Glitter in the Darkness? Non-fibrillar β-amyloid Plaque Components Significantly Impact the β-amyloid PET Signal in Mouse Models of Alzheimer's Disease

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Abbreviated title: β-amyloid PET signal source

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ABSTRACT

Objective: β-amyloid PET (Aβ-PET) is an important tool for quantification of amyloidosis in the brain of suspected Alzheimer’s disease (AD) patients and transgenic AD mouse models. Despite the excellent correlation of Aβ-PET with gold standard immunohistochemical assessments, the relative contributions of fibrillar and non-fibrillar Aβ components to the in vivo Aβ-PET signal remain unclear. Thus, we obtained two murine cerebral amyloidosis models that present with distinct Aβ plaque compositions and performed regression analysis between immunohistochemistry and Aβ PET to determine the biochemical contributions to Aβ-PET signal in vivo. Methods: We investigated groups of AppNL-G-F and APPPS1 mice at three, six and 12 months of age by longitudinal 18F-florbetaben Aβ-PET and with immunohistochemical analysis of the fibrillar and total Aβ burdens. We then applied group level inter-modality regression models using age and genotype matched sets of fibrillar/ non-fibrillar Aβ data (predictors) and Aβ-PET results (outcome) for both transgenic models. An independent group of double-hit APPPS1 mice with dysfunctional microglia due to knock-out of triggering receptor expression on myeloid cells 2 (Trem2−/−) served for validation and evaluation of translational impact. Results: Neither fibrillar nor non-fibrillar Aβ content alone sufficed to explain the Aβ-PET findings in either transgenic AD model. However, a regression model compiling fibrillar and non-fibrillar Aβ together with the estimate of individual heterogeneity and age at scanning could explain a 93% of variance of the Aβ-PET signal (P<0.001). Fibrillar Aβ burden had a 16-fold higher contribution to the Aβ-PET signal when compared to non-fibrillar Aβ. However, given the relatively greater abundance of non-fibrillar Aβ, we estimate that non-fibrillar Aβ produced 79±25% of the net in vivo Aβ-PET signal in AppNL-G-F mice, and 25±12% in the APPPS1 mice. Corresponding results in separate groups of APPPS1/Trem2−/− and APPPS1/Trem2+/+ mice validated the calculated regression factors and revealed that the altered fibrillarity due to Trem2 knockout impacts the Aβ-PET signal. Conclusions: Taken together, the in vivo Aβ-PET signal derives from the composite of fibrillar and non-fibrillar Aβ plaque components. While fibrillar Aβ has inherently higher PET tracer binding,
the greater abundance of non-fibrillar Aβ plaque in AD model mice contributes importantly to the PET signal.

**Key words:** amyloid, fibrillar, non-fibrillar, PET signal, mouse
INTRODUCTION

Positron emission tomography for β-amyloid (Aβ-PET) is now widely used for identification and quantification of amyloidosis in the brain of suspected Alzheimer’s disease (AD) patients (1), and has been incorporated into the current research framework for diagnostic recommendations in AD (2). Here, the Aβ status (A) identified by PET serves for diagnosis, together with biomarkers for tau (T) and neuronal injury (N) (2). Furthermore, Aβ-PET is used as an inclusion criteria of anti-amyloid immunotherapy clinical trials (3), and as a progression biomarker for therapy evaluation in these trials (4). In the preclinical setting, Aβ-PET has also become a useful tool for the dynamic assessment of neuropathology in transgenic Aβ mouse models (5,6). Despite the excellent correlation of Aβ-PET with immunohistochemical gold standard assessments of amyloidosis in patients (7,8) and mouse models of AD (6,9), there has remained an uncertainty about the relative contributions of fibrillar and non-fibrillar Aβ components in plaques to the Aβ-PET signal in vivo. This research gap needs to be closed as the two forms have differing neurotoxicity, and there is evidence that alterations in AD-related genes like triggering receptor expressed on myeloid cells 2 (TREM2) and Apolipoprotein E (APOE) alter the net Aβ plaque fibrillarity, which would consecutively bias the relationship between plaque density with Aβ-PET binding in vivo (5).

