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Gray matter brain alterations in type 1 diabetes – findings based on detailed phenotyping of neuropathy status

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Abstract

Aims: This study investigated brain structure in type 1 diabetes with diabetic peripheral neuropathy and type 1 diabetes with neuropathic pain and the associations to clinical, peripheral, and cognitive measurements.

Methods: Sixty individuals with type 1 diabetes and 20 healthy controls were included in the study. Nineteen individuals with type 1 diabetes and neuropathic pain, 19 with type 1 diabetes and diabetic peripheral neuropathy, 18 with type 1 diabetes without diabetic peripheral neuropathy, and 20 healthy controls were included in the brain analyses. We utilized structural brain magnetic resonance imaging to investigate total and regional gray matter volume.

Results: Significant lower gray matter volume was found in type 1 diabetes with neuropathic pain and in type 1 diabetes without diabetic peripheral neuropathy compared to healthy controls (p=0.024 and p=0.019, respectively). Lower insula volume was observed in all three diabetes groups (all $p \leq 0.050$). Thalamus and hippocampus volume were lower in type 1 diabetes with neuropathic pain, cerebellum volume was lower in type 1 diabetes with diabetic peripheral neuropathy, and somatosensory cortex volume was lower in type 1 diabetes without diabetic peripheral neuropathy (all $p \leq 0.018$). Attenuated memory was associated with lower gray matter volume in type 1 diabetes with diabetic peripheral neuropathy. No associations were found between gray matter volume and clinical/peripheral measurements.

Conclusion: We demonstrated lower gray matter volume in individuals with type 1 diabetes regardless of the presence of diabetic peripheral neuropathy and neuropathic pain. Hence, central gray matter alteration was not associated with peripheral alterations.

Keywords: Diabetes; Magnetic resonance imaging; Diabetic peripheral neuropathy; Brain structure; Brain volume alterations

1. Introduction

Diabetic peripheral neuropathy (DPN), a common complication of diabetes, has a prevalence of up to 50% of all individuals with diabetes.[1,2] DPN is characterized by dysfunction of the peripheral nervous system with loss of sensations and other sensory symptoms like tingling, prickling, and neuropathic pain.[2,3] The symptoms start distally in the toes and feet but progress proximally to the upper limbs.[1,3,4] Neuropathic pain is experienced by approximately 15-25% of all individuals with diabetes.[1,5,6]

Using non-invasive MRI, evidence has shown central nervous system (CNS) changes, including structural brain alterations in type 1 diabetes with and without DPN and neuropathic pain.[7–13] A meta-analysis revealed structural brain changes, especially in the thalamus in people with type 1 diabetes.[11] Other studies have investigated whether underlying diabetic complications like microangiopathy and DPN are related to changes in brain structure.[8,10,11,14,15] However, the results reported are not consistent on which regional brain structures are most vulnerable for alterations. Moreover, the underlying mechanisms behind structural brain alterations in diabetes are still not well-documented. Some studies have reported brain alterations in individuals with diabetes without any underlying complications.[14] Other studies have shown that structural brain changes occurred early in the neuropathic process also in persons with subclinical DPN.[16] Moreover, studies have reported that CNS alterations are associated with the severity of DPN.[15] Inconsistent findings in previous studies and the limited number of studies investigating brain alterations in groups of detailed phenotyping of neuropathy status still leave a gap between the understanding of DPN, neuropathic pain, and structural brain changes. More studies are needed to validate and further explore this relation.

Brain alterations in diabetes have also been associated with cognitive changes.[17] Even though studies have suggested cognitive impairment in neuropathy [18–20], the investigation of clear associations is still lacking in well-characterized neuropathy groups in type 1 diabetes.

The aim of this study was threefold. We utilized structural brain MRI to investigate our first and main aim, which was to examine total and regional GMV in well-phenotyped groups with type 1 diabetes (type 1

diabetes with neuropathic pain, type 1 diabetes with painless DPN, type 1 diabetes without DPN) and healthy controls. Secondly, we exploratively investigated the association of total GMV to clinical and experimental peripheral measurements. Moreover, we exploratively investigated whether the structural brain changes were related to cognitive alterations in the three diabetes groups. This study had a data-driven analysis approach to explore structural alterations in the whole brain.

