Stephen Robertson: a world-class win.

THE GREAT GENE QUEST

A scientist working on a shoestring budget in a cramped Dunedin office has taken a ground-breaking step towards healing babies born with brain damage.

by REBECCA MACFIE • photos by DAVID WHITE

In the week that New Zealanders watched victory dissolve into defeat on the waters of San Francisco Bay, Professor Stephen Robertson came to work as normal in his cramped Dunedin laboratory, filled with a sense of quiet satisfaction that his own small team had once again achieved a world-class win. There was no ticker-tape parade or civic ceremony to celebrate their achievement. As Robertson stood in the city's main street at lunchtime, barely a soul knew who he was, let alone that he had achieved something that could profoundly change lives.

The exhaustively peer-reviewed pages of *Nature Genetics* represent some of the most fiercely contested territory in the scientific world. It is the foremost international journal for genetic research and on September 20, for the fourth time in a decade, it revealed to the scientific community ground-breaking work led by Robertson.

The latest paper goes by a dizzying title: "Mutations in genes encoding the cadherin receptor-ligand pair DCHS1 and FAT4 disrupt cerebral cortical development". Significantly, Robertson's name appears at the end of a list of 32 local and international collaborators, indicating that this family man from Mosgiel was the intellectual leader of the five-year research effort that yielded what Melbourne paediatric neurologist Professor Ingrid Scheffer calls an "extensive, meticulous and fascinating" body of work that provides "seminal insights".

Robertson's group has made a pioneering discovery about two genes involved in brain development, and how they influence and regulate the way neural stem cells build the human brain as the fetus grows in the uterus. In 11 pages of dense, dry scientific language, the *Nature Genetics* article provides important clues as to how neural stem cells might be harnessed and deployed to repair the brains of babies damaged through the likes of birth trauma, asphyxia, congenital disease or even infant stroke (a condition more frequent in newborns than previously thought).

"This is a long-term goal, but understanding the biological pathways involved is the essential first step," says Scheffer, who is the professor of paediatric neurology at the University of Melbourne. "The aim would be to mobilise these special cells [neural stem cells] from within the infant brain to repair the lost cells."

ORCHESTRATING DEVELOPMENT

Robertson knows to keep the language sober and the expectations realistic, but he shares this startlingly ambitious goal. "The hope is that if we can understand some of the 'language' going on in the infant brain between stem cells, particularly in the context of an infant brain that has become damaged, perhaps we can leap in and encourage the brain to repair itself."

The package of knowledge behind all this is excruciatingly complex, but Robertson – the Cure Kids Professor of Paediatric Genetics at the University of Otago – is an expert at translating it. "The human brain develops from a single layer of cells that forms very early at the top end of the developing embryo. This layer of cells, called the neuroepithelium, houses a population of stem cells that have two major developmental tasks to perform to orchestrate brain development.

"The first is that they must renew themselves. But secondly, and crucially, they must also retain the capability to turn into any type of brain cell when required. As the brain develops, these two tasks need to be regulated: on one hand, the cells need to proliferate, and on the other, at appropriately timed points during brain development, they must change into mature brain cells – neurons and the like – to actually make a functioning and integrated brain."

There is much excitement in the research world about the possibility of growing adult stem cells and injecting them into the diseased brains of Alzheimer's or Parkinson's sufferers. But Robertson says the potential for intervention in infant brains is possibly far greater.

"We know that infants are born with this very substantial population of neural stem cells that are still very active and still migrating and doing their stuff to form the brain. The infant brain is very plastic; the adult brain is much less so."

Until now, little has been known about what prompts neural stem cells to take up their mature position and function in the developing brain. In a triumph of collaboration between Robertson's tight team of PhD students and post-doctoral researchers in Dunedin and some of the world's most respected neuroscientists – along with helpers from Canada, France, the Netherlands and the UK – something of that mystery has been unlocked. In essence, they have discovered a "radio signal" used by neural stem cells to communicate with each other as they conduct brain development.

"This is genuinely great science," says Professor Russell Snell, of the University of Auckland's neurogenetics group. "It's a beautiful story that starts with basic clinical science. The thing that makes Stephen Robertson unique is that he [is] a geneticist who sees patients with rare disorders, and he is also a research scientist. So he's not just a geek, but he is a geek as well. He has an incredibly strong drive to do good things for his patients."

"This is genuinely great science … It's a beautiful story."

