Institution: Swansea University		
Unit of Assessment: UoA3		
Title of case study: Opening a window on the metabolome: A novel sterol analysis technology for the diagnosis and treatment-monitoring of inborn errors of metabolism		
Period when the underpinning research was undertaken: 2007-2018		
Details of staff conducting the underpinning research from the submitting unit:		
Name(s):	**Role(s) (e.g., job title):**	**Period(s) employed by submitting HEI:**
William J Griffiths	Professor in Mass Spectrometry	Sept 2007–present
Yuqin Wang	Professor	Sept 2007–present
Period when the claimed impact occurred: 2014-2020		
Is this case study continued from a case study submitted in 2014? No		

1. Summary of the impact

Research by the Griffiths-Wang group at Swansea University has changed the way in which many sterols, including steroids and other cholesterol-like molecules, can be analysed. Owing to the nature of their structure, the diversity of occurrence in nature and the tendency of cholesterol to dominate in abundance, analysis of sterols has historically proved challenging. The Griffiths-Wang group have overcome these challenges by developing a novel "enzyme-assisted derivatisation for sterol analysis" (EADSA) technology, opening up new approaches to patient management for health care professionals worldwide. The monitoring of sterol metabolites has proven invaluable for disease diagnosis and prognosis and in monitoring the response to therapy for patients with rare inborn errors of metabolism, including lysosomal acid lipase deficiency (LALD), cholesterol 25-hydroxylase (CH25H) deficiency, spastic paraplegia type 5 (SPG5), Smith-Lemli-Opitz syndrome (SLOS) and acyl-CoA oxidase 2 (ACOX2) deficiency.

2. Underpinning research

Inborn errors of metabolism (IEM) provoke a broad spectrum of clinical presentations, from mild to lethal forms, and are mostly incurable but very often treatable. Although detection and diagnosis are key, the biochemical profile is needed for effective patient management. Since 2007, Swansea University's Griffiths-Wang group has investigated the biochemistry of cholesterol biosynthesis and metabolism and how it is implicated in disease. Cholesterol metabolites, including hormonal steroids and bile acids, have been analysed by mass spectrometry (MS) for several decades. One class of cholesterol metabolite called oxysterols is of particular clinical and pharmaceutical interest, as they can act as ligands to, or modulators of, nuclear receptors, G protein-coupled receptors (GPCRs) and *N*-methyl-D-aspartate receptors (NMDARs), and their abundances in body fluids can reflect underlying biochemical errors and ultimately disease. Oxysterols are difficult molecules to characterise as they are 'invisible' to most methodologies due to the absence of a strong chromophore or easily ionised functional group.

In 2008 the Griffiths-Wang group was the first to describe a method to investigate the oxysterol content of human plasma using high-performance liquid chromatography−mass spectrometry (HPLC−MS) that incorporated "enzyme-assisted derivatisation" and high-resolution MS. The methodology was shown to be straightforward, specific, highly sensitive and could be performed on an "omic" format **(R1).** The methodology opened a previously closed "window" into the combined metabolomic analysis of sterols, oxysterols, and steroid hormones. The technology was patented and formed the basis of the Swansea University spinout company Cholestenix Ltd (incorporated in November 2013), with a mission to deliver new innovative treatments and biomarkers for devastating neurodegenerative diseases. Using this method, Griffiths & Wang discovered novel ligands to nuclear receptors and to the Class F GPCR Smoothened **(R6, R7).**

In 2008, Griffiths, Wang and colleagues published a proof-of-concept study in a clinical setting that demonstrated the use of "enzyme-assisted derivatisation for sterol analysis" (EADSA) to detect inborn errors of metabolism. The method was used effectively in the 100% correct diagnosis of Smith-Lemli-Opitz syndrome using amniotic fluid. SLOS is a rare autosomal

recessive disease where severe cases present with profound intellectual disability and major physical abnormalities **(R2).**

In 2013-14, the efficacy of the technology in diagnosing disease in a clinical setting was further proven when EADSA was used to analyse the plasma of a young patient who presented with jaundice. The data confirmed the patient to be suffering from oxysterol 7α-hydroxylase deficiency, a very rare autosomal recessive disease which presents with liver disease in infants, and which can lead to hereditary spastic paraplegia type 5 (SPG5), a type of motor neuron disease, in adults. The technology was crucially used to monitor the response to therapy and subsequently demonstrated the improved biochemical phenotype of the patient when successfully treated with chenodeoxycholic acid (CDCA) **(R3).**

