CLINICAL STUDY

Apolipoprotein E genotype and mortality in Southern European and Anglo-Celt patients with type 2 diabetes: the Fremantle Diabetes Study

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Abstract

Objective: To determine whether cardiac and all-cause mortality are lower in Southern European (SE) patients than in Anglo-Celt (AC) patients with type 2 diabetes in an urban Australian setting, and, if so, whether ethnicity-specific differences in apolipoprotein E (APOE) genotypes are contributory.

Design: Longitudinal observational cohort study.

Methods: We analysed detailed data from 1057 patients from the community-based Fremantle Diabetes Study, 238 were of an SE migrant background and 819 of AC ethnicity. Cox proportional hazards modelling was used to identify independent predictors of cardiac and all-cause mortality.

Results: During 9.8 ± 3.5 years of follow-up, 411 (38.9%) participants died, 161 (39.2%) from cardiac causes. Significant positive baseline independent predictors of cardiac death were age, male gender, coronary heart disease, cerebrovascular disease, peripheral arterial disease, retinopathy and peripheral neuropathy ($P \leq 0.004$), while maternal history of diabetes was protective ($P = 0.014$). After adjusting for these variables, APOE4 carriage was predictive (hazard ratio (95% confidence interval) 1.61 (1.01–2.58); $P = 0.048$). SE ethnicity did not add significantly to the model either as a single variable or as an interaction term with APOE4 carriage ($P \geq 0.86$). Significant independent predictors of all-cause mortality were age, male gender, smoking, coronary heart disease, cerebrovascular disease, peripheral arterial disease, retinopathy, peripheral neuropathy and microalbuminuria ($P \leq 0.047$), while overweight/obesity, lipid-lowering therapy and recent exercise were protective ($P \leq 0.008$). APOE4 carriage, SE ethnicity and their interaction did not add to the model ($P \geq 0.32$).

Conclusions: SE ethnicity does not confer an independent survival advantage in community-based Australian type 2 diabetic patients, but APOE4 carriers are at higher risk of cardiac death.

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Introduction

Migrants are more likely to develop type 2 diabetes than people born in Australia, with the highest prevalence in those of Southern European (SE) ethnicity (1). SE males and females have more than double the diabetes prevalence of the Australian-born, principally Anglo-Celt (AC), population (1, 2). They are diagnosed at a younger age and have worse glycaemic control than AC patients with type 2 diabetes, despite earlier introduction of insulin therapy (3).

By contrast, Australian Bureau of Statistics data show that age- and sex-adjusted cardiovascular disease (CVD) mortality are lower in Greek and Italian migrants than in the Australian-born general population aged 45–64 years (4). This favourable outcome may relate to lifestyle factors, including diet (5, 6). However, polymorphisms of the apolipoprotein E (APOE) gene, which modulates CVD risk through lipoprotein metabolism

and other effects (7), show significant ethnic variability. This includes a low prevalence of the potentially adverse $\epsilon 4$ allele among SE populations (8).

The aim of the present study was, therefore, to determine whether, in a representative cohort of type 2 diabetic Australians and consistent with general population data (4), cardiac and all-cause mortality were lower in SE patients than in AC patients, and, if so, whether ethnicity-specific differences in APOE genotypes were contributory.

Patients and methods

Patients

The Fremantle Diabetes Study (FDS) was a longitudinal observational study conducted in a community of 120 097 people. The FDS protocol was approved by the Fremantle Hospital Human Rights Committee, and

all the subjects gave informed consent. Details of recruitment and sample characteristics (including non-recruited subjects) have been published elsewhere (9). Of 2258 patients identified between 1993 and 1996, 1426 (63%) were recruited and 1294 had type 2 diabetes. In the present sub-study, we included 1057 patients with type 2 diabetes (81.9% of the FDS cohort) who were of SE or AC ethnicity.

