

Institution: University of Oxford
Unit of Assessment: 4
<p>Title of case study:</p> <p style="text-align: center;">The Identification and Treatment of Patients with Congenital Myasthenic Syndrome due to <i>DOK7</i> Gene Mutations</p>
<p>1. Summary of the impact</p> <p>Congenital myasthenic syndromes (CMS) are diseases leading to muscle weakness. They are caused by various gene mutations. However, for many CMS patients with a 'limb girdle' pattern of weakness, the gene was unknown, and they were unresponsive to the usual CMS treatments. Research by David Beeson and colleagues has changed this state of affairs. First, they showed that this form of CMS is caused by a mutation in a gene called <i>DOK7</i>. Second, they identified the mechanism by which the mutation causes the disease. Third, they discovered that patients with <i>DOK7</i> mutations respond to a different class of drug, β2 adrenergic receptor agonists. <i>DOK7</i> mutations are now routinely tested for in clinical practice, and these drugs are standard therapy.</p>
<p>2. Underpinning research</p> <p>Congenital myasthenic syndromes (CMS) present with muscle weakness in infancy or childhood, and may worsen over time. Any muscle groups can be affected, and the sufferer often ends up wheelchair-bound. They are inherited disorders, caused by mutations in genes affecting how nerves communicate with muscles. The clinical picture, and the response to treatment, differs according to the particular gene affected.</p> <p>By the late 1990s, several gene mutations causing CMS were known, but other cases remained unexplained. In particular, there was a relatively common group, who were affected by limb girdle weakness at about 18 months of age, and whose weakness either did not improve, or got worse, when given the standard treatment for CMS (anticholinesterase drugs). Research by David Beeson and colleagues in Oxford since the early 2000s has now identified the cause of, and a treatment for, these cases of 'limb girdle CMS'.</p> <p>Identification of <i>DOK7</i> mutations as a cause of CMS</p> <p>The key research finding was reported in Beeson et al. (2006). They used a 'candidate gene' approach to identify that mutations in a gene known as <i>DOK7</i> cause the majority of cases of limb girdle CMS (Beeson et al., 2006, and see Section 4). They showed that the mutations affect the size and structure of the gap ('synapse') which links the nerve ending to the muscle at the neuromuscular junction, and also caused abnormal maturation and survival of these synapses. This paper therefore identified both the cause, and a biochemical mechanism. <i>DOK7</i> mutations were rapidly confirmed in similar cases by several other groups worldwide (e.g. Selcen et al., 2008, <i>Ann Neurol</i>, 64: 71-87; Anderson et al., 2008; <i>Muscle Nerve</i> 37: 448-456; Ben Ammar et al., <i>J Neurol</i> 2010; 257: 754-766). <i>DOK7</i> mutations are now recognised to be the second-equal commonest cause of CMS in the UK, responsible for about 21% of cases.</p> <p>In follow-up research, Beeson and colleagues defined the clinical spectrum of patients with <i>DOK7</i> mutations, providing useful differentiating features (Palace et al 2007); the findings were confirmed in an independent group of patients by European colleagues (Muller et al., 2007 <i>Brain</i>; 130:1497-1506.) Beeson and colleagues also analysed further the molecular mechanisms, by which the <i>DOK7</i> mutations affect the synapse (Hamuro et al., 2008) that underlie the disorder.</p> <p>Treatment implications of <i>DOK7</i> mutations</p> <p>The research has clear impact in terms of diagnostic testing (see Section 4), but Beeson and colleagues also noted that patients with <i>DOK7</i> mutations showed no benefit, or got worse, when given standard treatments for CMS. In contrast the patients appeared to respond to treatment with β2-adrenergic receptor agonists (e.g. ephedrine). Beeson and colleagues therefore performed a</p>

prospective follow-up study, which demonstrated dramatic beneficial effects of treatment with ephedrine on muscle strength and mobility (Lashley et al., 2010), a finding confirmed in an independent series from Europe (Schara et al., 2009 *Neuromusc Dis* 19:828-32). Routine use of ephedrine is limited by safety concerns, and so it is encouraging that other drugs in the same class (e.g. salbutamol) also show striking beneficial effects, both in a UK study (Burke et al., 2013) and an independent American study (Liewluck et al., 2011, *Muscle Nerve* 44:789-94). All 9 cases reported by Burke et al. (2013) made significant improvements, with children non-ambulant for many years regaining the ability to walk. Similarly marked improvements were also noted in the 15 cases studied by Liewluck et al. (2011). Beeson and colleagues are now studying whether initiating treatment earlier in life will have long term benefits, and alleviate the muscle wasting that occurs in older patients.

In summary, research by David Beeson and colleagues in Oxford has identified *DOK7* as one of the most commonly mutated genes in CMS in the UK. The discovery has enabled diagnostic testing for the condition; previously this was impossible. The research has also revealed the mechanism by which *DOK7* mutations produce the disease, and has shown that patients may be effectively treated with β 2-adrenergic receptor agonists.

