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The associations of apolipoprotein E and angiotensin-converting enzyme polymorphisms and cognitive function in Type 1 diabetes based on an 18-year follow-up of the DCCT cohort

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Abstract

Aims—Specific polymorphisms of the apolipoprotein E (APOE) and angiotensin-converting enzyme (ACE) genes appear to increase risk for Alzheimer's disease and cognitive dysfunction in the general population, yet little research has examined whether genetic factors influence risk of cognitive dysfunction in patients with Type 1 diabetes. The long-term follow-up of the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) population provides an opportunity to examine if specific genetic variations in APOE and ACE alter risk for cognitive decline.

Methods—Neurocognitive function in Type 1 diabetic subjects from the DCCT/EDIC study was assessed at DCCT entry and re-assessed approximately 18 years later, using a comprehensive

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Competing interests

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Supporting Information

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cognitive test battery. Glycated haemoglobin (HbA_{1c}) and the frequency of severe hypoglycaemic events leading to coma or seizures were measured over the 18-year follow-up. We determined whether the APO ε4 and ACE intron 16 indel genotypes were associated with baseline cognitive function and with change over time, and whether they conferred added risk in those subjects experiencing severe hypoglycaemic events or greater glycaemic exposure.

Results—None of the APOE or ACE polymorphisms were associated with either baseline cognitive performance or change in cognition over the 18-year follow-up. Moreover, none of the genotype variations altered the risk of cognitive dysfunction in those subjects with severe hypoglycaemic episodes or high HbA_{1c}.

Conclusions—In this sample of young and middle-aged adults with Type 1 diabetes, APOε4 and ACED alleles do not appear to increase risk of cognitive dysfunction.

Keywords

angiotensin-converting enzyme; apolipoprotein E; cognition; genetics; Type 1 diabetes

Introduction

Type 1 diabetes mellitus (Type 1 DM) leads to peripheral and autonomic neuropathy and micro- and macrovascular biomedical complications [1,2]. Increasing evidence also suggests that the central nervous system (CNS) may be affected adversely by diabetes and its treatment [3,4], but the underlying pathophysiological mechanisms remain poorly understood. Specific polymorphisms of the apolipoprotein E (APOE) and angiotensin-converting enzyme (ACE) genes have been reported to increase the risk for Alzheimer's disease as well as the risk for cognitive dysfunction in the general population and in individuals with Type 2 diabetes [5–10], but few studies have explicitly examined whether genetic factors influence risk of cognitive dysfunction in patients with Type 1 DM [11].

The APOE gene is involved in lipid metabolism and plays a role in remodelling neurons and glial cells in response to injury. Because lipids do not pass the blood–brain barrier, genetic variations that influence lipid metabolism could affect the ability of neurons to repair themselves after a wide range of assaults including those from glycaemic dysregulation. Indeed, the APO ε4 allele appears to increase risk of cognitive dysfunction after exposure to vascular disease and brain damage as a result of mild to moderate traumatic head injury [12]. In general, individuals homozygous for the ε4 allele have greater risk than those with APOε2 and ε3 alleles. Even ε4 heterozygotes show added risk for cognitive dysfunction in some studies [5]. In children, associations between polymorphisms in the APOE genotypes and cognitive function have not been found [13], but differences in brain structure associated with the ε4 allele have been identified [14]. Moreover, cognitively intact middle-aged adults with APO ε4 appear to show decreased density in frontal and temporal regions of the brain [15].

The only study of patients with Type 1DM has suggested that the presence of the APO ε4 allele was associated with worse cognitive performance, but this was limited to women; no genetic influence on cognition was found among men in the study [11]. Although it is plausible that the presence of an APO ε4 allele could exacerbate the neurocognitive consequences of glycaemic insults (severe hypoglycaemia and/or chronic hyperglycaemia), no interaction between genotype and previous exposure to severe hypoglycaemia was found in that study and interactions with persistent elevations in glycated haemoglobin (HbA_{1c}) were not tested. However, this was a small study with 96 individuals, so the potential of such known risk factors to amplify the effects of the APO ε4 allele would probably be missed because of inadequate power.

