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Review

The Respiratory Phenotype Pompe Disease Rodent Models

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Abstract: Pompe disease is a glycogen storage disease caused by a deficiency in acid α-glucosidase (GAA) – a hydrolase necessary for the degradation of lysosomal glycogen. This deficiency in GAA results in muscle and neuronal glycogen accumulation, which causes respiratory insufficiency. Pompe disease rodent models provide a means of assessing respiratory pathology and are important for pre-clinical studies of novel therapies that aim to treat respiratory dysfunction and improve quality of life. This review aims to compile and summarize existing manuscripts which characterize the respiratory phenotype of Pompe rodent models. Manuscripts included in this review were selected utilizing specific search terms and exclusion criteria. Analysis of these findings demonstrate that Pompe disease rodent models have respiratory physiological defects as well as pathologies in the diaphragm, tongue, phrenic and hypoglossal motor nucleus, phrenic and hypoglossal nerves, neuromuscular junctions, and airway smooth muscle and higher order respiratory control centers. Overall, the culmination of these pathologies contributes to severe respiratory dysfunction, underscoring the importance of characterizing the respiratory phenotype while developing effective therapies for patients.

Keywords: Pompe Disease; Breathing; Respiratory

1. Introduction

Pompe disease is a rare glycogen storage disease caused by an autosomal recessive mutation resulting in deficiency of acid α-glucosidase (GAA), the enzyme responsible for breaking down lysosomal glycogen [1,2]. As a result of this enzymatic deficiency, glycogen accumulates, which leads to progressive damage of the nervous system and of cardiac, skeletal and smooth muscle. Additionally, cellular processes, such as autophagy and metabolism, are affected by the glycogen accumulation [3-5]. Prior to the recent addition of Pompe disease to newborn screening, the incidence of Pompe disease was reported as 1:40,000 [6,7]. However, because newborn screening increased the accuracy of diagnosing Pompe disease, the reported frequency is now much higher, ranging from 1:27,800 to 1:8,700 [8]. Disease severity, age at symptom onset, and disease progression vary widely depending on the mutation severity, level of residual functional enzyme present and other modulating factors that have not been completely elucidated [9,10].

Patients are broadly characterized into two groups: infantile-onset Pompe disease (IPD) or late-onset Pompe disease (LOPD) [6,9,10]. Patients with IPD experience the most severe symptoms with onset in the first few months of life [6]. IPD patients have less than 1% of normal, functional GAA and do not survive beyond two years if untreated [4,11]. Cardiomegaly and respiratory insufficiency are often the first signs of IPD, which eventually progress to cardiorespiratory failure [3,12,13]. LOPD patients can begin presenting symptoms from adolescence through late adulthood with varying disease severity. Patients with LOPD typically maintain 1-40% of functional GAA [3]. Glycogen accumulation in components of the respiratory system causes progressive respiratory insufficiency, forcing approximately 75% of children and 33% of adults with Pompe disease to rely on mechanical ventilation [14-16].

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Enzyme replacement therapy (ERT) is the most common treatment for Pompe disease and involves the administration of recombinant human GAA (rhGAA). ERT improves survival by clearing glycogen in cardiac muscle and improving cardiac function. However, ERT does not effectively clear glycogen in respiratory skeletal muscle, airway smooth muscle, and neural control centers, many patients on ERT still suffer from respiratory dysfunction and require ventilation support [6,8,17-22]. Recent advances in gene therapy hold promise for treating Pompe disease, as gene therapy has the potential to ameliorate respiratory insufficiency [6,8,23-27]. However, before treating the respiratory pathology, thorough characterization of the respiratory phenotype in Pompe disease is necessary in order to develop precise, novel therapies.

Animal models have proven useful for studying the pathophysiology of Pompe disease. There are naturally occurring models of Pompe disease in certain breeds of cattle, dogs, quails, cats, and sheep [7,28-34]. Researchers have developed mice models of Pompe disease by disrupting exons 6 and 13. Mice models have greater phenotypic accuracy and are more convenient for laboratory research [7,35]. The model developed by *Bijvoet et al*., in which exon 13 is disrupted, has a near complete absence of GAA in all tissues and displays progressive accumulation of lysosomal glycogen in cardiomyocytes, hepatocytes, and skeletal muscle fibers[36]. However, this model does not have a severe muscle weakness at 9 months of age when the paper was published, despite having such a severe deficiency of GAA [35,36]. Similarly, the model developed by *Raben et al*., in which exon 6 is disrupted, has progressive accumulation of lysosomal glycogen in the muscles and motor neurons but does not display obvious muscle wasting and weakness until 8-9 months of age [37]. Several researchers have back-crossed this *Gaa*$^{-/-}$ to pure backgrounds, either 129SVE [38-45] or C57BL/6 [46,47]. The *Gaa*$^{-/-}$ mouse on the pure 129SVE background is thought to have less severe respiratory deficits as compared to the *Gaa*$^{-/-}$ mouse on the mixed B6/129 background [39,42,48], however, overall respiratory function has not been assessed in the *Gaa*$^{-/-}$ mouse on the C57BL/6 background. In addition to these global knockout mice, mice with tissue specific expression of hGAA were created to isolate the impact of GAA deficiency in specific tissues on respiration [48,49]. Since the development of these different mouse models, characterization of the respiratory phenotype of Pompe disease is more feasible. Further studying how Pompe disease affects each component of the respiratory system, as well as overall respiratory function, is essential while developing treatments that will more completely treat respiration and prevent respiratory failure. This review seeks to summarize the pathology and dysfunction observed in the respiratory system of pertinent rodent models used to characterize the respiratory phenotype of Pompe disease. A summary of these models will be useful in the future development of therapies that can address all the characteristic respiratory pathology of this disease.

