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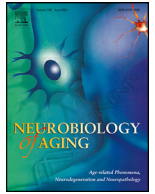


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Relationships between diabetes-related vascular risk factors and neurodegeneration biomarkers in healthy aging and Alzheimer's disease

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ABSTRACT

Vascular risk factors such as hyperglycemia and platelet hyperactivation play a significant role in type 2 diabetes (T2D), a risk factor for AD. We investigated the relationships between glycemia levels, platelet indices (platelet count; mean platelet volume (MPV)) and AD neuroimaging markers in 105 cognitively unimpaired adults, including 21 amyloid-negative older adults ($A\beta$ -neg controls), and 45 amyloid-positive patients with mild cognitive impairment or dementia ($A\beta$ -pos patients). We assessed between-group differences on the two T2D-related vascular risk factors, then the association between blood parameters and multimodal neuroimaging (structural MRI, ^{18}F -fluorodeoxyglucose, and ^{18}F -florbetapir-PET) in cognitively unimpaired adults and $A\beta$ -pos patients using multiple regressions. Compared to $A\beta$ -neg controls, $A\beta$ -pos patients showed lower platelet count and higher MPV. In cognitively unimpaired adults, increased glycemia levels were associated with atrophy and hypometabolism in AD-sensitive regions. In $A\beta$ -pos patients, increased MPV was associated with entorhinal and perirhinal cortex atrophy. Subclinical but high glycemia levels in healthy individuals and MPV in AD patients are associated with neurodegeneration in AD-sensitive brain regions but not with amyloid deposition.

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1. Introduction

The risk of developing Alzheimer's disease (AD)¹ appears to be increased by vascular risk factors, including cardiovascular diseases, hypertension or Type 2 diabetes (T2D) (Lee et al., 2020). T2D is characterized mainly by a chronic increase in blood glucose, that

is hyperglycemia, and is more prevalent with aging. This metabolic condition is considered as a risk factor for brain aging, cognitive decline and dementia (Biessels et al., 2006; Callisaya et al., 2019; Chen et al., 2019; Diniz Pereira et al., 2021). More specifically, T2D increases the risk of (1) developing mild cognitive impairment (MCI) (Ciudin, 2016), an intermediate stage between normal cognition and dementia; and (2) conversion from MCI to dementia (Alagiakrishnan & Sclater, 2012). Ongoing research aims at better understanding the link between AD and T2D. Notably, platelets have been proposed as a potential factor influencing the association between these 2 disorders as their function is altered in both pathologies (Laske et al., 2012; Randriamboavonjy et al., 2014). However, it is still not clear whether mild or “subclinical” increases

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¹ $A\beta$ -pos/neg, beta-amyloid positive/negative; AD, Alzheimer's disease; APOE, apolipoprotein E; BMI, body mass index; FDG, ^{18}F -fluorodeoxyglucose; MCI, mild cognitive impairment; MPV, mean platelet volume; MRI, magnetic resonance imaging; PCC, posterior cingulate cortex; T2D, type 2 diabetes; PET, positron emission tomography.

Table 1
Participant characteristics and between-group differences.

Variables	Cognitively unimpaired adults		Aβ-pos patients	Group comparisons between Aβ-pos patients and Aβ-neg controls
	All	Aβ-neg controls subgroup		
N	105	21	45	
Female % (N)	46.7(49)	52.4(11)	33.3(15)	<i>p</i> = 0.3336
Age (years; mean ± SD)	49.2 ± 20.1	66.4 ± 9.6	70.8 ± 8.6	<i>p</i> = 0.081
Education (years; mean ± SD)	13.0 ± 3.3	12.9 ± 3.5	11.6 ± 3.9	<i>p</i> = 0.209
BMI (kg/m ² ; mean ± SD)	23.6 ± 2.9	24.7 ± 3.5	24.4 ± 3.0	<i>p</i> = 0.697
APOE4 %(N)	32.4(34)	28.6(6)	62.2(28)	<i>p</i> = 0.022
Glycemia levels (mmol/L; mean ± SD)	5.3 ± .7	5.5 ± .5	5.5 ± .5	<i>p</i> = 0.853; η^2 = .001
Platelet count (G/L; mean ± SD)	245.6 ± 59.3	266.7 ± 62.3	222.2 ± 53.1	<i>p</i> = 0.004; η^2 = .128
MPV (fL; mean ± SD)	10.4 ± .8	10.1 ± .7	10.7 ± 1.1	<i>P</i> = 0.021; η^2 = .079

Abbreviations: Aβ-pos/neg, amyloid positive/negative; BMI, body mass index; MPV, mean platelet volume; η^2 , effect size; NS, not significant; SD, standard deviation.

in glycemia levels and platelet indices are associated with poorer brain health.

In non-diabetic and diabetic cognitively unimpaired older adults (> 60 years old), a direct association was found between the higher glycemia levels and the higher risk of dementia (Crane et al., 2013). Moreover, in non-diabetic cognitively unimpaired older adults, subclinical but high glycemia levels have been associated with (1) higher atrophy in structures sensitive to aging and neurodegenerative processes, such as the hippocampus and amygdala (Cherbuin et al., 2012; Kerti et al., 2013); and (2) decreased cerebral glucose metabolism in the precuneus (PrC), posterior cingulate cortex (PCC) and parietal regions, also affected in aging and AD (Burns et al., 2013; Ishibashi et al., 2015).

