



Plasma Amyloid Is Associated with White Matter and Subcortical Alterations and Is Modulated by Age and Seasonal Rhythms in Mouse Lemur Primates

Charlotte Gary^{1,2†}, Anne-Sophie Hérard^{1,2†}, Zoé Hanss^{1,2} and Marc Dhenain^{1,2*}

¹ Centre National de la Recherche Scientifique, Université Paris-Sud, Université Paris-Saclay, UMR 9199, Neurodegenerative Diseases Laboratory, Fontenay-aux-Roses, France, ² Commissariat à l'Energie Atomique et aux Energies Alternatives, Direction de la Recherche Fondamentale, Institut François Jacob, MIRCen, Fontenay-aux-Roses, France

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*Correspondence:

Marc Dhenain
Marc.Dhenain@cea.fr

[†]These authors have contributed
equally to this work.

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Accumulation of amyloid- β ($A\beta$) peptides in the brain is a critical early event in the pathogenesis of Alzheimer's disease (AD), the most common age-related neurodegenerative disorder. There is increasing interest in measuring levels of plasma $A\beta$ since this could help in diagnosis of brain pathology. However, the value of plasma $A\beta$ in such a diagnosis is still controversial and factors modulating its levels are still poorly understood. The mouse lemur (*Microcebus murinus*) is a primate model of cerebral aging which can also present with amyloid plaques and whose $A\beta$ is highly homologous to humans'. In an attempt to characterize this primate model and to evaluate the potential of plasma $A\beta$ as a biomarker for brain alterations, we measured plasma $A\beta_{40}$ concentration in 21 animals aged from 5 to 9.5 years. We observed an age-related increase in plasma $A\beta_{40}$ levels. We then evaluated the relationships between plasma $A\beta_{40}$ levels and cerebral atrophy in these mouse lemurs. Voxel-based analysis of cerebral MR images (adjusted for the age/sex/brain size of the animals), showed that low $A\beta_{40}$ levels are associated with atrophy of several white matter and subcortical brain regions. These results suggest that low $A\beta_{40}$ levels in middle-aged/old animals are associated with brain deterioration. One special feature of mouse lemurs is that their metabolic and physiological parameters follow seasonal changes strictly controlled by illumination. We evaluated seasonal-related variations of plasma $A\beta_{40}$ levels and found a strong effect, with higher plasma $A\beta_{40}$ concentrations in winter conditions compared to summer. This question of seasonal modulation of $A\beta$ plasma levels should be addressed in clinical studies. We also focused on the amplitude of the difference between plasma $A\beta_{40}$ levels during the two seasons and found that this amplitude increases with age. Possible mechanisms leading to these seasonal changes are discussed.

Keywords: Alzheimer, lemur, plasma amyloid, brain morphometry, aging, seasons

INTRODUCTION

Amyloid- β ($A\beta$) is a peptide generated after proteolytic cleavage of its precursor, the amyloid precursor protein (APP), by β - and γ -secretases (Haass, 2004). After their production in the brain, $A\beta$ peptides can aggregate into amyloid plaques that initiate neurotoxic events, neuronal loss, and tau-associated pathology leading to Alzheimer's disease (AD), the most common age-related neurodegenerative disorder. This aggregation of $A\beta$ is thought to result from increased local brain $A\beta$ concentration above a critical threshold (Burgold et al., 2014) because of an overproduction or a reduced clearance of $A\beta$. This clearance can be related to the degradation of $A\beta$ in the brain (Iwata et al., 2001) and/or to $A\beta$ exportation outside the brain through the blood-brain barrier (BBB) (Shibata et al., 2000) and/or via the interstitial fluid (ISF) bulk flow into the cerebrospinal fluid (CSF), and from there into the bloodstream (Silverberg et al., 2003). Amyloid is also found in peripheral tissues. For example, high $A\beta_{40}$ levels are reported in the aorta in patients with advanced atherosclerotic lesions (Roher et al., 2009) and in the plasma of patients with coronary artery diseases, cardiovascular diseases, or diabetes mellitus (Janelidze et al., 2016; Roeben et al., 2016). During physiological aging, some studies (Lopez et al., 2008; Huang et al., 2013), though not all (Roher et al., 2009), reported increased baseline plasma $A\beta$ levels too. These changes in peripheral $A\beta$ levels can be attributed to either clearance of cerebral $A\beta$, or to $A\beta$ synthesis at the level of peripheral sources such as skeletal muscle, platelets, and vascular walls which can produce appreciable amounts of $A\beta$ (Roher et al., 2009). Altered peripheral clearance such as that associated with renal function could also increase plasma $A\beta$ levels (Jin et al., 2017).

Despite the presence of $A\beta$ in brain pathology, the relationship between plasma $A\beta$ levels and other pathology markers is still unresolved. Recent longitudinal studies have demonstrated a clear link between plasma $A\beta$ and the risk of dementia in an AD context (Head et al., 2011; Chouraki et al., 2015) or a correlation with AD diagnosis and brain amyloid burden (Rembach et al., 2014), while some others have ruled out the predictive value of plasma $A\beta$ in AD risk determination (Lövheim et al., 2017). Some studies in AD and MCI patients suggested a relationship between high $A\beta_{42}$ and/or $A\beta_{40}$ levels and white matter lesions (Gurol et al., 2006; Janelidze et al., 2016). Studies in dementia-free persons reported mixed findings. Some reported that higher $A\beta_{42}$ and/or $A\beta_{40}$ levels are associated with larger white matter hyperintensities (WMH) and lacunar infarcts (van Dijk et al., 2004; Gurol et al., 2006; Hilal et al., 2017). Patients bearing MRI-defined brain infarcts had higher $A\beta_{42}$ levels (Toledo et al., 2011). In dementia-free persons, high plasma $A\beta_{40}$ was correlated with hippocampal atrophy and with increased rate of cerebral atrophy (Kaffashian et al., 2015). However, in some studies, opposite results were obtained and a reduced plasma $A\beta_{40}$ was associated with increased progression of WMH (Kaffashian et al., 2014).

