Institution: University College London

Unit of Assessment: 3B - Allied Health Professions, Dentistry, Nursing and Pharmacy: Pharmacy

Title of case study: Molecular genetic characterisation of human and animal disorders leading to improved diagnosis, prevention and treatment of inherited disorders

1. Summary of the impact

Research at the UCL School of Pharmacy has positively influenced healthcare in startle disease/hyperekplexia, a rare disease that affects humans and several animal species, including dogs, horses and cattle. The identification and functional characterisation of mutations in genes involved in human startle disease by researchers at the School has improved genetic diagnostics and patient care. Our research on startle disease in cattle and dogs has also led to new non-invasive diagnostic tests that have alleviated animal suffering and reduced negative economic impacts on farmers. Overall, our findings have been translated into tangible benefits for the human and animal populations affected by this disease and have changed the way in which the disease is diagnosed and treated. We have also significantly increased the awareness of this rare disorder by communicating with academics, healthcare and veterinary professionals, and the general public.

2. Underpinning research

Startle disease is a rare but potentially fatal neurological disorder (estimated <1,000 human cases total worldwide) characterised by an exaggerated startle reflex and muscle stiffness in response to tactile, acoustic or visual stimuli. This condition can cause infant death due to difficulties with breathing, aspiration pneumonia or severe bradycardia. Startle disease also occurs in livestock (cattle, horses) and dogs, with fatal consequences and significantly higher prevalence.

Impacts reported here result from genetics research and structure/function assays of glycine receptor and transporter function in startle disease undertaken from 2003-13 at the UCL School of Pharmacy by Robert J Harvey (Professor of Molecular Neuroscience and Genetics), Kirsten Harvey (Professor of Molecular Neuroscience and Cell Biology) and Brian R Pearce (Senior Lecturer in Pharmacology). These PIs either led research consortia or played critical roles within them. Key external collaborators included Professor Mark I Rees (University of Swansea) for human hyperekplexia, Michel George (University of Liège, Belgium), John Woolliams (Roslin Institute) for bovine startle disease and Diane Shelton (University of San Diego, USA) for canine startle disease.

In human startle disease, we characterised genetic, structural and functional defects in three genes encoding proteins involved in inhibitory glycinergic transmission: postsynaptic glycine receptor (GlyR) α1 and β subunits and a presynaptic Na^+/Cl^--dependent glycine transporter (GlyT2). For the GlyR α1 and β subunit genes (*GLRA1* and *GLRB)* we provided a genetic diagnosis for 44 cases of startle disease, and revealed that recessive mutations in these genes are more common than dominant mutations. We also revealed new pathogenic mechanisms affecting glycine receptors that are important to the understanding of other channelopathies **[1]**. For example, certain mutations in the GlyR α1 and β subunit genes result in GlyRs that open spontaneously in the absence of agonist **[1, 2]**. For GlyT2, we provided a genetic diagnosis for 20 cases, revealing new dominant and recessive mutations. New pathogenic mechanisms identified included splice-site mutations and missense mutations affecting residues implicated in Cl^- binding, conformational changes mediated by extracellular loop 4 and cation-π interactions **[3, 4]**. Importantly, we have also determined that low-dose clonazepam, a benzodiazepine that potentiates inhibitory $GABA_A$ receptor function, is therapeutically effective in children with either GlyR or GlyT2 mutations **[1-4]**.

In Belgian Blue cattle, novel whole-genome association techniques revealed that a missense mutation (L270P) in the GlyT2 gene (*SLC6A5*) causes congenital muscular dystonia type 2 (CMD2), which is highly reminiscent of startle disease in humans. Calves with CMD2 show muscle stiffness and tremor following acoustic, tactile or visual stimulation and die shortly after birth due to respiratory difficulties. We conducted key functional tests that revealed that glycine uptake by GlyT2 in recombinant systems was abolished by the L270P mutation **[5]**.

