Brain Amyloid Load and Its Associations with Cognition and Vascular Risk Factors in FINGER Study

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Abstract

Objective: Our aim was to investigate brain amyloid pathology in dementia risk population defined as CAIDE score (Cardiovascular Risk Factors, Aging and Dementia-Risk Score) at least six, but with normal cognition. Associations between brain amyloid load and cognitive performance and vascular risk factors were investigated. **Methods:** A subgroup of 48 individualss from the FINGER main study participated in brain [^{11}C]PIB-PET imaging and brain MRI, and neuropsychological assessment at the beginning of the study. Life-style/vascular risk factors were determined as body mass index, blood pressure, total and LDL cholesterol and HOMA index (glucose homeostasis model assessment). White matter lesions (WML) were visually rated from MR images by a semi-quantitative Fazekas score. **Results:** 20 participants (42%) had a positive PIB-PET at visual analysis. PIB positive participants performed worse in executive functioning tests, included more participants with ApoE4-allele (50%) and showed slightly better glucose homeostasis compared to PIB negative participants. PIB positive and negative participants did not significantly differ in other cognitive domain scores or other vascular risk factors. There was no significant difference in Fazekas score between the PIB groups. **Conclusions:** The high percentage of PIB positive participants provides evidence of a successful recruitment process of the at-risk population in the main FINGER intervention trial. The results suggest possible association between early brain amyloid accumulation and decline in executive functions. Apolipoprotein allele 4 was clearly associated with amyloid positivity, but no other risk factor was found to be associated with positive PIB-PET.

Introduction

Brain amyloid accumulation is an early and essential event in Alzheimer's disease (AD) process. Positron emission tomography (PET) has enabled pre-clinical detection of individuals with brain amyloid accumulation process and therefore with high risk of clinical AD later in life. It has also offered a tool for clinical trials to identify individuals with brain amyloid pathology, to follow up drug effects on amyloid load and to run trials even in very early, pre-clinical phase of the disease.

In addition to drug research, more and more focus is set on vascular and life-style risk factors that are associated with increased risk of dementia and AD (1). The CAIDE risk score (Cardiovascular Risk Factors, Aging and Dementia-Risk Score) is based on several important risk factors and was developed as a tool for predicting dementia risk (2). There is also some evidence of a negative interrelationship between brain amyloid load and nutrient intake (3) and lifetime cognitive activity (4), and a positive interrelationship between amyloid load and LDL cholesterol (5), insulin resistance (6), diastolic blood pressure (7), plasma homocysteine level (8) and brain white matter hyperintensities (9). However, there are also conflicting results that show no association between brain amyloid load and physical or cognitive activity, diabetes or glucose and insulin measures or homocysteine metabolism (10-15). Nevertheless, interventions that influence life-style and vascular risk factors could offer a tool for lowering the burden of AD (1).

The Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability (FINGER) study is a randomized 2-year multi-domain lifestyle intervention study in 1200 participants at increased risk of cognitive decline (16). First results of the multi-domain intervention suggest better preservation of cognition in the intervention group compared to the non-intervention group (17). A sub-group of 48 individuals participated in brain amyloid-PET and magnetic resonance imaging (MRI) at the beginning and after the 2-year intervention or follow-up period. At present, data collection and analysis of the imaging biomarkers and neuropsychological assessment at the baseline is complete. Our objective was to estimate the proportion of amyloid positive individuals in this at-risk population before any intervention and investigate whether there is an

association between brain amyloid accumulation and vascular risk factors, or whether brain amyloid load is associated with cognitive performance.

Participants and Methods

Participants

Altogether 48 individuals (26 male, 22 female, mean age 71.4 years, SD 5.2) from Turku cohort (n=67) of FINGER main study population were able to participate (i.e. suitable for MR imaging) this PET-sub-study that was conducted at Turku PET Centre in Southwestern Finland. All 48 participants fulfilled inclusion criteria of the FINGER main study (16), i.e. participants had increased risk for dementia according to CAIDE dementia risk score (range 6-14, mean 8.2, SD 2.2) and cognitive performance at mean level or slightly lower than expected for age (screened with Consortium to Establish a Registry for Alzheimer's Disease; CERAD test). Participants had to meet at least one of the following criteria: word list memory task results of 19 words or fewer; word list recall of 75% or less; or mini mental state examination (MMSE) of 26 points or less. Mean MMSE score was 27 (range 22-30, SD 1.8). FINGER-PET sub-study population did not differ from the rest of Turku cohort or the rest of FINGER main study population in education years, APOE4-carrier proportion, BMI, systolic blood pressure, total or LDL cholesterol and HOMA-IR (glucose homeostasis model assessment index) ($p>0.05$). FINGER-PET population was slightly older compared to the rest of FINGER population (mean 70.8, SD 5 versus 69.3; 4.7, $p=0.031$). However, this difference can be explained by slight delay in recruitment process of Turku cohort i.e. years of birth do not differ between study populations. Individuals with previously diagnosed dementia or suspected dementia after clinical assessment by study physician at FINGER screening visit (recommended for further investigations) were excluded. The proportion of participants with at least one ApoE4-allele was 29.2%.

