Heart failure (HF) is one of the commonest cardiovascular complications of Diabetes Mellitus (DM) with the prevalence of DM reported at around 50% in many pivotal heart failure studies. DM is an independent predictor of mortality in patients with HF; however molecular mechanisms that contribute to HF development in the diabetic population are poorly understood. Using a novel human relevant mouse model of DM (GENA348), identified through the MRC mouse mutagenesis programme with a point mutation in the pancreatic glucokinase (GLK) gene we investigated the molecular mechanisms that contribute to the HF phenotype in DM. GLK is the glucose sensor which regulates insulin secretion and GLK activity is reduced by 90% by the GENA348 point mutation resulting in severe hyperglycaemia. Similar mutations underlie Maturity Onset Diabetes of the Young Type 2 (MODY 2) in humans. Mean random blood glucose was found to be increased in the GENA348 mutant (HO) mice compared to wild type (WT) littermates (WT 6.9±0.3 mmol/l vs HO 20.6±0.8 mmol/l, p<0.001). Serial echocardiography was performed, at 3, 6 and 12 months. No significant changes in echocardiographic parameters were observed at 3 months, although by 6 months development of significant cardiac hypertrophy in HO mice was observed. At 12 months of age left ventricular dilatation was evident, characterised by an 8% increase in diastolic diameter (WT 4.08±0.10 vs HO 4.41±0.12, p<0.05). Systolic function was preserved although significant diastolic dysfunction was evident at 6 and 12 months with a 31% reduction in the E/A ratio. Histological staining illustrated significant cardiac hypertrophy with real time PCR data demonstrating a relative 150% increase in the hypertrophic marker BNP. Hypertrophic pathways were examined through western blot analysis revealing an age dependant increase in Akt phosphorylation (3 months- no increase, 6 months-140%, 12 months-460%). Serum levels of advanced glycation end products (AGE) were also elevated by 86% (WT 21±5.5 ng/ml vs HO 39±8.3 ng/ml, p<0.05) as was the protein expression level of the receptor for AGEs (RAGE). In vitro cellular experiments also revealed AGEs directly activate Akt through phosphorylation and increase levels of the receptor RAGE. AGE induced phosphorylation of Akt is inhibited in the presence of wortmannin, suggesting a PI3K dependent signalling mechanism. This was further confirmed in vivo where a bolus injection of wortmannin in 6-month old mutant mice returned Akt phosphorylation levels to those seen in WT mice. In conclusion, using the first human relevant mouse model of diabetes, GENA348 we demonstrate the development of a progressive cardiac phenotype including cardiac hypertrophy, LV dilatation and diastolic dysfunction similar to the clinical manifestations of diabetic cardiomyopathy. We propose that the RAGE/PI3K/Akt pathway contributes to the molecular mechanisms associated with the cardiac phenotype.
Results 5 candidate regions (MMP23B, VEGFA, DVL1, RIPK1, LPAL2) showed an over-accumulation of rare alleles in patients with CAD when compared to controls (FDR<5%). The number of analysed rare alleles at each of these loci ranged from 4 to 42. The most significant over-representation of rare variants were identified at MMP23B (matrix metallopeptidase 23B gene; p=1.3x10^-8), a gene previously unsuspected to play a major role in CAD and VEGFA (vascular endothelial growth factor A; p=2.6x10^-10). Only one of the identified genes (LPAL2; p=1.7x10^-8) lies within the locus that was previously shown to harbour rare variants associated with susceptibility to CAD.

Conclusions Rare alleles are associated with predisposition to CAD and this gene-centric analysis combining information from low-frequency variants of the same locus has a potential to uncover, at least a proportion of, the “missing heritability” of CAD.