Animal models are widely used to study cerebral pathologies and aging, to evaluate new drugs and to assess biomarkers. Among various species stands the mouse lemur (*Microcebus murinus*), a small (70–150 g) primate model of cerebral aging. It has a short lifespan of ~ 12 years in captivity (Languille et al.,

2012) and an $A\beta$ peptide that is completely homologous to that of humans (Silhol et al., 1996). Approximately 10% of lemurs over 6 years old develop intracerebral $A\beta$ deposits and their amyloid plaque load is usually low (Mestre-Frances et al., 2000; Kraska et al., 2011), though a previous study reported great heterogeneity of plasma $A\beta_{40}$ concentrations in old animals from low to high (Roy et al., 2015). Low plasma $A\beta_{40}$ levels in old animals have been associated with high cerebral intracellular labeling with antibodies specific of amyloid and APP (Roy et al., 2015). As mouse lemurs can spontaneously develop age-related cerebral atrophy (Kraska et al., 2011; Picq et al., 2012; Sawiak et al., 2014), they are widely used to evaluate parameters modulating cerebral atrophy (Djelti et al., 2016).

One of the biological features of mouse lemurs is that their metabolic and physiological parameters undergo seasonal changes that are strictly controlled by photoperiodic variations (Dal-Pan et al., 2011). Exposure to day lengths shorter than 12 h (i.e., 10 h light/day) results in complete sexual rest, fattening, lethargy, reduced behavioral activities, and torpor. Torpor is defined as a state during which body temperature and metabolic rate are significantly reduced. In contrast to hibernation, torpor is characterized by short-duration bouts that do not exceed 24 h, so that animals exhibit active states each day between torpor episodes (Perret and Aujard, 2001). Exposure to day lengths greater than 12 h (i.e., 14 h light/day) induces sexual activity, increases in behavioral activities, and high hormonal levels (Perret, 1997). Thus, two marked biological seasons called “winter” and “summer” occur in lemurs. They correspond respectively to periods of “lower” or “higher” activity levels.

In the present study, we assessed plasma $A\beta_{40}$ levels in 21 adult mouse lemurs (aged from 5 to 9.5 years-old at the beginning of the study) and confirmed previous studies demonstrating higher plasma $A\beta$ levels in the oldest animals. MR images were recorded for each animal in order to evaluate relationships between plasma $A\beta_{40}$ levels and cerebral pathology. A voxel-based morphometry (VBM) study revealed a relationship between lower plasma $A\beta_{40}$ levels and atrophy of white matter and subcortical brain regions. We also investigated the seasonal rhythmicity of plasma $A\beta_{40}$ in mouse lemurs by measuring its concentration during two consecutive seasons. Mouse lemurs displayed strong seasonal variations of plasma $A\beta_{40}$ levels with an increase in winter. We examined the amplitude of the difference between plasma $A\beta_{40}$ levels in the two seasons and found that this amplitude increased with aging.

MATERIALS AND METHODS

Animals and Biological Rhythms

This study was carried out in accordance with the recommendations of the European Communities Council directive (2010/63/EU). The protocol was approved by the local ethics committees CEtEA-CEA DSV IdF (authorizations 201506051 736524 VI, APAFIS#778). All mouse lemurs were born in a laboratory breeding colony (Brunoy, France, authorization n $^{\circ}$ E91-114-1), and maintained at steady ambient temperature (24–26°C) and relative humidity (55%). Their seasonal and daily rhythms were controlled by an artificial

photoperiod regimen of alternating 6 month seasons of short day lengths (10 h light/day, “winter”) and long day lengths (14 h light/day, “summer”). The shift from one season to the other was made without progressive transition. Animals were fed with fresh fruits, milky mixture (eggs, cereals, milk cheese, and honey bread) and meal worms.

Twenty-one mouse lemurs (5–9.5 years old) were involved in this study. They were split into two groups according to age at first blood sampling: middle-aged (fifteen animals, 5–6 years old) and old (six animals over 7 years old). Two blood samples were collected from each animal: the first one was taken during winter, 1 month after the shift in the season, and the second one was obtained 6 months later during summer. MRI images were recorded for each animal.

Blood Collection, Pre-treatment, and Plasma A β Detection

Plasma A β_{40} was measured as previously described (Roy et al., 2015). Briefly, blood was sampled at the beginning of the day after an overnight fast. This corresponds to the inactive period for the nocturnal lemurs. In mouse lemurs, blood has to be collected from the saphenous vein. The vein was pricked with a needle at a 45° angle and blood drops were collected in small heparinized hematocrit capillaries (60 μ l, Hirschmann-Laborgeraete, ref 91 00 260) in which the blood penetrated by capillarity. All capillaries were kept on ice and centrifuged ($\times 2,000$ g; 10 min) at +4°C immediately after collection. For each animal and at each time point, the plasma layer taken from the centrifuged hematocrit capillaries was then aliquoted into 200 μ l polypropylene tubes (Corning, Thermowell® Gold PCR tubes, Product #3745) to avoid freeze-thaw cycles. Volumes ranging from 120 to 200 μ l of plasma could be sampled for each animal. A cocktail of protease inhibitors (Complete Mini; Roche, Meylan, France) was added to each plasma sample at a final concentration of 1X. The aliquots were frozen at –80°C within 1 h of sampling and kept frozen until analysis. This preanalytical processing corresponds to previous recommendations (Watt et al., 2012) except for the use of heparin instead of EDTA as anticoagulant. We chose heparin because, to the best of our knowledge, the only anticoagulant-coated hematocrit capillaries commercially available use heparin as the anticoagulant. Using heparin probably resulted in lower A β values in our samples as compared to those that would have been obtained with EDTA (Watt et al., 2012), but this has not been reported to change intra-experiment values (Lachno et al., 2009).

