Institution: King's College London

Unit of Assessment: 1 - Clinical Medicine

Title of case study: Uncovering new titin mutations to develop better clinical tests for patients with genetic muscle disease

1. Summary of the impact

King's College London (KCL) researchers have had a tremendous impact on furthering the understanding of how titin mutations lead to severe hereditary and spontaneous muscle diseases, which has ultimately improved clinical guidelines, genetic diagnosis and counselling of patients and their families. New genetic tests, driven by KCL research pinpointing how specific mutations adversely impact the normal interaction of titin with other proteins and lead to a loss of muscle function, have been adopted by public health agencies across Europe. Based on these original research insights, novel potential treatment targets continue to be discovered, and drugs aimed at these targets are currently being developed.

2. Underpinning research

KCL research on titin mutations and genetic muscle disease is led by Professor Mathias Gautel (KCL, 2001-present).

Mutated proteins and muscle disease: Several muscle diseases, or 'myopathies', are caused by inherited or spontaneously arising genetic mutations that affect proteins involved in muscle contraction. Muscle fibres are organised into structures known as sarcomeres, which are made up of proteins that act to generate force and movement. Titin is the largest protein in the sarcomere – and indeed in the human body – where it links other proteins together and organises the sarcomere.

KCL research uncovers a single gene defect responsible for severe adult muscle disease: In a seminal publication in the journal *Science* in 2005, Professor Gautel, in collaboration with the clinical genetic teams of Professor Udd in Finland and Professor Sejersen in Sweden, discovered that mutations in the *titin* gene disrupt its normal role in muscle organisation and turnover. Such mutations were found to be directly responsible for hereditary myopathy with early respiratory failure (HMREF), a severe form of adult muscular disease, in families of diverse origins [1].

In 2005, KCL research identified that a particular region of the *titin* gene mutated in HMERF, known as the Protein Kinase domain, was extremely important for the normal function of titin [2]. In 2008, the team led by Professor Gautel determined that this Protein Kinase domain acted as a mechanical force sensor, allowing titin to sense and respond to the physical forces exerted on muscles [3].

Identification of a common genetic defect responsible for multiple muscle diseases: In 2007, in collaboration with Professor Ferreiro in France, KCL researchers identified a new titin mutation outside the Protein Kinase domain that resulted in a myopathy that affected patients from birth, rather than manifesting in adulthood [4]. This was the first evidence that distinct titin mutations could cause multiple types of muscle disease that could appear at different ages, either affecting patients from birth or developing over time with a progressive loss in muscle function. Titin mutations were also found to affect not only skeletal muscle, but also heart muscle, and thus may be implicated in heart failure [1,4].

Titin mutations prevent normal interactions with other muscle proteins: In 2008, Professor Gautel's team identified why mutations at certain positions in the *titin* gene led to muscle dysfunction. Many of these mutations adversely affected titin's ability to interact with other proteins [5]. One of these partners, obscurin, binds to titin to organise muscle structure and function. KCL research uncovered mutations that prevented obscurin interacting with titin, thereby causing inherited muscular diseases including Salih Myopathy and limb girdle muscular dystrophy 2J (LGMD2J) [5].

Identifying new titin binding partner interactions to reveal novel insights into other muscle diseases: Uncovering new titin binding partners enabled KCL researchers to identify novel candidate genes that could be implicated in other non-titin-related myopathies. Previous analysis of key titin

binding partners by the Gautel team revealed that the condition HMERF was due to a disruption in muscle protein mechanics that led to imbalanced muscle activity [1]. Building on this finding, the same titin-associated binding partners are now being studied at KCL in cases of acquired, non-hereditary muscle disease. In collaboration with Professor Larsson in Sweden, titin defects have already been linked to muscle diseases that occur as a common complication of critical care unit admission [6].

KCL research continues to elaborate new pathways involved in genetic muscle disease: Ongoing research carried out by Professor Jungbluth (Guy's Hospital, KCL, 2008–present), in collaboration with Professor Gautel, is continuing to uncover new genetic defects involved in inherited muscle disease [7]. Such insights are being used to guide genetic diagnosis and to develop new therapeutic interventions.

3. References to the research

1) Lange S, Xiang F, Yakovenko A, Vihola A, Hackman P, Rostkova E, Kristensen J, Brandmeier B, Franzen G, Hedberg B, Gunnarsson LG, Hughes SM, Marchand S, Sejersen T, Richard I, Edstrom L, Ehler E, Udd B, **Gautel M**. The kinase domain of titin controls muscle gene expression and protein turnover. *Science.* 2005;308:1599–603.