2. Results

2.1. Literature Search

The search terms identified 63 manuscripts. After using the exclusion criteria, a total of 27 manuscripts were included in this review. The manuscripts identified in this review describe pathology in the diaphragm, phrenic motor neurons, neuromuscular junctions, hypoglossal motor neurons, airway smooth muscle, and neural control centers (Fig 1). In addition, WBP in awake spontaneously breathing mice demonstrates that *Gaa*$^{-/-}$ mice have respiratory insufficiency. Neurophysiological recordings demonstrate *Gaa*$^{-/-}$ mice have blunted respiratory nerve output.

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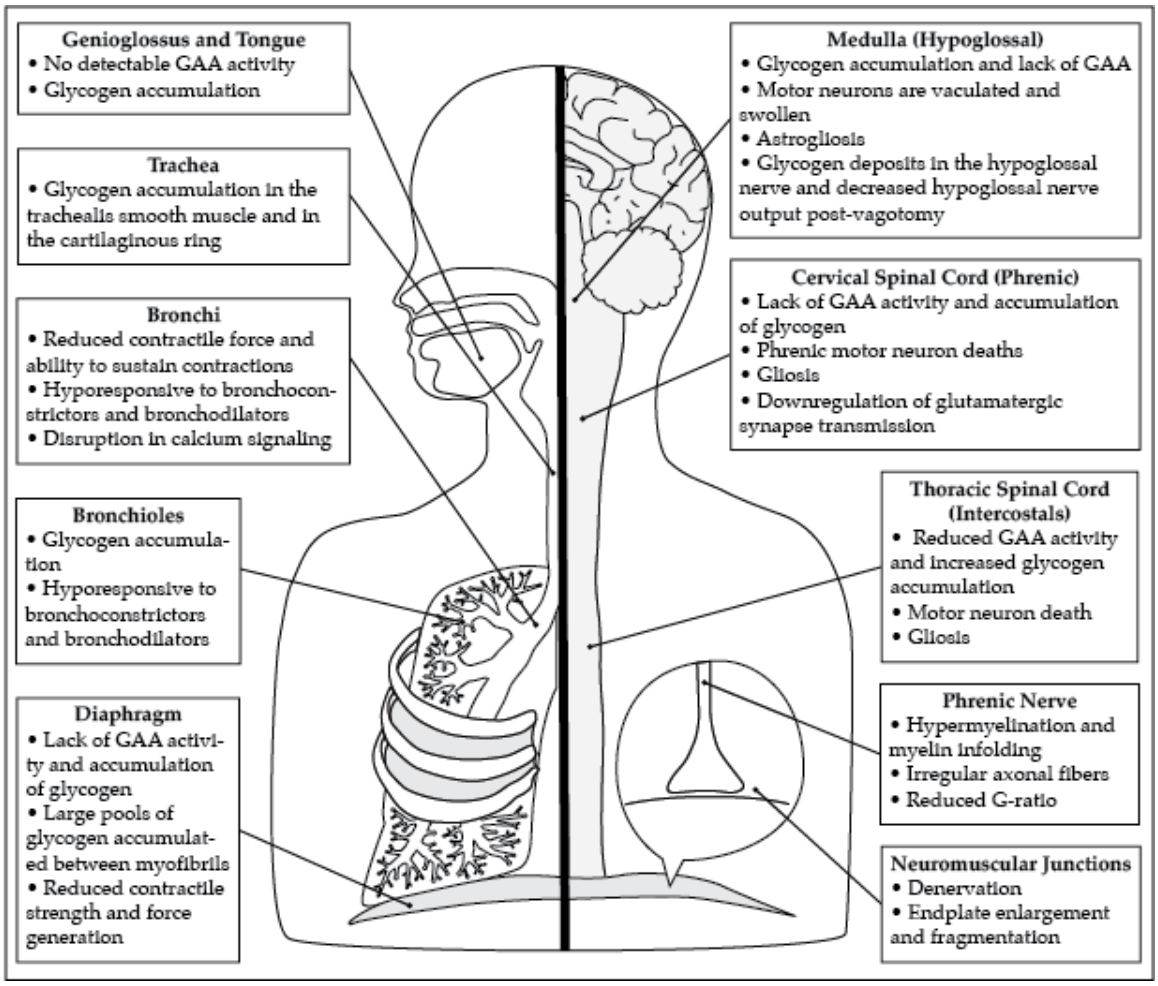

Figure 1. Pompe disease results in extensive pathology in both the muscular and neural components of the respiratory system. This figure summarizes some of the pathology present in the respiratory system that results in respiratory dysfunction and eventually respiratory failure.

