Institution: University of Leeds

Unit of Assessment: UoA4

Title of case study: Case Study 5: Improving screening, diagnosis and treatment of inherited blindness and deafness

1. Summary of the impact

Researchers at the University of Leeds (UoL) have identified mutations in key genes which are major causes of deafness and blindness. Mutations in *GJB2*, identified in a Leeds/London collaboration, are the most common cause of human inherited deafness, affecting millions worldwide, and Leeds researchers have also highlighted 13 key genes involved in inherited blindness, accounting for an estimated 5% of around 2 million people throughout the world with inherited eye diseases. This work has led to the availability of vital genetic testing, enabling early diagnosis, better management and improving outcomes for patients, as well as better counselling and prenatal screening for families.

2. Underpinning research

Researchers at the UoL use family studies to track down mutations causing human inherited diseases. Families of all ethnicities and inheritance types are studied. In particular high rates of consanguineous marriage in the West Yorkshire Pakistani community have created a local healthcare challenge in the form of increased risk of recessively inherited diseases. Such families are amenable to autozygosity mapping, a technique piloted and developed by UoL geneticists using a pipeline of in-house software. To date research in Leeds has identified more than 40 genes mutated in different inherited diseases, many of them tracked down by autozygosity mapping (www.autozygosity.org).

The first success for autozygosity mapping in Leeds was in non-syndromic deafness, through the Leeds Deafness Research Group set up by Professor Mueller (Consultant Clinical Geneticist at the Yorkshire Regional Genetics Service (YRGS), 1990-present, Professor 1993 until retirement through ill health in 2001, UoL) **[i-ii]** and Dr. Lench (Senior Lecturer, 1994-99, UoL). By pooling genetic data from families with collaborators at St Bartholomew's, London, they showed that mutations in the *GJB2* gene cause autosomal recessive deafness **[1]** (873 cites). Over the next four years, the Leeds group, working with international collaborators, characterised the two most common mutations found and defined the spectrum of *GJB2* mutations in patients with inherited deafness **[2]** (178 cites). Their work showed that *GJB2* mutations account for around 60% of all inherited prelingual non-syndromic neurosensory deafness.

Inspired by this, Professor **Inglehearn** (Senior Wellcome Fellow 1997-2002; Professor of Molecular Ophthalmology 2001-present, UoL) and Mr **McKibbin** (Consultant Ophthalmologist at St. James University Hospital, 2001-present; Honorary Senior Lecturer/Associate Professor, 2001-present, UoL) established the Leeds Vision Research Group **[iii-iv]**. They have used similar techniques in collaborative research, much of it led from Leeds, to identify 13 proteins (RP1, PRPF8, PRPF3, PAP1/RP9, ADAM9, CNNM4, LTBP2, LCA5/lebercilin, ATOH7, LRP5, TSPAN12, SLC4A11 and PXDN) mutated in eye diseases.

An early key Leeds finding was mutations in pre-mRNA splicing factors, including PRPF8 **[3]** (133 cites), PRPF3 and PAP1/RP9, in dominant retinitis pigmentosa. These were the first three of a series of spliceosome components implicated in inherited blindness, highlighting a new disease pathway.

Another mechanism uncovered by Leeds in collaboration with others is the finding that retinal degeneration can result from cilia defects. As co-leaders of an international consortium **Inglehearn** and colleagues identified mutations in lebercilin causing a rare inherited eye disease, Leber congenital amaurosis **[4]** (70 cites), with Leeds making the initial discovery. **Inglehearn** also supplied genetic data and a key genetically-linked family, contributing substantially to the identification of mutations in RP1 as a cause of dominant retinitis pigmentosa **[5]** (114 cites). Both

proteins are cilia components.

The Leeds Vision Research Group leads international research into retinal vascular diseases and has identified mutations in LRP5 **[6]** (147 cites) and TSPAN12 as common causes of familial exudative vitreoretinopathy (FEVR) – a condition which mimics retinopathy of prematurity seen in premature babies. These and other FEVR genes are components of the Norrin Beta-catenin signalling pathway, showing that this pathway regulates retinal blood vessel formation.