As mouse lemurs constitutively produce A β peptides, their plasma A β concentrations are much lower than those of transgenic mice overexpressing mutated forms of APP, so mouse lemur A β_{42} levels are usually below the limit of detection with classical enzyme-linked immunosorbent assay (ELISA) tests (Roy et al., 2015). We thus focused on A β_{40} as its high relative concentration compared to other A β species allowed measurements above the lower limit of quantification from very small volumes of plasma. It is the main A β component in the brain and is strongly correlated to A β_{42} in humans (Huang et al., 2013). Plasma A β_{40} levels were measured in duplicate

using ELISA “Human β amyloid 1–40” kits (Invitrogen, Saint Aubin, France) following the manufacturer’s protocol for non-diluted plasma samples. With this kit, 50 μ l of plasma is required to perform one measure. Antibodies used in these kits are coated with monoclonal antibodies against the N-terminal part of human A β and the secondary antibodies are rabbit antibodies specific for the C-terminal part of the human A β_{40} sequence. The calibration standards (synthetic A β_{40} peptides) were provided with the kits. The calibration standards and negative controls always performed within the manufacturer’s range and samples’ A β concentrations always stood within the calibration range.

MRI Acquisition and Analysis

Cerebral imaging was performed by MRI in all the animals involved in the study. Brain images were recorded on a 7.0 Tesla spectrometer (Agilent, USA) using a four channel phased-array surface coil (Rapid Biomedical, Rimpar, Germany) actively decoupled from the transmitting birdcage probe (Rapid Biomedical, Rimpar, Germany). Briefly, animals were anesthetized by isoflurane (4% for induction and 1–1.5% for maintenance). Respiratory rate was monitored to ensure animal stability until the end of the experiment. Body temperature was maintained by an air heating system. Two-dimensional fast spin echo images were recorded with an isotropic nominal resolution of 230 μ m (128 slices, TR/TE = 10,000/17.4 ms; rare factor = 4; field of view = 29.4 \times 29.4 mm², matrix = 128 \times 128, slice thickness = 230 μ m, acquisition time = 32 min).

Images were analyzed by VBM using SPM8 (Wellcome Trust Institute of Neurology, University College London, UK, www.fil.ion.ucl.ac.uk/spm) with the SPMmouse toolbox (<http://spmmouse.org>) dedicated to animal brain morphometry (Sawiak et al., 2014). VBM is a reference method, widely used to identify structural changes in the brain including in humans (Whitwell, 2009).

Brain images were segmented into three tissue probability maps (tpm) corresponding to tissues with cortical gray matter (GM), white matter and subcortical nuclei (WM-SC), and cerebrospinal fluid (CSF) characteristics, using locally developed priors. The intensity of the pixel in each probability map represents the probability of the pixel to be GM, WM, or CSF. Then brain images and tpm were spatially transformed to the standard space, defined by Sawiak et al., using a GM mouse lemur template (Sawiak et al., 2014). Affine regularization was set for an average-sized template, with a bias non-uniformity FWHM cut off of 10 mm, a 5 mm basis function cut off and a sampling distance of 0.3 mm. The resulting GM and WM-SC portions were output in rigid template space, and DARTEL (Ashburner, 2007) was used to create non-linearly registered maps for each subject and common templates for the cohort of animals. The warped GM and WM-SC portions for each subject were adjusted using the Jacobian determinant from the DARTEL registration fields to preserve tissue amounts (“optimized VBM,” Good et al., 2001) and smoothed with a Gaussian kernel of 600 μ m to produce statistical maps (T maps) for analysis.

A first general linear model (GLM) was designed to evaluate relative changes in GM and WM-SC tpm values, a parameter reflecting atrophy, as a function of age. The sex of the animals

and total intracranial volumes (TIV) were considered in the design matrix and were treated as covariates of no interest. More specifically, with the GLM, if the brain of one animal is defined by the number “*j*” and the location of a pixel is defined as “*k*.” The signal within a pixel (Y_j^k) can be explained by the following equation

$$Y_j^k = \beta_1^k + x_{j,1}\beta_2^k + S_{j,1}\beta_5^k + S_{j,2}\beta_6^k + TIV_j\beta_7^k + \epsilon_j^k \quad (GLM - 1)$$

With β_1^k = Mean image; β_2^k = Evolution of the signal according to the age of the animals ($n=21$ animals); β_5^k = Sex effect on signal for males; β_6^k = Sex effect on signal for females; β_7^k = effect of TIV on the signal for each animal. In this matrix, $x_{j,1}$ corresponds to the age of each animal *j*. $S_{j,1}$ and $S_{j,2}$ correspond to the sex of the animals. $S_{j,1} = 1$ if the animal *j* is a male and = 0 otherwise; $S_{j,2} = 1$ if the animal *j* is a female and = 0 otherwise.

