern similar to the GAAKO mice during quiet breathing without improvement, suggesting a neurologic rather than muscle etiology (65). Furthermore, glycogen accumulation was detected in the respiratory-related motoneurons in GAAKO mice (65,68).

We recently performed a comprehensive set of

behavioral tests to evaluate the impairments of motor coordination and balance, sensation, and memory in GAAKO mice in an attempt to understand the association of glycogen accumulation in the CNS with neurological defects (71). Neurological defects in muscle coordination and balance were demonstrated by four tests: the GAAKO mice showed shorter latency to fall in the rotarod test, asymmetric forelimb usage in the cylinder test, increased time to cross the narrow beam in the beam walking test, and gait abnormalities in the footprint test in comparison with age-matched wild-type (WT) mice. Sensory impairment was tested by the von Frey test, which showed that the withdrawal threshold increased dramatically in the GAAKO mice compared with the WT controls. Impairments in memory were assessed by the novel object recognition test and it showed that GAAKO mice spent less time exploring a novel object than the WT mice (71). These results strongly support that there is neurologic involvement in GAAKO mice and provide a quantitative method for evaluating CNS functioning in preclinical studies. We successfully used these behavioral tests to evaluate the effect of gene therapy on the correction of muscle and CNS defects in GAAKO mice (71).

Recent development of CNS-targeted gene therapy for PD

ERT with rhGAA has no effect on the CNS in patients with PD due to its inability to cross the blood-brain barrier (BBB) (74). In contrast, adeno-associated virus (AAV)-mediated gene therapy may provide an improved treatment option for PD due to the availability of novel AAV serotypes such as (75,76) AAV serotype 9 (AAV9) that can transduce muscle tissues with high efficiency and cross the BBB to deliver the therapeutic gene to the CNS (75-80). In the past 15 years, numerous studies have demonstrated the efficacy of gene therapy with AAV vectors expressing hGAA in GAAKO mice, however most prior studies focused on the correction of skeletal and cardiac abnormalities. Recently great efforts have been made to improve the correction of the CNS abnormalities in GAAKO mice using selective AAV serotypes, neuron-specific promoters, and various vector injection routes. In this review, we will focus on the recent articles related to CNS-targeted gene therapy in GAAKO mice (*Table 4*).

Several studies have demonstrated that administration of AAV vectors directly into the brain or spinal cord can effectively correct glycogen storage in the CNS but not in muscles of GAAKO mice (70,89). Intraspinal injection of an AAV5 vector expressing hGAA restored GAA activity and cleared neuronal glycogen accumulation in the region of spinal cord near the injection site and improved ventilation in GAAKO mice (82). Intrathecal administration of an AAVrh10- or AAV9-CAG-hGAA vector into GAAKO mice effectively reduced glycogen accumulation in the brain and spinal cord and achieved sustained neurologic and neuromuscular correction (70). Intracerebroventricular injection of a tyrosine-mutant AAV9/3 vector containing a neuronal cell-specific promoter to drive hGAA expression into neonatal GAAKO mice cleared the accumulated glycogen in the brain and spinal cord, but not in the muscle. Gene therapy with this vector substantially improved rotarod performance even though the strength of skeletal muscle was only slightly increased (89).

A recent study used a novel tandem promoter (LiNeuP) comprising a hepatocyte-specific promoter (hATT) and a neuron-specific (hSYN) promoter to prevent hGAA-induced immune responses in GAAKO mice (90). This study used an intravenous injection of the tolerogenic AAV9-LiNeuP-hGAA vector in GAAKO mice, which resulted in persistent hGAA expression in the liver and the CNS without provoking anti-hGAA immune responses. However, this treatment corrected glycogen storage only in the CNS and liver and had no effect on the skeletal muscles (90).

AAVB1 is a newly engineered vector that showed higher efficiency than AAV9 in transducing the brain, spinal cord, and muscle in mice (91). Intravenous administration of an AAVB1-GAA vector in GAAKO mice resulted in a significant increase in vector transduction in the thoracic spinal cord, compared to the AAV9-GAA treatment. However, the two treatments showed a similar level of vector transduction in the medulla, cervical, and lumbar spinal cord (88).