3. References to the research

- Beeson D, Higuchi O, Palace J, Cossins J, Spearman H, Maxwell S, Newsom-Davis J, Burke G, Fawcett P, Motomura M, Muller J, Lochmuller H, Slater C, Vincent A, Yamanashi Y. (2006) Dok-7 mutations underlie a neuromuscular junction synaptopathy. *Science* 2006; 313:1975-1978. PMID:16916845.
Identification of mutations in DOK7 as the cause of the major form of limb-girdle congenital myasthenic syndrome. 117 citations.
- Palace J, Lashley D, Newsom-Davis J, Cossins J, Maxwell S, Kennett R, Jayawant S, Yamanashi Y, Beeson D. Clinical features of the *DOK7* neuromuscular junction synaptopathy. *Brain* 2007; 130:1507-1515. PMID:17452375.
Outlining the phenotypic features that enable recognition in clinic of this syndrome and noting the potential of a beneficial response to ephedrine as a therapy. 49 citations.
- Hamuro J, Higuchi O, Okada K, Ueno M, Iemura SI, Natsume T, Spearman H, Beeson D, Yamanashi Y. Mutations causing *DOK7* congenital myasthenia ablate functional motifs in Dok-7. *J Biol Chem* 2008; 283:5518-5524. PMID:18165682.
Analysis of the disease mechanism for DOK7 mutations at the molecular level and thus providing insights into how synaptic transmission is impaired. 22 citations.
- Lashley D, Palace J, Jayawant S, Robb S, Beeson D. Ephedrine treatment in congenital myasthenic syndrome due to mutations in *DOK7*. *Neurology* 2010; 74:1517-1523. PMID:20458068.
A prospective open label study clearly demonstrating the benefits of ephedrine as a treatment for this group of patients. 32 citations.
- Cossins J, Liu WW, Belaya K, Maxwell S, Oldridge M, Lester T, Robb S, Beeson D. The spectrum of mutations that underlie the neuromuscular junction synaptopathy in *DOK7* congenital myasthenic syndrome. *Hum Mol Genet.* 2012; 21:3765-3775. PMID:22661499.
An extensive analysis of the many variants within the DOK7 gene in the UK population which defined 34 different pathogenic mutations, and 27 non-pathogenic variants, thus providing the basis for the definitive genetic diagnosis of 72 patients. 1 citation.
- Burke G, Hiscock A, Klein A, Niks E, Mansur A, Ng J, de Vile C, Beeson D, Muntoni F and Robb, S. Salbutamol benefits children with congenital myasthenic syndrome due to *DOK7* mutations. *Neuromuscul. Disord.* 2013; 23:170-175. PMID:2321935.
A clear clinical demonstration of the beneficial effects of salbutamol treatment in children with

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DOK7 mutations.

Key Grants funding this research

- MRC Senior Non-Clinical Fellowship G117/490 to Beeson: *Disease mechanisms and RNA-based therapies for pathogenic mutations at the neuromuscular synapse*, 2003-8 (£908K).
- MRC Programme Grant, R07835, to Beeson: *Genes, mechanisms, models and treatments for hereditary myasthenia*, 2008-13 (£1.48 million).

Other key researchers involved in Oxford were: Dr Judy Cossins (postdoc, funded by MRC) and Dr Jacqueline Palace (NHS); key collaborators: Professor Yuji Yamanashi (Tokyo Medical and Dental University) and Dr Clarke Slater (Newcastle University).

4. Details of the impact

This research has had a major impact on the diagnosis, testing, and treatment of CMS. Although CMS is a rare condition (approximately 70 extended families with *DOK7* mutations in the UK, with many more overseas), the ability to provide a clear diagnosis, leading to the appropriate treatment, has had, and will continue to have, a profound effect on the relevant population (the patients and families concerned).

Diagnostic testing

Diagnostic testing for *DOK7* mutations is now a routine part of clinical practice for patients with suspected CMS. The Genetics Laboratory at the Churchill Hospital and research laboratory at the Weatherall Institute of Molecular Medicine (Oxford University) offers a diagnostic service for samples from the UK and overseas. Similar genetic screening is offered at a number of sites overseas including Munich, Paris, Milan, and the Mayo Clinic and UCLA-Davis in the USA. In the UK approximately 100 samples a year are sent for *DOK7* screening of which about 10 will be positive. In addition, prenatal testing is available if requested.

