

# Amyloid-beta Uptake by Peripheral Blood Monocytes is Reduced by Ageing and Alzheimer's Disease

**Si-Han Chen**

Third Military Medical University Daping Hospital and Research Institute of Surgery

**Ding-Yuan Tian**

Third Military Medical University Daping Hospital and Research Institute of Surgery

**Ying-Ying Shen**

Third Military Medical University Daping Hospital and Research Institute of Surgery

**Yuan Cheng**

Third Military Medical University Daping Hospital and Research Institute of Surgery

**Dong-Yu Fan**

Third Military Medical University Daping Hospital and Research Institute of Surgery

**Hao-Lun Sun**

Third Military Medical University Daping Hospital and Research Institute of Surgery

**Chen-Yang He**

Third Military Medical University Daping Hospital and Research Institute of Surgery

**Pu-Yang Sun**

Third Military Medical University Daping Hospital and Research Institute of Surgery

**Xian-Le Bu**

Third Military Medical University Daping Hospital and Research Institute of Surgery

**Fan Zeng**

Third Military Medical University Daping Hospital and Research Institute of Surgery

**Juan Deng**

Third Military Medical University Daping Hospital and Research Institute of Surgery

**Zhi-Qiang Xu**

Third Military Medical University Daping Hospital and Research Institute of Surgery

**Yang Chen**

Third Military Medical University Daping Hospital and Research Institute of Surgery

**Yanjiang Wang** (✉ [yanjiang\\_wang@tmmu.edu.cn](mailto:yanjiang_wang@tmmu.edu.cn))

Department of Neurology and Center for Clinical Neuroscience, Daping Hospital, Third Military Medical University, Chongqing 400042, China <https://orcid.org/0000-0002-6227-6112>

**Keywords:** Alzheimer's disease, ageing, A $\beta$  uptake, innate immunity, monocyte

**Posted Date:** June 25th, 2020

**DOI:** <https://doi.org/10.21203/rs.3.rs-37808/v1>

**License:**  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

---

# Abstract

## Background

Deficits in the clearance of amyloid  $\beta$ -protein ( $A\beta$ ) play a pivotal role in the pathogenesis of sporadic Alzheimer's disease (AD). The roles of blood monocytes, the counterparts of microglia in the periphery, in the development of AD remain unclear. In this study, we sought to investigate the alterations in the  $A\beta$  phagocytosis function of peripheral monocytes during ageing and in AD patients.

## Methods:

A total of 104 cognitively normal participants aged 22 to 89 years old, 22 AD patients, 22 age- and sex-matched cognitively normal (CN) subjects, 15 Parkinson's disease patients (PD) and 15 age- and sex-matched CN subjects were recruited. The  $A\beta$  uptake by blood monocytes were measured and its alteration during ageing and in AD were investigated.

## Results

$A\beta_{1-42}$  uptake by monocytes was associated with  $A\beta_{1-42}$  levels in the blood.  $A\beta_{1-42}$  uptake by monocytes decreased during ageing, and further decreased in AD but not in PD patients. Among the  $A\beta$  uptake-related receptors and enzymes, the expression of Toll-like receptor 2 (TLR2) was reduced in monocytes from AD patients.

## Conclusion

Our findings suggest that monocytes regulate the blood levels of  $A\beta$  and might be involved in the development of AD. The recovery of the  $A\beta$  clearance function by blood monocytes represents a potential therapeutic strategy for AD.

## Background

Alzheimer's disease (AD) is the most common neurodegenerative disorder affecting 35 million elderly individuals (1). Its mechanism remains unclear, and no disease-modifying therapies are currently available. A large amount of evidence suggests that deficit in the clearance of amyloid  $\beta$ -protein ( $A\beta$ ), which leads to the cerebral accumulation of  $A\beta$ , plays a pivotal role in the development of sporadic AD (2).

Recent studies show that a series of AD risk gene mutations are associated with immune responses and endocytosis, suggesting that dysfunctional innate immunity, mainly involving microglia and peripheral myeloid cells, is a critical reason for AD (3–7). Indeed, studies have suggested that the reduced  $A\beta$  uptake capacity of microglia in the brain is a major mechanism underlying the development of AD (8, 9). However, the alterations in the functions and roles of peripheral myeloid cells in AD remains unclear.

While resident microglia play a key role in the clearance of A $\beta$  in the brain, approximately 40–60% of A $\beta$  from the brain is estimated to diffuse into the blood and be cleared in the periphery, indicating that the peripheral system also plays an essential role in clearing A $\beta$  from the brain(10–12). It remains undetermined how this brain-derived A $\beta$  is cleared in the periphery. Blood monocytes are the counterparts of microglia in the periphery. Some studies have demonstrated that monocytes are more effective at neuroprotection, neuroinflammation regulation and A $\beta$  clearance than microglia in AD (13–15). In addition, the depletion of blood monocytes exacerbates A $\beta$  accumulation in transgenic AD models (16, 17). Therefore, monocytes might play a critical role in the clearance of brain-derived A $\beta$  in the periphery.