3. References to the research

[1] Kelsell, D.P., … Lench*, N.J., … Mueller*, R.F., & Leigh, I.M. (1997). Connexin 26 mutations in hereditary non-syndromic sensorineural deafness. *Nature, 387*, 80-83. doi: 10.1038/387080a0
The first report of mutations in connexin26 (GJB2) causing prelingual deafness in both dominant and recessive families.
[2] Scott, D., Kraft, M., … Markham*, A.F., Mueller*, R.F., Lench*, N.J., … Smith, R.J., & Sheffield, V.C. (1998). Identification of mutations in the connexin 26 gene that cause autosomal recessive nonsyndromic hearing loss. *Human Mutation, 11*, 387-94. doi: 10.1002/(SICI)1098-1004(1998)11:5
Research showing the full spectrum of mutations in this gene and high frequency of these mutations as a cause of inherited deafness.
[3] McKie*, A.B., McHale*, J.C., Keen*, T.J., Tarttelin*, E.E., … A.C., Markham*, A.F. & **Inglehearn*, C.F**. (2001). Mutations in the pre-mRNA splicing factor gene PRPF8 cause autosomal dominant retinitis pigmentosa (RP13). *Human Molecuar Genetics, 10*, 1555-62. doi: 10.1093/hmg/ 10.15.1555
The finding of heterozygous mutations in the first of a series of mRNA splicing factors essential to every cell, yet which cause only inherited blindness.
[4] den Hollander, A., … Mohamed*, M.D., Arts, H.*, … Towns*, K., … **McKibbin*, M.,** … Ivings*, L., Williams*, G.A., Springell*, K., **Woods*, C.G.,** Jafri*, H., … **Inglehearn*, C.F.**, & Roepman, R. (2007). Mutations in LCA5, encoding the ciliary protein lebercilin, cause Leber congenital amaurosis. *Nature Genetics, 39,* 889-95. doi: 10.1038/ng2066
Research identifying mutations in an as yet uncharacterised protein causing inherited blindness plus evidence that it is a component of the primary cilium.
[5] Sullivan, L.S., … Hide, W.A., Gal, A., Denton, M., **Inglehearn*, C.F.**, Blanton, S., & Daiger, S.P. (1999). Mutations in a novel retina-specific gene cause autosomal dominant retinitis pigmentosa. *Nature Genetics, 22*, 255-59. doi: 10.1038/10314
Study describing mutations in an as yet uncharacterised protein, plus evidence that this is a component of the primary cilium.
[6] Toomes*, C., Bottomley*, H., … Bruffell*, K., Scott*, S., … Markham*, A. F., Downey*, L., & **Inglehearn*, C.F.** (2004). Mutations in *LRP5* or *FZD4* underlie the common FEVR locus on chromosome 11q13. *American Journal of Human Genetics, 74,* 721-30. doi:10.1086/383202
Paper showing that defects in LRP5, as well as causing osteoporosis/pseudoglioma syndrome, also cause potentially blinding retinal vascular defect FEVR.

Key Funding and Grants

[i] European Union Collaborative Award. (2000-2002). Human Hereditary Deafness. Mueller*, R.F. €245,774.

[ii] Royal National Institute for the Deaf. (2000-2003). Causes of Progressive age-related hearing loss. Co-PI- Mueller*, R.F. £110,422.

[iii] Wellcome Trust Senior Fellowship. (1997-2002). The identification and characterisation of genes involved in autosomal dominant retinitis pigmentosa. **Inglehearn*, C.F.** £963,917.

[iv] Sir Jules Thorn Trust award. (2010-1014). Identification of recessive disease genes in consanguineous families. Co-PI: **Inglehearn*, C.F.** £1.21m.

Note: All UoA4 researchers in **bold**; *research conducted by academics at the UoL.