2. Materials and methods

2.1 Design and participants

This study was a cross-sectional, observational, case-control study and part of the clinical study named MEDON (Methods of Early Detection of diabetic peripheral Neuropathy), where MRI results were one of the primary outcomes to investigate its prognostic value of DPN. Participants were recruited through the outpatient clinic at the Department of Endocrinology, Aalborg University Hospital, Denmark, between August 2019 and April 2021. The study included 80 participants in four groups: 1) 20 individuals with type 1 diabetes and neuropathic pain, 2) 20 individuals with type 1 diabetes and painless DPN, 3) 20 individuals with type 1 diabetes without DPN and neuropathic pain, and 4) 20 healthy controls. Each participant in one group was sex and age matched to a participant in each of the other three groups. The diabetes groups were clinically diagnosed with type 1 diabetes. All participants were included if they were between 18-70 years old and excluded if they met one of the following: abnormalities in the thyroid- or parathyroid metabolism, impaired liver- or kidney function, history or current alcohol abuse and/or drug abuse, cancer or history of chemotherapy, presence of chronic viral infections, severe skin disease, critical ischemia of the lower extremities, pregnancy, and factors that preclude MRI. All participants provided informed written consent before enrollment in the study. The study was conducted according to the Declaration of Helsinki. The North Denmark Region Committee on Health Research Ethics (N-20190003) granted the ethical approval, and the study was registered with clinicaltrials.gov (NCT04078516).

2.2 Phenotyping and clinical parameters

In the current MRI study, DPN was defined as confirmed DPN according to the Toronto consensus statement.[1] Individuals with DPN were pre-screened based on their medical journal and included if the vibration perception threshold was equal to or above 25 V. The test was performed using a biothesiometry on the participant's first toe. To confirm DPN, the participants were additionally tested for the presence of abnormal nerve conduction study (NCS) performed on the right leg with standardized skin temperature by the Department of Neurophysiology, Aalborg University Hospital, Denmark. The results were processed according to the local reference values. Measurements not detectable due to nerve damage were denoted with zero. This publication presents only the velocity (m/s), and amplitude (μV) of the sensory nerve, sural nerve.

Individuals with neuropathic pain were clinically confirmed by two independent medical doctors supported by the Douleur Neuropathique 4 Questions (DN4), a validated screening tool to identify diabetic neuropathic pain.[21] The 10 items questionnaire includes seven items related to pain description and abnormal sensations and three items of neurological examination in the painful area.[21,22] Participants with a score equal to or above 4 were classified as having neuropathic pain. Moreover, the peak and average pain intensity for the last 4 weeks was measured using a numeric rating scale (NRS) ranging from 0 to 10, where 0 represents no pain, and 10 represents the worst pain ever possible.[23] Information on demographical and clinical characteristics, including sex, age, diabetes duration and onset, and retinopathy/nephropathy status, were obtained. HbA1c was analyzed in blood samples at the Department of Clinical Biochemistry, Aalborg University Hospital, Denmark. The blood glucose level was measured between the cognitive test and the MRI scan.

2.3 Thermal detection threshold

A standardized battery of Quantitative Sensory Testing according to the protocol of the German Research Network on Neuropathic Pain (DFNS) was performed.[24,25] Only data for the warm and cold detection

threshold are reported in the current study. While the NCS performed tested the large nerve fiber neuropathy, the thermal detections are C- and A-delta fiber (small nerve fibers) mediated and were used as a measure of small nerve fiber neuropathy.[25] The test was performed using a thermal sensory testing device (Thermal Sensory Analyzer (TSA), Medoc, Israel). The thermode was placed 2-3 centimeters proximal to the second toe, and the detection thresholds were obtained by continuously increasing or decreasing the thermal stimuli. The examinations were conducted in a standardized room and skin temperature.

2.4 Cognitive questionnaire

Addenbrooke's Cognitive Examination-III (ACE-III), which is a validated cognitive test, was performed to assess cognitive function.[26] The total score is 100 points, which is distributed in five cognitive domains: attention (18 points), memory (26 points), verbal fluency (14 points), language (26 points), and visuospatial abilities (16 points). A higher score reflects better cognitive ability.[26] The cognitive test was performed before the MRI scan.