A smaller and less consistent body of research has also suggested that the ACE D allele confers risk of cognitive dysfunction and Alzheimer's disease in older adults [16–19]. While the majority of these studies suggest that the presence of the D allele increases risk, a smaller group of studies also suggest the presence of the I allele increases risk [20]. The apparent risk may relate to the effects of the ACE gene on cardiovascular status and effects on the rennin–angiotensin system. The presence of the D allele increases ACE activity, which is associated with increased prevalence of hypertension and cardiovascular disease. ACE activity also appears to directly affect the formation of amyloid and so may directly influence the progression of Alzheimer's disease [20]. It is not clear if ACE polymorphisms influence cognitive performance in younger adults or in those with diabetes. The vascular effects of ACE activity would be highly relevant to the risk of CNS disease in Type 1 DM, but no studies have examined this population.

We examined the association of the APOE and ACE polymorphisms on baseline cognitive performance and change in cognition in Type 1 DM patients followed in the Diabetes Control and Complications Trial (DCCT) and Epidemiology of Diabetes Interventions and Complications (EDIC) study over an average of 18 years. We addressed four primary research questions:

- i. Is the APO ϵ 4 or ACE D allele associated with level of cognitive functioning at DCCT baseline?
- ii. Is the APO ϵ 4 or ACE D allele associated with change in cognitive functioning over the 18-year follow-up?
- iii. Does the APO ϵ 4 or ACED allele modify the effects of either severe hypoglycaemic events or elevated HbA_{1c} on change in cognitive functioning?
- iv. Is there an interaction between either the APO ϵ 4 or ACED allele with gender or age such that women or older subjects with either polymorphism are at greater risk of cognitive decline than those without the polymorphism?

Secondarily, we carried out exploratory analyses to assess whether nine additional single-nucleotide polymorphisms (SNPs) in ACE and nine additional SNPs in the APOE region, selected to tag common variation in these genes, were associated with cognitive performance [21,22].

Patients and methods

Study sample

Between 1983 and 1989, 1441 subjects with Type 1 DM, 13 to 39 years of age, were enrolled in the DCCT and completed a comprehensive cognitive assessment battery. Approximately half of the sample ($n = 711$) was randomly assigned to intensive therapy; the remainder ($n = 730$) were assigned to conventional therapy with 1–2 daily insulin injections. Details of the diabetes management regimen can be found elsewhere [1]. At the end of the DCCT in 1993, intensive therapy was recommended for all subjects and, in 1994, 1375 (96%) of the 1428 surviving members volunteered to participate in the EDIC observational follow-up study [23]. In 2004, 1144 (85% of surviving, eligible participants) completed the cognitive test battery again. For these analyses, the sample was limited to the self-identified white subjects who were genotyped ($n = 1093$).

Cognitive test protocol

Cognitive testing, as originally described for the DCCT [24], was conducted at each site by personnel who were trained and certified by the DCCT/EDIC Central Neuropsychological Coding Unit. The test protocol, which required 4–5 h to complete, included the following

widely used, well-validated tests that were administered during the DCCT. Six subtests (Similarities, Comprehension, Digit Span, Digit Symbol, Block Design and Object Assembly) from the Wechsler Adult Intelligence Scale (WAIS), four subtests (Category Test, Tactual Performance Test, Trail Making Test and Finger Tapping Test) from the Halstead-Reitan Neuropsychological Battery, the Logical Memory and Visual Reproductions subtest from the Wechsler Memory Scale, the Digit Vigilance Test, the Grooved Pegboard Test, the Verbal Fluency (FAS) Test, the Four-Word Short Term Memory Test, the Symbol-Digit Learning Test and the Embedded Figures Test. Tests were administered in a fixed order. Capillary blood glucose levels were routinely monitored immediately before the testing and at its midpoint to rule out hypoglycaemia during testing. If a subject was found to have a blood glucose level ≤ 3.9 mmol/l, testing was stopped, the patient given a snack and, after waiting at least 15 min, testing was resumed when the blood glucose was ≥ 5.0 mmol/l. Tests were scored by technicians at the Central Neuropsychological Coding Unit who were masked to treatment assignment and other biomedical variables.

