Institution: UCL

Unit of Assessment: 05

Title of case study: Trimethylaminuria is a genetic disorder

1. Summary of the impact

Research by Professor Elizabeth Shephard at the UCL Research Department of Structural and Molecular Biology has led to identification of the genetic origin of Trimethylaminuria (TMAU), commonly known as fish-odour syndrome. This has led to genetic diagnosis and genetic counselling for TMAU in the UK, Europe, USA and Canada, and the publication of guidelines for treatment and diagnosis. Shephard has engaged closely with patient groups over the years to publicise her findings. There is now an increased understanding among medical practitioners and the public that the body odour produced is due to a metabolic defect of genetic origin, and is not due to poor hygiene.

2. Underpinning research

Trimethylaminuria (TMAU) is a rare but distressing disorder in which sufferers excrete large amounts of trimethylamine (TMA) in the breath, urine and sweat, resulting in an unpleasant body odour similar to rotting fish, or, in some individuals, rotting garbage. The incidence is about 1 in 40,000 and all ethnic groups are affected. The impacts reported here stem from basic molecular biology, biochemistry and molecular genetic research undertaken from the mid-1990s to the present at UCL, in collaboration with Ian Phillips, Queen Mary, University of London (now retired) and initially also with R. Smith (now retired), St. Mary's Hospital.

It was known that TMA could be converted to its *N*-oxide by a flavin-containing monooxygenase in liver and that individuals responded differently to a trimethylamine challenge. Thus a genetic origin for TMAU was suspected. This research took place without the benefit of the rapid sequencing and genome resources available today. A systematic cDNA cloning strategy identified five different flavin-containing monooxygenase (FMO) mRNAs (FMOs 1, 2, 3, 4 and 5) and the five genes were mapped to the long arm of chromosome 1 **[1]**. Analysis of expression patterns in human tissue **[2]** identified FMO3 as the best candidate for the disorder. Using a then-novel method for exon determination, the gene structure for *FMO3* was determined **[3]**. Amplification and DNA sequencing of exons revealed a mutation in an affected individual that changed pro153 to leu153. The child was homozygous for the mutation and the parents were heterozygous for this mutation. The mutant protein was expressed from its cDNA and shown not to be able to convert TMA to TMA *N*-oxide.

These results led, in 1997, to a paper in Nature Genetics reporting for the first time a genetic basis for TMAU **[4]**. This was accompanied by media coverage across the world and began the process of understanding of the genetic contribution to TMAU. Subsequently we, and others, identified additional mutations in the *FMO3* gene that cause TMAU in different families. Over thirty mutations are now known to cause TMAU. Some cases proved to be caused by compound heterozygosity **[5]**.

In 2003 we created an online database of *FMO3* mutations to inform researchers, patients and medical practitioners **[6]**. Since 2013 the database, which is curated at UCL, has been hosted by the Leiden Open Variation Database (LOVD) Server, which permits researchers to submit new mutations and thus increases the resource for genetic testing services and patients who elect for testing. This is an ongoing project.

In 2013 we were awarded funding under the MRC DFPS/DCS: MICA scheme for translational research to carry out a preclinical trial for a therapeutic we have devised, with industry, for TMAU. This preclinical trial project is in collaboration with an industrial partner.

3. References to the research

[1] Phillips IR, Dolphin CT, Clair P, Hadley MR, Hutt AJ, McCombie RR, Smith RL, Shephard EA. The molecular biology of the flavin-containing monooxygenases of man. Chem Biol Interact. 1995 Apr 28;96(1):17-32. http://dx.doi.org/10.1016/0009-2797(94)03580-2

[2] Dolphin CT, Cullingford TE, Shephard EA, Smith RL, Phillips IR. Differential developmental and tissue-specific regulation of expression of the genes encoding three members of the flavin-containing monooxygenase family of man, FMO1, FMO3 and FM04. Eur J Biochem. 1996 Feb 1;235(3):683-9. http://dx.doi.org/10.1111/j.1432-1033.1996.00683.x

[3] Dolphin CT, Riley JH, Smith RL, Shephard EA, Phillips IR. Structural organization of the human flavin-containing monooxygenase 3 gene (FMO3), the favored candidate for fish-odor syndrome, determined directly from genomic DNA. Genomics. 1997 Dec 1;46(2):260-7. http://dx.doi.org/10.1006/geno.1997.5031

[4] Dolphin CT, Janmohamed A, Smith RL, Shephard EA, Phillips IR. Missense mutation in flavin-containing mono-oxygenase 3 gene, FMO3, underlies fish-odour syndrome. Nat Genet. 1997 Dec;17(4):491-4. http://dx.doi.org/10.1038/ng1297-491