2.5 Structural brain MRI acquisition

Structural brain images were obtained using a 3T MRI GE scanner (Signa Premier, General Electrics, Milwaukee, WI, USA) at the Department of Radiology, Aalborg University Hospital, Denmark. A 48-channel head coil was used, and the head was fixed using foam pads. 3D T1-weighted structural brain scans (MPRAGE) were acquired with the following scanning parameters: echo time (TE) 3.6 ms, repetition time (TR) 8.4 ms, flip angle 8°, field-of-view (FOV) 25 cm, resolution 0.78 x 0.78 mm, and slice thickness 0.80 mm. The scan time was around 6 minutes.

2.6 Structural brain MRI processing

In order to investigate the total and regional GMV, voxel-based morphometry (VBM) analyses were carried out using the Computational Anatomy Toolbox (Cat12.7 version r1742), implemented through the Statistical Parametric Mapping (SPM) software (Wellcome Trust Centre for Neuroimaging, London, England)(SPM12, www.fil.ion.ucl.ac.uk/spm/). The default settings, described in detail in the CAT12 toolbox manual, were followed for the preprocessing step with a resolution fixed at 0.8 mm. Briefly, the processing steps were: The T1-weighted structural images were normalized to the Montreal Neurological Institute (MNI) template using DARTEL and then segmented into gray matter, white matter, and cerebrospinal fluid. Bias correction was performed to remove intensity non-uniformities. Then the images were normalized with a voxel size of 1.5×1.5 ×1.5 mm, followed by smoothing with 8 mm Gaussian Kernel. Whole-brain GMV, white matter volume, and cerebrospinal fluid were extracted for each participant.

2.7 Statistical analyses

Data are presented as mean±standard deviation (SD) or numbers (%) unless otherwise stated. The assumption of normal distribution was checked using Q-Q plots.

One-way ANOVA or Kruskal-Wallis test determined any group difference in the demographic, clinical, peripheral, and cognitive data. Chi-squared or Fisher's exact test was used to test for differences in sex, insulin treatment regime, and retinopathy/nephropathy status. Post-hoc analyses were performed to detect any group-wise difference with Bonferroni-correction used to correct for multiple comparisons.

One-way ANCOVA was used to compare group differences of GMV, white matter volume, and cerebrospinal fluid with total intracranial volume (TIV) and age as fixed factors. Group-wise comparisons were performed using Bonferroni corrected post-hoc tests. Also, GMV alterations were investigated in a subgroup analysis between a type 1 diabetes group without large or small nerve fiber neuropathy compared to healthy controls. Voxel-wise one-way ANOVA estimated the group difference in regional GMV with TIV and age as covariates.

This analysis was performed in SPM. An absolute threshold mask of 0.1 was applied. An initial $p<0.001$ and family-wise error (FWE) corrected $p<0.05$ at the cluster level was used for group comparisons.

In all participants with diabetes, partial correlation tests controlling for TIV and age or Spearman correlation tests were used to explore any association between GMV and clinical, cognitive, and peripheral parameters, including nerve conduction measurements and thermal detection thresholds. Since the possibility to correct GMV for TIV was eliminated in non-parametric correlation tests, GMV relative to TIV (denoted relative GMV) was used in the Spearman correlation tests. All correlation tests were controlled for multiple comparisons with Bonferroni correction.

Additionally, associations between relative GMV and nerve conduction tests and between total GMV and cognitive parameter (memory) controlling for TIV and age were exploratively investigated in each diabetes group.

$P<0.05$ was considered significant. IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY) was used for statistical analyses.

3. Results

One individual from the diabetes group with neuropathic pain and one from the diabetes group without DPN did not complete the MRI session due to claustrophobia. Furthermore, one subject from the diabetes group with painless DPN did not have any abnormal NCS results, while one subject in the diabetes group without DPN showed more than one abnormal NCS result. Hence, four subjects were excluded from the statistical analyses. The final cohort comprised 19 type 1 diabetes individuals with neuropathic pain, 19 type 1 diabetes individuals with DPN, 18 type 1 diabetes individuals without DPN or neuropathic pain, and 20 healthy controls. See also Figure 1.

