Cognitive functioning and hippocampal connectivity in patients with longstanding type 1 diabetes and apolipoprotein E ε4

Short title: Cerebral effects of APOE-ε4 in T1DM

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Abstract

Objective: While the Apolipoprotein E ε4 allele (ApoE-ε4) is related to cognitive and brain decline in the general population, its effect on the brain in type 1 diabetes (T1DM) remains unclear. Therefore, the aim was to determine the interaction between ApoE-ε4 and T1DM on cognitive performance and hippocampal structure and connectivity, as brain area most vulnerable to ApoE-ε4 effects in adult patients with T1DM.

Research Design and Methods: 104 T1DM and 49 controls underwent blood sampling for ApoE genotyping, neuropsychology, and neuroimaging to determine hippocampal volume and resting-state connectivity. The interaction between T1DM status and ApoE-ε4 presence was investigated, adjusted for age and mean systolic blood pressure.

Results: For 3 T1DM patients ApoE genotyping could not be performed. Significant interaction effects, indicating a differential effect of ApoE-ε4 between both groups, were found for overall cognitive functioning, and for the subdomains of information processing speed and attention. Additionally, interaction effects were present for right hippocampal connectivity with the right posterior cingulate and supramarginal gyri. Subsequent group analysis showed that T1DM patients with ApoE-ε4 performed worse on these cognitive domains with increased connectivity, relative to their counterparts without ApoE-ε4. In contrast, no cognitive effects, but decreased connectivity were observed in controls with ApoE-ε4. In T1DM patients, higher right hippocampus connectivity with the posterior cingulate gyrus was related to poorer overall cognitive functioning.

Conclusions: The results may suggest that ApoE-ε4 presence leaves our patients with T1DM more susceptible to cognitive decrements at a younger age, possibly through vascular pathways, warranting further longitudinal studies.

In individuals with type 1 diabetes mellitus (T1DM), speed-related cognitive decrements and focal brain alterations have been observed (1). These alterations are found across the life-span, and seem to be most pronounced in those with clinically manifest microangiopathy, in particular in patients with retinopathy (1). This suggests a major role for vascular damage in these cerebral alterations, a role which is further highlighted by a markedly increased risk of vascular dementia in aging patients with T1DM (2; 3).

An established genetic risk factor for dementia and cognitive decline is the ε4 allele of the Apolipoprotein E (ApoE) gene. ApoE has 3 different alleles, ε2, ε3, and ε4, and is involved in lipid metabolism and cerebral injury repair (4). The presence of one, and especially of 2 ε4 alleles significantly increases the risk of Alzheimer's disease (5), and of cognitive and brain decline (i.e. atrophy and white matter lesions) in younger individuals and in healthy aging (6; 7). Studies have shown that the hippocampus is one of the structures most vulnerable to the effects of ApoE-ε4 (4; 8). Due to the presence of insulin receptors on the hippocampus, this structure has been hypothesized to also be particularly susceptible to the effects of T1DM. Indeed, studies using T1DM animal models have been showing alterations in hippocampal apoptosis, neurogenesis, and pruning (9). In humans with T1DM, hyper- and hypoglycemia have been related to developmental alterations in the hippocampus (10).

A less well-known effect of ApoE-ε4 is an up to 3-times increased risk of vascular dementia and vascular cognitive impairment (11). Given the strong relationship between T1DM and vascular dementia (2; 3), it could be hypothesized that ApoE-ε4 presence in T1DM is related to a brain more vulnerable to decline. Studies, however, relating ApoE-ε4 to cognition in T1DM are scarce, and we are not aware of any studies having included neuroimaging. In middle-aged T1DM patients, the DCCT/EDIC study did not find a difference in cognitive performance between those with or without ApoE-ε4 (12). Another study did observe worse cognitive and intellectual performance in women with T1DM and ApoE-ε4 (13).

As the effects of ApoE-ε4 expression can already be observed in the general population before aging commences, further investigation of the effect of ApoE-ε4 expression in younger individuals with T1DM is of interest, also to provide insights for the aging population with T1DM. Therefore, we set out to detail the interaction between T1DM and ApoE-ε4 in a group of 104 adult individuals with T1DM and 51 controls. The first objective was to assess cognitive performance, where it was hypothesized that ApoE-ε4 expression would be related to mild cognitive deficits in T1DM. As the hippocampus has been found to be particularly vulnerable to the effects of ApoE-ε4 expression and potentially to the effect of T1DM, the second objective was to study hippocampal volume and how well the hippocampus is functionally connected to other brain regions (also called functional connectivity) in relation to ApoE-ε4. Lastly, if interactions proved to be present, associations between cognitive performance and hippocampal parameters were investigated.

