

a. FINAL PROJECT REPORT

a. Name:

Dr Barry Palmer

b. Project Title:

Polymorphic variants of X-linked genes in heart disease

Please copy the "Outcome(s)" statement, entered on your application form, in the space below.

This research will provide evidence for the utility of genetic tests for polymorphisms of X-linked genes, particularly for estimating the greater risk of HD events in males and defining risk groups based on combined genetic markers, biomarkers and clinical factors. In the long term this will improve patient outcome after acute HD events by reducing patient mortality and morbidity and improving the efficiency of treatment regimes.

Will your work contribute to this outcome(s) in the manner you envisaged? If not, what has changed?

This research project has provided evidence about the potential utility of genetic tests for polymorphisms (variants) of X-linked genes as tools for assessing patient risk for the development and progression of heart disease.

1. At least 1 genetic polymorphism in the ACE2 has potential for assessing male patient risk of death after an admission for Acute Coronary Syndromes (a heart attack or unstable angina).
2. The frequencies of the A and G variants at position 1075 of the ACE2 are significantly different in a group of male heart patients compared to a matched group of heart-healthy individuals. A genetic test for this polymorphism may have value in identifying those most at risk of developing coronary heart disease.
3. Female heart patients carrying a particular variant of the AT2R gene are more than twice as likely to have had heart valve disease and almost twice as likely to have suffered at least one previous heart attack.
4. Male patients with the G variant at position 97 of the KCNE5 (potassium channel regulator) gene, are significantly more likely to die within 5 years after an admission for Acute Coronary Syndromes, when statistical adjustments are made for patient age, plasma potassium levels and kidney function.

Please copy the "Specific Objective(s)" statement, entered on your application form, in the space below.

1. Genotype DNA samples from the Post-myocardial infarction (PMI), Heart-Healthy Volunteer (HV) & Acute Coronary Syndromes (ACS) cohorts for polymorphisms representing the major genetic variants of the ACE2, AT2R & KCNE5 genes.
2. Analyse genotype/haplotype data for associations with baseline characteristics, neurohormonal profiles, cardiac function, initial risk of acute MI and ACS and clinical outcome after acute events.

The study will evaluate whether polymorphisms in these genes contribute to the greater incidence of heart disease in male patients and if females with two copies of the same gene variants have poorer clinical outcome after disease events than those with two different variants of these genes. The ultimate outcome of this and related research is a panel of genetic tests that can be used in compiling patient risk profiles and individual treatment regimes.

Briefly describe how successful you were in achieving the stated objective(s). If the objective(s) was not achieved, explain why that is the case and describe what you did manage to achieve.

Progress on the specific objectives detailed above has been as indicated below:

1. Genotyping of DNA samples for the ACE2 A1075G polymorphism from the HV and ACS cohorts has been completed. Genotyping of DNA samples from the PMI cohort for the ACE2 G8790A has been completed. Samples from the ACS and HV cohorts have been genotyped for the AT2R A1675G and C3123A polymorphisms. Genotyping of DNA samples from the ACS cohort for the rs697829 single nucleotide polymorphism (SNP) from the KCNE5 gene has been completed. Genotyping of rs697829 for the HV cohort is in progress. These experiments used established PCR-RFLP and TaqMan assay technology. So far these genetic assays have given the most cost effective data yield.
2. A set of 48 probes to detect additional polymorphisms from the ACE2, AT2R & KCNE5 genes and also from other genes on the X-chromosome believed to be involved in the development or progression of heart disease has been designed using the SNPlex design system from Applied Biosystems. Genotyping ACS DNA samples using our X-chromosomal probe set began well, but after promising progress in the first two months of using this technique, technical difficulties were encountered. Trouble shooting to diagnose the problem with the technique has taken several months, while traditional PCR-RFLP genotyping for the polymorphisms outlined in Specific Objective 1, above, has continued in parallel. The complexity of the SNPlex protocol has made pinpointing the problematic step(s) difficult.
A new kit of SNPlex reagents was purchased in January 2009, which improved the quality of results, but has not completely solved the problem. We are working with Product Specialists from Applied Biosystems to try and pinpoint the problem with the SNPlex protocol. As a result only a small number of samples have been genotyped and validated using the SNPlex probe set. Additionally, time spent trouble-shooting the SNPlex experiments has meant not all the genotyping planned to be performed with PCR-RFLP and TaqMan protocols for the polymorphisms outlined in Specific Objective 1 have been completed.
3. Despite the delays and difficulties described above in 2., exciting and relevant results have been produced:

- Publication of the paper *Angiotensin-converting enzyme 2 A1075G polymorphism is associated with survival in an acute coronary syndromes cohort* in the American Heart Journal in October 2008.¹ This reported on data largely obtained with support from HRC, University of Otago and Paykel Trust grants. Employment of Research Technician, Ms. Cao, led to freeing up of Dr Palmer from data acquisition to allow submission, revision and ultimate acceptance of this article. A press release from University of Otago, Christchurch Communications Officer Ainslie Talbot describing this report led to significant media interest:

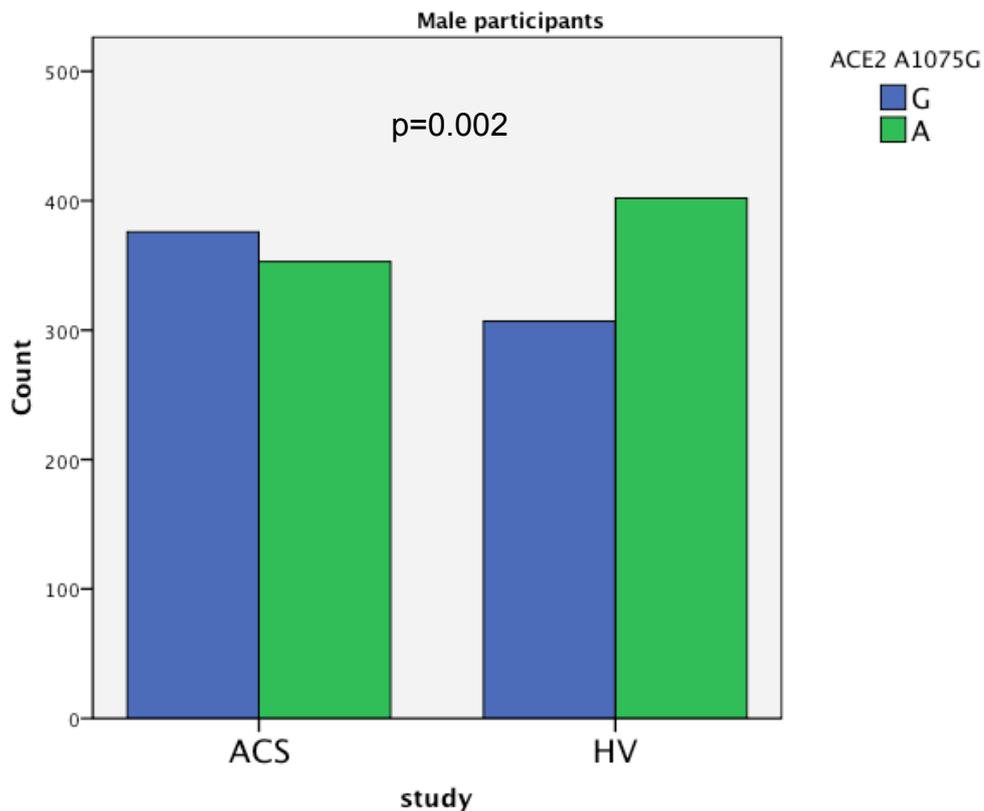
Palmer, B and Talbot, A. October 2008

- BRP interviewed for ONE News, “ACE2 gene & mortality risk for males,” 29-Oct 2008.
- Articles in The Press, Otago Daily Times, Gisborne Herald, NZ Education Review “ACE2 gene increases risk” 29 Oct-2 Nov 2008.
- BRP interviewed for RNZ Checkpoint, NewsTalk ZB, “University of Otago scientists have identified a gene variant that is a key indicator of whether a person is likely to survive a heart attack.” 29 Oct 2008.
- BRP interviewed for Plains FM, Herald “ACE2 gene increases risk” 31 Oct 2008.
- Statistical analysis of genotypic data for the AT2R A1675G and C3123A polymorphisms reveals that in female patients both are associated with history of previous heart attack ($p < 0.013$), cardiac valve disease ($p < 0.041$), severity of

infarct (heart attack), as measured by peak circulating creatinine kinase ($p < 0.032$). By contrast only an association with having a cardiac pacemaker fitted was found in male patients ($p < 0.045$).

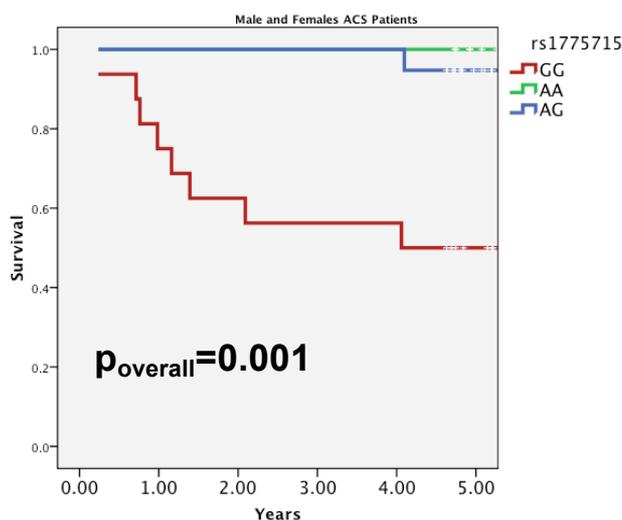