We resolved the genetic basis of two canine disorders - startle disease in Irish Wolfhounds **[6]** and

Episodic Falling Syndrome in Cavalier King Charles Spaniels, considered to be a form of startle disease by many veterinarians **[7]**. In a litter of Irish Wolfhounds with muscle stiffness and tremor in response to handling, along with severe breathing difficulties, sequencing of *GLRA1* and *GLRB* did not reveal any pathogenic mutations. However, analysis of *SLC6A5* revealed a microdeletion encompassing exons 2 and 3 in both affected dogs. This results in the loss of part of the large cytoplasmic N-terminus of GlyT2 and all subsequent transmembrane domains due to a frameshift. By contrast, we found that Episodic Falling Syndrome is caused by a microdeletion in a different gene (*BCAN*), encoding a neuronal extracellular matrix protein known as brevican **[7]**.

R Harvey's initial work with the Irish Wolfhound Foundation led to further grant funding to screen the >400 DNA samples in the Irish Wolfhound DNA bank, in order to obtain an estimate of the population frequency of this new disease gene variant. We detected a frequency of carrier animals of 2%, i.e. 1 carrier for every 50 dogs in the archive, demonstrating that in Irish Wolfhounds startle disease is an issue of significant concern. Wider testing of a larger population of Cavalier King Charles Spaniels without a history of Episodic Falling Syndrome in our research study also revealed that carriers were extremely common – 12.9% of dogs tested.

3. References to the research

[1] Chung SK, Vanbellinghen JF, Mullins JG, Robinson A, Hantke J, Hammond CL, Gilbert DF, Freilinger M, Ryan M, Kruer MC, Masri A, Gurses C, Ferrie C, Harvey K, Shiang R, Christodoulou J, Andermann F, Andermann E, Thomas RH, Harvey RJ, Lynch JW, Rees MI. Pathophysiological mechanisms of dominant and recessive *GLRA1* mutations in hyperekplexia. J Neurosci. 2010 Jul 14;30(28):9612-20. http://dx.doi.org/10.1523/JNEUROSCI.1763-10.2010

[2] James VM, Bode A, Chung SK, Gill JL, Nielsen M, Cowan FM, Vujic M, Thomas RH, Rees MI, Harvey K, Keramidas A, Topf M, Ginjaar I, Lynch JW, Harvey RJ. Novel missense mutations in the glycine receptor β subunit gene (*GLRB*) in startle disease. Neurobiol Dis. 2013 Apr;52:137-49. http://dx.doi.org/10.1016/j.nbd.2012.12.001

[3] Rees MI, Harvey K, Pearce BR, Chung SK, Duguid IC, Thomas P, Beatty S, Graham GE, Armstrong L, Shiang R, Abbott KJ, Zuberi SM, Stephenson JB, Owen MJ, Tijssen MA, van den Maagdenberg AM, Smart TG, Supplisson S, Harvey RJ. Mutations in the gene encoding GlyT2 (*SLC6A5*) define a presynaptic component of human startle disease. Nat Genet. 2006 Jul;38(7):801-6. http://dx.doi.org/10.1038/ng1814

[4] Carta E, Chung SK, James VM, Robinson A, Gill JL, Remy N, Vanbellinghen JF, Drew CJ, Cagdas S, Cameron D, Cowan FM, Del Toro M, Graham GE, Manzur AY, Masri A, Rivera S, Scalais E, Shiang R, Sinclair K, Stuart CA, Tijssen MA, Wise G, Zuberi SM, Harvey K, Pearce BR, Topf M, Thomas RH, Supplisson S, Rees MI, Harvey RJ. Mutations in the GlyT2 gene (*SLC6A5*) are a second major cause of startle disease. J Biol Chem. 2012 Aug 17;287(34):28975-85. http://dx.doi.org/10.1074/jbc.M112.372094

[5] Charlier C, Coppieters W, Rollin F, Desmecht D, Agerholm JS, Cambisano N, Carta E, Dardano S, Dive M, Fasquelle C, Frennet JC, Hanset R, Hubin X, Jorgensen C, Karim L, Kent M, Harvey K, Pearce BR, Simon P, Tama N, Nie H, Vandeputte S, Lien S, Longeri M, Fredholm M, Harvey RJ, Georges M. Highly effective SNP-based association mapping and management of recessive defects in livestock. Nat Genet. 2008 Apr;40(4):449-54. http://doi.org/dfhv58

[6] Gill JL, Capper D, Vanbellinghen JF, Chung SK, Higgins RJ, Rees MI, Shelton GD, Harvey RJ. Startle disease in Irish wolfhounds associated with a microdeletion in the glycine transporter GlyT2 gene. Neurobiol Dis. 2011 Jul;43(1):184-9. http://dx.doi.org/10.1016/j.nbd.2011.03.010