Methods

All the patients had a detailed baseline assessment between 1993 and 1996, and were requested to attend subsequent standardised annual reviews. At each visit, demographic and socio-economic data were recorded. These included ethnic background based on self-selection, country of birth and their father's/mother's

Table 1 Baseline characteristics of Southern European (SE) and Anglo-Celt (AC) type 2 Fremantle Diabetes Study patients. Data are mean $\pm$ s.d.

	SE (n=238)	AC (n=819)	P value
Age (years)	63.6 $\pm$ 10.3	64.8 $\pm$ 11.3	0.14
Male (%)	45.4	49.5	0.27
Overseas-born (n (%))	210 (88.2)	224 (27.4)	<0.001
Time since arrival in Australia (years)	35.1 $\pm$ 10.6	32.8 $\pm$ 13.5	0.002
Age on arrival in Australia (years)	29.5 $\pm$ 10.8	33.1 $\pm$ 12.8	0.06
Sex (% male)	46.2	54.0	0.12
Duration of diabetes (years)	5.3 (2.2–11.9)	4.1 (1.0–8.3)	<0.001
Glutamic acid decarboxylase antibody positive (%)	1.7	4.2	0.11
Maternal history of diabetes (%)	27.7	19.9	0.012
Fasting serum glucose (mmol/l)	9.0 (7.3–11.3)	8.2 (6.7–10.6)	0.004
HbA1c (%)	7.9 (6.7–9.1)	7.3 (6.3–8.7)	0.002
Diabetes treatment (diet/oral agents/insulin $\pm$ oral agents; %)	22.0/58.5/19.5	34.9/54.8/10.3	<0.001
Body mass index (kg/m ²)	30.3 $\pm$ 4.7	29.6 $\pm$ 5.6	0.039
Waist (% overweight or obese ^a)	90.7	85.7	0.049
Systolic blood pressure (mmHg)	151 $\pm$ 24	151 $\pm$ 23	0.79
Diastolic blood pressure (mmHg)	80 $\pm$ 11	81 $\pm$ 11	0.46
Taking antihypertensive medication (%)	42.9	53.0	0.006
Taking ACE-inhibitor (%)	21.8	22.0	1.00
Total serum cholesterol (mmol/l)	5.1 (4.3–6.2)	5.4 (4.4–6.6)	<0.001
HDL-cholesterol (mmol/l)	0.98 (0.69–1.38)	1.02 (0.75–1.38)	0.050
Total:HDL-cholesterol ratio	5.3 (3.7–7.5)	5.3 (3.8–7.4)	0.87
Serum LDL-cholesterol (mmol/l)	3.3 $\pm$ 0.9	3.5 $\pm$ 0.9	0.06
Serum triglycerides (mmol/l)	1.7 (1.0–2.9)	1.9 (1.1–3.4)	0.003
Taking lipid-lowering therapy (%)	10.5	10.5	1.00
Taking $\geq$ 75 mg/day aspirin (%)	16.9	22.3	0.07
Retinopathy (%)	26.1	13.5	<0.001
Neuropathy (%)	25.4	32.9	0.035
Microalbuminuria (%)	48.7	40.1	0.023
Coronary heart disease (%)	25.5	33.4	0.026
Cerebrovascular disease (%)	10.1	10.5	0.91
Peripheral arterial disease (%)	29.2	29.5	1.00
English fluency (% not fluent)	62.9	0	<0.001
Education beyond primary school (%)	32.8	85.4	<0.001
Married/de facto relationship (%)	76.4	62.4	<0.001
Smoking status (never/ex-/current; %)	60.3/27.0/12.7	41.1/43.7/15.1	<0.001
Any exercise in the last 2 weeks (%)	72.2	72.0	1.00
Alcohol consumption (standard drinks/day)	0 (0–1.2)	0 (0–0.4)	0.58
Genotype (%)			
E2/E2	0	0.9	0.36
E2/E3	10.1	12.5	0.36
E2/E4	1.8	2.6	0.63
E3/E3	75.3	64.7	0.003
E3/E4	12.3	18.0	0.054
E4/E4	0.4	1.3	0.47
Allele (%)			
ϵ 2	5.9	8.5	N/A
ϵ 3	86.6	79.9	N/A
ϵ 4	7.5	11.6	N/A
Carrier (%)			
E2 (E2/E2, E2/E3, E2/E4)	11.9	16.0	0.14
E3 (E2/E3, E3/E3, E3/E4)	97.8	95.2	0.09
E4 (E2/E4, E3/E4, E4/E4)	14.5	21.9	0.015

