

Vector developer

Genetics expert and microbiologist **Dr Guangping Gao** details the experiences that led to him devoting his scientific career to tackling Canavan's disease, and illuminates the successes he and his collaborators have achieved in this area

How has your professional background led to your interest in gene therapy? What motivates you in your work?

I was a graduate student at Miami Children's Hospital Research Institute and Florida International University during 1990-94, under the mentorship of Drs Reuben Matalon and Rajinder Kaul, diligently searching for the gene and causative mutations in Canavan's disease – a severe inherited neurodegenerative disease. While attending a fundraising event to celebrate the discovery of the Canavan gene and its mutations, reported in the journal *Nature Genetics* in October 1993, I met several paediatric patients with genetic diseases including Canavan's disease, Hunter syndrome, Sly syndrome and phenylketonuria. Seeing the unbearable suffering of those children and the helplessness of their parents deeply moved me. It was at that moment that I dedicated my life's work to searching for better therapeutic approaches and cures for these patients – and others. Over the past 22 years, I have primarily devoted myself to developing the key element of the gene therapy platform: an optimised vehicle for the safe and efficient delivery of therapeutic gene payload, known as a vector.

Could you explain how gene therapy works, and why might it be useful in tackling Canavan's disease?

The promise of gene therapy is to treat human diseases including inherited and acquired diseases at the genetic level, either by introducing good information where bad existed or by introducing genetic information that was absent. A major challenge towards this goal is to efficiently and safely deliver the therapeutic gene to the tissue and cell types that need it over a sustained period, ideally accomplishing lifetime gene correction with a single dose. The vehicle to deliver the gene payload, the vector, is the key element in gene therapy. I started my gene therapy research career as a vector platform developer in 1994. After more than 20 years of hard work by scientists in the field, we have now found an ideal vector system for gene therapy: adeno-associated virus (AAV). This is a common, benign, residential virus that can persist in primate tissues without integrating into host genomes and causing disease. Of all the different vectors brought to gene therapy to date, AAV vectors stand out for their high efficiency, stability and low toxicity.

Have you explored the possibility of combination therapies to combat Canavan's disease in human trials?

At this point, we are still trying to understand Canavan's disease mechanistically, and identify

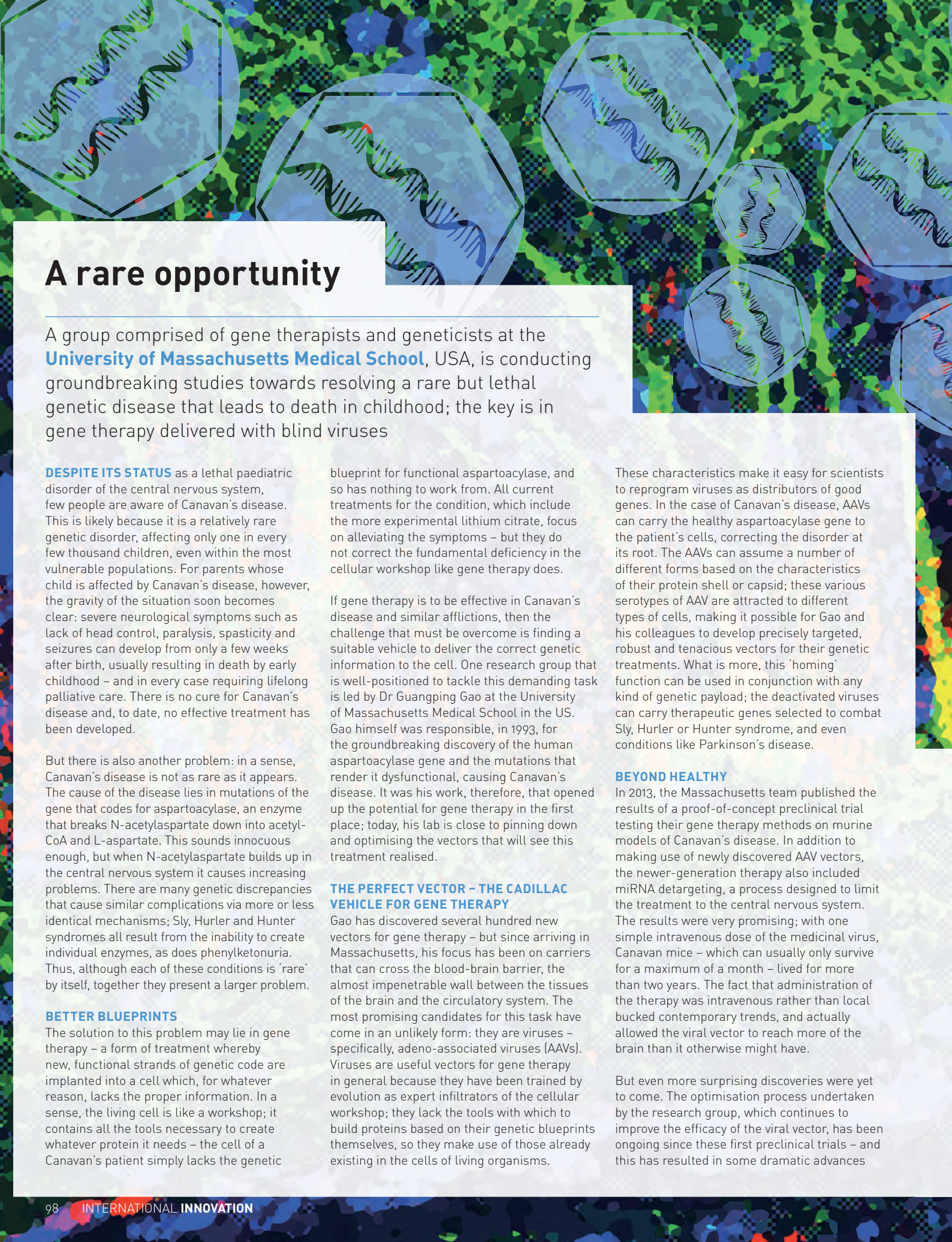
unknown disease mechanisms that can be targeted for treatment. The main problem with combination therapy is that current treatments for Canavan's disease treat the symptoms but not the actual disease – and combining this with gene therapy might have unpredictable effects. However, for human trials there will be medical support if needed – to treat seizures, for example.

