Institution: University of Bristol

Unit of Assessment: 5 – Biological Sciences

Title of case study: Avoiding unnecessary treatment of Rhesus negative pregnant women and improving outcomes for patients with rare kidney disease

1. Summary of the impact

Research on clinically important red blood cell membrane proteins has helped avoid unnecessary treatment of Rhesus negative pregnant women and enabled the early diagnosis of a rare kidney disease. During the late 1990s, researchers at the University of Bristol, in collaboration with the Blood Service in Bristol, cloned, sequenced and characterised many red blood cell membrane proteins important for transfusion, including the Rhesus proteins and Band 3/AE1 (SLC4AE1 gene). The work on Rhesus proteins facilitated the use of less invasive genetic screening methods to ascertain whether treatment was required to avoid Haemolytic Disease of the Foetus or Newborn (HDFN). In the UK, 5,000 women have been screened since 2001. Within the first six months of implementation of a Danish national screening program in January 2010, 862 women avoided unnecessary treatment. Reducing unnecessary treatment of mothers has saved resources and avoided unnecessary exposure to human derived blood products. In addition, research that has identified specific SLC4AE1 gene mutations that cause the rare kidney disease called distal renal tubular acidosis has enabled the early diagnosis and treatment of the disease, resulting in improved outcomes for patients.

2. Underpinning research

Key researchers and contributions

The research that underpins this impact represents a significant programme of work that spans over 40 years. Researchers in the School of Biochemistry (Prof. Michael Tanner (retired 2004), Dr Kay Ridgwell (1981-1996), Dr William Mawby (1978-2012), Dr Neil Avent (1988-1996), Dr Lesley Bruce (1990-2003) and Dr Ashley Toye (2005-current)) have cloned, sequenced and characterised red blood cell membrane proteins and their interactions to better understand their biology in health and disease [1,2]. Bruce and Toye have identified novel disease gene mutations [3], characterised the structure and function of these red blood cell proteins, studied interactions between proteins, and ultimately helped to understand the molecular basis of the diseases associated with mutations in these protein genes [4-6].

The team at Bristol collaborate with NHS Blood and Transplant (NHSBT). A key collaborator is Prof. David Anstee who is a Principal Investigator for NHSBT and Director of the International Blood Group Laboratories (IGBRL) and Bristol Institute for Transfusion Sciences (BITS). IBGRL is headed by Dr Geoff Daniels (Molecular Diagnostics Manager) and is internationally recognised for its work with complex blood grouping problems and is a designated collaborating centre for the World Health Organisation. This work is, by nature, collaborative with clinicians working from symptoms to understand the root cause of disease, while experts at the University of Bristol work from a fundamental understanding of proteins and how they function within the body in order to understand the molecular basis of disease. IBGRL refers undiagnosed patients with suspected red blood cell protein mutations to the University of Bristol research group. In addition, due to the renowned expertise of the Bristol group, external clinicians also refer interesting or complex haematology or kidney cases. The outputs of the research are generally co-authored with the clinicians as well as colleagues at NHSBT who have expertise in rare blood groups and clinical diagnoses.

Determining Rhesus blood group from DNA

In 1996, Tanner and colleagues examined the Rhesus (Rh) proteins in the membrane of human red blood cells and showed how they were organised at the surface of the red blood cell [1]. This knowledge directly fed into research on Haemolytic Disease of the Foetus and New Born (HDFN). Normally the majority of the population have two copies of the Rh30 gene called RhCE and RhD but around 15% of the population lack the RhD gene and so are described as Rh negative. When mothers who do not express the RhD protein (Rh negative) are exposed to the blood of their Rh positive foetus, they will develop an immune response to the Rh antigen. Any subsequent pregnancies with an Rh positive foetus are then at risk of HDFN as the mother's primed immune system will launch an attack on the red blood cells of the foetus, viewing the Rh antigen as a foreign body and potentially resulting in death of the baby. Research on this important group of

blood proteins, conducted by Tanner and his colleagues, meant that the RhD blood group of the foetus could be determined from foetal DNA, which can be found in the mother's blood using Polymerase Chain Reaction (PCR), thereby avoiding invasive procedures such as amniocentesis.

