



# TMEM198: A potential marker of ruptured plaque but not coronary ischaemia

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## Introduction

Acute coronary syndromes (ACS) account for 19% of all deaths in Australia (ref 1). Most ACS occur as a result of ruptured plaque (ref 2). Plaque rupture initiation may occur hours to days prior to symptomatic ACS presentation and fatal ACS autopsy data has identified multiple subclinical plaque ruptures that precede the fatal event (ref 3). Currently there is no diagnostic test that can easily identify subjects with preclinical or high risk plaque ruptures. If such a diagnostic was available this would allow subclinical screening of high risk subjects. We have identified a prospective protein marker that may identify subjects with plaque rupture and ACS.

## Aims

### Hypothesis:

We hypothesise that trans-coronary plasma protein levels are altered during the process of coronary plaque rupture and endothelial disruption, acting as blood markers of this process in subjects with established coronary artery disease.

### Aims:

1. Measure the plasma concentration of differentially expressed proteins in relation to time across the coronary tree (Aorta to Coronary Sinus) to identify a potential marker of plaque rupture in subjects undergoing elective coronary intervention.
2. Measure the plasma concentration of differentially expressed proteins across a ruptured plaque to identify a potential marker of plaque rupture in subjects presenting with acute coronary syndromes (ACS).

## Methods

In two different cohorts we recruited:

1. 16 subjects to have simultaneous Aortic and coronary sinus sampling performed following elective percutaneous coronary intervention (PCI), and,
2. 3 subjects with ST elevation myocardial Infarction (STEMI) to under-go aspiration of blood proximal and distal to the ruptured plaque.

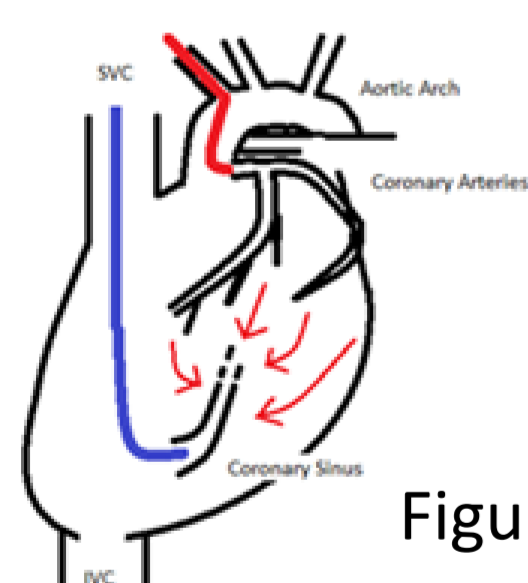


Figure One:

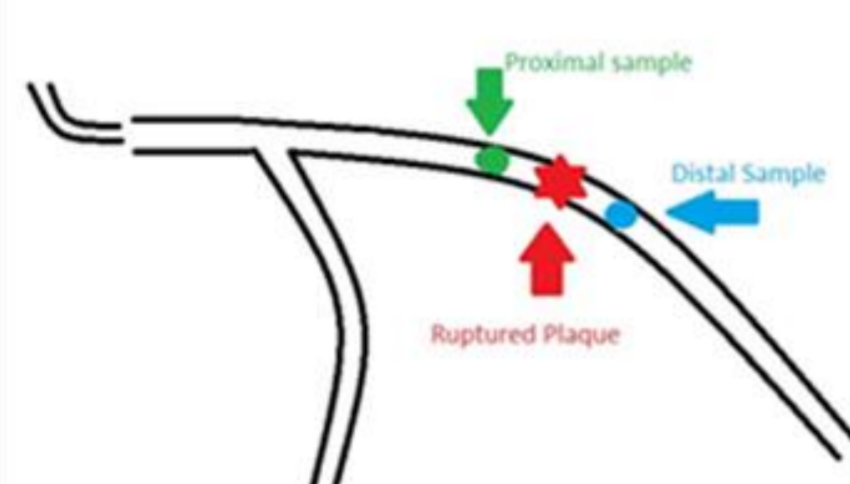


Figure Two:

Samples from each cohort underwent 2 dimensional liquid chromatography mass spectrometry with dimethyl labelling to identify a:

1. transcoronary protein gradient post PCI and
2. transplaque protein gradient post ACS.