In the present study, we aimed to investigate alterations in A $\beta$  uptake by peripheral monocytes during ageing in cognitively normal subjects and in sporadic AD patients and to evaluate the role of peripheral monocytes in A $\beta$  clearance.

## Materials And Methods

### Study subjects

A total of 104 cognitively normal participants aged 22 to 89 years old, 22 AD patients, 22 age- and sex-matched cognitively normal (CN) subjects, 15 Parkinson's disease patients (PD) and 15 age- and sex-matched CN subjects were recruited from Chongqing Daping Hospital between March 2017 and May 2019. Another 25 CN subjects who received epidural anaesthesia were enrolled for CSF collection.

Subjects were not eligible if they had a family history of dementia; had a concomitant neurologic disorder except for AD and PD; were in a state of obvious infection or inflammation potentially affecting the status of blood cells; had severe cardiac, pulmonary, hepatic, renal diseases, or any kinds of tumour; had any potent haematopathy, including acute monocytic leukaemia and myelodysplastic syndrome, during the recovery period of agranulocytosis; had autoimmune diseases, including rheumatoid arthritis and systemic lupus erythematosus; had an endocrine system disease, including Cushing syndrome and thyroid disorders; and declined to participate in the study.

The study was approved by the ethics committee of Chongqing Daping Hospital. Written consent was obtained from all participants or their legal representatives.

### Clinical Assessment

The clinical evaluation was performed by following the protocol described in our previous studies (18). In brief, demographic data including age, sex, education level, and occupation were collected on admission. The medical history including current medications, prior head trauma and surgery, prior gas poisoning, schizophrenia, hypothyroidism, coronary heart diseases, atrial fibrillation, cerebrovascular diseases, chronic obstructive pulmonary disease, chronic hepatitis, chronic renal insufficiency, hypertension,

diabetes mellitus, hypercholesterolemia and regular use of non-steroidal anti-inflammatory or prescription drugs, was collected from the medical records and a formal questionnaire.

Cognitive status was assessed using a neuropsychological battery that included Minimum Mental State Examination (MMSE), Activities of Daily Living (ADL) and Montreal Cognitive Assessment (MoCA). The subjects with abnormal performance in MMSE or MoCA assessment were further scheduled for neuropsychological tests, including Clinical Dementia Rating (CDR), Pfeiffer Outpatient Disability Questionnaire (POD) and Hachinski Ischemic Score (HIS) for assessing significant vascular disease. Subjects with abnormal cognition were further subjected to a brain CT/MRI investigation and blood tests for thyroxine, vitamin B12, folic acid and HIV/syphilis to rule out metabolic and infectious reasons for cognitive decline.

Dementia was diagnosed based on DSM-IV criteria. The diagnosis of probable AD was made according to the criteria of the National Institute of Neurological and Communicative Diseases and Stroke and the Alzheimer Disease and Related Disorders Association (NINCDS-ADRDA). Idiopathic PD was diagnosed according to the Parkinson's Disease Society Brain Bank criteria (19).

## Blood And Csf Sampling

**Blood sampling.** To avoid possible circadian rhythm effects, the sampling conditions, including sampling timing and fasting state, were consistent among AD and PD patients and they were matched pairs. A portion of fasting blood samples was aliquoted for measuring complete blood cell counts, and fasting glucose, thyroxine, creatinine, urea, uric acid, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and total cholesterol levels. For another portion of blood, plasma was separated within 30 minutes after sampling and stored at  $-80^{\circ}\text{C}$  for the further analysis of  $\text{A}\beta$ . For  $\text{A}\beta$  uptake-related assay, the blood samples were applied for the isolation of peripheral blood mononuclear cells (PBMCs) within two hours after blood drawing.

**CSF sampling.** For a subgroup of patients who underwent urological surgery, fasting blood and cerebrospinal fluid (CSF) were sampled at the same time during subdural anaesthesia before surgery. The CSF samples were collected, free from blood contamination, in polypropylene tubes by lumbar puncture, centrifuged at  $1800 \times g$  at  $4^{\circ}\text{C}$  for 10 minutes within 1 hour after collection, and stored at  $-80^{\circ}\text{C}$  until analysis.

## Isolation Of Blood Monocytes

Heparinized blood was diluted with PBS (1:1 ratio; vol/vol). PBMCs were isolated by density gradient centrifugation using Ficoll-Hypaque, and mononuclear sections were collected and washed with PBS three times. A portion of the PBMCs was used for the  $\text{A}\beta$  uptake assay, and the other portion of PBMCs was used for monocyte isolation by CD14 Microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany) and

passed through a MACS column for the positive selection of CD14<sup>+</sup> cells, according to the manufacturer's instructions. The remaining PBMCs were frozen at a concentration of  $1-2 \times 10^6$  cells per ml in 10% DMSO (Sigma-Aldrich, Saint Louis, USA)/90% foetal calf serum (vol/vol Gibco, California, Australia) for future use.