Cognitive domains

During the DCCT, 24 test variables had been chosen *a priori* to be of particular diagnostic value when applied to patients with Type 1 DM. For each of these 24 test variables, a standardized (Z) score was calculated, with the mean and standard deviation from the baseline assessment of the DCCT cohort used as reference [24]. To reduce the number of comparisons, the 24 standardized scores were grouped into one of eight cognitive domains consistent with standard neuropsychological assessment strategies [25]. For each domain, the simple average of the standardized scores was used to represent the change from baseline.

Genetic analyses

Genotyping of the APOE haplotype, rs7412 ($\epsilon 2/\epsilon 3$) and rs429358 ($\epsilon 3/\epsilon 4$) and ACE intron 16 indel (rs4340) was performed as previously described [21,26]. Genotyping and selection of nine additional SNPs in ACE allowed us to capture the common variation in that gene occurring with minor allele frequency $> 5\%$ and nine additional SNPs that extended outside the APOE locus. This was based on linkage disequilibrium criteria ($r^2 > 0.8$), which included the neighbouring genes APOC4 and APOC2 (see also Supporting Information, Table S1), and which are described elsewhere [22]. Quality control measures for the APOE and ACE markers used in the main analysis have been previously reported [21,26] as well as the quality control data for the additional 18 SNPs as described in the Supporting Information (Table S1) [27].

Biomedical evaluations

During the EDIC study, subjects completed an annual history, physical examination, electrocardiogram and laboratory testing, including serum creatinine and HbA_{1c}, using the same methods as during the DCCT [1]. Participants reported the presence of sensory symptoms of peripheral neuropathy as part of a neuropathy screening [23]. During the DCCT, severe hypoglycaemia over the previous 3 months was ascertained at quarterly interviews and through self-report between these visits. During the EDIC study, the severe hypoglycaemic events that occurred in the 3 months prior to the annual visit were documented on the annual history form. For this analysis, severe hypoglycaemic events are limited to those leading to coma and/or seizure [28]. There were 1282 episodes of coma or seizure reported over the 18-year follow-up (854 events in 247 intensive treatment group subjects and 428 events in 182 conventional treatment group subjects).

Statistical analyses

Clinical characteristics were compared using the Kruskal–Wallis test to assess the differences between genotype groups for ordinal and numeric variables. The contingency chi-square test was used for categorical variables [29].

Various transformations were used to normalize the eight cognitive domains as follows: domain 2 ($1.5^{\text{raw value}}$), domain 3 (winsorize tails to 1st and 99th percentiles), domain 4 (winsorize to 1st percentile), domain 5 [$\log(\text{raw score} - \text{minimum value})$], then winsorize to 1st percentile), domain 6 (winsorize to 2nd percentile), domain 7 ($\text{exponential}^{\text{raw value}}$), then winsorize to 99th percentile). Domains 1 and 8 remained untransformed.

The Kruskal Wallis test was used to assess the univariate relationship between ACE/APOE genotypes and each of the eight cognitive domains. For ACE we examined the three genotypes (I/I, D/D, I/D). For APOE we considered the presence of the $\epsilon 4$ and $\epsilon 2$ alleles separately and also modelled all six genotype groups simultaneously.

Separate analysis of covariance models were used to assess the effects of having at least one copy of the ACE or APO $\epsilon 4$ allele on the standardized quantitative score for each of the eight cognitive domain scores at DCCT baseline (see also Supporting Information, Table S2). Each model was adjusted for baseline age, gender, years of education and cohort assignment. We also examined 18 additional ACE/APOE markers that have been used in other genetic analyses of the DCCT/EDIC study outcomes [22].