Several studies have shown that increased glycemia levels contribute to increased platelet activity in non-diabetic and diabetic individuals (Kaur et al., 2018; Keating et al., 2003; Shilpi & Potekar, 2018; Zaccardi et al., 2017). Increased platelet activity in diabetic individuals is involved in the development of vascular complications and higher cardiovascular risk (Chen et al., 2017; Demirtunc et al., 2009). This increased platelet activity has been also largely described as an important vascular risk factor involved in AD progression (Liang et al., 2014; Wang et al., 2013; Wu et al., 2021; Yesil et al., 2012). Platelet indices – platelet count, mean platelet volume (MPV) – are derived from the whole blood count (Bancroft et al., 2000). In aging, platelet count decreases, while platelet activity increases (Montenont et al., 2019). MPV, representing the averaged size and the activity of platelets, is significantly higher in patients with T2D (Chen et al., 2017; Papanas et al., 2004; Sansanayudh et al., 2016) and in the presence of vascular risk (Renga & Scavizzi, 2017). Moreover, the high platelet indices, often evidenced in the presence of T2D or early T2D, were also found in patients with AD (Shilpi & Potekar, 2018). More specifically, the recent evidence suggests that platelet activation observed in T2D may contribute to AD development and may be potentially responsible for the link between AD and T2D (Randriamboavonjy et al., 2014). In AD, both increases (Chen et al., 2017; Yesil et al., 2012) and decreases of MPV (Liang et al., 2014; Wang et al., 2013) have been reported. These contradictory findings might be associated with the variability in samples characteristics (eligibility criteria) or methodological choices (covariates and delay between blood sampling and analysis) in previous studies, which are summarized in supplementary Table 1.

To date, no study used multimodal neuroimaging to investigate the brain changes associated with subclinical but the high glycemia levels and platelet indices (platelet count and MPV), both in cognitively unimpaired adults and in amyloid-positive (Aβ-pos) MCI and AD patients. Therefore, our main objective was to offer a comprehensive assessment of the links between these T2D-

related vascular risk factors (i.e. glycemia levels and platelet indices) on one side and brain structural, functional integrity and cerebral amyloid deposition on the other side, in healthy individuals and patients with cognitive impairment, along the AD continuum.

2. Methods

2.1. Participants

All participants were included in the “Imagerie Multimodale de la maladie d’Alzheimer à un stade Précoce” (IMAP+) study (Caen, France), for which the inclusion and the exclusion criteria have been fully detailed elsewhere (La Joie et al., 2013; Tomadesso et al., 2018). Briefly, participants were excluded if they presented a history or clinical evidence of diabetes (type 1 or 2), neurological or psychiatric disorders, alcohol use disorder or drug abuse, or abnormal somatic examination. In addition, 2 cognitively unimpaired adults were excluded from the present study as their glycemia levels (7.8 and 9.5 mmol/L, respectively) were above the normal range (normal range: 3.6–5.5mmol/L; cut-off for T2D diagnosis: > 7mmol/L).

One-hundred-and-five cognitively unimpaired adults aged from 18 to 85 years were recruited from the community. For subjects aged over 60 years, they had no memory complaint, never referred to a memory clinic, and performed in the normal range for their age and educational level (i.e. within 1.65 standard deviation [SD] of the norms for all neuropsychological test; see the flow diagram in Fig. 1). A group of 21 Aβ-neg older participants (> 55 years; see 2.3.2 PET data acquisition and pre-processing for details) was extracted from the 105 cognitively unimpaired adults and were matched for sex, years of education and body mass index (BMI) to the group Aβ-pos patients (see below). This sub-group will be referred to as “Aβ-neg controls.”

A total of 56 patients with cognitive impairment, along the AD continuum, were selected from the local memory clinics. They presented no major comorbidities, and had available blood parameters and neuroimaging data. Among the 56 patients, only Aβ-pos patients were included (see 2.3.2 for positivity/negativity classification) to ensure that they were on the AD continuum. Among the 45 Aβ-pos patients, 27 met clinical criteria for single or multiple domain amnesic MCI (Petersen & Morris, 2005), with predominant episodic memory deficits (-1.5 SD from age- and education-matched norms) and 18 patients fulfilled NINCDS-ADRDA criteria for probable AD, including (1) abnormal global cognitive functioning and (2) deficits in at least 2 cognitive domains. The MCI and AD groups were merged and only differ regarding the degree of functional deficits, therefore representing a relatively homogeneous population ranging on a continuum from mildly to moderately cog-

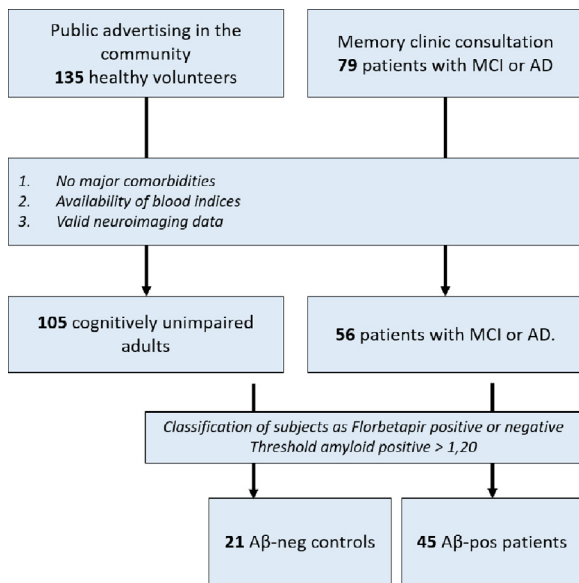


Fig. 1. Flow diagram of participants' selection and classification. Abbreviations: A β -neg, negative amyloid status; A β -pos, positive amyloid status; AD, Alzheimer's disease; MCI, mild cognitive impairment.

nitively impaired. We were mainly interested in comparing the role of subclinical high glycemia levels and platelet indices in healthy individuals and A β -pos patients without specific interest on the clinic stage. Demographic data and biological parameters per group are reported in [Table 1](#).