A second GLM was designed to evaluate the relationships between GM and WM-SC tpm values and plasma A β_{40} levels. Winter and summer plasma A β_{40} levels were added in the model. For this study, age, sex and TIV were considered in the design matrix and were treated as covariates of no interest. More specifically, with this second GLM, the signal within a pixel (Y_j^k) can be explained by the following equation

$$Y_j^k = \beta_1^k + x_{j,1}\beta_2^k + x_{j,2}\beta_3^k + x_{j,3}\beta_4^k + S_{j,1}\beta_5^k + S_{j,2}\beta_6^k + TIV_j\beta_7^k + \epsilon_j^k \quad (GLM - 2)$$

With β_1^k = Mean image; β_2^k = Evolution of the signal according to the age of the animals ($n = 21$ animals); β_3^k = Evolution of the signal according to winter plasma A β_{40} levels; β_4^k = Evolution of the signal according to summer plasma A β_{40} levels; β_5^k = Sex effect on signal for males; β_6^k = Sex effect on signal for females; β_7^k = effect of TIV on the signal for each animal. $x_{j,1}$, $x_{j,2}$ and $x_{j,3}$ correspond to the age, winter plasma A β levels and summer plasma A β levels for each animal *j*. $S_{j,1}$ and $S_{j,2}$ correspond to the sex of the animals. $S_{j,1} = 1$ if the animal *j* is a male and = 0 otherwise; $S_{j,2} = 1$ if the animal *j* is a female and = 0 otherwise.

As an example, on the basis of the GLM-2 model, for the first animal (a 7.8 year-old female, winter and summer plasma A β_{40} levels = 75.2 and 35.5 pg/ml with a TIV of 2150 mm³):

$$Y_1^k = \beta_1^k + 7.8 \beta_2^k + 75.2 \beta_3^k + 35.5 \beta_4^k + \beta_5^k + 2150 \beta_7^k + \epsilon_1^k;$$

for the second animal (a 5.6 year-old male, winter and summer plasma A β levels = 37.3 and 33.5 pg/ml with a TIV of 2058 mm³)

$$Y_2^k = \beta_1^k + 5.6 \beta_2^k + 37.3 \beta_3^k + 33.5 \beta_4^k + \beta_5^k + 2058 \beta_7^k + \epsilon_2^k$$

and so on for the other animals.

A contrast defines a linear combination of β as $c^T\beta$. For example, the test evaluating the positive relationship between plasma A β levels and the probability of pixels being GM is defined using a contrast $c^T\beta = \{0 \ 0 \ 1 \ 1 \ 0 \ 0 \ 0\}^T$. The Null hypothesis is

$H_0 : c^T\beta = 0$, whereas the alternative hypothesis is $H_1 : c^T\beta > 0$. This hypothesis is tested with:

$$T = \frac{c^T\beta}{\sqrt{\sigma^2 c^T (X^T X)^{-1} c}} = \frac{\text{contrast}}{\sqrt{\text{estimated variance}}}$$

This analysis allows the removal of confounding effects, such as age (β_2^k), sex (β_5^k and β_6^k), or TIV (β_7^k) from the raw data. In other words, volumetric scans were entered as the dependent variable. Depending on the tested hypothesis, aging or plasma A β levels were the independent variables. Aging, sex, and TIV were covariates.

One-tailed *t*-test contrasts were set up to find areas in which probability values from GM or WM-SC maps correlated with age or plasma A β levels. To control for multiple comparisons, an adjusted *p*-value was calculated using the voxel-wise false discovery rate (FDR-corrected $p < 0.05$), with extent threshold values of 500 voxels, meaning that clusters required 500 contiguous voxels to be selected as relevant (Genovese et al., 2002). Voxels with a modulated GM value below 0.2 were not considered for statistical analysis. The operator was blinded to the animal names during image processing. This type of regression technique produces *t*-statistic and color-coded maps that are the product of a regression model performed at every voxel in the brain. Contiguous groups of voxels that attain statistical significance, called clusters, are displayed on brain images.

One of the minor limitations of this study is that brain images were recorded at different time points surrounding the winter and summer blood sampling, so it was not possible to attribute MR images to a given season. However, to our knowledge, modulation of cerebral atrophy by seasonal effects has never been described in mouse lemurs. We thus consider that brain images correspond to the state of an animal the year of the study independently of the season.

Statistical Analysis

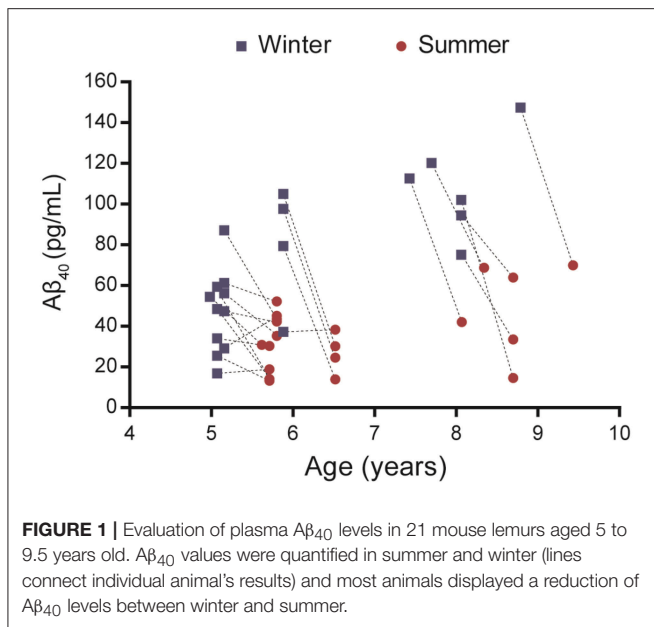
Paired Student's *t*-tests were used to evaluate seasonal effects on plasma A β_{40} levels. Pearson's tests were used to evaluate the correlation between plasma A β_{40} concentrations, or amplitude of seasonal variations of A β_{40} and age. Statistical analysis was done using Statistica 7.1 software (StatSoft, Maisons-Alfort, France). $P < 0.05$ was set as the level of statistical significance for each test.