Cognitive domain change scores were modelled using factorial analysis of variance models to identify any interactions with (i) treatment assignment, (ii) severe hypoglycaemia (0 vs. 1+ events), (iii) HbA_{1c} (above or below the median of 7.6%), (iv) the combined effect of hypoglycaemia and HbA_{1c} , (v) gender or (vi) age (above or below median age 46 years).

To address the potential for a Type 2 error (misinterpreting a null finding), we estimated our power to detect an effect using available data from the one other study of genetic effects on cognition in Type 1 DM [11]. With the available sample size, we have 80% power at $\alpha = 0.01$ to detect a locus that accounts for 1.2% of the trait variance in a quantitative trait at an α of 0.01 for an additive genetic model and a minor allele frequency of 0.3 (the $\epsilon 4$ allele frequency). This is comparable with that of Ferguson *et al.* [11] where the estimated η^2 value (which we presume is equivalent to r^2) was 26% for APOE on performance IQ and 25% on object assembly in women. The power of our study is also very consistent with other studies of non-diabetic samples examining the effects of APOE on cognitive function. Results were considered significant at the $P = 0.01$ level.

Results

Table 1 presents the clinical characteristics of participants at entry to the DCCT by genetic variations in both the APOE and ACE genotypes. The only statistically significant between-group difference at DCCT baseline was low-density lipoprotein (LDL) cholesterol across all six APOE genotypes as well as between the two $\epsilon 4$ groupings (3.0 ± 0.81 mmol/l for participants with $\epsilon 4$ vs. 2.7 ± 0.70 mmol/l for participants with no $\epsilon 4$; $P < 0.0001$). Because of the small number of individuals homozygous for the APO $\epsilon 4$ allele, we analysed the APOE data by comparing all variations and then comparing those subjects with and without the $\epsilon 4$ allele.

There were no significant main effects of either ACE or APOE variation on baseline cognitive performance after adjusting for baseline age, gender, years of education and cohort assignment.

By EDIC study year 12, the age of participants ranged from 29 to 62 years (mean $\pm$ SD 45.7 $\pm$ 6.8). Table 2 and Table 3 show the main and interaction effects of ACE D and APO ϵ 4 on change in cognition over time. Consistent with our earlier presentation of the cognitive findings [28], there is a statistically significant main effect for HbA_{1c} when modelled alone as well as in combination with severe hypoglycaemia. Higher levels of HbA_{1c} are associated with lower levels of cognitive functioning in relation to psychomotor efficiency and motor speed.

There were no significant main effects for either APO ϵ 4 or ACE D and no two-way interactions between genotype and either metabolic variable. There were also no statistically significant interactions between genotype, gender, age or treatment group (data not shown). There were also no differences in the impact of the ACE D gene on cognition between those who were on ACE inhibitors ($n = 431$ at year 12) and those who were not. Those taking ACE inhibitors had poorer cognitive function on one domain (domain 7) compared with those not taking an ACE inhibitor ($P < 0.0001$).

Separate analyses examined the effects of 18 additional ACE/APOE markers that have been used in other genetic analyses of the DCCT/EDIC study outcomes. Only two of the 144 associations examined were significant: ACE rs4305 and delayed recall ($P = 0.0089$) and ACE rs4354 and problem solving ($P < 0.0089$). For ACE rs4305, genotypes A/B and B/B performed worse than A/A (mean cognitive scores: -0.23 , -0.26 , -0.08 , respectively). In the second case, genotype A/B performed better than B/B (mean cognitive scores: 0.24 , 0.05 , respectively). However, when these P -values were conservatively adjusted with a Bonferroni correction, they were no longer significant.

Discussion

The primary goal of this study was to examine the extent to which the cognitive dysfunction frequently noted in adults with Type 1 DM [30] is linked to the presence of two genetic factors (APO ϵ 4 and ACE D alleles) that have previously been found to increase the risk of cognitive dysfunction in older healthy adults [9,16]. Contrary to our expectation, our study of young and middle-aged diabetic adults found no evidence of a relationship between cognition and the two genetic factors. This was true both for our analysis of diabetic subjects at entry into the DCCT, when they had a relatively brief duration of diabetes and minimal microvascular complications, as well as in analyses of the same subjects retested approximately 18 years later, at a time when a large proportion had some microvascular complications as well as exposure to severe hypoglycaemic events.

