Institution: Queen Mary University of London		
Unit of Assessment: 1		
Title of case study: Development of a New Genetic Diagnostic Panel for the Cost-Effective Diagnosis of Life-Threatening Adrenal Insufficiency		
Period when the underpinning research was undertaken: 01/2000 - 2020		
Details of staff conducting the underpinning research from the submitting unit:		
Name(s):	**Role(s) (e.g. job title):**	**Period(s) employed by submitting HEI:**
1) Lou Metherell	1) Professor of Endocrine Genetics	1) 10/1998 - present
2) Li Chan	2) Clinical Senior Lecturer/Honorary consultant	2) 12/2009 - present
3) Leo Guasti	3) Paediatric Endocrinology Academic Reader	3) 2006 - present
4) Claire Hughes	4) Consultant in Paediatric Endocrinology and Diabetes	4) 09/2008 - present
5) Rathi Prasad	5) Consultant in Paediatric Endocrinology and Diabetes	5) 05/2016 - present
6) Helen Storr	6) Professor and Honorary Consultant in Paediatric Endocrinology	6) 07/2007 - present
Period when the claimed impact occurred: 2014 - 2020		
Is this case study continued from a case study submitted in 2014? No		

1. Summary of the impact (indicative maximum 100 words)

In discovering multiple genetic mutations responsible for the life-threatening condition of adrenal insufficiency (AI), Queen Mary's Prof. Metherell and her research group have influenced international guidelines for AI diagnostics, updated clinical practice, and developed a cost-effective new genetic testing panel that is 20% cheaper and 30% more effective than existing alternatives. As a result, Queen Mary's Centre for Endocrinology has become a major global referral centre for AI — one that has tested samples from 33 countries to date and identified causal mutations for AI in 66% of cases. Based on Queen Mary's research on AI, European and American clinical guidelines now recommend genetic testing to ascertain the genetic cause(s) of AI, and testing is now offered in the UK via the United Kingdom Genetic Testing Network. Although a formal diagnosis is critical for a patient to access swift, appropriate treatment and support, approximately one-third of patients with AI do not know the genetic cause of their condition — or if they are likely to pass it on to their children. Metherell and her team have improved diagnosis and management of AI, increasing patient and clinical awareness of the condition and its risks.

2. Underpinning research (indicative maximum 500 words)

Adrenal insufficiency (AI) arises when the adrenal glands do not produce their normal output of hormones, in particular the main stress hormone, cortisol. The condition can be life-threatening, as it leaves sufferers unable to produce higher levels of cortisol when challenged by illness, pain or severe psychological stress. They also face uncertainty about whether they will pass their disorder on to any children they may have. Gaining a genetic diagnosis ensures that an optimal treatment and management plan can be put in place and helps with assessing the inheritance risk to enable accurate genetic counselling. However, AI patients face significant diagnostic delays, with fewer than 30% of girls and 50% of boys diagnosed in the first six months after onset of symptoms, 20% of patients suffering for more than five years before being diagnosed and approximately one-third of patients currently lacking a genetic diagnosis.

More than 25 genes are currently known to cause AI. In collaboration with Great Ormond Street Hospital (GOSH; John Achermann) and the University of Birmingham (Wiebke Arlt), Queen Mary's team led by Prof. Metherell has a) discovered more genes by refining genetic defect phenotypes; and b) described molecular diagnoses for a number of non-autoimmune AI disorders and associated syndromes by investigating the steroid metabolome of patients and cell lines with gene knockouts [3.1–3.6]. For some genes – *MC2R, MRAP, NNT, TXNRD2, MCM4* and *SGPL1* – these were the first descriptions of the defects causing AI, and led to more cases being identified. For other genes – *STAR* and *CYP11A1* – the Queen Mary team recognised 'silent' disease-causing mutations that cause AI disorders to present in non-classical ways. Other groups have since recognised identical or similar variants in patients.

Genes thought to cause AI disorders are typically sequenced individually — a time-consuming and complicated process. However, Metherell's team has shown that 'whole exome' and targeted approaches to genomic sequencing (Haloplex, designed at GOSH) can improve the cost-effectiveness of diagnosis and allow more genes to be screened [3.1]. This work has enabled the development of a genetic diagnostic panel that is now in clinical use.