RESULTS

Plasma A β_{40} Is Modulated by Age and Season

Plasma A β_{40} was evaluated in 21 middle-aged or old mouse lemurs in winter and summer (Figure 1, Supplementary Table 1). Visual observation of the data revealed that, in most animals, A β_{40} levels decreased from the winter to the summer season.

Statistical analysis showed that A β_{40} concentrations in winter were higher than in summer regardless of the age of the animals (paired Student's *t*-test $t = 5.32$, $df = 20$, $p = 0.00003$, Figure 2A) and were also correlated between the two seasons ($R^2 = 0.23$, $p < 0.026$, Figure 2B). When data were split into age categories,



we found higher plasma Aβ₄₀ concentrations in winter than in summer in both middle-aged and old animals ($p = 0.003$ and $p = 0.001$, respectively, **Figure 3A**).

Also, plasma Aβ₄₀ was higher in the old compared to middle-aged animals and we found age-related increases of Aβ concentrations of 94% ($p = 0.0005$) and 64% ($p = 0.024$) in winter and summer, respectively (**Figure 3A**). Finally, plasma Aβ₄₀ concentrations were correlated with age in both the winter ($R^2 = 0.60$, $p = 0.00004$) and summer ($R^2 = 0.24$, $p = 0.025$) periods (**Figure 3B**).

Plasma Aβ₄₀ Seasonal Variations Are Modulated by Aging

We then quantified the variation in plasma Aβ₄₀ concentrations in the two seasons for each animal. Increased plasma Aβ₄₀ seasonal variation ($\Delta A\beta_{40}$) was found in old animals (**Figure 4A**) and $\Delta A\beta_{40}$ was significantly correlated with aging ($R^2 = 0.36$, $p = 0.004$, **Figure 4B**).

Plasma Aβ₄₀ and Cerebral Atrophy

MRI images were used to evaluate, thanks to voxel-based analyses, the relationships between the age of the animals (GLM-1 including only an age effect and GLM-2 including age and plasma Aβ₄₀ effects) or plasma Aβ₄₀ levels (adjusted for the age of the animals - GLM-2) and cerebral atrophy [assessed from reduction in tissue probability maps (tpm)]. We did not detect any significant relationship between the age of the animals and gray matter, white matter or subcortical nuclei atrophy (analyses based on GLM-1 or GLM-2). We found a negative relationship between plasma Aβ₄₀ levels and white matter and subcortical nuclei atrophy when both summer and winter Aβ₄₀ were included in the model (GLM-2 model adjusted for the age of the animals, **Figure 5**) or when only summer Aβ₄₀ was taken into account (Supplementary Figure 1). In other words,

low Aβ₄₀ levels were associated with atrophy of white matter and subcortical brain regions. This relationship involved a large cluster encompassing bilaterally the following brain regions: corpus callosum, internal capsule, putamen, globus pallidus, thalamus, and geniculate nucleus. No other relationships, i.e., negative relationships between plasma Aβ₄₀ levels and white matter or subcortical nuclei tpm or any relationships between plasma Aβ₄₀ levels and GM tpm were detected. Also, with GLM-2, we did not detect any age effect when plasma Aβ₄₀ levels were used as variables of no interest.

DISCUSSION

This study evaluated plasma Aβ₄₀ levels in a cohort of 5–9.5 year-old mouse lemurs as well as the relationship between plasma Aβ₄₀ and cerebral atrophy. Mouse lemur primates express Aβ peptides homologous to those of humans (Silhol et al., 1996). However, amyloid plaque load is usually low in lemurs and only 10% of lemurs over 6 years old develop, mostly sparse, intracerebral Aβ deposits (Mestre-Frances et al., 2000; Kraska et al., 2011). In addition, the animals involved in this study were followed up by a veterinarian and they did not display any obvious pathology. Data from this study thus reflect “normal” aging conditions rather than pathological or AD-type conditions.

First, we found an age-related increase in plasma Aβ₄₀ levels. Second, we found that seasonal rhythms have a major impact on plasma Aβ₄₀ concentration with higher Aβ₄₀ levels in winter, the season of low activity and torpor. We also showed that the difference between plasma Aβ₄₀ levels in winter and summer ($\Delta A\beta_{40}$) increases with age. Finally, evaluation of cerebral atrophy showed that aging was not associated with cerebral atrophy in the middle aged/old animals from this study. Interestingly, when data were adjusted for age, low Aβ₄₀ levels were associated with white matter and subcortical nuclei atrophy. Thus, at a given age, animals with lower plasma Aβ₄₀ levels were more atrophied than animals with higher Aβ₄₀ values.

Our first result showing that old animals display higher plasma Aβ₄₀ levels than middle-aged ones confirms a trend reported in a previous study in lemurs (Roy et al., 2015). It is consistent with data in humans, in which aging increases plasma Aβ (Lopez et al., 2008; Huang et al., 2013), although this result is not reported by all studies (Roher et al., 2009). It is also consistent with the age-related increased synthesis of Aβ₄₀ reported in humans as well as primates (Fukumoto et al., 2004).

