Institution: Queen Mary University of London (QMUL)

Unit of Assessment: A1 (Clinical Medicine)

Title of case study: Monogenetic diseases

1. Summary of the impact

Research at the Centre for Cutaneous Research at Queen Mary has led to gene discovery and molecular diagnosis for a number of single gene skin disorders and associated syndromes including hearing loss, inflammatory bowel disease, cardiomyopathy and oesophageal cancer. It has identified *GJB2* mutations (encoding Cx26) as major cause of genetic hearing loss (20-50% of all cases) and *ABCA12* mutations with the (often fatal) recessive skin condition Harlequin Ichthyosis. Impacts include: 1) increased medical and scientific awareness/knowledge of the inherited basis of these conditions, 2) changes in clinical practice and molecular diagnosis, 3) improved information for patients, parents and the public.

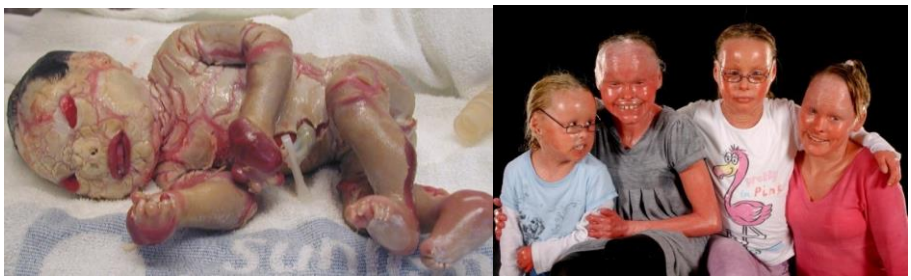
2. Underpinning research

Basic genetic research in the Centre for Cutaneous Research at Queen Mary includes:

GJB2 and hearing loss (discovery 1996-1997). In 1997, the team hypothesised that hearing loss in families with autosomal dominant skin disease may be due to an unidentified gene mapping in a locus for recessive non-syndromic hearing loss on chromosome 13 (termed DFNB1). Kelsell *et al* sequenced *GJB2* encoding Connexin26 and identified mutations linked to hearing loss in this family. They then sequenced recessive non-syndromic hearing loss families and identified further *GJB2* mutations. This group (including Professor Irene Leigh) were the first to link *GJB2* (encoding Cx26) mutations with hearing loss. The paper, published in *Nature* in 1997 and cited by over 1,000 publications since, described the first autosomal non-syndromic deafness gene ever identified [1]. The implication of this finding is that *GJB2* mutations account for between 20 - 50% of all genetic hearing loss in many distinct geographic and ethnic populations. Cochlear implants in children with *GJB2* hearing loss lead to major clinical improvement in hearing status, particularly if done in the early years of life (eg PMID: 11977173).

The linking of *GJB2* mutation and hearing loss led to further studies by the Kelsell group including *GJB2* mutation screening, the development of molecular assays for rapid mutation screening and tissue- / cell-based functional assays for specific mutations. Dominant mutations in *GJB2* and other connexin encoding genes including Cx31 were also shown to underlie cutaneous conditions with and without hearing loss [2]. In addition, the team provided evidence that the high carrier frequency of recessive *GJB2* mutation may be linked to heterozygous advantage, conferring protection from pathogen-borne disease. Functional studies are revealing new roles for loss of functional Cx26 leading to improved epithelial barriers and impaired bacterial entry into epithelial cell, thus another potential area of medical benefit.

Harlequin Ichthyosis and ABCA12 (discovery 2005-2006).



Harlequin Ichthyosis (HI) is the most severe form of congenital ichthyosis. Appearance at birth is usually characteristic (left panel above). The whole body is encased in an armour of thick white plates of scale separated by deep red fissures. The upper and lower eyelids are usually retracted, causing bilateral ectropion; the lips are parted, causing eclabium. The nose is flattened and appears rudimentary. Babies who survive infancy and into adulthood develop skin changes

resembling a severe congenital ichthyosiform erythroderma (right panel).

