


The advent of next-generation sequencing technologies available at an increasingly affordable price is revolutionizing the discovery and diagnosis of human disease genes. We are without doubt, witnessing the dawn of a new era of disease gene discovery that will radically impact the field of human genetics. Next-generation sequencing technologies are already having a significant impact on neurological disorders, with huge further potential for improved clinical service delivery.

In late 2010, Brain first published a paper (Wang et al., 2010) detailing the identification of a disease gene for spinocerebellar ataxia by sequencing the exomes of four affected family members. An exome comprises ~1% of the entire genome, encompassing all coding and coding-flanking regions, and is estimated to account for ~85% of all disease-causing mutations. Notably, Wang et al. (2010) demonstrated that when they allied linkage data with their exome sequencing results, exome data from only one affected individual were sufficient to find the mutation.

There have been further illustrations of the power of next-generation sequencing technologies in the past 2 years. Combining linkage and exome sequencing data was again effectively harnessed to identify mutations for familial amyotrophic lateral sclerosis (Johnson et al., 2010), a dominant form of limb girdle muscular dystrophy with a vacuolar pathology (Harms et al., 2012b), and paroxysmal kinesigenic dyskinesias (Wang et al., 2011). Homozygosity mapping in consanguineous families can also be partnered with exome sequencing for successful gene discovery, for example, for an autosomal recessive spinocerebellar ataxia (Doi et al., 2011), and also in the mammoth efforts of Najmabadi et al. (2011), who studied 136 consanguineous families leading to identification of 50 novel genes for intellectual disability or related neurological disorders. Targeted capture and next-generation sequencing of the transcriptome of a linkage region revealed the genetic cause of a dominant spinal muscular atrophy (Harms et al., 2012a), whereas sequencing of all X chromosome transcripts (Tsurusaki et al., 2011), or the entire mitochondrial genome (Kaufman et al., 2012), have also successfully been exploited. In the absence of multiple affected individuals from one family, small cohorts of unrelated patients diagnosed with the same disease have been studied together (Lee et al., 2012).

Reports such as these, and others using whole-genome sequencing [e.g. Lupski et al. (2010) to identify a Charcot–Marie–Tooth disease gene] are enticing examples of the ability of the application of next-generation sequencing to a small number of patients to find novel disease genes. Additionally, exome sequencing has indicated genetic association, such as rare CYP27B1 variants in multiple sclerosis (Ramagopalan et al., 2011).

This issue of Brain highlights the benefits of next-generation sequencing, in particular for large genes. Two teams apply exome sequencing, each to a cohort of three families with hereditary (cytoplasmic) myopathy with early respiratory failure [HMERF; Online Mendelian Inheritance in Man #603689; also known as hereditary inclusion body myopathy with early respiratory failure (Chinnery et al., 2001)]. Ohlsson et al. (2012; page….) and Pfeffer et al. (2012; page…) identify the same disease-causing mutation g.274375T>C in exon 343 (p.C30071R) in the giant gene titin, TTN, as the cause of the disease. The p.C30071R amino acid substitution is at a highly conserved residue in a myosin-binding fibronectin-III domain of A-band titin. TTN codes for a protein >1 μm in length, which spans from the M-line to the Z-disc in the sarcomere, and its sheer massive size (363 exons; complementatory DNA >100 kb) has been a serious impediment for screening using traditional methods. Mutations have previously been identified in TTN, including a p.R279W mutation in three families with HMERF (Lange et al., 2005), but the current studies are the first to exploit exome sequencing effectively to screen TTN for mutations. The current reports are vital examples of how next-generation sequencing can deal easily with the largest human gene and therefore potentially other large genes causing neuromuscular diseases (e.g. nebulin and the ryanodine receptor).

