Student: Lauren Teape

Project: The dawn of long noncoding RNAs as circulating cardiac biomarkers

Supervisors: Professor Vicky Cameron and Dr Anna Pilbrow

Sponsors: Canterbury Medical Research Foundation

Introduction:
Cardiovascular disease (CVD) is the leading cause of mortality in New Zealand, accounting for 30% of all deaths.

To predict who is at risk of a future cardiac event, GPs use the New Zealand 5 year cardiovascular disease risk score. However, this risk score fails to identify many at risk individuals, with more than 50% of heart disease deaths occurring in people with no previous symptoms or warning signs. Moreover this measure does not perform well in predicting CVD risk in Māori and Pacific peoples who have high prevalence of coronary heart disease. We therefore need new screening strategies in New Zealand to predict cardiac event risk.

Identifying circulating biomarkers could provide a new way of predicting impending cardiac events. It has recently been uncovered that most of the human genome encodes RNA transcripts as opposed to active protein-coding genes. These non-protein-coding transcripts, once thought of as "junk DNA," are now known to have varying biological functions and also evidently play important roles in disease inheritance.

International Genome Wide Association Studies have found over 90% of genetic variants associated with disease and complex traits are found in these non-protein coding regions. Thus new cardiac disease biomarkers may be found in the non-protein coding regions of the genome.
One class of non-protein coding regions of the genome is long non coding RNA (lncRNA). Some lncRNAs have been detected in the blood and seem to be associated with CVD.

This project investigates the potential for lncRNA detected in blood as predictive biomarkers for future cardiac events.

Aim:
To determine if plasma levels of lncRNA can predict future cardiac events in a prospective cohort of volunteers who were healthy at recruitment.

Method:
We measured lncRNAs in an initial screening cohort (n=85) and validation cohort (n=100), of which approximately half of each cohort went on to have a cardiac event since recruitment and half remained heart healthy. RNA was extracted from plasma samples and converted to complementary DNA.

Real-time Quantitative Polymerase Chain Reaction (RTqPCR) assays for eight lncRNA were established.

This method determines the amount of lncRNA there was originally in the plasma based on how quickly the lncRNA in the sample amplifies to exceed a certain level. Out of the eight lncRNAs, three were stably detected in plasma (KCNQ10T1, H19 and LIPCAR).

Statistical analyses using SPSS software tested associations between levels of these three lncRNAs comparing those who went on to have a cardiac event with heart healthy groups and also associations with clinical measures of cardiovascular disease.

Results:
Despite an initial promising result for the lncRNA H19, in the independent validation cohort H19 was not higher in volunteers that had cardiac events since recruitment compared to those who had not ($p=0.373$). KCNQ10T1 and LIPCAR were also not at significantly different levels between those people who had cardiac events and who did not in both screening and validation cohorts ($p=0.943$, $p=0.497$).

However, an interesting association was found in the screening cohort between KCNQ10T1 and type II diabetes. Higher KCNQ10T1 levels in those volunteers who had type II diabetes were found ($n=10$) compared to those who did not have diabetes ($n=75$) ($p=0.048$).

This association is to be tested in another cohort containing more diabetes patients as well as pre-diabetes patients.

Conclusion:
While LIPCAR, KCNQ10T1 and H19 were detected in plasma, these are not suitable plasma biomarkers for future cardiovascular events. No association was found between levels of these three lncRNA and future cardiac events.

However, higher levels of KCNQ10T1 in type II diabetes patients indicate this lncRNA might be a potential biomarker for type II diabetes. Previous studies have linked KCNQ10T1 excess insulin secretion.

Excess insulin secretion is common in people with type II diabetes as this usually results in resistance to insulin and high blood sugar levels. Thus there are plausible biological connections that may underpin the observed association between high KCNQ10T1 levels and type II diabetes.

Current diabetes screening is able to identify many individuals with pre-diabetes but fails to accurately predict who will actually go on to develop type II diabetes. Further work beyond this project will determine if KCNQ10T1 might compliment the current methods for diabetes risk prediction and diagnosis.

This would allow preventative action to be put in place to minimise development of type II diabetes in at risk patients. To summarise, while none of the three lncRNAs examined in this project proved to be predictive markers for future cardiac events, the lncRNA KCNQ10T1 was found associated with type II diabetes. A serendipitous discovery that, if validated, may be an exciting advance in prediction and prevention of type II diabetes.