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Review

The respiratory neuromuscular system in Pompe disease☆

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ABSTRACT

Pompe disease is due to mutations in the gene encoding the lysosomal enzyme acid α-glucosidase (GAA). Absence of functional GAA typically results in cardiorespiratory failure in the first year; reduced GAA activity is associated with progressive respiratory failure later in life. While skeletal muscle pathology contributes to respiratory insufficiency in Pompe disease, emerging evidence indicates that respiratory neuron dysfunction is also a significant part of dysfunction in motor units. Animal models show profound glycogen accumulation in spinal and medullary respiratory neurons and altered neural activity. Tissues from Pompe patients show central nervous system glycogen accumulation and motoneuron pathology. A neural mechanism raises considerations about the current clinical approach of enzyme replacement since the recombinant protein does not cross the blood-brain-barrier. Indeed, clinical data suggest that enzyme replacement therapy delays symptom progression, but many patients eventually require ventilatory assistance, especially during sleep. We propose that treatments which restore GAA activity to respiratory muscles, neurons and networks will be required to fully correct ventilatory insufficiency in Pompe disease.

1. Overview of Pompe disease

The clinical features of Pompe disease were originally described by J.C. Pompe (1932) and subsequently the disease pathophysiology is considered the prototypical lyosomal storage disease (Cori, 1954; Hers, 1963). This neuromuscular disorder results from mutations in the *GAA* gene which has been mapped to the long arm of chromosome 17 (17q25.2–q25.3). More than 350 different mutations have been described, and the genotype–phenoype relationship is a subject of active investigation (Kroos et al., 2012a,b). The gene encodes a lysosomal enzyme – acid α-glucosidase or GAA – that is required for glycogen degradation. It is estimated that approximately 10% of total intracellular glycogen is normally present within lysosomes (Calder and Geddes, 1989; Geddes and Stratton, 1977). The glycogen enters the lysosome via incorporation into an autophagic vacuole or by invagination of the lysosomal membrane (i.e. microautophagy) (Geddes and Stratton, 1977). The 952 amino acid GAA enzyme is synthesized and processed via an intracellular pathway that enables post-translational modifications (Hirschhorn, 2001). After synthesis, GAA is glycosylated in the endoplasmic reticulum producing a 110 kDa precursor molecule (Hirschhorn, 2001). The molecule then acquires mannose 6-phosphate residues in a post-endoplasmic reticulum compartment and ultimately enters the lysosome via receptor-mediated transport (Raben et al., 2002). It appears that the most relevant receptor is the mannose 6-phosphate receptor, although a mannose 6-phosphate-independent pathway has been described (Klumperman et al., 1991; Tsuji and Suzuki, 1987). Once inside the lysosome, the GAA precursor molecule is cleaved to produce catalytically active 95-, 76-, and 70-kDa forms of GAA. Approximately 10% of glycosylated GAA precursor molecules are not cleaved in the lysosomes, but rather are secreted into the cytoplasm (Raben et al., 2002).

Pompe disease is associated with an absence or reduction of functional GAA which results in extensive glycogen accumulation in skeletal muscle, visceral organs and the central nervous system (CNS) (DeRuisseau et al., 2009; Raben et al., 2002; Sidman et al., 2008). The disease occurs in approximately 1 per 40,000 births, and based on appearance of symptoms, patients are typically classified as either early (infantile) or late-onset

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(juvenile/adult). These classifications, however, actually represent a continuum that relates to the extent of residual enzyme deficiency (Byrne et al., 2011b). Thus, early-onset Pompe disease results from complete or near complete deficiency of functional GAA protein, while late-onset patients maintain some residual enzyme activity (Hirschhorn, 2001; Raben et al., 2002). Heterogeneity of symptoms in Pompe disease is primarily explained by the specific gene mutation (Kroos et al., 2012a,b). For example, the most severely affected Pompe patients have a mutation in both *GAA* alleles that severely blunts or even eliminates the formation of functional GAA protein. Other mutations may result in variable levels of functional GAA protein and later onset of symptoms.

2. Respiratory insufficiency in Pompe disease

Respiratory insufficiency is extremely common in both the infantile and late-onset forms of Pompe disease (Burghaus et al., 2006; Mellies and Lofaso, 2009; Mellies et al., 2005; Pellegrini et al., 2005). Infants typically present at 4–6 months of age, and "respiratory difficulty" is often noted as the first symptom (van den Hout et al., 2003). Considerable CO_2 retention (e.g. $PaCO_2 > 60$ mmHg) can be present during spontaneous breathing in Pompe infants (Hogan et al., 1969), and cardiorespiratory failure is the leading cause of mortality (van den Hout et al., 2003). Late-onset patients show progressive respiratory muscle weakness and approximately 75% of children and adolescents with Pompe disease eventually require mechanical ventilation (Haley et al., 2003; Marsden, 2005). Subtle symptoms of night-time respiratory difficulty can include daytime somnolence or morning headache as well as laboratory data including polycythemia or elevated CO_2. Of adults with Pompe disease, roughly 33% require mechanical ventilator support, and respiratory-related problems (e.g. pneumonia, bronchitis) are prevalent (Hagemans et al., 2005). Hypoventilation during sleep may occur even if the patient is still fully mobile and commonly precedes daytime respiratory failure. Respiratory-related symptoms also include restrictive alveolar disease and impaired cough. Impaired cough results in retained secretions and an inability to clear both the normal volume of pulmonary secretions as well as those associated with acute infections. Many adult patients present initially with respiratory insufficiency, and acute respiratory failure is often precipitated by pulmonary infections.