## A $\beta$ Uptake Assay

Isolated PBMCs were resuspended in RPMI medium with 10% foetal calf serum and 1% penicillin/streptomycin and adjusted to a concentration of  $2 \times 10^6$  cells/mL. To test the uptake of A $\beta$ , PBMCs were incubated with FITC-A $\beta_{1-42}$  (2  $\mu$ g/mL) (GL Biochem, Shanghai, China) overnight at 37 °C in a 5% CO<sub>2</sub> incubator. Following incubation, the cell suspensions were discarded, and adherent cells were detached from the well plate by 0.25% trypsin and washed with fluorescence-activated cell sorting (FACS) buffer twice. Then, the cell suspensions were preincubated with Human TruStain FcX (Biolegend, CA, USA) on ice for 20 minutes to avoid producing a high background by the non-specific binding of the Fc receptor expressed on immune cells to the Fc fragment of the fluorophore-labelled antibody. Then, the cell suspensions were washed and stained with the following fluorophore-labelled antibodies according to the corresponding manufacturer's instructions (BD, NJ, USA): APC-anti human CD14, PE-anti human CD16, APC-mouse IgG2a,  $\kappa$  isotype control, PE-mouse IgG2a, and  $\kappa$  isotype control. Following incubation, the cells were washed twice with FACS buffer and fixed with 1% paraformaldehyde. Flow cytometry was performed on a NovoCyte Flow Cytometer (ACEA Biosciences, CA, USA) after appropriate compensation. Monocytes were gated using forward and side scatter, and monocyte subsets were identified by differential expression of CD14 and CD16, as indicated in (Figure 1 g, h). The data were analysed by NovoExpress software based on forward and side scatter and the mean fluorescence intensity (MFI). To maintain consistent testing conditions, a gating strategy was designed and applied equivalently across all study samples.

## Imaging Flow Cytometry

Imaging flow cytometry (IFC) was performed according to a previous report(20). In brief, the procedures for labelling surface markers were the same as those used for conventional flow cytometry, which is described above. IFC was performed on a two-camera ISX with INSPIRE acquisition software (Amnis, NJ, USA). Excitation lasers used for analysis included a 5 mW 405 nm, a 100 mW 488 nm and a 150 mW 642 nm. A 2.5 mW 785 nm laser was used for internal calibration to provide a scatter signal and measure speed beads. FITC and PE were excited by the 488 nm laser, and the emission was captured in the ranges of 505–560 nm (Ch02) and 560–595 nm (Ch03). APC was excited by the 647 nm laser, and the emission was captured in the wavelength range of 642–745 nm (Ch05). In total, 25,000 events were acquired, and all images were captured with the 20  $\times$  objective and a cell classifier (threshold) applied to the bright field channel (Ch01) to exclude small particles (Figure. 1a, b). Monocytes were identified using Amnis IDEAS

software. Cells with high-intensity labelling of the CD14 marker were chosen as monocytes (Figure. 1c-R3).

## A $\beta$ Assay

Plasma and cerebrospinal fluid A $\beta_{1-42}$  levels were measured using an ultra-sensitive single molecule array (SIMOA) on the Simoa HD-1 Analyzer (Quanterix, Lexington, Massachusetts), as previously described (21). SIMOA technique implied immunocapture of the target protein on magnetic beads, which are trapped in femto-liter volume wells, followed by the addition of enzyme-labelled detection antibody and accurate digital quantification. The high analytical sensitivity of this technique allows for pre-dilution of CSF and plasma samples, thus contributing to reducing matrix interferences. It has been widely used and been validated useful in numerous studies(22).

## Measurement Of A $\beta$ Uptake-related Receptors In Monocytes

The staining of A $\beta$  uptake-related receptors, including Toll-like receptor 2 (TLR2), triggering receptor expressed on myeloid cells 2 (TREM2), CD36, CD33, and macrophage scavenger receptor 1 (SCARA1), was performed by flow cytometry. Following CD14 positive selection by magnetic activated cell sorting,  $1.0 \times 10^5$  monocytes were preincubated with Human TruStain FcX (Biolegend, CA, USA) on ice for 20 minutes. For cell surface staining, cells were incubated with monoclonal antibodies against APC-anti human CD33, BB515-anti human CD282, Percp-CyTM5.5-anti human CD36, BV421-anti human MSR1 (Biolegend, CA, USA), and PE-anti human TREM2 for 15 minutes, washed via centrifugation twice and fixed with 1% paraformaldehyde. Cells were acquired on a FACS Navios (Beckman, CA, USA), and analyses were performed using FlowJo v10 software.

