Can studies of neuroinflammation in a TSPO genetic subgroup (HAB or MAB) be applied to the entire AD cohort?

Running title: Does TSPO genotypes associated with AD?

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ABSTRACT:

Rationale: Neuroinflammation plays a significant role in Alzheimer’s disease (AD), and TSPO PET imaging allows us to quantify this process. However, the binding of second generation TSPO tracers depends on the TSPO genotype coded by the rs6971 single nucleotide polymorphism (SNP), with 40-50% increase in BP in high affinity (HAB) compared to mixed affinity (MAB) binders; while low affinity binders (LAB) are unsuitable for evaluation. Hence several studies are either using HAB alone or HAB and MAB subjects. To translate the findings of neuroinflammation studies to the entire population, it is crucial to establish the influence of TSPO genotypes on AD. Here we investigated whether different TSPO genotypes influence their cognitive function, amyloid load and disease progression over time.

Methods: We evaluated 798 subjects (225 normal, 388 MCI and 185 AD subjects) at baseline and during follow-up from ADNI cohort. All subjects were screened for TSPO genotype and underwent detailed clinical and neuropsychological assessments yearly for four years. 291255 subjects also had T1, T2 MRI and amyloid PET with 11C-PIB or 18F-Florbetapir.

Results: We have demonstrated that all TSPO binding groups (HAB, MAB, and LAB) have same level of amyloid load in AD and MCI subjects. We have also demonstrated that the prevalence of HAB is 50.3%, MAB is 41.2% and 8.5% for LAB, without statistical difference among AD, MCI and control groups. During longitudinal follow up, mean change of neuropsychometric test scores of MMSE, ADAS Cog 11, ADAS MOD, and GDS over time were similar in AD and MCI subjects among three TSPO binding groups. The analysis of the covariates showed that diagnostic group (normal, MCI, AD), ApoE4 status, and gender had a significant effect on ADAS MOD decline (>3 points of the scale) but not age or TSPO genotype.

Conclusion: This study suggests that information obtained from evaluating a subgroup of AD or MCI subject using second generation TSPO tracers could be translated to the entire AD and MCI population. This will allow us to study lower numbers of AD subjects in evaluating new anti-neuroinflammatory/anti-microglial agents in intervention studies, and in observational studies evaluating the role of neuroinflammation.

Keywords: Alzheimer’s Disease, TSPO, MCI, cognitive function, PET study
INTRODUCTION

