Institution: The University of Oxford

Unit of Assessment: 1

Title of case study:

DEFINING CRANIOFACIAL DISORDERS FOR IMPROVED CLINICAL MANAGEMENT

Summary of the impact:

As a result of research from Oxford's Professor Andrew Wilkie, accurate genetic diagnostic tests are now available for over 23% of all craniosynostosis cases nationally and internationally, leading to improved family planning and clinical management of this common condition worldwide. The premature fusion of cranial sutures, known as craniosynostosis, is a common developmental abnormality that occurs in 1 in 2,500 births. Over the past 20 years, the University of Oxford's Clinical Genetics Lab, led by Professor Wilkie in collaboration with the Oxford Craniofacial Unit, has identified more than half of the known genetic mutations that cause craniosynostosis and other malformations of the skull.

Underpinning research:

Craniosynostosis is the most common form of non-cleft craniofacial malformation, affecting one in 2,500 children and around 52,000 births worldwide each year. While the majority of craniosynostosis cases occur as isolated incidents, around 15% of all cases relate to over 150 different syndromes, many of which are the result of genetic mutations.

Since 1993, the University of Oxford's Clinical Genetics Laboratory, led by Professor Andrew Wilkie, has been collaborating closely with plastic surgeons at the Oxford Craniofacial Unit to research genetic mutations behind rare craniofacial malformations. Over the past 20 years the laboratory has identified the genetic causes of 14 craniofacial disorders, including many of the most common and important conditions, such as Apert syndrome (*FGFR2* gene)[1], Pfeiffer syndrome (*FGFR2* gene), the otopalatodigital syndromes (*FLNA*)[2], craniofrontonasal syndrome (*EFNB1*) and recently two new syndromes caused by mutations in *ERF*[3] and *TCF12*[4]. Each of these syndromes is a serious disorder, which commonly presents at birth and affects the development of multiple organ systems, with potential lifelong consequences for health, and in some cases, mental development. By identifying the genetic mutations behind more than half of all known syndromes, the Wilkie group have made the largest single contribution to the genetics of human craniofacial disorders in the world.

In 2010 the Clinical Genetics Laboratory made a major contribution to our understanding of the overall burden of genetic disorders in craniosynostosis. Through a comprehensive genetic testing programme conducted on 326 children with craniosynostosis a study identified 84 children (and 64 of their relatives) with genetic alterations. This study was the first to demonstrate the full potential and accuracy of genetic testing among patients with craniofacial syndromes in a large prospective cohort[5].

Recent studies from the Clinical Genetics Laboratory have highlighted the importance of accurate genetic diagnosis in improving the management of craniosynostosis. This principle is exemplified in the case of mutations in two genes, termed *ERF*[3] and *TCF12*[4], which each account for 1-2% of all craniosynostosis cases. The Oxford laboratory's analysis of genetic data, from more than 400 families collected over a 20-year period, has indicated that these new disorders show markedly different clinical features. Patients with *ERF* mutations often present later in childhood but can develop serious complications with raised intracranial pressure leading to brain damage in untreated cases[3]. In contrast, patients with mutations in the *TCF12* gene present early in life and require surgery within the first 6-18 months[4]. Importantly, several of the parents tested in this

study, who showed no clinical signs of craniosynostosis, were found to carry the *TCF12* mutation; indicating that accurate diagnosis and risk estimation for such families is only possible through genetic testing[4]. These contemporary studies[3,4] demonstrate the importance of accurate genetic testing in management, as well as in establishing prognosis.

References to the research:

1. Wilkie, A. O. *et al.* Apert syndrome results from localized mutations of FGFR2 and is allelic with Crouzon syndrome. *Nat. Genet.* **9**, 165–172 (1995).
Primary paper showing results from research into FGFR2 gene mutation in Apert Syndrome.

2. Robertson, S. P. *et al.* Localized mutations in the gene encoding the cytoskeletal protein filamin A cause diverse malformations in humans. *Nat. Genet.* **33**, 487–491 (2003). ***Paper outlining gene mutations for otopalatodigital syndromes.***

3. Twigg, S. R. F. *et al.* Reduced dosage of ERF causes complex craniosynostosis in humans and mice and links ERK1/2 signaling to regulation of osteogenesis. *Nat. Genet.* **45**:308-313 (2013). doi: 10.1038/ng.2539.
Paper showing mutations in the ERF gene in patients with craniosynostosis.

