Institution: The University of Manchester

Unit of Assessment: 1

Title of case study: Defining the phenotype of severe growth disorders, discovering new genes that control human growth and enhancing clinical practice

1. Summary of the impact

One in ~1,000 children has significant short stature that needs medical evaluation, one in ~4,000 has growth hormone deficiency and one in ~≥10,000 has a genetic growth disorder. Research at the University of Manchester (UoM) has impacted on clinicians worldwide who manage growth disorders. UoM researchers have: characterised growth disorder phenotypes to ensure the right tests are used for the right child and verified the accuracy of diagnostic biochemical tests; discovered new genes associated with a primordial growth disorder and introduced new molecular diagnostic tests for international use; and generated clinical practice guidelines adopted by the worldwide paediatric endocrine community.

2. Underpinning research (*See section 3 for references 1-6; UoM researchers are given in bold.)*

Key UoM researchers:

- **Peter Clayton** (Lecturer, 1992-1994; Senior Lecturer, 1994-2001; Professor, 2001-date)
- **Graeme Black** (Lecturer, 1995-1997; Senior Lecturer, 1997-2003; Professor, 2003-date)
- **Andrew Read** (Reader in Medical Genetics, 1992-1995; Professor of Human Genetics 1995-2004; Honorary Professor of Human Genetics 2004-date)
- **Forbes Manson** (Lecturer, 2002-2005; Senior Lecturer, 2005-date)
- **Dan Hanson** (Research Associate, 2008-date)
- **Leena Patel** (Lecturer, 1992-1994; Clinical Lecturer, 1994-1995; Senior Lecturer, 1995-1998; Clinical Senior Lecturer, 1998-date)

This work was initiated at UoM in 1997 as a collaboration between **Clayton** and Oliveira (Brazil) to work on a cohort of highly consanguineous very short people. UoM's collaborators in the US (Salvatore, John Hopkins, USA) had identified that this cohort had an autosomal recessive form of severe isolated growth hormone deficiency (GHD) due to an inactivating mutation in the GH releasing hormone receptor gene (GHRH-R).

The research characterised the effects of untreated severe GHD on phenotype, biomarkers of GH action for diagnostic purposes (1) and metabolic consequences (2), based on field-work in Brazil and analysis of anthropometric data and samples brought to Manchester.

While working with this cohort in the field in Brazil, **Clayton** recognised that some adults and children (clustered in one section of the pedigree of the whole cohort) did not have GHD but were still very short. **Clayton** identified the features of another growth disorder – the 3-M syndrome – a primordial growth disorder characterised by small size at birth followed by poor post-natal growth resulting in a height in childhood similar to untreated GHD, but in this case with intact GH secretion. The 3-M syndrome was known to be an autosomal recessive disorder but in the early 2000s no gene had been identified.

Clayton and UoM colleagues went on to find the first gene (*CUL7*) associated with 3-M in 2005 at a similar time to Cormier-Daire's group in Paris; the work was published by all who had contributed to finding this first gene (3). UoM researchers started to build a cohort of patients with 3-M syndrome in Manchester from the local population and from regional, national and international referrals. However no mutations in CUL7 were found in these patients. This outcome led to a new round of autozygosity mapping, leading to the discovery of the second gene (*OBSL1*) (4) in 2008 and the third gene (*CCDC8*) (5) in 2010 (the latter using the new technique of exome sequencing). As a result a new pathway controlling growth has been defined. The 3-M syndrome is a 'pure' growth disorder without other major system disorders (unlike many other primordial growth disorders), and is therefore an excellent model to define growth mechanisms. Skin fibroblast cell lines from patients with each of the mutations have been used to define their impact on cell growth, growth factor signalling, gene expression, and metabolomic profiles, all leading to a systems approach to identify the 3-M interactome, and hence new candidates for primordial growth disorders (6).

3. References to the research

1. Aguiar-Oliveira MH, Gill MS, de ES, Barretto A, Alcântara MRS, Miraki-Moud F, Menezes CA, Souza AHO, Martinelli CE, Pereira FA, Salvatori R, Levine MA, Shalet SM, Camacho-Hubner C, **Clayton PE**. Effect of Severe Growth Hormone (GH) Deficiency due to a Mutation in the GH-Releasing Hormone Receptor on Insulin-Like Growth Factors (IGFs), IGF-Binding Proteins, and Ternary Complex Formation Throughout Life. *Journal of Clinical Endocrinology & Metabolism*. 1999;84(11):4118-26. DOI: 10.1210/jc.84.11.4118
Provided evidence in an 'exemplar' cohort of the impact of severe GH deficiency on serum biomarkers, providing evidence of which marker was the most useful in clinical practice.