How have your research collaborations furthered the development of your work?

Productive collaboration with other researchers with different expertise has been essential for the progress of this research project. My PhD mentor, Dr Reuben Matalon, has helped us with animal models, disease pathomechanisms and clinical aspects of Canavan's disease, while my postdoctoral mentor, Dr James Wilson, has played a critical role in the discovery of novel AAVs. Meanwhile, Dr Zuoshang Xu has helped tremendously in developing vector delivery platforms for gene transfer in the central nervous system – and the world-leading expert in miRNA biology, Dr Phil Zamore, has been instrumental in the development of our miRNA-regulated AAV vectors for systemically-delivered but central nervous system-restricted gene transfer.

What challenges have you faced during the course of your research?

Many gene therapies for diseases of the central nervous system face similar problems. For example, the understanding of disease pathomechanism, the availability of the therapeutic gene and an appropriate animal model are the mainstay of every gene therapy. For Canavan's disease, as for other metabolic disorders of the central nervous system, achieving a widespread distribution of the AspA gene is challenging. With its intravenous delivery AAV can help to overcome this problem to a great extent; however, there is still room for improvement. Another aspect is the question of whether AspA delivery to cells that naturally express this gene is actually necessary – or if any cell type is sufficient, as long as N-acetylaspartic acid can be cleaved by AspA. This will have significant consequences on our vector design, AAV serotype selection, dose, and route of gene therapy administration.



A rare opportunity

A group comprised of gene therapists and geneticists at the **University of Massachusetts Medical School**, USA, is conducting groundbreaking studies towards resolving a rare but lethal genetic disease that leads to death in childhood; the key is in gene therapy delivered with blind viruses

DESPITE ITS STATUS as a lethal paediatric disorder of the central nervous system, few people are aware of Canavan's disease. This is likely because it is a relatively rare genetic disorder, affecting only one in every few thousand children, even within the most vulnerable populations. For parents whose child is affected by Canavan's disease, however, the gravity of the situation soon becomes clear: severe neurological symptoms such as lack of head control, paralysis, spasticity and seizures can develop from only a few weeks after birth, usually resulting in death by early childhood – and in every case requiring lifelong palliative care. There is no cure for Canavan's disease and, to date, no effective treatment has been developed.

But there is also another problem: in a sense, Canavan's disease is not as rare as it appears. The cause of the disease lies in mutations of the gene that codes for aspartoacylase, an enzyme that breaks N-acetylaspartate down into acetyl-CoA and L-aspartate. This sounds innocuous enough, but when N-acetylaspartate builds up in the central nervous system it causes increasing problems. There are many genetic discrepancies that cause similar complications via more or less identical mechanisms; Sly, Hurler and Hunter syndromes all result from the inability to create individual enzymes, as does phenylketonuria. Thus, although each of these conditions is 'rare' by itself, together they present a larger problem.