4. Details of the impact

Identification of previously unknown gene mutations in Leeds has had a major impact on diagnosis,

management and screening for affected families. Findings have also opened the way for better understanding of the causes of inherited blindness and deafness and potential treatments.

Impact on health and welfare: Inherited deafness

Approximately seven million people worldwide have inherited deafness, and of these around three million have deafness due to *GJB2* mutations. Prior to the discovery that *GJB2* mutations cause inherited deafness, it was impossible to carry out genetic testing in patients. Our work alongside international collaborators has enabled clinical genetics laboratories to provide testing and unequivocal genetic counselling for deaf individuals around the world during the period 2008-13.

As well as publishing, Professor Mueller worked with YRGS colleagues to develop a diagnostic service screening for *GJB2* mutations. There has been high demand for this locally due to the frequency of *GJB2* mutations within the local Pakistani population. Furthermore, in 2002 they submitted details of the reagents and protocols used in screening GJB2 to the UK Genetic Testing Network portfolio. Other laboratories around the world used the published information to set up their own *GJB2* screening services. In all, 353 laboratories offering molecular genetics testing are listed on the Orphanet web site, which is primarily European, while the Genetests website lists over 600 such laboratories, with a greater emphasis on laboratories from the US and elsewhere (data confirmed 19/9/13). There is little overlap, with laboratories tending to list on one site or the other, and neither list is comprehensive. It therefore seems likely that over 1000 laboratories around the world offer genetic testing. Of these, 268 offer a screen for *GJB2* **[A]**. We surveyed 15 of these, including 4 UK, 2 US, 10 European and 1 Israeli laboratories, which stated that they carry out a total of around 1340 *GJB2* tests per year. Using figures based largely on 2012 **[B]** we infer that as many as 24,000 GJB2 screens are carried out internationally per year **[B]**.

For patients with genetic disease, a delay in diagnosis is one of the principal barriers to appropriate care **[C]**. The availability of genetic testing significantly improves outcomes for deafness, one of the most common abnormalities present at birth. A child with undetected hearing loss is at risk of failing to develop normal speech and language and acquiring the cognitive abilities needed to access education **[D]**. Presymptomatic identification of children with inherited hearing loss, only possible through genetic testing in high-risk families, permits the fitting of hearing aids at a very early age, which significantly improves outcome **[E]**. It also facilitates the learning of speech skills and training in lip reading and/or British Sign language.

Impact on health and welfare: Retinal dystrophy

Around two million people are affected by retinal dystrophy worldwide and the 13 genes identified in Leeds over the period 1999 to 2013, which include RP1 (1999), PRPF8 (2001), LRP5 (2004) and LCA5 (2007), may account for around 5%. With partial gene lists it is difficult to determine the cause in such a heterogeneous disease, so finding new genes not only benefits patients with mutations in these genes but also makes it easier to interpret results in all patients, including those with mutations in other known retinal dystrophy genes. Our work, done in international collaboration, has led to vital genetic tests enabling diagnosis and appropriate counselling. We worked directly with YRGS to develop a screening service, providing primers, protocols and expertise. In 2008 we also submitted a gene dossier of diagnostic information on LRP5 mutations to the UK Gene Testing Network (the document can be retrieved from http://ukgtn.nhs.uk/uploads/tx_ukgtn/EVR_FZD4_LRP5_NDP_GD_Oct_09.pdf) to facilitate the development of genetic testing for FEVR throughout the UK. The Orphanet and Genetests websites list 45 laboratories worldwide that test for retinal dystrophies **[A]**. We contacted these and 6 responded, including 2 UK and 4 European, stating that they do 2995 tests per year for PRPF8 (659), RP1 (1329), LRP5 (360) and LCA5 (647) **[B]**. These figures imply that perhaps as many as 22,000 screens for these genes were done in 2012 internationally **[B]**.