INTERNATIONAL NETWORK

Robertson's latest journey of discovery began in about 2007, on the back of his rising international reputation as a clinical and research geneticist (of which more later). Through his networks with other clinicians around the world who see patients with genetic disorders, his lab had accumulated a bank of 3000 vials of DNA from people born with rare conditions. As he met up with his peers at international meetings and kept in contact via email, it became clear that a new and extremely unusual genetic syndrome was being identified. The sufferers of the condition had a raft of symptoms: clawed hands, widely spaced eyes, tiny ears and deafness, intellectual disability – and a large band of "grey" matter stuck in the middle of the brain, rather than forming the outermost layer on the cerebral cortex where it is supposed to be.

Robertson had already seen this abnormality in the construction of the brain in earlier work, and it was known to give rise to a seizure disorder in females. Yet here it was again, presenting in these patients with a completely different clinical syndrome. For some reason the neural stem cells in the developing brain of these children had failed to migrate to their correct position and take on their specialist roles.

The parents of children born with this mysterious condition didn't even have the benefit of a clear diagnosis or a name to describe it (although in the past couple of years it has become known as Van Maldergem syndrome, after the Belgian geneticist who described the first case). At the time, Robertson had working in his Dunedin lab a talented young PhD student from Horowhenua, Mary Gray. In 2008, she set to work studying this rare syndrome, and quickly deduced that the gene responsible for the condition must lie on chromosome 11. But which of thousands might it be?

"I remember her coming into the office and saying to me, 'This is where it's got to be.' But, wow, it was like looking at an aerial photograph and trying to spot a single letter box," recalls Robertson.

He knew what a slow and painstaking task it would be to track down the culprit gene the old-fashioned way. He'd done it himself in 2002 when, as a PhD student at Oxford, he'd spent three years searching for the gene responsible for the tragic malformation and death of seven babies born to a West Auckland family. But by 2008, genetic research technology had advanced dramatically, massively speeding up the process of identifying the genes and mutations implicated in congenital disorders.

So Gray and Robertson opted to use what's called Massively Parallel Genomic Sequencing technology, which meant sending the DNA of chromosome 11 to a specialist company in Connecticut, where it was shredded and rinsed clean of the 98% of DNA that does not code for proteins (the stuff that was once referred to as "junk DNA" but is now known to be functional), and then decoded.

A few weeks later the information was mailed back on a couple of CDs to Robertson's Hanover St lab. Gray went to work, hunting through the lines of code for the genetic "spelling mistakes" that were causing the syndrome. Before long, she implicated the gene responsible, identifiable by mutations that were repeated among three unrelated families with the syndrome.

A STROKE OF LUCK

This alone would have been worthy of a hearty celebration and publication in a scholarly journal. But Robertson and Gray sensed there was much more to learn; they wanted to know not only *what* had happened in the culprit gene to cause this condition but *how*.

The gene coded for a protein named Dachsous1. Robertson says he "didn't know it from a bar of soap" at the time. He had always been focused on a set of genes known as filamins, so getting to grips with Dachsous required masses of study to find out what was already known about it.

By a great stroke of luck, it turned out that it had been intensively studied in fruit flies, but "jumping from what is known about this gene in flies to understanding its role in a mammal, let alone a human, is a big, big leap. That was a mind-blowing moment."

What the fly geneticists had discovered was that the Dachsous1 gene sits embedded in the membrane on the outside of cells

Clockwise from left: Tania Gunn; Francis Collins; Robertson in his office; Magdalena Götz, Andrew Wilkie; Robertson and family on holiday in Wales in 2011.

DAVID WHITE; GETTY IMAGES; PROF STEPHEN ROBERTSON

and binds onto a gene that codes for a protein called FAT4 – encoded by the "biggest, ugliest gene you've ever seen" – located on the surface of an adjacent cell. Robertson figured both Dachsous1 and FAT4 were playing a role in the cause of Van Maldergem syndrome, but looking for the mutation on FAT4 that might be causing the trouble was going to be like "looking for a bolt out of place on the Starship Enterprise".

Once again, they sent a batch of DNA off for sequencing, this time to a collaborator in London. When it came back, Gray went to work looking for misspellings on the FAT4 gene. And there they were: the DNA of the three remaining unrelated families with Van Maldergem syndrome all had mutations distributed throughout the gene.