Neuroinflammation plays a significant role in Alzheimer's disease (AD) and other neurodegenerative diseases. Studies are evaluating the influence of neuroinflammation on neurodegeneration, and therapeutic agents against these targets are still under clinical evaluation to prevent neurodegeneration in AD. Before commencing on a large phase 3 study, it is essential to determine whether these agents can suppress brain inflammation. Neuroinflammation is characterised by an increase of 18 kDa translocator protein (TSPO) expression, and is assessed in vivo by the use of TSPO PET (Positron Emission Tomography). The TSPO overexpression following microglial activation, can be imaged via binding of PET ligands such as $^{11}$C-(R)-PK11195 ($1\text{-}[2\text{-chlorophenyl}]-\text{Nmethyl-N-[1-methyl-propyl]-3-isoquinoline\ carboxamide}$). It is shown that AD subjects and a proportion of MCI subjects show a 20–35% increase in microglial activation in frontal, temporal, parietal, occipital and cingulate cortices ($p<0.05$). Currently, several second generation of TSPO ligands ($^{11}$C-PBR28, $^{11}$C-DPA713, and $^{18}$F-GE180) have been developed with the aim of achieving higher affinity and better signal-to-noise ratio than $^{11}$C-(R)-PK11195 PET. However, their uptake is dependent on TSPO phenotypes: high affinity binders (HAB), low affinity binders (LAB) and mixed affinity binders (MAB); with 40-50% increase in binding potential in high affinity (HAB) compared to mixed affinity (MAB) binders, while low affinity binders (LAB) are unsuitable for evaluation. It is now clear that a non-synonymous polymorphism of TSPO, coded by the rs6971 single nucleotide polymorphism (SNP) affects the binding affinity of these tracers. This SNP in exon 4 of the TSPO gene causes an alanine-to-threonine substitution in position 147. The Ala/Ala TSPO genotype (wild-type) results in HAB, while the Ala/Thr results in MAB, and Thr/Thr results in LAB. The MAB have 40-50% lower binding potential for TSPO than HAB. Due to the lack of binding in LAB, they are unsuitable for evaluation. Hence, several studies are evaluating only HAB subjects in natural progression and intervention studies. Apart from the binding of the PET tracer, TSPO gene mutation has been previously associated to functional alteration resulting in the reduction of peripheral lymphocyte allopregnanolone production. Given that neurosteroids such as allopregnanolone may play a role in neuronal survival, favouring neurogenesis and preventing apoptosis, it is important to establish whether there is a link between 147/Thr TSPO genotype and AD development and progression, especially if TSPO genotypes will become a discriminating factor for selecting AD patients for neuroinflammation studies. To date, no study has evaluated the clinical impact of TSPO genotypes on AD. If the amyloid load and deterioration of cognitive function (clinical progression) were similar in the three TSPO subgroups of AD and MCI subjects, information obtained from HABs alone could then be generalised to all AD or MCI patients. Here we hypothesised that the distribution of amyloid deposition and the progression of disease would be the same in all TSPO subgroups within the same diagnostic group. In the present study, we investigated the influence of different TSPO genotypes on amyloid load and neuropsychometric performance at baseline and during follow-up in AD, MCI and healthy control subjects.

MATERIALS AND METHODS
Our study population was drawn from the ADNI database (www.loni.ucla.edu/ADNI). The institutional review board (IRB) approved this study and all subjects signed a written informed consent. A subset of 798 ADNI subjects were selected, and was genotyped with TSPO rs6971 as a part of this study. This comprised 225 normal, 388 MCI and 185 AD subjects. MCI subjects were enrolled if they had MMSE scores between 24-30 (inclusive) for more details see ADNI website. Participants underwent apolipoprotein E (ApoE) genotyping and were monitored longitudinally at 6- to 12-month intervals for cognitive function with multiple cognitive tests including the Mini Mental State Examination (MMSE) and Alzheimer Disease Assessment Scales (ADAS cognitive, ADAS-Cog; and modified, ADAS-MOD, Clinical Dementia Rating (GDR) and Geriatric Depression Scale (GDS). Among the cohort of 798 subjects, 255 subjects had T1 and T2 weighted MRI and PET evaluation for amyloid burden. 188 subjects enrolled had $^{18}$F-Florbetapir and 67 subjects had $^{11}$C-PIB PET.

Image Analysis

A complete description of MRI and amyloid data acquisition is available on the ADNI website (http://www.adni-info.org/Scientists/ADNIStructuralProcedures.aspx). Image analysis was done as described previously with Statistical Parametric Mapping 8 using MRI for creating the object map. A threshold for amyloid positivity of 1.36 and 1.4 for the mean cortical ROIs normalised to cerebellar SUVR was used based on the range of control in previous studies for $^{18}$F-Florbetapir and $^{11}$C-PIB, respectively.

Genetics

Individuals were genotyped using the Illumina 610-quad array, as described in Saykin et al, 2010. No quality control had been performed on the publicly available ADNI Genome-wide association study (GWAS) dataset, therefore the data were subjected to QC-filtering prior to analysis. This included retaining individuals with missing genotype rates < 0.05, with mean autosomal heterozygosity between 0.30 and 0.36, and with mean X-chromosome heterozygosity either <0.02 for males, or between 0.28 and 0.42 for females.