2.2 Diaphragm and Phrenic Motor Neuron Pathology

The diaphragm is the primary muscle of inspiration. Lysosomal glycogen accumulation in the diaphragm results in weakness and respiratory dysfunction. *Gaa*$^{-/-}$ mice on both the B6/129 and the pure 129SVE background lack GAA protein and GAA activity in the diaphragm[50-54]. As a result, the diaphragms of these *Gaa*$^{-/-}$ mice and the mice created by *Bijvoet et al*. have significant glycogen accumulation as demonstrated by positive periodic acid-Schiff (PAS+) staining and mass spectrometry [36,37,46,47,50,51,53-57]. Mass spectrometry provides evidence that *Gaa*$^{-/-}$ mice are born with glycogen accumulation in the diaphragm that progressively increases throughout life[55]. Accumulation of glycogen disrupts the structure of the skeletal muscle myofibrils that make up the diaphragm[36]. These structural abnormalities lead to a progressive decrease in the contractile strength of the diaphragm, impeding inspiration [42,48,58,59].

Gaa$^{-/-}$ mice have reduced GAA activity in motor neurons within the 3rd - 5th segment of the cervical spinal cord where phrenic motor neurons are located [51,60,61]. Furthermore, PAS^{+} staining, a marker for glycogen accumulation, is present throughout the cervical spinal cord of *Gaa*$^{-/-}$ mice and is most evident in the ventral grey matter, where motor neurons, including phrenic motor neurons, are located[40,45,48,61]. Phrenic motor neurons, identified by retrograde labeling with cholera toxin-B (CTb), exhibit significant pathology, such as significant vacuolization and swelling of somas[45,48]. In the cervical spinal cord, *Gaa*$^{-/-}$ mice have an overall reduction in positive motor neurons stained with choline acetyltransferase (ChAT) and, more specifically, a reduction CTb-labeled phrenic motor neurons [48,51]. Particularly in late stages of the disease, *Gaa*$^{-/-}$ mice exhibit significant microgliosis and astrogliosis throughout the grey matter of the cervical spinal cord, as indicated by positive

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Ionized Calcium Binding Adaptor Molecule 1 (IBA-1) and Glial Fibrillary Acidic Protein (GFAP), respectively [40,45,51]. The reduction of motor neurons and excessive glial activation is a hallmark of neurodegeneration and neuroinflammation [40,45].

Transcriptome analysis of the cervical spinal cord of *Gaa*$^{-/-}$ mice reveals significant alterations in mRNA expression in genes related to neurodegeneration, neuronal loss, neuroinflammation, signal transduction, synaptic plasticity, and cell metabolism [40]. *Gaa*$^{-/-}$ mice have increased mRNA expression associated with p53, apoptotic, and natural killer cell cytotoxicity pathways, suggesting neurodegeneration in the cervical spinal cord [40]. The mRNA expression changes are verified by positive terminal deoxynucleotidyl transferase dUTP nick and labeling (TUNEL) which shows DNA fragmentation induced by apoptosis and further suggests neurodegeneration[40]. Early in the disease progression, at around 6 weeks of age, there is no significant TUNEL staining in the ventral horn of the cervical spinal cord where the phrenic motor neurons are located [45]. By 6-8 months, when *Gaa*$^{-/-}$ mice begin to demonstrate respiratory dysfunction, there is significant TUNEL staining in the region of the phrenic motor nucleus. However, late in the disease progression, most *Gaa*$^{-/-}$ mice had positive TUNEL staining in cervical motor neurons in the region of the phrenic motor nucleus, however, one mouse had a reduction in motor neurons and only subtle TUNEL staining [45]. These results suggest that the presence of neurodegeneration changes throughout the progression of disease and that neurodegeneration in the phrenic motor nucleus is related to the decline in respiratory function. Changes in mRNA expression associated with signal transduction are also evident in the *Gaa*$^{-/-}$ mice cervical spinal cords. Pathways related to glutamatergic synaptic transmission, which is prevalent in respiratory-related synapses, are down-regulated. Dysfunction in glutamatergic synaptic transmission could account for decreased respiratory nerve output in *Gaa*$^{-/-}$ mice [40].

Reduced phrenic nerve output could also be a result of pathology of phrenic motor neuron axons and their neuromuscular junctions within the diaphragm. The phrenic nerve of *Gaa*$^{-/-}$ mice have irregular fibers that are larger and have increased hypermyelination, myelin swelling and myelin infoldings [43]. The phrenic nerves have a significantly lower G-ratio, or the ratio of the diameter of the axon to the thickness of the myelin [43]. *Gaa*$^{-/-}$ mice also have significant denervation of the diaphragm, as shown by the lack of overlap between presynaptic axon terminals and post synaptic motor endplate terminals [43,61]. The diaphragm post-synaptic motor endplates of *Gaa*$^{-/-}$ mice are abnormally large as compared to WT and have fragmented acetylcholine receptor clusters [43].

