Defective N-linked protein glycosylation pathway in congenital myasthenic syndromes

Congenital myasthenic syndromes are heterogeneous genetic disorders characterized by compromised neuromuscular transmission for which defects in multiple mechanisms are responsible. Clinical manifestations vary by syndrome subtype, but generally affected individuals present with fatigable muscle weakness, which can affect ocular, bulbar, limb and respiratory muscles. The onset of disease is usually, but not invariably, in infancy or childhood. The severity of disease varies greatly from death in early childhood to mild muscular weakness throughout life (Engel, 2012). To date, mutations in 16 different genes have been implicated in the development of congenital myasthenic syndromes. These genes encode the presynaptic protein (CHAT), synaptic components (COLQ, AGRN, LAMB2), and postsynaptic proteins (CHRNA1, CHRNB1, CHRNG, CHRN, RAPSN, MUSK, DOK7, GPT1, SCN4A, PLEC and DPAGT1) (Chaouch et al., 2012; Engel, 2012; Lorenzoni et al., 2012). The functional properties of these genes have highlighted a number of mechanisms that involve the synthesis or packaging of acetylcholine load into synaptic vesicles, the Ca²⁺-dependent evoked release of acetylcholine from nerve terminals, and the efficiency of released load in generating a postsynaptic depolarization. The functional efficiency of neurotransmission depends on the endplate architecture, density and state of acetylcholinesterase (AChE) in the synaptic space, as well as the density, affinity for acetylcholine and kinetic properties of the acetylcholine receptor. However, for many disease-associated proteins, their role at the neuromuscular junction and contribution to the development of these syndromes remains unknown (Chaouch et al., 2012; Engel, 2012; Lorenzoni et al., 2012).

Diagnostic precision is important in assessing the prognosis and managing these disorders and genetic testing is now available for a number of genes implicated in these disorders. The current technology is slow and expensive but research groups and some diagnostic laboratories are moving towards more efficient, cost-effective screening methods, such as next generation sequencing in targeted gene panels and subsequent exome sequencing in the negative families. There are still a number of genes to be identified as those identified to date account for <20% of cases (Abicht et al., 2012; Beeson, 2012); and many patients showing the characteristic predominant weakness of proximal muscle groups still lack a genetic diagnosis. But even with the application of new methods for genetic analysis, functional studies are needed to show that the genetic defects are pathogenic (Fig. 1). Although exome sequencing has been applied successfully in congenital myasthenic syndromes, there are limitations, such as difficulties in sequencing repetitive and GC-rich regions, lack of intronic sequence and problems in identifying large deletions, insertions and duplications that will require the use of multiplex ligation-dependent probe amplification or custom comparative genomic hybridization arrays.

In this issue of Brain, David Beeson and Hans Lochmüller’s groups report the identification of mutations in two enzymes involved in glycosylation, ALP2 and ALG14, as causes of recessive congenital myasthenic syndrome. More importantly this work underscores the role of the N-linked glycosylation pathway in this disorder (Cossins et al., 2013). Genetic linkage analysis, whole exome, whole genome sequencing and segregation analysis were used to determine the underlying defect in patients with the syndrome. ALG13 and ALG14 constitute uridine diphosphate (UDP) N-acetylgalactosamine (GalNAc) transferase, and ALG2 is an alpha-1,3-mannosyltransferase; both enzymes catalyse early steps in the asparagine-linked glycosylation pathway. They show that defects in these genes are pathogenic both genetically and
functionally, and that both proteins are enriched in the normal muscle motor endplate. One family with congenital myasthenic syndrome was identified with a homozygous ALG14 missense mutation where small interfering RNA silencing of ALG14 resulted in reduced cell surface expression of muscle acetylcholine receptors expressed in cultured HEK293 cells. Mutations in the ALG2 gene were identified in two congenital myasthenic syndrome kinships, where in the context of the missense ALG2 mutation, p.Val68Gly, expression was severely reduced in both patient muscle tissue and in cell cultures. Although limited functional work on these proteins is provided, the preliminary data suggest that both ALG2 and ALG14 are likely to cause impairment but without total loss of function.

The discovery of mutations in the GPFT1 and DPAGT1 genes as a cause of congenital myasthenic syndrome first outlined the role of the asparagine-linked protein glycosylation pathway in this disorder (Senderek et al., 2011; Belaya et al., 2012). The identification of ALG14 and ALG2 mutations further emphasizes the importance of this pathway, required for glycosylation of acetylcholine receptor subunits and efficient export of acetylcholine receptors to the cell surface (Cossins et al., 2013). The mechanism behind DPAGT1-associated congenital myasthenic syndrome is similar to that of ALG2 and ALG14 with reduced levels of acetylcholine receptors at the endplate region. GPFT1 defects are also likely to have a similar mechanism and lead to reduced levels of UDP-GlcNAc, a substrate for GPFT1 in the early stages of the glycosylation pathway.

The congenital myasthenic syndromes that result from mutations in these four genes display a similar phenotype, namely a limb-girdle pattern of muscle weakness where eye, facial and bulbar muscles are largely spared; and this contrasts with many other myasthenic syndromes. A number of patients present with permanent and early-onset weakness, which is similar to congenital muscular dystrophy. Judith Cossins et al. (2013) studied a family that was initially diagnosed with Ullrich’s congenital muscular dystrophy, until the ALG2 mutation was identified. A decremental response of compound muscle action potentials to repetitive nerve stimulation on electromyography was seen in these cases providing evidence for impaired neuromuscular transmission. The importance of a genetic diagnosis is emphasized by the history in sisters with ALG14 mutations, who were previously thought to have autoimmune seronegative myasthenia gravis and, as a result, underwent extensive and largely ineffective treatment (Cossins et al., 2013).

The identification of ALG2, ALG14 and DPAGT1 mutations using a method focused around exome sequencing and the confirmation of functional abnormalities with these defects further emphasizes the use of this technology in genetically undefined, but clinically well characterized neurological disorders. The glycosylation pathway has many steps (Cossins et al., 2013) and it is likely that other genes, which cause congenital myasthenic syndrome when defective, will be identified. The analysis of muscle biopsies for tubular aggregates will also be important in assessing other defective genes; aggregates are invariably present in the biopsies from the four genes identified to date and these aggregates may be the accumulated end result of abnormal glycosylation. Given that many essential proteins are modified by the addition and processing of glycans, the predicted biological consequence of impaired function should involve several systems of an organism (Larkin and Imperiali, 2011). This is the case in the first patient identified with an ALG2 (Thiel et al., 2003) and the first four patients identified with DPAGT1 mutations (Wurde et al., 2012). How some defects in these genes lead to more specific, limb-girdle pattern of neuromuscular dysfunction, and the structural abnormalities that these glycosylation gene defects cause also needs to be investigated since this could form the basis for the identification of effective therapeutic agents.

**Funding**

Research in the author’s Laboratory is supported by the Medical Research Council UK, NORD, FP7 NeurOmics grant and the National Institute of Health Research (NIHR) UCLH/UCL Biomedical Research Centre.

Henry Houlden

Department of Molecular Neuroscience, The MRC Centre for Neuromuscular Diseases and The Neurogenetics Laboratory, UCL Institute of Neurology, Queen Square, London WC1N 3BG.

Tel.: +44(0)20 3448 4068, Email: hhoulden@ucl.ac.uk

doi:10.1093/brain/awt042

**References**