BETTER BLUEPRINTS

The solution to this problem may lie in gene therapy – a form of treatment whereby new, functional strands of genetic code are implanted into a cell which, for whatever reason, lacks the proper information. In a sense, the living cell is like a workshop; it contains all the tools necessary to create whatever protein it needs – the cell of a Canavan's patient simply lacks the genetic

blueprint for functional aspartoacylase, and so has nothing to work from. All current treatments for the condition, which include the more experimental lithium citrate, focus on alleviating the symptoms – but they do not correct the fundamental deficiency in the cellular workshop like gene therapy does.

If gene therapy is to be effective in Canavan's disease and similar afflictions, then the challenge that must be overcome is finding a suitable vehicle to deliver the correct genetic information to the cell. One research group that is well-positioned to tackle this demanding task is led by Dr Guangping Gao at the University of Massachusetts Medical School in the US. Gao himself was responsible, in 1993, for the groundbreaking discovery of the human aspartoacylase gene and the mutations that render it dysfunctional, causing Canavan's disease. It was his work, therefore, that opened up the potential for gene therapy in the first place; today, his lab is close to pinning down and optimising the vectors that will see this treatment realised.

THE PERFECT VECTOR – THE CADILLAC VEHICLE FOR GENE THERAPY

Gao has discovered several hundred new vectors for gene therapy – but since arriving in Massachusetts, his focus has been on carriers that can cross the blood-brain barrier, the almost impenetrable wall between the tissues of the brain and the circulatory system. The most promising candidates for this task have come in an unlikely form: they are viruses – specifically, adeno-associated viruses (AAVs). Viruses are useful vectors for gene therapy in general because they have been trained by evolution as expert infiltrators of the cellular workshop; they lack the tools with which to build proteins based on their genetic blueprints themselves, so they make use of those already existing in the cells of living organisms.

These characteristics make it easy for scientists to reprogram viruses as distributors of good genes. In the case of Canavan's disease, AAVs can carry the healthy aspartoacylase gene to the patient's cells, correcting the disorder at its root. The AAVs can assume a number of different forms based on the characteristics of their protein shell or capsid; these various serotypes of AAV are attracted to different types of cells, making it possible for Gao and his colleagues to develop precisely targeted, robust and tenacious vectors for their genetic treatments. What is more, this 'homing' function can be used in conjunction with any kind of genetic payload; the deactivated viruses can carry therapeutic genes selected to combat Sly, Hurler or Hunter syndrome, and even conditions like Parkinson's disease.

BEYOND HEALTHY

In 2013, the Massachusetts team published the results of a proof-of-concept preclinical trial testing their gene therapy methods on murine models of Canavan's disease. In addition to making use of newly discovered AAV vectors, the newer-generation therapy also included miRNA detargeting, a process designed to limit the treatment to the central nervous system. The results were very promising; with one simple intravenous dose of the medicinal virus, Canavan mice – which can usually only survive for a maximum of a month – lived for more than two years. The fact that administration of the therapy was intravenous rather than local bucked contemporary trends, and actually allowed the viral vector to reach more of the brain than it otherwise might have.

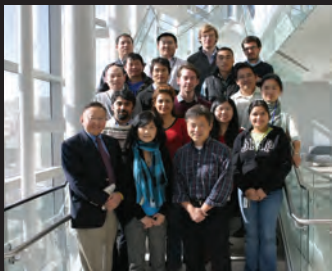
But even more surprising discoveries were yet to come. The optimisation process undertaken by the research group, which continues to improve the efficacy of the viral vector, has been ongoing since these first preclinical trials – and this has resulted in some dramatic advances



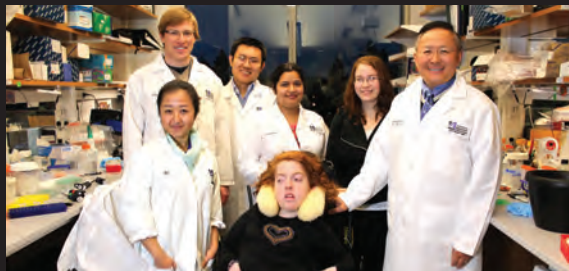
Dr Reuben Matalon – long time mentor and collaborator, and a grandfather of Canavan's disease research.