[5] Dolphin CT, Janmohamed A, Smith RL, Shephard EA, Phillips IR. Compound heterozygosity for missense mutations in the flavin-containing monooxygenase 3 (FM03) gene in patients with fish-odour syndrome. Pharmacogenetics. 2000 Dec;10(9):799-807. http://doi.org/bxqpnn

[6] Hernandez D, Addou S, Lee D, Orengo C, Shephard EA, Phillips IR. Trimethylaminuria and a human FMO3 mutation database. Hum Mutat. 2003 Sep;22(3):209-13. http://doi.org/d7hfqf

Key Grants:

- I.R. Phillips (Queen Mary and Westfield), E. A. Shephard (UCL), R. Smith (St Mary's Medical School) – Wellcome Trust: The flavin-containing monooxygenase family of man and the molecular genetics of fish-odour syndrome; The Wellcome Trust; (1995–8) (£168,799).
- E. A. Shephard (UCL), I. R. Phillips (Queen Mary) – Wellcome Trust: The flavin-containing monooxygenase family: regulation, function and inherited disorders (1998–2004) (£636,853).
- E.A. Shephard (UCL) – MRC DFPS/DCS: MICA. Therapy for the body and breath malodour disorder Trimethylaminuria (TMAU) (2013–5) (£358,123.90; FEC £445,436). Industrial contribution in kind, £510,000.

4. Details of the impact

The identification of the gene for FMO3 and the elucidation of TMAU as a genetic disorder have transformed our understanding of why some individuals have an extremely unpleasant 'fishy' body odour. Prior to our 1997 paper **[4]**, which proved that TMAU is caused by mutations in the *FMO3* gene, affected individuals were presumed to have poor hygiene. Individuals with TMAU can suffer social isolation, depression, rejection and higher than normal suicide rates. In some cases, because the odour is episodic, medical practitioners assumed the symptoms to be imaginary and resorted to psychiatric referral and prescription of antidepressants or antipsychotics. This creates an even worse outcome, because several of these drugs are substrates for FMO3, and the limited enzyme activity that the patient has is then occupied by drug detoxification.

Impact on clinical practice

Our work on TMAU has led to increased understanding by the medical profession of the genetic basis of the disorder of body odour. In 2007, at the invitation of the National Institutes of Health, USA (NIH), we produced a TMAU resource for the medical profession, which was updated in 2011 **[a]**. The resource provides information on the disorder, its treatment and its diagnosis. A spokesman for the patient advocacy group, MEBO, states that "this is the resource which we recommend to everyone who wants information on TMAU" **[b]**. Shephard also contributed to the NHS Choices page on trimethylaminuria in 2013 **[c]**.

The identification of a genetic origin has enabled increased understanding of the condition by sufferers and those around them. For example, MEBO report that: "*One Kenyan TMAU sufferer was being persecuted by neighbours and feared having to go to the police. She was able to send Dr Shephard's scientific TMAU paper to the management committee of her housing block to support her explanation of her condition*" **[d]**.

Genetic testing

Following our discovery that TMAU is due to mutations in the *FMO3* gene, private testing facilities began to offer a diagnostic test. Subsequently, the NHS added the test to their list of genetic tests, and testing is also available elsewhere in the world. Various companies have reported the numbers of patients tested as follows for the period 2008-13:

- Sheffield Diagnostic Genetics Service: 236 patients tested (since 2007)
- Prevention Genetics, Wisconsin: 79 unique patients
- University of Colorado, Denver: 1-2 patients per month
- Mount Sinai Hospital, Toronto, Canada: 1 patient tested
- GENETAQ, Molecular Genetics Centre, Malaga, Spain: 13 families tested **[e]**.

Urine and genetic testing for TMAU are available on the NHS, but this is not the case in most countries, where patients must pay for costly tests. With the advent of cheaper DNA sequencing, some patients may elect to have their *FMO3* gene sequenced. In collaboration with the patient group MEBO (see below), Shephard assists with the sequencing reads and explanation to patients **[b]**. In 2011 we published the European guidelines (Clinical Utility Gene Card) for genetic testing of TMAU **[f]**. This provides guidelines for the medical profession and genetic testing industry.