2) Grater F, Shen J, Jiang H, **Gautel M**, Grubmuller H. Mechanically induced titin kinase activation studied by force-probe molecular dynamics simulations. *Biophys J.* 2005;88:790–804.

3) Puchner E, Alexandrovich A, Kho AL, Hensen U, Schäfer LV, Brandmeier B, Gräter F, Grubmuller H, Gaub HE, **Gautel M**. Mechanoenzymatics of titin kinase. *Proc Natl Acad Sci.* 2008:105:13385–90.

4) Carmignac V, Salih MAM, Quijano-Roy S, Marchand S, Al Rayess MM, Mukhtar MM, Urtizberea JA, Labeit S, Guicheney P, Leturcq F, **Gautel M**, Fardeau M, Campbell KP, Richard I, Estournet B, Ferreiro A. C-terminal titin deletions cause a novel early-onset myopathy with fatal cardiomyopathy. *Ann. Neurol.* 2007;61:340–51.

5) Fukuzawa A, Lange S, Holt MR, Vihola A, Carmignac V, Ferreiro A, Udd AB, **Gautel M**. Interactions with titin and myomesin target obscurin and its small homologue, obscurin-like 1, to the sarcomeric M-band: implications for hereditary myopathies. *J Cell Sci.* 2008;121:1841–51.

6) Ochala J, Gustafson AM, Lano Diez M, Renaud G, Li M, Aare S, Qaisar R, Banduseela VC, Hedstrom Y, Tang X, Dworkin B, Ford GC, Nair S, Perera S, **Gautel M**, Larsson L. Preferential skeletal muscle myosin loss in response to mechanical silencing in a novel rat intensive care unit model: underlying mechanisms. *J Physiol.* 2011;589:2007–26.

7) Cullup T, Kho AL, Dionisi-Vivi C, Brandmeier B, Smith F, Urry Z, Simpson MA, Yau S, Bertini E, McClelland V, Al-Owain M, Koelker S, Koerner C, Hoffmann GF, Wikburg FA, ten Hoedt AE, Rogers RC, Manchester D, Miyata R, Hayashi M, Said E, Soler D, Kroisel PM, Windpassinger C, Filloux FM, Al-Kaabi S, Hertecant J, Del Campo M, Buk S, Bodi I, Goebel HH, Sewry CA, Abbs S, Mohammed S, Josifova D, **Gautel M**, **Jungbluth H**. Recessive mutations in EPG5 cause Vici syndrome, a multisystem disorder with defective autophagy. *Nature Genetics.* 2013;45:83–7.

Since 2007, charitable and industrial funding of more than £4.5 million has been awarded through competitive tender to directly support the research of Professor Gautel in the Cardiovascular Division of KCL. This includes:

- Medical Research Council 5-year programme grant (2007–2012; 2012-2017) **£4.2M**
- British Heart Foundation Chair Award (2008–2013; 2013-2018) **£2.7M**
- Wellcome Trust Project Grant (2011–2013) **£360,909**
- Leducq Transatlantic Network (2012–2017) **£547,867**

4. Details of the impact

Improved care for patients with genetic muscle disease: The finding that mutations in the Protein Kinase domain of the titin protein were directly responsible for the muscle disease, HMERF, has led to a transformation in the perception of the role of titin in acquired and hereditary muscle diseases, which has had a significant impact on clinical practice. Since the prevalence of titin mutations is high in dilated cardiomyopathy (a condition where the heart muscles become weak and cannot pump enough blood around the body), occurring in 25% of inherited cases and 18% of spontaneously arising cases. KCL-informed screening for titin mutations is now routinely performed in national prenatal genetic diagnosis clinics across Europe, including France [8], Finland [9], Italy [10] and Sweden [11].

Improved genetic counselling: Since titin kinase mutations can be inherited in multiple different patterns that lead to a broad spectrum of disease symptoms, the improved understanding of underlying mechanism by the Gautel lab has been key for estimating exactly how their combination will affect muscle function (see [1–5, 7] above), identifying early stages of disease, and providing optimal genetic counselling and treatment recommendations for patients and families. This research is also facilitating reproductive counselling, where genetic screening is performed for *in vitro* fertilised preimplantation human embryos originating from carrier or affected individuals to prevent children inheriting the same muscle diseases.

Based on our prominence in the field of titin mutations and muscle disease, the Gautel group at KCL also serves as a research-based reference laboratory for screening and characterising unique titin mutations [12].