Linking Rhesus proteins with the band 3 complex

More recently, the Tanner group has studied a range of patient samples to understand how certain genetic mutations result in haemolytic anaemia and how this impacts on the structure of the red blood cell membrane. The culmination of these studies demonstrated that the Rhesus proteins complex is associated with the band 3 protein complex in healthy red blood cells [2]. This observation explained why certain genetic mutations impacted on what were sometimes thought to be unrelated genes to influence blood group expression.

Identifying Band 3/AE1 mutations

In addition to being highly expressed on red blood cells, a truncated version of band 3 is expressed in the kidney (expressed from the same gene via a kidney specific promoter). In 1997, the Bristol researchers showed that a number of specific mutations in the band 3/AE1 gene, which is a bicarbonate chloride transporter involved in cellular gas transport in red blood cells and acid/base homeostasis in the kidney, result in distal renal tubular acidosis (dRTA). dRTA is a rare human disease characterised by failure of specialised cells in the kidneys to secrete acid, which leads to calcium deposits in the kidneys, low blood potassium levels and metabolic bone disease. Eventually this disease can cause death due to kidney failure if left undiagnosed, but can easily be treated with bicarbonate if diagnosed. To date only a small number of patients, of European origin, are known to be affected (approx. 20 families are known) with the dominant form of the disease, but a recessive form of the disease is more prevalent in South East Asia.

The Bristol group has worked extensively on this disease and has made the following contributions to the existing body of knowledge:

- In 1997, they were the first to indentify the molecular basis of this disease [3]. Mutations in the band 3/AE1 gene cause all dominantly inherited forms of dRTA known to date, and development of the disease is inevitable in individuals with these mutations.
- In 2000, they disproved the idea that there was no recessive form of the disease when they described novel AE1 gene mutations found in families in Asia that did cause recessive dRTA [4]. Their results suggested that dRTA might arise by a different mechanism for each of these novel mutations.
- They were also the first to show that AE1 mutations that cause hereditary spherocytosis or Southeast Asian Ovalocytosis in red blood cells, generally do not cause dRTA, unless present in homozygous or compound heterozygotes [4].
- In 2002, they used a novel kidney cell model to show for the first time that the dominant mutations caused protein mis-trafficking [5] and this work was later expanded to a polarized cell model system (Toye et al., *J Cell Science*).
- In 2008, Toye and his colleagues provided the second description of an individual homozygous for AE1 gene mutation with both haemolytic anaemia and dRTA [6]. This showed that certain AE1 gene mutations can result in both types of disease in one individual. This work also confirmed that the band 3 protein is in complex with the Rhesus protein complex in human erythrocytes.

3. References to the research

[1] Avent, N.D., Liu, W., Warner, K.M., *et al*. (1996) 'Immunochemical analysis of the human erythrocyte Rh polypeptides', *The Journal of Biological Chemistry,* 271 (24): 14233-9. DOI:10.1074/jbc.271.24.14233 (55 citations*)

[2] Bruce, L.J., Beckmann, R.,Ribeiro, M.L., *et al*. (2003) 'A band 3-based macrocomplex of integral and peripheral proteins in the RBC membrane', *Blood*, 101 (10): 4180-8. DOI: 10.1182/blood-2002-09-2824 (233 citations)

[3] Bruce, L.J., Cope, D.L., Jones, G.K., *et al.* (1997) 'Familial distal renal tubular acidosis is associated with mutations in the red cell anion exchanger (Band 3, AE1) gene', *The Journal of Clinical Investigation,* 100 (7): 1693-707. DOI: 10.1172/JCI119694 (246 citations)

