Institution: Newcastle University

Unit of Assessment: UoA1

Title of case study:
Diagnostic test for the rare muscular disorder limb-girdle muscular dystrophy type 2A

1. Summary of the impact

Limb-girdle muscular dystrophy type 2A is a rare (about six cases per million individuals) and incurable muscular disorder with a genetic basis. Although diagnosis is a multi-step process, which includes symptom assessment and histopathology of affected muscle, it invariably involves measurement of the amount of protein calpain 3 in muscle biopsy samples. This is performed in diagnostic laboratories worldwide using the two monoclonal antibodies CALP-12A2 and CALP-2C4, which were developed by researchers at Newcastle University in the late 1990s. In 2009 Newcastle University signed a licensing agreement with the international bioscience company Leica Biosystems that currently sells the antibodies to institutions worldwide.

2. Underpinning research

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

- Dr Louise Anderson, Lecturer in the Department of Neurobiology (1992–2005).
- Professor John Harris, Professor of Experimental Neurology.
- Professor Kate Bushby, initially a Clinical Research Associate, then Senior Lecturer (1997–1999), Reader in Human Genetics (1999-2001), and subsequently Professor of Neuromuscular Genetics.

Background

In the early 1990s the genetic defects underlying the limb-girdle muscular dystrophies, a group of rare muscle disorders, were discovered. One disease subtype, limb-girdle muscular dystrophy type 2A, was found to be caused by mutations in the CAPN3 gene, which encodes the skeletal muscle protein calpain 3. Genetic analyses of samples from several limb-girdle muscular dystrophy type 2A patients revealed a number of distinct disease-causing mutations within CAPN3, making a simple genetic test difficult to develop. An effort was mounted to come up with a protein-based diagnostic test for the disease.

A biomarker for limb-girdle muscular dystrophy type 2A

Anderson, Bushby and Harris, in a collaboration with researchers at the University of California Los Angeles, published a key paper in 1997 showing that the protein calpain 3 was absent in muscle biopsies taken from three patients with limb-girdle muscular dystrophy type 2A but present in all 12 control samples of healthy muscle. The researchers also found that calpain 3 was clearly detectable in samples from nine patients suffering from muscle diseases other than limb-girdle muscular dystrophy type 2A, suggesting that the marker was highly specific - and therefore potentially of diagnostic use (R1).

Development and validation of monoclonal antibodies against calpain 3

In the 1997 study (R1) that identified calpain 3 protein as a potential diagnostic marker for limb-girdle muscular dystrophy type 2A, the researchers used affinity-purified polyclonal antisera (a preparation containing several kinds of antibody, each with a slightly different specificity) to detect the protein. Following that study, Anderson led a project at Newcastle University to generate highly specific monoclonal antibodies against two regions of calpain 3. Three monoclonal antibodies, clones CALP-2C4, -11B3 and -12A2, were made, and they were described in a paper published in the American Journal of Pathology in 1998 (R2). The antibodies were validated with samples from 33 healthy controls, 70 disease controls (muscle diseases other than limb-girdle muscular dystrophy type 2A) and nine people with limb-girdle muscular dystrophy type 2A. The controls

(healthy and other disease) all yielded a normal calpain profile, and seven of nine limb-girdle muscular dystrophy type 2A samples showed a clear reduction in amount of full-length calpain 3. In the other two LGMD2A samples there were normal levels of full-length protein but reduced levels of two calpain 3 degradation products (R2).

3. References to the research

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

R1. Spencer MJ, Tidball JG, **Anderson LV**, **Bushby KM**, **Harris JB**, Passos-Bueno MR, Somer H, Vainzof M, Zatz M (1997). Absence of calpain 3 in a form of limb-girdle muscular dystrophy (LGMD2A). *J Neurol Sci.* 146(2):173-8. DOI: 10.1016/S0022-510X(96)00304-8. **32 citations.**

Harris, Bushby and Anderson were involved in the conception, organisation and prosecution of the study that led to output R1. They were also involved in drafting the manuscript. Harris is corresponding author.

R2. **Anderson LV**, **Davison K**, **Moss JA**, Richard I, Fardeau M, Tomé FM, Hübner C, Lasa A, Colomer J, Beckmann JS (1998). Characterization of monoclonal antibodies to calpain 3 and protein expression in muscle from patients with limb-girdle muscular dystrophy type 2A. *Am J Pathol.* 153(4):1169-79. DOI: 10.1016/S0002-9440(10)65661-1. **117 citations.**

Anderson was the principal researcher and is the corresponding author.

<u>Select research grants</u>

- Muscular Dystrophy Group of Great Britain. 1991 - 1994. £76 000. *Expression of dystrophin and related proteins in normal and diseased tissues*
- Muscular Dystrophy Group of Great Britain. 1960s – 1990s. ~ £100 000 per year. Centre grant, supporting the staffing costs (including Anderson) at the Muscular Dystrophy Centre, based at Newcastle General Hospital.
- Novocastra Laboratories. Royalties (received from 1997) from sales of dystrophin and other antibodies produced by Newcastle were recycled back into research leading to development of the calpain 3 monoclonal antibodies.

4. Details of the impact

<u>Background to limb-girdle muscular dystrophy type 2A</u>

Limb-girdle muscular dystrophy type 2A is a rare progressive muscle-wasting disease caused by mutation(s) in the CAPN3 gene that result in reduction of protein expression or loss of protein function. The disease, which usually becomes symptomatic at between 10 and 30 years of age, manifests as progressive weakening of muscles in the hips and shoulders, causing a slow reduction in mobility, balance, and the ability to lift objects. A patient typically becomes wheelchair bound 20 – 30 years after disease onset. A study carried out in north-east England estimated the prevalence there of all the limb-girdle muscular dystrophies to be 2.27 per 100,000 individuals; limb-girdle muscular dystrophy type 2A is the most common type with a prevalence of 0.6 per 100,000 (Norwood et al. (2009) PubMed ID: 19767415). A separate study carried out in north-east Italy yielded an estimated prevalence of 0.95 per 100,000 individuals (Fanin et al. (2005) PubMed ID: 15725583).