All participants underwent a brain [^{11}C]PIB-PET and structural MRI scans at Turku PET Centre. A cognitive test battery (an extended and adapted version of neuropsychological test battery, NTB)(18) was carried out including 14 tests that form three different cognitive domains (memory,

executive function and processing speed domains), which together constitute the total composite mNTB score (calculated as Z scores standardized to the baseline mean and SD, with higher scores suggesting better performance). Test results from NTB memory domain sub-tests that are related to delayed memory performance (CERAD word list recall and Wechsler Memory Scale (WMS) delayed verbal memory) were separately analyzed. Vascular risk factors were determined as systolic blood pressure, body mass index (BMI) and total and LDL blood cholesterol concentration as described previously (19). Insulin resistance was determined as glucose homeostasis model assessment (HOMA-IR), which was counted by the equation: fasting insulin (μU/ml) times fasting glucose (mmol/l) divided by 22.5, as previously described (20).

Neuropsychological assessment and laboratory testing were performed as a part of the FINGER main study, nearby PET and MR imaging. Neuropsychological assessment was carried out on average 29 days before PIB-PET scan (SD 98, range 219 days before to 180 days after PET scan).

Standard Protocol Approvals, Registrations and Patient Consents

FINGER (ClinicalTrials.gov identifier for the main FINGER trial: NCT01041989) was approved by the coordinating ethics committee of the Hospital District of Helsinki and Uusimaa. Participants gave written informed consent.

MR and PET imaging

All participants underwent a brain 3T MRI (Philips Ingenuity TF PET/MR, Amsterdam, Netherlands). The imaging protocol included T1–weighted sequences in sagittal orientation (T1-3D turbo field echo, isotropic 1mm x 1mm x 1mm resolution, total of 160 slices, field of view (FOV) 240mm x 240mm, repetition time 8.1ms, echo time 3.7ms). Parallel imaging (sensitivity encoding, SENSE) was used with factor 2. Scan duration was 3 minutes 59 seconds. FLAIR sequences were imaged in coronal orientation (0.65mm x 0.98mm resolution, reconstructed to 0.45mm). Slice

thickness was 4mm with gap between slices 1mm. Total of 36 slices were collected (FOV 230mm x 183mm, repetition time 10s, inversion time 2800ms, echo time 125ms). Scan duration was 3 minutes 30 seconds. White matter lesions (WML) were assessed from FLAIR images using a semi-quantitative visual rating scale (Fazekas score 0-3) by an experienced neuroradiologist (RP) blinded to clinical data.

[^{11}C]PIB was produced as described earlier(21). On average 406,3MBq (SD 107,7) of [^{11}C]PIB was injected intravenously and a dynamic scan from 60 – 90min (3 x 10min frames) after injection was performed with a Philips Ingenuity TF PET/MR scanner (Philips, Amsterdam, the Netherlands). Images were reconstructed using a line-of-response row-action maximum likelihood algorithm (LOR-RAMLA) with MR-based attenuation correction (MRAC) employing segmentation-based algorithm with three tissue classes, including the head coil template used in MR imaging protocol (22). The data were reconstructed using two iterations and 33 subsets. The image matrix size was 128x128x90 with voxel size of 2mm in each direction. All quantitative corrections for PET data were applied, including scatter, randoms, attenuation, detector deadtime and normalization. Neither time-of-flight information nor resolution modeling was applied in this study.

PIB-images with summated data over 60 to 90 minutes were visually interpreted by two experienced readers (JOR and NK) and judged as visually positive or negative after two-party consensus agreement. Participants graded as PIB-positive showed cortical retention of [^{11}C]PIB at least in one cortical region typically affected by amyloid in AD, whereas participants graded as PIB-negative had only nonspecific [^{11}C]PIB retention in white matter. The scans were quantitatively assessed using automated region-of-interest (ROI) analysis. FreeSurfer (version 5.3;http://surfer.nmr.mgh.harvard.edu/) was used to generate a high-definition cortical parcellation on the basis of the individual cortical folding patterns (23), resulting in a number of cortical gray matter ROIs in both hemispheres, which were subsequently combined over hemispheres to form aggregate ROIs. ROIs relevant for AD were established in the prefrontal, parietal, lateral temporal,