Statistical Analysis

Statistical analysis was performed using SPSS Statistics 21.0. Effects of demographic variables were evaluated with factorial ANOVA. Associations between groups were evaluated using Pearson correlation analysis. A two-sample t-test for continuous variables and chi-squared test for categorical variables were used to compare baseline variables. Kaplan-Meyer survival analysis was done using ADAS-MOD increase of >3 as the significant cognitive decline event. Log-Rank Mantel Cox test was used to compare survival curves for each genotype. Cox proportional hazards regression was used to identify baseline variables, which were associated with cognitive decline. The sample size of our cohort was sufficient to warrant a power of regression analysis more than 90% with an alpha error of 0.05.
Detailed demographic data and baseline parameters are given in Table 1. The mean age of the entire cohort was 75.3 years (±6.8) while the mean age of the control, MCI and AD groups were 75.9 (±5.0), 74.8 (±7.3) and 75.4 (±7.5), respectively. No statistically significant differences were observed in the 3 groups with respect to the age (p>>0.05).

**Baseline data**

The prevalence of genotypes among diagnostic groups is reported in Table 2. With the chi-squared test, no difference was observed in the prevalence of these genotypes among AD, MCI and control groups (p=0.691). The prevalence of the Thr allele (both in homozygotes and in heterozygotes) is reported in Table 3. There was no statistical difference in prevalence of the Thr allele among groups (p>>0.05): the odds ratio and confidence intervals (CI) at 95% were 1.24 (0.89-1.73) for control vs MCI groups, 0.87 (0.61-1.24) for MCI vs AD groups, and 1.09 (0.73-1.60) for control vs AD groups.

The mean baseline parameters clustered by TSPO genotypes are reported in Table 4. No differences were found in terms of sex, age, MMSE, ADAS-Cog, ADAS-MOD, and GDS among the different TSPO genotypes (p>>0.05) (Figure 1). 255 subjects had amyloid scans, and this subgroup did not significantly differ from the rest of the cohort in sex and age even when stratified for both diagnostic groups and TSPO genotypes. There was no difference in the prevalence of TSPO genotypes in the amyloid subgroup. Within the same diagnosis group, similar distributions of amyloid deposition in Thr/Thr, Ala/Thr and Ala/Ala were found. Examples of individual parametric images of amyloid deposition among diagnostic groups and TSPO genotypes are shown in Figure 2.

As anticipated, a significant difference in the prevalence of subjects with the ApoE4 genotype was found among groups (p<0.001): patients with AD presented the greatest percentage of ApoE4 (17.9%) followed by MCI (12.4%). 84.2% AD and 63.5% MCI patients were amyloid-positive while 31.3% of controls were amyloid positive (p<0.01). TSPO genotypes did not correlate with ApoE4 genotypes. At baseline, as expected, a significant difference was demonstrated between diagnostic groups in MMSE, ApoE4, amyloid status, ADAS-Cog and ADAS-MOD, CDRs and GDS (p<0.01) (Table 1).