## Measurement Of A $\beta$ -degrading Enzymes In Monocytes

Western blotting was performed as previously described. After being thawed, PBMCs were washed in 10 ml of PBS. Cells were lysed in RIPA buffer. Samples (15–30  $\mu$ g) were subjected to electrophoresis on SDS-PAGE (8–12% acrylamide) gels. The blots were probed with antibodies against cathepsin D (1:1000, monoclonal, Arigobio), cathepsin S (1:1000, monoclonal, Arigobio), and  $\beta$ -actin (1:1000, monoclonal, Sigma-Aldrich). The protein bands were scanned using Odyssey scanner software (Li-COR Bioscience, CA, USA) and quantified by Quantity One 6.0. The band density was normalized to that of  $\beta$ -actin in the same sample.

## Statistical analysis

For each statistical analysis, appropriate tests were selected based on whether or not the data were normally distributed. Differences in demographic characteristics were assessed by the Chi-square test. Statistical comparisons between two groups were made by using a paired *t* test, the Wilcoxon matched-pairs test or an unpaired *t* test, where appropriate. Specifically, a Spearman correlation or covariate correlation analysis was utilized to analyse the association of A $\beta$ <sub>1-42</sub> uptake with ageing or A $\beta$ <sub>1-42</sub>, A $\beta$ <sub>1-40</sub>, A $\beta$ <sub>1-42</sub>/ A $\beta$ <sub>1-40</sub> levels in the plasma and CSF, respectively. The trajectory of A $\beta$ <sub>1-42</sub> uptake with age was modelled using Fit spline/ Lowess (cubic) spline. All statistical analyses were performed with GraphPad Prism v5.0 software. The data are expressed as the mean  $\pm$  SD, and significance was achieved at *p* < 0.05.

## Results

### A $\beta$ <sub>1-42</sub> uptake by monocyte subsets

In humans, peripheral monocytes can be divided into three subsets based on the expression of the cell-surface markers CD14 (a pattern recognition receptor for lipopolysaccharide) and CD16 (Fc $\gamma$  III receptor), including non-classic monocytes with low CD14 expression and high CD16 expression (CD14<sup>dim</sup>CD16<sup>+</sup> or CD14<sup>-</sup>CD16<sup>+</sup>); intermediate monocytes with high or intermediate CD14 and CD16 expression (CD14<sup>+</sup>CD16<sup>+</sup>); and classic monocytes with high CD14 expression but very low or negative CD16 expression (CD14<sup>+</sup>CD16<sup>-</sup>) (23). We found that all subsets of monocytes could intracellularly take up A $\beta$ <sub>1-42</sub> (Figure. 1d-e and Supplemental Figure. 1). The CD14<sup>+</sup>CD16<sup>+</sup> subset had the highest uptake of A $\beta$ <sub>1-42</sub> among the three subsets, with no significant difference in A $\beta$ <sub>1-42</sub> uptake between the CD14<sup>+</sup>CD16<sup>+</sup> and CD14<sup>dim</sup>CD16<sup>+</sup> subsets (Figure. 1i).

#### Correlation and trajectory of A $\beta$ <sub>1-42</sub> uptake by monocyte subsets relative to age

Then, we measured A $\beta$ <sub>1-42</sub> uptake by monocytes in 104 CN subjects aged 22 to 89 years with no differences in sex among the different age groups (Supplemental Table. 1). A $\beta$ <sub>1-42</sub> uptake by the total monocyte population was correlated with age; that is, the older the age was, the lower the A $\beta$ <sub>1-42</sub> uptake level ( $\gamma$  = -0.0.366, *P* = 0.0001). Among the three subsets, A $\beta$ <sub>1-42</sub> uptake was correlated with age in the CD14<sup>+</sup>CD16<sup>-</sup> ( $\gamma$  = -0.374, *P* < 0.0001) and CD14<sup>dim</sup>CD16<sup>+</sup> subsets ( $\gamma$  = -0.285, *P* = 0.003), but not in the CD14<sup>+</sup>CD16<sup>+</sup> ( $\gamma$  = -0.100, *P* = 0.311; Figure. 2a-d). A $\beta$ <sub>1-42</sub> uptake by total monocytes and the CD14<sup>+</sup>CD16<sup>-</sup> subset decreased rapidly in the 20–40 years of age group, but the reduction rate became relatively slow after 40 years of age. A $\beta$ <sub>1-42</sub> uptake by CD14<sup>dim</sup>CD16<sup>+</sup> subset decreased rapidly in the 40–60 years of age group, but the reduction rate became relatively slow after 60 years of age. This result suggests that the decrease in A $\beta$  uptake is a life-long process that may be different among the three monocyte subsets and have existed prior to the cerebral accumulation of A $\beta$  (Figure. 2e).