lateral occipital, anterior cingulate, posterior cingulate, and mesial temporal cortices, and precuneus. In addition, FreeSurfer was used to generate ROIs in the cerebellar cortex. The composite score was determined as the average of the prefrontal, parietal, lateral temporal, anterior cingulate, posterior cingulate and precuneus ROIs. Prior to extraction of mean ROI values the PET data were corrected for head motion induced misalignment between frames as well as for possible mismatch between the MRI and PET scanning positions. Image realignment and coregistration were carried out using SPM8 version of Statistical Parametric Mapping toolbox (Wellcome Department of Cognitive Neurology, London, UK) implemented in Matlab 7.13.0 (Mathworks inc., Natik, MA, USA). Mutual information was used as the optimization criterion in image alignment, while the PET data were resliced into MRI voxel-size (1mm x 1mm x 1mm) yielding voxel-by-voxel correspondence with the FreeSurfer-based ROI-mask images. ROI-based quantitation was obtained as region-to-cerebellar cortex ratio over 60-90 minutes scan duration.

Statistical analyses

All analyses were conducted using IBM SPSS Statistical software (version 22). Differences between the frequencies of dichotomous variables (APOE4 positive/negative, Fazekas score and sex) in PIB positive and negative groups were tested using Pearson Chi-Square test. Two-sample t-test was used to test between-group differences in age, years of formal education and PIB composite score. Analysis of covariance (ANCOVA) adjusted for APOE-group (E4-negative/E4-positive) and age was used to evaluate differences in risk factors between PIB-groups. Moreover, two-way analysis of variance (ANOVA) was used to test the modifying effect of APOE-group on the differences between PIB-groups in risk factors using APOE-group by PIB-group interaction effect. Differences in neuropsychological test scores between the PIB positive and negative groups were evaluated with ANCOVA adjusted for age. In PIB positive group correlations between quantitated PIB composite score and cognitive test score were evaluated using Pearson correlation coefficients. All p-values were calculated with two-sided tests and p-values less than 0.05 were considered as statistically significant. No corrections for multiple comparisons were made.

Results

At visual PIB-PET analysis 20 participants (42%) were graded as PIB-positive, whereas 28 participants (58%) did not show cortical retention of [^{11}C]PIB. In agreement with the visual analysis, there was a difference in PIB composite score between PIB positive (mean 1.83, SD 0.39) and negative groups (mean 1.28, SD 0.07, $p<0.0001$). PIB positive and negative groups did not differ by age, sex or educational level (Table 1). PIB-positive group showed higher frequency of individuals carrying ApoE allele 4 (50%) compared to PIB-negative group (14%) ($p=0.007$). In fact, 71% of all ApoE4-positive participants were PIB-positive as well. ApoE4-positive and ApoE4-negative participants did not differ in total or LDL cholesterol, blood pressure, BMI or HOMA-IR ($p>0.37$). There was no modifying effect of APOE-group on the differences between PIB-groups in any of the evaluated risk factors (All APOE-group by PIB-group interaction effects, $p>0.37$).

PIB positive group had lower HOMA index than PIB negative group ($p=0.04$, adjusted for ApoE4 positivity/negativity and age), i.e. PIB positive group had better glucose homeostasis. PIB positive and negative groups did not show any significant difference in systolic blood pressure, BMI or total or LDL cholesterol adjusted for age and APOE-group (Table 1). There was no significant difference in Fazekas score between the groups (Table 2). PIB-negative group performed significantly better in executive function domain than PIB positive group (Table 3). There was no significant difference between the groups in processing speed or memory domain z-scores, or in delayed memory test results.

Discussion

Our study population consisted of individuals with vascular and life-style risk factors for dementia and a marked percentage of participants had a positive PIB-PET scan, which in turn was associated with worse executive cognitive functioning. Proportion of PIB positive individuals in this study (42%) exceeds the expected percentage of amyloid positive healthy elderly individuals in general population, which is estimated to be 20-30% for the age group of the present study population (24).

