Institution: University of Nottingham

Unit of Assessment: 5 - School of Life Sciences

Title of case study:
Development of Genetic Tests for Inherited Human Disorders

1. Summary of the impact

Research by Professor David Brook on inherited disorders has made a major contribution to the human genetics field. The work involved gene identification and mutation detection for genotype/phenotype correlation analysis in patients, which has led to the development of diagnostic tests for inherited conditions including myotonic dystrophy type 1 (DM1), Holt-Oram Syndrome (HOS), and campomelic dysplasia (CD). The tests have benefitted patients in the UK and throughout the rest of the world and in many cases they have been used as the definitive diagnostic measure. The assays developed have also been used in affected families for prenatal diagnosis to enable informed reproductive decisions.

2. Underpinning research

The main focus of research in Professor Brook's laboratory at the University of Nottingham over the past twenty years has been the genetics of inherited disorders, which represent a major health burden globally. Conditions investigated include myotonic dystrophy type 1(DM1), Holt-Oram syndrome (HOS) and campomelic dysplasia (CD). DM1 is caused by the expansion of an unstable CTG repeat sequence in the DMPK gene and the effect of the unstable CTG repeat on the DM1 phenotype was reported in 1993[1] by Brook and colleagues. In 1995 mutations in SOX9, an SRY related gene[2,3] were shown to be responsible for CD. Following a range of approaches and detailed analysis of patient samples, HOS was shown to be caused by mutations in TBX5[4] by the Brook lab in 1997. In recent years this work has been extended to include the identification and analysis of other genes that cause congenital heart disease.

The underpinning research involved positional cloning studies, cell biological and molecular genetic analysis of DNA and tissue samples from patients with HOS[5] and CD[3]. Following gene identification, extensive molecular genetic analysis of DM1 patient DNA samples provided a platform to understand some of the genotype/phenotype relationships in this complex disorder[6]. As a consequence diagnostic genetic tests and prenatal diagnoses can be conducted to produce relevant information, and optimise informed decision-making. The Brook laboratory generated key reagents in the form of oligonucleotide primer sequences enabling diagnostic tests for all three disorders and plasmids containing DNA fragments for Southern blot analysis of the DM1 mutation, which have been distributed from Nottingham to numerous laboratories around the world. Working closely with Dr Gareth Cross (Head of Molecular Genetics diagnostic laboratory, City Hospital, Nottingham), Brook established the diagnostic test for HOS in the NHS, which has been adopted worldwide. Assessment of patient samples was crucial to determine genotype/phenotype correlations in all three disorders[1,3,5].

The research was funded by a series of grants from the Medical Research Council, the Wellcome Trust, the British Heart Foundation, the Muscular Dystrophy Campaign and the Muscular Dystrophy Association, USA and independent fellowships (see section 3). The research has been conducted over the past twenty years by Brook and a team of more than 25 colleagues including postdoctoral researchers, experimental officers, technicians and PhD students. Work on DM1 and HOS continues in the Brook lab to this day.

Collaborators have been involved in most aspects of the projects. The DM1 project was conducted with Professor Peter Harper and colleagues at the Institute of Medical Genetics, Cardiff and Dr David Housman's laboratory at MIT, USA. The HOS project was mostly conducted in Nottingham with some interaction with Professor Arnold Munnich and Dr Damien Bonnet, Paris. The CD work was conducted in collaboration with Professor Peter Goodfellow, Cambridge and multiple clinicians have been involved in all three projects.