## **Correlation between $A\beta_{1-42}$ uptake by monocytes and $A\beta_{1-42}$ , $A\beta_{1-40}$ , $A\beta_{1-42}/A\beta_{1-40}$ in the plasma and CSF**

After controlling the cofounding factors of age, sex, vascular risk factors and *APOE*  $\epsilon 4$  carriers, the partial correlation analysis showed that  $A\beta_{1-42}$  uptake by total monocytes was significantly correlated with plasma  $A\beta_{1-42}$  levels ( $\gamma=-0.668$ ,  $P = 0.002$ ) in CN subjects ( $n = 25$ , mean age  $60.54 \pm 17.21$  years). Among them,  $A\beta_{1-42}$  uptake by both  $CD14^+CD16^-$  ( $\gamma=-0.576$ ,  $P = 0.010$ ) and  $CD14^{dim}CD16^+$  ( $\gamma=-0.506$ ,  $P = 0.027$ ) subsets, but not the  $CD14^+CD16^+$  subset ( $\gamma=-0.390$ ,  $P = 0.099$ ), was correlated with plasma  $A\beta_{1-42}$  levels (Figure. 3a-d). Moreover,  $A\beta_{1-42}$  uptake by total monocytes was significantly correlated with plasma  $A\beta_{1-40}$  levels ( $\gamma=-0.615$ ,  $P = 0.005$ ) (Figure. 4a). Among them,  $A\beta_{1-42}$  uptake by  $CD14^+CD16^-$  ( $\gamma=-0.640$ ,  $P = 0.003$ ) and  $CD14^+CD16^-$  ( $\gamma=-0.458$ ,  $P = 0.048$ ) subsets, but not the  $CD14^{dim}CD16^+$  ( $\gamma=-0.327$ ,  $P = 0.172$ ) subset, was correlated with plasma  $A\beta_{1-40}$  levels (Figure. 4b-d).  $A\beta_{1-42}$  uptake by  $CD14^{dim}CD16^+$  ( $\gamma=-0.470$ ,  $P = 0.042$ ) subset, but not the total monocyte ( $\gamma=-0.235$ ,  $P = 0.334$ ),  $CD14^+CD16^-$  ( $\gamma=-0.039$ ,  $P = 0.874$ ) and  $CD14^+CD16^+$  ( $\gamma=-0.080$ ,  $P = 0.746$ ) subsets, was correlated with plasma  $A\beta_{1-42}/A\beta_{1-40}$  level (Supplemental Figure. 2a-d). However,  $A\beta_{1-42}$  uptake by monocytes was not correlated with CSF  $A\beta_{1-42}$ , CSF  $A\beta_{1-40}$  and CSF  $A\beta_{1-42}/A\beta_{1-40}$  levels. (Supplemental Figure. 3–5). The  $A\beta_{1-42}$ ,  $A\beta_{1-40}$ ,  $A\beta_{1-42}/A\beta_{1-40}$  and T-tau/ $A\beta_{1-42}$  levels in the plasma were not associated with those in the CSF (Supplemental Figure. 6).

### **$A\beta_{1-42}$ uptake by monocytes from AD and PD patients and CN subjects.**

To investigate whether the alteration in  $A\beta_{1-42}$  uptake by monocytes is specific to AD patients, 22 AD patients, 15 age- and sex-matched PD patients and their matched CN controls were enrolled (Supplemental Table. 2, 3). There were no significant differences in sex, age, years of education, *APOE*  $\epsilon 4$  carrier, comorbidities including hypertension, hyperlipidaemia, and diabetes mellitus, or medications between the matched groups. We found that  $A\beta_{1-42}$  uptake by total monocytes and the various subsets was lower in AD patients than in CNs (Figure. 5a-d). There were no significant differences in  $A\beta_{1-42}$  uptake by total monocytes and their subsets between PD patients and CN controls (Supplemental Figure. 7a-d). These results suggest that  $A\beta_{1-42}$  uptake by monocytes might be specifically decreased in AD patients.

### **Expression of $A\beta_{1-42}$ uptake-related receptors and $A\beta$ -degrading enzymes in monocytes of AD patients**

The expression of TLR2 was lower in AD patients than CNs. However, no differences were observed in the expression of receptors, including TREM2, CD36, CD33, and SCARA1, between AD patients and CN (Figure. 6a-k). There were no significant differences in the protein levels of  $A\beta$ -degrading enzymes, including cathepsin D and cathepsin S, between AD patients and CN controls (Figure. 6l-m).

## **Discussion**

Ageing is an important factor for the development of AD (24). We found that A $\beta$ <sub>1-42</sub> uptake by monocytes decreased as patient age increased. Consistently, a previous study showed that the internalization of A $\beta$ <sub>1-42</sub> by aged human blood cell-derived monocytes was lower than that by human umbilical cord blood cell-derived monocytes (25). It is worth noting that the decrease in A $\beta$  uptake by classic monocyte subset and non-classic subset began at the age of 20 years and 40 years old respectively, suggesting that a decrease in A $\beta$  uptake ability is a life-long process. Despite the impact of ageing, the A $\beta$  uptake ability of monocytes is further decreased in AD patients, implying that compromised A $\beta$  uptake by monocytes is involved in AD pathogenesis (26–28).