2.3 Pathology of the Tongue and Hypoglossal Motor Neurons

Maintaining a stable, open airway is important for proper respiratory function. The genioglossus muscle helps position the tongue during breathing and is important in maintaining upper airway patency during inspiration [62]. The hypoglossal nerves, which innervate the genioglossus, transmit an impulse to open the airway immediately before inspiration when the phrenic nerve innervates the diaphragm [62]. All *Gaa*$^{-/-}$ mice models present pathology in the tongue, particularly in the genioglossus muscle [36,39,44,50]. No detectable GAA activity is present in *Gaa*$^{-/-}$ mice tongues [44,50], which is accompanied by excessive glycogen buildup [36,39,44,50,61]. As in the phrenic motor neurons, the hypoglossal motor neurons have extensive pathology [39,41,62]. The hypoglossal motor neurons of *Gaa*$^{-/-}$ mice lack GAA protein[39,44] and have PAS+ staining [39,44,45,62]. Additionally, the hypoglossal motor neurons are also morphologically abnormal with swollen somas and glycogen-filled vacuoles [39,41,44] (Fig 2). PAS+ staining is also present in ependymal cells around the central canal as well as astrocytes in the hypoglossal motor pool [62]. Evidence of astrogliosis indicated by positive GFAP staining is also observed in hypoglossal motor nucleus [61]. The pathology present in the tongue, genioglossus, and hypoglossal could result in decreased airway patency and stability during inspiration.

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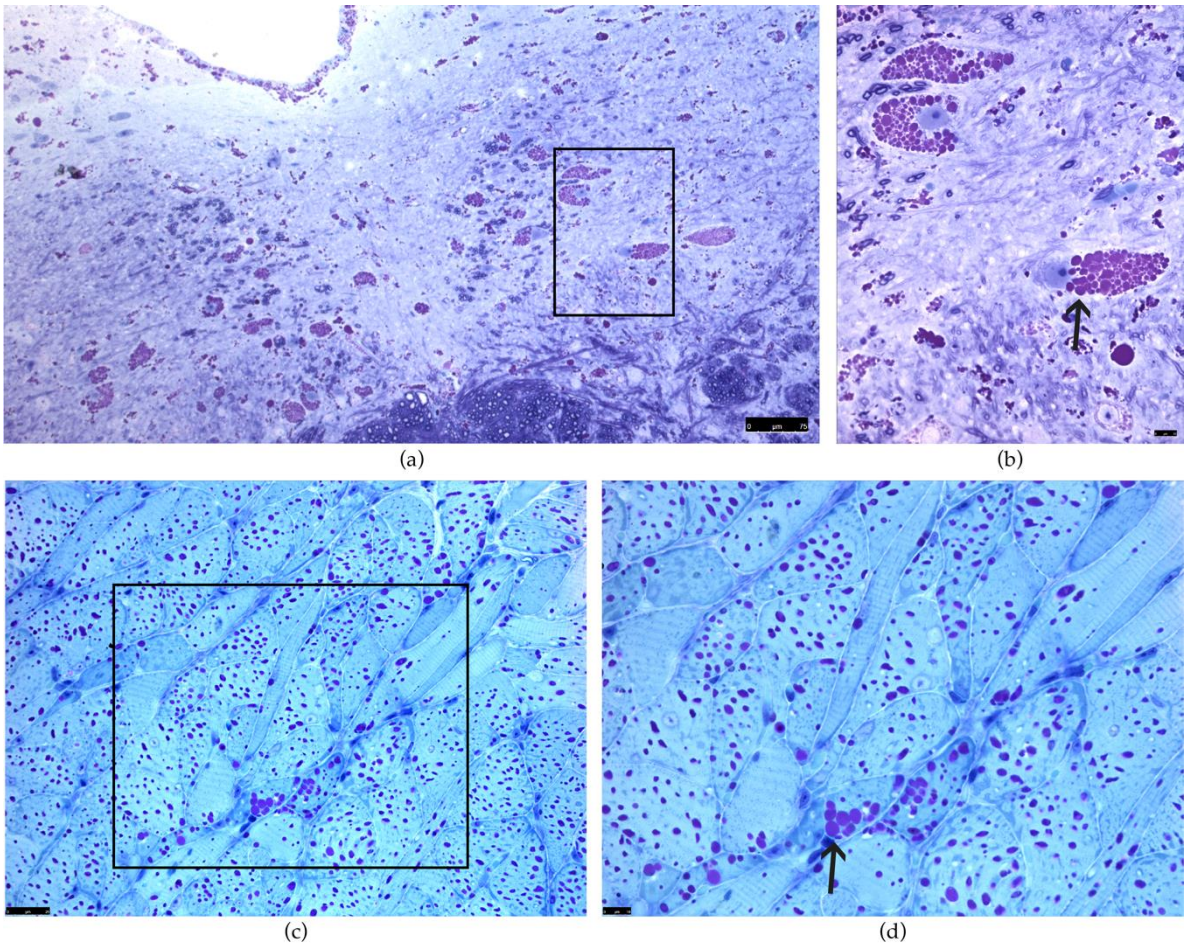

Figure 2. This figure shows the glycogen accumulation in the (a, b) hypoglossal motor nucleus and (c,d) the tongue demonstrated using periodic acid Schiff staining. The purple circle (arrows), are lysosomes filled with glycogen. The glycogen accumulation causes disruption in the architecture and cellular processes both in the tongue muscle and the hypoglossal motor neurons which control the tongue. As a result, both muscular and neural pathology contributes to the dysfunction in the upper airway which prevents proper airway stabilization prior and during inspiration. Panel b and d are higher magnification images of the selected regions (rectangles) in panel a and c.