Research Design and Methods

Participants

This study was approved by the Medical Ethics Committee of the VU University Medical Center and conducted in accordance with the Declaration of Helsinki. Patients and healthy controls provided written informed consent. Between April 2007 and October 2009 153 (104 with T1DM and 49 controls) were included. Patients were recruited through outpatient clinics of the VU University Medical Center and affiliated hospitals, and through advertisements in (diabetes-specific) printed media. Controls were recruited through patients and the same advertisements. Inclusion criteria were age between 18 and 56 years, right handedness, and proficiency in Dutch. Patients with T1DM needed to have a disease duration of 10 years or more, be either free of clinically manifest microangiopathy or have at least proliferative retinopathy (see below). Exclusion criteria were insufficient visual acuity to perform neuropsychological testing, BMI above 35 kg/m^2, MRI-contraindications, cardiovascular or cerebrovascular disease, (a history of) alcohol or drug abuse, psychiatric comorbidity warranting treatment, centrally acting medication use, head trauma, hepatitis, anemia, thyroid dysfunction, pregnancy, or epilepsy. Hypertension and lipid lowering medication use were exclusion criteria for controls only.

Biomedical and anthropometric variables

Proliferative retinopathy was established by fundus photography and rated according to the EURODIAB classification (ACM)(14). Albuminuria was ascertained by a 24-hour urine albumin:creatinine ratio equal to or above 2.5 mg/mmol for men and 3.5 mg/mmol for women. Neuropathy information was obtained from the medical records, based on information from an annual clinical check-up that patients receive, or self-reported, if this information was not available (n=12)(15). Those with proliferative retinopathy could have other microvascular

complications, whereas their counterparts without microangiopathy could not have any clinically manifest microvascular complications. Severe hypoglycemic events were self-reported during life-time, according to DCCT-guidelines (16).

During the study days, blood glucose was actively kept between 4 and 15 mmol/l. Individuals were instructed to inject 2 units of their rapid acting insulin analogue in case of blood glucose levels up to 20 mmol/l, and 4 units in case of levels exceeding 20 mmol/l. In case of hypoglycemia, the ingestion of 20 g of carbohydrates was required. After 30 minutes blood glucose was measured again, and the protocol was repeated until blood glucose levels were in the required range. Testing recommenced 30 minutes after glucose levels were within the required range. Hypoglycemic events 24-hours prior to testing resulted in rescheduling. Mean blood pressure was measured 3 times with 5-minute intervals in a seated position after a 15-minute rest.

Apolipoprotein E genotyping

DNA was isolated from 10 ml EDTA blood by the QIAamp DMN blood isolation kit from Qiagen. The genotype was then determined by the Light Cycler ApoE mutation detection kit (Roche Diagnostics GmbH, Mannheim, Germany). For the purpose of this study, patients and controls with at least 1 ε4 allele were categorized as ApoE-ε4 positive, the other participants as ApoE-ε4 negative. This created 4 groups, those with T1DM and controls who were ApoE-ε4 negative or positive.

Neuropsychological assessment

A detailed description of the cognitive tests used and domains evaluated can be found elsewhere (17). IQ was estimated using the Dutch version of the National Adult Reading Test (18). ‘Memory’ included the Rey Auditory Verbal Learning Test (19), the Digit Span forward

and backward of the Weschler Adult Intelligence Scale III revised (WAIS-III-R) (20), and the WAIS-III-R Symbol Substitution Incidental Learning Test (20). 'Information processing speed' consisted of the Concept Shifting Test (CST) part A and B (21), the WAIS-III-R Symbol Substitution Test (20), the Stroop Color-Word Test part 1 and 2 (22), the Simple Auditory and Visual Reaction Time Tests (23), and the Computerized Visual Searching Task (23). 'Attention' was created using parts of the D2-test (24). 'Executive functions' was constructed by part C of the CST (21), part 3 of the Stroop Color-Word Test (22), D2-test total errors (24), the Wisconsin Card Sorting Test (25), and the Category Word Fluency Test (26). 'Motor speed' included part 0 of the CST (21), and the Tapping Test with the dominant and non-dominant hand (23). 'Psychomotor speed' was created using the Letter-Digit Modalities Test (27).