A human autopsy validated 18F-florbetaben PET study showed preliminary evidence that diffuse plaques may make only a minor contribution to the net Aβ-PET signal (10). However, autopsy controlled data with 18F-flutemetamol in vivo (11) and comprehensive in vitro data (12) indicated that the binding of that structurally distinct tracer to diffuse plaques also contributes to the net PET signal. Furthermore, our recent preclinical study revealed a discernible Aβ-PET signal in AppNL-G-F mice (13), although this model displays only very limited fibrillar Aβ pathology (14). Therefore, we aimed to quantify the contributions of fibrillar and non-fibrillar plaque components to the Aβ-PET signal in vivo in AD model mice.
We recently demonstrated that the \( \text{App}^{NL-G-F} \) and APPPS1 mouse models exhibit differences in \( \text{A}\beta \) plaque fibrillarity \((14)\), such that a comparative study of these mice could help to determine the effect of fibrillarity on \( \text{A}\beta \)-PET signal \textit{in vivo}. Thus, we combined a standardized preclinical \( ^{18}\text{F-florbetaben} \) PET study with immunohistochemical characterization of fibrillar vs. non-fibrillar \( \text{A}\beta \) in \( \text{App}^{NL-G-F} \) and APPPS1 mice examined at different pathological stages. We then developed a regression model for immunohistochemistry and \( \text{A}\beta \)-PET to establish the relative proportions of fibrillar and non-fibrillar sources in the \( \text{A}\beta \)-PET signal \textit{in vivo}. Furthermore, we validated the calculated regression factors in an independent cohort APPPS1/Trem2\(^{-/-}\) and APPPS1/Trem2\(^{+/-}\) mice and tested a hypothesis that the non-fibrillar \( \text{A}\beta \) pool contributes more to the \( \text{A}\beta \)-PET signal in APPPS1/Trem2\(^{-/-}\) mice than in APPPS1/Trem2\(^{+/-}\) mice.

**MATERIAL AND METHODS**

**Experimental Design**

All experiments were performed in compliance with the National Guidelines for Animal Protection, Germany and with the approval of the regional animal committee (Regierung Oberbayern), and were overseen by a veterinarian. Animals were housed in a temperature- and humidity-controlled environment with 12 h light-dark cycle, and with free access to food (Sniff, Soest, Germany) and water. We conducted longitudinal \( ^{18}\text{F-florbetaben} \) PET imaging in cohorts of female \( \text{App}^{NL-G-F} \) (n=18) and APPPS1 (n=14) mice \( \text{A}\beta \)-PET at three, six and 12 months of age, together with an age- and sex-matched group of wild-type (n=8) mice. 56\% of the transgenic mice had their baseline examination at three months of age, and the remaining 44\% of the mice were imaged from six to 12 months of age. All mice of each model originated from the same breeding colony. To exclude batch effects within each modality, we used separate cohorts of mice \((14)\) for immunohistochemistry analyses of fibrillar and non-fibrillar \( \text{A}\beta \) plaque components in wildtype and AD model mice (n=3-4) at three, six, and 12 months of age. We then applied inter-modality
regression models to separate the relative contributions of fibrillar and non-fibrillar Aβ plaque components to Aβ-PET signals in the two transgenic strains.

Animal Models

APP/PS1 (APPPS1-21) mice show extensive fibrillar Aβ plaque pathology, first evident at 6–8 weeks of age (15). In contrast, AppNL-G-F(AppNL-G-F/NL-G-F) is a murine model with relatively limited fibrillar Aβ plaque pathology, but showing Aβ PET signal from eight weeks of age in homozygous mice (16,17). Wild-type controls were C57BL/6 mice.

