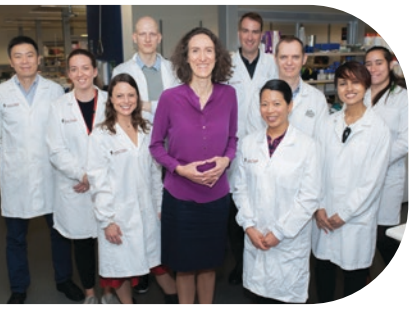
Doctors in Training Grant

FINAL REPORT



Dr Annabelle Enriquez (seventh from left) with Professor Sally Dunwoodie (centre) and the team at the Victor Chang Cardiac Research Institute

Dr Annabelle Enriquez

Master of Medicine
(Developmental Biology)

University of New South Wales
Sydney, New South Wales, Australia

Thanks to the MIGA Doctors in Training Grants Program, I've been able to pursue part-time research studies while continuing part-time training in Clinical Genetics. I am now completing my clinical training, and am approximately halfway through my research degree, having recently transferred from a Masters to a PhD in Medicine.

I have been undertaking my research under the supervision of Professor Sally Dunwoodie at the Victor Chang Cardiac Research Institute (VCCRI). The Institute's mission is 'the relief of pain and suffering, and the promotion of wellbeing, through an understanding of the fundamental mechanisms of cardiovascular biology in health and disease'. Professor Dunwoodie is an internationally-renowned developmental biologist and a world leader in the discovery of genetic and environmental causes of congenital vertebral defects. She also leads the largest Australian Genome Sequencing Initiative seeking to undercover the genetic causes of congenital heart disease.

The goal of my research is to identify the causes of congenital anomalies, specifically the genetic changes that lead to cardiac and vertebral malformation.

Congenital anomalies, or structural organ defects, affect 3-6% of live human births. In Australia, they are the most common cause of death among babies, accounting for about a third of perinatal deaths. Those who survive usually go on to have permanent disability and/or lifelong disease. Congenital anomalies affect more children than most chronic childhood diseases including autism, cancer and type 1 diabetes. The most common birth defect is congenital heart disease (CHD), affecting 1 in 100 live births. CHD refers to a heterogeneous collection of cardiac defects that range in severity from self-resolving to life-threatening. Interventions include serial surgeries and life-long medical treatment, resulting in considerable economic burden and significant demand for medical resources. Congenital vertebral malformations (CVM) are also variable, and although less prevalent than CHD, CVM are still encountered in approximately 1 in 1,000 live births. CVM may also lead to a spectrum of clinical effects that include chronic pain, respiratory restriction, neurological compromise, permanent disability and premature death. Congenital anomalies occur in isolation or in conjunction with defects in other organs, aggravating the significant burden of disease on families and society in general. Despite these devastating consequences, the underlying cause of these anomalies is known in only about 20% of cases. We now recognise that congenital anomalies may be caused by genetic or environmental factors, or a combination of both. Much research remains to be done to elucidate these causes.

Dr Annabelle Enriquez
Master of Medicine, Sydney

My research has given me the opportunity to be involved in the care of patients with congenital anomalies from the bedside, to the laboratory, and back to the bedside. As a trainee in Clinical Genetics at the Children's Hospital at Westmead and Liverpool Hospital, I have met several families who have had at least one member affected with a congenital anomaly in one or more organs.

These families are referred to our team for a genetic assessment to identify the underlying cause of these anomalies. Only by identifying the molecular cause of these anomalies are we able to predict prognosis, guide future disease surveillance or explore targeted therapy. Without a genetic diagnosis, we are unable to offer families an accurate recurrence risk or options for pregnancy planning. More often than not, parents continue to blame themselves for somehow causing this anomaly in their child. Given the paucity of known genetic causes, there is currently no meaningful genetic test available that we can offer. Enrolling in research is the only avenue available for these families in their search for a diagnosis. Families seen in other hospitals in the country and around the world are also able to participate in our research if they fulfil our selection criteria.