**Longitudinal data**

The mean decline of MMSE at 36 months among the diagnostic groups was -0.08 (±0.60), -0.62 (±2.05), -1.16 (±1.88) for normal, MCI and AD subjects, respectively (p<0.05). The mean annual decline of ADAS-Cog and ADAS-MOD in the three diagnostic group was 0.13 (±1.23), 0.99 (±2.68) and 2.45 (±5.63) and 0.23 (±1.8), 1.20 (±3.1) and 1.05 (±8.6) for normal, MCI and AD subjects, respectively (for each scale p<0.05). No statistical differences (p>0.05) were found among different genotypes in the diagnostic groups (normal, MCI, and AD) in terms of MMSE, ADAS-Cog, ADAS-MOD mean annual decline as shown in Figure 3. No statistical differences in mean variation of GDS (p>0.05) were found among different TSPO genotypes. The prevalence of HAB was 50.3%, MAB was 41.2% and 8.5% for LAB, without statistical difference among the AD, MCI and control cohorts.
The linear regression analyses of the TSPO genotypes and the mean annual decline of MMSE, ADAS-Cog and ADAS-MOD are reported in Table 5. The multi-regression analysis of mean annual decline of MMSE, ADAS-Cog and ADAS-MOD are showed in Table 6. The TSPO genotype was not associated with the mean decline of MMSE, ADAS-Cog and ADAS-MOD. The mean annual decline of ADAS-MOD was significantly correlated to age, presence of ApoE4, diagnostic group (normal, MCI and AD) and gender. The survival analysis of ADAS-MOD changes (>3 points of the scale) performed in the entire population stratified for genotypes did not reveal any significant differences (p>>0.05) with Mantel-Cox test (Figure 4). The analysis of the covariates showed a significant effect on ADAS-MOD (>3 points of the scale) only for diagnostic groups (normal, MCI and AD), ApoE4 and gender (p<0.05) but did not show any effect on age and TSPO genotypes. Mantel-Cox test including TSPO genotypes as covariate in 134 amyloid positive subjects did not reveal any significant association (H.R. 1.01 C.I. 95% 0.71-1.32 0.80-1.30, p=0.92). Repeat analysis adjusting for the presence of ApoE4 did not improve the results of TSPO genotypes (ApoE4 association showed a trend in cognitive decline even if it did not reach the statistical significance H.H. 1.26 C.I. 95% 0.99-1.60, p=0.06).

DISCUSSION

This is the first study to evaluate the influence of TSPO genotypes on the clinical phenotypes of AD and disease progression. Here we demonstrated for the first time that the pathological features, clinical phenotypes and rate of cognitive decline in AD and MCI are similar in three TSPO subgroups. This finding is of clinical relevance considering the increasing interest in the role of microglia/neuroinflammation in AD pathophysiology that has led to development of several novel TSPO radiotracers aimed at improving the understanding of the disease process, improving the diagnosis, and obtaining a marker of disease progression 5, 11, 22. In order to make the assessments more robust, some studies have chosen only to use subjects who are high affinity TSPO binders, representing around 50% of Caucasians 12. This approach will result in a selection bias of patients. Moreover, if changes in levels of microglial activation are to be used as a surrogate endpoint in future interventional AD trials, 50% of subjects could not be enrolled on the basis of their TSPO genotype. Our data suggest that evaluating a subgroup of AD subjects with HAB will still allow their clinical and amyloid PET results to be translated to the rest of the AD patients; and considering the sample size in this cohort we can conclude that this finding is robust. Amyloid burden, although measured with two different methods and multiple cameras, was prevalent in the three diagnostic groups at a level consistent with the literature 23. Additionally, the prevalence of ApoE4 was the same among the three TSPO subgroups in AD, MCI and control subjects. The prevalence of three TSPO phenotypes in AD and MCI was consistent with the prevalence of the TSPO phenotypes in the general population: 49% for HAB, 9% for LAB, and 42% for MAB 10, 12.

Longitudinal analysis has been addressed with two different methods in order to improve the quality of the data. We firstly analysed the data using linear regression with mean decline over time of the cognitive test scores (MMSE and ADAS-Cog and ADAS-MOD) as the parameter
estimates, and TSPO genotypes as the dependent variable. We also performed a multi-regression analysis with survival function using several independent variables to assess whether other factors (age, ApoE4, gender, diagnostic groups) could better explain cognitive decline. Consistent with baseline analysis, the regression analyses confirmed that the rate of cognitive decline was similar over time (up to 36 months) between genotypes and supported by similar mean annual decline in change of ADAS-Cog and ADAS-MOD score or MMSE. The multi-regression analysis showed similar results within diagnostic groups with different TSPO genotypes and, interestingly, for the ADAS-MOD, mean annual decline showed an inverse correlation with the age, as previously reported by Holland et al. 24. The Kaplan-Mayer analysis confirmed baseline and linear regression analysis results. The covariate analysis revealed that a significant association existed only between ADAS-MOD increase (>3 points), diagnostic group (MCI, AD, control), ApoE4 status, and gender. There were inconsistencies in the literature regarding the gender differences and the annual rate of cognitive decline, but it has been suggested that men (with larger brain volume) have higher cognitive reserve to withstand more insult (pathological changes) compared to woman 25, which is in agreement with our finding. The latter findings have already been published and were not the objective of this study 26-28. Finally, even if underpowered, the sub-analysis of TSPO genotypes and the hazards of cognitive worsening in amyloid positive subject did not show any significant correlation also after adjusting for ApoE4 homozygotes presence thus suggesting that TSPO genotypes seems not to have any significant interaction with amyloid deposition.