2.4 Pathology of Airway Smooth Muscle and Associated Control Centers

Airway smooth muscle, such as tracheal and bronchial smooth muscle, also plays a critical role in maintaining an open stable airway during inspiration and expiration. *Gaa*$^{-/-}$ mice have lysosomal glycogen accumulation in the tracheal and bronchial smooth muscle [36,63]. Excessive lysosomal glycogen in *Gaa*$^{-/-}$ tracheal and bronchial smooth muscle cells disrupts cellular architecture, causing nuclear displacement, and severe cytoplasmic inclusion [63]. Following the administration of the bronchoconstrictive agent methacholine, *Gaa*$^{-/-}$ mice have decreased overall respiratory resistance, central airway resistance, and tissue resistance of the smallest airway [63]. The bronchial smooth muscle also has a reduced contractile force following exposure to methacholine, as measured by *ex vivo* bronchial ring isometric contraction [63]. The airway smooth muscle is also hyporesponsive to the bronchodilator albuterol [63]. Following the administration of albuterol to reverse the response to methacholine, the central airway resistance and bronchial force of the *Gaa*$^{-/-}$ mice did not decrease [63]. These results provide evidence that airway smooth muscle cannot properly contract or sustain contractions. This airway smooth muscle pathology is thought to be a result of morphological pathology as well as calcium signaling disruption. Although intracellular calcium ($[Ca^{2+}]_i$) release from the bronchial smooth muscle to initiate a contraction is normal and the $[Ca^{2+}]_i$ reaches the same peak as wild type controls, the sustained $[Ca^{2+}]_i$ is significantly lower [63]. The dysfunction in the

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airway smooth muscle results in decreased airway patency and stability, which can lead to respiratory insufficiency.

The nucleus ambiguus of *Gaa*$^{-/-}$ mice, which houses the motor neurons controlling the pharyngeal and laryngeal muscles, contains PAS+ staining [62]. The motor neurons of the nucleus ambiguus of *Gaa*$^{-/-}$ mice have vesicle accumulation leading to enlarged somas compared to WT controls [41]. As with lower airway smooth muscle, upper airway smooth muscle dysfunction could result from decreased neural stimulation which reduces airway patency and stability.

2.5 Pathology of Intercostal Muscles and their Neural Control Center

The intercostal muscles are a primary muscle group used during expiration and also facilitate inspiration in cases of increased respiratory demand. *Gaa*$^{-/-}$ mice experience pathology in the motor neurons that control the intercostal muscles. Lower GAA activity and PAS+ staining indicates glycogen accumulation in the ventral grey matter of the 5th to 8th thoracic spinal segment, specifically within the region of the intercostal motor pool [44,45,60]. In 6 week old *Gaa*$^{-/-}$ mice, IBA-1 and GFAP staining already indicate evidence of gliosis and thus neuroinflammation. At 6 weeks, mice do not have positive TUNEL or cleaved caspase 3 (CC3) staining, which are both markers for apoptosis, indicating there is not yet neurodegeneration in the region of the intercostal motor pool[45]. However, at 10 months of age, *Gaa*$^{-/-}$ mice have a significant reduction in ChAT positive motor neurons relative to WT in the region of intercostal motor pool and still have a significant increase in IBA-1 and GFAP glial cells with activated morphology [45,51]. Thus, it is possible neuroinflammation is prefacing neurodegeneration in the intercostal motor pools. Neurodegeneration and neuroinflammation in putative intercostal motor neurons could result in decreased signaling to the intercostal muscles and thus dysfunction during expiration and inspiration during respiratory challenge, such as the respiratory insufficiency caused by Pompe disease.

2.6 Additional Neural Control Centers

Higher order neural control centers in the brain stem control respiratory motor output, rhythm generation, and respiratory reflexes. The nucleus of the solitary tract (NTS) is the primary integration site of afferent input regarding cardiorespiratory function. Information received by the NTS is sent to other respiratory control centers in the brainstem [62]. *Gaa*$^{-/-}$ mice have glycogen accumulation in the NTS [62]. In addition, positive CC3 and TUNEL staining is present in the NTS of *Gaa*$^{-/-}$ mice, indicating cells in the NTS are undergoing apoptosis. Positive GFAP staining also provides evidence of gliosis which suggests neuroinflammation. The neurodegeneration and neuroinflammation in the NTS could result in dysfunction in the integration of afferent signaling [45] and impaired vagal reflexes observed in hypoglossal and phrenic nerve recordings of *Gaa*$^{-/-}$ mice [62]. Vagotomy normally causes a robust increase in phrenic and hypoglossal burst amplitude [62]. However, in *Gaa*$^{-/-}$ mice, the phrenic and hypoglossal burst amplitudes do not increase following vagotomy [62]. The dorsal vagal motor nucleus is negative for both CC3 and TUNEL staining and the motor neurons in the dorsal vagal motor nucleus do not have the characteristic enlarged somas and vacuolization seen in motor neurons in affected respiratory centers [41,45]. The lack of pathology in the dorsal vagal motor nucleus suggests that the lack of vagal reflexes is probably indeed a result of NTS dysfunction being compensated for in vagal intact mice [45,62].

