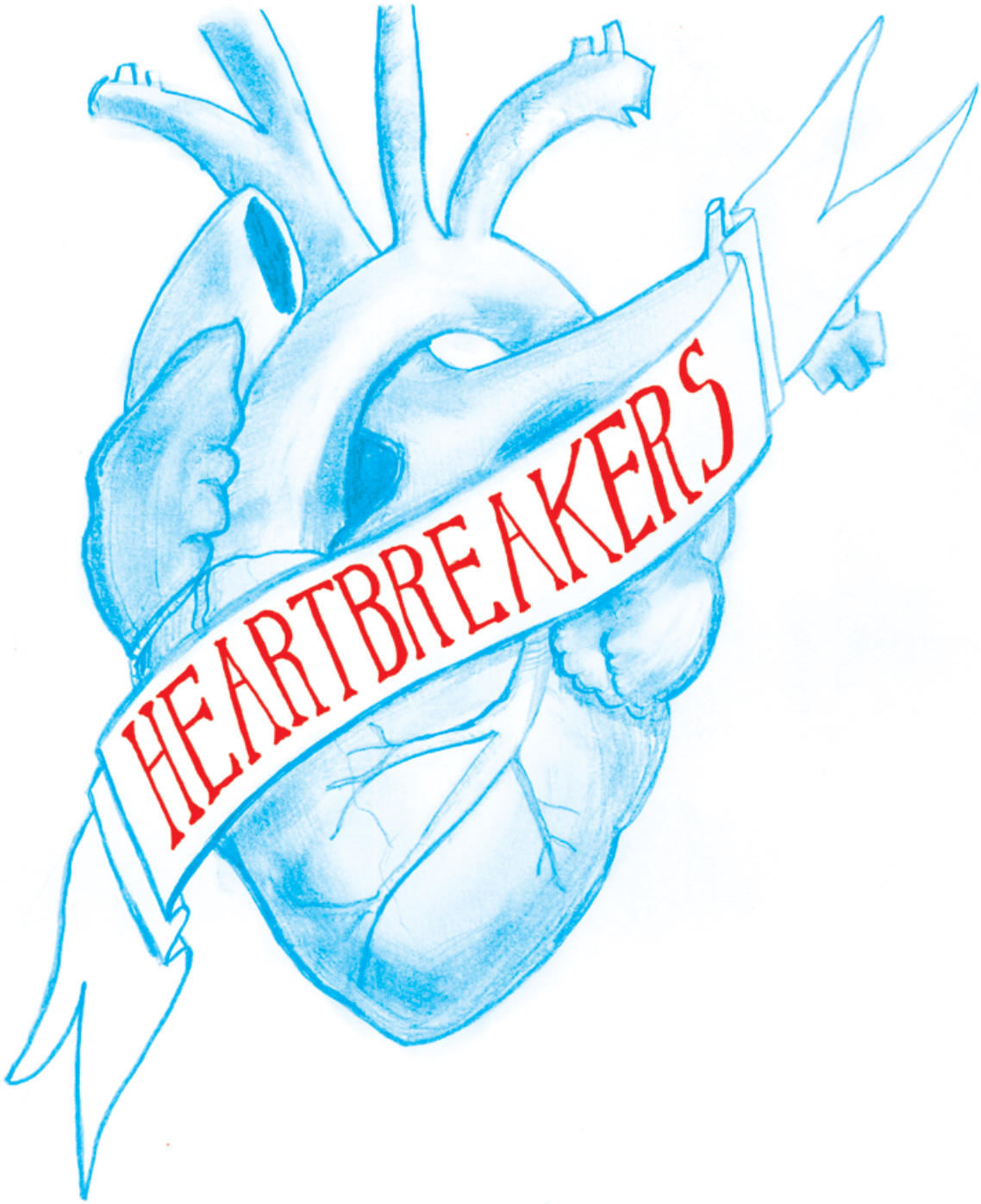

Illustration by Jean Claude Vancell

Every person possesses the same genes within every cell. Their DNA provides the information to first create an entire functioning body and then keep it running. While all humans share more than 99.9% of their DNA, it is the subtle differences in our DNA that ensure individuality. Many differences are superficial effects, like hair colour, but some can have disastrous health effects. ***Scott Wilcockson*** *talks to* ***Dr Stephanie Bezzina-Wettinger*** *(Faculty of Health Sciences, University of Malta) about her research on these subtle differences and how they can contribute to heart attacks.*

By this time next year, around 17 million people will have been lost to heart or cardiovascular disease. Almost half of these deaths will have been due to coronary heart disease, commonly resulting in heart attacks (myocardial infarction)—the world's leading cause of death. While mortality rates are steadily declining throughout Europe, the death rate in some countries, including Malta, remains much higher than the EU average.