PET Imaging

PET Data Acquisition, Reconstruction and Post-Processing: For all PET procedures, radiochemistry, data acquisition, and image pre-processing were conducted according to an established, standardized protocol (6). In brief, we obtained 18F-florbetaben Aβ-PET recordings (average dose: 12.1±1.8 MBq) with an emission window of 30–60 min after injection.

PET Image Analysis: We performed all analyses using PMOD (version 3.5; PMOD technologies). Normalization of attenuation-corrected emission images to standardized uptake value ratio (SUVr) images was performed using previously validated periaqueductal gray (PAG) (18) and white matter (WM) reference regions for the AppNL-G-F and APPPS1 mouse models, respectively (5). We analyzed the wild-type mice separately with both reference regions to serve as controls for the transgenic models. Bilateral neocortical volumes of interest (15 mm3) matching the region of interest in the immunohistochemistry analysis were applied for calculation of SUVrForebrainWM or SUVrForebrainPAG.
Immunohistochemical Analysis

Groups of APPPS1 and App^{NL-G-F} mice at an age of three months (n=4), six months (n=3) and 12 months (n=4) were transcardially perfused with ice cold PBS (0.1 M) followed by 4% PFA, after cryopreserved in 30% sucrose. The mouse tissue used for immunohistochemical analysis included some of the APPPS1 and App^{NL-G-F} mouse brains utilized in our previous publication (14). All stainings and analyses were performed newly for the purpose of the present study. We collected 30 µm-thick coronal sections for free-floating immunostaining. We used the 3552 antibody (1:5000; (19)) to label total Aβ and Thiazine red (2 µM, Sigma) to stain the fibrillar Aβ. 24 images were acquired in four coronal sections (6 images per section) in regions matching PET using a confocal microscope (20x dry objective, Leica TCS SP5). Given the prominent differences in the levels of fibrillar Aβ between the APPPS1 and App^{NL-G-F} mice, the confocal settings were optimized for each mouse model to acquire the Thiazine red signal. For the three- and six-month-old App^{NL-G-F} mice, the “averaging” and “accumulation” confocal functions were set to “2”, to better detect the Thiazine red signal. An in-house programmed macro from ImageJ (NIH) was used to analyze the total and fibrillar Aβ coverage.

As a validation analysis, we reanalyzed data from a previous study that included immunohistochemistry markers for fibrillar (x-34) and total Aβ (3552) components of Aβ plaques (5). Immunohistochemistry was obtained from APPPS1xTrem2^{+/+} and APPPS1xTrem2^{−/−} mice (three and six months: n=4, 12 months: n=8). Aβ-PET data were analyzed by the processing pipeline described above and at the same time-points for both genotypes (APPPS1xTrem2^{+/+}: three months: n=3, six and 12 months: n=10; APPPS1xTrem2^{−/−}: three months: n=7, six months: n=9, 12 months: n=7). Furthermore, for validation purpose we obtained Aβ coverage for fibrillar (methoxy-x04 or x-34) and total Aβ (3552) components at 13 months of age in the PET cohorts. In all datasets, non-fibrillar Aβ was calculated by subtraction of fibrillar Aβ from total Aβ (area-_%non-fibrillar = area-_%total − area-_%fibrillar).
Statistics

Graph Pad Prism (Version 8.43, GraphPad Software, LCC) was used for all statistical tests. A $P$-value of less than 0.05 was considered to be significant for rejection of the null hypothesis.

Group Level Analysis: Non-fibrillar Aβ, fibrillar Aβ, and the Aβ-PET z-score were compared between $App^{NL-G-F}$ and APPPS1 mice at different ages by an unpaired Student's t-test. Mean values of each of the three read-outs from the $App^{NL-G-F}$ and APPPS1 groups at different ages were subject to a linear regression analysis. The area between the regression plots served as an index of the potential bias in the estimates of Aβ pathology by Aβ-PET.