2. Gleeson HK, Souza AHO, Gill MS, Wieringa GE, De A. Barretto ES, Barretto-Filho JAS, Shalet SM, Aguiar-Oliveira MH, **Clayton PE**. Lipid profiles in untreated severe congenital isolated growth hormone deficiency through the lifespan. *Clinical Endocrinology*. 2002;57(1):89-95. DOI: 10.1046/j.1365-2265.2002.01568.x
Provided evidence in an 'exemplar' cohort of the impact of severe GH deficiency on serum lipid profiles in both adults and children as a potential marker of cardiovascular risk.

3.Huber C, Dias-Santagata D, Glaser A, O'Sullivan J, Brauner R, Wu K, Xu X, Pearce K, Wang R, Uzielli MLG, Dagoneau N, Chemaitilly W, Superti-Furga A, Santos HD, Megarbane A, Morin G, Gillessen-Kaesbach G, Hennekam R, Burgt IVd, **Black GCM, Clayton PE**, **Read A**, Merrer ML, Scambler PJ, Munnich A, Pan Z-Q, Winter R, Cormier-Daire V. Identification of mutations in CUL7 in 3-M syndrome. *Nature Genetics*. 2005;37(10):1119-24. DOI: 10.1038/ng1628
The first description of a gene causing the 3-M syndrome in patients for all over the world. This also demonstrated that 3-M and Gloomy Face syndromes were in fact the same condition.

4.**Hanson D**, Murray PG, Sud A, Temtamy SA, Aglan M, Superti-Furga A, Holder SE, Urquhart J, Hilton E, **Manson FDC**, Scambler P, **Black GCM, Clayton PE**. The Primordial Growth Disorder 3-M Syndrome Connects Ubiquitination to the Cytoskeletal Adaptor OBSL1. *The American Journal of Human Genetics*. 2009;84(6):801-6. DOI: 10.1016/j.ajhg.2009.04.021
The first description of the second gene causing 3-M syndrome. 2007-2010 ***Medical Research Council*** *- Clinical Training Fellowship for Dr P Murray - The Role of Disordered Ubiquitination in Pre- and Post-natal Growth Restriction.* ***Clayton*** *&* ***Black****. Award £157,482.*

5.**Hanson D***,* Murray PG, O'Sullivan J, Urquhart J, Daly S, Bhaskar Sanjeev S, Biesecker Leslie G, Skae M, Smith C, Cole T, Kirk J, Chandler K, Kingston H, Donnai D, **Clayton PE, Black GCM**. Exome Sequencing Identifies CCDC8 Mutations in 3-M Syndrome, Suggesting that CCDC8 Contributes in a Pathway with CUL7 and OBSL1 to Control Human Growth. *The American Journal of Human Genetics*. 2011;89(1):148-53. DOI: 10.1016/j.ajhg.2011.05.028
The first description of the third gene causing 3-M syndrome, including functional data on the physical relationships between the three proteins and the recognition of a new growth pathway.

6.**Hanson D***,* Murray PG, Coulson T, Sud A, Omokanye A, Stratta E, Sakhinia F, Bonshek C, Wilson LC, Wakeling E, Temtamy SA, Aglan M, Rosser EM, Mansour S, Carcavilla A, Nampoothiri S, Khan WI, Banerjee I, Chandler KE, **Black GCM, Clayton PE**. Mutations in CUL7, OBSL1 and CCDC8 in 3-M syndrome lead to disordered growth factor signalling. *Journal of Molecular Endocrinology*. 2012;49(3):267-75. DOI: 10.1530/JME-12-0034
Phenotypic detail on 3-M patients with different mutations, including in vitro cellular responses to the growth factors GH and IGF-I.