The IMAP+ study, sponsored by the Caen University Hospital, was approved by the local ethics committee (Comité de Protection des Personnes Nord-Ouest III, Caen, France; trial registration number: IDRCB: 2011-A01-493-38; clinicaltrials.gov identifier: NCT01638949). All participants gave their written informed consent prior to examinations.

2.2. Blood sampling and covariates assessment

Blood samples were collected in all individuals in the morning after an overnight fast. Blood samples were collected in fluorinated tubes and measured by spectrophotometry for glycemia levels (mmol/L) and in K3 salt of ethylenediaminetetraacetic (EDTA)-containing tubes for platelet indices (platelet count in G/L; MPV in femtoliter, fL). All samples were processed within 6 hours after blood collection. All blood parameters were within the normal range (i.e. subclinical levels) (see previous section for exclusion details). *APOE* genotype was also obtained from blood samples and individuals were classified as *APOE4* non-carriers (participants with no copies of the epsilon 4 allele) or *APOE4* carriers (individuals with at least one copy of the epsilon 4 allele). Body mass index (BMI) was computed with the formula [weight (kg)/height (m)²] and obtained from participants' self-reported weight and height.

2.3. Neuroimaging assessment

Participants underwent T1-weighted magnetic resonance imaging (MRI), ¹⁸F-fluorodeoxyglucose (FDG)- and ¹⁸F-florbetapir (AV45, Amyvid)- positron emission tomography (PET) scans, to measure gray matter (GM) volume, brain glucose metabolism, and amyloid burden, respectively. MRI and PET scans were performed in all participants at the Cyceron Center (Caen, France) on a Philips Achieva 3.0 T scanner and a Discovery RX VCT 64 PET-CT device (General Electric Health-care), respectively.

2.3.1. MRI data acquisition and pre-processing

High-resolution T1-weighted anatomical volumes were acquired using a 3D fast-field echo sequence (3D-T1-FFE sagittal; repetition time = 20 ms; echo time = 4.6 ms; flip angle = 10°; 180 slices with no gap; slice thickness = 1 mm; field of view = 256 × 256 mm²; in-plane resolution = 1 × 1 mm²).

Neuroimaging data pre-processing was performed using the Statistical Parametric Mapping version 12 software (SPM12; Wellcome Trust Centre for Neuroimaging, London, UK). T1-weighted images were segmented using multimodal segmentation (T1-weighted MRI and FLAIR) and spatially normalized to the Montreal Neurological Institute (MNI) space. Then, normalized GM segments were modulated to correct for non-linear warping effects, and the resultant images were smoothed using an 8-mm full-width half-maximum (FWHM) Gaussian kernel. MRI images were finally masked to exclude non-GM voxels as well as the cerebellum from the analyses (La Joie et al., 2012; Villain et al., 2008).

2.3.2. PET data acquisition and pre-processing

Both FDG- and florbetapir-PET scans were acquired with a resolution of 3.76 × 3.76 × 4.9 mm³ (field of view = 157 mm). Forty-seven planes were obtained with a voxel size of 1.95 × 1.95 × 3.2 mm³. A transmission scan was performed for attenuation correction before the PET acquisition. For FDG-PET, participants were fasted for at least 6 hours before scanning. After a 30-minute resting period in a quiet and dark environment, ~180MBq of FDG were intravenously injected as a bolus. A 10-minute PET scan was acquired 50 minutes after the injection. For florbetapir-PET, each participant underwent a 20-minute PET scan, beginning 50 minutes after the intravenous injection of ~4MBq/kg of florbetapir.

PET data were coregistered onto their corresponding MRI, and normalized using the deformation parameters obtained from the MRI procedure. Resultant images were quantitatively normalized using cerebellar GM as a reference region. PET images were then smoothed using a 10-mm FWHM Gaussian kernel (La Joie et al., 2012; Villain et al., 2008) and finally masked to exclude non-GM voxels as well as the cerebellum from the analyses. The standardized uptake value ratio (SUV_r) was used to classify subject as A β -pos or A β -neg, using a threshold derived from 45 healthy individuals (aged 21–39 years) from the IMAP cohort, who are all included in the present study (André et al., 2019; Besson et al., 2015). The positivity threshold was calculated as the 95th percentile of the distribution among 45 healthy controls, corresponding to a SUV_r of 1.20. Individuals with values above this threshold were considered as A β -pos and those below this threshold were considered as A β -neg.