By 2005, the Kelsell Group at Queen Mary (including Professor Edel O'Toole) had identified and recruited a number of families worldwide with apparent recessive HI. Using the facilities at Barts and The London Genome Centre, they implemented a high density Single Nucleotide Polymorphism (SNP) array / homozygosity approach to map the condition to a region on chromosome 2q35 [3]. Positional candidate gene sequence analysis led them to be the first group to identify ABCA12 mutations as the cause of this disease. They and others screened HI families with identifiable biallelic ABCA12 mutations, which account for over 98% of cases. Kelsell's centre is now a global referral centre for the genetic diagnosis of HI; they have now identified ABCA12 mutations in over 130 families [4-6]. Furthermore, a functional programme of work has been established with the development of HI keratinocyte cell lines and in vitro 3D human HI skin models to dissect the cellular pathways disrupted by loss of ABCA12 and the development of assays which may lead to new therapies.

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3. References to the research

1. **Kelsell DP**, Dunlop J, Stevens HP et al. Connexin 26 mutations in hereditary non-syndromic sensorineural deafness. *Nature* 1997; 387, 80-83. PMID: 9139825
2. Scott CA, **Kelsell DP.** Key functions for gap junctions in skin and hearing. Biochemical Journal 2011; 438: 245-54. *PMID*: 21834795.
3. **Kelsell DP**, Norgett EE, Unsworth H et al. Mutations in ABCA12 underlie the severe congenital skin disease Harlequin Ichthyosis. *American Journal of Human Genetics* 2005; 76: 794-803. PMID: 15756637.
4. Thomas AC, et al and **Kelsell DP**. Novel and recurring ABCA12 mutations associated with Harlequin Ichthyosis: implications for prenatal diagnosis. *British Journal of Dermatology* 2008; 158: 611-3. PMID: 17986308.
5. Scott CA et al and **Kelsell DP**. Targeted sequence capture and high-throughput sequencing in the molecular diagnosis of ichthyosis and other skin diseases. *Journal of Investigative Dermatology* 2012; 133: 573-6. PMID: 22992804.
6. Rajpopat S, et al and **Kelsell DP**, O'Toole EA. Harlequin ichthyosis: a review of clinical and molecular findings in 45 cases. *Archives of Dermatology* 2011; 147: 681-6. PMID: 21339420.

4. Details of the impact

Improved clinical practice, diagnostic accuracy and public awareness in relation to GJB2 and hearing loss

Since *GJB2* accounts for up to 50% of genetic hearing loss, most clinical genetic services and commercial testing companies now offer *GJB2* testing, including 23andMe, University of Chicago Genetic Services, University of Iowa, ARUP Laboratories, Greenwood Genetic Centre, Knight Diagnostic Centre, Harvard Medical School, Athean Diagnostics and Centogene. In addition, bloodspot-based genetic testing for *GJB2* alleles can provide a means for rapid confirmation in the subset of infants who fail bedside newborn hearing tests [7]. In a US study in 2000, eight participating laboratories provided *GJB2* testing to approximately 230 families per month [8]. These studies have led to a number of additional impacts:

1. Improved medical and scientific awareness and knowledge of *GJB2* mutations as a major underlying cause of congenital hearing loss [9].
2. Changes in clinical practice and molecular diagnosis:
   a. Early diagnosis allows for early intervention such as cochlear implants in patients with *GJB2* associated hearing loss. In the UK for example, 1,650 *GJB2* tests were performed in

2010-2011 according to the Clinical Molecular Genetics Society Audit [10]. Delayed diagnosis of hearing loss in infants may have a harmful effect on social, emotional, cognitive, and academic development. A genetic diagnosis provides key information for genetic counseling, including prognosis and recurrence risk. For example, not all GJB2-associated hearing loss presents at birth [11].

b. A positive diagnosis helps rule out other diseases, including Ushers Syndrome.

3. Genetic testing as described above is now recommended as the gold standard by the European Molecular Genetics Quality Network, and laboratories that follow this guidance can seek a formal certificate of quality [12].
4. Improved clinical and genetics information on *GJB2*-associated hearing loss for patients with hearing loss and their families via websites, leaflets and similar material [13].

Improved diagnosis, management, prenatal diagnosis and awareness of Harlequin ichthyosis

Kelsell *et al*'s 2005 discovery of recessive ABCA12 mutations as the major cause of this devastating and often life threatening skin condition has led to a number of impacts:

1. Molecular diagnosis. The team has developed into a major global referral centre for HI in the eight-year period since they discovered the HI gene. Over 130 families from the UK, Europe, Africa and Asia have been sent for ABCA12 genetic analysis to this laboratory for molecular diagnosis. In 98% of these cases, ABCA12 mutations were identified. For many of the samples from overseas there is often no financial support for the HI families to cover the genetic test and in these cases Kelsell's team do the tests at no cost to the family. Importantly, they have developed genetic screening strategies such as copy number arrays, targeted and exome high throughput sequencing to increase accuracy of mutation detection and increase turnaround time (within three months), which is critical for many HI families. Last year, they performed genetic analysis on eleven HI families, mainly from Pakistan, India and Iran. For UK, Europe and the US, the ABCA12 genetic test is now an established service in general clinical genetic / skin disease laboratories.
2. Pre-natal diagnosis (in excess of 20 HI families by this laboratory) based on the genetic findings. As predicted, in around 75% of cases, the test indicated that the mother was carrying a child that would not develop HI, and this was of great reassurance to the family. Furthermore, the team has performed what is believed to be the first case of pre-implantation genetic diagnosis for this condition, allowing the single genetically unaffected embryo to be selected from nine fertilised eggs, hence producing a child who was not only phenotypically normal but who was not even a carrier of the condition. This clinical application was published as an online communication by Barts Charity [14].
3. Data to support specific changes to clinical management. As the team had access to a large cohort of HI families (due to families requesting genetic testing), the team were able to perform the first large-scale study of HI, providing important prognostic information for clinicians and affected families. Previously the only clinical information were individual case reports describing neonatal lethality. The large number of HI families in this study published in the Archives of Dermatology (reference 6 above) provided huge insights into biology of this severe skin disease including that nearly 50% of HI babies in our study survived (eg retinoids give 86% survival).
4. Patient and public awareness. Kelsell *et al*'s work on HI has been a component in three TV documentaries (ITV, Channel 5 and Discovery) shown worldwide [15] and also as an interactive learning tool within Centre of the Cell, QMUL's interactive science education centre [16].

5. Sources to corroborate the impact

GJB2 and hearing loss

7. Schimmenti LA, Warman B, Schleiss MR *et al*. Evaluation of newborn screening bloodspot-based genetic testing as second tier screen for bedside newborn hearing screening. *Genetic*

Medicine 2011; 13:1006-10.

8. Kenneson A, Myers MF, Lubin IM, Boyle C. Genetic laboratory practices related to testing of the GJB2 (Connexin-26) gene in the United States in 1999 and 2000. *Genetic Testing* 2003; 7: 49-56.
9. Ardle BM, Bitner-Glindzicz M. Investigation of the child with permanent hearing impairment. *Archives of Diseases of Childhood (Education and Practice Edition)* 2010; 95: 14-23.
10. Clinical Molecular Genetics Society Audit 2010-2011: http://www.cmgs.org/CMGS%20audit/2011%20audit/CMGSAudit10_11_FINAL.pdf
11. Minami SB, Mutai H, Nakano A, Armoto Y et al. GJB2-associated hearing loss undetected by hearing screening of newborns. *Gene* 2013 Sep 5, epub ahead of print. doi: 10.1016/j.gene.2013.08.094.
12. Guidelines for testing of DFNB1 as part of EMQN. Hoefsloot LH, Roux A-F and Bitner-Glindzicz M on behalf of the contributors to the EMQN DFNB1 best practice meeting. *European Journal of Human Genetics advance online publication*, 22nd May 2013. doi: 10.1038/ejhg.2013.83.
13. Example of patient support organisation website for GJB2-related deafness. https://www.counsyl.com/diseases/gjb2-related-dfnb-1-nonsyndromic-hearing-loss-and-deafness/

ABCA12 and Harlequin Ichthyosis

14. First recorded case of pre-implantation genetic diagnosis of HI, leading to confirmation that fetus was unaffected: www.bartsandthelondoncharity.org.uk/News/Detail/Look-what-we-got-when-we-googled-genetics
15. TV Documentaries, featuring Kelsell *et al*'s research. For example: ITV1 'Real Lives' October 2005; 'The Girls with Too Much Skin' www.channel5.com/shows/extraordinary-people/episodes/extraordinary-people-the. This programme was short-listed for the Media Award for factual Programming at RADAR's People of the Year Human Rights Awards 2008. Another documentary of this research including gene identification and skin models of Harlequin Ichthyosis was filmed October 2012 and will be shown in late 2013 on the Discovery Channel.

    Other media coverage: BBC Online http://news.bbc.co.uk/1/hi/health/4337009.stm
16. Public understanding of science. The School of Medicine and Dentistry's Centre of the Cell 'Find a Gene' interactive exhibit featuring HI: www.centreofthecell.org/centre/?page_id=129