Raw scores were transformed into z-scores based on the mean and standard deviation of the control participants. If necessary, scores were inversed so that positive z-values indicated better performance. The average of all cognitive tests formed the domain of 'overall cognitive functioning'. However, for this domain the standard deviation of all controls as one group was 0.44, instead of 1 for the other domains. Renormalizing overall cognitive functioning would create a standard deviation of 1, but would increase the individual's domain-score by a factor of 0.44. To be able to report the real mean overall cognitive functioning scores, we therefore decided not to renormalized this domain.

MRI protocol

Imaging was performed using a 1.5T whole-body Siemens Sonata (Siemens, Erlangen, Germany), using an 8-channel phased-array head coil. For this study, T1 Magnetization Prepared Rapid Acquisition Gradient Echo (T1-MPRAGE; repetition time (TR) 2700 milliseconds; echo time (TE) 5.17 milliseconds; inversion time (TI) 950 milliseconds; flip angle 80^{o}; 248x330 mm^2 field of view (FoV); 1.0x1.0x1.5 mm voxel size; 160 contiguous

coronal partitions), a T2* Echo Planar Imaging (EPI) functional sequence during rest (10 minutes, 202 volumes, TR 2850 milliseconds; TE 60 milliseconds; flip angle 90^{o}; 384x384 mm^2 FoV; isotropic 3.3 mm voxels; 36 axial slices), a 3D Fluid Attenuated Inverse Recovery (3D-FLAIR; TR 6500 milliseconds; TE 385 milliseconds; variable flip angle; 1.3 mm isotropic voxels) were included. The 3D-FLAIR was used to determine prevalence of white matter lesions based on visual inspection by a trained neuroradiologist.

Hippocampus volume

Segmentation of the bilateral hippocampi was executed in native T1-MPRAGE space using FMRIB's Software Library (FSL) version 6.0.1's Brain's Integrated Registration and Segmentation Tool (FIRST) (28). Segmentations were manually checked for errors, which did not occur. Segmentations were binarized and used to extract mean left and right hippocampal volume using FSL-STATS. Volume in native space was then normalized for head size by multiplying by the V-scaling parameter obtained from FSL-SIENAX.

Hippocampus functional connectivity

As preprocessing, from the raw fMRI-scan, the first 10 images were discarded to obtain a steady-state signal. The remaining images were slice timing corrected, smoothed with a 6mm FWHM Gaussian kernel, motion corrected, and transformations to T1-MPRAGE and MNI152 standard space were calculated using FSL-FEAT. To control for additional motion, all images were then processed using ICA-AROMA to perform ICA-based motion correction (29), and white matter and cerebrospinal fluid signal was regressed out. The resulting images were then band-pass filtered using a 0.01 to 0.1 Hz kernel, and non-linearly warped to 4mm MNI152 standard space.

To determine hippocampus functional connectivity, the binarized hippocampus segmentations were transformed from native T1-MPRAGE to fMRI-EPI space, and hippocampal time-series were extracted in fMRI native space, as this better matches the individual anatomical shape, reducing the partial volume effect, than extracting the time-series from standard space. Then, in MNI152 standard space using FSL-FEAT, the hippocampus functional time-series was correlated with the time-series of every other voxel, thus creating an individual statistical connectivity map between the seed and all other brain voxels.

Statistical analyses

Normality of all variables was checked using the histograms and QQ-plots in SPSS version 25 (IBM-SPSS, Chicago, IL). Demographic variables were analyzed with an one-way ANOVA, Kruskal-Wallis Test, or χ^2 test.

To analyze the differential effect of ApoE-ε4 presence (1 or 2 alleles together) between controls and patients with T1DM, an interaction term ApoE-ε4 x Group was created and, together with group (T1DM vs. controls) and ApoE-ε4 (positive vs. negative) entered into a regression model. This model was then adjusted for age and mean systolic blood pressure. Accordingly, the interaction between group and ApoE-ε4 presence was analyzed for cognitive performance, and normalized hippocampus volume. A $P < 0.05$ for the interaction effect was considered statistically significant.

To determine the interaction between group and ApoE-ε4 presence for hippocampus functional connectivity, a second level functional analysis in FSL-FEAT was performed, including all individuals' first level connectivity maps. Statistically significant interactions were determined by a cluster-wise threshold set at $z < 3.1$ with a Family Wise Error corrected $P < 0.05$. This, according to previously published data, sufficiently controls for multiple

comparisons (30). In case of statistically significant interaction effects, mean functional connectivity z-values were extracted from clusters.

