Institution: University of Southampton		
Unit of Assessment: 01 Clinical Medicine		
Title of case study: 01-04 Genetic epigenetics: Discovering, diagnosing and treating imprinting disorders (IDs) - a transformational program		
Period when the underpinning research was undertaken: January 2000 to March 2018		
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:**
Prof Deborah JG Mackay	Reader then Professor of Medical Epigenetics	September 1998 – present
Prof I Karen Temple	Visitor (Category C RAE 2001); Senior Lecturer then Professor of Medical Genetics	November 1990 – October 2000 November 2000 – present
Period when the claimed impact occurred: August 2013 to July 2020		
Is this case study continued from a case study submitted in 2014? N		

1. Summary of the impact

Karen Temple and Deborah Mackay's groundbreaking research in medical epigenetics defined the phenotypes and elucidated the molecular mechanisms of genomic imprinting disorders, which as a result have grown from unknown or barely known entities in the 1990s to a spectrum of well-characterised congenital conditions affecting 1 in 3000 people worldwide.

Their work has led to improved tools for clinical and molecular testing. After Temple described Temple Syndrome *(TS)* as a key differential diagnosis for Silver-Russell Syndrome (SRS), TS testing was performed in 62% of SRS referrals between 2014 and 2019, resulting in improved treatment, patient care and economic wealth for testing kit manufacturers. New European diagnosis and testing guidelines, written by Temple and Mackay, have been accessed more than 55,000 times and used by health professionals around the world. Patients and families now access diagnosis and treatment not previously available, including over 2000 patients from over 100 countries being tested for transient neonatal diabetes.

2. Underpinning research

Imprinting disorders (**IDs**) are congenital conditions involving imprinted genes, whose expression is regulated by the parent from whom they are inherited. While some patients have genetic mutations in these genes, the majority have epigenetic mutations changing not their genetic sequence but the way their genes are expressed. IDs affect approximately 1 in 3000 children worldwide, with major impacts on growth, metabolism, development, behaviour, cancer risk and overall quality of life; but an early diagnosis can mitigate these impacts through appropriate treatment and surveillance.

In 2000 the Wessex Imprinting Group, led at the University of Southampton by Deborah Mackay, discovered the genetic locus and (epi)genetic mutations causing Transient Neonatal Diabetes Mellitus (TNDM), defining it as an imprinting disorder and developing effective diagnosis for patients [**3.1, 3.2**].

In 2006 Temple and Mackay established the International Transient Neonatal Diabetes Register, with the aim of learning about the history and long-term effects of TNDM and how best to manage it. With 90 participants, the Register proved a valued resource for researchers to follow up on patients with this ultra-rare disorder. Study of TNDM patients on the Register showed for the first time that some had imprinting errors across different parts of the genome, termed multi-locus imprinting disorders (MLID). By developing an international cohort of MLID patients, Temple and Mackay identified an underlying single-gene cause of MLID, the *ZFP57* mutation, defining a new category of genetic, epigenetic disease [**3.3**].

Subsequently, their MRC-funded study discovered gene mutations in mothers that cause MLID, recurrent miscarriage and developmental problems in their offspring [maternal-effect mutations; **3.4, 3.5**], thus defining a new mechanism of genetic disease in children with significant developmental problems. The research pinpoints genetic and epigenetic factors that are critical for early development and showed that maternal-effect mutations produce a broad spectrum of viable and nonviable outcomes, suggesting that MLID testing may be relevant for children with developmental delay as well as for subfertile but otherwise healthy couples.

Temple and Mackay have identified new clinical disorders, most notably Temple Syndrome (TS, 2014) [**3.6, 3.7**], associated with growth restriction, hypotonia, obesity and early puberty. TS, whose genetic locus is on chromosome (chr) 14, has clinical overlap with better-known IDs, Silver-Russell (chr11) and Prader-Willi (chr15) syndromes, each affecting 1 in 15,000 newborns, but has key differences from each. Early and accurate diagnosis enables the personalised management required to improve outcomes for patients with each disorder.

Overall, Temple and Mackay have played a key driving role in medical epigenetics, expanding imprinting disorders from a group of obscure clinical syndromes to a spectrum of disorders impacting growth, development, behaviour, metabolism and reproductive health, and offering fundamental insights into developmental biology.