There are abnormalities in other respiratory control centers in the brainstem, specifically in the pre-Botzinger complex and the noradrenergic neurons in the A1/C1 group of the ventral medulla [41]. Neurons in the pre-Botzinger complex, which are responsible for generating respiratory rhythms, experience vesicle accumulation in the somas of *Gaa*$^{-/-}$ but not WT mice [41]. Neurons in the A1/C1 group, which are speculated to modulate and activate breathing especially during hypoxia, also have abnormal morphology and vesicle accumulation in *Gaa*$^{-/-}$ but not WT mice [41,64,65].

2.7 Respiratory Pathophysiology in the Gaa$^{-/-}$ *mouse model*

To assess overall respiratory physiological function, researchers have used whole body plethysmography (WBP) and neurophysiological nerve recordings in Pompe rodent models. WBP

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quantifies minute ventilation, lung volumes and changes in flow, in awake, spontaneously breathing mice [41,50,66]. WBP studies confirm that rodent models of Pompe disease have respiratory dysfunction [39,42,48,50,51,58,60,67]. Although results of WBP at baseline vary, most studies found that *Gaa*$^{-/-}$ mice have abnormal parameters of WBP. While breathing room air (FiO_2 0.21; N_2 balance), these mice have greater expiratory time (Te)[42] and lower tidal volume (TV) [48,60], frequency (f) [42,48], tidal volume to inspiratory time ratio (TV/Ti) [48,58], minute ventilation (VE) [48], minute ventilation to expired CO_2 ratio(VE/V_{CO2})[48,58], peak inspiratory flow (PIF) [48,58], and peak expiratory flow (PEF) [48,67]. Furthermore, during a hypercapnic challenge ($FiCO_2$: 0.07; FiO_2 0.21; nitrogen balance), *Gaa*$^{-/-}$ mice on both the B6/129 and the 129SVE backgrounds have a reduced response to respiratory challenge [39,48,50,58-60,67]. For example, these mice have lower VE, TV, and PIF relative to WT during hypercapnic challenge, although these factors did increase relative to baseline [39,48,50,58-60,67]. The respiratory cycle in *Gaa*$^{-/-}$ mice also has higher variability than WT [62]. These abnormalities are predictive of pathology in inspiratory and expiratory muscles, upper and lower airway smooth muscles, and respiratory neural control centers. Lower VE and VE/V_{CO2} in *Gaa*$^{-/-}$ mice compared to WT mice suggest that the Pompe rodents are hypoventilating [48]. In addition, *Gaa*$^{-/-}$ mice have decreased PaO_2, providing further evidence of hypoventilation under normoxia [48]. Interestingly, *Gaa*$^{-/-}$ mice also have higher hematocrit and hemoglobin levels, which may be an attempt to compensate for decreased oxygenation [48].

Neurophysiology is utilized to assess respiratory nerve output. The goal of neurophysiology is to record phrenic (diaphragm) and hypoglossal (tongue) motor output across a range of levels of respiratory drive [39,62,68]. Neurograms of hypoglossal and phrenic nerves show dysfunction in hypoglossal and phrenic efferent nerve output [48,62]. Hypoglossal neurograms reveal a double bursting pattern in the *Gaa*$^{-/-}$ mouse's hypoglossal nerves [62]. Although there is no significant difference in frequency in *Gaa*$^{-/-}$ mice compared to WT, there is increased respiratory variability [62]. The amplitude of the hypoglossal nerve signal is lower in *Gaa*$^{-/-}$ mice than WT following vagotomy although there was no significant difference before vagotomy. Normally, after vagotomy, the hypoglossal and phrenic amplitudes significantly increase. Although the WT hypoglossal and phrenic burst amplitudes increased after vagotomy, the *Gaa*$^{-/-}$ hypoglossal and phrenic burst amplitudes did not significantly increase [62]. Additionally, there was no pre-inspiratory hypoglossal activity in *Gaa*$^{-/-}$ mice. Whereas the hypoglossal normally bursts before the phrenic to open up and stabilize the airway before inspiration, *Gaa*$^{-/-}$ mice do not have that pre-inspiratory airway stabilization, which could result in problems stabilizing the upper airway, especially during increased respiratory drive. Whereas the hypoglossal normally bursts before the phrenic to open up and stabilize the airway before inspiration, *Gaa*$^{-/-}$ mice do not have that pre-inspiratory airway stabilization [62]. Phrenic nerve efferent output is decreased in *Gaa*$^{-/-}$ mice shown by a reduced phrenic inspiratory burst amplitude, frequency, and slope of integrated inspiratory burst relative to WT mice [48,62].