Furthermore, proportion of ApoE4-allele carriers seemed to be somewhat higher in our study population (29%) compared to proportion in general population in Finland (21%) (25). ApoE4-allele positivity was associated with increased likelihood for positive PIB-PET, while no associations were found between higher vascular risks and PIB-positivity. ApoE4-allele is a well-known risk factor for sporadic AD with odds ratios 3.2 and 14.9 for participants with one or two E4 alleles, respectively (26), and it also increases the risk for amyloid positivity in PIB-PET in healthy elderly individuals. ApoE4 allele is a risk factor for certain vascular diseases as well, although much weaker than for AD. That is, ApoE4-allele is associated with slightly increased risk of hypertension (27) and higher total and LDL-cholesterol (28). Moreover, ApoE4-allele might be an independent risk factor for coronary artery disease and more severe atherosclerosis (28). The overrepresentation of ApoE4-allele carriers in our study population might indeed be explained by recruitment of individuals with higher CAIDE risk score, and thus with vascular risk factors. It appears that the recruitment process and inclusion criteria for the FINGER intervention study have been successful and the study population is enriched with individuals at-risk for AD and even at preclinical stage of the disease.

Our data indicates that vascular risk factors were not linked to amyloid accumulation at the start of the trial. Because of small sample size we may have missed some relationships between vascular factors and amyloid load. Moreover, our population was not divided into separate groups by use of medication for hypercholesterolemia, hypertension or diabetes, which have been shown to modify the interrelationship between cholesterol and blood pressure and amyloid load (29). Our results showed slightly better insulin homeostasis in PIB positive group. Epidemiological studies have found that type 2 diabetes and insulin metabolism are associated with AD (30) and there is also evidence of impaired cognitive functioning in individuals with insulin resistance (31). However, results on pathological correlates of diabetes and insulin resistance on brain amyloid are somewhat controversial; experimental studies indeed provide evidence that insulin is closely interrelated to

brain amyloid pathology in AD (30), but human amyloid-PET studies in cognitively healthy individuals have shown either an association between insulin resistance and increased brain amyloid load (6), or have reported no interrelationship between impaired peripheral glucose metabolism and brain amyloid load (13, 14). Elderly individuals with diabetes have been shown to have less brain β-amyloid pathology in autopsy although diabetes was been found to be related to increased overall risk for dementia (32). Indeed, β-amyloid pathology and impaired glucose homeostasis might be competing risks of otherwise independent processes for cognitive decline. On the other hand, it is possible that there are different pathways for brain amyloid pathology and AD, where factors like ApoE-allele, vascular damage, glucose homeostasis and cholesterol levels modulate pathological mechanisms. That is, we need to study larger, cognitively healthy populations that are separated into subgroups by risk factor profiles, in order to clarify their interrelationships with early amyloid pathology.

Our results revealed that PIB positive group performed slightly worse in executive functioning compared to PIB negative group, but the groups did not differ in performance in other cognitive domains, including delayed verbal memory subtests. Although impaired performance in memory tasks is one of the clinical hallmarks of amnestic MCI and early AD symptomatology, we did not find any difference in memory performance between PIB positive and negative groups or relation between episodic memory and amyloid load in this risk population. Corresponding to inclusion criteria in the FINGER main study, both PIB positive and negative groups included individuals with slightly lower memory performance than expected for age. That is, we cannot rule out a possibility that amyloid accumulation is associated with slightly impaired memory function in PIB positive group while some other factors mediate a subtle memory decline in PIB negative group. Previous studies on the relation between brain amyloid load and cognition in healthy elderly individuals have revealed inconsistent results. Some of the studies have shown an association between brain amyloid accumulation and decline in several cognitive domains or specifically in episodic memory (33, 34),

while others have reported no such associations (24, 35, 36). There might be several factors in study population compositions that could have caused discrepancies between the studies. Indeed, it has been reported that age and educational level are the strongest predictors of cognitive performance in healthy elderly individuals, while only subtle effects of amyloid load were seen after controlling for these two factors (37). Moreover, sex and ApoE-allele status were shown to modulate amyloid-cognition relation so that amyloid accumulation was shown to be related to worse visuospatial performance in healthy individuals without ApoE4-allele (37).

In our study PIB positive and negative groups did not differ by age, education or sex, that is, it is unlikely that difference in executive performance between the groups could be explained by these factors. Furthermore, although vascular and metabolic deficits and insulin resistance have been linked to worse executive performance in general population (38, 39), our PIB positive group with worse executive functioning did not differ from PIB negative group in vascular markers, and showed in fact slightly better insulin homeostasis than PIB negative group. Higher proportion of ApoE4-allele carriers in PIB positive group probably does not account for lower executive functioning as existence of ApoE4-allele is not found to affect executive performance in healthy elderly individuals, although some relation to impaired verbal memory might exist (40). Therefore, it seems probable that brain amyloid accumulation largely explains impaired executive functioning in this risk population, although we cannot rule out a possibility that some associations between vascular risks and cognition could be found in larger population with wider variability in risk and cognitive profile. PIB-PET study population is representative of the FINGER main study population, with no differences in vascular risk factor profile, proportion of APOE4-allele carriers or inclusion and exclusion criteria. As a conclusion, recruitment process for FINGER intervention study has been successful and population is enriched with PIB positive, and thus AD-risk individuals**.** Participants are representative of a part of the general Finnish elderly population with several risk factors for dementia, but without pronounced cognitive impairment. Our study failed to

show associations between amyloid load and vascular or metabolic risk factors, but as there are several factors that may modulate this interrelationship, such as ApoE-allele, lifetime changes in risk factor profile and medication used for vascular and metabolic diseases, studies in larger subgroups are warranted.

