Using Genetics to Diagnose Rare Metabolic Diseases

Dr Michael Wangler

Scientia

USING GENETICS TO DIAGNOSE RARE METABOLIC DISEASES

Identifying the cause of an illness in a sick baby or child is not always easy, particularly if the disease is rare. Throughout his career, **Dr Michael Wangler**, at the Baylor College of Medicine and Jan and Dan Duncan Neurological Research Institute, has investigated rare childhood diseases. Combining his expertise in paediatrics and genetics, Dr Wangler utilises genomics, metabolomics and the humble fruit fly to identify the genes responsible for rare and undiagnosed diseases to improve both diagnosis and treatment.

Genetics and Genomics and Disease

Many genetic disorders are due to a mutation in one or more genes coding for a protein. Dr Michael Wangler at the Baylor College of Medicine and Jan and Dan Duncan Neurological Research Institute seeks to identify genetic mutations in individuals with previously undiagnosed medical conditions. For a sick baby or child without a diagnosis, not knowing the root cause of the illness understandably creates considerable stress for worried parents. Clinicians work to provide a diagnosis as doing so can help them identify appropriate medical care. Unfortunately, diseases with a genetic cause can be extremely difficult to diagnose. One of the key tools in this process is DNA sequencing.

The human body is a collection of trillions of cells, in which collections of specialised cells are organised into tissues and organs. Within each cell is the 'blueprint' or code held by deoxyribonucleic acid (DNA). Genes are sections of DNA and each gene encodes specific proteins that are required to perform all the functions of the human body.

Genetically coded proteins are extremely important to the structure and function of cells, organs and tissues and perform the bulk of the work within individual cells. Many proteins are involved in body chemistry or metabolism. Metabolism refers to the various biochemical processes which take place in the body. This includes the breakdown of carbohydrates, fats and proteins in food and the release, use or storage of energy. Metabolic disease or disorder occurs when one or more of the biochemical processes are disrupted such as those due to mutations in metabolic genes.

A large proportion of the human genome is comprised of DNA that exists outside of genes and does not encode protein, so the term 'exome' refers to the ~3% of the genome containing the protein-coding genes. Whole exome sequencing (WES) is a key diagnostic tool to sequence the DNA for the protein-coding genes for an individual. WES is more focused than sequencing the entire genome, and it not only facilitates diagnosis for individual patients but also can contribute to the identification of new causes of

genetic disease particularly when there are additional causes for a particular disorder.

One group of diseases that Dr Wangler has focused on for years is peroxisomal disorders. Peroxisomes form a key part or organelle of our cells, and they are generated and function due to the action of particular genes. Genetic changes causing defects in the production of peroxisomes can affect the liver and brain amongst other organs, whilst mitochondrial defects can affect the brain, muscles, vision and hearing. Together with his team, Dr Wangler contributes to the expanding knowledge of genetic disorders by identifying genetic changes and the associated defects they cause in cell organelles.

An alteration or 'variant' of a gene alters the code it holds. Every individual has hundreds of variants in their genome that affects proteins but not every variant is pathogenic – the term used to describe a mutation's ability to cause a disease or disorder. If a variant affects the code for a protein and leads to a significant change, it can alter the structure of the resultant protein. The result can be a defective protein, or the protein may not even be produced.

The mutation of even a single gene can have severe clinical outcomes. Cystic fibrosis and haemophilia are both well-known examples of diseases caused by a pathogenic variant of a gene. WES can be a powerful tool for diagnosis in patients. However, it is not simple to interpret. The careful review of the data provided by WES in the detection of disease can uncover other changes in the DNA sequence known as 'variants of unknown significance' which are not obviously benign or pathogenic. If WES identifies two or more genes that could be the cause of the disease, it is a challenge to ascertain if one or even both are responsible for the disease.

Metabolomics and the Humble Fruit Fly

To understand which genetic variants are pathogenic and to further study diseases like peroxisomal disorders, Dr Wangler and his team have developed *Drosophila* models which are used to test the function of rare variant genes which they have identified as potential causes of disease. This process assists in the verification of findings and is a key focus in the work of Dr Wangler and his team. *Drosophila*, or to give it the full Latin name, *Drosophila* melanogaster, is better known as the fruit fly. At first glance, the fruit fly is not an obvious substitute for the human body – but looks can be deceiving. Genetically, the genomes of flies and humans share many essential genes, particularly for the function of the nervous system. *Drosophila* shares approximately 60% of its DNA with humans, and internally, many of the organ systems and cells function in the same way.