4. Details of the impact (*See section 5 for corroborating sources S1-S10.)*

Context

Clayton's research group has demonstrated, using a very rare population in Brazil as an exemplar, the effect of severe growth hormone deficiency on hormone profiles and phenotype and, using discovery science, how new molecular aetiologies for growth disorders can be found. The impact of this in clinical practice is that standards have been set against which milder forms of GH deficiency commonly treated in first world countries should be compared. The research has brought about improvements in the management of growth disorders and has paved the way for new diagnostic tests.

Pathways to impact

Clayton's research provided important insights to inform the construction of consensus guidelines for less severe growth disorders – in particular which tests should be carried out and in which circumstances. He was selected by International Societies to chair and/or sit on the steering/organising committees of five growth-related workshops (S1-S5). These meetings brought together experts representing the global paediatric endocrine community (with European, North American, Canadian, South American and Asia-Pacific Societies invited), the Pharma Industry and Regulatory Bodies (US FDA) to generate guideline publications for clinical use.

Reach and significance of the impact

Improved management of growth disorders

The consensus guidelines (S1-S5) have been cited ~700 times, have been used internationally to guide clinicians in pragmatic management, to support licensing applications (e.g. recombinant human (r-h)GH licence for treating short small for gestational age children in Japan), to guide Medical Insurance Company funding for r-hGH treatment and have been scrutinised extensively by Pharma. Estimates of the worldwide spend on r-hGH are difficult to make, but it is known that r-hGH prescriptions in the UK total ~£30m per year (5000 children on treatment) and in the US (for paediatric indications) ~$1bn (~50,000 on treatment).

Clayton's work in severe growth hormone deficiency provided him with the appropriate expertise to write (with **Patel**) evidence for NICE technology appraisal guidance 188, *Human growth hormone (somatropin) for the treatment of growth failure in children* (2010; review of NICE TA42, 2002) (S6). **Clayton**'s evidence for TA188, a review on treating a child with GHD, was prepared on behalf of the British Society for Paediatric Endocrinology and Diabetes (BSPED) and informed the NICE guidance (S6, TA188, p. 45; BSPED submission acknowledged; source authors not directly credited in the NICE TA process). **Clayton** also produced evidence on behalf of BSPED for the NICE TA42 process in 2002 (S6, TA42). The NICE guidance determines the criteria for children who should receive r-hGH, how much benefit should be derived from the treatment and guidelines on how the treatment should be monitored. This relates to ~5,000 children treated with r-hGH in the UK, including ~800 new prescriptions each year (S7).

The international paediatric endocrine community has always considered evaluation of the long-term safety of recombinant human GH as a major priority (considering issues such as the potential link between use of rhGH and cancer and the occurrence of Creutzfeldt-Jacob disease associated with the use of pituitary-derived GH), and thus safety has always been a part of Consensus guidelines (in particular S2). Long-term (post-GH) pharmacovigilance had not been directly addressed; a consortium from 8 European countries were awarded an EU grant (2009-2011) to focus on this issue (S8). A cohort of ~25,000 r-hGH users, now adults, are under surveillance, including ~4,000 in the UK.

R-hGH is a 'high-cost' drug: ensuring r-hGH is used as effectively as possible is a priority. One approach is to predict response at initiation of treatment. It is recognised that pharmacogenomics can be used in the management of growth disorders treated with GH (see, e.g., **Clayton P** et al., *Eur J Endocrinol*. 2013 Jul 29;169(3):277-89). MerckSerono has invested an estimated €15m in PREDICT (S9), an international (14 countries) research programme to investigate further the role of pharmacogenomics in managing growth disorders. **Clayton** is Co-Chief Investigator with Chatelain from Lyon, France. PREDICT is relevant to all children worldwide treated with r-hGH, and aims to identify the ~10% of children in whom r-hGH is ineffective and should be stopped, thereby significantly enhancing the cost-benefit ratio. (The estimated incremental cost per quality adjusted life-year (QALY) for r-hGH treatment in the UK is currently £23k for GHD and £33k for the short SGA child).

Making molecular diagnoses in primordial growth disorders

Following its identification of genes associated with 3-M, Manchester has become a recognised centre for referral of potential 3-M cases both for a clinical opinion and a molecular diagnosis (from the UK and ~10 countries worldwide). Manchester receives requests at ~1 per month, has assessed ~100 families, and has now developed new techniques (Haloplex Next Gen Exome

Sequencing on targeted genes) for routine clinical use. The commercial sector has recognised the need for molecular testing in 3-M, and companies in the US are now offering to sequence 3-M genes (www.ctgt.net/ Sequencing Costs: CUL7 $1830, OBSL1 $1680, CCDC8 $595).