Individual Level Analysis: We applied regression models using the Aβ-PET z-score of all investigated mice of both models as an outcome variable. Fibrillar Aβ and non-fibrillar Aβ estimates deriving from all age and genotype-matched mouse groups were used as predictors, and heterogeneity of individual mice with respect to PET results and age were used as additional covariates. Here, we defined heterogeneity as the deviation of individual mice of each genotype from their group mean at each time-point. The regression coefficients for fibrillar Aβ and non-fibrillar Aβ were extracted to calculate their relative contributions to the Aβ-PET signal. Bootstrapping was performed with 1000 random samples.

Validation Analysis: The derived regression coefficients were applied to immunohistochemistry analysis of independent samples of APPPS1/Trem2$^{+/+}$ and APPPS1/Trem2$^{-/-}$ mice. The predicted Aβ-PET z-scores were compared with the actual Aβ-PET z-scores in vivo, and the deviation between the predicted and actual Aβ-PET z-scores was compared with separate consideration of both plaque components and sole consideration of fibrillar Aβ. The bias resulting from consideration only of fibrillar Aβ was calculated as a function of longitudinal changes in the Aβ-PET signal in the contrast of APPPS1/Trem2$^{+/+}$ and APPPS1/Trem2$^{-/-}$ mice.
RESULTS

Separate Quantification of Fibrillar or Non-fibrillar Aβ Plaque Deposition Fails to Explain the Aβ-PET Signal

First, we performed a direct standardized comparison of non-fibrillar and fibrillar Aβ estimates by immunohistochemistry and Aβ-PET between AppNL-G-F and APPPS1 mouse models at different ages. Non-fibrillar Aβ area coverage of AppNL-G-F mice exceeded that of APPPS1 mice at three and six months of age, whereas APPPS1 mice had higher non-fibrillar Aβ area coverage at 12 months of age (Fig. 1A; Fig. 2). Fibrillar Aβ area coverage was significantly higher in APPPS1 mice than in AppNL-G-F mice at all ages studied (Fig. 1B; Fig. 2). Immunohistochemically assessed area coverage values did not differ between the immunohistochemistry cohorts and the PET cohorts at 12/13 months of age (all P>0.05, Supplemental Fig. 1). Aβ-PET z-scores of AppNL-G-F and APPPS1 mice are provided and illustrated in Figs. 1C,D. There were no interindividual SUVr differences between mice imaged three times at 3, 6 and 12 months when compared to mice only imaged twice at 6 and 12 months (all P >0.05, Supplemental Fig. 2). Aβ-PET showed significantly higher standardized differences in APPPS1 mice when compared to AppNL-G-F mice at six and 12 months, whereas there were no significant differences at three months of age (Fig. 1C). Plotting of Aβ-PET results as a linear function of non-fibrillar or fibrillar Aβ at different ages indicated a mismatch between the two mouse models (Fig. 1E). Plotting fibrillar Aβ as a linear function of non-fibrillar Aβ coverage underpinned that APPPS1 mice had a higher proportion of fibrillar Aβ compared to AppNL-G-F (Fig. 1E). The comparison of the linear functions of both mouse models (the area transected by the regression lines) indicated that Aβ-PET underestimated the proportion of non-fibrillar Aβ in AppNL-G-F mice (-2.08 z-score units), but overestimated the proportion of fibrillar Aβ in AppNL-G-F mice (+2.36 z-score units). Thus, neither fibrillar nor non-fibrillar Aβ alone could explain the combined Aβ-PET findings.
Non-fibrillar Aβ Contributes Significantly to the Aβ-PET Signal

Next, we hypothesized that a combined model of non-fibrillar and fibrillar Aβ components could improve the explanation of variance in the Aβ-PET signals. To test this, we established a multiple regression model using all available combinations of age and genotype matched Aβ-PET-immuno/histochemistry estimates with inclusion of all AppNL-G-F and APPPS1 mice.