In humans, peripheral monocytes can be divided into non-classic, intermediate and classic monocyte subsets (23). These monocyte subsets have different functions in AD, which are not fully understood. As reflected by our findings, the intermediate subset had the highest A $\beta$  uptake ability, while there was no significant difference in A $\beta$  uptake between the classic and non-classic subsets in cognitively normal controls. The A $\beta$  uptake ability was mainly decreased in the classic and non-classic subsets during ageing but decreased in all subsets in AD patients. These results suggest that there is an overall decrease in A $\beta$  uptake by all three monocyte subsets in AD patients, implying that the mechanisms underlying the alteration in A $\beta$  uptake ability by monocytes in AD patients are different from those associated with ageing.

The mechanisms underlying the decreased A $\beta$  uptake ability by monocytes during ageing and AD remain to be investigated. In AD, the decrease could be partially due to deficits in A $\beta$  recognition by monocytes, as reflected by the reduced expression of TRL2 in monocytes. TRL2 is a type I transmembrane pattern recognition receptor and acts as a natural innate immune receptor to clear A $\beta$ <sub>1-42</sub> and delay cognitive decline in a mouse model of AD (29). However, we did not find any differences in the expression of A $\beta$ -degrading enzymes between AD patients and CN controls, suggesting that dysfunctional A $\beta$  recognition could be particularly important for the decrease in A $\beta$  uptake by monocytes in AD patients.

The decrease in A $\beta$  uptake by monocytes seems specific to AD, as it was not changed in PD patients compared with each matched CN controls in our study. Additional evidence indicates that the A $\beta$  uptake ability is correlated with blood A $\beta$  levels in CN subjects, that is, the greater the A $\beta$  uptake ability is, the lower the blood A $\beta$  levels. These findings suggest that monocytes might play a critical role in clearing A $\beta$  from the blood. We did not find a correlation between A $\beta$  uptake by monocytes and A $\beta$ <sub>1-42</sub> levels in the CSF. This might be due to the lack of a correlation between plasma and CSF A $\beta$ <sub>1-42</sub> levels in our CN subjects.

## Conclusion

In conclusion, our findings are of significance to the understanding of the pathogenesis of sporadic AD. A $\beta$  in the brain can be transported to the peripheral blood (30–32), and the clearance of A $\beta$  in the periphery has been suggested to substantially contribute to the clearance of A $\beta$  from the brain (10, 33). Therefore, the decrease in A $\beta$  uptake by monocytes could play a significant role in the development of

sporadic AD. The recovery of the A $\beta$  clearance function of blood monocytes may represent a potential strategy for the prevention and treatment of AD.

## List Of Abbreviations

AD

Alzheimer's disease; A $\beta$ :amyloid  $\beta$ -protein; CN:cognitively normal control; PD:Parkinson's disease; CSF:cerebrospinal fluid; MMSE:Mini-Mental State Examination; ADL:Activities of Daily Living; CDR:Clinical Dementia Rating; MoCA:Montreal Cognitive Assessment; POD:Outpatient Disability Questionnaire; HIS:Hachinski Ischemic Score; PBMC:peripheral blood mononuclear cell; TLR2:Toll-like receptor 2; TREM2:triggering receptor expressed on myeloid cells 2; SCARA1:macrophage scavenger receptor 1.

## Declarations

## Ethics approval and consent to participate

Ethics committee of Chongqing Daping Hospital has approved the use of human subjects for this study. This study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

### Consent for publication

Not applicable

### Availability of data and material

The datasets generated or analyzed during the current study are available from the corresponding author on reasonable request.

### Competing interests

The authors declare no conflicts of interests, and approval the contents of this study.

## Funding

This study was supported by National Natural Science Foundation of China (81930028, 91749206, 81625007 and 31921003)

## Authors' contributions

Conceptualization: Yan-Jiang Wang and Yang Chen; Methodology: Si-Han Chen and Ding-Yuan Tian; Formal analysis and investigation: Ying-Ying Shen, Yuan Cheng, Dong-Yu Fan, Hao-Lun Sun, Chen-Yang He, Sun-Pu Yang; Writing - original draft preparation: Si-Han Chen; Writing - review and editing: Xian-Le Bu and Fan Zeng; Funding acquisition: Yan-Jiang Wang; Resources: Yan-Jiang Wang; Supervision: Zhi-Qiang Xu and Juan-Deng.

## Acknowledgements

.The authors would like to thank the patients and their families for their participation and dedication to research.