2.4. Statistical analyses

Normality was assessed using the Shapiro-Wilk test. Non-normally distributed variables were Box-Cox- and log-transformed for glycemia levels and platelet count, respectively. All analyses were done both with the raw (Supplementary Tables 2, 3) and the transformed data. As results remained similar, only transformed data are presented here. To investigate potential underlying mechanisms, all analyses were replicated adjusting for blood pressure (BP) and medications (statins, beta-blockers, anti-hypertensive...), and mediation analyses were performed in both cognitively unimpaired adults and A β -pos patients. As results remained unchanged (Supplementary Table 4, 5) and no mediation was found (data not shown), only results without these covariates are presented here.

Between-group comparisons for demographics were assessed using Student's t-test for continuous variables and chi-square tests for categorical variables, with statistical significance set at $p < 0.05$.

2.4.1. Association between age, glycemia levels, and platelet indices in cognitively unimpaired adults

To assess the effect of age on glycemia levels and the platelet indices, multiple regression analyses were performed between age and each blood parameters separately (i.e. glycemia levels and platelet indices) within the whole group of cognitively unimpaired adults. Sex, years of education, BMI, and APOE4 status were included as covariates in the regression models, and results were considered significant at $p < 0.05$. To assess the robustness of the results, analyses were replicated after removing the Aβ-pos cognitively unimpaired adults ($n = 36$).

2.4.2. Differences in glycemia levels and platelet indices between Aβ-neg controls and Aβ-pos patients

To assess the differences between Aβ-pos patients and Aβ-neg controls on each blood parameters, we performed ANCOVAs, correcting for age, sex, years of education, BMI, and APOE4 status. We also tested multiple regressions between each blood parameter to investigate the potential relationships in Aβ-pos patients.

2.4.3. Voxelwise regression analyses between blood parameters and neuroimaging in cognitively unimpaired adults and Aβ-pos patients separately

Within the group of cognitively unimpaired adults and Aβ-pos patients separately, voxelwise multiple regression analyses were performed between each blood parameter and each neuroimaging modality (MRI, FDG-PET and florbetapir-PET) in SMP12, controlling for age, sex, years of education, BMI, APOE4 status, and the other blood parameters (i.e. analyses on platelet indices were controlled for glycemia levels, and reversely). To highlight the spatial overlap between the results from these voxelwise multiple regression analyses and AD-related neurodegeneration, two-sample t-tests (Aβ-neg controls vs. Aβ-pos patients) were conducted on GM volume and FDG-PET to derive atrophy and hypometabolism patterns in patients. For all neuroimaging analyses, results were considered significant at $p < 0.005$ (uncorrected) at the voxel level, and a threshold of $p < 0.05$ (corrected) at the cluster level, determined by Monte-Carlo simulation using the AlphaSim program (AFNI).

3. Results

3.1. Association between age, glycemia levels, and platelet indices in cognitively unimpaired adults

When adjusting for sex, years of education, BMI, and APOE4 status, the age was positively associated with glycemia levels (Fig. 2), but not with platelet indices ($r = .009, p = 0.931$ and $r = -.101, p = 0.363$ for platelet count and MPV; respectively). The association between the age and the glycemia levels remained significant without controlling for sex, years of education, BMI, and APOE4 status. This association remained also significant when removing the Aβ-pos cognitively unimpaired adults ($n = 36$) (Supplementary Table 6).

3.2. Differences in glycemia levels and platelet indices between Aβ-neg controls and Aβ-pos patients

There was no group difference in demographics between Aβ-neg controls and Aβ-pos patients, except for the APOE4 status ratio, with Aβ-pos patients showing a higher proportion of APOE4 carriers (Table 1). Aβ-pos patients presented lower platelet count (Fig. 3a) and higher MPV (Fig. 3b) than Aβ-neg controls. The between-group difference in platelet count and MPV remained significant without controlling for APOE4 status (Supplementary Tables 4, 5). By splitting the group of Aβ-pos patients into Aβ-pos

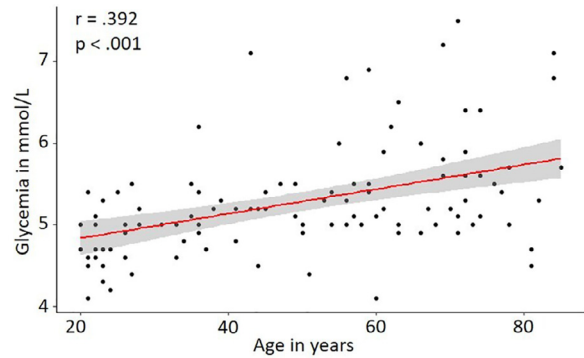


Fig. 2. Association between age and glycemia levels in cognitively unimpaired adults. Partial correlation coefficient and p -value adjusted for sex, education, BMI and APOE4 status are indicated on the graph.

MCI and Aβ-pos AD patients, higher MPV and lower platelet count were found in MCI, but not in AD patients (Supplementary Table 7). There was no difference in terms of glycemia levels between Aβ-neg controls and Aβ-pos patients (Table 1). Finally, no association was found between the glycemia levels and the platelet indices ($r = .214, p = 0.268$ and $r = -.078, p = 0.905$ for platelet count and MPV; respectively).