[4] Bruce, L.J., Wrong, O., Toye, A.M., *et al.* (2000) 'Band 3 mutations, renal tubular acidosis and

South-East Asian ovalocytosis in Malaysia and Papua New Guinea: loss of up to 95% band 3 transport in red cells', *Biochemical Journal,* 350 Pt 1: 41-51. <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1221222/pdf/10926824.pdf> (139 citations)

[5] Toye, A.M., Bruce, L.J., Unwin, R.J., *et al*. (2002) 'Band 3 Walton, a C-terminal deletion associated with distal renal tubular acidosis, is expressed in the red cell membrane but retained internally in kidney cells', *Blood,* 99(1): 342-7. DOI: 10.1182/blood.V99.1.342 (81 citations)

[6] Toye, A.M., Williamson, R.C., Khanfar, M., *et al.* (2008) 'Band 3 Courcouronnes (Ser667Phe): a trafficking mutant differentially rescued by wild-type band 3 and glycophorin A', *Blood*, 111(11): 5380-9. DOI: 10.1182/blood-2007-07-099473 (29 citations)

*all citation rates are from Google Scholar as of 4th September, 2013.

4. Details of the impact

Research on clinically important red blood cell membrane proteins has helped avoid unnecessary treatment of Rhesus negative pregnant women and enabled the early diagnosis of a rare kidney disease.

Research on Rhesus proteins reduces unnecessary treatment of Rh negative mothers

The research conducted at Bristol [1] enabled the Rh blood group of a foetus to be determined from foetal DNA in the mother's blood. This facilitated non-invasive screening techniques to avoid the unnecessary treatment of mothers carrying an Rh negative foetus [a]. This PCR Rhesus genotyping technique was developed in Bristol by Prof. Neil Avent (currently at Plymouth University), alongside Dr Geoff Daniels and colleagues from NHSBT and IGBRL. Traditionally, Rh negative mothers, who have not previously been sensitised to RhD antigen, are treated with a routine antenatal anti-D prophylaxis (RAADP). However, anti-D treatment is only necessary in cases where the foetus is RhD positive, which means that about 40% of Rh negative mothers receive anti-D unnecessarily [b]. In England and Wales, this translates to just over £1.3 million in unnecessary spending based on the estimated annual cost for antenatal treatment of £3.37 million [c]. Denmark and the Netherlands both implemented a national screening program, in January 2010 and July 2011 respectively, which uses the foetal DNA to determine the Rh blood group early in pregnancy. Within the first six months of implementation in Denmark, the unnecessary treatment of RhD negative women was avoided in 862 (37.3%) pregnancies [d]. Results are not yet available for The Netherlands. Nationwide screening has not been introduced in the UK, but the service of predicting foetal Rh D blood group was introduced in May 2001 [e]. Since its introduction, the International Blood Group Reference Laboratory (IBGRL) has tested 5,000 UK women [a]. Though the number of women carrying Rh negative foetuses is unavailable, there has been a steady reduction in referrals to IBGRL for foetal D blood grouping using invasively derived foetal material, such as amniocentesis [b].

The cost savings estimate of avoiding unnecessary treatment in pregnant women are incredibly complex as it is dependent on many factors including the sensitivity of the screening, royalty fees, cost of the anti-RhD treatments, and whether one or two anti-RhD injections were avoided. Estimates have ranged from being cost neutral to annual savings of more than £500K annually [c]. Other benefits, however, include: i) a reduction in the number of invasive procedures, such as amniocentesis, which carries a risk of miscarriage, ii) reduced risk of infection (e.g. hepatitis C) associated with anti-RhD as it is a blood product, and iii) reduced use of anti-RhD with the ethical benefit of reducing the need to hyperimmunise male volunteers to provide source serum for its production [c].