In simple terms, the suspect gene is inactivated in the DNA of fruit fly embryos and the resulting flies are examined for the effects compared to normal flies. If the resulting disease in the fly can be rectified by inserting a normal, healthy version of the human gene into the fly but not by inserting the human variant version, it corroborates the variant as being the genetic cause of the illness.

In research published in 2016, and again in 2019, Dr Wangler and his team identified variants of *DNM1L*, a gene which has been indicated to be crucial in mammalian development, in peroxisomal and mitochondrial function and used *Drosophila* mutant studies to determine which variants were pathogenic.

With his team, Dr Wangler also conducts metabolomic research using animal models, studying small molecules like metabolites and their substrates, and the effects that genetic changes can have on their production and interactions.

Using mice in addition to fruit flies, Dr Wangler has investigated the metabolism and genetics of peroxisome production. Conducting such investigations is complex, and

incorporates gene manipulation, biochemical analysis and monitoring of the animal reactions in various tests and comparing them to normal animals. Dr Wangler's research into peroxisomal biogenesis disorders (PBD) uncovered an unforeseen link between peroxisomes and carbohydrate metabolism. Critically, peroxisomes are known to be vital to fat metabolism. The discovery of the link between peroxisome function and carbohydrate metabolism suggests it could be a new target for the treatment of PBD.

Metabolomic Profiling in Patients

In further research into metabolic diseases, Dr Wangler and his team studied 19 patients with mild to intermediate peroxisomal biogenesis disorders-Zellweger spectrum disorders (PBD-ZSD). The team combined biochemical investigation for the diagnosis of peroxisomal dysfunction with untargeted metabolomic small molecule profiling. More than 650 compounds were detected, and the results identified a reduction in plasma sphingomyelin as a consistent feature indicating it has the potential to be a novel biomarker for PBD-ZSD. Findings also indicated that characteristic metabolite changes decrease with age, suggesting that untargeted metabolomic profiling is useful in detecting abnormal peroxisome function in young patients but the usefulness decreases with the age of the patient.

Further to this, in a study published in 2019, Dr Wangler and colleagues used fruit flies to verify a diagnosis of peroxisomal disorder when the patient's metabolic profile did not indicate PBD, possibly due to their age. The team successfully identified a new *DNM1L* variant gene and verified the effect using the *Drosophila* functional studies. The researchers identified the patient, age 27, as the first adult reported with a pathogenic variant of DNML1 to exhibit neurological symptoms. The team concluded that *DNM1L*-related disorders are associated with different variations of the gene and a broad range of symptoms and severity.

Future Research Plans

Currently, Dr Wangler and his team are involved in several projects. They are part of an international collaboration with the Model Organisms Screening Center for the Undiagnosed Disease Network (UDN). In this project, DNA sequencing of undiagnosed patients is performed in the search for additional cases of rare disorders and further develops *Drosophila* models for testing the functionality of possible disease-causing variants.

Work to date completed by Dr Wangler and his team using WES and genomic and metabolomic studies to investigate the genetic causes of rare diseases holds much-needed promise for the diagnosis and treatment of genetic disorders in humans. Further work will undoubtedly drive forward this complex yet critical area of research.

Dr Michael Wanger with his team.

Meet the researcher

Dr Michael Wangler

Department of Molecular and Human Genetics, Baylor College of Medicine
Jan and Dan Duncan Neurological Research Institute
Houston, TX
USA

Dr Michael Wangler is a licensed, practising physician, board-certified in both paediatrics and medical genetics. With a career focussed on rare childhood disease, he uses genetics to understand human health. Currently, Dr Wangler is an Assistant Professor in the Department of Molecular and Human Genetics at Baylor College of Medicine, and the Jan and Dan Duncan Neurological Research Institute. Dr Wangler specialises in research into the underlying mechanisms of Mendelian disease, and the clinical and genetic aspects of rare human disease. Dr Wangler has contributed to numerous peer-reviewed scientific articles regarding gene function in peroxisomal disorders and undiagnosed disease. His various achievements include the Molecular and Human Genetics Most Outstanding Fellow Award in 2011 and, since 2014, a seat on the Scientific Advisory Board of the Global Foundation for Peroxisomal Disorders.

CONTACT

E: michael.wangler@bcm.tmc.edu
W: http://wanglerlab.org/
@mfwangler

KEY COLLABORATORS

Hugo J. Bellen DVM, PhD
Shinya Yamamoto DVM, PhD
Nancy Braverman, MD
Joe Hacia, PhD
Sarah Elsea, PhD
Brendan Lee, MD, PhD
Ann Moser, BA
Richard Gibbs, PhD
Jennifer Posey, PhD
James Lupski, MD, PhD
Dustin Baldridge. MD, PhD

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FURTHER READING

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Baylor College of Medicine