\( ^{11} \text{C-PBR28} \) has 80-fold higher affinity for TSPO than the first generation TSPO tracer, \( ^{11} \text{C-PK11195} \). \( ^{11} \text{C-(R)-PK11195} \) has significantly lower signal-to-noise ratio compared to \( ^{11} \text{C-PBR28} \). However, it was found that patients exhibited different binding affinities for TSPO, falling into high affinity, low affinity, or mixed affinity binder groups 29. This variability in binding affects modelling of radioligand binding as a proxy for microglial activation, to the extent where some studies have chosen only to use high affinity binders to make their analysis more robust. Another second-generation ligand is \( ^{11} \text{C-DAA1106} \), which has much higher affinity for TSPO than \( ^{11} \text{C-(R)-PK11195} \), is also influenced by TSPO polymorphisms 30. As more and more TSPO tracers are being used to evaluate neuroinflammation, our results demonstrating that TSPO genotype did not influence the amyloid pathology or disease progression in AD is very relevant in evaluating AD patients with second generation TSPO tracers.

At molecular level, the TSPO protein is involved in steroidogenic cells controlling the uptake of cholesterol and thus the synthesis of neuroactive steroids 31. Allopregnanolone, an endogenous neurosteroid synthesised in the central and peripheral nervous systems in myelinating glial cells, astrocytes, and several neuronal cell types, declines with age and neurodegenerative disease 14. Costa et al. demonstrated that the TSPO Ala147Thr spontaneous amino acid substitution within TSPO is able to affect pregnenolone production in a peripheral cell model, represented by circulating lymphomonocytes 13. However, it is not known to what extent Thr 147 TSPO genotype could affect neurosteroidsynthesis such as allopregnanolone at CNS level, and whether it could play a role in neurodegenerative pathophysiology. Considering our data, it seems very unlikely that Thr 147 TSPO genotype could affect the course of AD.
Considering the number of subjects analysed in our study at baseline and during follow-up, it seems very unlikely that Thr 147 TSPO genotype could affect the course of the disease in AD. A possible limitation of our study is the limited time of follow-up. However, it is reasonably long given the AD patient life span (a mean of 10 years) and the annual rate of conversion of MCI to AD (10-15%). One could argue that, for novel therapeutic targets, the influence of genotype is still unknown. However, unless new potential treatments will interfere with TSPO genotypes and cholesterol metabolism, it is very unlikely this will have an effect. Even though we have not evaluated the influence of HAB, MAB, and LAB in other neurodegenerative diseases, it is unlikely that this difference in binding is going to be clinically significant in other conditions.

CONCLUSION

In conclusion, we have demonstrated that all binding groups (HAB, MAB, and LAB) have the same level of amyloid deposition in AD and MCI subjects. The rate of cognitive decline was also similar in the three TSPO subgroups of AD and MCI subjects. We have demonstrated that the prevalence of HAB was 50.3%, while MAB was 41.2% and 8.5% for LAB in AD, MCI and control subjects. This implies that any information about the AD process or effects of intervention explored in a subgroup of high affinity or mixed affinity binder subjects using second generation TSPO could be translated to the whole AD or MCI population. This will allow us to study lower numbers of AD subjects in evaluating new anti-neuroinflammatory/anti-microglial agents in intervention studies, and in observational studies evaluating the role of neuroinflammation.