Genomic DNA from affected and unaffected individuals in enrolled families undergo whole genome sequencing (WGS). At the start of the millennium, it took 23 laboratories 13 years and $3 billion to sequence the entire human genome. There are now powerful sequencing machines that can sequence up to 18,000 entire human genomes annually; we have been fortunate to have one such system, the HiSeq X Ten sequencing platform, next door to the VCCRI at the Garvan Institute. We have been able to perform WGS using this system. The challenge is finding the single genetic variation that has led to the congenital anomaly in each family (of those that are caused by single mutations). Currently, over 24,000 human genes are recognised, yet less than 4,000 genes are associated with a human disease phenotype. At VCCRI, I have learned to analyse the huge amounts of WGS data under the guidance of some of the best biomedical researchers in the country. Bioinformatic filtering significantly narrows the number of gene candidates, often allowing us to pinpoint the likely gene responsible. However, before we can establish a clinical connection between the genetic variation identified and the disease in question, we need to confirm the sequencing findings by performing further studies *in vivo* and *in vitro*. I have been able to go back to the clinic to obtain more biological samples from the family members to help validate our candidate gene. In the Dunwoodie laboratory, I have also learned how animal models are used to recapitulate the human phenotype. I have also been learning about embryonic development not just from ground-breaking work in our laboratory, but also from hearing about cutting-edge research being performed by other scientists who have spoken at the VCCRI or the Garvan Institute. Moreover, being part of Professor Dunwoodie's research group has led to fantastic collaborations with other clinicians and scientists all over the world.

Such collaborations led to our landmark discovery that was published this year in the New England Journal of Medicine. In our paper, we describe how we identified the genetic cause of multiple congenital anomalies in four distinct families.

The genes we identified are involved in a biological pathway that has never before been implicated in congenital anomalies, or even in embryonic development.

The genetic defects in this pathway led to a deficiency in the molecule nicotinamide adenine dinucleotide (NAD). NAD is an essential molecule in living things, required in hundreds of cellular reactions leading to energy metabolism, DNA repair, and effective gene transcription. It is so important that there are at least two pathways leading to its synthesis: the de novo pathway that is affected in our patients, and a salvage pathway using vitamin B_3 or niacin. Furthermore, in our mouse models, we found that giving vitamin B_3 to the pregnant mice prevented the pups from developing congenital anomalies.

Thus, not only have we discovered an important cause of congenital anomalies, we may have also found an easily accessible way to prevent these in humans carrying the same genetic defect, though this will require confirmation through further research and rigorous clinical trials. We are continuing with further laboratory and clinical research to enable translation of these findings more broadly.

These research findings have already had an immediate and profound impact on the families involved. Knowing the specific genetic cause has enabled me to give an accurate recurrence risk to the affected families. I was also able to organise early prenatal diagnosis for one of the mothers who feared that she might have another child with multiple congenital anomalies. Having the genetic cause ascertained empowers family members in the current and successive generations when making reproductive decisions, which is invaluable. I have discussed vitamin B_3 supplementation for the mothers who may be planning more pregnancies. I am also exploring the value of vitamin B_3 supplementation in the surviving individuals with congenital anomalies, as the vitamin may at least attenuate the further development of symptoms.

In addition to the direct benefits to the families enrolled in our research, identification of causative genetic pathways will have significant clinical impact in other families affected with congenital anomalies. I have had the opportunity to share our research findings with other clinicians who care for patients with similar genetic defects. We are also studying variations in other genes associated with NAD production and function. Our results will lead to the availability of diagnostic genetic testing, which may allow other families worldwide to obtain a genetic diagnosis. In some families, the emotional and psychological cross of having a child with a congenital malformation (CM) outweighs the clinical burden of disease. Many families endure a diagnostic odyssey as they try to find the reason why their child, or children, are affected. A definitive molecular diagnosis is important for overall patient management, even when it does not necessarily change treatment. It exonerates the parents, as they usually feel guilt for somehow causing the CM. It provides relief from the uncertainty, allowing families to move on, plan for the future and possibly explore therapeutic options.

This work will also underpin further efforts to unravel the complex interplay between genetic and environmental factors affecting embryogenesis, making an important contribution towards shaping guidelines regarding pre-conception and antenatal care, as well as increasing therapeutic strategies to reduce the incidence and severity of birth defects.

My research to date has been rewarding in so many ways, and I would like to express my sincere gratitude to MIGA for supporting my training.

Doctors in Training Grant

PRELIMINARY REPORT

Victor Chang Cardiac Research Institute

Dr Annabelle Enriquez

Master of Medicine
(Developmental Biology)

University of New South Wales
Sydney, New South Wales, Australia

In 2015 I enrolled in a Master of Medicine degree through the University of New South Wales, with the aim of studying genetic defects that may affect the development of the spine and the heart.

In clinical genetics, a significant proportion of the patients we see have birth defects affecting one or more body parts, resulting from genetic and/or environmental factors. Congenital malformations occur in 3-6% of human births, and are the leading cause of infant death. Despite the high prevalence and devastating impact of these defects, the underlying genetic causes of most malformations are unknown. This lack of a genetic diagnosis is incredibly frustrating for both families and the healthcare teams responsible for their care. Without a genetic diagnosis, we cannot predict prognosis, identify the best treatment, test other family members nor help plan future pregnancies.