3.3. Association between blood parameters and neuroimaging in cognitively unimpaired adults

Glycemia levels negatively correlated with GM volume in the PCC and the right hippocampus, and with brain glucose metabolism in the PrC, cuneus, PCC, angular cortex, and medial temporal gyrus in cognitively unimpaired adults (Fig. 4A). Interestingly, these regions overlapped with the pattern of neurodegeneration in AD patients, including both the hippocampus for atrophy, and the PrC, cuneus, PCC, angular cortex, and medial temporal gyrus for hypometabolism (Fig. 3B). Amyloid burden was not associated with glycemia. We did not find any significant association between platelet indices (platelet count or MPV) and neuroimaging in cognitively unimpaired adults.

3.4. Association between glycemia levels and platelet indices and neuroimaging in Aβ-pos patients

In Aβ-pos patients, MPV negatively correlated with GM volume in the entorhinal and perirhinal cortices (Fig. 5A), whereas no association was found with the brain glucose metabolism or amyloid burden. The cerebral correlates of MPV in Aβ-pos patients overlapped with the pattern of atrophy evidenced in AD patients in the entorhinal cortex (Fig. 5B). Glycemia and platelet count were not associated with neuroimaging in Aβ-pos patients.

3.5. Complementary analyses

To further investigate the association between T2D-related vascular risk factors with MCI and AD patients, analyses were assessed between glycemia levels, platelet indices, and memory using the 16-word delayed recall from Grober & Buschke (Dion et al., 2014) in cognitively unimpaired adults and Aβ-pos patients (Supplementary Table 8). No significant association was found, suggesting that these blood parameters are not associated with memory decline.

To ensure that covariates (i.e. sex, years of education, BMI, APOE4 status) had no impact on age-effect results, interaction analyses were carried out between each T2D-related vascular risk factors and each covariates in cognitively unimpaired adults (Supplementary Figure 1). Interaction analyses were also performed with

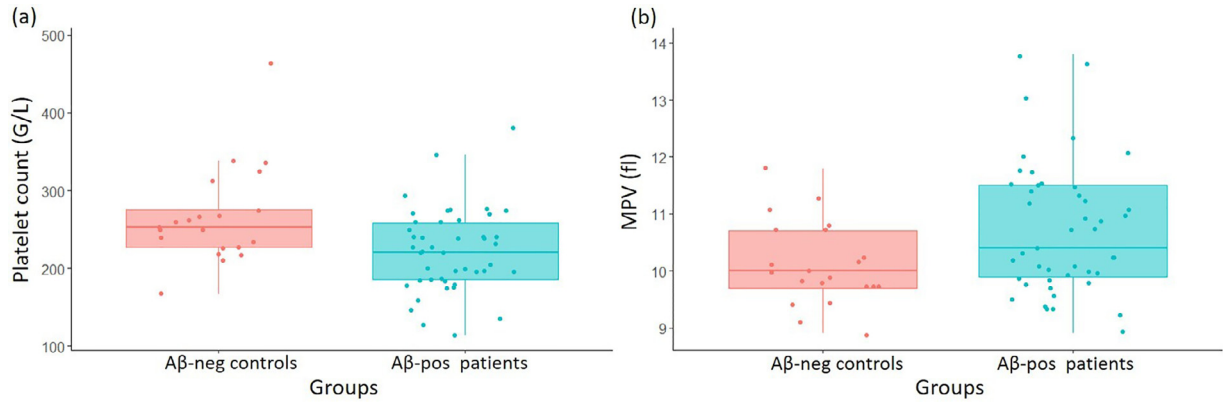


Fig. 3. Comparison of platelet count (a) and MPV (b) between A β -pos patients and A β -neg controls.

Abbreviations: A β -pos, amyloid positive; A β -neg, amyloid negative; AD, Alzheimer's disease; MCI, mild cognitive impairment; MPV, mean platelet volume.

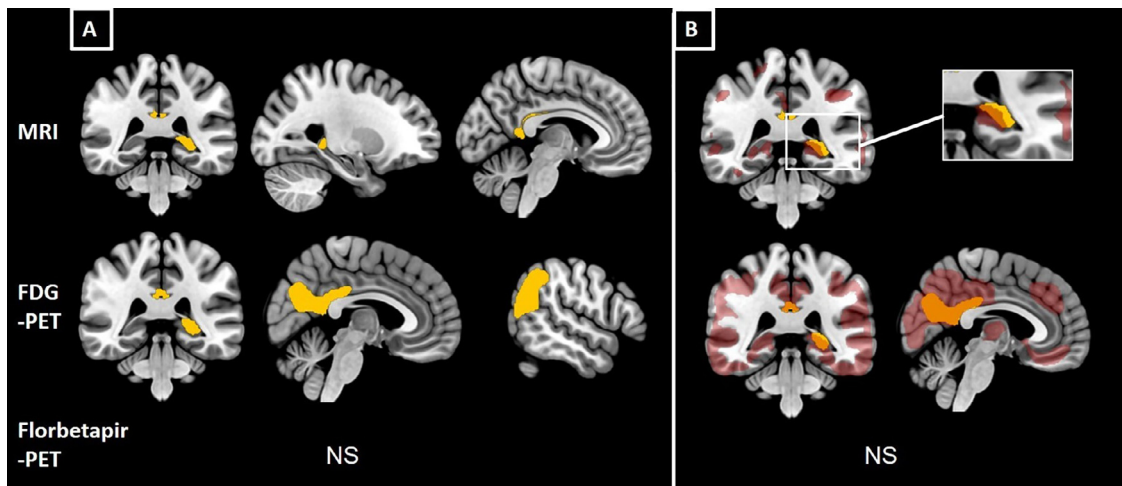


Fig. 4. Voxelwise associations between glycemia and neuroimaging in cognitively unimpaired adults (A) and their overlap with AD-related alterations (B) for structural MRI (upper row), FDG-PET (middle row), and florbetapir-PET (lower row), controlling for age, sex, education, BMI and APOE4 status. Areas in yellow indicate regions that are negatively correlated with glycemia at $p < 0.005$ uncorrected at the voxel level and $p < 0.05$ corrected at the cluster level (see Methods for details). Areas in red represent atrophy and hypometabolism patterns of alteration in MCI/AD patients. Orange areas represent the overlap between glycemia-related and AD-related brain changes.