To further confirm the importance of neuropathology in Pompe disease, measures of overall respiratory function were performed in *Gaa*$^{-/-}$ mice that have muscle specific expression of hGAA, isolating the respiratory dysfunction resulting from neural pathology [48]. These mice have similar diaphragm contractile force as WT mice, confirming that the diaphragm muscle is functioning properly [48]. However, despite proper function of the diaphragm, *Gaa*$^{-/-}$ mice with muscle specific correction of GAA expression still have respiratory dysfunction demonstrated by WBP. At baseline, the *Gaa*$^{-/-}$ mice and the *Gaa*$^{-/-}$ mice with GAA activity in the muscle have similar minute ventilations. However, the *Gaa*$^{-/-}$ mice with muscle specific correction of GAA expression have a minute ventilation that is between the *Gaa*$^{-/-}$ mice and WT [48]. Additionally, phrenic nerve recordings reveal that the *Gaa*$^{-/-}$ mice with GAA expression in the muscles have reduced phrenic inspiratory burst amplitude, frequency, and slope of integrated inspiratory burst relative to WT [48]. These results confirm that when the muscle is spared and produces GAA, respiratory defects are still evident. This underscores the importance of the neural involvement in the respiratory dysfunction of Pompe disease rodent models.

3. Discussion

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This review describes the mechanisms of respiratory dysfunction in Pompe rodent models. WBP is a useful tool to assess respiratory function in awake, spontaneously breathing mice [48]. Similar to patients with Pompe disease, rodent models of Pompe disease have respiratory dysfunction as shown through the use of WBP [39,42,48,50,51,58,60,67]. Although the results of WBP varied in different studies, most studies noted significant dysfunction during baseline and challenged breathing [39,42,48,50,58-60,67]. The respiratory dysfunction seen in *Gaa*$^{-/-}$ mice is a result of pathology throughout the respiratory control system and involves both muscles and neuronal tissue [48].

Although respiratory dysfunction was traditionally thought to be a result of muscle pathology and weakness, *Gaa*$^{-/-}$ mice have pathology in both respiratory muscles and the neurons that control those muscles[48]. Accumulation of glycogen is seen throughout respiratory muscles and control neurons. Rodent models that have muscle specific expression of hGAA still have respiratory dysfunction, underscoring the importance of neuronal involvement in Pompe disease [48]. Therefore, targeting muscle pathology alone is not sufficient to curing dysfunction in respiration.

During inspiration, the primary muscle in use is the diaphragm which is controlled by phrenic motor neurons. The diaphragm of Pompe mice have accumulation of glycogen and decreased contractile force when stimulated [36,37,42,46-48,50,51,53,55-59]. In addition, the phrenic nerves have a decreased ability to stimulate the diaphragm due to glycogen accumulation in the phrenic motor neuron cell bodies and axons, neurodegeneration of the phrenic motor neurons, and denervation of the diaphragm [40,43,45,48,51,60-62]. Pompe rodent models also have downregulation of glutamatergic synapse transmission, which is prevalent in respiratory related neurons[40]. Ampakines which enhance glutamatergic receptors can increase ventilation and phrenic and hypoglossal nerve output and decrease respiratory cycle variability [41]. The phrenic nerve also has irregular axon fibers, irregular myelination, and a lower G-ratio [43]. The neuromuscular junctions of Pompe rodent models have irregular distribution of acetylcholine receptor clusters as well as limited colocalization of presynaptic and post synaptic labels [43,61].

Neural control of airway muscles is also important for breathing. The hypoglossal motor neurons help maintain a stable, open airway and coordinate the tongue during breathing [62]. Pompe rodent models display pathology in hypoglossal motor neurons and the hypoglossal nerve, as indicated by positive PAS staining, hypoglossal neurograms and nerve burst amplitude [62]. Hypoglossal motor neurons have abnormal vesicle accumulation and larger somas compared to wild type motor neurons [39,41,44,45,62]. Neurograms of the hypoglossal nerve indicate irregular activity of the hypoglossal nerve of *Gaa*$^{-/-}$ mice compared to WT mice [62].

Pompe rodent models also display pathology in the upper airways and the motor nuclei that control the pharynx and larynx, as well as in the tracheal and bronchial airway smooth muscle. Glycogen accumulation contributes to pathology in the bronchial and tracheal smooth muscle tissue, and the bronchial smooth muscle of Pompe mice have reduced contractile ability [63]. The nucleus ambiguus, containing motor neurons that play a role in pharynx and larynx function, have glycogen accumulation, have vesicle accumulation and soma enlargement [41,62].

Higher order neural control centers coordinate breathing, generate the rhythm of breathing, and producing respiratory reflexes [41]. The Pre-Botzinger complex is responsible for generating the rhythm of breathing. Abnormal vesicle accumulation in the Pre-Botzinger complex could result in dysfunction in rhythmogenesis and the increased respiratory variability. Vesicle accumulation and soma enlargement in the A1/C1 group of neurons in the ventral medulla, which help activate the XII and regulate and activate breathing, especially during hypoxia, could account for the late and decreased activation of hypoglossal before inspiration [41]. Glycogen accumulation, along with neurodegeneration and neuroinflammation, was noted in the nucleus of the solitary tract, however, this pathology is not present in the dorsal vagal motor nucleus, indicating that solitary tract dysfunction likely results in observed vagal reflex dysfunction [62].