Simplified regression models with either fibrillar or non-fibrillar Aβ as predictors of the Aβ-PET z-score explained 50% and 32% of the variance in Aβ-PET, respectively (both $P<0.001$, Table 1). Combined consideration of fibrillar Aβ and non-fibrillar Aβ as predictors of the Aβ-PET z-score increased the explained variance to 57% ($P<0.001$, Table 1, Fig. 3A), and fibrillar ($\beta=0.563$, $P=1.11e^{-27}$) and non-fibrillar Aβ ($\beta=0.309$, $P=9.38e^{-11}$) were both strong and independent predictors of the Aβ-PET z-score.

A model including fibrillar and non-fibrillar Aβ components with the estimate of individual heterogeneity yielded 85% explanation of variance of the Aβ-PET signal (Fig. 3B), and further inclusion of age further increased the explanation of variance of the Aβ-PET signal (93%, Fig. 3C). Thus, age-related factors influence importantly immuno/histochemistry and PET signals (i.e. age-dependent perfusion or partial volume effects). We considered “fibrillar x non-fibrillar x heterogeneity” and “fibrillar x non-fibrillar x heterogeneity x age” to be the most accurate models and we calculated the mean regression coefficients from these two models to obtain the contributions of fibrillar Aβ and non-fibrillar Aβ to the PET signal. One percent area covered by fibrillar Aβ explained 3.17 PET z-score units and one percent area covered by non-fibrillar Aβ explained 0.20 PET z-score units, thus indicating a 16-fold higher contribution of fibrillar compared to non-fibrillar Aβ. The opposite edges of the 95% confidence intervals, as assessed by bootstrapping, indicated a possible range between 11-fold and 26-fold for the relationship between fibrillary and non-fibrillar contributions to the Aβ-PET signal. Application of this multiplicative factors to the direct comparison of group averaged immuno/histochemistry and Aβ-PET scores in
App\textsuperscript{NL-G-F} and APPPS1 mice at different ages confirmed the suitability of this model, as indicated by 98% explanation of the variance using weighted factors, compared to only 84% for isolated fibrillar and 55% for non-fibrillar plaque components (Fig. 3D).

**Impact of Fibrillar and Non-fibrillar Plaque Components in Mice with Dysfunctional Microglia**

Finally, we validated our results in independent cohorts of APPPS1 mice, and made an additional investigation of the impact of Trem2 deficiency on the A\textsubscript{\beta}-PET signal in these mice, given that Trem2 is a known driver of changes in the plaque fibrillarity. Application of the regression factors to immuno/histochemistry data indicated an excellent prediction of the actual A\textsubscript{\beta}-PET signal in independent cohorts of APPPS1/Trem2\textsuperscript{-/-} and APPPS1/Trem2\textsuperscript{+/+} mice (Fig. 4A). APPPS1 mice with Trem2 loss of function showed a higher contribution of non-fibrillar parts plaque components to the A\textsubscript{\beta}-PET signal (30% at three months, 26% at six months, and 24% at 12 months) when compared to APPPS1 mice with intact Trem2 (4% at three months, 15% at six months, and 21% at 12 months; Fig. 4A). A combined consideration of fibrillar A\textsubscript{\beta} and non-fibrillar A\textsubscript{\beta} predicted the actual PET signal more precisely that did sole consideration of fibrillar A\textsubscript{\beta} (Fig. 4B). Previously calculated increases in A\textsubscript{\beta}-PET signal with age in these mice indicated a considerable bias when considering only the fibrillar A\textsubscript{\beta} component (Supplemental Fig. 3). In summary, microglial dysfunction altered the relative proportions of fibrillar and non-fibrillar A\textsubscript{\beta}, thus directly influencing the A\textsubscript{\beta}-PET signal as a function of mouse age.