The clinical and demographic characteristics of the participants are summarized in Table 1. Overall, the four groups were comparable with respect to sex, age, and BMI ($p\geq0.318$). Age at diabetes onset and insulin

treatment regime were comparable in the three diabetes groups (all $p \geq 0.183$). The overall p-value for nephropathy status was $p=0.011$. However, pairwise comparisons showed no significant differences (all $p \geq 0.12$). As expected, HbA1c was significantly lower in healthy controls than the other three diabetes groups (post-hoc, all $p<0.001$). Moreover, both HbA1c and diabetes duration were higher in the diabetes group with DPN compared to those without DPN (post-hoc, all $p \leq 0.020$). The results of all peripheral measurements (NCS and thermal detection thresholds) were poorer in the group with diabetes and neuropathic pain and in the group with diabetes and DPN as compared to those with diabetes without DPN and healthy controls (post-hoc, all $p \leq 0.017$). As expected, those with neuropathic pain had higher pain scores compared to the other three groups (post-hoc, all $p<0.001$). A lower score for memory was observed in the group with diabetes and DPN compared to healthy controls (post-hoc, $p=0.035$). No differences between the other groups or other cognitive domains were found (post-hoc, all $p \geq 0.057$).

3.1 Total gray matter volume

Table 1 also summarizes TIV, total GMV, white matter volume, and cerebrospinal fluid adjusted for TIV and age. The total adjusted GMV was significantly different between the groups (overall $p=0.010$). The pairwise comparisons demonstrated lower GMV in both diabetes groups with neuropathic pain and without DPN compared to healthy controls (post-hoc, $p=0.024$ and $p=0.019$, respectively). Also, total GMV in adults with DPN was lower than healthy controls but not statistically significant (post-hoc, $p=0.435$). See Figure 2.

To further investigate whether GMV alterations were independent of small nerve fiber neuropathy, a subgroup analysis further explored the finding of reduced GMV adjusted for TIV and age. Thus, 11 subjects with type 1 diabetes without any small or large nerve fiber abnormalities were compared to healthy controls, whereas seven participants experiencing one or more abnormalities in small nerve fiber measurements (warm and cold detection thresholds) were excluded. The subgroup analysis showed lower adjusted GMV (mean±SE: 631.7±5.5 mL) compared to healthy controls (mean±SE: 655.6±4.1 mL), ($p=0.002$), data not shown

in Table 1. This confirmed that GMV is affected in individuals with type 1 diabetes regardless of small and large nerve fiber peripheral neuropathy.

3.2 Associations between gray matter volume and clinical, peripheral, and cognitive parameters

Associations between unadjusted GMV and clinical and cognitive parameters (HbA1c, diabetes duration, and memory) were investigated with TIV and age as controlling factors. For this, all participants with type 1 diabetes (n=56) were included. Moreover, correlation tests were performed between relative GMV and peripheral measurements (nerve conduction amplitude and velocity and thermal thresholds). Association between relative GMV and warm detection threshold was seen (r=-0.308, p=0.021) but did not survive the Bonferroni corrected p-value (p=0.147)). There were no associations between GMV and the other peripheral measurements nor between GMV and clinical or cognitive measurements (all $p \geq 0.932$, not Bonferroni-corrected).

The Bonferroni corrected statistical significance was accepted at $p \leq 0.005$. There was an association between GMV and memory score in the group with diabetes and DPN (r=0.676, p=0.003), also significant after the Bonferroni correction (p=0.027). This association was not found in the diabetes group with neuropathic pain or the group without DPN (p=0.899 and p=0.174, respectively). No correlations were found between the GMV and nerve conduction tests in any subgroups (all p=0.125).

3.3 Regional gray matter volume

Regions with altered regional GMV are described in Table 2 and Figure 3. Overall, lower regional GMV was found in individuals with type 1 diabetes and neuropathic pain compared with healthy controls in bilateral thalamus (dorsal part), hippocampus, planum temporale, transverse temporal gyrus, parietal operculum, superior temporal gyrus, and central operculum (all $p_{FWE}<0.018$). Moreover, lower GMV was found in bilateral frontal regions as well as in the insula and operculum (all $p_{FWE}<0.050$).