Briefly, all samples were collected into EDTA vacutainers, stored on ice prior to centrifuging at 4dec C 4000rpm for 10mins with plasma supernatant stored at -80deg prior to proteomic analysis. Protein was extracted via acetone method, reduced and alkylated, resuspended in urea prior to trypsin digestion. Peptides were dimethyl stable isotope labelled with isotopomers of formaldehyde and cyanoborohydride. Samples were then combined and 2-dimensional liquid chromatography (strong cation exchange followed by online reverse phase) was performed. Mass Spectrometry was performed using an Orbitrap Elite.

## Results

- Following a biomarker discovery project (CSANZ ref380) we identified 25 proteins that were differentially expressed across the coronary circulation
- Transmembrane protein 198 (TMEM198) was identified as a prospective marker of plaque rupture as it decreased by log2 fold change -6.39, (adjusted  $p < 0.046$ ) and was not affected by the degree of post procedural troponin elevation (adjusted  $p = 0.70$ ) suggesting the decrease occurred in the epicardial coronary artery rather than the myocardium.
- In a separate cohort of 3 subjects presenting with STEMI undergoing plasma aspiration proximal and distal to a ruptured plaque, TMEM198 decreased across the plaque (log fold change -7.56, adjusted  $p < 0.001$ ) again suggesting that the epicardial artery and more specifically the site of plaque rupture was the site of differential expression.

Figure Three:

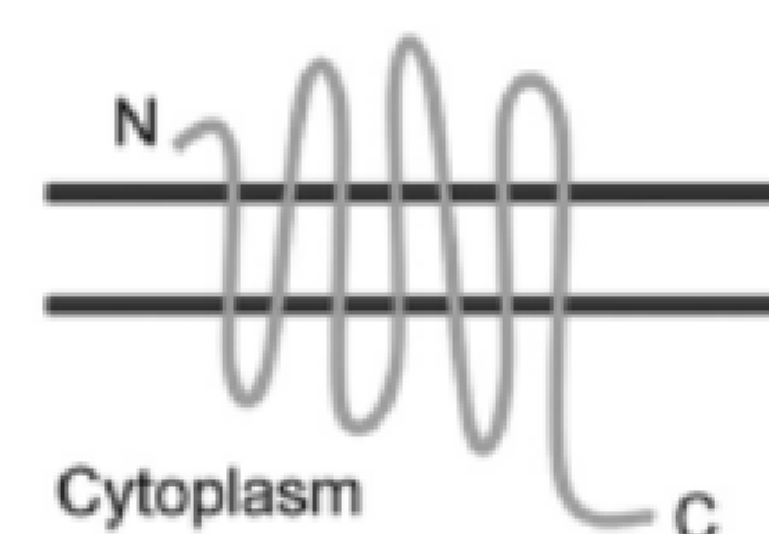


Figure Four:

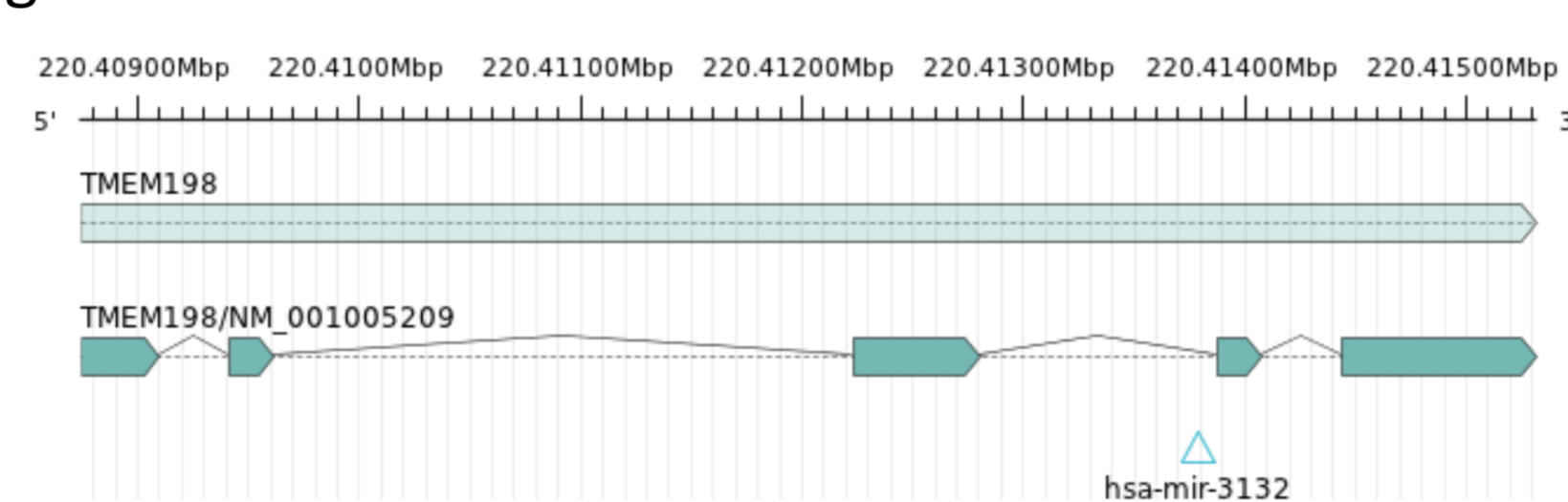
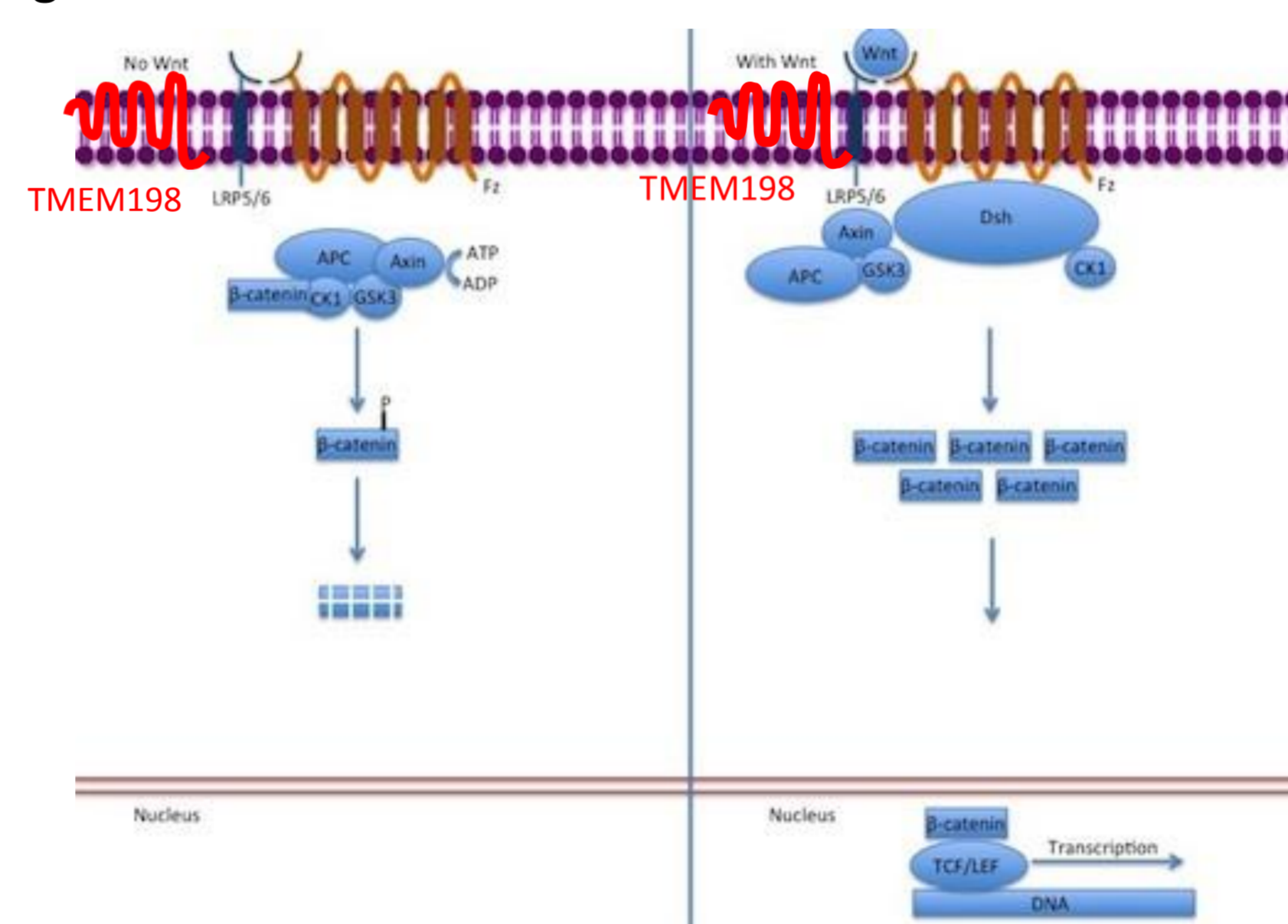