Because some research suggests that the ϵ 4 allele may confer added risk in response to other processes that can lead to brain injury [12,31,32], we also tested the hypothesis that genetic factors could modify the effects of common glycaemic exposures (recurrent, severe hypoglycaemic events or chronically elevated HbA_{1c}) and thus influence cognitive performance in those important subgroups. Again, we found no evidence of such an effect. Recording of severe hypoglycaemic events varied in frequency from quarterly during DCCT to once each year (history limited to the prior 3 months) in the EDIC study. While this yielded substantial prospectively gathered information, we may still have underestimated the effects of hypoglycaemia on cognition, especially during the EDIC study when subjects were seen less frequently.

These null results deserve some comment. The evidence for genetic effects on cognitive function and/or Alzheimer's disease in the non-diabetic population is more robust for APO ϵ 4 than that for the ACE D allele and this is particularly true when examining older individuals, especially those with or at high risk for Alzheimer's disease [9,17]. Only a few

studies have included groups of subjects who are less than 60 years of age and the evidence for the effects of each genotype is less persuasive in such younger populations [8]. The relative youthfulness of our sample (mean sample age at follow-up was 47 years) may explain our null results, as well as the fact that, across studies, even among older adults, the magnitude of these effects tends to be quite small [9]. It is possible that such effects would be found in older Type 1 DM adults and only then would they magnify the effects of glycaemic exposure. Of interest, the relationship of APO $\epsilon 4$ to LDL cholesterol level and higher HbA_{1c} to poorer cognitive function is consistent with the literature and this helps support the validity of the primary findings.

Our results are inconsistent with the single published study examining the relationship between APOE polymorphisms and cognition in patients with Type 1 DM [11]. In that cross-sectional study, genetic-cognition effects were limited to women with the $\epsilon 4$ allele who had an added risk of cognitive dysfunction compared with women without the allele or to diabetic men with or without the allele. With a far larger sample size (1093 subjects; 47% female; 290 with at least one $\epsilon 4$ allele, as compared with 96 subjects; 21 with at least one $\epsilon 4$ allele [11]), we have far more statistical power to identify these genetic/cognition/gender relationships and, given our sample size and our extended follow-up into the middle adult years, we still find no specific risk of cognitive dysfunction for women with at least one $\epsilon 4$ allele.

One important limitation of our study is that there were relatively few people in this study who were homozygous for APOE $\epsilon 4$. Such individuals are at greatest risk and so an effect could have been missed which would have been uncovered with a larger pool of high-risk individuals. However, increased risk for Alzheimer's disease has clearly been shown in $\epsilon 4$ heterozygotes and 26.5% of DCCT/EDIC study white probands carry at least one $\epsilon 4$ allele (Table 1). Moreover, the DCCT/EDIC study is relatively large compared with most available clinical populations and the clinical assessments are very detailed, thereby making it less likely that effects have gone undetected in the age range and follow-up period studied.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations

ACE	angiotensin-converting enzyme
APOE	apolipoprotein E
CNS	central nervous system
DCCT	Diabetes Control and Complications Trial
EDIC	Epidemiology of Diabetes Interventions and Complications
HbA_{1c}	glycated haemoglobin
LDL	low-density lipoprotein
SNP	single-nucleotide polymorphism
Type1 DM	Type 1 diabetes mellitus

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Table 1
Characteristics of participants at entry into the DCCT by genetic variations in APOE and ACE

