Uncovering the genetics of neuromuscular foetal akinesias using next generation sequencing

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Foetal akinesia/hypokinesia is the absence or reduction of movement *in utero*. Pena-Shokeir syndrome or foetal akinesia deformation sequence (FADS) are used to describe the range of abnormalities associated with a lack of foetal movement (including: multiple joint contractures, facial anomalies, pulmonary hypoplasia, polyhydramnios; Hall, 2009). Other disease entities overlap phenotypically with FADS, including lethal congenital contracture syndromes (Markus *et al.*, 2012), multiple pterygium syndromes and arthrogryposis multiplex congenita (Quinn *et al.*, 1991). It has been proposed that ~50% of foetal akinesia cases arise due to a primary muscle defect (Quinn *et al.*, 1991).

A recent review highlighted that 29 neuromuscular disease genes are associated with absent or reduced movement *in utero* (Ravenscroft *et al.*, 2011), with additional disease genes identified more recently (Logan *et al.*, 2011, Markus *et al.*, 2012). These genes encode proteins that function at all stages of the neuromuscular pathway; including the motor neuron, neuromuscular junction and skeletal muscle. Given the clinical and genetic heterogeneity associated with these diseases and the severity, many cases and families remain without a genetic diagnosis though many demonstrate apparent recessive inheritance. The arrival of next generation sequencing (NGS) has revolutionized the field of disease gene discovery, facilitating gene discovery in isolated cases and small families (Lalonde *et al.*, 2010).

We have conducted whole exome sequencing, one form of NGS, of nine foetal akinesia cases from seven families (two of which are consanguineous). In three of these families we have not yet been able to identify the causative variants. In one family diagnosed with lethal multiple pterygium syndrome, we identified compound heterozygous mutations in the glycogen branching enzyme 1 gene (*GBE1*). Subsequent, re-evaluation of the foetal muscle biopsies and enzyme assays performed on patient fibroblasts confirmed the defect in GBE1 function. In the three remaining families, all with foetal akinesia and nemaline myopathy (a well-recognized entity; Lammens *et al.*, 1997) we have identified variants in two skeletal muscle protein genes, not previously associated with disease. Subsequent screening confirmed that the variants segregated with disease within the families and for one of the genes also identified further variants in similarly affected patients from around the world.

Thus NGS is unlocking the genetic basis for the foetal akinesias, where previously this was a near impossibility. However NGS is not a magic bullet, since three families remain without a genetic diagnosis. This study highlights both the power and limitations of NGS. The identification of the disease genes facilitates prenatal and preimplantation genetic diagnosis for subsequent pregnancies; perhaps the ultimate outcome for these families, since developing therapies to treat these disorders will be problematic. Characterization of the disease genes in normal muscle development and function will provide further insights into fundamental muscle physiology.

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