Ohlsson et al. (2012) sequenced 50 Mb captured exomes for two affected and two unaffected family members. Pfeffer et al. (2012) initially sequenced two exomes from affected individuals using a 38 Mb capture system, which gave poor coverage of TTN, so they subsequently sequenced a further exome from a different affected individual using a 62 Mb capture system. This sequenced 99.8% of the TTN coding region, at a minimum 10-fold coverage, and mean depth of 105-fold. After filtering and analytical steps revealed the TTN variant as a likely disease-causing candidate,
both groups confirmed that it segregated in other members of their three families. Collectively, the current studies tested 39 affected individuals across six different families, with the three Swedish families (Ohlsson et al., 2012) sharing a 6.99 Mb region at chromosome 2q which contained the TTN mutation, and the three families from North East England (Pfeffer et al., 2012) sharing a 2.93 Mb region within this 6.99 Mb haplotype. This strongly indicates a founder mutation in all six families, which was possibly donated to North East England by one of the Vikings who visited those parts.

Although, key features of the clinical and pathological presentation are shared among the majority of affected individuals with this TTN mutation, there is wide variation in the age of onset, with a range of 18–71 years. The skeletal muscle weakness exhibited by affected individuals is typically symmetrical and slowly progressive. Trunk, pelvic girdle, neck flexor and ankle dorsiflexors are regularly severely affected, particularly in later stages of the disease. Some patients exhibit predominantly distal muscle weakness, whereas others have mainly proximal, axial or respiratory muscle weakness, or indeed a mixture. Respiratory insufficiency is readily apparent while still ambulatory is notable, with a considerable number of patients requiring night-time ventilation from as early as their mid-30s. MRI studies by Pfeffer et al. (2012) reveal prominent semitendinosus, peroneus longus and obturator externus involvement in patients (including those who are presymptomatic), with the two former muscles being the first to show fatty infiltration. Prominent calf hypertrophy is seen in some patients, whereas cardiomyopathy is not detected in any patients.

Pathologically, significant fibre size variability, multiple internal nuclei, numerous split/fragmented fibres, eosinophilic inclusions (inclusion bodies), blue-rimmed vacuoles, ‘rubbed out’ areas (unstained by NADH-tetrazolium reductase), Z-disc streaming and widespread myofibrillar disruption are common features. Most inclusions show positive staining for a variety of proteins, including desmin, myotilin and B-crystallin. Great disparity is however seen between, as well as within muscle biopsies, with certain areas having a concentration of pathological features, whereas in other areas these are less frequent, or indeed absent, and for some biopsies the finding is non-specific.

Other phenotypes have also been shown to be caused by TTN mutations, namely tibial muscular dystrophy (a late onset distal myopathy), limb-girdle muscular dystrophy type 2J (early onset) (Hackman et al., 2002), an early onset myopathy with fatal cardiomyopathy (Carmignac et al., 2007), as well as hypertrophic (Sato et al., 1999) and dilated (Gerull et al., 2002) cardiomyopathy. A recent large study that specifically captured all mutations, namely tibial muscular dystrophy (a late onset distal myopathy), cause familial dilated cardiomyopathy. Nat Genet 2002; 30: 61–340–51.


Nevertheless, accurate molecular diagnosis provides patients with more informed genetic counseling (including prenatal, pre-implantation and presymptomatic diagnoses), essential clinical care that can improve the quality and length of life (such as nocturnal ventilation), and possibly targeted therapeutic approaches in the future. Therefore, it is imperative that next-generation sequencing technologies are embraced to screen suspected patients for TTN mutations. Pfeffer et al. (2012) propose that, due to the phenotypic variability of the disease caused by the g.274375T>C TTN mutation, it is probably an under-recognized entity, and may potentially be diagnosed as a myofibrillar myopathy due to pathological and MRI findings. It was also noted that two patients were misdiagnosed with obstructive sleep apnoea (Pfeffer et al., 2012). Time will now tell the prevalence of the g.274375T>C mutation, and indeed of other mutations in TTN, and the resulting disease phenotype/s of patients (e.g. Vasli et al., 2012). It is therefore likely that along with all the benefits that next-generation sequencing will bring to this field, it may initially complicate our level of understanding before ultimately providing clarity.

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