3. References to the research

Key Publications (UoN authors in bold, key author(s) underlined)

1. Harley H G, Rundle S A, Macmillan J, Myring J, **Brook J D,** Crow S, Reardon W, Fenton I, Shaw D J and Harper P S (1993). Size of the unstable CTG repeat sequence in relation to phenotype and parental transmission in myotonic dystrophy. Am J of Human Genet, 52, 1164-1174. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1682262/pdf/ajhg00064-0145.pdf
2. Foster J W, **Dominguez-Steglich M A**, Guioli S, Kwok C, Weller P A, Stevanovic M, Weissenbach J, **Mansour S, Young I D,** Goodfellow P N, **Brook J D** and Schafer A J (1994) Campomelic dysplasia and autosomal sex reversal caused by mutations in an *SRY*-related gene. Nature 372, 525-530. DOI:10.1038/372525a0
3. Kwok C, Weller P A, Guioli S, Foster J W, **Mansour S,** Zuffardi O, Punnett H H, **Dominguez-Steglich M A, Brook J D, Young I D,** Goodfellow P N and Schafer A J (1995). Mutations in SOX9 the gene responsible for campomelic dysplasia and autosomal sex reversal. Am J of Human Genet 57, 1028-1036. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1801368/pdf/ajhg00037-0044.pdf
4. **Li Q-Y, Newbury-Ecob R A, Terrett J**, Wilson D I, Curtis A R J, Yi C H, Gebuhr T, Bullen P J, Robson S C, Strachan T, Bonnet D, Lyonnet S, **Young I D, Raeburn J A,** Buckler A J, Law D J and **Brook J D** (1997) Holt-Oram syndrome is caused by mutations in *TBX5,* a member of the *Brachyury (T)* gene family. Nature Genetics 15, 21-29. DOI:10.1038/ng0197-21
5. **Cross S J, Ching Y-H, Li Q L, Armstrong-Buisseret L**, Spranger S, Munnich A, Bonnet D, Pentinnen M, Jonveaux P, Mortier G, van Ravenswaaij C, **Brook J D, and Newbury-Ecob R** (2000). The mutation spectrum in Holt-Oram syndrome. J of Med Genet 37, 785-787. DOI: 10.1136/jmg.37.10.785
6. **Hamshere M G,** Harley H, Harper P, **Brook J D** and **Brookfield J F Y** (1999). Myotonic dystrophy: The correlation of (CTG) repeat length in leukocytes with age at onset is significant only for patients with small expansions. J of Med Genet 36, 59-61 DOI: 10.1136/jmg.36.1.59

Research Funding (1993 to present)

Myotonic Dystrophy: Grants with total funding of £1,276,072 including:
J D Brook; Understanding the Molecular Basis of Myotonic Dystrophy.
Muscular Dystrophy Association USA $220,675 (1992-1995)
J D Brook and S Reddy; Assessing the contribution of multiple genes to the myotonic dystrophy phenotype. Muscular Dystrophy Association USA $200,000 (1998-2000)
M G Hamshere and **J D Brook**; Understanding the relationship between CTG expansion, nuclear retention, CUG binding proteins and alternative splicing in myotonic dystrophy.
Muscular Dystrophy Campaign £55,910 (1999-2002)
J D Brook, R K Patient and **M Gering**; The role of muscleblind and CUG binding proteins in myotonic dystrophy. Muscular Dystrophy Campaign £23,836 (2002-2004).
J D Brook; Screening for drugs to treat myotonic dystrophy.
Medical Research Council £160,000, (2009-2011).

Holt-Oram Syndrome and Congenital Heart Disease research total funding £3,307,982 including:
R A Newbury-Ecob and **J D Brook**; The Holt-Oram Syndrome; A clinical and molecular genetic study. British Heart Foundation £57,222 (1993-1995)
J D Brook and R A Newbury-Ecob; Identification and cloning of a gene for Holt Oram Syndrome.
British Heart Foundation £119,416 (1995-1998)
J D Brook and R A Newbury-Ecob; Molecular Genetic studies of Cardiac Development.
British Heart Foundation £653,689 (1997-2002)
J D Brook, F Bu'Lock and N Rutter; The molecular genetics of congenital heart disorders.
British Heart Foundation £1,079,803 (2003-2007).
J D Brook, F Bu'Lock, **S Loughna**, J Eason and **M Loose**; A gene regulatory network for the developing heart and congenital heart disease.
British Heart Foundation £1,095,435, (2008-2012)

Campomelic Dysplasia: A Human Frontiers Science Program fellowship to Marina Dominguez-Steglich with **J D Brook** as primary sponsor, supported part of the campomelic dysplasia work.