We recently reported that a single intravenous injection of a novel AAV-PHP.B vector expressing hGAA into 2-week-old GAAKO mice resulted in widespread GAA expression in all the affected tissues including the CNS (71). Glycogen contents were reduced to WT levels in the brain and heart, and significantly decreased in the skeletal muscles by the AAV treatment. Histology showed no visible glycogen accumulation in any region of the brain and spinal cord of the AAV-treated mice. Improvement of neuromuscular and neurological function was demonstrated following the AAV treatment (71). This is the first study to demonstrate that the reduction of glycogen in the CNS can improve neurological functions in PD (71). Despite

Table 4 Gene therapy approaches to target CNS impairment in Pompe disease mice

AAV serotype	Promoter	Improvement	Injection	Ref
AAV8	CMV enhancer/chicken β-actin promoter (CB)	• GAA expression, increased activity, and reduction of glycogen accumulation in the brain	Intravenous	(81)
AAV5	Chicken β-actin	• Increased GAA activity and glycogen reduction in the cervical spinal cord • Improvement of respiratory function	Intraspinal	(82)
rAAV9	Desmin (DES), CMV	• AAV genome vector detection in the spinal cord • Positive immunolabeling of GAA in the spinal cord • Cardiac and respiratory function improvement	Intrapleural	(83)
AAV1, AAV9	CMV promoter	• AAV genome vectors detection in the brain, medulla, and spinal cord • Decreased glycogen accumulation in the motoneurons which expressed GAA	Intralingual	(84)
rAAV9	Desmin (DES)	• AAV genome vectors detection in the spinal cord	Intravenous	(85)
AAV9	DES:LSP (copackaged)	• Improvement of GAA activity in the spinal cord	Intravenous	(86)
AAV8	human alpha-1 anti-trypsin (hAAT) promoter	• Uptake of secreted GAA from the liver by the brain and spinal cord • Reduction of glycogen in the brain and spinal cord by secretable GAA • Increased motor neuron survival in the spinal cord • Normalized astrogliosis in the spinal cord gray matter	Intravenous	(87)
AAV9, AAVrh10	CMV enhancer/chicken β-actin promoter (CB)	• Restored GAA activity by long-term treatment of AAV-GAA • Reduced glycogen in the brain and spinal cord • Improved behavioral defects • Correction of reactive astrocytosis • Normalized the myelin composition	Intrathecal	(70)
AAVB1, AAV9	Desmin (DES)	• GAA expression in spinal cord	Intravenous	(88)
*AAV9/3	Synapsin I (Syn-I)	• Increased GAA expression and activity in the brain and spinal cord • Decreased glycogen contents in the brain and spinal cord • Improved astrogliosis and myelination • Slightly increased muscle strength	Intracerebroventricular	(89)
AAV8, AAV9	Tandem liver-muscle (LiMP) and liver-neuron promoter (LiNeuP)	• GAA expression and reduction of glycogen accumulation in the brain and spinal cord • Prevented the immune responses to hGAA	Intravenous	(90)
AAV-PHP.B	CMV enhancer/chicken β-actin promoter (CB)	• Increased GAA expression and activity in the brain • Reduction of glycogen contents decreased in the brain and spinal cord • Improvement of behavioral defects	Intravenous	(71)

*, a tyrosine-mutant AAV9 vector containing the AAV3 derived viral genome (inverted terminal repeats).

these promising results in mice, recent studies showed that the AAV-PHP.B vector failed to robustly transduce the CNS in WT primates (non-human animal models) (92,93). With the rapid advancement of AAV vector technology, development of a novel AAV vector that can transduce both muscle and the CNS in humans is vital for effective gene therapy. At this point, more studies are warranted in animal models to allow for clinical translation into patients.

Conclusions

Until recently, PD was considered a muscle disorder due to deficiency of acid alfa glucosidase. With advances in research and availability of ERT, patients with PD are living longer. A new phenotype is emerging across the disease spectrum among the survivors. It is now clear that there is a neurological component to the disease. Data

from animal studies and clinical research suggest that the functional deficits observed in patients with PD are attributed to dysfunction of both muscle and the nervous system. However, the true extent of CNS pathology and its clinical impact is still not well understood. Hence, there is need for a comprehensive approach, which includes detailed neurological examination, routine neuroimaging and measurement of developmental outcomes, to monitor patients over time. Animal models provide a valuable tool for understanding disease progression in the CNS and for exploring novel therapies for PD. Our data from GAAKO mice demonstrate that AAV-mediated gene therapy improves neurological defects, but additional research is necessary to translate these findings into human trials (71). Delineation of CNS involvement in patients with PD, through systematic integration of the knowledge obtained from animal models and clinical studies in patients, could enable development of successful therapies that can specifically target CNS pathology and improve outcomes.

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Footnote

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