Variable	ACE				APOE				APOE			
	n	II	ID	DD	ε2/ε2	ε2/ε3	ε2/ε4	ε3/ε3	ε3/ε4	ε4/ε4	ε4	No ε4
Genotypes												
Total (n) (%)	1093	252 (23.1)	539 (49.3)	302 (27.6)	5 (0.5)	137 (12.5)	23 (2.1)	661 (60.5)	247 (22.6)	20 (1.8)	290 (26.5)	803 (73.5)
Sex												
Male	578	132	292	154	2	72	14	347	130	13	157	421
Female	515	120	247	148	3	65	9	314	117	7	133	382
Treatment												
Intensive	564	138	281	145	3	73	12	329	136	11	159	405
Conventional	529	114	258	157	2	64	11	332	111	9	131	398
Cohort												
Primary	531	115	271	145	2	68	9	322	120	10	139	392
Secondary	562	137	268	157	3	69	14	339	127	10	151	411
Education (years)	1093	13.7 ± 2.6	14.2 ± 2.5	14.1 ± 2.6	12.4 ± 2.6	14.3 ± 2.5	13.4 ± 2.6	14.1 ± 2.6	14.1 ± 2.5	14.3 ± 3.6	14.0 ± 2.6	14.1 ± 2.6
Age (years)	1093	26.8 ± 7.2	27.1 ± 6.9	27.0 ± 6.6	26.4 ± 8.1	27.1 ± 6.7	28.8 ± 7.1	27.0 ± 6.9	26.9 ± 6.7	26.3 ± 7.8	27.0 ± 6.8	27.0 ± 6.9
Duration (years)	1093	6.2 ± 4.2	5.8 ± 4.2	6.0 ± 4.2	5.4 ± 3.4	6.1 ± 4.4	7.5 ± 4.8	5.9 ± 4.2	5.7 ± 4.1	6.0 ± 4.4	5.9 ± 4.2	5.9 ± 4.2
HbA _{1c} (%)	1093	9.0 ± 1.6	8.9 ± 1.6	8.9 ± 1.6	8.4 ± 1.0	9.1 ± 1.5	9.5 ± 1.6	8.9 ± 1.6	9.0 ± 1.6	9.0 ± 1.5	9.0 ± 1.6	9.0 ± 1.6
LDL cholesterol (mmol/L)	1093	2.8 ± 0.8	2.8 ± 0.7	2.8 ± 0.8	1.8 ± 0.9	2.5 ± 0.6	2.7 ± 0.7	2.8 ± 0.7	3.0 ± 0.8	3.2 ± 1.1 [†]	3.0 ± 0.8	2.7 ± 0.7 [†]
Domain	1093											
Problem solving		-0.03 ± 0.04	0.00 ± 0.03	0.01 ± 0.03	0.46 ± 0.14	-0.05 ± 0.05	0.32 ± 0.12	-0.02 ± 0.02	0.04 ± 0.04	-0.08 ± 0.12	0.06 ± 0.05	-0.02 ± 0.02
Learning		0.05 ± 0.05	-0.04 ± 0.04	0.05 ± 0.04	-0.29 ± 0.30	0.06 ± 0.06	-0.15 ± 0.15	0.00 ± 0.03	-0.01 ± 0.05	0.18 ± 0.14	-0.01 ± 0.05	0.01 ± 0.03
Immediate memory		0.07 ± 0.04	-0.00 ± 0.03	-0.01 ± 0.04	0.13 ± 0.39	0.06 ± 0.05	-0.17 ± 0.15	-0.01 ± 0.02	0.05 ± 0.04	0.06 ± 0.16	0.03 ± 0.04	0.01 ± 0.02
Delayed recall		-0.02 ± 0.04	-0.01 ± 0.03	0.05 ± 0.04	-0.34 ± 0.13	0.02 ± 0.05	-0.07 ± 0.15	-0.01 ± 0.02	0.07 ± 0.04	-0.22 ± 0.15	0.04 ± 0.04	-0.01 ± 0.02
Spatial information		-0.06 ± 0.04	0.04 ± 0.03	-0.04 ± 0.04	0.40 ± 0.27	-0.12 ± 0.06	0.25 ± 0.15	-0.02 ± 0.03	0.05 ± 0.05	-0.03 ± 0.16	0.06 ± 0.04	-0.03 ± 0.02
Attention		-0.00 ± 0.04	0.02 ± 0.03	-0.00 ± 0.04	-0.08 ± 0.18	-0.01 ± 0.05	-0.20 ± 0.11	0.02 ± 0.02	0.01 ± 0.04	-0.03 ± 0.21	-0.01 ± 0.04	0.02 ± 0.02
Psychomotor efficiency		-0.03 ± 0.03	0.00 ± 0.02	0.02 ± 0.03	0.35 ± 0.10	0.01 ± 0.04	0.09 ± 0.08	-0.01 ± 0.02	0.00 ± 0.03	0.03 ± 0.08	0.01 ± 0.02	-0.00 ± 0.02
Motor speed		0.01 ± 0.05	0.03 ± 0.04	-0.01 ± 0.05	-0.39 ± 0.37	0.06 ± 0.08	0.10 ± 0.23	0.00 ± 0.04	0.02 ± 0.05	-0.04 ± 0.22	0.02 ± 0.05	0.01 ± 0.03