4. Sharma, V. P. *et al.* Mutations in TCF12, encoding a basic helix-loop-helix partner of TWIST1, are a frequent cause of coronal craniosynostosis. *Nat. Genet.* **45**:304-307 (2013). doi: 10.1038/ng.2531.
Paper showing mutations in the TCF12 gene in patients with craniosynostosis.

5. Wilkie, A. O. M. *et al.* Prevalence and complications of single-gene and chromosomal disorders in craniosynostosis. *Pediatrics* **126**, e391–400 (2010). doi: 10.1542/peds.2009-3491.
Paper outlining the burden of genetic abnormalities in craniosynostosis.

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Details of the impact:

Although the majority of craniosynostosis cases are considered non-syndromic (occurring as an isolated incident), 15% of craniosynostosis cases are associated with specific syndromes and around 23% of all cases (syndromic or non-syndromic) now have a genetic diagnosis, 38% of which were identified by the Clinical Genetics Laboratory in Oxford. This research has led to the development of genetic tests for accurate diagnosis, improved clinical management, and enhanced quality of life for patients and their families worldwide.

<u>Accurate Genetic Diagnosis</u>
The Clinical Genetics Laboratory's determination of 14 genes relating to craniofacial syndromes has led to the development of the first genetic diagnostic tests for these syndromes, now available in multiple laboratories around the world. The *GeneTests* website, hosted by the *National Centre for Biotechnology Information*, USA, lists 34 laboratories in 15 different countries offering tests for mutations in the *FGFR2* gene alone[6]. In the UK eight genes discovered by the Wilkie lab have now been approved for genetic testing, including *ERF* and *TCF12,* through the *UK Genetic Testing Network* gene dossiers process[7]. These tests provide a range of safe and accurate diagnostic options including: pre-implantation genetic diagnosis, prenatal diagnosis, and ultrasound scanning. Data from the *Clinical Molecular Genetics Society Audit*, showed there were 127 prenatal diagnoses in the UK for craniosynostosis between 2010 and 2011[8]. The Oxford Medical Genetics

Laboratories tested 260 new patient samples (for craniosynostosis syndromes) between 2010 and 2012 (174 from the United Kingdom and 86 abroad)[9], representing a small sample of the total tests carried out worldwide each year.

Improved Clinical Management

The great significance of prenatal diagnosis for craniofacial syndromes on a case-by-case basis is shown in a 2007 study of five cases of suspected Apert syndrome, confirmed prenatally by *FGFR2* mutation analysis. While three of these pregnancies were terminated, it is important to note that two families chose to continue with pregnancy. In such cases, the accurate prenatal diagnosis allows swift clinical responses, such as early referral to specialists, better communication with families, a more precise long-term prognosis, and improved clinical management[10].

The effect of accurate genetic diagnosis for improved clinical outcomes is exemplified in a further study from the Clinical Genetics Laboratory, which was published in 2009. This study shows that patients with Saethre-Chotzen syndrome (related to the *TWIST1* mutation) have a 42% recurrence rate of intracranial hypertension following standard surgical intervention. As a result, it is now recommended that all patients with syndromic features should be tested for *TWIST1* mutations in order to prevent the recurrence of intracranial hypertension following surgery[11]. The following supplementary commentary from senior plastic surgeon, Scott P. Bartlett, M.D. Division of Plastic Surgery, Children's Hospital of Philadelphia, reflects increasing realisation among clinicians of the importance of genetic screening as a management tool[12]: *"This is an important article. I hope those who deal with this area will read it closely and share it with members of their craniofacial team who do not have access to this publication."* [12]

Improved Quality of Life

Craniosynostosis is a common condition causing illness and uncertainty for thousands of patients and their families worldwide. Accurate genetic diagnosis offers definitive explanations for these often misdiagnosed, misunderstood, and mismanaged disorders.