The group developed the technology further with the introduction of isotope-coded derivatisation reagents, allowing the multiplexed analysis of samples with the detection of sterols, including steroids and bile acids with either a hydroxy or ketone function by HPLC-MS, a key aspect of the Swansea technology. The ultimate method allows the quantitative measurement of the widest range of cholesterol precursors and metabolites, in terms of exact molecular structures, in a single analysis (**R4, R5**). The EADSA method was exploited to identify novel oxysterols in plasma from patients suffering from cerebrotendinous xanthomatosis (CTX, a rare autosomal recessive neurometabolic disease leading to progressive spastic-ataxic gait disorder and cognitive decline due to abnormal bile acid and cholesterol metabolism), SPG5 and SLOS **(R4, R7)**, and then to identify unusual intermediate metabolites in patients diagnosed with a number of different lysosomal storage disorders, to define the biochemical phenotypes of those disorders **(R5).**

The derivatization technology described in these papers was patented by Swansea University and licensed to global chemical suppliers (Avanti Polar Lipids Inc. and Cayman Chemical Company).

Furthermore, SU-led research on motor neuron disorders (including SPG5, CTX and amyotrophic lateral sclerosis) using cerebrospinal fluid (CSF) and plasma from patients showed that specific cholesterol-derived acids selectively regulate the balance between survival and death of motor neurons, revealing therapeutic potential towards this devastating group of diseases **(R6).**

3. References to the research

All papers represented below are published in peer reviewed journals. All have been supported by BBSRC funding and represent collaborative work either with clinical or industrial partners. **R1** is cited in 5x patents technologies, R6 is cited in 1x patent (linked patents: WO2014132052A2. EP3044192B1, WO2017037465A1. WO2018007803A1, US9851368B2). R1 and R2 were submitted to REF2014.

R1. Discovering Oxysterols in Plasma: A Window on the Metabolome. **Griffiths WJ**, …, **Wang Y.** J. Proteome Res. **2008** Aug; 7(8): 3602-3612; PMCID: PMC2567817; DOI: 10.1021/pr8001639.

R2. Potential of Sterol Analysis by Liquid Chromatography–Tandem Mass Spectrometry for the Prenatal Diagnosis of Smith-Lemli-Opitz Syndrome. **Griffiths WJ, Wang Y**, …, Shackleton C. Clin Chem. **2008** Aug; 54 (8): 1317-1324; PMCID: PMC2533047; DOI: 10.1373/clinchem.2007.100644.

R3. Liver disease in infancy caused by oxysterol 7 α-hydroxylase deficiency: successful treatment with chenodeoxycholic acid. Dai D, …, **Wang Y, Griffiths WJ,** Clayton PT. J Inherit Metab Dis. **2014** Sep; 37(5): 851-61; PMID: 24658845. DOI: 10.1007/s10545-014-9695-6.

R4. Quantitative charge-tags for sterol and oxysterol analysis. Crick PJ, …, **Wang Y, Griffiths WJ.** Clin Chem. **2015** Feb; 61(2): 400-11; PMID: 25512642. DOI: 10.1373/clinchem.2014.231332.

R5. Identification of unusual oxysterols and bile acids with 7-oxo or 3β,5α,6β-trihydroxy functions in human plasma by charge-tagging mass spectrometry with multistage fragmentation. **Griffiths WJ,** …, **Wang Y**. J Lipid Res. **2018** Jun; 59(6): 1058-1070; PMCID: PMC5983402; DOI: 10.1194/jlr.D083246.

R6. Cholestenoic acids regulate motor neuron survival via liver X receptors. Theofilopoulos S, **Griffiths WJ**, …, **Wang Y**. J Clin Invest. **2014** Nov; 124(11): 4829-42; PMCID: PMC4347238; DOI: 10.1172/JCI68506.
R7. Bile acid biosynthesis in Smith-Lemli-Opitz syndrome bypassing cholesterol: Potential importance of pathway intermediates. Abdel-Khalik J, …, **Griffiths WJ, Wang Y.** J Steroid Biochem Mol Biol. **2021** Feb; 206:105794. PMCID: PMC7816163; DOI: 10.1016/j.jsbmb.2020.105794. Epub 2020 Nov 24.

Grants supporting the underpinning research at Swansea University:
G1. GlaxoSmithKline, GBP50,000, Dec 2007–Nov 2010.
G2. BBSRC (BB/C515771/2), GBP42,226, May 2008–Sept 2009 PI Griffiths.
G3. BBSRC (BB/K019112/1), GBP652,377, July 2013–Aug 2016 PI Ghazal, CoI Griffiths.
G4. BBSRC (BB/I001735/1), GBP326,463, Sept 2011–Aug 2014 PI Griffiths, CoI Wang.
G5. BBSRC (BB/L001942/1), GBP382,198, Jan 2014–April 2017 PI Wang, CoI Griffiths.
G6. BBSRC (BB/N015932/1), GBP460,386, Oct 2016–Oct 2019 PI Griffiths CoI Wang.