Abbreviations: A β -pos, amyloid positive; APOE, apolipoprotein E; BMI, body mass index; MRI, magnetic resonance imaging; FDG-PET, fluorodeoxyglucose- positron emission tomography; NS, not significant.

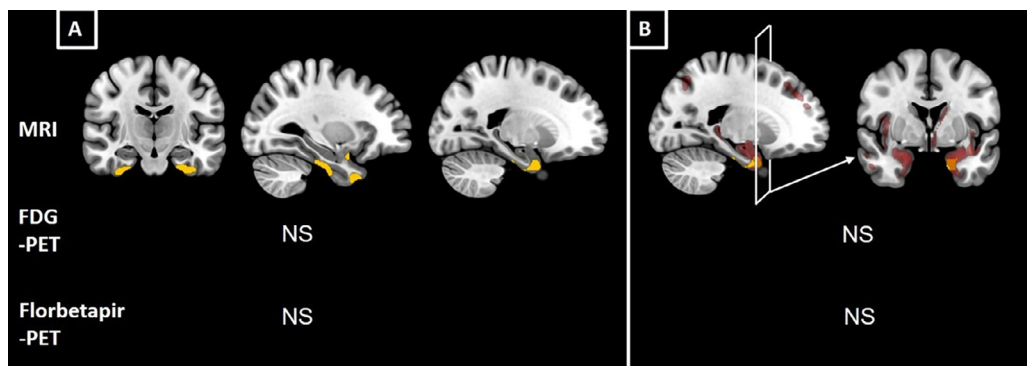


Fig. 5. Voxelwise associations between MPV and neuroimaging in A β -pos patients (A) and their overlap with AD-related alterations (B) for both structural MRI (upper row), FDG-PET (middle row), and florbetapir-PET (lower row), controlling for age, sex, level of education, BMI and APOE4 status. Areas in yellow indicate regions that are negatively correlated with MPV at $p < 0.005$ uncorrected at the voxel level and $p < 0.05$ corrected at the cluster level (see Methods for details). Area in red indicate patients' pattern of atrophy. Orange regions correspond to the overlap of MPV-related and AD-related alterations.

Abbreviations: A β -pos, amyloid positive; APOE, apolipoprotein E; BMI, body mass index; MPV, mean platelet volume; MRI, magnetic resonance imaging; FDG-PET, fluorodeoxyglucose- positron emission tomography; NS, no significant.

APOE4 and sex for voxelwise analyses (data not shown) to investigate potential modifying effect on the relationships between the blood parameters and the neurodegeneration in cognitively unimpaired adults and $A\beta$ -pos patients. No significant interaction was found, suggesting that these associations are not impacted by sex, years of education, BMI or *APOE4*.

4. Discussion

The main goal of the present study was to provide a comprehensive understanding of the cerebral changes associated with T2D-related vascular risk factors, namely subclinical but the high glycemia and the platelet activity levels, in healthy individuals and AD-dementia.

As shown in previous studies, we found that glycemia levels increase with age (O'Sullivan, 1974) but not platelet indices (Sansanayudh et al., 2016). The covariates used in the present study (i.e. sex, *APOE4* status, years of education and BMI) did not seem to play a role in these associations as they did not moderate the relation between the age and the blood parameters (Supplementary Figure 1).

In our study, glycemia levels were not associated with platelet indices and did not differ between $A\beta$ -pos patients and $A\beta$ -neg controls. Evidence suggests a link between the glycemia levels and the platelet activity and, more specifically, that glycemia levels contribute to increased platelet activity in non-diabetic and diabetic individuals (Kaur et al., 2018; Keating et al., 2003; Shilpi & Potekar, 2018; Zaccardi et al., 2017). Moreover, several studies showed that glycemia levels and activated platelets are involved in both T2D and AD pathological mechanisms (Burns et al., 2018; Horstman et al., 2010; Wang et al., 2013). Both, T2D and high glycemia levels have been associated with the higher incidence of dementia and cognitive decline (Biessels et al., 2006; Crane et al., 2013). In our study, we did not find any association between high glycemia levels or MPV and memory either in cognitively unimpaired adults or $A\beta$ -pos patients (Supplementary Table 8). However, both glycemia levels and platelet indices were in the normal range, that is subclinical, even if glycemia levels were in the upper part of the normal range (glycemia normal range: 3.6–5.5 mmol/L; cut-off for T2D > 7 mmol/L; mean: 5.5 mmol/L; platelet count normal range: 150–450 G/L; mean 222 G/L; MPV normal range: 7.5–12.0 fL; mean: 10.7 fL). The absence of association with cognition or of difference between $A\beta$ -pos patients and $A\beta$ -neg controls could be attributed to the fact that our cohort is non-diabetic, with subclinical glycemia levels.