Clinical services

Based upon the research findings by Beeson and others in Oxford, the Department of Health National Specialist Commissioning Advisory Group commissioned a National Diagnostic and Advisory Service for CMS (as part of 'Rare inherited neuromuscular disease') located at the John Radcliffe and Churchill Hospitals in Oxford. The service has since been integrated into the NHS, and is now part of the NHS Nationally Commissioned 'Rare Neuromuscular Disorders' service, linked with Great Ormond Street Hospital, London. A national outpatient service is provided in addition to the national diagnostic genetics laboratory mentioned above. This clinical service is closely linked to the Neurosciences Group research laboratory at the Weatherall Institute of Molecular Medicine allowing the rapid translation of findings to the national patient cohort. Patients referred to this service are offered clinic appointments at which they are advised about the molecular basis for their condition and offered appropriate therapy. Patients are subject to regular follow up and their response to treatment assessed. Patient and family satisfaction was assessed in 2012, and 82% were 'completely satisfied' with the service.

Treatment

Resulting from Beeson's work and the papers from others, which confirmed the Oxford group's observations (mentioned in Section 3), β_2 adrenergic receptor agonist treatment is now routinely offered to patients with *DOK7* mutations (in contrast to the treatments recommended for other forms of CMS); it is also endorsed by experts internationally. (NB: The disorder is too rare for there to have been randomised controlled trials, and neither are evidence-based guidelines [e.g. from NICE] available at present.)

Patient involvement

Educational pamphlets and DVDs giving advice on the condition, as well as advice about *DOK7*, reflecting the work of Beeson and colleagues, have been distributed by the Myasthenia Gravis Association and the Muscular Dystrophy Campaign.

5. Sources to corroborate the impact

Contacts to corroborate impact on diagnosis and treatment:

1. Professor Hans Lochmuller, Chair of Experimental Myology, Institute of Genetic Medicine, Newcastle University, UK. Prof. Lochmuller is a leading UK figure in this field. Letter on file, confirming significance of Beeson's work in identifying *DOK7* mutations, and the impact this diagnosis has for treatment. *E.g. 'The major gene underlying this disorder was identified by Professor Beeson's group in Oxford...The grouping of these patients based on genetic diagnosis enabled recognition that they show a beneficial response to β 2-adrenergic receptor agonists...Based upon the underlying knowledge of the condition...these CMS patients can now be given effective treatment, leading to many patients reporting a dramatically improved quality of life...'*
2. Professor Andrew G. Engel, William McNight-3M Professor of Medicine, Mayo Clinic College of Medicine, 200 First Street SW, Rochester, Minnesota 55905, USA. Letter on file, from the senior academic clinician at the leading US centre for CMS. He writes: *'...Beeson identified mutations in DOK7 as the disease gene...followed by several publications...and by reports of effectiveness of adrenergic agonists as a successful modality of therapy.'*
3. Dr. Daniel Hantai, Institut de Cerveau et de la Moelle epiniere, Paris. Letter on file from a European expert confirming significance of Beeson's work. Includes: *'Thanks to your breakthrough, our genetic centre at the Hospital Pitie-Salpetriere in Paris, is also screening for DOK7 mutations in the diagnosis of congenital myasthenic syndromes.'*
4. Dr. Angela Abicht, Medizinisch Genetisches Zentrum, Munich. Letter on file confirming use of *DOK7* mutation screens in this German referral centre.

International workshop recommendations regarding diagnosis and treatment:

5. 186th ENMC International Workshop: Congenital myasthenic syndromes 24-26 June 2011, Naarden, The Netherlands. Summarised in *Neuromuscular Disorders* 2012; 22:566-76. *E.g., p569: 'Two patients with CMS were initially thought to have a metabolic myopathy...Mutations in DOK7 were subsequently confirmed...Bruno Eymard [Paris] emphasised the risks of mislabelling patients with a specific diagnosis...'* *E.g., p573: 'Stephanie Robb [London] reported their experience using oral salbutamol in 8 DOK7 children...all 8 children reported functional benefit. Improvement was progressive over several months.'*
6. Global list of 12 official laboratories carrying out genetic testing for *DOK7* mutations: <http://www.ncbi.nlm.nih.gov/gtr/tests/?term=DOK7%5Bgene%5D>

Websites giving information to patients and families:

7. <http://www.mga-charity.org> and <http://www.myasthenickids.org> both include an Information leaflet *'The Congenital Myasthenic Syndromes: information for Children and Adults Diagnosed with Congenital Myasthenic Syndromes'*, dated February 2010, which describes the *DOK7* mutation as the latest type to be identified, and its implications for treatment.

Patient satisfaction:

8. NHS Specialised Services Commissioning: Rare Neuromuscular Disorders Group Congenital Myasthenic Syndromes Annual Report September 2012. Available on request. Appendix 6 gives results of an anonymised survey showing 82% of patients attending the CMS clinic in Oxford *'completely satisfied'*, and 84% had their *'questions and concerns addressed completely'*.
9. Letters to Beeson from two patients (2010, 2012), available on request. One states: *'I finally have a definitive diagnosis [of a DOK7 mutation]...since treatment the results are unbelievable...my heartfelt thanks...'*