In all T1DM patients, linear regression was used to determine whether ApoE-ε4-mediated cognitive alterations were related to alterations in hippocampal functional connectivity and hippocampus normalized volume. This analysis was adjusted for age, mean systolic blood pressure, sex, and estimated IQ.

Results

Participants

For 3 patients with T1DM ApoE genotyping was unsuccessful, due to insufficient material. As can be found in Table 1, almost every participant had at least 1 ApoE-ε3 allele, with the ε3/ε3 combination being the most common. ApoE-ε4 was present in 33 (32.7%) cases with T1DM and in 15 (30.6%) controls, whereas only 8 participants (4 T1DM) had 2 ApoE-ε4 alleles. As can be found in Table 2, controls without ApoE-ε4 were significantly younger than the other groups (P=0.003). HbA_{1c} (P<0.001), systolic blood pressure (P=0.045), and pulse pressure (P=0.001) were higher in both groups with T1DM compared with controls. Lipid profile was more favorable in those with T1DM showing higher HDL cholesterol (P=0.002) and lower triglyceride levels (P=0.001), possibly due to the use of lipid lowering medication in this group. Those with T1DM and ApoE-ε4 had on average an earlier disease onset age (P=0.031), reported a higher number of severe hypoglycemic events (P=0.042), but had similar pre-MRI and pre-neuropsychology blood glucose levels (P>0.05), compared to their counterparts without ApoE-ε4. There was no difference in microvascular complication prevalence between both groups with T1DM, nor was there for white matter lesions (all P>0.05).

ApoE-ε4 affects cognition in T1DM patients but not in controls

Overall cognitive functioning showed a statistically significant interaction with presence of the ApoE-ε4 allele, after correction for age and mean systolic blood pressure (β=-0.365, P=0.012, Figure 1). Subsequent post-hoc testing of the cognitive subdomains demonstrated significant interaction effects, after age and mean systolic blood pressure were added, for information processing speed (β=-0.332, P=0.023, Figure 1) and attention (β=-0.390, P=0.010, Figure 1).

Subsequent regression analyses were performed to determine cognitive domain differences in controls and T1DM patients based on the presence or absence of ApoE-ε4,

correcting for age and mean systolic blood pressure. As shown in Figure 1, the presence of ApoE-ε4 was associated with poorer overall cognitive functioning (β=-0.263, *P*=0.006), information processing speed (β=-0.260, *P*=0.008), and attention (β=-0.220, *P*=0.022) in T1DM patients, but not in controls. The association of ApoE-ε4 with the first 2 domains (*P*<0.05), but not for attention (*P*=0.065), remained significant after additional correction for diabetes onset age, retinopathy, and sex. In all T1DM patients, there was no interaction between sex and ApoE-ε4 status for these cognitive domains (all *P*>0.05).

ApoE-ε4 is related to increased hippocampal functional connectivity in T1DM, but decreased in controls, without affecting hippocampal volume

There were no significant interaction effects between ApoE-ε4 and Group for left or right normalized hippocampus volumes (all *P*>0.05, Figure 1). Contrary, uncorrected for confounding factors, a voxel-wise interaction was statistically significant for resting-state functional connectivity between the right hippocampus and the right posterior part of the cingulate and precentral gyri (P_{FWE}=0.031, 16 voxels, 1024mm^3, z_{max}=4.31, Cohen's δ=-0.78, Figure 1, Figure 2A). The interaction between ApoE-ε4 and group status also reached significance for connectivity between the right hippocampus and the right supramarginal and angular gyri (P_{FWE}=0.005, 23 voxels, 1472mm^3, z_{max}=3.84, Cohen's δ=-0.65, Figure 1, Figure 2B). These interactions survived correction for age only, but not age and mean systolic blood pressure (*P*=0.074 and *P*=0.061 respectively). No interaction effects were found for the left hippocampus, nor were there interactions between sex and ApoE-ε4 status in T1DM (all *P*>0.05).

In the regression analysis (Figure 1), the presence of ApoE-ε4 in T1DM was, after correction for age and mean systolic blood pressure, related to increased functional connectivity between the right hippocampus and the right posterior part of the cingulate and

precentral gyri (β=0.356, *P*<0.001), and between the right hippocampus and the right supramarginal and angular gyri (β=0.290, *P*=0.004). Both remained significant after additional correction for diabetes onset age, retinopathy, and sex (*P*<0.001 and *P*=0.001). For both clusters, functional connectivity was decreased in controls with ApoE-ε4 (all *P*<0.05).