[7] Gill JL, Tsai KL, Krey C, Noorai RE, Vanbellinghen JF, Garosi LS, Shelton GD, Clark LA, Harvey RJ. A canine *BCAN* microdeletion associated with episodic falling syndrome. Neurobiol Dis. 2012 Jan;45(1):130-6. http://dx.doi.org/10.1016/j.nbd.2011.07.014

Peer reviewed funding:

Medical Research Council (J004049). PI: RJ Harvey, Co-PI: K Harvey. *Mechanisms of inhibitory $GABA_A$ and glycine receptor clustering in health and disease*. 2012-2015. £395,365; **Action**

Medical Research (1966). PI: RJ Harvey, Co-PIs: R Thomas, MI Rees (University of Swansea). *Identification of new genetic causes of hyperekplexia/startle disease using exome sequencing*. 2012-2014. £124,835; **Medical Research Council** (G0601585). PI: RJ Harvey, Co-PIs: K Harvey, MI Rees (University of Swansea). *Dysfunction of GABA and glycine transporters in human neurological disease*. 2007-2011. £639,143.

4. Details of the impact

Impacts on human health: As a result of the underpinning research described above, genetic testing for startle disease genes identified in our research studies is now offered by the Center for Genomics and Transcriptomics (CeGaT), Leiden University Medical Center and GENDIA (for GENetic DIAgnostic Network), an international network consisting of more than 100 laboratories located in the USA, Europe, Asia and Australia **[a]**. In the course of our research programme, 77 individuals with startle disease have received a definitive genetic diagnosis from 634 screens of index patients through multiple candidate genes.

Definitive genetic diagnosis allows improved clinical management of the condition, improved patient outcomes and reduction of risk. For example, genetic changes in the GlyT2 gene cause severe breathing problems during early infancy. With a definitive genetic diagnosis, parents are now being trained in effective resuscitation techniques and provided with heart rate and breathing monitors. For example, the charity Action Medical Research highlight the case of one child with startle disease: *"since the diagnosis the entire family has been educated about startle disease so that they're all equipped to deal with a seizure and give her the medication she needs. They also know how to give cardiopulmonary resuscitation in case of an emergency following an apnoea attack"* **[b]**. Children with GlyT2 and GlyR β subunit gene mutations also appear to have recurrent infantile apnoea episodes, developmental delay and a mild to severe delay in speech acquisition. Knowing the genetic causes of illness enables clinicians to predict more accurately what sort of educational needs they are likely to have in the future, thus helping parents gain access to appropriate support services.

Harvey has also undertaken public engagement work on this topic, giving a total of 25 invited lectures/seminars on startle disease during the period 2008-13 to audiences in Austria, Belgium, France, Germany, Italy, Spain, Switzerland, the Netherlands, UK and USA. He has also spoken on startle disease at a Café Scientifique event for members of the public. He has also increased awareness of startle disease via media work **[c]**.

Overall, our work into startle disease has led to significant changes in the way that the condition is understood, detected and diagnosed. Furthermore, based on a deep understanding of the genetic basis of the disease, our work has clearly indicated that clonazepam is the treatment of choice, which is again of significant use for clinicians treating this disorder.

Impacts for cattle farming: Startle disease in cattle (also known as CMD2, or congenital muscular dystonia type 2) is lethal within days after an animal's birth. Discovery of the causative mutation led to the implementation of genetic testing for startle disease in Belgian Blue cattle, which has led to healthier animal populations and economic benefits to farmers. The British Blue Cattle Society report that as a result of our work, they brought into place new policies from 1 January 2013, requiring all artificial insemination sires to be tested for genetic details, and that the results of these tests should be displayed on the society's website **[d]**. An educational programme was also instigated to inform the society's membership about the importance of identifying genetic defects and restricting breeding of such animals. To April 2013, 521 cattle had been tested, and of these 12 were found to be carriers. The introduction of this genetic test has had an immediate impact for cattle breeders with cattle affected by CMD2 **[e]**, and has reduced the frequency of carriers in the population, enabling the breed to become healthier.