Figure Five:



## References

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## Discussion

TMEM198 is decreased across the coronary circulation following elective PCI. TMEM198 is decreased across ruptured plaque following plaque rupture. These findings suggest TMEM198 may be involved in the process of plaque rupture or is a prospective marker of this process.

- TMEM198 is a transmembrane protein (7 transmembrane domains with 30AA extracellular and 110AA cytoplasmic domain). Figure 3.
- TMEM198 promotes phosphorylation (cytoplasmic domain) and activation of LRP6 (LDL receptor protein 6) by casein kinase-1 (CK1). LRP6 is involved with "wnt" pathway activation which is associated with various human diseases. LRP6 activation of the wnt pathway leads to effects on gene expression through B-catenin cytoplasmic accumulation and translocation to the nucleus. (ref 4).

The significance of TMEM198 is likely to be due to its effect on LRP6 binding and wnt pathway activation. Interestingly rare autosomal-dominant loss of function mutations in the LRP6 gene resulting in impaired wnt signaling have been associated with early onset coronary artery disease. 3 mutations have been identified that occur at a site of ligand binding. One group has suggested that a rare genetic variant of LRP6 increases the risk of the metabolic syndrome (ref 3). Wnt signaling is involved in insulin sensitivity and loss of function may lead to the development of diabetes. The same group have shown that LRP6 is involved with uptake of lipoproteins including LDLR mediated LDL uptake. In addition LRP6 leads to PDGF degradation and decreased VSMC proliferation.

Our demonstration of decreased TMEM198 across the ruptured plaque may indicate sequestration/activation (through LRP6 binding) or degradation. Increased activation of TMEM198 with binding to LRP6 may lead to increased LDL uptake and may explain the changes in lipoproteins (eg decreased LDL) that occur after ACS.

TMEM198 gene (Ch2q35) encodes a micro rna (mir3132). The miRNA 3132 is expressed in the heart but no other tissue. It is not known what function mir3132 plays or if it interacts with TMEM198. (Figure Four).

## Conclusion:

Whether TMEM198 is a bystander marker of plaque rupture or some other process, or actively involved in the plaque rupture process is not known. These findings are intriguing as there are no reliable markers of plaque rupture. If TMEM198 plays a pathological role it is likely to be through LRP6 binding and the wnt pathway.

Further investigation of the changes in TMEM198 associated with coronary syndromes, plaque rupture and the wnt/LRP6 pathway is required and is the focus of ongoing research with a verification and validation study of this provisional data underway.