Since mouse lemurs are seasonal animals (Dal-Pan et al., 2011), it was important to determine the possible influence of seasonal variation on plasma Aβ₄₀ levels. We found strong seasonal variations of plasma Aβ₄₀ levels with increased concentrations during winter. To our knowledge, this is the first study reporting seasonal variations of Aβ in biological fluids. In humans, some studies (Huang et al., 2013), but not all (Lachno et al., 2009), showed diurnal variations of plasma Aβ, suggesting a circadian regulation of Aβ levels. Circadian variations of Aβ were also reported in mice, showing that interstitial fluid (ISF) Aβ levels are ~25% higher during the dark period, when

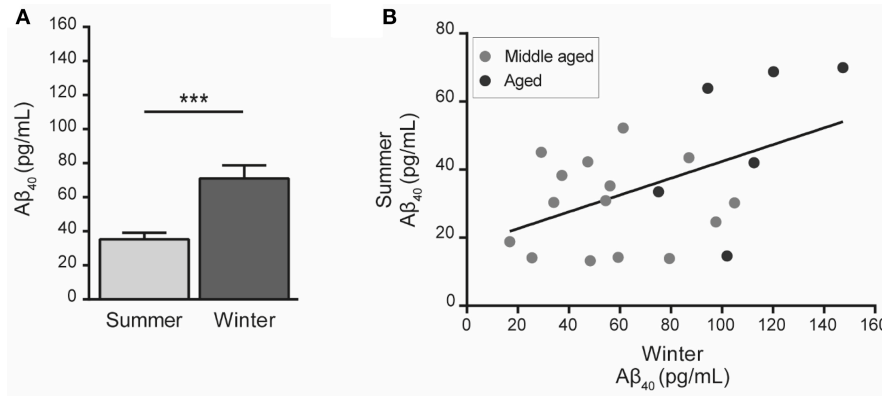


FIGURE 2 | Plasma Aβ₄₀ levels in mouse lemurs regardless of the age category. **(A)** Pooled data from middle-aged and old animals showed higher Aβ₄₀ concentration in winter than in summer (paired Student's *t*-test $t = 5.32$, $df = 20$, $p = 0.00003$). *** $p < 0.001$. **(B)** Correlation between Aβ₄₀ concentrations in summer and winter periods (Pearson's test, $R^2 = 0.23$, $p < 0.026$).

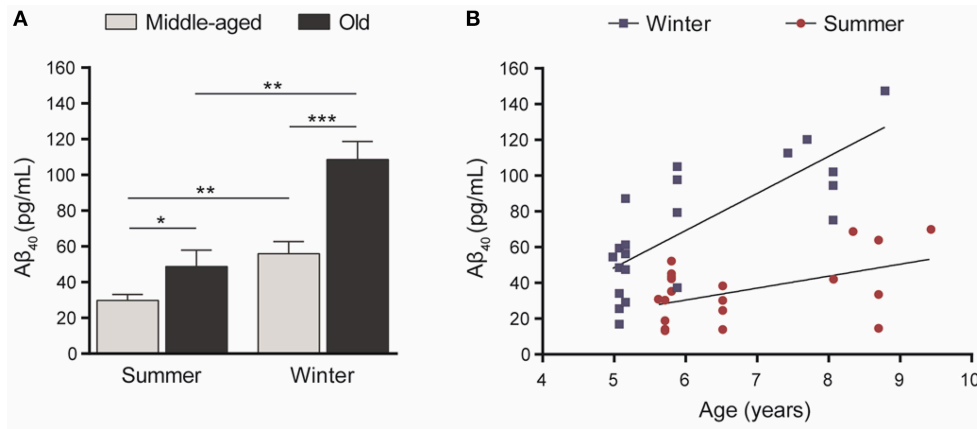


FIGURE 3 | (A) Plasma Aβ₄₀ levels in middle-aged (5–6 years old at the first sampling time, $n = 15$) or old (7.5–9.5 years old, $n = 6$) mouse lemurs. The plasma Aβ₄₀ concentration was higher in old animals than in middle-aged ones in summer ($t = 2.45$, $df = 19$, $p = 0.024$) and winter ($t = 4.19$, $df = 19$, $p = 0.0005$). Also, the plasma Aβ₄₀ concentration was higher in winter than in summer in middle-aged ($t = 3.51$, $df = 14$, $p = 0.003$) and old animals ($t = 6.62$, $df = 5$, $p = 0.001$). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. **(B)** Age-associated evolution of plasma Aβ₄₀ as a function of season. Plasma Aβ₄₀ levels increased as a function of age during both seasons (Pearson's tests, winter: $R^2 = 0.60$, $p = 0.00004$; summer: $R^2 = 0.24$, $p = 0.025$).

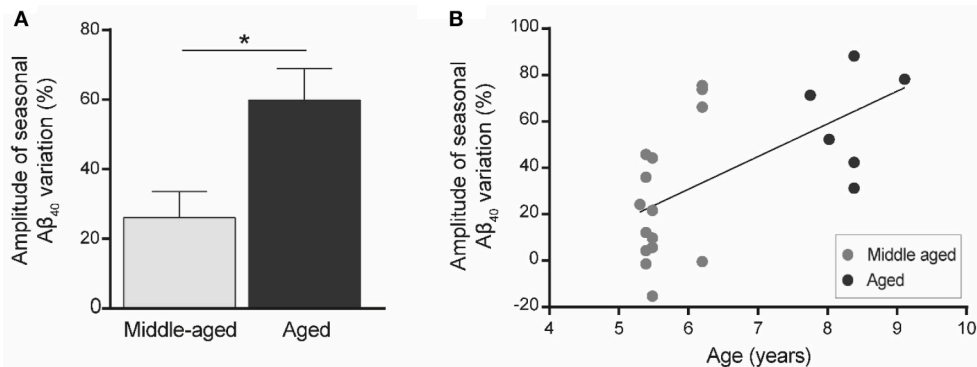


FIGURE 4 | Amplitude of seasonal variations of plasma Aβ₄₀ ($\Delta A\beta_{40}$) in mouse lemurs ($n = 21$ animals). **(A)** Seasonal variations of plasma Aβ₄₀ were greater in old compared to middle-aged animals. * $p < 0.05$. **(B)** Seasonal variation of plasma Aβ₄₀ is correlated with age in mouse lemurs (Pearson's test, $R^2 = 0.36$, $p = 0.004$).