Data are mean ± SD or n.

* HbA_{1c} value derived from assessment to determine study eligibility.

[†] $P < 0.01$ by the χ^2 -test or Fisher's exact test for categorical traits or the Kruskal–Wallis test for ordinal and numeric traits.

ACE, angiotensin-converting enzyme; APOE, apolipoprotein E; DCCT, Diabetes Control and Complications Trial; HbA_{1c}, glycated haemoglobin; LDL, low-density lipoprotein; SD, standard deviation.

Table 2
Main and interaction effects of ACE D on change in cognitive functioning, analysed by level of severe hypoglycaemia by HbA_{1c}

Domain	Problem solving	Learning	Immediate memory	Delayed recall	Spatial information	Attention	Psychomotor efficiency	Motor speed
Model 1								
ACE D (yes vs. no)	0.14 (0.70)	1.21 (0.27)	0.31 (0.58)	0.21 (0.65)	0.00 (0.99)	0.57 (0.45)	3.60 (0.06)	0.10 (0.75)
Severe hypo (0 vs. 1+)	0.44 (0.51)	1.38 (0.24)	1.28 (0.26)	0.05 (0.82)	2.18 (0.14)	0.18 (0.67)	2.98 (0.08)	2.34 (0.13)
Severe hypo × ACE D	0.00 (0.99)	0.08 (0.78)	0.33 (0.57)	0.51 (0.47)	0.41 (0.52)	0.98 (0.32)	0.07 (0.79)	0.07 (0.79)
Model 2								
ACE D (yes vs. no)	0.57 (0.45)	1.20 (0.27)	0.35 (0.55)	0.05 (0.82)	0.13 (0.72)	0.28 (0.60)	2.06 (0.15)	0.16 (0.69)
HbA _{1c} (low vs. high) [†]	0.57 (0.45)	0.64 (0.42)	3.52 (0.06)	1.23 (0.27)	3.66 (0.06)	6.33 (0.01)	21.96 (< 0.01)	18.53 (< 0.01)
HbA _{1c} × ACE D	2.50 (0.11)	0.76 (0.38)	0.49 (0.48)	1.19 (0.28)	2.53 (0.11)	1.04 (0.31)	1.50 (0.22)	1.37 (0.24)
Model 3*								
ACE D (yes vs. no)	0.48 (0.49)	1.44 (0.23)	0.62 (0.43)	0.01 (0.93)	0.08 (0.77)	0.12 (0.73)	1.89 (0.17)	0.23 (0.63)
Severe hypo (0 vs. 1+)	0.07 (0.79)	1.33 (0.25)	0.83 (0.36)	0.00 (0.96)	3.08 (0.08)	0.05 (0.83)	2.38 (0.12)	1.77 (0.18)
HbA _{1c} (low vs. high) [†]	0.43 (0.51)	0.79 (0.37)	4.16 (0.04)	1.45 (0.23)	2.81 (0.09)	6.18 (0.01)	22.11 (< 0.01)	20.45 (< 0.01)
Severe hypo × HbA _{1c} × ACE D	5.37 (0.02)	0.73 (0.39)	0.03 (0.85)	0.74 (0.39)	2.67 (0.10)	0.62 (0.43)	0.00 (0.99)	0.16 (0.69)

Data are *F*-statistics (*P*-values) for main effects and interaction effects.