4. Details of the impact

The difference between incidence and detection of inborn errors of metabolism is dependent on the ability of the clinician to first suspect a rare disease and secondly confirm both the phenotype and genotype. For the patients and families of those suffering with rare conditions, a diagnosis means an opportunity for early treatment e.g., by CDCA in the case of CTX and oxysterol 7α-hydroxylase deficiency, and an end to the uncertainty and lack of information around treatment measures and outcomes, opening avenues for connection with support groups. Many inborn errors of metabolism do not yet have a specific therapy, so for the clinician a confirmed phenotype allows potential treatments to be tried and tested.

The EADSA technology has been used to identify key sterol metabolites and bile acid precursors in patient samples. The unique technology and expertise of Griffiths and Wang, which they offered without charge, has helped to confirm biochemical phenotypes of, and improve the selection of treatments for and the management of 70 patients with a range of rare inborn errors of cholesterol metabolism across Europe, Asia and the U.S (**Table 1**).

Table 1 *The number of individual patients per disease benefitting from biochemical diagnosis from the Griffiths-Wang group, 2014-2020. The prevalence, where known, is included for context of the rarity of each condition (data from OMIM).*

Disease	Prevalence/100,000 (confirmed cases)	Patients
Spastic Paraplegia Type 5 (SPG5)	Unknown	13
SPG5 (under therapy with CDCA)	-	1
Acyl-CoA Oxidase 2 (ACOX2) Deficiency	Unknown	3
ACOX2 Deficiency (under therapy with CDCA)	-	13
Lysosomal Acid Lipase Deficiency (LALD) - Wolman	<1 /100,000	2
Niemann Pick Type B (NPB)	0.4 /100,000	3
Niemann Pick Type C (NPC)	1 /100,000	11
Smith-Lemli-Opitz Syndrome (SLOS)	3.7 /100,000	10
Cerebrotendinous xanthomatosis (CTX)	1 – 0.2 /100,000	14
Lathosterolosis	Unknown	1
NSDHL Deficiency (CHILD)	Unknown	1
Cholesterol 25-hydroxylase (CH25H) Deficiency	Unknown	3
CH25H Deficiency (following transplantation)	-	3
Total no. patients worldwide benefitting from the research.		**70**

These patients can now be more confidently diagnosed and better treated, and their disease is better managed through the application of this novel patented technology. Specific examples of the impact the research has had within disease states are presented below:

SPG5

SPG5 is a rare condition with unknown prevalence that is caused by mutations in the *CYP7B1* gene, which controls the production of the enzyme oxysterol 7α-hydroxylase, a key enzyme in steroid metabolism in the brain. A deficiency in this enzyme leads to a wide range of signs and symptoms, most notably neurodegeneration and movement problems. In infants, the deficiency may present with liver disease, in which case it is referred to as oxysterol 7α-hydroxylase deficiency.

In 2016 Professors Griffiths and Wang *"greatly assisted in characterizing the biochemical phenotype (sterol profile) of a Cypriot patient with spastic paraplegia, in particular a patient with novel, bi-allelic CYP7B1 mutations causing SPGA5A*". This was the first Cypriot patient identified with this condition **[C1].**

In Athens, the Griffiths-Wang laboratory was instrumental between 2016 and 2017 in the successful treatment of a 22-year-old patient with SPG5. The laboratory measured sterols in monthly blood and urine samples over a period of 18 months to track the patient's response to treatment with chenodeoxycholic acid (CDCA). The clinicians confirmed that *"the procedure performed helped considerably in the treatment of the patient and the biochemical profile in response to CDCA treatment"* **[C2].**

Between 2013 and 2014 in collaboration with clinicians at Great Ormond Street Hospital and UCL Institute of Child Heath, who were treating a young patient presenting with liver disease, and suffering from oxysterol 7α-hydroxylase deficiency, the Swansea group monitored the biochemical response to treatment with CDCA. The therapy was successful. **[C3].**

ACOX2 Deficiency

ACOX2 deficiency is an exceptionally rare disease with less than five cases reported world-wide, it arises from mutations in the *ACOX2* gene coding a peroxisomal enzyme involved in the sidechain shortening of bile acid precursors, a necessary step in the biosynthesis of primary bile acids. ACOX2 deficiency is an autosomal recessive disorder presenting to differing extents with cholestatic liver disease and neurologic dysfunction in infants and children.