**DISCUSSION**

We provide the first *in vivo* analysis to elucidate the contributions of fibrillar and non-fibrillar plaque components to the A\textsubscript{\beta}-PET signal. Our data clearly show that non-fibrillar plaque fractions
have a significant impact on the net $^{18}$F-florbetaben binding to Aβ plaques of Aβ mouse models in vivo. Although the resulting Aβ-PET tracer signal is inherently 16-fold higher when comparing equal amounts of fibrillar and non-fibrillar Aβ, the larger proportions of non-fibrillar plaque components counterbalance the net contribution. We validated our regression model in an independent cohort of APPPS1 mice, and extended the potential translational impact of our findings by showing that microglial dysfunction can influence the longitudinal Aβ-PET signal via changing the relative proportions of fibrillar to non-fibrillar plaque components.

In various analyses of single amyloidosis mouse models, there was a strong agreement between Aβ-PET and different immuno/histochemistry markers for Aβ (9,20). It is widely acknowledged that the Aβ-PET signal with $^{18}$F-florbetaben primarily derives from aggregated fibrillar Aβ, since this and other clinically approved Aβ-PET tracers were derived from the chemical scaffold of thioflavin-T, which only colors fibrillar Aβ (21). However, a recent small animal Aβ-PET investigation from our lab (18) indicated that there could be discrepancies between immuno/histochemistry and in vivo Aβ-PET signals, if one attributes the entire PET signal to fibrillar Aβ. In fact, App$^{NL-G-F}$ mice exhibited an only moderate Aβ-PET signal, although their plaques were mainly composed of non-fibrillar Aβ (14). Therefore, we applied in this study a standardized $^{18}$F-florbetaben PET examination comparing App$^{NL-G-F}$ and APPPS1 mice in conjunction with combined histochemical and immunohistochemical examination to elucidate the separate contributions of fibrillar and non-fibrillar Aβ sources to the in vivo Aβ-PET signal. We performed PET acquisitions in both Aβ models and wild-type mice with identical housing conditions and using the same tomograph and image reconstruction parameters, thus minimizing the potential methodological bias. Nonetheless, we acknowledge that scanning of mice on different days of the week and social hierarchy factors, along with technical factors due to different cage positions might still impact the detection of plaque pathology by Aβ-PET. We had to choose between conducting longitudinal PET examination and immuno/histochemistry analysis in separate groups (cross-sectional design), rather than conducting the study in which PET
examination directly preceded immunohistochemistry in the same mice. Since animal batch
effects may introduce a bias into cross-sectional PET quantifications between different ages of a
given mouse model, we elected to conduct longitudinal PET imaging together with cross sectional
immuno/histochemistry to exclude batch effects, at least for PET. To account further for the
heterogeneity (22) and asymmetry (13) of amyloidosis in individual mice of a lineage, we used a
bilateral target in a regression model including each available combination of PET-
immuno/histochemistry results for each model at each of three ages, controlled for the individual
heterogeneity. Regression coefficients for the proportions of fibrillar and non-fibrillar Aβ in different
models were robust, and revealed that fibrillar Aβ makes an intrinsically 16-fold higher contribution
to the Aβ-PET signal when compared to non-fibrillar Aβ in the studied Aβ mouse models. Our
preclinical in vivo results concur with the post-mortem validation of human 18F-flutemetamol PET
data, where ligand binding to diffuse plaques was the most likely explanation for positive in vivo
signals in patients who later proved to have only sparse neuritic plaques at autopsy (11,23). The
same research group recently validated the contributions of diffuse and neuritic plaques to the 18F-
flutemetamol and 11C-PiB autoradiography signals in vitro study (12). However, our study
comprises the first translation of such findings into the in vivo setting, and enables the arithmetic
conversion of Aβ-PET signals into fibrillar and non-fibrillar Aβ components. We note that structure
of the stilbene 18F-florbetaben is different to the structures of the benzothiazoles 18F-flutemetamol
and 11C-PiB, which could result in different proportions of fibrillar and non-fibrillar binding
capacities in vivo. As usual, the limited resolution of small animal PET systems in relation to the
mouse brain size and resulting partial volume effects present a limitation for the transfer of present
findings into the human context and we want to emphasize that detailed regression factors cannot
be transferred directly. Yet, the demonstration of an inherently 16-fold higher contribution of fibrillar
plaque to the PET signal is concurs with a biophysical chemistry study investigating binding
mechanisms of Aβ ligands by molecular docking, molecular dynamics and generalized Born-
based free energy calculations (24). Here, core sites of Aβ fibrils, which are more abundant in
The Aβ-PET signal with \(^{18}\text{F}\)-florbetaben \textit{in vivo} arises from a combination of fibrillar and non-fibrillar plaque components. Fibrillar Aβ has inherently higher tracer binding, but the greater proportion of non-fibrillar Aβ relative to fibrillar Aβ in most plaques mean that the non-fibrillar signal
source is a relevant component of the total signal. Since experimental AD therapy regimens can shift the proportion of fibrillar vs. non-fibrillar Aβ, any longitudinal changes in Aβ-PET signal as a read-out of therapy monitoring must be interpreted with caution; a detailed understanding of the biochemical basis of Aβ-PET signal is critical for the correct use of PET for monitoring novel AD therapies.