By now, Robertson sensed that they were onto something more than just the cause of a rare congenital syndrome and the underpinning role of the two culprit genes. He went back to the literature on fly biology, and found that the interaction between FAT4 and Dachsous1 was known to produce an instruction – something akin to a language or a radio signal – that was involved in the development of the embryo of all multicellular organisms. There was already considerable knowledge about that signal, which went by the peculiar name of "Hippo signalling" and had long been thought to be involved in regulating the size of internal organs such as the kidney and liver. But what Robertson and Gray didn't know was what it had to do with the formation of the brain.

Then, in another stroke of luck, a Japanese paper published in 2009 implicated

Dachsous1 and FAT4 in the developing brains of mice. It appeared that if either of the two genes was damaged or disabled, the expression of the other became defective. Smack in the centre of the tiny brain of an embryonic mouse, the two genes were playing a commanding role in the way neural stem cells did their work.

Robertson and his team knew the two genes were implicated in Van Maldergem syndrome, and now they knew they also played a key role in the development of a mouse brain. But how to connect these fragments of knowledge and give them meaning for human health?

BEAUTIFUL TOOLS

It was time to search for a collaborator in what Robertson likens to a quest to figure out the "electrical engineering" of neural stem cells: "I had this feeling that we were onto an understanding of a human disorder that tells us something about what channels these neural stem cells are tuned into and what language they use to regulate themselves."

He scoured the literature and settled on a German neuroscientist, Magdalena Götz, internationally recognised for her work in discovering the nature of neural stem cells and the leader of a large research group. Götz had developed "beautiful tools where she can interfere with genes in the brains of embryonic mice in a very precise way – to disable genes and then observe the effect of this manipulation on their subsequent function and behaviour. This is in real time, in a live mouse embryo."

Robertson emailed her with news that his small Dunedin lab had identified the role of two genes in a rare human condition, and asked whether she was keen to collaborate. Götz responded immediately, and a powerful research partnership began. Götz and her senior research fellow, Sylvia Cappello, would inject a fluorescent protein into the embryonic brain of mice, alongside a chemical that disabled either Dachsous1 or FAT4, then put the embryo back into the uterus and watch how the intervention affected the behaviour of the fluorescent stem cells.

In this way, they were able to replicate Van Maldergem syndrome in mice. Cappello then went further, observing the "radio signal" that seemed to be orchestrating the damage, and showing that by interfering at the right time with that signal, the damage to the brain could be corrected.

"It was a heady moment to realise that on one hand we could produce a developmental disorder within the brain of a mammal with one intervention, and then with the other, reverse it," says Robertson. These experiments provided proof that they had found a mode of communication used by these two key genes to regulate the work of stem cells in the developing brain.

"It does show that it is possible to lead these international collaborations from New Zealand."

Götz says her lab could not have made this breakthrough without the benefit of Robertson's new knowledge of the effect of the two genes on human development; Robertson says his lab could not have modelled the effect in mice so quickly and precisely without Götz's techniques.

"It does show that it is possible to lead these international collaborations from New Zealand," he says.

CURING KIDS

As Russell Snell points out, Robertson's latest research achievement is not a one-off. "He regularly does this sort of work, and he does it on a shoestring. Yes, he has funding, but nowhere near the funding of the large US and European labs."

For Robertson, it all began as a young paediatric registrar at Starship Children's Hospital in the 1990s. Having grown up in a large Catholic family in Hawke's Bay, he had gone to the University of Otago to study medicine and graduated with distinction, winning the Prince of Wales Prize for the most outstanding student completing an undergraduate degree, and coming top of his medical school class.

Genetics wasn't even taught at the university while he was training, and only a small number of genetic disorders had been solved at the molecular level – the race to find the gene for cystic fibrosis was won in the late 1980s by an American group led by Francis Collins while Robertson was a student. But it was a field of research that was about to undergo explosive development.

The idea of curing children had enormous appeal, and in his second year out of medical school he went to the newly opened Starship as a house surgeon before being fast-tracked into a registrar role.

Before long, he met the family who would change his life and launch his career as a world-class geneticist.

June Miru and her family had come into the care of Starship paediatrician Tania Gunn in 1989. The close-knit whanau had begun to fear that they were afflicted by a *makutu* – a curse. June, the matriarch, had lost her first-born boy in 1963 – a child so malformed that she wasn't permitted to see him. She went on to give birth to another son and four daughters – all healthy. Then in 1988, her daughters began having babies. And the babies, if they were boys, were mostly dying at birth, their tiny little bodies cruelly twisted and tangled, with lungs compressed within malformed rib cages, with defective hearts and, in some cases, with their abdomens open and their intestines on the outside.