Geometric mean (s.d., range), median (interquartile range) or % except where indicated.

^aWaist circumference $\geq$ 94 cm in males and $\geq$ 80 cm in females.

birth and language spoken at home. The AC patients had family origins in the UK/Ireland, while most SE patients were born in Italy (74%), Portugal, Spain or Greece. Health data were also documented, but a detailed dietary history was not collected. Each patient underwent a physical examination and provided fasting blood and urine samples for automated biochemical analyses in a single laboratory (9). DNA was isolated from leukocytes, and APOE genotype was determined via PCR amplification and restriction enzyme digestion using validated methods (10, 11). Three co-dominant alleles ($\epsilon 2$, $\epsilon 3$ and $\epsilon 4$) encode for three different isoforms (E2, E3 and E4) with six different genotypes.

Complications were identified using standard definitions (12). Coronary heart disease was defined as a self-reported history of, or prior hospitalisation for, myocardial infarction, angina, coronary revascularisation and/or definite myocardial infarction on a Minnesota-coded electrocardiogram. Cerebrovascular disease was defined as self-report of/prior hospitalisation for stroke/transient ischaemic attack. Peripheral arterial disease was taken as an ankle:brachial index ≤ 0.90 . Retinopathy (any grade) was assessed from direct/indirect ophthalmoscopy or documented retinal examination performed outside the study. Neuropathy was defined as a score $> 2/8$ using the Michigan Neuropathy Screening Instrument clinical portion. Albuminuria was taken as urinary albumin:creatinine ≥ 3.0 mg/mmol.

Death data were obtained from WA Data Linkage System (13) under approval from the Confidentiality of Health Information Committee. All-cause mortality was determined from study entry to end-June 2006. Causes of death were reviewed independently by two investigators (D G B and T M E D) and classified as cardiac or other under the system used in the UK Prospective Diabetes Study (14).

Data analysis

The genotype frequencies were tested for deviation from Hardy–Weinberg equilibrium using the χ^2 test. Kaplan–Meier survival curves defined by ethnic background or APOE genotype/carrier were compared using the log-rank test. Cox proportional hazards modelling was used to determine independent predictors of cardiac and all-cause mortality. After adjusting for the most parsimonious models, ethnic background or APOE genotype/carrier status was added separately. The validity of the proportional hazards assumption was assessed from $\ln(-\ln(\text{survival}))$ curves and examination of time-dependent covariates.

Results

Baseline characteristics

Baseline characteristics of patients by ethnicity are summarised in Table 1. The SE patients were of similar age to the AC patients but had significantly longer diabetes duration and were younger at diagnosis. The SE patients were more likely to report a maternal history of diabetes. They were also less likely to speak English fluently and to have had education beyond primary school. The SE patients had a greater body mass index and were more likely to be centrally obese than the AC group.

Fasting serum glucose and HbA1c were significantly higher among the SE patients who were twice as likely to be insulin treated. Mean blood pressures were similar, but the AC patients were more likely to be taking antihypertensive medication. The AC patients had significantly higher serum total cholesterol and triglycerides, but only 10.5% of either group was taking lipid-lowering medication. Current smoking habit, alcohol intake and exercise history were similar in the two groups, but the SE patients were more likely to have

Table 2 Baseline serum lipid levels by APOE genotype and carrier status for the SE and AC type 2 diabetic patients combined.