### Rights and permissions

**Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

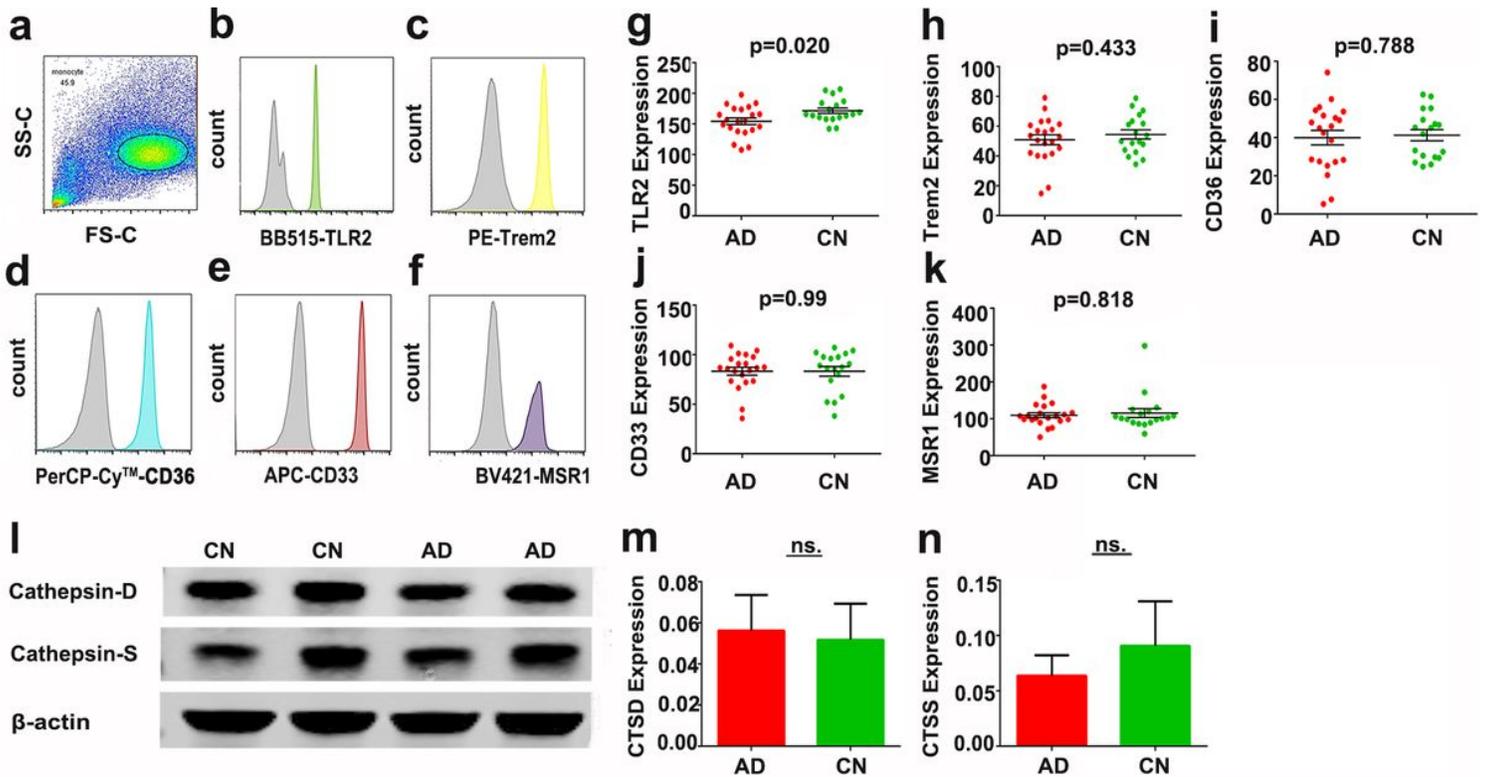
## References

1. Prince M, Bryce R, Albanese E, Wimo A, Ribeiro W, Ferri CP. The global prevalence of dementia: a systematic review and metaanalysis. *Alzheimer's dementia: the journal of the Alzheimer's Association*. 2013;9(1):63–75.e2.
2. Mawuenyega KG, Sigurdson W, Ovod V, Munsell L, Kasten T, Morris JC, et al. Decreased clearance of CNS beta-amyloid in Alzheimer's disease. *Science*. 2010;330(6012):1774.
3. Bradshaw EM, Chibnik LB, Keenan BT, Ottoboni L, Raj T, Tang A, et al. CD33 Alzheimer's disease locus: altered monocyte function and amyloid biology. *Nature neuroscience*. 2013;16(7):848–50.
4. Carmona S, Zahs K, Wu E, Dakin K, Bras J, Guerreiro R. The role of TREM2 in Alzheimer's disease and other neurodegenerative disorders. *Lancet Neurol*. 2018;17(8):721–30.
5. Vasquez JB, Fardo DW, Estus S. ABCA7 expression is associated with Alzheimer's disease polymorphism and disease status. *Neurosci Lett*. 2013;556:58–62.
6. Crehan H, Hardy J, Pocock J. Blockage of CR1 prevents activation of rodent microglia. *Neurobiol Dis*. 2013;54:139–49.