Higher right hippocampus functional connectivity was related to lower cognitive functioning in all T1DM patients

In order to identify if the right hippocampus affected by ApoE-ε4 was related to cognitive decrements in T1DM, forward linear regression was used. The forward block consisted of right hippocampus normalized volume and functional connectivity between this structure and the right posterior part of the cingulate and precentral gyri and the right supramarginal and angular gyri. Independent predictors of cognitive decrements were then corrected for age and systolic blood pressure.

Within the T1DM group as a whole, poorer overall cognitive functioning was related to higher functional connectivity between the right hippocampus and the right posterior part of the cingulate and precentral gyri (β=-0.230, *P*=0.018, Figure 1). Additionally, correcting for diabetes onset age, retinopathy, and sex or excluding the outlier did not alter statistical significance. Information processing speed and attention were not related to hippocampus variables. There were no correlations in the group with T1DM and ApoE-ε4 only.

Conclusions

The aim of this study was to evaluate the effects of ApoE-ε4 on cognitive functioning and the hippocampus in adult individuals with T1DM. We found significant interactions between group allocation (controls versus T1DM) and ApoE-ε4 (presence versus absence) for cognitive functioning and functional connectivity of the right hippocampus. Regarding cognition, performance worsened in individuals with T1DM and ApoE-ε4 compared to their counterparts who were ApoE-ε4 negative. In controls of this study, there was no noticeable effect of ApoE-ε4 on cognition. Contrary to the decrease in cognitive performance, functional connectivity of the right hippocampus with the right posterior part of the cingulate and precentral gyri and with the right supramarginal and angular gyri was increased in those with T1DM and ApoE-ε4. In controls, a decreasing effect of ApoE-ε4 presence was found. In T1DM, poorer overall cognition was independently related to increased functional connectivity of the right hippocampus with the right posterior part of the cingulate and precentral gyri.

In this group of relatively young patients with T1DM, ApoE-ε4 presence had a moderately negative effect (-0.25 standard deviation) on overall cognitive performance. Further analysis demonstrated that this was driven by stronger negative effects on information processing speed (-0.52 standard deviation) and attentional performance (-0.37 standard deviation). As in control subjects with ApoE-ε4 of a similar age no associations with cognition were found, it can be hypothesized that ApoE-ε4 presence leaves our patients with T1DM more susceptible to cognitive decrements at a younger age. These results are in line with a previous case-control study, albeit that study showed an effect in women only, something not observed in the current study (13). Our results differ from the DCCT/EDIC analysis, which did not show any longitudinal effect of ApoE-ε4 on cognitive functioning in patients of roughly the same age at year 18 of follow-up (12). These different results may be driven by the lack of control data in the DCCT/EDIC trial, due to different inclusion criteria between studies, especially

relating to microvascular disease, or that ApoE-ε4 presence causes an initial effect on cognition, without longitudinal effects in middle-aged patients. Importantly, it may be different for an older population of patients, where ApoE-ε4 may cause an additional vulnerability along with aging. Future longitudinal studies should determine the effect of ApoE-ε4 in aging T1DM groups.

Previously, we have shown that performance was diminished in the above mentioned cognitive domains in all T1DM patients, independent of the presence of microvascular complications, albeit effect sizes were largest in those with proliferative retinopathy (15). The current results, which were found to be independent of proliferative retinopathy, could indicate that ApoE-ε4 specifically targets those cognitive functions that are already weaker in adult individuals with T1DM, or it may be indicative that in younger T1DM patients ApoE-ε4 specifically affects speed-related domains. As speed-related cognitive decline is a hallmark of vascular cognitive impairment and vascular dementia, it could then be hypothesized that in these adult patients with T1DM, the ApoE-ε4 genotype exerts its negative effect through vascular pathways (11; 31), and not through the Alzheimer or amyloid deposition pathway (4). This would need to be studied in more detail in future projects including larger samples of T1DM patients with ApoE-ε4, specifically by including markers of cerebral vascular functioning, such as perfusion, vasoreactivity, or neurovascular coupling. Unfortunately, these measures were not available in this study. However, in the current T1DM group increased cerebral vascular burden has been found in comparison with controls (32), whereas no evidence of cerebral amyloid pathology was demonstrated (33), possibly supporting the vascular hypothesis. However, as in older people with T1DM memory decrements have been observed (34), the Alzheimer/amyloid pathway of ApoE-ε4-related cognitive decrements may become more prominent as people with T1DM age. An alternative pathway by which ApoE-ε4 could affect cognition is through altered cerebral glucose metabolism. Decreased glucose metabolism

has been found in T1DM patients (35), and has also been demonstrated in people with ApoE-ε4, even in those who are cognitively normal (36). Future studies should verify the effects of these different pathways on cognition and cerebral structures in T1DM.