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Table 1. Demographic and risk factor data (mean, SD or n, %) in PIB positive and negative groups.

	PIB positive		*PIB negative*			*p-value*
Age, years mean (SD)	72.5 (3.7)		70.6 (6.0)			0.19[a]
Men, n (%)	8 (40.0)		14 (50.0)			0.50[b]
Education, years mean (SD)	8.9 (2.06)		9.7 (2.93)			0.32[a]
ApoE e4 carriers, n (%)	10 (50.0)		4 (14.3)			**<0.01**[b]
	PIB positive mean (SD)		*PIB negative mean (SD)*	*Mean difference (95% CI)*	*Adjusted[c] mean diff. (95% CI)*	*Adjusted p-value[c]*
Systolic blood pressure, mmHg	135.4(14.5)		138.1 (14.5)	2.70 (-5.82–11.22)	1.77 (-8.37–11.91)	0.73
BMI, kg/m^2	26.2 (2.71)		27.9 (3.69)	1.67 (-0.28–3.63)	1.58 (-0.76–3.93)	0.18
Total cholesterol, mmol/l*	5.3 (1.0)		5.0 (0.9)	-0.49 (-0.98–0.00)	0.04 (-0.63–0.71)	0.91
LDL cholesterol, mmol/l*	3.3 (0.9)		3.0 (0.9)	-0.29 (-0.83–0.25)	0.07 (-0.57–0.70)	0.84
*HOMA-IR***	1.3 (0.7)		2.1(1.3)	0.73 (0.04–1.41)	0.90 (0.04–1.77)	**0.04**

[a]Two-sample t-test

[b]Pearson Chi-square test

[c]Analysis of covariance; adjusted for ApoE4 positivity/negativity and age. ApoE-group not available in one PIB-positive participant.

*N=47, data not available in one PIB-negative participant

**N=42, data not available in three PIB-negative and three PIB-positive participants

Abbreviations: BMI = body mass index, HOMA-IR = glucose homeostasis model assessment –index, CI = Confidence interval

Table 2. Fazekas scores within the PIB positive and negative groups, n (%)

	Fazekas score*			
	0	**1**	**2**	**3**
PIB positive	4 (20%)	6 (30%)	6 (30%)	4 (20%)
PIB negative	9 (32%)	10 (36%)	6 (21%)	2 (7%)

*Pearson Chi-square test, no significant difference in Fazekas score distribution between the groups (p = 0.46)

Table 3. Neuropsychological assessment (mNTB, modified neuropsychological test battery) results including mean (SD) z-scores from three cognitive domains and their average score (Total score). A negative score indicates worse performance. Moreover, MMSE (mini-mental state examination) score and delayed memory subtest results are presented.

PIB positive mean (SD)	*PIB positive mean (SD)*		*PIB negative mean (SD)*	*Mean difference (95% CI)*	*Adjusted[a] mean diff. (95% CI)*	*Adjusted p-value[a]*
mNTB Total score	-0.17 (0.60)		0.08 (0.57)	0.25 (-0.09–0.59)	0.14 (-0.16–0.44)	0.36
mNTB Executive functioning	-0.28 (0.63)		0.18 (0.67)	0.47 (0.08–0.85)	0.39 (0.01–0.76)	**0.04**
mNTB Processing speed	-0.13 (0.87)		0.18 (0.87)	0.26 (-0.27–0.79)	0.18 (-0.30–0.66)	0.46
mNTB Memory	-0.09 (0.73)		-0.05 (0.60)	0.04 (-0.35–0.43)	-0.09 (-0.42–0.25)	0.61
MMSE	27.0 (1.79)		27.0 (1.77)	0.09 (-0.96–1.14)	-0.03 (-1.09–1.03)	0.95
CERAD Word list recall	5.5 (2.5)		5.1 (1.5)	-0.31 (-1.48–0.87)	-0.63 (-1.71–0.45)	0.25
WMS Delayed verbal memory	7.7 (3.7)		8.8 (2.94)	1.14 (-0.86–3.13)	0.80 (-1.17–2.77)	0.42

[a]Analysis of covariance; adjusted for age

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