DISCLOSURE

C.H. collaborates with Denali Therapeutics, participated on one advisory board meeting of Biogen, and received a speaker honorarium from Novartis and Roche. C.H. is chief advisor of ISAR Bioscience. P.B., A.R. and M.B. received speaking honoraria from Life Molecular Imaging and GE healthcare. M.B. is an advisor of Life Molecular Imaging. No other potential conflicts of interest relevant to this article exist.

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KEY POINTS

QUESTION: Does non-fibrillar β-amyloid (Aβ) contribute to the in vivo Aβ-PET signal?

PERTINENT FINDINGS: Fibrillar Aβ has 16-fold higher tracer binding, but the greater proportion of non-fibrillar Aβ relative to fibrillar Aβ in most plaques mean that the non-fibrillar signal source is a relevant component of the total Aβ-PET signal.

TRANSLATIONAL IMPLICATIONS: Shifts of the proportion of fibrillar vs. non-fibrillar Aβ need to be considered when interpreting the longitudinal Aβ-PET signal for monitoring of therapeutic effects.
REFERENCES


Figure 1: Quantitation of non-fibrillar Aβ (A), fibrillar Aβ (B), and the Aβ-PET signal z-scores (C) in the neocortex of AppNL-G-F and APPPS1 mice at three, six and 12 months of age, together with (D) axial images of group-wise PET z-scores projected upon a MRI standard template. (E) Correlation plots of the associations between immuno/histochemistry markers and PET at different ages (group level) in the contrast of both mouse models. *P<0.05; **P<0.01; *** P<0.001.
Figure 2: Representative images of immuno/histochemistry. Total Aβ was assessed by 3552 staining and fibrillar Aβ was assessed by Thiazine red. Hoechst (HOE, blue) was used for nuclear visualization. Scale bars: 500 µm.
Figure 3: (A-C) Regression plots illustrate the correlation between the actual and the predicted Aβ-PET z-score when using fibrillar Aβ and non-fibrillar Aβ as predictors, and individual heterogeneity and age as covariates. Regressions were calculated with a total of n=261 permutations between immuno/histochemistry and PET endpoints using all available combinations with matched age and genotype. (D) Application of the average regression factors for fibrillar (B=3.17) and non-fibrillar Aβ (B=0.20) on the combined immuno/histochemistry data of both models (group means per age).
Figure 4: (A) Z-scores of the measured Aβ-PET signal (red) and the predicted fibrillar (orange) and non-fibrillar (blue) source components in an independent cohort of APPPS1 mice with dysfunctional (Trem2−/−) and intact (Trem2+/+) microglia. Pie charts show the fibrillar and non-fibrillar contributions to the measured Aβ-PET signals predicted by immuno/histochemistry. A representative double staining of a APPPS1/Trem2−/− mouse shows more non-fibrillar Aβ (3552-positive, blue) surrounding the core (x-34-positive, yellow/orange) when compared to a APPPS1/Trem2+/+ mouse, both at three months of age. (B) Bias of the predicted z-scores, when only fibrillar or the combination of fibrillar and non-fibrillar plaque contributions were considered. The analysis displays the deviation of predicted values and the actual Aβ-PET signal in part B at the three different ages.