Pompe disease causes pathology throughout the respiratory system which combine to result in the devastating respiratory dysfunction and eventual respiratory failure experienced by Pompe patients and rodent models. Using treatments that only target portions of the respiratory system will not be sufficient to prevent respiratory failure. Thus, it is critical that the respiratory phenotype of Pompe disease is thoroughly elucidated while treatments are being developed so that those

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treatments can completely address all aspects of the respiratory phenotype and improve quality and longevity of life.

4. Materials and Methods

Manuscripts for this review were identified in PubMed using the search term (((((("Glycogen Storage Disease Type II" [MeSH]) OR (Pompe Disease[Title/Abstract] OR Type 2 Glycogen Storage Disease[Title/Abstract] OR Glycogen Storage Disease Type 2[Title/Abstract] OR Glycogen Storage Disease Type II[Title/Abstract] OR Acid Alpha Glucosidase Deficiency[Title/Abstract] OR Acid Maltase Deficiency[Title/Abstract]))) AND (("Respiration" [MeSH]) OR (Breath*[Title/Abstract] OR Plethysmography[Title/Abstract] OR Diaphragm[Title/Abstract] OR Phrenic[Title/Abstract] OR Respiratory[Title/Abstract] OR PreBötzinger Complex[Title/Abstract] OR lung[Title/Abstract] OR trachea[Title/Abstract] OR Pharynx[Title/Abstract] OR Larynx[Title/Abstract] OR Apnea[Title/Abstract] OR Nucleus Ambiguus[Title/Abstract] OR Hypoglossal[Title/Abstract]))) AND (("Mice" [MeSH]) OR (Mice[Title/Abstract] OR Mouse[Title/Abstract] OR Rat[Title/abstract] OR rodent[Title/Abstract] OR Murine[Title/Abstract]))) AND English[Language]. Exclusion criteria was designed prior to going through the manuscripts. Manuscripts were excluded if they did not focus on characterizing the respiratory dysfunction and pathology in components of the respiratory system in Pompe rodent models. Review articles were also excluded. Articles focusing on the efficacy of a treatment were only included if while doing so dysfunction or pathology in the respiratory system were evaluated in untreated Pompe rodents and compared to an untreated WT control. Figure 3 shows how manuscripts were excluded and how many manuscripts were excluded using each exclusion criteria, leading to the final set of included manuscripts. Additional manuscripts were used to better explain techniques such as neurophysiology or whole body plethysmography and to explain the importance of different components of the respiratory system.

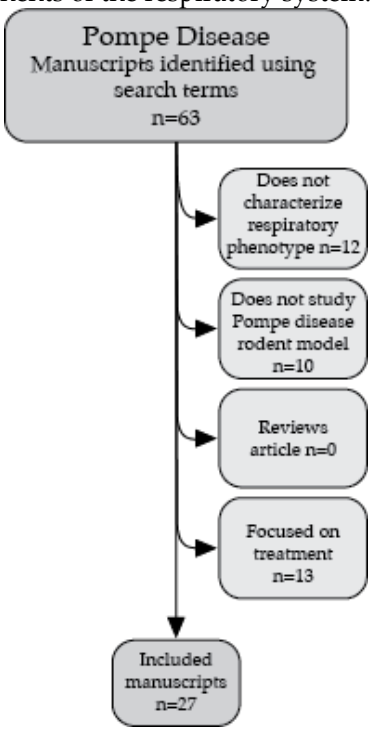

Figure 3. This figure shows the specific exclusion criteria used to find the final set of included manuscripts. Manuscripts were excluded if they did not characterize the respiratory phenotype, did not use rodent models of Pompe disease, were review articles, or focused on assessing the efficacy of treatments.

5. Conclusions

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In conclusion, the respiratory pathology noted in the Pompe disease rodent models involves dysfunction of the diaphragm, neuromuscular junctions, phrenic and hypoglossal nerves and motor neurons, airway smooth muscle, accessory respiratory muscles, and various neurological control centers. Current literature describing pathology in Pompe disease models utilize *Gaa*$^{-/-}$ mice with mice of varying backgrounds. Characterization of the respiratory phenotype of Pompe disease rodent models is necessary to improve understanding of disease respiratory pathology, providing essential information for the development of novel therapies that address the shortfalls of current treatments and contribute to improved quality of life.

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Abbreviations

GAA	acid α-glucosidase
XII	hypoglossal
IPD	Infantile Onset Pompe Disease
LOPD	Late Onset Pompe Disease
ERT	Enzyme Replacement Therapy
WBP	Whole Body Plethysmography
CtB	Cholera Toxin B
ChAT	Choline Acetyltransferase
IBA-1	Ionized Calcium Binding Adaptor Molecule 1
GFAP	Glial Fibrillary Acidic Protein
PAS+	Positive Periodic Acid Schiff
TUNEL	Terminal Deoxynucleotidyl Transferase dUTP Nick and Labeling
CC3	Cleaved Caspace 3
WT	Wild Type
TV	Tidal Volume
Te	Expiratory Time
f	Frequency
TV/Ti	Ratio of Tidal Volume to Inspiratory Time
PEF	Peak Expiratory Flow
PIF	Peak Inspiratory Flow
VE	Minute Ventilation
VE/V_{CO2}	Ratio of Minute Ventilation to Expired CO_2
NTS	Nucleus of the Solitary Tract

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