3. References to the research

3.1 Gardner RJ, Mackay DJG … **Temple IK**, Robinson DO. An imprinted locus associated with Transient neonatal diabetes mellitus. Hum Mol Genet: 9: 589-96; 2000 https://doi.org/10.1093/hmg/9.4.589

3.2 Mackay DJ, **Temple IK**, Shield JP, Robinson DO. Bisulphite sequencing of the transient neonatal diabetes mellitus DMR facilitates a novel diagnostic test but reveals no methylation anomalies in patients of unknown aetiology. Hum Genet: 116(4):255-6;2005. https://doi.org/10.1007/s00439-004-1236-1

3.3 Mackay DJ … **Temple IK**. Hypomethylation at multiple imprinted loci in individuals with transient neonatal diabetes is associated with ZFP57 mutations Nature Genetics 2008 Aug;40(8):949-51. https://doi.org/10.1038/ng.187

Funding for **3.1**-**3.3**: Medical Research Council Project grant: Identifying factors required for genomic DNA methylation using the imprinting control protein ZFP57. GBP600,000 (2012-2015)

3.4 Docherty LE … **Temple IK**, **Mackay DJ**. Mutations in NLRP5 are associated with reproductive wastage and multi-locus imprinting disorders in humans. Nat Commun. 2015 Sep 1;6:8086. https://doi.org/10.1038/ncomms9086

3.5 Begemann M … **Temple IK**, **Mackay, DJ**. (2018). Maternal variants in NLRP and other maternal-effect proteins are associated with multi-locus imprinting disturbance in offspring. J Med Genet. 2018 Jul;55(7):497-504. https://doi.org/10.1136/jmedgenet-2017-105190

3.6 Temple IK, Shrubb V, Lever M, Bullman H, **Mackay DJ**. Isolated imprinting mutation of the DLK1/GTL2 locus associated with a clinical presentation of maternal uniparental disomy of chromosome 14. J Med Genet. 2007 Oct;44(10):637-40. https://dx.doi.org/10.1136%2Fjmg.2007.050807

3.7 Ioannides Y, Lokulo-Sodipe K, **Mackay DJ**, Davies JH, **Temple IK**. Temple syndrome: improving the recognition of an underdiagnosed chromosome 14 imprinting disorder: an analysis of 51 published cases. J Med Genet. 2014 Aug;51(8):495-501. https://doi.org/10.1136/jmedgenet-2014-102396

4. Details of the impact

Impact on patients and families

Temple and Mackay's research has made early diagnosis of IDs a standard practice. As an example, the Wessex Regional Genetics Laboratory has increased its reporting of ID tests from 378 in 2012/13 to 535 in 2018/19, an increase of 42% [**5.1**]. Early diagnosis enables access to the stratified treatment that patients require for optimal outcomes.

Diagnosis of Temple Syndrome (TS)

TS was described as a clinical entity by Temple in 2014 [**3.7**], and has been recognised as a key differential diagnosis for Silver-Russell Syndrome (SRS) [**5.2**], notably in the 2017 international clinical consensus guidelines for SRS [**5.3**]. Children diagnosed with TS benefit from early intervention to mitigate complications such as early puberty and resistant central obesity, with critical benefits for growth and wellbeing. The positive impact was observed by a Director at the MAGIC Foundation in the USA – the global leader in endocrine health, advocacy, education, and support – who stated in 2020: "The chromosome 14 patients I know *that were diagnosed at one to three years old* and have been following the SRS Consensus Statement recommendations… are far leaner, their parents are educated and, in many cases, the whole family exercises together." [**5.4**].

Genetic testing for TS was developed by Temple and Mackay and made available to the NHS in 2008. From 2008 to 2013, only 1% of SRS referrals received TS testing on chr14; but since its description in 2014 it has grown in recognition, and in 2019 TS testing was performed in 62% of SRS referrals [**5.1**]**.** Thus, a diagnosis before 2014 is now widely available. This has been welcomed by patient support groups, with the Membership & Parent Support Manager of the Child Growth Foundation commenting: "Receiving a diagnosis of Temple Syndrome … can be very important to families. Without a diagnosis, many families constantly question 'why' and can be concerned for the future for their child, due to the unknown. A diagnosis can help with management and treatment of the condition, as well as peace of mind for all concerned." [**5.5**]

In 2017 Temple established a multidisciplinary clinic in Southampton treating UK patients with TS, SRS and other IDs. This clinic, which enrols five to 10 new cases per year and sees over 50 cases regularly, is part of the prestigious European Reference Network for Endocrine Disease, and provides integrated precision management of nutrition, growth, body composition, puberty timing, orthopaedics and additional specialist referrals [**5.6**].