<table>
<thead>
<tr>
<th>Model</th>
<th>$R^2$</th>
<th>$R^2$ (corr.)</th>
<th>Reg. coeff Fibrillar (B)</th>
<th>Reg. coeff Non-fibrillar (B)</th>
<th>$P$-value</th>
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<tr>
<td>Fibrillar</td>
<td>0.499</td>
<td>0.497</td>
<td>4.414 (3.915-4.926)</td>
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<td>$P&lt;0.001$</td>
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<tr>
<td>Non-fibrillar</td>
<td>0.324</td>
<td>0.322</td>
<td></td>
<td>0.477 (0.378-0.569)</td>
<td>$P&lt;0.001$</td>
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<td>Fibrillar x non-fibrillar</td>
<td>0.574</td>
<td>0.571</td>
<td>3.521 (2.954-4.041)</td>
<td>0.259 (0.178-0.341)</td>
<td>$P&lt;0.001$</td>
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<tr>
<td>Fibrillar x non-fibrillar x heterogeneity</td>
<td>0.854</td>
<td>0.852</td>
<td>3.521 (2.954-4.041)</td>
<td>0.259 (0.178-0.341)</td>
<td>$P&lt;0.001$</td>
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<tr>
<td>Fibrillar x non-fibrillar x heterogeneity x age</td>
<td>0.928</td>
<td>0.927</td>
<td>2.810 (2.620-3.032)</td>
<td>0.146 (0.103-0.193)</td>
<td>$P&lt;0.001$</td>
</tr>
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Table 1: Coefficients of determination ($R^2$) and regression coefficients (B) are given for prediction of the Aβ-PET signal by fibrillar and non-fibrillar Aβ components, with additional factoring for heterogeneity (in mice with equal genotype at a single time-point) and age. Numbers in brackets represent the 95%-confidence interval as assessed by bootstrapping with 1000 random samples.
Supplement

Supplemental Figure 1: Comparison of Aβ area coverage of the immunohistochemistry (IHC) cohort and the PET cohort for both Aβ mouse models. Note that different histology markers were used for assessment of fibrillar Aβ (Thiazine red, x-34, methoxy-x04). Non-fibrillar Aβ was consistently assessed by 3552. P-values derive from a Student’s t-test. Error bars represent SD.
Supplemental Figure 2: (A) PET SUVr quantification in Aβ mouse models and wild-type (WT) mice. Previously validated model-specific periaqueductal grey and white matter reference tissues were used for SUVr calculation. Z-score calculation of these SUVr values (TG – WTmean / WTSD) enabled the direct comparison of both Aβ mouse models in the regression models of the main manuscript. (B) Comparison of Aβ-PET standardized uptake value ratios (SUVr) between mice with baseline examination at 3 months compared to mice with baseline examination at 6 months. P-values derive from a Student’s t-test. **P<0.01; ***P<0.001. Error bars represent SD.
**Supplemental Figure 3:** Bias of Aβ-PET rate of change when fibrillar Aβ is considered solely. Fits represent polynomic functions of the bias in longitudinal changes of Aβ-PET in APPPS1/Trem2^{−/−} mice (purple) in contrast to APPPS1/Trem2^{+/−} mice (grey). Dotted lines represent functions of s.e.m. The plot was created analogous to (Parhizkar, Arzberger et al. 2019).