3. References to the research (indicative maximum of six references)

[3.1] Maharaj, A., Buonocore, F., Meimaridou, E., Ruiz-Babot, G., Guasti, L., Peng, H. M., Capper, C. P., Burgos-Tirado, N., Prasad, R., Hughes, C. R., Maudhoo, A., Crowne, E., Cheetham, T. D., Brain, C. E., Suntharalingham, J. P., Striglioni, N., Yuksel, B., Gurbuz, F., Gupta, S., Lindsay, R., Couch, R., Spoudeas, H. A., Guran, T., Johnson, S., Fowler, D. J., Conwell, L. S., McInerney-Leo, A. M., Drui, D., Cariou, B., Lopez-Siguero, J. P., Harris, M., Duncan. E. L., Hindmarsh, P. C., Auchus, R. J., Donaldson, M. D., Achermann, J. C. & Metherell, L. A. (2018). Predicted Benign and Synonymous Variants in CYP11A1 Cause Primary Adrenal Insufficiency Through Missplicing. *Journal of the Endocrine Society*, *3* (1), 201-221. https://doi.org/10.1210/js.2018-00130

[3.2] Prasad, R., Hadjidemetriou, I., Maharaj, A., Meimaridou, E., Buonocore, F., Saleem, M., Hurcombe, J., Bierzynska, A., Barbagelata, E., Bergadá, I., Cassinelli, H., Das, U., GOSgene, Krone, R., Hacihamdioglu, B., Sari, E., Yesilkaya, E., Storr, H. L., Clemente, M., Fernandez-Cancio, M., Camats, N., Ram, N., Achermann, J. C., van Veldhoven, O. P., Guasti, L., Braslavsky, D., Guran, T. & Metherell, L A. (2017). SGPL1 mutations cause primary adrenal insufficiency and steroid resistant nephrotic syndrome. *The Journal of Clinical Investigation, 127* (3), 942-953. https://doi.org/10.1172/JCI90171

[3.3] Prasad, R., Chan, L. F., Hughes, C. R., Kaski, J. P., Kowalczyk, J. C., Savage, M. O., Peters, C. J., Nathwani, N., Clark, A. J., Storr, H. L. & Metherell, L. A. (2014). Thioredoxin reductase 2 (*TXNRD2*) mutation associated with familial glucocorticoid deficiency (FGD). *The Journal of Clinical Endocrinology & Metabolism*, *99* (8), E1556-1563. https://doi.org/10.1210/jc.2013-3844

[3.4] Meimaridou, E., Kowalczyk, J., Guasti, L., Hughes, C. R., Wagner, F., Frommolt, P., Nürnberg, P., Mann, N. P., Banerjee, R., Saka, H. N., Chapple, J. P., King, P. J., Clark, A. J. L. & Metherell, L. A. (2012). Mutations in NNT, encoding nicotinamide nucleotide transhydrogenase, cause familial glucocorticoid deficiency. *Nature Genetics*, *44*, 740-742. https://doi.org/10.1038/ng.2299

[3.5] Hughes, C. R., Guasti, L., Meimaridou, E., Chuang, C. H., Schimenti, J. C., King, P. J., Costigan, C., Clark, A. J. & Metherell, L. A. (2012). MCM4 mutation causes adrenal failure, short stature, and natural killer cell deficiency in humans. *The Journal of Clinical Investigation*, *122* (3), 814-820. https://doi.org/10.1172/JCI60224

[3.6] Metherell, L. A., Chapple, J. P., Cooray, S., David, A., Becker, C., Ruschendorf, F., Naville, D., Begeot, M., Khoo, B., Nurnberg, P., Huebner, A., Cheetham, M. E. & Clark, A. J. (2005). Mutations in MRAP, encoding a new interacting partner of the ACTH receptor, cause familial glucocorticoid deficiency type 2. *Nature Genetics, 37,* 166-170. https://doi.org/10.1038/ng1501

Evidence of the quality of the research

[EQR.1] Metherell, L. A. (2013-2016). Antioxidant defence in adrenocortical cells [MR/K020455/1]. *MRC*. Research Grant. GBP392,467.

[EQR.2] Metherell, L. A. (2009-2012). ACTH receptor pathway defects as the cause of Familial Glucocorticoid Deficiency type 3 (FGD3) [G0801265]. *MRC*. New Investigator Award. GBP495,810.