When comparing individuals with type 1 diabetes and DPN and healthy controls, lower GMV was observed in bilateral cerebellum and left insula in the diabetes group (all p_{FWE}<0.018). The diabetes group without DPN had lower GMV in bilateral frontal regions, insula, and operculum compared to healthy controls (all p_{FWE}<0.004), similar to the regions seen in the group with diabetes and neuropathic pain. Moreover, the same group also demonstrated lower GMV in the somatosensory cortex (p_{FWE}=0.010). Finally, GMV in frontal regions was lower in type 1 diabetes without DPN compared to those with DPN (p_{FWE}=0.037). No significant differences were found between the other diabetes groups, and no areas showed increased regional GMV in any of the diabetes groups compared to healthy controls.

4. Discussion

This study investigated the structural brain changes in well-characterized groups of type 1 diabetes with and without DPN and neuropathic pain. Lower total GMV was found in participants with type 1 diabetes and neuropathic pain and in type 1 diabetes without DPN compared to healthy controls. However, total GMV alterations were not related to clinical and peripheral nerve measurements in individuals with type 1 diabetes. Moreover, cognitive alterations in the form of memory were lower in those with DPN compared to healthy controls, and this alteration was associated with lower total GMV in the same diabetes group. Compared to healthy controls, all three groups with diabetes showed lower regional GMV in one or more brain regions, including insula adjacent to operculum, putamen, thalamus/hippocampus, and frontal regions, mainly superior frontal cortex, cerebellum, and somatosensory cortex.

Several studies have demonstrated lower GMV in individuals with type 1 diabetes compared to healthy controls, both in total GMV and in different regional areas.[11–14,27] Investigations of the impact of underlying complications on GMV loss are limited. In our study, no differences in total GMV were observed between the

three type 1 diabetes groups. However, significantly lower total GMV was demonstrated in those with diabetes and neuropathic pain and in those with diabetes without DPN compared to healthy controls.

Our findings suggest that total GMV loss in diabetes is not specific for DPN or neuropathic pain but is a general phenomenon in individuals with type 1 diabetes. This was additionally supported by our subgroup analysis, where participants with type 1 diabetes without DPN and subclinical DPN still showed lower GMV. The lower total GMV in diabetes without DPN suggests that brain structure alterations could be present before the development of DPN, which is also suggested in other studies with similar but also different diabetes duration than our cohort.[11,12,28] Also, studies investigating GMV in youths with type 1 diabetes have shown regional gray matter alterations, suggesting that the alterations might start early and that other factors might contribute to the process of GMV loss.[11,29]

A previous study investigated individuals with type 1 diabetes and DPN compared to healthy controls and found lower total GMV, which was more pronounced in subgroups of participants with neuropathic pain.[13] These findings are in accordance with our study. Even though the group with type 1 diabetes and DPN did not show significantly lower GMV in our study, the mean value was lower. Compared to Hansen et al. 2021[13], our study had a smaller sample size which might explain that our findings are not similar to the differences reported byby Hansen et al. Moreover, another study found lower total GMV in a group with painful and painless DPN compared to type 1 diabetes without DPN and healthy controls.[14] In our study, the type 1 diabetes with painless DPN and type 1 diabetes with neuropathic pain were analyzed separately, which may explain the difference in results between the studies. Moreover, these somehow conflicting results also suggest the importance of phenotyping and grouping the type 1 diabetes individuals with DPN and neuropathic pain, which might reflect different results.

The total GMV loss observed in our study was not associated with any of the clinical parameters. Furthermore, while other studies found correlations between GMV and peripheral measurements, our study did not find any. These conflicting results may partly be explained by the different GMV estimates used. While we used total GMV for the correlation tests, other studies have used peripheral GMV (not including deep gray matter volumes) and regional GMV. Moreover, the peripheral measurements used in our study were based

on the raw values of NCS of peripheral sensory nerve only. In contrast, other studies have calculated a composite score that also includes one or more of the motor nerves. Hence, more studies are needed in the future to investigate these inconsistencies.