4. Details of the impact

Myotonic Dystrophy is the most common muscular dystrophy in adults affecting around 1/8000 individuals. It is also by far the most variable neuromuscular disorder in terms of severity, age at onset and different body systems affected. In addition to the health-related issues in DM1 there is a very high social cost of the condition. 50-70% of DM1 patients of working age, are likely to be unemployed. Figures produced by the Muscular Dystrophy Association USA indicate that the cost of DM to the US economy in terms of medical and non-medical expenses and lost income is roughly $450 million dollars per annum[A]. In comparison HOS and CD are both very rare developmental disorders, each affecting around 1/100,000 individuals, but both are associated with severe physical abnormality.

The major beneficiaries of the research conducted by Brook, are the affected individuals and families who can now be provided with very accurate genetic information and the NHS and global health care providers who supply this information. The pathway from research to impact for all three disorders is the same; original research resulted in gene identification[2,4], followed by detailed analysis of mutations in patient samples[1,3], publication in scientific literature of the genotype/phenotype relationships[5,6] with concomitant distribution of reagents and relevant knowledge. Publication of the work described has resulted in the availability of genetic tests and knowledge of genotype/phenotype relationships for DM1, HOS or CD, which did not previously exist. The effect of the availability of these tests is described below.

Impact 1 - DM1

The CTG repeat expansion test for DM1 established by Brook is the definitive test for the disease and to some extent disease severity and its benefits are:

- symptomatic and pre-symptomatic testing with near 100% accuracy;
- prediction of potential severity of disease phenotype;
- provision of choice and ability to inform with pre-natal and pre-implantation diagnosis;
- predictive testing of asymptomatic adults in families positive for the disease facilitating personal life decisions;

An indication of the broad impact of this work is revealed in figures published by four sources. The Clinical Molecular Genetics Society audit shows that 1196 DM1 tests were reported in the UK[B] for 08/09. A further 1334 tests were conducted in 09/10, 1225 in 10/11 and 1252 in 11/12. During the period 2008 to 2012, 88 pre-natal DM1 diagnostic genetic tests were conducted in the UK[B]. The UK Genetic Testing Network refers to 15 NHS laboratories that provide these tests, both pre- and post-natally at a cost of £65 - £460[C]. The Genetic Testing Registry (a publicly funded medical genetics information resource in the USA), and www.orpha.net (portal for rare diseases and orphan drugs)[C] describe 201 clinical laboratories providing the test in North America, South America, Europe, Australasia and Asia. Between 2008 and 2013, 3267 tests were carried out by 11 of these overseas (non-UK) laboratories[D].

Healthcare providers worldwide, including those in the UK, Australia, South Africa and the USA as well as multiple international charities provide an array of leaflets describing Brook's genetic test for a definitive diagnosis and severity of DM1[E]. At the individual level the impact of the test is highly significant, as illustrated on the Muscular Dystrophy Campaign web site. Here a patient describes diagnosis with DM leading to subsequent use of the test for pre-implantation genetic diagnosis screening for a disease-free embryo[F]. The co-ordinator of the Myotonic Dystrophy Support Group in the UK[F] and the Executive Director of the Myotonic Dystrophy Foundation in the USA[F] affirm that Brook's research and his development of a genetic test for the unstable CTG repeat has had a profound impact upon individuals with Myotonic Dystrophy[G]. With the aid of the test, these individuals are given both knowledge of their disease and choice.