In the absence of hippocampus volume changes, functional connectivity alterations between the right hippocampus and the posterior part of the cingulate and precentral gyri and the supramarginal and angular gyri were observed. Within the functional brain network, the hippocampus is part of the default mode network, which additionally encompasses bilateral lateral temporal and parietal regions, the precuneus, the posterior and anterior cingulate cortex and bilateral frontal parts (37). Both the posterior cingulate gyrus and the supramarginal and angular gyri regions are part of this default mode network, thus indicating that ApoE-ε4 presence in adult T1DM is related to an alteration of the functional connectiveness of the right hippocampus within the default mode network. As default mode network functional connectivity alterations have not been found previously in this sample (15), it may be an effect specifically of ApoE-ε4. It remains unclear why this effect was lateralized to the right hippocampus, but such lateralized effects, towards either side, have been found before (38; 39).

This increased functional connectivity was shown to have clinical significance as it correlated to poorer overall cognitive functioning in all patients with T1DM. Although it is known that the relationship between functional connectivity and cognition is more complex, it is commonly thought that increased functional connectivity may serve as a compensation mechanism for loss of structural integrity and to keep up cognitive performance (40). Indeed, we previously found that in T1DM increased occipital functional connectivity was related to better overall cognitive functioning and information processing speed (15). Such a relationship has also been found in another population of ApoE-ε4 carriers (39). Contrary, we found that increased right hippocampal functional connectivity with the right posterior part of the cingulate and precentral gyri to be related to lower overall cognitive functioning, indicating

that the increased functional connectivity in those with ApoE-ε4 may not be compensatory in nature, but rather detrimental. It could also indicate, however, that the increased functional connectivity was not sufficient (anymore) to prevent diminished overall cognitive functioning. This correlation does show that altered hippocampus functional connectivity is related to ApoE-ε4-driven cognitive decrements in individuals with T1DM and that it thus captures clinically important information. However, future studies should further evaluate the relationship between functional connectivity and cognition in relation to ApoE-ε4 and T1DM.

As patients with ApoE-ε4 reported a slightly higher median number of severe hypoglycemic events than those without ApoE-ε4 (4 versus 1.5), we determined the correlation between cognition and hippocampal connectivity in T1DM, but did not find any significant associations (data not shown). This suggests that in our group of adult patients, hypoglycemia did not influence the results, or the effects were too subtle to detect.

Limitations include the younger age of the controls without ApoE-ε4, higher systolic blood pressure in T1DM, and earlier disease onset age for those with ApoE-ε4. However, all the results are independent from these variables. With 101 T1DM patients, we were able to detect within-T1DM interactions with a medium effect of Cohen's f of 0.28, which limited our possibility to assess interactions between ApoE-ε4 and sex, proliferative retinopathy and early onset age. Given the low number of participants with 2 ApoE-ε4 alleles (Table 1), we could not discriminate between those with 1 or 2 alleles. Because of the wide age-range of this study, the time-scale of the found mechanism may vary. Information on racial background, and why patients were using antihypertensive and lipid lowering medication was not collected. We were also not able to determine the longitudinal effects of ApoE-ε4 presence on the brain.

In conclusion, we found that ApoE-ε4 presence in T1DM was related to lower cognitive performance in speed-related domains, but higher functional connectivity of the right hippocampus with the right posterior part of the cingulate and precentral gyri and the right

supramarginal and angular gyri. Furthermore, higher right hippocampal functional connectivity with right posterior cingulate and precentral gyri was related to lower overall cognitive functioning. It can thus be hypothesized that ApoE-ε4 presence leaves our patients with T1DM more susceptible to cognitive decrements at a younger age, possibly through vascular pathways. It shows the importance to study the effect of ApoE-ε4 presence in T1DM, especially in the more vulnerable aging population, warranting longitudinal studies, including larger samples and older people with T1DM.

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Duality of Interest

The authors declare no conflict of interest pertaining to this study.