Identification of mutations in the band 3/AE1 gene improves diagnosis and clinical treatment of patients with rare kidney disease

Bristol's identification of mutations in the band 3/AE1 gene and their understanding of the molecular basis for dRTA [3-6] has been of paramount importance to clinicians as it provides them with a specific diagnosis and treatment plan for their patients. Once diagnosed this disease can be easily and cheaply treated using oral bicarbonate. The Bristol-based research has also led to the adoption of new diagnostic tests for suspected dRTA patients. A nationally approved test became

available in June 2011, which screens for common AE1 mutation sites and full genetic analysis is now provided for NHS clinicians to confirm or identify specific AE1 gene mutations [f].

Furthermore, there are individuals studied by the Bristol team [5-6], who have had their clinical treatment regimen altered as a result of the research findings, leading to improved outcomes for these patients. French clinicians studying rare haemolytic anaemias were drawn to the deleterious effects of a homozygous mutation in the anion transporter, identified by Toye [6]. This enabled them to make an early diagnosis of dRTA, which has a worse prognosis than the original haemolytic anaemia being investigated [g]. The patient was treated with an alkaline diet as a result. In another case, a heterozygous mutation was discovered in the glucose transporter-1, which led clinicians to suggest the use of a ketogenic diet to prevent the neurological disorders that accompany the haematological manifestations [g].

In another case, Dr Toye facilitated the diagnostic work up on a family with dRTA who were tested for mutations in the AE1 gene and this was later confirmed by genetic analysis at Addenbrooke's Hospital. The younger siblings confirmed to have the R589H gene mutation were treated with bicarbonate supplementation [h]. Toye's novel research on glomerular expression of AE1, also motivated testing of the affected members of this family for proteinuria, and these patients are being followed closely to determine whether there are additional affects on the kidneys that have not yet been realised [h].

5. Sources to corroborate the impact

[a] Senior Research Fellow and Molecular Diagnostics Manager, IBGRL.

[b] Finning, K., Martin, P. & Daniels, G. (2004) 'A clinical service in the UK to predict fetal Rh (Rhesus) D blood group using free fetal DNA in maternal plasma', *Annals of the New York Academy of Sciences,* 1022:119-23. DOI: 10.1196/annals.1318.019. Statistics for number of Rhesus negative mothers receiving unnecessary treatment in the UK.

[c] Szczepura, A., Osipenko, L., and Freeman, K. (2011) 'A new fetal RHD genotyping test: Costs and benefits of mass testing to target antenatal anti-D prophylaxis in England and Wales, *BMC Pregnancy & Childbirth,* 11:5. <http://www.biomedcentral.com/1471-2393/11/5>. Provides financial estimates for antenatal treatment of RhD negative women.

[d] Clausen, F.B., Christiansen, M., Steffensen, R., *et al.* (2012) 'Report of the first nationally implemented clinical routine screening for fetal RHD in D- pregnant women to ascertain the requirement for antenatal RhD prophylaxis', *Transfusion,* 52 (4):752-758. DOI: 10.1111/j.1537-2995.2011.03362.x. Provides statistics for the number of RhD negative women prevented from having unnecessary treatment due to national screening efforts in Denmark.

[e] IBGRL (2012) *IBGRL Blood Group Genotyping Service.* URL: http://ibgrl.blood.co.uk/ReferenceServices/Genotyping/Genotyping.htm [Accessed online 13Jun2013]. Evidence of date of introduction of the fetal genotyping test at IBGRL.

[f] Addenbrooke's Hospital (June 3 2011) *New tests available: Recessive distal renal tubular acidoses,* Cambridge University Hospitals NHS Foundation Trust [Latest News]. <http://www.cuh.org.uk/addenbrookes/services/clinical/genetics/genetics_labs/news/new_tests.html>. Evidence of introduction of new test to screen for common AE1 mutations.

[g] Clinician, Institut National de la Santé et de la Recherche.

[h] Professor and Honorary Consultant Paediatric Nephrologist, University of Bristol.