* Because there were no significant two-way interactions, they were not included in the model.

[†] Higher HbA_{1c} was associated with a greater cognitive decline in psychomotor efficiency and motor speed.

ACE, angiotensin-converting enzyme; HbA_{1c}, glycated haemoglobin

Main and interaction effects of APO $\epsilon 4$ on change in cognitive functioning, analysed by level of severe hypoglycaemia, HbA_{1c} and severe hypoglycaemia by HbA_{1c}

Table 3

Domain	Problem solving	Learning	Immediate memory	Delayed recall	Spatial information	Attention	Psychomotor efficiency	Motor speed
Model 1								
APO $\epsilon 4$	0.40 (0.53)	0.34 (0.56)	0.13 (0.71)	0.01 (0.92)	1.29 (0.26)	0.38 (0.54)	0.00 (0.99)	1.04 (0.31)
Severe hypo (0 vs. 1+)	0.30 (0.59)	1.01 (0.32)	2.31 (0.13)	0.31 (0.58)	1.24 (0.26)	1.31 (0.25)	3.35 (0.07)	2.15 (0.14)
Severe hypo $\times$ APO $\epsilon 4$	0.18 (0.67)	0.71 (0.40)	0.01 (0.92)	0.73 (0.39)	0.00 (0.97)	4.58 (0.03)	0.06 (0.80)	0.01 (0.94)
Model 2								
APO $\epsilon 4$	0.21 (0.65)	0.10 (0.75)	0.27 (0.61)	0.01 (0.90)	1.62 (0.20)	0.87 (0.35)	0.02 (0.88)	1.12 (0.29)
HbA _{1c} (low vs. high) [†]	0.05 (0.82)	0.03 (0.86)	1.44 (0.23)	1.58 (0.21)	0.39 (0.53)	5.70 (0.02)	19.93 (< 0.01)	29.90 (< 0.01)
HbA _{1c} $\times$ APO $\epsilon 4$	0.01 (0.91)	0.87 (0.35)	0.58 (0.45)	2.18 (0.14)	0.97 (0.33)	0.29 (0.59)	0.02 (0.89)	0.09 (0.77)
Model 3*								
APO $\epsilon 4$	0.30 (0.59)	0.29 (0.59)	0.19 (0.66)	0.00 (0.98)	1.40 (0.24)	0.38 (0.54)	0.05 (0.83)	1.37 (0.24)
Severe hypo (0 vs. 1+)	0.34 (0.56)	0.75 (0.39)	2.31 (0.13)	0.42 (0.52)	1.55 (0.21)	1.91 (0.17)	2.84 (0.09)	2.30 (0.13)
HbA _{1c} (low vs. high) [†]	0.03 (0.86)	0.00 (0.99)	1.86 (0.17)	1.88 (0.17)	0.25 (0.62)	5.65 (0.02)	20.45 (< 0.01)	31.58 (< 0.01)
Severe Hypo $\times$ HbA _{1c} $\times$ APO $\epsilon 4$	0.36 (0.55)	0.14 (0.71)	0.14 (0.71)	1.67 (0.20)	0.88 (0.35)	3.58 (0.06)	0.48 (0.49)	1.27 (0.26)

Data are *F*-statistics (*P*-values) for main effects and interaction effects.

* Because there were no significant two-way interactions, they were not included in the model.

[†] Higher HbA_{1c} was associated with a greater cognitive decline in psychomotor efficiency and motor speed.

APO, apolipoprotein; HbA_{1c}, glycated haemoglobin