To try to address some of these deficits in knowledge, I chose to begin a Master of Medicine, focusing on identifying the genes responsible for some of these malformations.

Specifically, I wanted to learn how to utilise the latest advances in gene technology, in the hopes of finding answers for the families I see day to day.

Additionally, I wanted to understand how the environment of the developing fetus can influence gene function, and lead to disease. Currently, I am recruiting patients and analysing their genomic data to try and identify the genetic causes of their congenital malformations. My aim is to identify gene mutations that cause congenital vertebral and heart defects using high-throughput DNA sequencing (next generation sequencing), specifically employing whole exome sequencing or whole genome sequencing. My research allows me to offer families a chance to find a diagnosis, when previously there were no further genetic tests we could pursue.

My research is based in the laboratory of Professor Sally Dunwoodie, part of the Developmental and Stem Cell Biology Division at the Victor Chang Cardiac Research Institute. Prof Dunwoodie is one of the most successful developmental biologists in the country, having published paradigm-shifting papers on the development of the embryo. The Dunwoodie laboratory is a world leader in identifying the genetic and environmental causes of congenital vertebral malformations.

This training provides me with the opportunity to use cutting-edge technology to tackle the genetic causes of developmental malformations. We now have the ability to look at the entire sequence of the human genome, an almost impossible challenge just twenty years ago. Our major challenge now is learning how to read and understand this enormous amount of genetic information. We know that not all variations in the genetic code lead to disease. Out of three billion base pairs, finding one specific mutation that causes a congenital malformation is akin to finding a needle in a huge haystack. At the Victor Chang Cardiac Research Institute, I have the opportunity to learn how to analyse and manipulate genetic data with some of the best developmental biologists and bioinformaticians in Australia. The MIGA Grant has enabled me to purchase computing equipment that is powerful enough to interrogate the astronomical amounts of genomic data generated from sequencing our patients' DNA. This will hopefully enable me to identify some of the genes that are essential for the development of the heart and the vertebral column in the embryo.

Given that we have entered the post-genome era, it is important to be able to deal with and interpret the vast swathes of genomic data that can be generated more easily and cheaply than ever. The problem lies not so much in generating the data but making sense of it for clinical benefit.

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PRELIMINARY REPORT

Dr Annabelle Enriquez
Master of Medicine, Sydney

The institute was established in memory of the late Dr Victor Chang with the vision of reducing the incidence, severity and impact of heart diseases

I am using bioinformatics to sift through genomic data to identify potential gene candidates. Once a candidate gene has been identified, confirming its involvement in developmental malformations is critical as it allows us to better translate the genetic findings into clinical practice.

By being part of the Dunwoodie laboratory, I am learning how animal models are used to confirm and validate the role of gene mutations in causing malformations. Furthermore, working at the Victor Chang Cardiac Research Institute has allowed me to hear about other groundbreaking research by local and international scientists.

This training will arm me with the skills to make a real difference in a clinical setting and furthermore, the knowledge gained from these studies has the power to reveal the identity of genetic defects that afflict a large number of patients. The aim of a genetics service is to help individuals and their families live and reproduce as normally as possible.

The findings of my research will have an immediate and profound benefit for the families involved. An accurate genetic diagnosis provides a cause for the congenital malformation. Identifying a genetic cause removes the uncertainties and exonerates the parents, particularly the mothers, of the burden of guilt that they may have somehow caused the birth defect.

It makes it possible for us to determine prognosis. Additionally, it allows the family to move on and make informed reproductive choices. A confirmed genetic mutation enables us to give an accurate recurrence risk and may be used for prenatal testing and pregnancy planning. Antenatal genetic diagnosis of an affected baby can then facilitate earlier intervention if necessary. We can also offer testing to relevant family members to see if they are also carrying the genetic change and are at risk of having an affected child. Hence it has benefits for current family members and for future generations.

For my research, I have been identifying and enrolling suitable families from my local hospitals and across Australia. Finding causative genes will allow us to offer diagnostic testing to other families presenting to a genetics service, both locally and globally. As a direct result of previous research on congenital vertebral malformations in the Dunwoodie laboratory, there are now genetic tests available worldwide for these malformations. I expect a similar progression to occur from my research results.

Moreover, finding a genetic cause will allow researchers to search for rationally designed, targeted treatment. My research is working towards the ultimate goal of disease prevention. It will also underpin further efforts to unravel the complex interplay between genetic and environmental factors that causes birth defects, making an important contribution towards shaping guidelines regarding pre-conception and antenatal care both in Australia and abroad, as well as increasing therapeutic options to reduce its incidence and severity.

I'm very grateful to MIGA for supporting my research training, which will allow me to be involved in most of the aspects of the bench to bedside cycle in caring for patients and their families.