[EQR.3] Hughes, C. (2009-2011). Investigation of the genetic aetiology and pathogenetic mechanism of disease in patients with late-onset FGD [G0901980]. *MRC*. Fellowship. GBP146,589.

4. Details of the impact (indicative maximum 750 words)

Queen Mary's Centre for Endocrinology has greatly improved diagnosis and understanding of AI by characterising recessive gene mutations and developing a novel and cost-effective genetic screening panel that is helping patients to receive swift, appropriate, effective treatment and genetic counselling for this potentially life-threatening disorder.

Influencing international clinical guidelines and developing patient resources

Metherell was the first to link several genes – including *CYP11A1, STAR, MC2R, MRAP, MCM4, NNT, TXNRD2* and *SGPL1* – with AI and associated syndromes. This work led the European Society of Endocrinology and American Association for Clinical Chemistry to recommend that AI is diagnosed through genetic testing in 2015 [5.1].

Metherell has also contributed to:

- The International Classification of Paediatric Endocrine Diagnoses (ICPED) Consortium (Chapter 8, 2015) [5.2]
- The US National Institutes of Health patient guide [5.3] to understanding familial glucocorticoid deficiency, published in 2018 [3.2-3.5]
- The patient resource page Orphanet, through which she has been contacted by concerned parents seeking genetic testing for their children [5.4].

Changing clinical practice to ensure efficacy of treatment

As a result of these guidelines, genetic testing is now offered via the United Kingdom Genetic Testing Network (UKGTN) at Exeter [5.5], with approximately 10 tests offered per year. Understanding the precise genetic defects underlying an expression of AI is crucial to ascertain the correct course of treatment and ensure that it is neither excessive nor insufficient. Standard practice upon clinically diagnosing AI is to begin a hydrocortisone and fludrocortisone treatment – but for certain gene defects, such as *MC2R* and *MRAP*, fludrocortisone may be unnecessary, and can be stopped without risk of developing further co-morbidities.

At the other extreme, standard practice may be insufficient. Genetic diagnosis of a *SGPL1* defect, for instance, requires discussion between clinicians and patients, and patients must be monitored to ensure that no other features arise as a consequence of their AI (with kidney disease and neurological conditions being of particular concern). In this way, determining the genetic cause(s) of AI is essential in helping AI patients achieve optimal health and quality of life while avoiding unnecessary risk.

Developing a fast, accurate, cost-effective genetic testing panel, which has turned Queen Mary into a global referral centre

Metherell's team has become a major global referral centre for AI [5.5], and has developed genetic screening strategies for AI to a) increase the accuracy of mutation detection, and b) reduce testing turnaround time to under three months — critical for clinical management for many AI families.

The pre-existing genetic test for the five most common genes of interest in AI costs GBP750 per individual, but is only able to diagnose half of cases. By contrast, Queen Mary's approach costs GBP600 per individual and diagnoses at least two-thirds of cases using targeted and whole exome high-throughput sequencing. This ensures timely and suitable treatment, while preventing costly further investigations when diagnosis cannot be made on phenotyping alone.

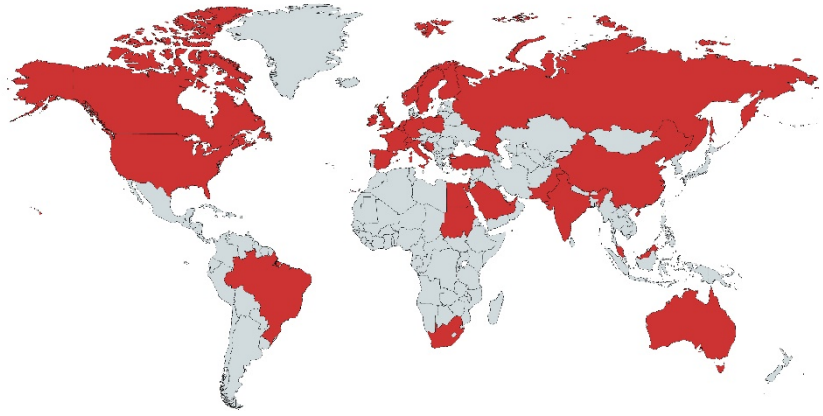

Figure 1: World map representing countries from which Queen Mary's Centre for Endocrinology has tested patient samples.