KCL research improves clinical diagnostic techniques: KCL's original biomechanical research into muscle function led to the identification of new markers for damaged heart muscles. Methods to detect these markers were incorporated into a novel clinical test capable of rapidly diagnosing patients who had suffered a heart attack. Patents on this technique were granted in 2012/3 (USA, Japan, Europe), assigned to KCL and invented by Professor Mayr (KCL, 2006–present), Dr Jacquet (KCL, 2005–2010), Professor Marber (KCL, 1996–present) and Professor Gautel [13].

KCL research shapes international clinical guidelines: KCL research (see [5] above) has informed European clinical guidelines for the diagnosis of muscle disease. This includes the 165th ENMC International workshop guidelines written by the European Neuro Muscular Centre [14], an international research support organisation that informs networks such as the Association Française contre les Myopathies (France), Deutsche Gesellschaft für Muskelkranke (Germany), Telethon Foundation (Italy), Muscular Dystrophy Campaign (UK), Muskelsvindfonden (Denmark), Prinses Beatrix Fonds & Vereniging Spierziekten Nederland (The Netherlands), Schweizerische Siftung für die Erforschung der Muskelkrankheiten (Switzerland) and Österreichische Muskelforschung (Austria). These clinical guidelines therefore have a very wide reach across Europe and beyond.

KCL research (see [1] above) has also been incorporated into clinical genetic guidelines for the diagnosis of the titin-induced myopathy, Udd Distal Myopathy, published online in *GeneReviews* by the University of Washington, Seattle [15]. *GeneReviews* are expert-authored disease descriptions focused on clinically relevant and medically actionable information on the diagnosis, management, and genetic counseling of patients and families with specific inherited conditions, and are widely used as a clinical guideline reference.

Developing partnerships to establish new treatments for genetic muscle disease: KCL's original research into titin mutations and their impact on muscle function led Rigel Pharmaceuticals, a San Francisco-based "clinical-stage" drug development company, to partner with the Gautel lab to initiate a drug discovery programme focussed on titin. This collaboration has facilitated the discovery of several small molecules that target titin kinase, which will be assessed for their effect on titin-related muscle and metabolic diseases [16].

KCL research continues to uncover new therapeutic targets: Ongoing KCL research continues to identify important new aspects of muscle biology, in particular new protein interactions, which can be

used to inform patient therapies. In collaboration with University College London, the Gautel laboratory has now identified over 500 new titin mutations. In the future, it is anticipated that complementary biophysical and structural studies carried out in collaboration with other international researchers will continue to uncover new therapeutic interventions (see [6] above).

5. Sources to corroborate the impact

Genetic testing centres that incorporate clinical assays based on KCL research

8) National Early Onset Myopathy Clinic at the INSERM *Institut de Myologie*, Hôpital La Salpetrière & Université Pierre et Marie Curie, Paris, France. Contact name available separately.

9) Folkhälsan Institute of Genetics, University of Helsinki, Finland. Contact name available separately.

10) Institute of Neurology, Catholic University School of Medicine, Rome, Italy. Contact name available separately.

11) Department of Clinical Neuroscience, Karolinska Institute, Stockholm, Sweden. Contact name available separately.

Use of novel data in reference laboratory screening for titin mutations

12) Recessive TTN truncating mutations define novel forms of core myopathy with heart disease. Chauveau C, Bonnemann CG, …….**Gautel M**, Ferreiro A. *Human Mol Genetics.* 2013; published October 8, 2013 http://hmg.oxfordjournals.org/content/early/2013/10/07/hmg.ddt494.full.pdf+html

Diagnostic tests developed by KCL research group

13) CMYBP-C and MLC2 as diagnostic markers of cardiac injury. Mayr M, Jacquet S, Marber M and Gautel M. Patent Application No. 20120156702 http://www.google.com/patents/US20120156702

European disease guidelines based on KCL research

14) Udd B, 165th ENMC International Workshop: distal myopathies 6–8th February 2009 Naarden, The Netherlands*.* 2009. *Neuromuscul. Disord.*, 19: 429–38. PMID: 19477645. Cites ref [5], p.432.

15) Suominen T, Udd B & Hackman P: Gene Reviews™ Udd Distal Myopathy; Editors: Pagon RA, Adam MP, Bird TD. University of Washington, Seattle, 1993–2013. PMID: 20301498. Cites ref [1]. http://www.ncbi.nlm.nih.gov/books/NBK1323/

Pharmaceutical collaboration with KCL research group

16) Rigel Pharmaceuticals, Inc., San Francisco, USA. Contact name available separately.