Gunn had taken the Miru family under her wing and, along with Professor David Becroft and the up-and-coming registrar Stephen Robertson, wrote a paper describing the syndrome.

The cause was clearly genetic and passed on by the mother. Robertson wrote to an eminent geneticist at Oxford, Andrew Wilkie, and asked nervously if he would help him find the answer to the Miru family's torment. Wilkie made a start, but it soon became clear to Robertson that he didn't have much time to invest in solving the mystery. Instead, Wilkie suggested to Robertson that he come to Oxford and have a go at finding the gene himself. "I thought, 'Wow! Going to Oxford, finding a disease gene!' – it pressed all my buttons."

But Robertson had no training in genetics. Everything he knew was self-taught. If he was to crack the answer for the Miru family, he needed to be properly schooled in the discipline. So, with his wife, GP Robyn Blake, and their infant son Nick in tow, he went to the Murdoch Institute in Melbourne – an eminent outfit bankrolled by Rupert Murdoch's philanthropic mother, Dame Elizabeth Murdoch – and studied genetics as a sub-specialty for three years.

Wilkie continued to chip away at the Miru family's problem in the meantime, but progress was slow. Again, Wilkie put it to Robertson that he should come to Oxford and hunt for the gene.

OFF TO OXFORD

So, funded by a Nuffield Scholarship, he, Robyn and their growing family (by then Nick had a baby brother, Mark), headed to the UK in 1999 with the objective of getting an Oxford PhD in genetics and delivering an explanation to June Miru and her family.

"I was absolutely obsessed," recalls Robertson of his intensive three years there. "In some ways it felt as if I was looking under every stone in a river bed. It didn't feel

June Miru and her daughter Noki-Jane Miru Saunders; the wider Miru whanau, pictured below in 2003, lost seven babies as the result of a gene mutation.

terribly creative at times and sometimes it felt quite desperate."

His hunting ground was two million base pairs of DNA, and within that, about 60 genes. He scoured the world and found other families suffering the same disorder as the Mirus; most had had only one baby with the syndrome and some, frozen with fear, had no more. What made the Mirus unique was that they had continued to have babies, providing a rich statistical foundation from which to search for genetic clues.

There were false starts when he would go home and tell Robyn that he thought he'd found the responsible gene, only to discover he was wrong. He remained in close contact with June Miru and the family, phoning regularly and keeping them up to date – even when he felt as if he was banging his head against a brick wall. While his hunt for the offending gene continued, June's youngest daughter, Noki-Jane, lost her second baby boy to the syndrome.

But June says she never doubted that this "young pup", whom she came regard as a son, would deliver the answer. "He knew we were sitting waiting."

Eventually – and increasingly desperate as his three-year scholarship was running out – Robertson began looking at a gene he'd previously ignored. In 1998, a mutation in a gene that codes for a protein called filamin A had been discovered to be the cause of a syndrome characterised by epileptic seizures. Robertson had initially discounted it, thinking that if it caused seizures it would have nothing to do with the complex skeletal syndrome suffered by the Miru family.

Using the laborious techniques available at the time, he noticed a suspicious-looking aberration in this gene in one of the sufferers of the syndrome, but he was nervous about believing what he was seeing. One evening, while having a pint with his laboratory colleagues, he grabbed a copy of the Salvation Army magazine *Watchtower* that was lying on a table, jotted his findings

DAVID WHITE; JANE USSHER

down in a margin and asked the others if they "smelt a rat".

"They looked back at me over the top of their lagers and said, 'Oh, you doofus! You've found the bloody thing.'"

Robertson's findings – co-authored by Andrew Wilkie – were published in 2003 in *Nature Genetics*. More importantly, he was able to call the Miru family together and give them the gift of an explanation, a name for the gene responsible (FLNA), and the ability to test the women of the families to ascertain if they were carriers of the mutation and to test their unborn babies to find out if they were affected.

Not long after, the family presented him with a small trophy that he still keeps on his desk in his tiny Dunedin office. The inscription reads: "The What Took You So Long Award" – a dry and understated token of gratitude for the dry and understated man who came into their lives and lightened their misery. "He lifted a veil for us as a family," says June Miru.

The inscription on the small trophy reads: "The What Took You So Long Award."

