Institution: Newcastle University

Unit of Assessment: UoA 4

Title of case study:
Diagnosis and treatment of congenital myasthenic syndrome in patients with Dok-7 mutations

1. Summary of the impact

Congenital myasthenic syndromes (CMS) are inherited neuromuscular disorders caused by defects at neuromuscular junctions, which are often a result of acetylcholine receptor gene mutations. A subset of CMS patients (around 14% in the US and Europe) have limb-girdle myasthenia (LGM). This disease can be highly disabling with symptoms including increasing weakness of skeletal muscles. As a result of collaborative work between Newcastle and Oxford, it was determined that many LGM patients have a mutation of the Dok-7 gene (unrelated to the acetylholine receptor), and do not, therefore, respond to standard CMS treatments. Since then, a number of additional mutations have been discovered, and genetic testing is now available for the majority of known LGM-causative genes. Crucially, Dok-7 patients, and those with other non-receptor related mutations, can now be diagnosed accurately and treated effectively, with ephedrine and salbutamol (in the US, albuterol). This significantly improves these patients' quality of life by enabling them to walk and breathe unassisted.

2. Underpinning research

Key Newcastle researchers
(Where people left or joined the university in the period 1993-2013, years are given in brackets)

CR Slater (1985-2005), Reader in Neurosciences (1985-2000), then Professor of Neuroscience (2000-2005); PRW Fawcett (1982-2009), Honorary Lecturer and Clinical Neurophysiologist; and H Lochmüller (2007 onwards), Professor of Experimental Myology.

Background
Congenital Myasthenic Syndromes (CMS) are clinically and genetically heterogeneous inherited disorders. They are caused by defects of several types at the neuromuscular junction (which connects the nervous system to the muscles). It is estimated that one in 500,000 people in Europe has CMS, and around 14% of CMS patients in Europe and the US are now known to have limb girdle myasthenia (LGM). Symptoms of LGM include increasing weakness of skeletal muscles such as the limb girdle and the muscles used in speech, swallowing and breathing. This weakness can result in impairment of speech, swallowing difficulties, curvature of the spine (scoliosis) and respiratory problems. Symptoms most often present soon after birth or during childhood, before adolescence.

Research
During the 1990's, Slater and the Muscular Dystrophy group at Newcastle, studied the structure and function of neuromuscular junctions in a cohort of control patients. This research aimed to elucidate the nature of the neuromuscular transmission defects in patients with CMS.

The findings of that research formed the reference point for further studies at Newcastle with a cohort of eight CMS patients who had a suspected diagnosis of congenital LGM [R1]. Patients with LGM were known to display different disease characteristics to other CMS patients, and thus it was hypothesised that the term 'limb-girdle myasthenia' encompassed a heterogeneous group of conditions. However, no one had previously tried to determine underlying neuromuscular transmission defects in these patients. The Newcastle group used a combination of structural, ultrastructural, electrophysiological and immunolabelling methods to acquire comprehensive sets of patient data from biopsy samples. This enabled them to provide the first account of neuromuscular junction properties in patients with LGM [R1]. The study revealed that although these patients had significantly smaller neuromuscular junctions than controls, with abnormally

small amounts of transmitter being released in response to nerve activity, the level of excitation of muscles was still great enough to maintain muscle fibre size, that is, there was no muscle wasting. Therefore, it was concluded that the clinical weakness in patients with LGM was primarily associated with a neuromuscular transmission defect that resulted from the structural abnormalities of the neuromuscular junctions, rather than from defects related to the underlying processes of neuromuscular transmission itself [R1]. This had important implications for the treatment of affected patients.

Patients with an unrelated autoimmune condition, myasthenia gravis, exhibit similar symptoms to CMS patients; clinical weakness results from neuromuscular transmission defects caused by an antibody-mediated autoimmune response to acetylcholine receptors (AChR). These patients benefit from esterase inhibitors. However, in spite of the similarity of symptoms, none of the LGM patients in the Newcastle cohort had anti-AChR antibodies and only one out of eight patients showed a long-term benefit from esterase inhibitors. However, four out of the eight patients did benefit from treatment with ephedrine (similar to adrenalin) [R1].

Because the Newcastle research had revealed that the primary underlying reason for clinical weakness in their cohort of LGM patients was abnormally small neuromuscular junctions [R1], it was concluded that this disorder may arise from the defective formation or maintenance of the synaptic structure of the neuromuscular junction [R1, R2]. The genetic basis for the clinical weakness of the LGM cohort was not known at the time of the original Newcastle study. However, in a collaborative study with Prof Beeson and colleagues at Oxford, where DNA from patients in the Newcastle LGM patient cohort was screened, it was subsequently revealed that seven out of the eight 8 patients had mutations of the gene that encodes Dok-7 [R2]. This is a protein that plays an essential role in the formation and maintenance of neuromuscular junctions [R2]. Following these findings a number of case studies indicated a benefit of ephedrine in patients with Dok-7 mutations and the Newcastle group took part in a collaborative, prospective study on the therapeutic effects of ephedrine in eight patients with Dok7 mutations. They documented an improvement of clinical symptoms and reduced side effects over a period of 12–24 months [R3].