Author Contributions

EvD participated in the design of the study, collected the data, performed the data analysis, and wrote the manuscript. RIJ was involved in grand writing, participated in the design of the study, supervised the general flow of the study, and made critical revisions to the manuscript. FB clinically rated all MRI-scans made in this study, supervised the neuroradiological part of the study, participated in the design of MRI part of this study, and made critical revisions to the manuscript. ACM rated all fundus photographs and made critical revisions to the manuscript. MD was the principal investigator of this study and, as such, was responsible for the design of

the study, grant writing, and all other aspects of this study. She passed away before this manuscript was written. FJS participated in the design of the study, supervised the psychological part of this study, and made critical revisions to the manuscript. MK participated in the design of the study, supervised the neuropsychological part of this study, and made critical revisions to the manuscript. EvD and RIJ are guarantors of this study and, as such, had full access to all the data and take responsibility for the integrity of the data and accuracy of the data analysis.

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Table 1. Results of ApoE genotyping.

	Controls (n = 49)	T1DM patients (n = 101)
ApoE genotyping (%)		
ε2/ε2	0 (0)	0 (0)
ε2/ε3	7 (14.7)	9 (8.9)
ε2/ε4	0 (0)	4 (4.0)
ε3/ε3	26 (53.1)	59 (58.4)
ε3/ε4	11 (22.4)	25 (24.8)
ε4/ε4	4 (8.2)	4 (4.0)

Table 2. Participant characteristics.

	Controls without ApoE-ε4	Controls with ApoE-ε4	T1DM without ApoE-ε4	T1DM with ApoE-ε4	Overall P	P controls*	P T1DM*
N	34	15	68	33	-		
Age (years)	34. 38 ± 9.82	41.93 ± 12.68	41.79 ± 9.14	39.55 ± 8.33	0.003	0.069	0.999
Sex (women/men; [% men])	10/5 (33.3)	20/14 (41.2)	42/26 (38.2)	20/13 (39.4)	0.966	0.754	0.999
Estimated IQ†	107.29 ± 11.66	111.13 ± 11.81	110.65 ± 11.18	104.91 ± 13.59	0.105	0.999	0.148
Depressive symptoms‡	4.00 (0 – 19)	5.00 (0 – 37)	7.00 (0 – 37)	7.00 (0 – 42)	0.147	0.735	0.772
Body mass index (kg/m^2)	24.31 ± 3.84	24.17 ± 3.04	25.34 ± 3.97	24.92 ± 3.63	0.517	0.999	0.999
Current nicotine use (%)	10 (29.4)	1 (6.7)	9 (13.2)	7 (21.2)	0.148	0.137	0.385
Current alcohol use (%)	24 (70.6)	13 (86.7)	59 (86.8)	23 (69.7)	0.101	0.298	0.057
Systolic blood pressure (mmHg)	123.41 ± 12.66	124.10 ± 7.79	131.07 ± 15.54	130. 17 ± 15.85	0.045	0.999	0.999
Diastolic blood pressure (mmHg)	77.71 ± 7.37	76.57 ± 6.91	77.02 ± 9.10	76.21 ± 8.46	0.904	0.999	0.999
Pulse pressure (mmHg)§	45.72 ± 9.50	47.03 ± 4.94	54.04 ± 12.11	53.95 ± 12.35	0.001	0.999	0.999
Hypertension (%)‖	-	-	30 (44.1)	15 (45.5)	-	-	0.999
Total cholesterol (mmol/l)	4.32 ± 0.84	4.85 ± 0.94	4.67 ± 0.76	4.54 ± 0.70	0.106	0.217	0.999
HDL cholesterol (mmol/l)	1.48 ± 0.39	1.50 ± 0.42	1.83 ± 0.46	1.75 ± 0.53	0.002	0.999	0.999
LDL cholesterol (mmol/l)	2.35 ± 0.72	2.79 ± 0.84	2.42 ± 0.65	2.38 ± 0.54	0.227	0.295	0.999
Non-HDL (mmol/l)¶	2.84 ± 0.86	3.44 ± 1.02	2.84 ± 0.75	2.79 ± 0.58	0.056	0.107	0.999
Triglycerides (mmol/l)	1.10 (0.50 – 2.00)	1.35 (0.50 – 3.20)	0.80 (0.30 – 2.40)	0.80 (0.40 – 2.50)	0.001	0.255	0.886
Lipid lowering medication (%)	-	-	17 (25.0)	7 (21.2)	-	-	0.805
White matter lesion prevalence (%)	5 (14.7)	4 (26.7)	11 (16.2)	8 (24.2)	0.548	0.427	0.417
TSH (mU/l)	1.42 ± 0.69	1.79 ± 1.00	1.67 ± 0.88	1.74 ± 0.80	0.361	0.999	0.999
Corrected thyroid dysfunction (%)#	-	-	8 (11.8)	2 (6.1)	-	-	0.492
HbA1c (%)	5.26 ± 0.24	5.31 ± 0.23	7.81 ± 0.93	8.15 ± 1.43	<0.001	0.999	0.516
HbA1c (mmol/mol)	34.04 ± 2.62	34.50 ± 2.46	61.81 ± 10.11	65.52 ± 15.60	<0.001	0.999	0.516
Diabetes duration (years)	-	-	27.85 ± 10.73	29.00 ± 10.28	-	-	0.611
Diabetes onset age (years)	-	-	12.00 (1 – 35)	8.00 (1 – 35)	-	-	0.031
Blood glucose before MRI (mmol/l)	-	-	9.93 ± 4.24	9.68 ± 4.34	-	-	0.780
Blood glucose before NPA (mmol/l)	-	-	8.13 ± 3.71	9.20 ± 4.62	-	-	0.216
Albumin:creatinine ratio (mg/mmol)	-	-	0.53 (0 – 19.13)	0.39 (0 – 33.17)	-	-	0.881
Proliferative retinopathy (%)**	-	-	31 (45.6)	20 (60.6)	-	-	0.204
Neuropathy (%)††	-	-	17 (25.0)	8 (24.2)	-	-	0.999

