Editorial

# Cardiovascular Genetics – Two Steps Forward, One Step Back

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The recent publication of 2 studies of the genetics of coronary heart disease[1,2] provides an opportune moment to reassess strategies and progress towards the time when the genetic investment in this common cause of death and disability will be repaid with better detection and prevention.

These 2 studies are important because they are large and their findings appear to be consistent. The genetic literature has been plagued by small and discrepant reports of genetic discovery. So much so that journal editors are beginning to demand more stringent definitions of statistical significance[3] to avoid publishing false positive results.

However, one must not assume that inconsistency equates to error, as there is sufficient genetic heterogeneity in different populations around the world to explain why one study might be positive and another negative. Even within the recent reports, the authors found that the association of myocardial infarction with a sequence variant on chromosome 9p21 was evident in Caucasian but not in black subjects.[2]

The size of a study is important as it determines statistical power to detect genetic associations.[4] The numbers needed to study depend on whether one takes a pessimistic or optimistic view of the likely frequency and magnitude of effect of influential genetic variants. The pessimistic view that subscribes to infrequent variants of small individual effect has led to predictions of sample sizes in the hundreds of thousands. This view no doubt was a stimulus for the studies of Helgadottir et al and McPherson et al to include a combined total of about 40,000 subjects.

However, these studies revealed surprisingly that the genetic variants were relatively common, with homozygotes (carrying 2 copies of the variant) comprising about 20% of the Caucasian population. The results also showed that the effect size was not inconsequential, with these homozygotes displaying an apparent 30% to 100% increase in risk of myocardial infarction.

This genetic risk is less of a risk than smoking, hypertension, diabetes or even a positive family history of coronary heart disease in a first-degree relative, which are associated individually with a 200% to 500% increase in risk, and much higher when combined. Nevertheless, the genetic variant might be something worth testing, and may provide information independent of traditional coronary risk factors, with which it does not appear to be associated.[2]

Notwithstanding the excitement over these 2 new studies, we need to reconcile the fact that despite an accumulation of other genome wide studies of coronary disease, this is the first time that the locus on chromosome 9 has emerged as potentially important. Some of the previous negative studies[5-7] and meta-analyses[8,9] have also involved thousands of subjects. Most of these studies have involved Caucasian subjects. It seems unlikely that they just missed chromosome 9 altogether because of design deficiencies. The explanation remains a mystery, and should temper calls for the widespread adoption of the new markers as validated clinical tests.

The other important finding in the studies of Helgadottir et al and McPherson et al is that the associated DNA variants (single nucleotide polymorphisms – SNPs) map to a region between genes. Even the genes in the region are not what one might consider candidates for coronary disease.

The two genes in the vicinity – *CDKN2A* and *CDKN2B* – encode isoforms of cyclin-dependent kinase inhibitors. They are involved in cell growth signalling, and *CDKN2A* is believed to act in a tumour suppressor capacity, being frequently mutated or deleted in a wide variety of tumours. The expression of *CDKN2B* is induced by TGF beta, also suggesting its role in growth inhibition. One could perhaps propose some disruption of the function of these genes in a way that might lead to some of the atherogenic cellular proliferation and plaque instability that have been observed in coronary arteries.[10,11]

These genes might be somewhat of a surprise, but the fact that the associated SNPs are in non-coding DNA perhaps should not be. Whereas Mendelian major genetic mutations affect coding sequences, resulting in premature truncation of proteins or substitution of key amino acids, it is emerging that the DNA variants that underpin common conditions exist in the non-coding DNA that determines gene expression. This can alter tissue specific or developmental-stage specific expression and exert quantitative rather than qualitative effects on gene products.[12]

The challenge is to recognise the impact of a SNP on non-

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coding DNA. That would be simpler if we were able to read the language of these sequences on either side of genes, and between the coding sequences within genes. Unfortunately, we are still learning and much of that discovery will come from taking sequences and their variants and testing their physiological effects.

This is not a trivial exercise, as it requires some means of expressing the genes in an environment that mimics their normal control mechanisms. Our ignorance of the details of such control mechanisms places investigators in something of a Catch-22 situation. For example, some gene regulatory elements can be thousands of bases away from the gene itself.[13] Taking such possibilities into account requires the expression of the gene with long stretches of DNA on either side in the so-called minigenes.[14] This important field is in its early days, yet holds the key to understanding genetic mechanisms in common disease.

It also needs to be emphasised that the location of genetic variants relies more on statistical methods than it does on molecular biology. These days, gene chips with 1 million SNPs are becoming routine. The problem is sorting the wheat from the chaff, and here genetic statisticians are essential. Many do not realise that the cutting edge of biostatistics is trying to keep up with the leading edge of molecular biology.

Indeed, the development of statistical modelling of such data is *bone fide* research in itself. Gone are the days of "off the shelf" statistical programs for analyses. Unless a research team has dedicated expert biostatisticians to model the precise data set, then progress is severely limited.

On this background, what are the implications for Singapore? First, studies need to be population-based, for it is in this setting that the relevance of genetic testing begins and ends. Second, the studies need to be large. Third, as there are likely to be differences between population groups, each major racial and ethnic group needs to be studied separately.[15] Fourth, modern molecular, high throughput technology is needed to scan the genome. Fifth, biostatisticians must be involved to analyse the data in meaningful and informative ways. Sixth, strong links between molecular biologists and physiologists and clinicians must exist to validate and understand the biological consequences of DNA variants.

In many of these considerations, Singapore is very well positioned internationally. The Singapore Consortium of Cohort Studies led by Professor Chia Kee Seng[16] addresses the first 3 requirements, and the Matrix and Genome at the Singapore Biopolis are meeting the fourth and, to a degree, the fifth requirements. However, as faced in other countries, there is a pressing need to provide clear and rewarding career pathways in genetic biostatistics for undergraduate students in mathematics and statistics.

The translational type of research relies on more focused laboratory and hospital groups, such as those of Dr Lee Yung Seng in genetic determinants of childhood obesity,[17-19] Dr Heng Chew Kiat in coronary artery disease risk profile[20-22] and Professor Vernon Oh in hypertension.[23] Professor Oh and Associate Professor Ling Lieng Hsi are developing new areas such as diastolic heart failure. Dr Martin Lee works on transcriptional and post-transcriptional molecular switches for orphan nuclear receptors, which may regulate transcription factors and their targets in cardiovascular tissues.[24] This is just to name a few.

There is a real potential that, with appropriate funding, collaboration and organisation, Singapore could make major contributions to the genetics of common conditions such as hypertension and coronary heart disease. Perhaps the most exciting potential comes from being able to address genetics in members of 2 of the largest population groups in the world, the Indians and the Chinese. Hopefully, the genetic discoveries in Singapore will significantly mitigate the devastating effects of the coronary heart disease epidemic destined to sweep across China and India.

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