- The G allele of the ACE2 A1075G SNP was found to be present at higher frequency in the ACS cohort compared to similarly aged heart-healthy HV cohort participants in males ($n = 729$ ACS, $n = 709$ HV; $p = 0.002$, Figure 1), but not in females. This implicates the A1075G SNP in the development of male coronary disease and may go some way to explain the gender imbalance in heart attack and angina patients in New Zealand and many other countries. This data will be prepared for publication in a suitable international journal.

Figure 1. Frequency of ACE2 A1075G alleles in male ACS and HV cohort members.



- Analysis of existing SNPlex data suggests that the rs1775715 from an intron in the kinesin family member 5B (KIF5B) gene is associated with survival in male and female patients (n=42, p=0.001, Figure 2.) Further genotyping and statistical analysis is needed to conform this promising finding.

Figure 2. Survival of ACS cohort patients stratified by KIF5B rs1775715 genotype.



Reference

1. Palmer, B.R, Jarvis, M.D., Pilbrow A.P., Ellis, K.L., Frampton, C.M., Skelton, L., Yandle, T.G., Doughty, R.N., Whalley, G.A., Ellis, C.J., Troughton, R.W., Richards, A.M., and Cameron, V.A. (2008) Angiotensin-converting enzyme 2 A1075G polymorphism is associated with survival in an acute coronary syndromes cohort. *American Heart Journal* 156(4) 752-758.

Please confirm delivery of the outputs listed on your application form. If these outputs were not to be delivered, please explain why.

- Provision of this grant greatly assisted the publication of the paper *Angiotensin-converting enzyme 2 A1075G polymorphism is associated with survival in an acute coronary syndromes cohort* in the *American Heart Journal* in October 2008.¹ This reported on pilot data mentioned in our application.
- Statistical analysis of data generated by this project and preparation of manuscripts describing findings related to the AT2R and KCNE5 genes is in progress.