	<i>n</i>	Total cholesterol (mmol/l)	HDL-cholesterol (mmol/l)	Total:HDL-cholesterol	LDL-cholesterol (mmol/l)	Triglycerides (mmol/l)
Genotype						
E2/E2	7	6.1 (4.3–8.6)	1.02 (0.80–1.30)	5.9 (4.4–8.0)	3.8 $\pm$ 2.4	3.2 (2.1–4.9)
E2/E3	119	5.1 (4.1–6.2)*	1.06 (0.78–1.45)	4.8 (3.4–6.8)*	3.0 $\pm$ 0.8 ^{†,‡}	2.0 (1.1–3.4)
E2/E4	24	5.4 (4.1–7.1)	1.00 (0.72–1.38)	5.2 (3.5–7.6)	3.3 $\pm$ 0.8	2.1 (0.9–5.0)
E3/E3	668	5.3 (4.4–6.4)	1.01 (0.74–1.38)	5.2 (3.8–7.2)	3.4 $\pm$ 0.8 [†]	1.8 (1.0–3.1)
E3/E4	166	5.6 (4.6–6.8)*	1.00 (0.75–1.33)	5.6 (4.1–7.7)*	3.7 $\pm$ 1.0 [†]	2.0 (1.2–3.3)
E4/E4	11	6.0 (4.8–7.6)	0.93 (0.55–1.57)	6.4 (4.0–10.2)	4.1 $\pm$ 1.2 [‡]	2.4 (1.3–4.5)
Trend <i>P</i> value		0.001	0.58	0.001	< 0.001	0.012
Carrier						
E2 (E2/E2, E2/E3, E2/4)	150	5.2 (4.1–6.5) [§]	1.05 (0.77–1.43)	4.9 (3.4–7.0)	3.1 $\pm$ 0.9	2.0 (1.1–3.8) [§]
E3 (E2/E3, E3/E3, E3/E4)	953	5.3 (4.4–6.5)	1.02 (0.75–1.38)	5.2 (3.8–7.2)	3.4 $\pm$ 0.9	1.9 (1.1–3.2) [§]
E4 (E2/4, E3/4, E4/4)	201	5.6 (4.5–6.9)	1.00 (0.73–1.35)	5.6 (4.0–7.8)	3.7 $\pm$ 1.0	2.0 (1.1–3.6)

**P* = 0.002; [†]*P* < 0.001 ; [‡]*P* = 0.004; (Bonferroni correction for multiple comparisons applied); Student's *t*-test for carrier versus non-carrier: [§]*P* < 0.05 , ^{||}*P* < 0.01 , [¶]*P* < 0.001 .

never smoked. The proportion with any manifestation of microangiopathy was greater among the SE patients (64.3 vs 56.7% in AC; $P=0.043$), while the proportion with any macrovascular complication was higher among the AC patients (52.7 vs 45.0%; $P=0.039$).

Apolipoprotein E genotype and outcome

APOE genotype was available for 995 participants (94.1%). The genotype frequencies were in Hardy–Weinberg equilibrium ($P=0.51$). The $\epsilon 2$ and $\epsilon 4$ alleles were more common among the AC patients, while the allele $\epsilon 3$ was more prevalent among the SE patients. The E3/E3 genotype was significantly more common among the SE patients, whereas E4 carriers were significantly more likely to be in the AC patients. E2 carriers had the least atherogenic serum lipid profile, while E4 carriers had a significantly higher mean serum total and low-density lipoprotein (LDL) cholesterol than non-E4 carriers (see Table 2).