Albuminuria (%)‡‡	-	-	9 (13.2)	5 (15.2)	-	-	0.999
Severe hypoglycemic events§§	-	-	1.50 (0 – 50)	4.00 (0 – 30)	-	-	0.042

Data were presented as means with standard deviation, absolute numbers with percentages, or medians with minimum and maximum. MRI: Magnetic Resonance Imaging; NPA: Neuropsychological Assessment.

* P-values of the post-hoc between group (with and without ApoE-ε4) analyses in controls and T1DM patients separately.

† Estimated IQ was measured using the Dutch version of the National Adult Reading Test.

‡ Depressive symptoms were measured using the Center for Epidemiological Studies scale for Depression.

§ Pulse pressure was calculated as the difference between systolic and diastolic blood pressure for each measurement and then averaged.

‖ Hypertension was defined as a systolic blood pressure of 140 or above, a diastolic blood pressure of 90 or above, or the use of antihypertensive medication. Controls with hypertension were excluded.

¶ Non-HDL was calculated by subtracting HDL from total cholesterol levels.

T1DM patients with thyroid dysfunction could only participate when using proper medication and normal TSH.

** Proliferative retinopathy was established by fundus photography, evaluated by an ophthalmologist (ACM) according to the EURODIAB criteria.

†† Neuropathy was based on the medical records, or, in case these were unavailable, on self-report.

‡‡ Albuminuria was defined as an albumin:creatinine ratio of 2.5 mg/mmol or higher for men and 3.5 mg/mmol or higher for women.

§§ Severe hypoglycemic events were self-reported during lifetime and consisted of those events which resulted in loss of consciousness, seriously deranged functioning, coma, or seizure owing to low glucose levels.

Figure 1. Bar graphs of mean values with standard error of the mean for each of the 4 groups. Displayed are values for the cognitive domains, for hippocampus volume and functional connectivity. White bars indicate controls without ApoE-ε4, white striped bars indicate controls with ApoE-ε4, black striped bars indicate patients with T1DM without ApoE-ε4, and black bars indicate T1DM with ApoE-ε4. In the last graph, a scatter plot represents the correlation between overall cognition and right hippocampus functional connectivity in all T1DM patients. Individuals with T1DM without ApoE-ε4 are represented as black circles and those with ApoE-ε4 are represented as black squares. Due to the averaging process for overall cognitive functioning, all controls as one group had a standard deviation of 0.44 instead of 1 for the other domains. Although the graph presents the 'real' mean scores for overall cognitive functioning, caution when comparing this domain mean score with the other domains is necessary as the standard deviations are on a different scale. * Statistically significant interaction effect. ** Statistically significant difference between the 2 respective groups. FC: functional connectivity, PCC: posterior part of the cingulate and precentral gyri, SML: supramarginal and angular gyri.

Figure 2. Schematic view of the location of the 2 clusters of resting-state functional connectivity with the right hippocampus that showed a statistically significant interaction at a cluster threshold of z = 3.1, and Family Wise Error corrected $P < 0.05$. The clusters are presented in radiological orientation (right is left; left is right) and overlaid on a 4 mm isotropic voxels MNI152 standard brain. Coordinates are given in MNI152 standard space.