CONFLICT OF INTEREST

Fan Z: Nothing to disclose

Harold D: Nothing to disclose

Pasqualetti G: Received funding from University of Pisa and from PAIM (Pisa, Italy) and Italian Society of Pharmacology for research travel cost

Williams J: Nothing to disclose

Brooks, DJ: Professor Brooks was also the chief medical officer for GE healthcare, and he has received consultancy fees/honoraria from the following: Acadia Pharmaceuticals Inc, Amsterdam Molecular Therapeutics BV, AstraZeneca, Biogeldec, NeuroNova AB, Eli Lilly and Company, Medtronic Inc, Shire Pharmaceuticals Inc, Synosia Therapeutics AG, GlaxoSmithKline, UBC Biosciences Inc, Veralis (R&D) limited, Genentech Inc, Navidea.

Edison P: Received grant funding from Medical Research Council, UK, Alzheimer’s Research, UK, Alzheimer’s Society, UK, Novo Nordisk, and GE Healthcare.
ACKNOWLEDGEMENT

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REFERENCES


Figure 1. Baseline cognitive test

Figure 1 demonstrates baseline mini mental state examination (MMSE) and Alzheimer Disease Assessment Scale (ADAS-Cog and ADAS-MOD) scores (mean value) across TSPO genotypes (Thr/Thr, Ala/Thr, Ala/Ala) and diagnostic groups (normal, MCI, AD subjects). No significant differences were observed in each diagnostic group by binding status (TSPO genotype); p>0.05. Error bars show 95% CI.
Figure 2. Parametric RATIO images of amyloid deposition in individual subjects

Figure 2 demonstrates the target to cerebellar RATIO images of amyloid deposition. Each row represents an individual subject of each diagnosis group – normal (top row), MCI (middle row) and AD (bottom row) and each column represents an individual scan of different genotypes – Thr/Thr (left column), Ala/Thr (middle column) and Ala/Ala (right column).
Figure 3. Longitudinal cognitive changes

Figure 3 demonstrates mean annual decline of mini mental state examination (MMSE) and Alzheimer Disease Assessment Scale (11 items and modified [MOD]) stratified for diagnostic group and genotypes (Thr/Thr, Ala/Thr, Ala/Ala). No significant differences have been found in each neuropsychological test across the three TSPO genotypes (p>0.05). Error bars show 95% CI.
Figure 4. Survival analysis

Figure 4 demonstrates Kaplan-Mayer curves of the survival analysis of Alzheimer's Disease Assessment Scale modified (MOD) increase >3.0 over time of the three cohorts stratified by TSPO genotypes. mo = months.
Table 1 shows the demographic details at the baseline, along with scores of MMSE, ADAS-Cog, ADAS-MOD, GDRs and GDS. Please see the text for details of the amyloid positivity. ApoE4 denotes ApoE4/ApoE4 homozygous. ns: not significant.* The amyloid PET scan was performed only in 255 patients and the percentage reported was calculated in this subgroup.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>Sex (male%)</th>
<th>Age, Year (SD)</th>
<th>%Amyloid+ (number of subjects)</th>
<th>ApoE4</th>
<th>MMSE (±SD)</th>
<th>ADAS-Cog (±SD)</th>
<th>ADAS-MOD (±SD)</th>
<th>GDRs (±SD)</th>
<th>GDS (±SD)</th>
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<tbody>
<tr>
<td>Normal</td>
<td>225</td>
<td>52.0</td>
<td>76(±5.0)</td>
<td>31%(99)</td>
<td>1.8</td>
<td>29(±1)</td>
<td>6.2(±3)</td>
<td>9(±4.1)</td>
<td>0.0(0.0)</td>
<td>0.8(1.1)</td>
</tr>
<tr>
<td>MCI</td>
<td>388</td>
<td>63.9</td>
<td>75(±7.3)</td>
<td>64%(137)</td>
<td>12.4</td>
<td>27(±1.8)</td>
<td>12(±4.4)</td>
<td>18(±6.6)</td>
<td>0.49 (0.02)</td>
<td>1.6(1.4)</td>
</tr>
<tr>
<td>AD</td>
<td>185</td>
<td>52.9</td>
<td>75(±7.5)</td>
<td>84%(19)</td>
<td>17.9</td>
<td>23(±2.0)</td>
<td>18(±6.7)</td>
<td>28(±9.5)</td>
<td>0.74 (0.25)</td>
<td>1.6(1.4)</td>
</tr>
<tr>
<td>Total</td>
<td>798</td>
<td>57.4</td>
<td>75(±6.8)</td>
<td>255</td>
<td>10.6</td>
<td>26(±2.7)</td>
<td>11(±6.4)</td>
<td>18(±9.5)</td>
<td>0.41 (0.30)</td>
<td>1.3(1.4)</td>
</tr>
</tbody>
</table>