One possible explanation for the different findings across the studies is that the results might be cohort-specific. Moreover, it is also suggested that other etiologies may contribute to structural alterations noted in diabetes, which is not directly related to neuropathy status. For instance, several studies have suggested that gray matter regions are vulnerable to hyperglycemia and severe hypoglycemia.[30–32] Also, hyperglycemia may result in an accumulation of potentially toxic glucose metabolites, oxidative stress, accelerated formation of advanced glycation end-products, and microvascular changes in the brain.[33] Hyperglycemia-induced alterations may lead to accelerated aging of the brain, such as cortical atrophy.[34] To reflect this HbA1c was available in the current study. We found higher HbA1c in all three diabetes groups compared to healthy controls. Moreover, higher HbA1c was also found in those diabetes individuals with painless DPN compared to those without DPN. Since the highest HbA1c value was observed in diabetes with DPN, one might expect GMV loss, especially in this particular group. However, this group did not show any alterations in the GMV. Moreover, we did not find any associations between GMV and HbA1c, suggesting that HbA1c did not influence our GMV data. However, a possible impact of HbA1c cannot be excluded. Furthermore,HbA1c is an indirect measurement for hyperglycemia. Since we did not collect data on hyperglycemia and hypoglycemic events, we cannot conclude that GMV alterations are related or not related to such mechanisms.

The investigation of regional GMV alterations showed that all three type 1 diabetes groups had regional GMV loss compared to healthy controls. All three diabetes groups showed lower GMV of the insula. Insula has been suggested to play a crucial role in the somatosensory manifestations [35] and various homeostatic functions, including the control of the autonomic function.[36–39] Insula has especially been recognized to be more vulnerable to hypoglycemic events, which has been associated with atrophy in insula.[40] This could

be one possible explanation for why this area was generally affected in diabetes and why this was not specific for any of the phenotyped groups.

The frontal regions were another interesting area with lower GMV in type 1 diabetes, especially the superior frontal cortex in those with neuropathic pain and those with diabetes without DPN. The GMV in the superior frontal cortex was also more decreased in participants with diabetes without DPN compared to those with diabetes and DPN, which suggests that this area is vulnerable to changes regardless of the neuropathic complications.

Three other brain regions were observed to be specific for each of the three type 1 diabetes cohorts investigated in this study, of which lower volumes of the dorsal thalamus and hippocampus observed in the diabetes group with neuropathic pain are of particular interest. The dorsal thalamus comprises several nuclei, which play a central role in relaying sensory processing and may play an important role, particularly in those experiencing pain, which is in agreement with other studies.[41] However, in contrast to our study, other studies have found lower thalamus volume in the diabetes group with DPN but also in those without DPN with similar diabetes duration.[11,13,15] Hence, the alteration of thalamus volume may also be suggested to be a more generalized finding in type 1 diabetes.

Selvarajah and coworkers reported volume loss in the primary somatosensory cortex in people with type 1 diabetes and DPN.[14] In our study, we found lower GMV in the somatosensory cortex in the type 1 diabetes group without any neuropathic complications but not in those with DPN. This suggests that the somatosensory cortex is maybe not restricted to DPN but could also be affected before clinical DPN is verified. In our study, the confirmation of DPN was based on the abnormalities in the NCS. However, this diagnostic tool is based on the measurements of the large nerve fibers.[1] Hence, subclinical neuropathy will not be detected with this method. More studies are needed to clarify this relation in detail.

Our study found attenuated memory in the diabetes group with DPN compared to healthy controls. Lower memory scores were associated with lower GMV in those with DPN. However, such correlations were not observed in the other diabetes groups. Even though type 1 diabetes with neuropathic pain and type 1

diabetes without DPN showed significantly lower GMV, their memory function was preserved compared to healthy controls. This also suggests that those with DPN are more vulnerable to cognitive changes, which is also suggested by previous studies.[18,19] Previous studies have suggested that compensatory mechanisms are developed in diabetes, in which functional brain activity is increased in areas like subcortical regions and cerebellum to maintain normal cognitive function.[42,43] It might be speculated whether such compensatory adaptions are specifically altered in those with DPN. However, it is not possible to conclude anything specific based on the current observation. Hence, caution must be applied, and further studies on MRI and cognitive functions are needed to confirm these findings.