The following testimonials represent a small percentage of the thousands of individual lives affected by this research:

Quote from the mother of a boy accurately diagnosed with a mutation in *ERF:*
"When Charlie was finally diagnosed it came as a relief that somebody had listened to us and was able to put a name to the problem. I always suspected that something was wrong despite tests coming back normal." [13]

Email to Professor Wilkie from a young woman with a mutation in *TCF12:*
"After letting things settle and sink in over the weekend, I would just like to say a quick a huge thank you for speaking with me on Friday, and completely putting my prospective future of having children in a completely different light. For this, I cannot thank you enough."[14]

Email to Professor Wilkie from a family involved in the group's *ERF* study:
"I have finally read the reports and information on the Internet and have been meaning to email you just to thank you and your team for your hard work in helping find answers for families like us. Thank you to you and your team for giving us a name for (my sons) problems… in some ways it has definitely made a difference in how we feel about it all, a lot of things with (my son) make sense now and the problems he has had in the past and still experiencing now..." [15]

Sources to corroborate the impact:

6. NCBI, GeneTests, FGFR2-Related Craniosynostosis.[online] Seattle. University of Washington (2013) Available at: http://www.ncbi.nlm.nih.gov/sites/GeneTests/lab/clinical_disease_id/2760?db=genetests [Accessed 4 April 2013] ***GeneTests website containing information about all genetic tests for craniofacial malformations currently available in the US and around the world.***

7. NHS, UK Genetic Testing Network. (2012) [online] Available at: http://www.ukgtn.nhs.uk/gtn/Home [Accessed 4 April 2013] ***Gene Dossiers for all approved genetic tests can be found on the UK Genetic Testing Network website.***

8. Clinical Molecular Genetics Society Audit of Data 2010-11. [online] London, CMGS, (2012). Available at: http://www.cmgs.org/CMGS%20audit/2011%20audit/CMGSAudit10_11_FINAL.pdf [Accessed 4 April 2013] ***CMGS audit published in 2012, showing there were 127 prenatal diagnoses in the UK for craniosynostosis between 2010 and 2011.***

9. Oxford University Hospitals NHS. Oxford Medical Genetics Laboratories audit of revenue from genetic testing of craniofacial and skeletal disorders, for the period 1 March 2011 to 28 Feb 2012 (available on request). ***Audit from the National Specialised Commissioning Group outlining total revenue from Oxford Medical Genetics Laboratories genetic testing of craniofacial and skeletal disorders from 1 March 2011 to 28 Feb 2012.***

10. David, A. L. *et al.* Diagnosis of Apert syndrome in the second-trimester using 2D and 3D ultrasound. *Prenat. Diagn.* **27,** 629–632 (2007). ***Study of five cases of suspected Apert syndrome, confirmed prenatally by FGFR2 mutation analysis.***

11. Woods, R. H. *et al.* Reoperation for intracranial hypertension in TWIST1-confirmed Saethre-Chotzen syndrome: a 15-year review. *Plast. Reconstr. Surg.* **123,** 1801–1810 (2009). doi: 10.1097/PRS.0b013e3181a3f391. ***A study showing that patients with Saethre-Chotzen syndrome have a 42% recurrence rate of intracranial hypertension following standard surgical intervention, emphasising the clinical importance of genetic testing.***

12. Bartlett, S. P. & Foo, R. Discussion. Reoperation for intracranial hypertension in TWIST1-confirmed Saethre-Chotzen syndrome: a 15-year review. *Plast. Reconstr. Surg.* **123,** 1811–1812 (2009). doi: 10.1097/PRS.0b013e3181a3f213. ***Supplementary commentary for (Woods, R. H. et al 2009) supporting genetic screening as a clinical tool.***

13. Nottinghamshire 10 year old diagnosed with super-rare condition. IRIS Magazine:[online] March 2011 Available at: http://www.askiris.org.uk/uploads/docs/Iris_12pp_Mag_3_Mar_aw.pdf [Accessed 4 April 2013] ***Article about the impact of correct genetic diagnosis (mutation in ERF gene) for a 10-year-old boy who had been previously misdiagnosed with Crouzon syndrome.***

14. Patient diagnosed with a TCF12 mutation. Email statement addressed to Professor Andrew Wilkie received 25th March 2013 (available on request). ***Email statement from a patient who was diagnosed with a TCF12 gene mutation, thanking Professor Wilkie for genetic counseling.***

15. Mother of a child with ERF craniosynostosis. Email statement addressed to Professor Andrew Wilkie, received 20th March 2013 (available on request). ***Email statement from the mother of a patient, which supports the work of Professor Wilkie's group.***