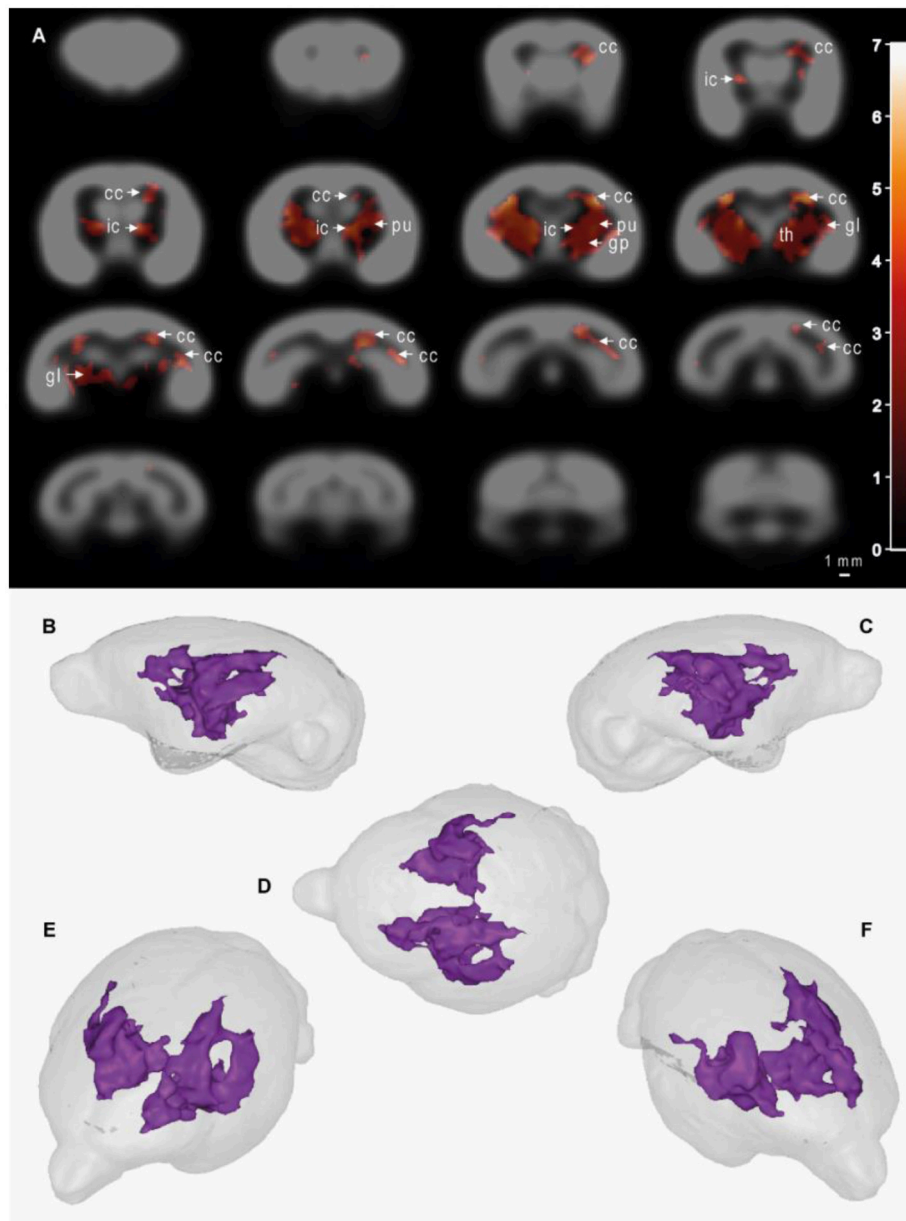


FIGURE 5 | Cerebral alterations correlated with plasma $A\beta_{40}$ levels in mouse lemurs. Statistical parametric maps depicting regions in which the probability of voxels belonging to white matter or subcortical nuclei increases with $A\beta_{40}$ plasma levels (A). In other words, the voxels in which low $A\beta_{40}$ levels were significantly associated with atrophy of white matter and subcortical brain regions are shown as colored patterns on brain templates (i.e., gray scale images of the brain). Slices are spaced 1 mm apart along the rostro-caudal axis (VBM parameters: FDR-corrected $p < 0.05$; extent threshold $k = 500$; cluster mapping represents t values). Maps were derived from MRI recorded on 21 animals aged from 5 to 9.5 years old. The color bar represents the value of the t statistic (no unit). The cluster measured 104 mm^3 , so 8,516 pixels. cc, corpus callosum; ic, internal capsule; pu, putamen; gp, globus pallidus; th, thalamus; gl, geniculate nucleus. (B–F) 3D representations of affected areas: sagittal left (B), sagittal right (C), dorsal (D), oblique front left (E) and oblique front right (F) views. Scale bar = 1 mm.

rodents are more active, than during the light period (Kang et al., 2009). Fluctuations of plasma $A\beta$ have been reported during a longitudinal study in humans (Roher et al., 2009), but their seasonal origin has not been established. Deciphering the biological parameters leading to the reported seasonal changes remains difficult. These changes could be associated with the regulation of the production and/or clearance of cerebral $A\beta$,

as well as with regulation of the production and/or clearance of peripheral sources of $A\beta$.