Around 20 families a year from all over the world now send samples to Queen Mary for genetic analysis and diagnosis, and causal mutations have been identified in 66% of cases. To date, samples from 33 countries (represented in Figure 1) have been tested [5.6]. Approximately one-third of samples come from the UK, and the rest from overseas. For many overseas samples, there is no financial support to cover genetic testing. In these cases, tests are run at no cost to the family.

Metherell's gene discoveries have also been incorporated into a Haloplex targeted array designed at GOSH for diagnosis of AI. The array was first used for a Turkish cohort study in 2016 [5.7], in which 95 patients from 85 families and their unaffected siblings and parents were recruited from 19 tertiary paediatric endocrinology clinics in Turkey – the largest ever nationwide study of the molecular genetics of childhood AI. A molecular diagnosis was achieved for 80% of the children.

Improving management of AI and reassuring worried families

AI patients require a definitive formal diagnosis to begin timely, appropriate treatment and genetic counselling, access support and relevant clinical trials, and understand how their disease will progress. The importance of diagnosis was confirmed by Prof. Catherine Choong, consultant endocrinologist at Perth Children's Hospital who said, "given that these are rare conditions, identification of the genetic variants by Professor Metherell has allowed us to monitor the literature for emergent phenotypes thereby improving the clinical surveillance and management of these children and families" [5.8]. Additionally, Metherell's research has enabled clinicians to stratify levels of risk both during and after delivery of affected and unaffected children with "significant benefit to the health and safety of the parent and the neonate," according to Prof. Choong [5.8].

5. Sources to corroborate the impact (indicative maximum of 10 references)

[5.1] Bornstein, S. R., Allolio, B., Arlt, W., Barthel, A., Don-Wauchope, A., Hammer, G. D., Husebye, E. S., Merke, D. P., Murad, M. H., Stratakis, C. A. & Torpy, D. J. (2016). Diagnosis and Treatment of Primary Adrenal Insufficiency: An Endocrine Society Clinical Practice Guideline. *The Journal of Clinical Endocrinology & Metabolism, 101*, 364-389. https://doi.org/10.1210/jc.2015-1710

[5.2] International Classification of Paediatric Endocrine Diagnoses. (2015). *Chapter 8: Adrenal Glands (section 8A.2b.1)*. http://icped.org/revisions/0/2015/diagnoses/#!/8A.2b reviewed and updated 2015.

[5.3] National Institutes of Health (NIH). U.S. National Library of Medicine: MedlinePlus. (2018). *Familial glucocorticoid deficiency.* https://medlineplus.gov/genetics/condition/familial-glucocorticoid-deficiency/

[5.4] Orphanet. (2015). *Familial glucocorticoid deficiency*. https://www.orpha.net/consor/cgi-bin/OC_Exp.php?Expert=361

[5.5] NHS UK Genetic Testing Network. (2015). *Endocrine Disorders 65 Gene Panel Validation*.

[5.6] Queen Mary University of London. *A.D.R.E.N.A.L*. https://www.qmul.ac.uk/adrenal/. Accessed 7 December 2020.

[5.7] Guran, T., Buonocore, F., Saka, N., Ozbek, M. N., Aycan, Z., Bereket, A., Bas, F., Darcan, S., Bideci, A., Guven, A., Demir, K., Akinci, A., Buyukinan, A., Aydin, B. K., Turan, S., Agladioglu, S. Y., Atay, Z., Abali, Z. Y., Tarim, O., Catli, G., Yuksel, B., Akcay, T., Yildiz, M., Ozen, S., Doger, E., Demirbilek, H., Ucar, A., Isik, E., Ozhan, B., Bolu, S., Ozgen, I. T., Suntharalingham, J. P. & Achermann, J. C. (2016). Rare Causes of Primary Adrenal Insufficiency: Genetic and Clinical Characterization of a Large Nationwide Cohort. *The Journal of Clinical Endocrinology & Metabolism, 101* (1), 284-292. https://doi.org/10.1210/jc.2015-3250
[5.8] C. Choong. Consultant Endocrinologist. *Government of Western Australia, Department of Health, Child and Adolescent Health Service - Endocrinology and Diabetes Services* (testimonial letter, 17 February 2019). [Corroborator 1]