Unfortunately, our modern way of living threatens to turn the tide against reducing deaths. A growing love for junk food and a loathing for vegetables is leading to high rates of obesity and diabetes, while alcohol, tobacco, and other drugs are abused of regularly. Altogether, these lifestyle factors account for around a third of all cardiovascular disease in the developed world. But this information is nothing new. We have known about this for years.

PREDISPOSED TO A BROKEN HEART?

These lifestyle factors act on our genetic make-up. Like most other conditions, cardiovascular disease has a genetic element. Back in 1994, a study into death by coronary heart disease in twins showed that genetics plays a role in our susceptibility and accounts for 40–60% of the variability between individuals. Research being carried out by Dr Stephanie Bezzina-Wettinger and colleagues is looking into how genetics can have a hand in driving heart attacks in the Maltese population.

'Many years before a heart attack, the artery wall develops what is known as an atheroma plaque,' Bezzina-Wettinger explains. 'This is where cells from the blood start to accumulate, begin taking up fat, and secrete a lot of inflammatory molecules [which have a number of roles, including attracting more blood cells and promoting blood clotting]. At some point the plaque can rupture, liberating its contents into the bloodstream [which] can trigger blood clotting. This can then either heal [...] or end up causing a [heart attack] because heart tissue dies off [as] it is starved of oxygen and nutrients.'

Now a large collaborative study headed by Bezzina-Wettinger is investigating the genetics that leave the Maltese susceptible to plaque formation, known as atherosclerosis, and subsequent heart attack. The Maltese Acute Myocardial Infarction (MAMI) study is focused on three key topics: inflammation, fatty lipid and cholesterol deposition, and blood clotting. The idea is to search for genes that could in some way contribute to each of these three processes.

IT'S ALL IN THE GENES

So genes are the instructional element of DNA and can be imagined as a specific sequence of letters. These letters are read to provide the blueprints for the construction of proteins that regulate every aspect of our biology. While we all share the same set of genes, the exact sequence of letters can vary from person to person resulting in different variants of the same genes. 'All of us have literally tens of thousands of [gene] variants,' clarifies

Dr Stephanie Bezzina-Wettinger

Bezzina-Wettinger, and the effect that these variants have on us, if any at all, depends on multiple different factors. 'The genetics of [heart attacks] is very complicated. We talk about it as a complex disease, [...] one caused by a mixture of genes and [variants] that interact with one another as well as with the environment, such as lifestyle and physiological factors like diabetes.' These interactions can make identifying the gene variants to blame for complex diseases harder than usual and this is further complicated by the fact that many of these gene variants are quite common. These variations are known as polymorphisms. This is the idea that a single type of object or animal can come in multiple different forms or shapes. The most dramatic biological example is the existence of males and females. Genetic polymorphism refers to the same gene existing in a population in multiple variations. For example, there are a small group of polymorphic genes that determine your hair colour and so, whether your hair is blonde, brown, black, or red depends on which gene variants you receive from your parents. Bezzina-Wettinger explains that many of the genes we know are linked to heart attacks are polymorphic. These are the focus of her research.

Malta has its unique genetic mix so these studies could not be blindly applied to the Maltese population.

DISSECTING THE MALTESE HEART

Previous studies carried out on other nationalities worldwide have identified groups of gene variants associated with an increased risk of heart attack. Malta has its unique genetic mix so these studies could not be blindly applied to the Maltese population. Bezzina-Wettinger explains that 'we knew a lot about the epidemiology but in terms of the genetics, there was nothing when we started.' To study the Maltese population they had to start from scratch.

Collecting all the material necessary for population-based studies can seem like a mammoth task and the MAMI study is a substantial project that involves collaboration over many disciplines. The team consists of clinicians, technicians, and cardiologists from Mater Dei Hospital, as well as geneticists from the University of Malta. Just over 1,000 participants were involved, including around 400 who

had had a heart attack, 400 'control' participants who did not, and 200 relatives of heart attack victims. A lot of data was obtained for every single patient, starting off with each answering an extensive questionnaire that delved into all aspects of their daily lives. This was accompanied by a number of biochemical tests to gauge their general health, such as whether liver and kidney were functioning correctly. These tests provided an overview of the general lifestyle and health of the patients involved in order to determine the lifestyle risk factors that predispose the Maltese people to heart attack.

The study's early results have shown that the Maltese are not immune to the conventional risk factors for heart attack including smoking, diabetes, high blood pressure, and high cholesterol. Regular consumption of alcohol can be associated with decreased risk of heart attacks, but before running to the bar to get another drink, you should only be having a few drinks a week. Too much alcohol has the exact opposite effect. Binging on six drinks or more on a daily basis greatly increases your risk. Moderation is key.