*p<0.01, ns*
TABLE 2: Prevalence of the TSPO genotypes

<table>
<thead>
<tr>
<th>SNP</th>
<th>Thr/Thr</th>
<th>Ala/Thr</th>
<th>Ala/Ala</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>NORMAL</td>
<td>21(9.3)</td>
<td>98(43.6)</td>
<td>106(47.1)</td>
<td>225</td>
</tr>
<tr>
<td>MCI</td>
<td>28(7.2)</td>
<td>156(40.2)</td>
<td>204(52.6)</td>
<td>388</td>
</tr>
<tr>
<td>AD</td>
<td>19(10.3)</td>
<td>75(40.5)</td>
<td>91(49.2)</td>
<td>185</td>
</tr>
<tr>
<td>Total</td>
<td>68(8.5)</td>
<td>329(41.2)</td>
<td>401(50.3)</td>
<td>798</td>
</tr>
</tbody>
</table>

Pearson Chi-Square $P=0.570$

Prevalence of the three TSPO (Thr/Thr, Ala/Thr, Ala/Ala) genotypes among the diagnostic groups (normal, MCI, AD) and Pearson chi-squared test results.
TABLE 3: Odds Ratio incidence of TSPO and risk of dementia

<table>
<thead>
<tr>
<th>SNP</th>
<th>OR</th>
<th>CI (95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Thr/Thr, Ala/Thr)</td>
<td>Ala/Ala</td>
</tr>
<tr>
<td>Normal</td>
<td>119</td>
<td>106</td>
</tr>
<tr>
<td>MCI</td>
<td>184</td>
<td>204</td>
</tr>
<tr>
<td>AD</td>
<td>94</td>
<td>91</td>
</tr>
</tbody>
</table>

Odds Ratio (OR) and 95% of confidence interval (C.I.) of TSPO (Thr147) genotypes, heterozygote and homozygote, among diagnostic groups (normal, MCI, AD).
<table>
<thead>
<tr>
<th>TSPO genotypes</th>
<th>Number</th>
<th>Sex (%)</th>
<th>Age, Year (SD)</th>
<th>%Amyloid+ (tot. number)</th>
<th>ApoE4</th>
<th>MMSE</th>
<th>ADAS-Cog</th>
<th>ADAS-MOD</th>
<th>GDRs</th>
<th>GDS</th>
</tr>
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<tbody>
<tr>
<td>Thr147Thr</td>
<td>68</td>
<td>60.2</td>
<td>76.6(6.1)</td>
<td>62.5(24)</td>
<td>16.2</td>
<td>26.7(2.7)</td>
<td>11.8(7.0)</td>
<td>18.2(10.5)</td>
<td>0.41(0.32)</td>
<td>1.5(1.5)</td>
</tr>
<tr>
<td>Ala147Thr</td>
<td>329</td>
<td>56.2</td>
<td>75.2(6.7)</td>
<td>52.2(113)</td>
<td>8.5</td>
<td>26.8(2.7)</td>
<td>11.7(6.5)</td>
<td>18.3(9.6)</td>
<td>0.40(0.30)</td>
<td>1.3(1.3)</td>
</tr>
<tr>
<td>Ala147Ala</td>
<td>401</td>
<td>59.1</td>
<td>75.2(7.0)</td>
<td>50.8(118)</td>
<td>11.5</td>
<td>26.7(2.6)</td>
<td>11.6(6.3)</td>
<td>18.1(9.2)</td>
<td>0.42(0.29)</td>
<td>1.5(1.4)</td>
</tr>
<tr>
<td>Total</td>
<td>798</td>
<td>58.0</td>
<td>75.3(6.8)</td>
<td>52.5(255)</td>
<td>10.7</td>
<td>26.7(2.7)</td>
<td>11.6(6.4)</td>
<td>18.1(9.5)</td>
<td>0.41(0.30)</td>
<td>1.4(1.4)</td>
</tr>
</tbody>
</table>