Impact 2 - HOS

The TBX5/HOS mutation assay was developed in the Brook lab and transferred to the NHS molecular genetics laboratory at the Nottingham University Hospital Trust to provide a diagnostic service for this disorder. Between 2008 and 2012 79 post-natal and 2 pre-natal tests to confirm HOS were carried out in the UK[B]. More than 70 mutations in the TBX5 gene cause HOS and the Brook lab continue to find further TBX5 mutations (Granados-Riveron *et al*; 2012; doi: 10.1111/j.1747-0803.2011.00573.x) adding to the list of disease causing mutations and increasing

the sensitivity of HOS genetic testing. Based on work from the Brook lab HOS genetic testing is available worldwide. The UK Genetic Testing Network describes 2 NHS laboratories providing testing for HOS at a cost of £100 - £400[C]. The Genetic Testing Registry and www.orpha.net cite 44 laboratories in North America, Europe and Asia offering diagnostic tests for this condition[C]. Between 2008 and 2013, 140 tests were carried out by 5 of these laboratories[D].

The Co-Ordinator of the Holt-Oram Syndrome Support Group confirms that the availability of a genetic test has had considerable benefit to individual patients with HOS[G]. The test was first described in pre-implantation genetic diagnosis in 2004[H]. In 2012 deCODE genetics added TBX5 to their repertoire of tests for atrial fibrillation, demonstrating the test's importance not only for HOS diagnosis, but also for other conditions that feature congenital heart disorders[I].

Impact 3 - CD

In collaboration with others, the Brook lab discovery of mutations in SOX9 causing CD has greatly improved the ability to diagnose the disorder. The SOX9 test is the only genetic test for CD, the availability of which importantly provides influence on family planning due to the significant risk of newborn death from breathing problems due to small chest and lung size[J]. The Genetic Testing Registry and www.orpha.net cite 39 labs in Europe and North America offering CD diagnostic tests[C]. Three of these global labs have performed 159 SOX9 genetic tests since 2008[D]

Impact 4 – Growth of Genetics Service and Scientific Training

Researchers who trained in the Brook lab moved into positions in pharmaceutical companies: Terrett and Li with GSK and Ronksley with Pfizer, indicating that the lab provides highly skilled individuals for industry. Genetic tests are provided by both public sector and private organisations depending upon the healthcare system in various countries. Consequently, these and other tests require employment of a range of healthcare professionals involved in prenatal diagnosis and pre-symptomatic genetic testing. In the UK the increase in clinical genetics services reflects the development of genetic tests for inherited disorders. Thus, in 2004 there were 148 Consultant Clinical Geneticists employed by the NHS. By 2011 this had increased by 29% to 209[B]. The number of staff employed in NHS DNA service laboratories has also increased significantly in recent years, rising from 139 in 1993 to 434 in 2008 and further to 638 in 2012[B].

Thus, the research by Brook and colleagues has proved to be of substantial benefit to patients with DM1, HOS and CD, their families and the healthcare professionals who care for them.

5. Sources to corroborate the impact

A. http://mda.org/research/cost-of-illness
B. Letter and staff number information from the CMGS Audit Sub-committee Chair corroborating UK test numbers and numbers of staff employed in genetic testing.
C. Document listing web sites providing information on diagnostics testing laboratories.
D. E-mail communication regarding numbers of genetic tests carried out by different companies and health services.
E. Worldwide healthcare provider and charitable organisation leaflets and web links describing the availability of a genetic test for DM1
F. http://www.muscular-dystrophy.org/about_muscular_dystrophy/yourstories/lifestyle/2968_our_quest_for_a_baby
G. Corroborative statements from the Myotonic Dystrophy Support Group, Myotonic Dystrophy Foundation and Holt-Oram Syndrome Support Group.
H. He J, McDermott DA, Song Y, Gilbert F, Kligman I, Basson CT. (2004). Preimplantation genetic diagnosis of human congenital heart malformation and Holt-Oram syndrome. Am J Med Genet A. 126A(1):93-8. doi: 10.1002/ajmg.a.20487
I. Word document containing an article from the Heart.org
J. Document listing web links to information about CD and diagnosis with a genetic test

Corroborative documents and copies of webpages are held on file and are available on request.