<u>Why diagnosis is important</u>

While limb-girdle muscular dystrophy type 2A is incurable, diagnosis does benefit the patient in other ways. Health monitoring can be customised to the patient: the 2A subtype, compared with the other limb-girdle muscular dystrophies, is associated with a relatively low incidence of cardiac and respiratory problems, and so the affected person can be reassured and given low-key health surveillance. Diagnosis also means that the patient can be given access to the disease-specific registry, and potentially a chance to participate in clinical trials of future therapies. Further, as it is known that limb-girdle muscular dystrophy type 2A is an autosomal recessive disorder, precise

genetic counselling can be given to the patient and family members and prenatal testing can be done if requested. In addition, diagnosis often has a positive psychological effect on patients, who welcome official recognition of their condition and the ability to share their experiences with others with the disease (e.g. Bird (1999) PubMed ID: 12194381).

Diagnosis of limb-girdle muscular dystrophy type 2A

Typically the diagnostic approach for the disease is multi-faceted. Initial tests include symptom assessment (pattern of affected muscles), measurement of creatine kinase levels in the blood and muscle histopathology.

If limb-girdle muscular dystrophy type 2A is suspected on the basis of these test results, calpain 3 immunoblot analysis is performed on a muscle biopsy sample (Ev a). A reduction in the level of calpain 3, when considered in the context of amounts of other muscle proteins (for example dysferlinopathy is associated with reductions in the amount of calpain 3 and dysferlin), is usually sufficient for confident diagnosis of limb-girdle muscular dystrophy type 2A to be made. About one-fifth of limb-girdle muscular dystrophy type 2A patients have normal amounts of calpain 3 protein, and for them sequencing of the CAPN3 gene needs to be performed after the protein test for a diagnosis to be reached.

In 2007 the European Federation of Neurological Societies published a guideline on diagnosis and management of limb-girdle muscular dystrophies. It states (citing research that used the Newcastle University CALP antibodies):

"Immunoblotting [for calpain 3] has been the accepted test required for the diagnosis of LGMD2A" (Ev b)

Use of the Newcastle calpain 3 antibodies in Europe and the United States

All muscle biopsies from suspected limb-girdle muscular dystrophy cases in England, Wales and Scotland are tested at the NHS Specialised Service Rare Neuromuscular Disorders laboratory in Newcastle, which was established in 2001 (Ev c). The Newcastle unit performed calpain 3 immunoblots using the Newcastle CALP antibodies on 186 samples in the year 2008/9 and on 164 samples in the year 2009/10, yielding molecular confirmation of limb-girdle muscular dystrophy type 2A in nine and six individuals respectively in those years (Ev d). There is also evidence of the usage of CALP antibodies in Europe; in Paris 90 diagnostic tests were performed in the last year, (47 had calpain defects) (Ev e) and in Milan 52 samples were tested in the last two years (3 were calpain-deficient) (Ev f).

The antibodies are also used in the United States to diagnose the disease. Dr Melissa Spencer is a Professor of Neurology and the Co-Director of the Center for Duchenne Muscular Dystrophy at UCLA, Chair of the Scientific Advisory Board of the Coalition to Cure Calpain 3, and an authority in US on the science and treatment of the disease. She has stated that:

"Dr Anderson generated and characterized several monocolonal antibodies … and these antibodies are widely used in calpain 3 research and are considered to be the 'gold standard".

"While there are a number of distinct mutations in the CAPN3 gene which can cause LGMD2A, not all of which result in a reduction or abolition of calpain 3 protein, or an altered proteolytic degradation profile, blotting for the protein in muscle biopsy lysates using the antibodies developed by Louise Anderson at Newcastle University is now a routine part of the process - in the US and worldwide - leading to diagnosis of LGMD2A." (Ev g)

Worldwide sales of the Newcastle CALP antibodies

The Newcastle University CALP antibodies were initially sold through the local university spin-out company Novocastra Laboratories. However, since 2009 a licensing agreement has been in place with the international bioscience company Leica Biosystems and they now sell significant quantities of the antibodies worldwide – including in North America, Europe, Asia and Australasia (Ev h).

Unit sales for the UK and Ireland

[Data and text removed for publication]

5. Sources to corroborate the impact

Ev a. GeneReviews (NCBI Bookshelf): Calpainopathy. http://www.ncbi.nlm.nih.gov/books/NBK1313/#lgmd2a.Diagnosis

Ev b. Norwood F, De Visser M, Eymard B, Lochmüller H, Bushby K (2007). EFNS guideline on diagnosis and management of limb girdle muscular dystrophies. *European Journal of Neurology* 14(12):1305-1312.

Ev c. NHS Specialised Services: Rare Neuromuscular Disorders. http://www.specialisedservices.nhs.uk/service/rare-neuromuscular-disorders

Ev d. Statement from the NHS Specialised Service Rare Neuromuscular Disorders Diagnostic Laboratory, Newcastle, UK.

Ev e. Statement from the Laboratory of Biochemistry and Molecular Genetics, Cassini Hopital Cochin, Paris, France.

Ev f. Statement from the Neuromuscular Diseases and Neuroimmunology Unit, Fondazione IRCCS Istituto Neurologico, Milan, Italy.

Ev g. Statement from a Professor at the UCLA Department of Neurology

Ev h. Leica Biosystems: confidential sales information. (Data tab.)