FAR-REACHING IMPLICATIONS

Sadly, Tania Gunn, who had initially taken the family into her care, died of cancer just months before Robertson made his breakthrough.

The implications of the discovery went far beyond the Miru family and others with the same syndrome. The offending gene turned out to be a "many-faced beast, implicated in a whole raft of congenital syndromes". Where the filamin proteins had previously been thought to play a rather minor role in cell development, Robertson's disclosures contributed to showing that they were a crucial regulator. The discovery launched Robertson's research career on two parallel paths: learning more about the gene's role in skeletal malformations and in neurological abnormalities.

In the middle of all this, he was asked to come home to New Zealand and establish a new research facility in Dunedin, jointly funded by Cure Kids and the University of Otago. Still only in his mid-thirties and still polishing off his PhD, he was being asked to cold-start a genetics research lab on the opposite side of the planet from Oxford. "It all felt a bit premature," he recalls.

But he and Robyn were keen to raise the family (which by then included Isabelle, born in Oxford) in a smaller centre, and the decision was made.

Even before he got the new Dunedin lab properly set up, he made another major breakthrough. He and research technician Tim Morgan were working with borrowed and shared equipment when, in a collaboration with American scientists, they discovered a mutation in another filamin-coding gene (FLNB) that caused children to be born with their joints dislocated from their sockets. The work was published in *Nature Genetics* in 2004.

Clinical geneticists from around the world began asking him to enrol their patients in his research programme, and rapidly the fridges in the Dunedin lab were filled with thousands of DNA samples.

At the same time, Cure Kids decided not only to part-fund the Otago chair in paediatric genetics (one of three research chairs funded by the charity), but to establish an "enablement" fund of $1 million over five years that can be spent on blue-skies research. Most New Zealand scientists have to scrabble around for funding that is often short-term but, Robertson says, the stability of the Cure Kids funding gives him and his colleagues space to think and "tinker". "They understand that science works by fiddling around in your tool shed, kicking the tyres on ideas, trying things out … There is so little provision for that in New Zealand."

Robertson (at rear) and his research team, with Mary Gray (in pink). Others, from left, are Sarah Holman, Tim Morgan, Zandra Jenkins, Dee Yang, Phil Daniel, Sarah Cardoso, Sophia Cameron-Christie and Heather Tiffin.

ROBERTSON FAMILY ARCHIVE

In 2009, his group headlined in *Nature Genetics* again, this time with research that disrupted contemporary thinking about the role of a mutation in what was known as a "cancer gene", called WTX, which was thought to predispose carriers to the childhood cancer Wilms tumour. Robertson's work showed that being born with a WTX mutation resulted in a severe bone disorder, rather than cancer.

The work won him the Health Research Council's Liley Medal in 2010. By this time there was rising international confidence that his small genetics lab at the bottom of the world could deliver, and the work on Van Maldergem syndrome that would eventually lead to the breakthrough published in *Nature Genetics* last month was under way.

THE TYRANNY OF DISTANCE

When Robertson left Oxford in 2003, his mentor Andrew Wilkie told him that in moving back to distant New Zealand he was electing to make his research career 50% more difficult. A fair comment? Robertson adopts his characteristically quizzical expression, head tilted a little to the side, and acknowledges that Wilkie might have been exaggerating a little but was possibly right. Despite email and the internet, there is still the tyranny of distance and the absence of a large local community of research peers. "I can't just go out into the corridor and find a Magdalena [Götz]."

But there are compensations. What his lab lacks in scale it makes up for in close and productive relationships – both within the university and with the members of the public who financially support his research through Cure Kids. New Zealanders are generous and supportive, he says, and they are "just one step away" – he spends large amounts of time explaining his work to groups.

There's also the lifestyle. On Robertson's computer screen is a glorious photo of the bright red Brewster Hut, set on a high tussock-clad ridge near Haast where he tramped recently. A few days after our interview, he and the children were off to Queenstown to support Robyn while she competed in the Spring Challenge multisport event. And the following week he was in one of his regular clinical sessions seeing children with rare genetic disorders – the coal-face work that takes up 30% of his time and keeps him constantly in touch with the real purpose of his geeky scientific endeavours.

Whether he's in Dunedin or Oxford, that, in the end, is what matters to Robertson. "Working with children is something I find enormously rewarding – knowing that we can actually make a difference to a life that is unfolding." ▮