A 3-M website has been established to provide information on UoM work on 3-M (http://3msyndrome.com/), and over the 8 months during which detailed visit statistics have been collated, there has been a total of 1,896 views from 40 countries, with 30-40 visits per month and 70-160 page views. Contact from parents also led to the genesis of grouped growth data on their 3-M children.

The interest in making specific genetic diagnoses has led Pharma companies that market r-hGH to develop programmes that evaluate genetic contributions to short stature of as yet undefined aetiology (ISS). One example is Ipsen's EPIGROW, a pharmacoepidemiological study across 8 European countries to identify genes related to ISS. **Clayton** was invited to be the Chief Investigator and at completion of the study (S10) has received all the data from the study for further data mining under a Material Transfer Agreement between Ipsen and UoM.

Sources to corroborate the impact

S1. Consensus Guidelines for the Diagnosis and Treatment of Growth Hormone (GH) Deficiency in Childhood and Adolescence: Summary Statement of the GH Research Society. *Journal of Clinical Endocrinology & Metabolism*. 2000;85(11):3990-3. *Report of a Workshop (**Clayton** a principal author) (2000)*

S2. Critical Evaluation of the Safety of Recombinant Human Growth Hormone Administration: Statement from the Growth Hormone Research Society. *Journal of Clinical Endocrinology & Metabolism*. 2001;86(5):1868-70. *Report of a Workshop (**Clayton** on the writing panel) (2001).*

S3. Clayton PE, Cuneo RC, Juul A, Monson JP, Shalet SM, Tauber M. Consensus statement on the management of the GH-treated adolescent in the transition to adult care. *European Journal of Endocrinology*. 2005;152(2):165-70.

S4. Clayton PE, Cianfarani S, Czernichow P, Johannsson G, Rapaport R, Rogol A. Management of the Child Born Small for Gestational Age through to Adulthood: A Consensus Statement of the International Societies of Pediatric Endocrinology and the Growth Hormone Research Society. *Journal of Clinical Endocrinology & Metabolism*. 2007;92(3):804-10.

S5. Ho KKY. Consensus guidelines for the diagnosis and treatment of adults with GH deficiency II: a statement of the GH Research Society in association with the European Society for Pediatric Endocrinology, Lawson Wilkins Society, European Society of Endocrinology, Japan Endocrine Society, and Endocrine Society of Australia. *European Journal of Endocrinology*. 2007;157(6):695-700. ***Clayton** on writing committee.*

S6. Human Growth Hormone (somatropin) for the treatment of growth failure in children NICE Technology appraisal guidance 42, 2002.
Human Growth Hormone (somatropin) for the treatment of growth failure in children. Review of NICE Technology appraisal guidance 42, Guidance 188, 2010.*NICE guidelines for the use of r-hGH in children, to which **Clayton** contributed.*

S7. Kirk J. Indications for growth hormone therapy in children. *Archives of Disease in Childhood*. 2012;97(1):63-8. *This is an article on the current use of r-hGH in the UK, giving an indication of the number of children in one European country for which clinical guidelines are relevant to their management.*

S8. 2009-2011 FP7 European Union - Safety and Appropriateness of Growth hormone treatments in Europe – Award €2,989,154 (UK share €519,882, of which €134,792 to UoM)) with UK investigators including **Clayton** (UoM), Butler (University College London), Swerdlow (Institute of Cancer Research, London).

S9. Clinical Trials references for the 1 month initial PREDICT study, the long-term follow-up phase and the validation study (available from UoM on request).

S10. **Clayton P**, Bonnemaire M, Dutailly P, Maisonobe P, Naudin L, Pham E, Zhang Z, Grupe A, Thiagalingam A, Denèfle P, Group tES. Characterizing Short Stature by Insulin-like Growth Factor Axis Status and Genetic Associations: Results From the Prospective, Cross-sectional, Epidemiogenetic EPIGROW Study. *Journal of Clinical Endocrinology & Metabolism*. 2013;98(6):E1122-E30.