4.1 Limitations and methodological considerations

Our study cohort was well-matched on sex and age in all groups, and the neuropathy status was further well-phenotyped. However, the current study was not without limitations. First, this study was a part of a larger study, and sample size calculation was not based on the MRI method. However, previous studies have demonstrated that a sample size lower than 20 is sufficient to detect structural brain alterations. Hence we believe that these data are robust. Secondly, the study was a cross-sectional study, and no determination of causality can be concluded. Thirdly, there was a significant difference between groups in diabetes duration and the presence of retinopathy. Retinopathy has in previous studies shown to have an association with GMV.[13] Due to the nature of the study population, it was not possible to match the groups based on disease duration, retinopathy, and other diabetic complications, which could have impacted the current study results. Fourthly, other factors like hyperglycemia and hypoglycemia may also have influenced structural brain changes [33], but our study did not provide these data. Finally, this study included only structural MRI, and more studies investigating other MRI functional and metabolite-based techniques could be of interest to further understand the central alterations. Also, MRI studies combining both the CNS and peripheral nerve imaging are needed to deeper investigate the connection between central and peripheral nerve alterations.

5. Conclusions

This study investigated gray matter volume in three well-characterized groups of diabetes (type 1 diabetes and neuropathic pain, type 1 diabetes and diabetic peripheral neuropathy, type 1 diabetes without DPN), and healthy controls. Compared to healthy controls, the three diabetes groups showed lower total gray matter volume, significant for the groups of diabetes with neuropathic pain and diabetes without diabetic peripheral neuropathy. Moreover, regional reductions of gray matter volume were present in all three groups with type 1 diabetes. While the decreased volume of the insula was consistent across all three diabetes groups, other specific regional changes were identified for each group, including thalamus and hippocampus for those with neuropathic pain, cerebellum for those with diabetic peripheral neuropathy, and somatosensory cortex in those with type 1 diabetes without peripheral neuropathy. No associations were found between total gray matter volume and clinical parameters/peripheral measurements. However, a lower memory score was associated with lower gray matter volume in type 1 diabetes with DPN. Our study can further confirm the findings of existing studies that the gray matter brain structure is affected in type 1 diabetes. However, there is still a lack of conformity and consistency in the literature when investigating structural brain changes and the potential underlying mechanisms, including peripheral neuropathy, neuropathic pain, and other disease characteristics. This suggests that the findings are cohort-specific and shed light on the importance of well-defined cohorts in future studies. More studies are needed not only to confirm our data but also to promote the knowledge in this field to prevent the progression of brain alterations in diabetes.

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7. Conflict of interest

None of the authors has potential conflicts of interest to be disclosed. All authors have approved the final version of the article.

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Table legends

Table 1:
Data are presented as mean±standard deviation unless otherwise stated. The overall *p*-values are presented. Statistical differences between groups are denoted with A, B, and C, where pairs of different letters (such as A and B) indicate pairwise statistical differences. [a]Median(IQR). [b]Mean±standard error. *Indicates significant difference. †No pairwise statistical significance.
Abbreviations: CSF: cerebrospinal fluid, DN4: Douleur Neuropathique 4 Questions, DPN: diabetic peripheral neuropathy, GMV: gray matter volume, NP: neuropathic pain, NRS: Numeric rating scale, T1DM: type 1 diabetes mellitus, TIV: total intracranial volume, WMV: white matter volume.

Table 2:
Voxel-based morphometry analysis of gray matter volume.
Abbreviations: DPN: diabetic peripheral neuropathy, HC: healthy controls, L: left, MNI: Montreal Neurological Institute, NP: neuropathic pain, R: Right, T1DM: type 1 diabetes mellitus.

Figure legends

Figure 1: Flow diagram of the included participants.
Abbreviations: DPN: diabetic peripheral neuropathy, NP: neuropathic pain, T1DM: type 1 diabetes mellitus.

Figure 2:
Total GMV adjusted for age and total intracranial volume are presented with standard error in individuals with T1DM and neuropathic pain, T1DM and DPN, T1DM without DPN, and healthy controls. Significant *p*-values from the post-hoc analysis are reported.
Abbreviations: DPN: diabetic peripheral neuropathy, GMV: gray matter volume, NP: neuropathic pain, T1DM: type 1 diabetes mellitus.

Figure 3:
Regional gray matter alterations-A summary of the main findings, presented in Table 2. Red indicates insula with potential adjacent to other regions. Blue indicates frontal regions. Green indicates other regions mentioned in the figure.
Abbreviations: DPN: diabetic peripheral neuropathy, GMV: gray matter volume, NP: neuropathic pain, T1DM: type 1 diabetes mellitus.