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Title: Improving Cardiovascular Risk Prediction in the General Population

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Sponsor: New Zealand Heart Foundation

Introduction:

Coronary heart disease can result in a heart attack and is a leading cause of death in New Zealand, accounting for over 40% of deaths per year. To screen patients for their risk of having a cardiovascular event such as a heart attack or stroke, a GP will refer to the New Zealand Cardiovascular Risk Charts. These charts use traditional risk factors associated with heart disease; age, sex, lipid levels, diabetes, smoking and blood pressure to estimate the likelihood of a cardiovascular event occurring in the next 5 years.

However, traditional risk factor profiling fails to identify many individuals who go on to have an event, with more than 50% of heart disease deaths occurring in people with no previous symptoms or warning signs and who, using current screening, would be considered to be at only moderate cardiovascular risk. There is a need to improve our screening model so that we can better identify people for whom an acute cardiovascular event is imminent.

A newer risk prediction model could include biomarkers that can be measured in blood in addition to traditional risk factors. To identify potential markers, we investigated proteins in blood that differ in abundance between individuals who are genetically susceptible to coronary heart disease, compared with those who aren't.

Within the last decade, new genomics technologies have identified key regions of the human genome (the DNA carried on our chromosomes) associated with risk of coronary heart disease. One region of DNA on chromosome 9 (Chr9p21) is most strongly associated with inherited risk for coronary heart disease. The DNA at Chr9p21 comes in two forms: a high-risk form and a low-risk form. People who have inherited two copies of the high-risk form, one from each parent, are at a 60% greater risk of developing coronary heart disease than people who possess two copies of the low-risk form.

Based on previous work in our laboratory, we hypothesised that carrying the risk form of the Chr9p21 DNA region may be associated with altered blood levels of three proteins related to cardiovascular health; TGF-B, VCAM-1 and calreticulin. We also hypothesised that altered blood levels of these proteins may be associated with an increased risk of an impending cardiovascular event within the next 3 to 5 years.

My key resource for this studentship is a group of ~3000 blood samples taken from a cohort of healthy older people recruited for the Canterbury Healthy Volunteers study. At the time these participants donated blood they had no signs of cardiovascular disease. However, in the years following sample collection, some of these participants developed cardiovascular disease and experienced a cardiovascular event. Testing these samples retrospectively allows us to compare biomarker levels in blood between people who experienced an adverse cardiovascular event within 3 years and people who had remained event-free for at least 5 years.

Aims:

1. To determine whether genetic variation at the Chr9p21 risk region is associated with adverse cardiovascular outcomes in healthy volunteers with no previous history of heart disease.
2. To determine whether genetic variation at the Chr9p21 risk region is associated with a differing levels of TGF-B, VCAM-1 and calreticulin in blood, even in healthy volunteers with no previous history of heart disease.
3. To determine whether variation in protein levels of TGF-B, VCAM-1 and calreticulin are associated with subsequent adverse cardiovascular outcomes in healthy volunteers with no previous history of heart disease.

Methods:

Genotyping for the Chr9p21 risk region in >1,500 healthy volunteers was carried out using real-time quantitative polymerase chain reaction (PCR), a method of amplifying the DNA region of interest to determine its sequence. From this cohort, 108 individuals were selected for biomarker analysis (54 with the low risk form of DNA and 54 with the high-risk form of DNA at Chr9p21). ELISA assays were optimised for three potential protein biomarkers (TGF-B, VCAM-1, calreticulin). Levels of these protein biomarkers were then analysed in healthy volunteer participants and tested for associations with Chr9p21 genotype and cardiovascular outcomes.

Results:

TGF-B levels were strongly associated with subsequent adverse cardiovascular events, independent of traditional risk factors ($p=0.023$) or Chr9p21 genotype, with lower levels of TGF-B associated with increased risk. Neither VCAM-1 or calreticulin levels were found to be associated with adverse cardiovascular events.

In this cohort of healthy older people, carrying the high-risk form of DNA at Chr9p21 was not associated with an increased risk of adverse cardiovascular events over a median 4.7 years of follow-up. Nor was genetic variation at Chr9p21 was associated with any changes in levels of TGF-B, VCAM-1 or calreticulin.

Discussion:

We have demonstrated that protein levels of TGF-B are strongly associated with adverse cardiovascular outcomes in previously asymptomatic individuals. We have also shown that TGF-B predicted heart disease risk independently of other established risk factors, such as age, gender, cigarette smoking and high blood pressure. These exciting findings suggest that adding TGF-B into existing risk factor scores may improve our ability to predict near-future adverse cardiovascular events.

TGF-B is an anti-inflammatory protein with numerous functions across the body. In blood vessels, low levels of TGF-B can promote atherosclerosis and in heart tissue low levels of TGF-B prevent cardiac repair. These processes might be the reason that low TGF-B levels in blood are associated with an increased likelihood of a person having an adverse cardiovascular event.

The next step for the research would be to analyse whether inclusion of TGF-B would add value to existing cardiovascular risk scores. If so, then testing levels of TGF-B could be performed using a blood sample taken at the GP's office. Improving cardiovascular risk prediction will allow GPs to make better-informed decisions for individual patient care and allow people who are at a high-risk of an imminent cardiovascular event to be identified, so that precautionary interventions can be made to reduce possible harm.

In summary, our data suggests that measuring circulating protein levels of TGF-B may improve prediction of near-future cardiovascular events in the general population.