Several hypotheses can be proposed to explain our results. We can rule out that an increased concentration of plasma $A\beta_{40}$ in winter is linked to an increased production of $A\beta$. Indeed, in mouse lemurs, winter is associated with torpor and a reduction of general metabolism (Perret and Aujard, 2001;

Dal-Pan et al., 2011), and thus with low neuronal activity. This hypoactivity should lead to a reduction of amyloid production as A β production is linked to neuronal activity (Cirrito et al., 2005). Also, we expect that the low general metabolism should lead to reduced A β synthesis from peripheral sources of A β such as the skeletal muscle, platelets or vascular walls (Roher et al., 2009). Another explanation for increased plasma A β_{40} in winter is increased clearance from the brain to the blood. In many animals, torpor leads to a reduction of the size of the neurons and of dendrite density and thus to increased ISF space (Magariños et al., 2006; von der Ohe et al., 2006; Andrews, 2007). Also, increased ISF space facilitates the clearance of various metabolites, including A β , to outside of the brain (Xie et al., 2013). Thus, one likely explanation for the increased plasma A β concentration in winter is a torpor-induced increase in ISF space leading to increased A β clearance in the blood. Reduced seasonal changes of peripheral A β clearance can also explain our results. As renal function was recently shown to participate in A β clearance (Jin et al., 2017), we cannot exclude that a lower renal function associated with the hypoactivity occurring in winter participated in the induction of high plasma A β levels. In humans, physical activity reduces plasma A β levels (Stillman et al., 2017), so the lower plasma A β level in summer, i.e., the active season for mouse lemurs, could be related to an increased physical activity. Follow-up studies on new cohorts of animals will have to be initiated to further evaluate the origin of seasonal changes in plasma A β_{40} .

Then, we evaluated age-related changes of seasonal variation of plasma A β_{40} (i.e., the difference between plasma A β_{40} levels in winter and summer: $\Delta A\beta_{40}$). $\Delta A\beta_{40}$ increased with age. This result was not expected as the amplitude of most seasonal cycles (weight, metabolism, hormones, and so on) tends to decrease with aging in mouse lemurs (Perret and Aujard, 2006). Also, in humans, the amplitude of circadian plasma A β concentrations reduces with aging (Huang et al., 2013). In summer, old mouse lemurs already had plasma A β_{40} levels 64% higher than levels of middle-aged animals. In winter, this difference increased to 94%, leading to the increased $\Delta A\beta_{40}$ in old lemurs. Because of the reduction of the general metabolism occurring in lemurs during winter, we do not expect any increased cerebral or peripheral A β synthesis in the old animals in winter. An additional explanation for the increased $\Delta A\beta_{40}$ in old lemurs might be that in winter brain-to-blood A β clearance is enhanced and clearance from the blood is reduced. Mechanisms leading to these changes remain to be explored.

Our result showing that low A β_{40} levels are associated with atrophy in several white matter and subcortical brain regions was unexpected. This result was not linked to a confounding effect of aging as our analysis was adjusted for the age of the animals (taken as a variable of no interest). Aging did not modulate cerebral atrophy in our study either. Thus, the relationship between plasma A β_{40} and atrophy was independent of aging. However, our study suggests that plasma A β_{40} measures alone should not be taken as a surrogate marker for cerebral atrophy as it evolves with the age of the animals. In other words, plasma A β_{40} increases with aging and at a given age

lower A β_{40} levels are associated with cerebral atrophy. In a previous study, we showed that low plasma A β_{40} levels in mouse lemurs are associated with high cerebral intracellular labeling with antibodies specific to amyloid and APP (Roy et al., 2015). Thus, a link between amyloid overproduction, low A β_{40} plasma levels and white matter and subcortical atrophy cannot be excluded. In humans, data evaluating the relationships between plasma A β and cerebral impairments are controversial. Several studies suggest a relationship between high plasma A β_{40} levels and cerebral pathologies such as white matter lesions (van Dijk et al., 2004; Gurol et al., 2006; Janelidze et al., 2016), hippocampal atrophy and increased rate of cerebral atrophy (Kaffashian et al., 2015). It is possible that these studies were enriched with AD patients and did not reflect non-pathological conditions. One study focusing on dementia-free older adults reported that low plasma A β_{40} levels were associated with increased progression of white matter hyperintensities (WMH) due to vascular alterations. The authors suggested that the association of low plasma A β_{40} levels with increased WMH may reflect increased deposition of A β_{40} in cerebral vessel walls after its clearance, resulting in impaired cerebral blood flow (Kaffashian et al., 2014). In our study we did not observe any obvious white matter hyperintensity, but the image resolution in mouse lemurs impedes the detection of such lesions. We cannot, however, rule out vascular alterations in our mouse lemurs. To date, our animals are involved in a longitudinal follow-up study, hindering access to tissue. To identify the cause of plasma A β_{40} lowering in the atrophied animals, new investigations are now required. Developing techniques for perfusion measurements combined with brain vessel histopathology should be an elegant way to link plasma A β and brain morphology variations and to determine the potential of plasma A β_{40} as a biomarker of brain pathology.

To conclude, we confirmed an age-related increase of plasma A β and showed for the first time that plasma A β_{40} is modulated by seasonal variations. Seasonal effects should thus be taken into account when studying plasma A β , in preclinical as well as in clinical studies. Since seasonal changes are amplified in mouse lemurs, they appear to be a useful model for evaluating the impact of seasons on amyloid metabolism. Further mechanistic studies should now be initiated to understand the origin of the link between plasma A β_{40} concentrations and cerebral atrophy as well as of the plasma A β changes associated with seasonal variations. We also observed that in normal conditions and after adjustment for age, low plasma A β_{40} is correlated with brain tissue atrophy in several white matter and subcortical regions including the corpus callosum, internal capsule, putamen, globus pallidus, thalamus and geniculate nucleus. The origin of these changes remains to be evaluated but supports a strong link between plasma A β_{40} concentrations and brain morphometry.

AUTHOR CONTRIBUTIONS

CG and MD designed the study; CG and ZH performed the blood sampling; CG and A-SH performed the biochemical analysis; CG,

A-SH, and MD were responsible for statistical analyses and wrote the manuscript; A-SH and MD performed the MRI voxel wise analysis; ZH revised the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fnagi.2018.00035/full#supplementary-material>

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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