In our study, MPV, a marker of platelet activation, was found to be higher in $A\beta$ -pos patients than in $A\beta$ -neg controls. This result aligns with prior studies showing that MPV is increased in AD (Chen et al., 2017; Yesil et al., 2012) and T2D patients (X. Chen et al., 2017). In our study, this finding could have reflected the fact that the proportion of *APOE4* carriers was higher in $A\beta$ -pos patients compared to $A\beta$ -neg controls, as this allele is known to be strongly associated with greater risk for the presence of brain amyloid pathology (Wisniewski & Drummond, 2020). Therefore, we have repeated our analysis controlling for this factor, and we showed that *APOE4* status did not impact the difference in MPV between $A\beta$ -pos patients and $A\beta$ -neg controls (Supplementary Tables 4, 5). Other studies reported a MPV decrease in AD patients (Liang et al., 2014; Wang et al., 2013). These conflicting findings might be the result of methodological differences between studies (Supplementary Table 1 for details). Briefly, the amount of EDTA, the most commonly used *in vitro* anticoagulant, in sample tubes could increase the platelet size just as the time between the blood collection and the sample analysis (Bowles et al., 2005). In our study, all samples were analysed within a 6-hour

delay after blood sampling; consequently, we are expecting no or limited impact on platelet size. Secondly, eligibility criteria and covariates differ across studies. Among them, hypertension was reported as a strong predictor of high MPV (Ntaios et al., 2011; Sansanayudh et al., 2016), while drugs such as statins or antiplatelet agents were previously associated with decreased MPV levels (Coban & Afacan, 2008; Jagroop et al., 2004). Our results remained unchanged after adjusting for BP and medications (Supplementary Tables 4, 5) and BP was not found to be a significant moderator of the relationships between glycemia, MPV levels and brain integrity (data not shown). Although the influence of other factors, and especially vascular risk factors, could be further explored in future studies, these additional analyses suggest that our findings were not biased by medication or BP.

Here, we found that both higher glycemia levels and MPV, even at a subclinical level, were associated with atrophy and/or cerebral glucose hypometabolism in regions known to be sensitive to AD. More specifically, in $A\beta$ -pos patients, higher MPV was associated with atrophy in the entorhinal and the perirhinal cortices, bilaterally (Fig. 5). Interestingly, it is well established that temporal structures (hippocampus, amygdala, and entorhinal cortex) are atrophied in patients with MCI or AD (Chételat, 2011; Chételat & Baron, 2003). Therefore, for the first time, our findings highlight an association between higher MPV and atrophy in regions particularly affected by AD, in $A\beta$ -pos patients. Moreover, in cognitively unimpaired adults, glycemia levels were associated with (1) atrophy in the hippocampus and PCC; and (2) hypometabolism in the angular gyrus, medial temporal gyrus, PrC, cuneus and PCC. Interestingly, regions associated with glycemia levels in cognitively unimpaired adults overlapped with regions particularly sensitive to atrophy/hypometabolism in AD patients (Fig. 3). Previous human and animal studies have also reported an association between glycemia levels and atrophy in the hippocampus and PCC (Cherbuin et al., 2012; Honea et al., 2022; Shaw et al., 2017). Moreover, our findings are consistent with previous studies describing an association between increased glycemia levels and hypometabolism of the PrC, PCC, and parieto-temporal cortex (Ishibashi et al., 2016, 2017; Viglianti et al., 2017). Similarly, in older adults with a first-degree family history of AD, higher glycemia levels were associated with lower regional cerebral metabolic rate for glucose in brain regions preferentially affected by AD (Burns et al., 2013) and remained significant when assessed longitudinally (Burns et al., 2018). Therefore, the increased vulnerability of these structures with increased glycemia levels in healthy individuals could be one of the key mechanisms underlying the association between the glycemia and the AD.

While the pathological processes underlying the association between the high glycemia levels and the hypometabolism in AD-sensitive regions are not well understood, several hypotheses have been proposed (Kuehn, 2020). For instance, changes in glucose transporters function and expression or dysfunction in glycolysis (conversion of glucose to energy) could affect brain glucose homeostasis (Hipkiss, 2019; Szablewski, 2016) and thus affect cerebral metabolism. In addition, the genetic variants *APOE2* and *APOE4*, which have been shown to respectively increase and decrease glycolysis, could have an indirect impact on brain glucose metabolism (Wu et al., 2018). Moreover, previous studies found that sex could affect the association between glycemia levels and CSF biomarkers of AD (Ou et al., 2020). Yet, we did not find any interaction with either *APOE4* or sex on the association between glycemia levels or MPV and neurodegeneration (i.e. atrophy and hypometabolism) in cognitively unimpaired adults and $A\beta$ -pos patients (data not shown).