Diagnosis of Transient Neonatal Diabetes Mellitus (TNDM)

TNDM, defined by the Wessex Imprinting Group led at Southampton by Temple, is now clinically well-recognised through online resources including GeneReviews, which consistently receives over 2,000 views per year by clinicians, scientists and families globally (2,921 views in 2019 [**5.7**]). The Group remains in periodic contact with patients and families worldwide through their International TNDM Register, which is supported by families [**5.8**].

The Register provided patients for the landmark study, Lango et al 2015 [**5.9**], which showed that 38% of all neonatal diabetics have the imprinting disorder TNDM. As a direct result of this research, all patients with neonatal diabetes receive early testing (including TNDM testing) as standard, with 2,298 patients referred from 103 countries as of July 2019 [**5.10**]. Accurate diagnosis enables appropriate management, including the cessation of insulin treatment in early childhood when it is no longer required, and screening to prevent complications of diabetes when it recurs in later life

Multi-locus imprinting disorder (MLID), described by Temple and Mackay, is recognised as a component of TNDM among other imprinting disorders. *Zinc finger protein 57 homolog ZFP57* sequencing is now included in genetic testing for neonatal diabetes in the UK, France, Germany, Italy , Spain and Switzerland, as well as in Chicago, USA [**5.11**].

Patient advocacy groups

As medical advisers to the UK Child Growth Foundation and the Global Alliance on SRS, Temple and Mackay receive more than 100 enquiries a year from clinicians and families for advice on IDs. Temple actively supports the MAGIC Foundation USA. At panel clinics in 2018

and 2019 she gave clinical support to more than 100 families and in 2019 she spoke to the MAGIC Congress on how a TS diagnosis alters management . There is now a TS subgroup of the MAGIC Foundation; parents have reported how a diagnosis has changed their child's treatment and meant that doctors took their concerns seriously, seeking help from experts [**5.12**].

Impact on international clinical practice

In 2012 Temple and Mackay were founders of the EU Consortium on Imprinting Disorders (EUCID) and continue to be working group leaders. EUCID is an EU network dedicated to implementation, harmonisation and education in diagnosis and management for IDs. EUCID developed clinical consensus guidelines for SRS (incorporating Temple syndrome) in 2017 and Beckwith Wiedemann Syndrome (BWS) in 2018; Temple and Mackay were senior authors on both. These guidelines have been accessed more than 55,000 times and used by health professionals around the world to guide testing and treatment for patients [**5.3, 5.13**].

Mackay co-wrote European guidelines for diagnosis of SRS and BWS and implemented a European quality management scheme for SRS and BWS testing, active since 2015, with 40 participating labs annually from 14 nations on three continents. Ongoing annual reports from EMQN demonstrate that both diagnosis and interpretation have rapidly increased in quality [**5.14**].

Our research, as outlined in Section 2, demonstrated the clinical need for ID testing that drove development of multiplex ligation-dependent probe amplification diagnostic kits by MRC-Holland. The kit contains sufficient reagents to perform 100 tests. Two decades ago, TNDM, TS and MLID were undefined disorders; one decade ago, testing was limited to a handful of specialist research centres; now, commercial tests are available for all known IDs, making diagnosis available to patients internationally. Over 2016-2019 MRC-Holland sold 4,000 tests for TNDM, 31,000 for TS and 8,200 for MLID, with an estimated value of GBP378,000. Notably the sale of the MLID test has increased 40-fold since its introduction in 2017 [**5.15**].