The next step will be to look into the genetics. To do this, the team are using Next Generation Sequencing technology that vastly decreases sequencing costs. Since the advent of the Human Genome Project in the 1990s, our ability to sequence DNA is becoming increasingly easier, cheaper, and readily accessible, along with the power to process, store, and analyse the vast amounts of data this technology produces. Bezzina-Wettinger's team are using a method known as Whole Exome Sequencing that focuses specifically on genes and misses out the DNA in between. 'We take one group of proteins that we think are involved [in heart attacks] and look at all their genes—there can be something like 100–200—and then we look for the variants.' This technology has enabled Dr Ritienne Attard, a former Ph.D. student of Bezzina-Wettinger, to compare the variation in sequences between members of the same family. By doing this, Attard hopes to identify gene variants that could predispose individuals to heart attack.

ADDING COMPLEXITY: THE GENE-LIFESTYLE LINK

What you have to keep in mind when studying the genetics of complex diseases, like heart attack, is that you can be looking at very common gene variants. 'If you study the genetics alone you don't really get anything conclusive [....] You could start to see a genetic [variant] that has no effect in the general population. But, for example, in smokers with this [variant, you find] they will have a higher risk. So we do see these kinds of gene-lifestyle interactions'. This phenomenon is prevalent in all complex diseases, including the plethora of cardiovascular diseases, type 2 diabetes, and many common cancers. Thus we can introduce the idea of one being predisposed to a particular illness.

The initial results of the MAMI genetics study show just this. Bezzina-Wettinger describes one of the team's results whereby a single letter is changed in the sequence of one gene. 'In [people] who are non-smokers, non-diabetic, and have low cholesterol, the risk is the same as the base line population. But in smokers, diabetics, or [people with] high cholesterol the risk goes up 6.6-fold. Now, only a part of this is due to the [lifestyle factors] because someone with the same lifestyle factors but without this [gene variant] only has a four-fold increased risk.' The difference between these two is down to the gene variant which does nothing at all in a healthy individual. Inherited genes play a role, but there is a chance to change the outcome with the lifestyle you choose for yourself.

THE BEATING HEART OF MALTA

So is the Maltese population particularly predisposed to heart attacks? Answering this question is not straightforward. The genetic information obtained so far relates to single families and is not representative of the entire population. While the genetic variants, or polymorphisms,

Inherited genes play a role, but there is a chance to change the outcome with the lifestyle you choose for yourself.

Photo by Yevgen Belich / Shutterstock.com

that have so far been studied are common, the frequency can vary greatly from one population to the next. In addition, lifestyle factors can also vary. For example, one nation's average diet can be very different to that of the neighbouring country. Bezzina-Wettinger adds that 'the prevalence of diabetes is higher here and this [provides] a background within which some of these gene variants have an effect.' The results of the MAMI study have yet to be fully published and proposed future work includes the sequencing of the exomes of all 1,000 participants. This will markedly increase the team's ability to identify the gene variants that increase risk to disease and link them to the environment and lifestyle of the Maltese population.

According to the World Health Organisation, there are over 300 risk factors associated with coronary heart disease including depression, low socioeconomic status, and illicit drug use. This information could be useful to policy-makers when aiming to reduce the incidence of heart attack in the general population. However, we know the major risk factors in the developed world with well-founded gene-lifestyles links (smoking, obesity, alcohol use, high blood pressure, and high cholesterol) and so these will remain key targets in the fight to reduce heart attack incidence. The advent and growth of this field of research now presents new possibilities when it comes to patient treatment or the application of preventative measures.

Accompanying the arrival of cheap Next Generation Sequencing technology, there has been an explosion in the field of personalised medicine. This is the idea of tailoring a patient's treatment to the individual. Everyone has a unique genetic makeup that can influence how they react to treatments and drugs. This is already being applied in some cases, particularly in certain types of cancer, to determine the best course of treatment for that individual. However, Bezzina-Wettinger believes we are not there yet when it comes to heart attacks. 'The very fact that the influence of a gene can change depending on the lifestyle of that individual makes it far more complex... Eventually the major drivers [of heart attack] will be identified and treatments, either direct or preventative, will be developed.' So while the age of personalised medicine may have already begun, our knowledge of the genetics of disease still has a way to go yet.

Annoyingly, complex diseases are quite complicated.

This research by the University of Malta forms part of the IAAMI and NGS projects conducted in collaboration with Mater Dei Hospital and funded through the National Research and Innovation Programme (Malta Council for Science and Technology). The study's principal investigator is Dr Stephanie Bezzina-Wettinger with Dr Rosienne Farrugia and Dr Ritienne Attard as close associates.