Above the processes linking specifically glycemia levels to hypometabolism, the mechanisms underlying the association be-

tween glycemia, platelet activity and changes in brain integrity more generally are not fully understood. Increasing evidence suggests that the high glycemia levels and the activated platelets play an important role in inflammation and neuroinflammation (Horstman et al., 2010; Rawish et al., 2020). While the active contribution of platelet in inflammatory processes is well-acknowledged (Randriamboavonjy et al., 2014; Rawish et al., 2020), the involvement of glycemia levels in inflammation remains scarcely documented. However, a previous study showed an association between experimentally increased glycemia levels and increased cytokine levels in healthy control subjects and in individuals with impaired glucose tolerance (Esposito et al., 2002). Interestingly, the overlap between MPV-related and AD-related atrophy aligns with previous human and animal studies investigating inflammation in temporal lobe, and particularly in the hippocampal and entorhinal cortices (Hauss-Wegrzyniak et al., 1998, 2002; Sala-Llonch et al., 2017). Thus, it is reasonable to hypothesize that these 2 blood parameters could be associated with neurodegeneration through increased inflammation, both mechanisms being implicated in the development and progression of AD (Sala-Llonch et al., 2017). Vascular factors (e.g. hypertension or vascular diseases) also play an important role in the development of inflammation (Willerson & Ridker, 2004). In our study, individuals with vascular diseases were excluded and as previously mentioned, we did not find an impact of BP on the results (Supplementary Tables 4, 5).

We found no association between glycemia levels, platelet indices, and amyloid load. Previous studies assessing the links between the high glycemia or diabetes and the brain amyloid-PET positivity showed inconsistent findings. Some studies found an association between the glycemia levels and the cerebral amyloid burden in older adults (Honea et al., 2022; Morris et al., 2016; Taylor et al., 2017) consolidating the hypothesis that diabetes contributed to increased amyloid deposition (Chui et al., 2006). However, our results, in line with other studies, suggest that diabetes or related parameters were not associated with amyloid deposition (Arvanitakis et al., 2006; Thambisetty et al., 2013). To our knowledge, our study is the first to examine the association between platelet parameters and PET-based markers in cognitively unimpaired adults and $A\beta$ -pos patients. The lack of link in our study is in line with the inconsistency in literature and could reflect the weak association between these parameters and amyloid load. It could also reflect the low variability of amyloid deposition in $A\beta$ -pos patients, difference between our samples (i.e. cognitively unimpaired adults and $A\beta$ -pos patients) and the study populations and lastly blood parameters thresholds (i.e. subclinical but high glycemia and platelet indices levels). Alternatively, it might indicate that subclinical but high glycemia and MPV levels are related to AD independently from amyloid accumulation. Interestingly, previous studies demonstrates a link between tau and hyperglycemia and platelets (Kim et al., 2013; Neumann et al., 2011), suggesting that tau might be a potential factor relating these T2D-related vascular risk factors to AD. Further examination of these potential mechanisms linking subclinical glycemia levels, platelet activity and dementia, including the role of tau accumulation, is needed.

5. Strengths, limitations, and perspectives

The present study has several strengths, including the use of multimodal neuroimaging combined with the measurement of complementary vascular risk factors, allowing to reach a comprehensive understanding of the associations between T2D-related parameters and brain alterations. Moreover, these associations were evaluated throughout the lifespan and in $A\beta$ -pos patients, provid-

ing a more comprehensive picture of their role from healthy individuals to AD.

Our study has also some limitations. First, we used BMI as an indicator of obesity because it is easy to collect and widely used in previous studies and medical practice (Cherbuin et al., 2012; Yesil et al., 2012). In our study, its impact on the results when included as a covariate remains moderate. However, some argue that BMI is not a sensitive index, especially in older ages, when higher BMI would be protective (Woolley et al., 2019). Secondly, our study is limited by the small sample size of our groups and requires replication in larger samples. Finally, the cross-sectional and observational design of our study prevents the inference of any causal relationship between brain changes and T2D-related vascular risk factors. Previous longitudinal and interventional studies have investigated various modifiable risk factors, notably vascular risk factors, via several lifestyle interventions in non-demented older adults (MAPT (NCT00672685), FINGER (NCT01041989) trials...). Yet, clinical trials using conjointly multimodal neuroimaging modality, blood sampling and lifestyle interventions designed to protect against T2D or improving cardiovascular health are still needed to further our understanding of the impact of vascular risk factors related to T2D on dementia progression.

6. Conclusion

Overall, our study showed that subclinical but high glycemia levels were associated with neurodegeneration in AD-sensitive brain regions in healthy individuals. In addition, subclinical but high MPV was associated with decreased structural integrity in AD-sensitive brain regions in $A\beta$ -pos MCI/AD patients. Thus, these 2 T2D-related vascular risk factors were associated with higher vulnerability in brain regions affected in AD in a non-diabetic population. By contrast, neither glycemia levels nor MPV levels were associated with amyloid burden, suggesting a possible relation with AD pathology through other mechanisms than amyloid accumulation. Overall, it would be interesting to further examine glycemia and platelet levels with other hallmark of AD to better understand the pathophysiological mechanisms underlying neurodegenerative processes of AD. Our findings highlight the importance of monitoring T2D-related vascular risk factors, easy to measure in a single blood sample, and consider them as potential vascular risk factors for AD.

Credit author statement

GC, GP conceived and designed the study. JG, EK, FM, BL, AC, AQ, SE, MD, VDLS collected the data, which were analyzed by CP, FF and interpreted by CP, GC, GP. CP wrote the article, which was critically reviewed by all authors.

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viewed the contents of the manuscript being submitted, approve of its contents and validate the accuracy of the data.

Disclosure statement

The authors have no actual or potential conflicts of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.neurobiolaging.2022.06.004.

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