Approximately 60% of patients with late-onset Pompe disease have a mild reduction in vital capacity (<80% predicted), and 30–40% have moderate reduction (<60% predicted) (Hirschhorn and Huie, 1999; Mellies et al., 2001). In one sample of 8 adult-onset Pompe patients, vital capacity and peak inspiratory pressure averaged 31% and 26% of predicted values. In this patient cohort, daytime hypoventilation was evident by arterial blood gas values (PaO_2: 56, $PaCO_2$: 67 mmHg) (Mellies et al., 2005). Interestingly, severe respiratory insufficiency can occur in Pompe disease without evidence for significant limb muscle weakness. For example, there is only a weak relationship between indices of respiratory and locomotor function in adults with Pompe disease, and severe respiratory insufficiency can be present without any evidence of limb girdle muscle weakness (Pellegrini et al., 2005). The physiological reasons for this observation are not clear, but could relate to increases in the metabolic activity of respiratory muscles and neurons as compared to other skeletal motor systems. In any case, it appears that the respiratory neuromuscular system is particularly susceptible to dysfunction in Pompe disease which is a unique aspect compared to other forms of muscular dystrophy in which loss of ambulation precedes ventilatory insufficiency.

3. Respiratory muscle function in Pompe disease

It is well accepted that skeletal muscle weakness is prominent in Pompe disease (Mellies and Lofaso, 2009; Mellies et al., 2001; Prigent et al., 2012). Muscular pathology is evident on histological exam, and electron microscopy reveals extensive accumulation of glycogen in muscle cell lysosomes in Pompe patients (Baudhuin et al., 1964; Hudgson and Fulthorpe, 1975). In the early phases of the disease, glycogen is also found dispersed in the cytoplasm and intrafibrillary spaces. In advanced Pompe disease, ruptured lysosomal fragments can be seen in skeletal muscle, and some myofibrils are nearly completely replaced by glycogen (Griffin, 1984). The end result of striated muscle glycogen accumulation is a loss of myofibrils and weakness (Hirschhorn, 2001).

Clinical and animal data both indicate that respiratory muscle function is impaired in Pompe disease. Prigent and colleagues (2012) evaluated trans-diaphragmatic pressure in a large sample of adults with Pompe disease using gastric and esophageal manometry. Magnetic stimulation of the phrenic nerve was used to evoke trans-diaphragmatic twitch pressure, which provides an indicator of diaphragm strength. The study confirmed diaphragmatic weakness, but it should be emphasized that diaphragmatic twitch pressures could also be influenced by conduction impairments along the motor nerve, as well as alterations in the neuromuscular junction. Reductions in expiratory pressures were also observed, and thus expiratory muscle function may also be impaired in Pompe disease (Prigent et al., 2012). This suggestion is strengthened by whole body MRI imaging data showing apparent pathology in lumbar extensor and abdominal muscles of Pompe patients (Carlier et al., 2011). That study also revealed that patients with the most impaired respiratory function had the most substantial alterations in the intercostal muscles. Respiratory muscle dysfunction and histopathology is also prominent in Pompe animal models (Mah et al., 2007, 2010). For example, the in vitro contractile force generated by the $Gaa^{-/-}$ mouse diaphragm is substantially blunted compared to wild-type control mice, and histological and biochemical evaluation shows profound glycogen accumulation. Thus, the literature has unequivocally established that diaphragm weakness is a hallmark feature of Pompe disease, and it is likely that the accessory respiratory muscles are also impaired. In this review, however, we emphasize that ventilatory failure in Pompe disease reflects a complex interplay between neural and muscular function (see Section 8).

4. The upper airway and Pompe disease

In addition to the primary and accessory respiratory "pump" muscles which actively change the volume of the thoracic or abdominal cavities, breathing also involves activation of pharyngeal and laryngeal muscles (Feldman and Del Negro, 2006). Hypoglossal (XII) motoneurons are of particular importance to upper airway patency since they regulate the shape, stiffness and position of the tongue (Bailey and Fregosi, 2004; Fregosi and Fuller, 1997; Gestreau et al., 2005; Remmers, 1978). Contraction of the extrinsic tongue muscles can dilate and/or stiffen the pharyngeal lumen, thereby minimizing airway narrowing and/or collapse in the face of negative inspiratory pressures (Fuller et al., 1999). Importantly, the tongue muscles appear to be particularly susceptible to pathology in Pompe disease. For example, Carlier et al. (2011) evaluated MRI images of Pompe patients, and concluded that while the majority of facial muscles were unaffected in Pompe, the tongue was always affected. Specifically, T1 weighted images showed "massive fat content" in the tongue, but the facial muscles were "systematically spared". Moreover, the appearance of tongue pathology was unrelated to overall disease severity (i.e., the tongue