Conclusion

We have confirmed the potential importance of genetic variation at the *PPARA* locus in modulating susceptibility to cardiovascular disease, and have shown that this association is relevant to individuals with type 2 diabetes.

Methods

In the Tayside region of Scotland detailed clinical information on all individuals with diabetes mellitus is recorded on a continuously updated electronic clinical information system known as DARTS (Diabetes Audit and Research in Tayside Scotland) [16]. Validated, region-wide electronic record-linkage techniques facilitate the identification of individuals with diabetes in the Tayside population with a sensitivity of 97% [16]. Relevant clinical data is linked to databases containing all inpatient hospital admissions in Tayside from 1980 with diagnostic codes from ICD-9/10 (International Classification of Diseases, ninth and tenth revisions), and records of death certificates from the registrar general. This automated electronic follow-up is manually validated through a continuous cycle of review by dedicated study clinicians. Incident cardiovascular events in this population have been described previously [27].

Following written informed consent from individuals registered on DARTS, blood samples for genetic studies have been collected, thereby creating a genetic sub-study known as Go-DARTS. Rigorous compliance with NHS data protection and encryption standards is maintained and the study was approved by the local research ethics committee.

The PPARα L162V and G2528C genotypes were determined in 1,810 individuals all of whom were Caucasian with type 2 diabetes diagnosed between the age of 35 and 70 years. Taqman (Applied Biosystems) allelic discrimination assays were used. The primers and probes used for the allelic discrimination assays were as follows: L162V Forward primer-CAGAAACAAATGCCAGTATTGTCG, Reverse primer-GGCCACCTTACCTACCGTTGTG, L162 probe (FAM labelled) – TTCACAAGTGC**C**TTTCTGTCGGGATGT, V162 probe (TET labelled) – TTCACAAGTGC**G**TTTCTGTCGGGATGT.

G2528C Forward primer-TCCTTAAATATGGTGGAACACTTGAAG, Reverse primer-TCACAACCACCAGTTTTGCAT, G2528 probe (FAM labelled) – ATATCTAGTTT**G**GATTCAAAAGCTTCATTTCCCA, C2528 probe (TET labelled) – ATATCTAGTTT**C**GATTCAAAAGCTTCATTTCCCA.

Statistics

For each clinical measure the mean was determined from multiple measures obtained up to a maximum period of three years (up to two years prior to enrolment, and up to one year following enrolment). LDL cholesterol was estimated through the use of the Freidwald equation. Linear regression was used to determine the association of genotype with each measure corrected for age at genotyping. For determining the association of genotype with age of diagnosis, this was corrected for gender and presence of a history of smoking by determining residuals and adding these to the overall mean age of diagnosis. Cox's proportional hazards was used to model time to first event. All individuals were followed from the point of genotyping until a non-fatal myocardial infarction occurred, or a composite of non-fatal myocardial infarct or all cause death. Censoring occurred either at the end of the study or death from any cause. Both variants were included in the model and it was found that a co-dominant model for each variant produced the best fit. The following variables were also included in the model; age at genotyping, gender, history of smoking, treatment with insulin, a previous history of a myocardial infarction, prevalent angina and prevalent cerebrovascular disease. Haplotype frequency estimates together with haplotype effects were determined using the THESIAS Program [28,29]. In the case of survival analysis by haplotype using THESIAS only age at genotyping, prevalent angina and a previous history of a myocardial infarction were included in the model.

List of abbreviations

DARTS Diabetes Audit and Research in Tayside Study

Go-DARTS Genetics of DARTS

PPARα Peroxisome Proliferator Activated Receptor alpha

PPARA Gene for PPARα

PPARD Gene for PPARδ

CVD Cardiovascular disease

LOCAT Lopoid Coronary Angiography Trial

NPHS2 Second Northwick Park Heart Study

HR Hazard Ratio

CI Confidence Interval

Competing interests

The author(s) declare that they have no competing interests.

Authors' contributions

ASFD and CNAP wrote the manuscript and performed the analysis, CNAP and ADM conceived the study and partic-