During 10 308 patient-years of follow-up to death or end-June 2006 (a mean of 9.8 ± 3.5 years), 411 (38.9%) participants died, 161 (39.2%) were due to cardiac causes. The likelihood of cardiac and all-cause death was not significantly increased in SE patients versus AC patients or in E4 carriers versus non-carriers ($P \geq 0.19$, log-rank test; see Fig. 1). We considered stratifying the analysis by ethnicity but had limited power to examine the effect of APOE status on outcome. For example, for the dependent variable with fewer endpoints (cardiac mortality), there was 80% power to detect a relative risk ≥ 2.7 in APOE4 carriers among the 238 SE participants. We therefore analysed the two groups combined but included the interaction between APOE4 carrier status and ethnicity. After adjusting for independent risk factors, the presence of APOE4, but not the ethnic background, was a significant determinant of cardiac mortality (see Table 3). The interaction between SE ethnicity and APOE4 was not significant when added to this model ($P=0.86$). Ethnic background, APOE4 carriage and their interaction were not independently associated with all-cause death after adjusting for the significant independent determinants (see Table 3).

Discussion

The present data show that SE ethnicity does not confer an independent survival advantage in community-based Australian type 2 diabetic patients relative to that associated with AC ethnicity. APOE4 carriers were, however, at higher risk of cardiac death. As expected, APOE4 carriers in our cohort had relatively atherogenic serum lipid profiles. However, there is emerging evidence that other factors, such as increased oxidative status and vascular inflammation (7), may contribute to the adverse cardiovascular effects of this genotype.

Figure 1 Kaplan–Meier plots of cardiac (upper panel) and all-cause (lower panel) death in SE and AC patients.

Most SE patients in our community-based Australian cohort were first-generation migrants who did not speak English fluently and who had not been educated beyond primary school level. These factors are likely surrogates for a traditional lifestyle including a Mediterranean diet. Indeed, there is evidence from national surveys that a Mediterranean diet continues to be a part of SE lifestyle for some time after arrival in Australia (15). In addition, they had a lower prevalence of APOE4 carriage, consistent with previous studies in SE populations (8), and generally less atherogenic serum lipid profiles. These factors and general population data from both Australia (4) and Europe (16) suggest that the SE patients should have had a lower risk of death than their AC counterparts.

We did not, however, find that the SE patients had such a survival advantage, regardless of whether death was due to cardiac or other causes. Since significant determinants of cardiac and all-cause mortality included both micro- and macrovascular disease, we postulate that any relative genetic or environmental (including dietary) CVD benefit enjoyed by the SE patients was offset by their significantly worse glycaemic control and higher prevalence of both retinopathy and nephropathy. There is epidemiological evidence of

Table 3 Hazard ratios (HRs) and 95% confidence intervals (CI) for independent baseline determinants of cardiac (upper panel) and all-cause (lower panel) mortality. The most parsimonious models are shown together with with HRs for *APOE4* carriage, Southern European (SE) ethnicity and their interaction after adjustment for the significant variables in the models.

Baseline variable	HR (95% CI)	P value
Cardiac mortality		
Age (increase of 10 years)	2.03 (1.61–2.57)	<0.001
Male sex	1.99 (1.38–2.89)	<0.001
Coronary heart disease	2.29 (1.62–3.24)	<0.001
Cerebrovascular disease	1.92 (1.24–2.97)	0.004
Peripheral arterial disease	2.01 (1.41–2.86)	<0.001
Retinopathy	2.66 (1.82–3.87)	<0.001
Neuropathy	1.88 (1.30–2.70)	0.001
Maternal history of diabetes	0.50 (0.29–0.87)	0.014
After adjustment for the above variables		
<i>APOE4</i> carriage	1.61 (1.01–2.58)	0.048
SE ethnicity	1.02 (0.61–1.71)	0.93
<i>APOE4</i> carriage × SE ethnicity	0.91 (0.30–2.71)	0.86
All-cause mortality		
Age (increase of 10 years)	2.32 (1.99–2.69)	<0.001
Male sex	1.81 (1.42–2.31)	<0.001
Overweight/obese (waist circumference)	0.66 (0.49–0.89)	0.006
Taking lipid-modifying medication	0.53 (0.34–0.85)	0.008
Current smoker	1.48 (1.08–2.02)	0.015
Any exercise in past 2 weeks	0.67 (0.53–0.86)	0.001
Coronary heart disease	1.54 (1.23–1.93)	<0.001
Cerebrovascular disease	1.37 (1.04–1.87)	0.047
Peripheral arterial disease	1.55 (1.23–1.95)	<0.001
Retinopathy	1.67 (1.28–2.18)	<0.001
Neuropathy	1.61 (1.27–2.04)	<0.001
Ln(urinary albumin:creatinine ratio) ^a	1.16 (1.07–1.25)	<0.001
After adjustment for the above variables		
<i>APOE4</i> carriage	1.18 (0.85–1.63)	0.32
SE ethnicity	0.96 (0.705–1.31)	0.79
<i>APOE4</i> carriage × SE ethnicity	1.18 (0.60–2.33)	0.64