Work with patients and patient groups

The unusual nature of TMAU has led to close links between researchers and patients developing in the course of the research described above, which continue today. In 2010, the patient advocacy group MEBO was founded, and Shephard has acted as scientific advisor to this group since 2011 **[g]**. MEBO provides support and advice for those who suffer from TMAU with registered branches in the US, UK and Europe. It now has around 1,200 members worldwide. In January 2012, Shephard assisted the organisation with a submission to fast-track TMAU for Social Security support in the US **[h]**. Later that year she was appointed to the Institutional Review Board (USA) for ethical assessment of a breath test device as part of a MEBO-initiated project. One member of the group has reported on the following positive impacts that Shephard's work has had on the community: "*her approachability as a scientific advisor for MEBO Research; her educational articles which have helped our TMAU community internationally; her willingness to build bridges between the layperson sufferer and the biomedical scientist, and her support in empowering the TMAU community to take action to help itself*" **[i]**.

Shephard has presented lectures and webinars on her research for MEBO to explain the science behind TMAU to those with the condition **[i]**. She presented at the MEBO Research Body Odor and Halitosis meet-ups in Washington (April 2011) and Miami (June 2012) **[j]**. Shephard also posts on the MEBO website and assists with lay postings on the disorder on the organisation's blog. She has also helped patients to write articles about their experience, for example, one such article was published in the Daily Mirror and internationally. In 2012 she assisted another patient with an article for the Royal College of General Practitioners' InnovAiT journal **[k]**.The disorder has been the subject of several TV programmes (e.g. BBC 2011, Food programme), radio (e.g. BBC World Service, March 2012 **[l]**) and the press. In each of these instances, Shephard has acted as advisor or interviewee. This coverage has had a huge 'advertising' impact, helping to increase public awareness of the disorder.

5. Sources to corroborate the impact

[a] NCBI resource for Trimethylaminuria: http://www.ncbi.nlm.nih.gov/books/NBK1103/ (Page

viewed 7,748 times in the period May 2012-April 2013)

[b] Letter of testimony available from MEBO's Managing Director which corroborates details of the organisation and endorses the importance of Shephard's work. Contact details provided.

[c] NHS Choices page on trimethylaminuria. Emails attesting to Shephard's involvement in writing these pages can be provided on request.
http://www.nhs.uk/conditions/trimethylaminuria/Pages/Introduction.aspx

[d] Letter of testimony from MEBO member available on request. Contact details provided.

[e] Genetic testing is now offered by a number of organisations. Emails confirming patient numbers are available on request:
- Sheffield Diagnostic Genetics Service, Sheffield, United Kingdom. http://www.sheffieldchildrens.nhs.uk/downloads/labgenetics/LaboratoryHandbook.pdf
- Prevention Genetics, http://www.preventiongenetics.com/
- http://www.ucdenver.edu/academics/colleges/medicalschool/programs/genetics/DiagnosticTests/Documents/UCDDNALabTESTS+CPTs+Prices.pdf (see page 16)
- Mount Sinai Hospital, Toronto, Canada: http://www.bloodbornebodyodorandhalitosis.com/2013/04/tmau-testing-at-mount-sinai-hospital.html
- GENETAQ, Molecular Genetics Centre, Malaga, Spain http://www.genetaq.com/en/catalogue/test/trimethylaminuria-fmo3-gene-mutation-analysis-r51g

[f] Clinical utility gene card for Trimethylaminuria: http://www.eurogentest.org/index.php?id=668

[g] MEBO page giving details of Shephard's role as scientific advisor to the group: http://www.bloodbornebodyodorandhalitosis.com/2013/06/details-of-uk-medical-research-council.html

[h] Information on the MEBO Blog about Shephard's involvement in the application: http://www.bloodbornebodyodorandhalitosis.com/2012/01/nomination-of-tmau-as-disability-in.html

[i] MEBO Research – includes links to webinars by Shephard, and other resources she has collaborated in: http://www.meboresearch.org/trimethylaminuria.html

[j] MEBO public engagement activities:
- Talk given at MEBO meeting, April 2011 http://www.youtube.com/watch?v=GDNM4drd5eI (2,234 views at 15 July 2013)
- Talk given at MEBO meeting, June 2012 http://www.rareconnect.org/en/community/trimethylaminuria/article/2nd-tmau-webinar (425 views at 15 July 2013)
- Webinar with TMAU patients via MEBO, Eurodis and Rareconnect, September 2012 http://www.bloodbornebodyodorandhalitosis.com/2012/09/tmaufmo3-webinar-recording-professors.html (521 views at 15 July 2013)

[k] Assisted patient with article. http://ino.sagepub.com/content/6/4/256.full. Copy available on request.

[l] BBC World service programme, March 2012: http://www.rareconnect.org/en/community/trimethylaminuria/article/tmau-audio-story-on-bbc-world-service (1,248 plays at 15 July 2013)