In 2018, Griffiths and Wang used their EADSA technology to define the biochemical phenotype of Turkish patients suspected to have ACOX2 deficiency and "*for the first time, a previously unknown syndrome caused by recessive Acyl CoA Oxidase 2 deficiency has been identified with the great help of Professor William J. Griffiths from Swansea University Medical School in UK. Prof. Griffiths and his laboratory have greatly contributed to both the diagnosis and follow-up of the new disease by performing urine, serum and plasma bile acid analysis"* **[C4].**

Lysosomal Acid Lipase Deficiency (LALD, Wolmans) Cholesterol 25-Hydroxylase Deficiency and Other Sterol-Related Disorders.

Caused by mutations in the *LIPA* gene, LALDs are rare inherited disorders about which little is known of the signs, symptoms, and progression. Wolmans disease is a LALD characterised by a complete absence of the *LIPA* protein. LALDs result in problems with lipid metabolism and the accumulation of harmful amounts of lipids in cells and tissues throughout the body, which subsequently causes liver disease.

In 2017 in collaboration with the Genomic Medicines team at St Mary's Hospital in Manchester, the first ever patients with a double deletion of *LIPA* and of the adjacent gene *CH25H*, coding the enzyme cholesterol 25-hydroxylase, were identified using EADSA. Professors Griffiths and Wang were the first to biochemically confirm the enzyme deficiency in these children, and they have continued to "*look in detail at their sterolomic and oxysterol profiles of the LALD* (and double deletion) *patients before and after treatment with both Enzyme Replacement Therapy and Hemopoietic Stem Cell transplantation. This is proving a critical part in assessing the benefits of treatment"* **[C5].** Work with the team in Manchester has extended beyond LALDs and currently includes other rare lysosomal disorders Niemann-Pick type B and C

diseases. Niemann-Pick disease can affect the brain, nerves, liver, spleen, bone marrow and lungs. There is no cure, but treatments are being developed. People with these conditions experience progressive loss of function of nerves, the brain and other organs. Other inborn errors of cholesterol metabolism being studied include, cerebrotendinous xanthomatosis (CTX), NSDHL deficiency and lathosterolosis. Less than five cases of lathosterolosis have been reported world-wide, the disorder is characterised by facial dysmorphism, congenital anomalies, failure to thrive, developmental delay and liver disease, there are treatments but no cure.

SLOS

SLOS is a severe autosomal recessive disorder resulting from defects in a cholesterol-synthesising enzyme that lead to a build-up of the cholesterol precursor 7-dehydrocholesterol in tissues and blood plasma. SLOS affects an estimated 1 in 20,000 to 60,000 newborns. Mildly affected individuals may have only minor physical abnormalities with learning and behavioural problems, but severe cases can be life-threatening and involve profound intellectual disability and major physical abnormalities, and there is no cure. Despite this, it is important for parents to have a diagnosis for their child and for clinicians to initiate treatments. Professors Griffiths and Wang have discovered a novel bile acid biosynthesis pathway in SLOS which may be responsible for the dysmorphology observed in this disease **[R7]**. Regarding these discoveries, collaborators at the Great Ormond Street Institute of Child Health stated "*Laboratories such as mine that can pick up unusual metabolites in children with significant disease really value the ability of Bill's lab to identify the abnormal compound(s) we have detected*" **[C6].**

Our EADSA methodology has also been used to diagnose SLOS at Hong Kong Queen Mary Hospital, where the medical technologist stated, "*I can successfully detect 7-DHC in MS and I am so excited now*!", confirming that elevated 7-DHC provides a diagnosis of SLOS **[C7].** The definitive diagnosis and monitoring of response to therapy provide peace of mind and hope to parents of those with IEM and allow clinicians to search for ever better therapies.

5. Sources to corroborate the impact

C1 Testimonial from Senior Consultant in Clinical Genetics at The Cyprus Institute of Neurology and Genetics Cypriot, Aug 2020.
C2 Testimonial from The Rare Diseases Clinic, Athens Medical Centre, supporting work carried out between Mar 2016 and Aug 2017.
C3 Communications with Professorial Research Associate, UCL Great Ormond Street Institute of Child Health.
C4 Testimonial from Division of Paediatric Gastroenterology, Faculty of Medicine, Gazi University, Ankara, Turkey, Dec 2020.
C5 Testimonial from Medical Director, Consultant in Paediatric Inherited Metabolic Disease, NIHR Manchester Children's Clinical Research Facility, Oct 2020.
C6 Testimonial from Professorial Research Associate, UCL Great Ormond Street Institute of Child Health, Sept 2020.
C7 Communication with medical technologist, Queen Mary Hospital, Hong Kong.