Qualitative research with retinal dystrophy patient groups by **McKibbin** indicates that families place a high value on knowing the cause of their condition and regard this information as potentially life-

changing **[F]**. Genetic testing currently identifies mutation(s) in 50-70% of patients, including ~5% with mutations in the genes identified in Leeds. Knowing the exact gene and mutation allows clinicians to give their patients a clear prognosis, including likely progression and complications and clarifying mode of inheritance.

In addition, in a growing number of cases, treatment can be improved in light of the genetic diagnosis. Some retinal dystrophies are treatable with dietary or other interventions. Diagnosis of a ciliopathy **[4, 5]** points to a need to assess kidney function **[G]**; an FEVR diagnosis **[6]** has implications for risk of osteoporosis and indicates bone density scanning **[H]**. Genetic diagnosis can also lead to gene or mutation-specific therapies. There are now 16 different therapies for inherited blindness undergoing clinical trials around the world **[I]**.

Importance for families

The complex nature of genetic disease means that families and clinicians place a premium on simple diagnostic tests **[J]**. Couples planning a family can be given accurate recurrence risks, and unaffected members can request confidential testing of genetic status. Genetic counselling is of particular relevance in the Pakistani community, where arranged marriage to a relative is the norm, increasing risk of recessive disease. Raised awareness of the consequences of consanguinity may lead to changes in reproductive practise and thus reduce incidence of these conditions. The Leeds Genetics grouping held quarterly meetings in Bradford 2010-12 with patients, parents and families, patient advocacy groups and interested healthcare professionals to discuss these issues.

5. Sources to corroborate the impact

[A] Orphanet. A database of genetic tests provided by European laboratories. http://www.orpha.net/consor/cgi-bin/ClinicalLabs.php?lng=EN. Genetests. A partial list of additional genetic screening services throughout the world: http://www.genetests.org

[B] Postal survey of all laboratories offering screening for *GJB2* mutations listed on the Genetests and Orphanet Websites together with individual responses. Includes service review documents from the Head of YRGS Laboratory, on numbers tested in Yorkshire

[C] Rare Disease UK. (2010). Experiences of rare diseases: An insight from patients and families (pp. 8, 9). Retrieved from http://www.raredisease.org.uk/documents/RDUK-Family-Report.pdf

[D] Public Health England's NHS Newborn Hearing Screening Programme. Retrieved from http://hearing.screening.nhs.uk/nationalprog

[E] Anderson, I., Weichbold, V., D'Haese, P.S., Szuchnik, J., Quevedo, M.S., … Dieler, W.S., & Phillips, L. (2004). Cochlear implantation in children under the age of two-what do the outcomes show us? *International Journal of Pediatric Otorhinolaryngology, 68*, 425-31. doi: 10.1016/j.ijporl.2003.11.013

[F] Bong, C., Potrata, B., Hewison, J., & **McKibbin, M.** (2010). Attitudes of patients and relatives/carers towards genetic testing for inherited retinal disease. *Eye, 24*, 1622-25. doi: 10.1038/eye.2010.91

[G] Waters, A.M., & Beales, P.L. (2011). Ciliopathies: an expanding disease spectrum. *Pediatric Nephrology, 26*, 1039–56. doi: 10.1007/s00467-010-1731-7

[H] Qin, M., Hayashi, H., Oshima, K., Tahira, T., Hayashi, K., & Kondo, H. (2005). Complexity of the genotype-phenotype correlation in familial exudative vitreoretinopathy with mutations in the LRP5 and/or FZD4 genes. *Human Mutation, 26,* 104-12. doi: 10.1002/humu.20191

[I] Boye, S.E., Boye, S.L., Lewin, A.S., & Hauswirth, W.W. (2013). A comprehensive review of retinal gene therapy. *Molecular Therapy, 21*, 509-19. doi: 10.1038/mt.2012.280

[J] Testimonials are available on the value of Leeds' gene discovery research to patient groups, Clinicians and NHS Genetics laboratories (24.6.13- 8.11.13).