Continued research at Newcastle has provided further insights into the pathological mechanism of LGM, using cellular and zebrafish models. This resulted in the discovery of additional causative genes, including GFPT1 [R4]. The findings confirm the heterogeneous nature of LGM and provide an important basis for differential diagnosis within LGM patients. Further funding from the Medical Research Council (MRC) and other funding bodies has been received to pursue this research.

3. References to the research

(Newcastle researchers in bold. Citations from Scopus, July 2013)

R1. **Slater CR**, **Fawcett PRW**, **Walls TJ**, Lyons PR, Bailey SJ, Beeson D, **Young C**, Gardner-Medwin D (2006). Pre- and postsynaptic abnormalities associated with impaired neuromuscular transmission in a group of patients with 'limb-girdle myasthenia'. Brain, 129:2061–76. DOI: 10.1093/brain/awl200 **Cited by 51**

R2. Beeson D, Higuchi O, Palace J, Cossins J, Spearman H, Maxwell S, Newsom-Davis J, Burke G, **Fawcett P**, Motomura M, Müller JS, Lochmüller H, **Slater C**, Vincent A, Yamanashi Y (2006). Dok-7 mutations underlie a neuromuscular junction synaptopathy. Science, 313:1975–78. DOI: 10.1126/science.1130837 **Cited by 113**
(Newcastle authors were responsible for determining the functional and morphological properties of neuromuscular junctions.)

R3. Schara U, Barisic N, Deschauer M, Lindberg C, **Straub V**, Strigl-Pill N, Wendt M, Abicht A, **Müller JS**, **Lochmüller H** (2009). Ephedrine therapy in eight patients with congenital myasthenic syndrome due to DOK7 mutations. Neuromuscular Disorders, 19:828–32. DOI: org/10.1016/j.nmd.2009.09.008 **Cited by 14**

R4. Senderek J, **Müller JS**, Dusl M, Strom TM, Guergueltcheva V, Diepolder I, **Laval SH**, Maxwell S, Cossins J, Krause S, Muelas N, Vilchez JJ, Colomer J, Mallebrera CJ, Nascimento A, Nafissi S, Kariminejad A, Nilipour Y, Bozorgmehr B, Najmabadi H, Rodolico C, Sieb JP, Steinlein OK, Schlotter B, Schoser B, Kirschner J, Herrmann R, Voit T, Oldfors A, Lindbergh C, Urtizberea A, von der Hagen M, Hübner A, Palace J, **Bushby K**, **Straub V**, Beeson D,

Abicht A, **Lochmüller H** (2011). Hexosamine biosynthetic pathway mutations cause neuromuscular transmission defect. American Journal of Human Genetics, 88:162–72. DOI: org/10.1016/j.ajhg.2011.01.008 **Cited by 31**

Selected funding awards

- 2008–2013 *MRC Centre for Neuromuscular Disease in Children & Adults*. MRC – £618,566
- 2009–2011 *Understanding the role of Dok-7 at the neuromuscular junction*. Association Francaise Contre les Myopathies – £45,974
- 2000–2002 *Competitive delocalisation of membrane proteins at the neuromuscular junction*. Wellcome Trust – £139,261

4. Details of the impact

Breathing problems in congenital myasthenic syndrome (CMS) can become so severe that patients require ventilators or die, while limb girdle weakness can result in patients having to use wheelchairs. There is no cure, but these conditions can now be treated.

Impact on patients: diagnosis and treatment

As a result of the research at Newcastle and the collaborative work with Oxford, it was revealed that seven out of the eight CMS patients, in the Newcastle cohort, who were not responding to standard treatment with esterase inhibitors, had a non-AChR related mutation of the Dok-7 gene. Since then, a number of additional mutations have been discovered, including GFTP1 [R4, Section 3; EV a]. Clinical molecular genetic testing is now available for the majority of known CMS-causative genes, and patients are being offered specifically tailored treatments (depending on their genetic mutation) and genetic counselling [EV a]. This is crucial because inaccurate treatment of these disorders is often not only ineffective, but can worsen symptoms [EV b]. Based on the work on genetic diagnosis of limb girdle myasthenia (LGM), the Department of Health National Commissioning Group introduced genetic testing for LGM in April 2007, via the NHS Genetic Testing Service (http://ukgtn.nhs.uk/find-a-test/search-by-disorder-gene/test-service/myasthenia-limb-girdle-familial-312/). There are three centres in the UK that specialise in neuromuscular disease: Newcastle, London and Oxford. Genetic testing takes place at the Oxford Congenital Myasthenia Service, a national referral centre for children and adults in whom a CMS is suspected [EV b, EV c]. Their data, published in 2013, show that 225 CMS patients, which equates to 80%–90% of all CMS patients in the UK, now have a confirmed genetic diagnosis [EV d]. Around 50 patients are now diagnosed with CMS in the UK every year [EV e], and 72 individuals have been diagnosed with the Dok-7 mutation since 2007 [EV f]. Professor Beeson, of the Oxford Congenital Myasthenia Service, has stated that, prior to the underpinning research outlined in Section 3:

"…Dok-7 patients were frequently seen to lose ambulation and to require mechanical ventilation due to ineffective treatment. However, thanks to the collaborative work between Newcastle and Oxford, all genetically diagnosed CMS patients can now be given effective treatment, leading to most patients reporting dramatically improved quality of life." [EV e]

Since 2009 clinics for patients with CMS have been run six times per year in Newcastle and across the North of England, providing counselling and treatment. Further research was conducted on the basis of the suggested benefits of ephedrine for patients with Dok-7 mutations (R3, Section 3). Published in 2013, that research confirmed that these patients also respond favourably to salbutamol [EV g]. Ephedrine and salbutamol are now both provided routinely through specialised neuromuscular NHS services [EV b, EV c]. This has had a major positive impact on patients' well-being and quality of life; the mobility of patients is improved (they can attend school or work) and the need to use a ventilator can be avoided.

In a recent study on nine Dok-7 patients (aged 6-15 years), all patients benefited from increased motor function within one month of starting treatment, and continued to improve for up to 17 months. In addition, three patients who had not been able to walk for many years resumed walking with assistance after just 2-4 weeks of starting treatment [EV g]. Salbutamol has a better safety profile than ephedrine; ephedrine can have adverse effects on the cardiovascular and central

nervous systems, particularly with long-term use in children [EV g]. In the US, albuterol (a salbutamol equivalent) has replaced ephedrine as a treatment for CMS patients with Dok-7 mutations. In 2010 a clinical trial in the US began recruiting CMS patients, including those with Dok-7 mutations, to determine optimal doses of albuterol for clinical practice [EV h].

Reach of impact

International recommendations for diagnosis, treatment and further research have been agreed and published through workshops of the European Neuromuscular Centre (ENMC); the most recent one was held in 2011 [EV f]. These were convened and led by Professor Hanns Lochmüller. Professor Lochmüller also leads the activity on 'patient registries and biobanks' for TREAT-NMD (http://www.treat-nmd.eu/), and has an international (Europe, South America, Asia) cohort of approximately 900 CMS patients, 680 of which were included in a recent mutation screening study, where 31 patients were found to have Dok-7 mutations [EV i]. Full genetic testing for CMS is currently available at four centres in the US and Europe: Rochester (New York), Paris, Munich and Oxford. These have collectively diagnosed 1109 patients, and identified 157 (14%) patients with Dok-7 mutations [EV f]. Most patients diagnosed with a mutation that causes LGM are now given salbutamol or ephedrine [EV j], as it is withheld only in patients with very mild symptoms, which is rare. This means that patients with LGM are now able to walk and breathe unaided. Diagnosis and treatment has therefore had a profound effect on their quality of life (EV e).

5. Sources to corroborate the impact

EV a. Abicht A, Müller JS, Lochmüller H (updated 2012). Congenital Myasthenic Syndromes. In: Pagon RA, Bird TD, Dolan CR, et al., editors. GeneReviews™ [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2013. Available at: http://www.ncbi.nlm.nih.gov/books/NBK1168/

EV b. National Specialised Commissioning Team. Service Specification for Rare Neuromuscular Disorders (2012-13). Pdf available at: http://www.specialisedservices.nhs.uk/document/10429

EV c. NHS referral service: http://www.ouh.nhs.uk/services/referrals/neurosciences/myasthenia.aspx

EV d. Finlayson S, Beeson D, Palace J (2013). Congenital myasthenic syndromes: an update. Practical Neurology, 13:80–91. DOI:10.1136/practneurol-2012-000404

EV e. Corroborative statement: Oxford Congenital Myasthenia Service Centre. (Copy held at Newcastle)

EV f. 186th ENMC International Workshop: Congenital myasthenic syndromes 24-26 June 2011, Naarden, The Netherlands. Chaouch A, Beeson D, Hantaï D, Lochmüller H (2012). Neuromuscular Disorders. 22:566-76. DOI: 10.1016/j.nmd.2011.12.004.

EV g. Burke G et al. (2013) Salbutamol benefits children with congenital myasthenic syndrome due to DOK7 mutations. Neuromuscular Disorders 23:170–175. DOI: org/10.1016/j.nmd.2012.11.004

EV h. http://clinicaltrials.gov/show/NCT01203592

EV i. Abicht A et al (2012) Congenital myasthenic syndromes: achievements and limitations of phenotype-guided gene-after-gene sequencing in diagnostic practice: a study of 680 patients. Human Mutation. 33:1474-84. DOI: 10.1002/humu.22130

EV j. Information booklet published by the Myasthenia Gravis Association; compiled by the Oxford National Commissioning Group (NCG) for CMS, the Muscle Team at the Institute of Human Genetics, Newcastle upon-Tyne and the Neuromuscular Team at Great Ormond Street Hospital for Sick Children. Available at: http://myasthenickids.org/wp-content/uploads/2013/01/CMS-Print-Web-Version-2.pdf