^aA 2.72-fold increase in ACR corresponds to an increase of 1.0 in ln(ACR).

independent associations between increased HbA1c levels (17, 18), retinopathy (19) and microalbuminuria (20) and the risk of CVD and death.

Consistent with a range of past general population studies (7) and adequate statistical power, our carriers of *APOE4* had an independent and significant 58% increased risk of cardiac death. In an Italian study of 433 type 2 clinic patients, *APOE* polymorphisms were not markers of premature cardiovascular or all-cause mortality (21). Although the authors postulated that the effect of *APOE* mutations was attenuated by environmental factors, notably a healthy diet, the only independent predictors of death among a range of plausible candidate variables were age and HbA1c for CVD mortality, and age, HbA1c and diabetes duration for all-cause mortality. This suggests that they were quite different from our community-based sample, while their mean duration (~13 years) raises the possibility of a survivor bias. Our data, including those for the SE patients, are consistent with the results of a smaller

study of Finnish men with type 2 diabetes in which *APOE4* carriage was positively associated with coronary heart disease (22).

A recent large general UK population study found that, after adjustment for a restricted number of conventional risk factors, most notably the LDL/high-density lipoprotein cholesterol ratio, a positive relationship between the E4 genotype and coronary heart disease events became non-significant (23). However, this finding is at odds with two recent large meta-analyses of data from diverse populations (24, 25), while we adjusted for a much greater range of potential confounders than in the UK study (23), including serum lipids, and still found that the E4 genotype was predictive. It is also possible that the results of the present study and Finnish (22) studies may mean that the lipid- and/or non-lipid-associated effects (7) of *APOE* genotype on CVD risk are greater among people with diabetes; there was a >45% increased risk associated with E4 genotype in these two studies compared with 6–42% in the general population (23–25).

It is possible that impaired healthcare access due to ethnicity-specific issues such as language difficulties may have contributed to our findings. Since socio-economic variables such as income, English fluency and educational attainment were highly correlated with ethnicity, it was not possible to assess their independent influence on healthcare access. However, the Medicare system, introduced in 1984 and underpinned by a tax levy, provides universal comprehensive coverage for all Australians. In the 2 years prior to recruitment, the SE patients made more, rather than fewer, visits to their general practitioners than the AC patients (6 (2–12) vs 4 (1–8), $P=0.012$). This observation, and the fact that baseline CVD risk factor profiles and treatments in the two groups were not discordant, suggests that our findings were not confounded by inequitable healthcare access.

The present data show that a range of baseline phenotypic differences between the SE and AC patients underlie a complex relationship between ethnicity and outcome. While *APOE4* carriage was an independent predictor of cardiac death regardless of ethnicity, a younger presentation with type 2 diabetes and relatively poor glycaemic control may overcome beneficial genetic and lifestyle effects of SE ethnicity on CVD observed in the general Australian population (4).

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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