5. Sources to corroborate the impact

5.1 Corroborating statement from the Head of Molecular Diagnostics, Wessex Regional Genomics Laboratory

5.2 Chromosome 14q32.2 Imprinted Region Disruption as an Alternative Molecular Diagnosis of Silver-Russell Syndrome. Geoffron et al. J Clin Endocrinol Metab, July 2018, 103(7):2436–2446 https://doi.org/10.1210/jc.2017-02152

5.3 Wakeling EL, et al. Diagnosis and management of Silver-Russell syndrome: first international consensus statement. Nat Rev Endocrinol. 13,105-124 2016. https://doi.org/10.1038/nrendo.2016.138; accessed >24,000 times and cited in 108 papers: www.nature.com/articles/nrendo.2016.138/metrics

Personal communications of evidence for Temple Syndrome:

5.4 Director, RSS/SGA Research & Education Fund of MAGIC foundation USA, the global leader in endocrine health, advocacy, education, and support.

5.5 Secretary of the UK Child Growth Foundation, 'a leading UK charity focusing on the support and management of rare growth conditions affecting children and adults'.

5.6 The European Reference Networks (ERN) are virtual networks involving Reference Centres across Europe which aim to tackle complex or rare diseases and conditions. They provide infrastructure for secure, EU-level virtual multidisciplinary teams to collaborate and advise, ensuring that it is 'medical knowledge and expertise that travel, rather than the patients' (https://endo-ern.eu/ern/). The multidisciplinary service for SRS has been implemented in Southampton by Temple, underpinned by Southampton adoption as a Reference Centre in the European Reference Network for endocrine disease (Endo-ERN).

5.7 Corroborating contact: GeneReviews Project Manager can corroborate readership of chapter authored by Temple and Mackay https://www.ncbi.nlm.nih.gov/books/NBK1534/

5.8 TNDM Register webpage:
www.southampton.ac.uk/geneticimprinting/informationclinicians/transientneonatalregister.page

UK NICE webpage: www.evidence.nhs.uk/search?q=transient+neonatal+diabetes

Orphanet pages www.orpha.net/consor/cgi-bin/OC_Exp.php?lng=EN&Expert=99886

5.9 The effect of early, comprehensive genomic testing on clinical care in neonatal diabetes: an international cohort study. De Franco E, Flanagan SE, Houghton JA, Lango Allen H, Mackay DJ, Temple IK, Ellard S, Hattersley AT. Lancet. 2015;386:957-63. https://doi.org/10.1016/S0140-6736(15)60098-8.

5.10 https://www.diabetesgenes.org/about-neonatal-diabetes (specialist genetic testing centre for neonatal diabetes, hosted by the University of Exeter);

5.11 ZFP57 sequencing:
a) https://www.orpha.net/consor/cgi-bin/ClinicalLabs_Search_Simple.php?lng=EN (12 labs in 5 EU nations performing testing for ZFP57);
b) https://dnatesting.uchicago.edu/tests/comprehensive-neonatal-diabetes-mutation-analysis (Neonatal diabetes genetic testing at the University of Chicago centre, listing ZFP57)

5.12 Patient advocacy groups:
a) Global alliance for Silver-Russell syndrome (https://silverrussellsyndrome.org).
b) UK Child Growth Foundation (http://www.childgrowthfoundation.org).
c) Magic Foundation (https://www.magicfoundation.org)
d) Maternal UPD14/ Temple syndrome/ DUP 14 Materno Support group: A group created to give support and find people or families with children with Temple syndrome (www.facebook.com/groups/311930455805623/)

5.13 Brioude F et al. Clinical and molecular diagnosis, screening and management of Beckwith–Wiedemann syndrome: an international consensus statement. Nat Rev Endocrinol 14, 229-249 2017. https://doi.org/10.1038/nrendo.2017.166. Accessed >21,000 times, and cited in 91 papers: www.nature.com/articles/nrendo.2017.166/metrics

5.14 Eggermann K, et.al. EMQN best practice guidelines for the molecular genetic testing and reporting of chromosome 11p15 imprinting disorders: Silver-Russell and Beckwith-Wiedemann syndrome. Eur J Hum Genet. 2016 May 11. https://doi.org/10.1038/ejhg.2016.45.

European Molecular Quality Management schemes for BWS and SRS (www.emqn.org/schemes/beckwith-wiedermann-silver-russell-syndromes)

5.15 Corroborating statement from Head of Sales and Distribution, MRC-Holland.