There are also commercial benefits for farmers in terms of reduced economic impacts. In a 2011 investigation on behalf of the Animal Health and Veterinary Laboratories Agency (AHVLA), we identified six CMD2 carriers and two affected animals on a single UK farm as well as several animals 'in calf' that could produce carriers or affected calves **[e]**. The direct impact of the loss of the carrier bull to the pedigree market, the death of two affected calves and loss of market value for other calves was estimated to be £17,500 in this case alone. Losses would have been ongoing

without our intervention and guidance on future breeding strategies, since carriers of this disorder are phenotypically normal and impossible to identity by visual inspection **[f]**.

Impacts on dog breeding: Disorders that resemble startle disease have also been reported in several dog breeds, although the incidence was unknown. Following our work to resolve the genetic basis of startle disease in Irish Wolfhounds and Episodic Falling Syndrome in Cavalier King Charles Spaniels, benefits to the University and animal breeders/owners were secured by the patenting **[g]** and commercial licencing of DNA diagnostic tests which are currently available via Laboklin **[h]**. These tests have enabled dog breeders to avoid 'at risk' matings, or eliminate these disorders by using non-carrier dogs for future breeding. For example, in 2011 we identified two affected Irish Wolfhounds and 13 carriers of a GlyT2 gene mutation, enabling a US breeder to eliminate startle disease within one generation. This intervention was described by the breeder as having saved her "*from ruin*". The importance of the new genetic test was also highlighted: "*With your hard work in coming up with a viable test for us, we will know what paths to take and which paths lead to disaster*" **[i]**. The diagnostic test for startle disease was described by the Secretary of the Irish Wolfhound Foundation as "*the first clinically useful test for a genetic disease in our breed*" **[j]**. Wider testing of a Cavalier King Charles spaniels by Laboklin has also revealed that Episodic Falling Syndrome is much more common than previously anticipated. In the 2012-13 period, Laboklin have tested 440 dog DNA samples, revealing 38 affected animals and 139 carriers **[h]**.

5. Sources to corroborate the impact

[a] Centres offering genetic testing:
- Center for Genomics and Transcriptomics (CeGaT) http://www.cegat.de/List-of-genes-%28by-disease%29_l=1_41.html (hyperekplexia – *GLRA1*, *GLRB, SLC6A5*)
- Leiden University Medical Center (https://www.lumc.nl/)
- GENDIA http://www.gendia.net/tests_tab1.html#h (hyperekplexia – *GLRA1*, *GLRB, SLC6A5*)

[b] http://www.action.org.uk/touching_lives/october_2012/startle

[c] For example: BBC Health article: http://www.bbc.co.uk/news/health-18911272. Copy of Café Scientifique programme available on request.

[d] Details of genetic tests carried out on artificial insemination sires are listed on the British Blue Cattle Society's website: http://www.britishbluecattle.org/health/genetic_defects.html. Impacts have also been corroborated by Secretary, British Blue Cattle Society. Available on request.

[e] Report jointly written with the Animal Health and Veterinary Laboratories Agency:
Gill JL, James VM, Carta E, Harris D, Topf M, Scholes SF, Hateley G, Harvey RJ. Identification of congenital muscular dystonia 2 associated with an inherited GlyT2 defect in Belgian Blue cattle from the United Kingdom. Anim Genet. 2012 Jun;43(3):267-270. http://doi.org/b82xxd

[f] Letter of testimony to corroborate these claims from Veterinary Investigation Officer at the Animal Health and Veterinary Laboratories Agency. Copy available on request.

[g] Patent EP2522744: A canine *BCAN* microdeletion associated with Episodic Falling Syndrome. Harvey, Gill, Clark with Laboklin GmbH.

[h] http://www.laboklin.co.uk/laboklin/showGeneticTest.jsp?testID=8227D
http://www.laboklin.co.uk/laboklin/showGeneticTest.jsp?testID=8191DD
These impacts can be corroborated by Laboklin. Contact details provided.

[i] Letter of testimonial provided by owner of Kellcastle Irish Wolfhounds. Available on request.

[j] Letter of testimonial provided by the Secretary of the Irish Wolfhound Foundation. Copy available on request.
http://www.iwfoundation.org/healthstudies_detail.html?item_id=17
http://www.iwfoundation.org/news_detail.html?item_id=31