Table 1 shows the demographic details at the baseline, along with scores of MMSE, ADAS-Cog, ADAS-MOD, GDRs and GDS. Please see the text for details of the amyloid positivity. ApoE4 denotes ApoE4/ApoE4 homozygous. ns: not significant. * The amyloid PET scan was performed only in 255 patients and the percentage reported was calculated in this subgroup.
TABLE 5. Regression analysis of TSPO genotypes and mean annual decline of cognitive function

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Independent variable (TSPO Genotypes)</th>
<th>B</th>
<th>SE</th>
<th>Beta</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean MMSE decline</td>
<td>Thr/Thr, Ala/Thr, Ala/Ala</td>
<td>0.23</td>
<td>0.15</td>
<td>0.054</td>
<td>0.13</td>
</tr>
<tr>
<td>Mean ADAS -Cog change</td>
<td>Thr/Thr, Ala/Thr, Ala/Ala</td>
<td>-0.16</td>
<td>0.16</td>
<td>-0.035</td>
<td>0.32</td>
</tr>
<tr>
<td>Mean ADAS-MOD change</td>
<td>Thr/Thr, Ala/Thr, Ala/Ala</td>
<td>0.09</td>
<td>0.230</td>
<td>0.01</td>
<td>0.70</td>
</tr>
</tbody>
</table>

B=unstandardized coefficient, SE= standard errors, Beta= standardized coefficient.
### Multi-regression analysis

<table>
<thead>
<tr>
<th>Model</th>
<th>Mean MMSE Decline</th>
<th>Mean ADAS-Cog decline</th>
<th>Mean ADAS-MOD decline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>SE</td>
<td>Beta</td>
</tr>
<tr>
<td>Age</td>
<td>0.01</td>
<td>0.01</td>
<td>0.04</td>
</tr>
<tr>
<td>ApoE4</td>
<td>-0.34</td>
<td>0.20</td>
<td>-0.06</td>
</tr>
<tr>
<td>Diagnostic groups (normal, MCI, AD)</td>
<td>-1.16</td>
<td>0.14</td>
<td>-0.30</td>
</tr>
<tr>
<td>Gender</td>
<td>-0.09</td>
<td>0.20</td>
<td>-0.02</td>
</tr>
<tr>
<td>TSOP genotypes (Thr/Thr, Ala/Thr, Ala/Ala)</td>
<td>0.26</td>
<td>0.15</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Multi-regression analysis of age, ApoE4 status, diagnostic groups (normal, MCI, AD), gender, and TSPO genotypes (Thr/Thr, Ala/Thr, Ala/Ala) as independent variables and Mean annual decline of mini mental state examination (MMSE) and Alzheimer Disease Assessment Scale (ADAS-Cog items and ADAS-MOD) (B=unstandardized coefficient, beta= standardized coefficient, SE= standard errors).
Can studies of neuroinflammation in a TSPO genetic subgroup (HAB or MAB) be applied to the entire AD cohort?

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