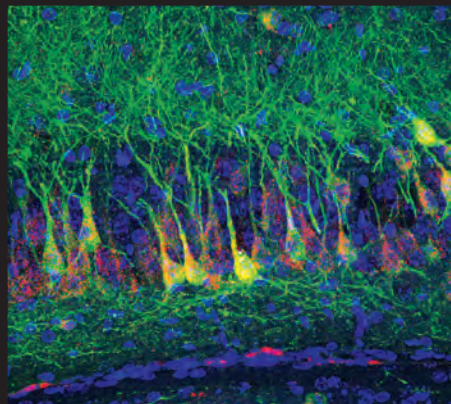
Dr Guangping Gao – a world-leading gene therapist for Canavan's disease.



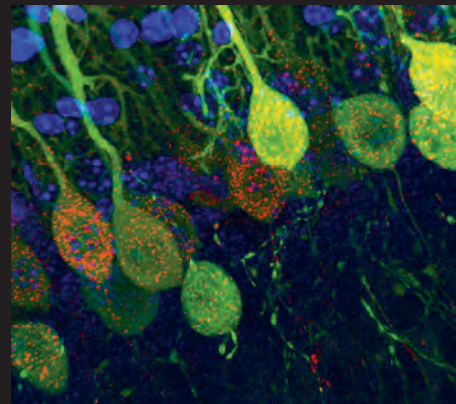
Gao lab members.



Canavan gene therapy research team with a patient and family member/researcher.



Above are 3D images of neurons (green) of the hippocampus and cerebellum in a 26-day-old mouse with Canavan's disease. The blue colored structures are cell nuclei. Yellow dots show vesicles at an early stage of autophagy while red displays the progression of those vesicles to a later time point. The labelling of this highly dynamic process in different colors was accomplished by adeno-associated virus mediated delivery of a multicolor autophagy sensor and helps to investigate the potential contribution of autophagy to the Canavan's disease pathomechanism.



INTELLIGENCE

NOVEL GENE THERAPY STRATEGIES FOR CANAVAN'S DISEASE

OBJECTIVES

- To further understanding about the pathophysiology of Canavan's disease
- To define the latest therapeutic window for Canavan's disease
- To optimise the transgene cassette to express hASPA more efficiently and safely

KEY COLLABORATORS

Dr Reuben Matalon, University of Texas Medical Branch, USA

Dr James Wilson, University of Pennsylvania, USA

Dr Zuoshang Xu; **Dr Phil Zamore**, University of Massachusetts Medical School, USA

PARTNERS

University of Massachusetts Medical School
University of Texas Medical Branch

FUNDING

National Institutes of Health (NIH): National Institute of Neurological Disorders and Stroke • NIH: National Institute of Allergy and Infectious Diseases • The Will Foundation • Jacob's Cure • National Tay-Sachs & Allied Diseases Association (NTSAD)

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DR GUANGPING GAO is the Director of the Gene Therapy Center at the University of Massachusetts Medical School, a world-leading expert in virus-based gene therapy and scientific founder of Voyager Therapeutics. He has published more than 170 papers in peer-reviewed journals and 30 patented inventions.

that are due to be published this year. Not only has the power of the vector increased to such a degree that one-third of the original dose is now required to achieve the same effect, but Gao's results suggest that this treatment actually improves motor function and overall activity. In two separate mouse models of Canavan's disease, the genetic treatment first reversed the disease phenotype and then improved the abilities of the treated mice above and beyond those of mice that never had the disease at all.

PROGRESS INTO PRACTICE

As well as conducting this vital research, which has made substantial progress over the last 10 years, Gao has also been involved more recently in founding a company to put these treatments into action. Voyager Therapeutics was established in early 2014, cofounded by Gao, three other world leading scientists and Mark Levin of Third Rock Ventures, and will aim to capitalise on the promising treatment by bringing it to the market. Although the

therapy was developed with Canavan's disease in mind, there is no reason why AAVs could not be used to combat similar genetic disorders, or even a broader range of conditions; Voyager Therapeutics already has Parkinson's disease, Friedreich's Ataxia, amyotrophic lateral sclerosis, and Huntington's diseases in its sights.

Canavan's disease may be rare – but this is no comfort for parents whose child is affected by the disease. Gao's personal contribution to the early identification and treatment of the disease has been colossal, both through his important early work identifying the gene at fault and his more recent endeavours to develop vectors for treatment. Now that his effective gene therapy is on the verge of being realised, there is every reason to hope that in the future, Canavan's disease will not just be rare – it will be non-existent.