7. Kunkle BW, Grenier-Boley B, Sims R, Bis JC, Damotte V, Naj AC, et al. Genetic meta-analysis of diagnosed Alzheimer's disease identifies new risk loci and implicates Abeta, tau, immunity and lipid processing. *Nat Genet.* 2019;51(3):414–30.
8. Krabbe G, Halle A, Matyash V, Rinnenthal JL, Eom GD, Bernhardt U, et al. Functional impairment of microglia coincides with Beta-amyloid deposition in mice with Alzheimer-like pathology. *PloS one.* 2013;8(4):e60921-e.
9. Grathwohl SA, Kälin RE, Bolmont T, Prokop S, Winkelmann G, Kaeser SA, et al. Formation and maintenance of Alzheimer's disease beta-amyloid plaques in the absence of microglia. *Nature neuroscience.* 2009;12(11):1361–3.
10. Xiang Y, Bu XL, Liu YH, Zhu C, Shen LL, Jiao SS, et al. Physiological amyloid-beta clearance in the periphery and its therapeutic potential for Alzheimer's disease. *Acta Neuropathol.* 2015;130(4):487–99.
11. Yuede CM, Lee H, Restivo JL, Davis TA, Hettinger JC, Wallace CE, et al. Rapid in vivo measurement of beta-amyloid reveals biphasic clearance kinetics in an Alzheimer's mouse model. *J Exp Med.* 2016;213(5):677–85.
12. Qosa H, Abuasal BS, Romero IA, Weksler B, Couraud PO, Keller JN, et al. Differences in amyloid-beta clearance across mouse and human blood-brain barrier models: kinetic analysis and mechanistic modeling. *Neuropharmacology.* 2014;79:668–78.
13. Butovsky O, Kunis G, Koronyo-Hamaoui M, Schwartz M. Selective ablation of bone marrow-derived dendritic cells increases amyloid plaques in a mouse Alzheimer's disease model. *Eur J Neurosci.* 2007;26(2):413–6.
14. Koronyo-Hamaoui M, Ko MK, Koronyo Y, Azoulay D, Seksenyan A, Kunis G, et al. Attenuation of AD-like neuropathology by harnessing peripheral immune cells: local elevation of IL-10 and MMP-9. *Journal of neurochemistry.* 2009;111(6):1409–24.
15. Koronyo Y, Salumbides BC, Sheyn J, Pelissier L, Li S, Ljubimov V, et al. Therapeutic effects of glatiramer acetate and grafted CD115(+) monocytes in a mouse model of Alzheimer's disease. *Brain.* 2015;138(Pt 8):2399–422.
16. Simard AR, Soulet D, Gowing G, Julien J-P, Rivest S. Bone marrow-derived microglia play a critical role in restricting senile plaque formation in Alzheimer's disease. *Neuron.* 2006;49(4):489–502.
17. Hawkes CA, McLaurin J. Selective targeting of perivascular macrophages for clearance of beta-amyloid in cerebral amyloid angiopathy. *Proc Natl Acad Sci U S A.* 2009;106(4):1261–6.
18. Li WW, Wang Z, Fan DY, Shen YY, Chen DW, Li HY, et al. Association of Polygenic Risk Score with Age at Onset and Cerebrospinal Fluid Biomarkers of Alzheimer's Disease in a Chinese Cohort. *Neurosci Bull.* 2020.
19. Hughes AJ, Daniel SE, Kilford L, Lees AJ. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. *J Neurol Neurosurg Psychiatry.* 1992;55(3):181–4.

20. Qian F, Montgomery RR. Imaging Immunosenescence. *Methods in molecular biology*. (Clifton NJ). 2015;1343:97–106.
21. Wilke C, Bender F, Hayer SN, Brockmann K, Schöls L, Kuhle J, et al. Serum neurofilament light is increased in multiple system atrophy of cerebellar type and in repeat-expansion spinocerebellar ataxias: a pilot study. *Journal of neurology*. 2018;265(7):1618–24.
22. Lue LF, Guerra A, Walker DG. Amyloid Beta and Tau as Alzheimer's Disease Blood Biomarkers: Promise From New Technologies. *Neurology therapy*. 2017;6(Suppl 1):25–36.
23. Passlick B, Flieger D, Ziegler-Heitbrock HW. Identification and characterization of a novel monocyte subpopulation in human peripheral blood. *Blood*. 1989;74(7):2527–34.
24. Hou Y, Dan X, Babbar M, Wei Y, Hasselbalch SG, Croteau DL, et al. Ageing as a risk factor for neurodegenerative disease. *Nature reviews Neurology*. 2019;15(10):565–81.
25. Darlington D, Li S, Hou H, Habib A, Tian J, Gao Y, et al. Human umbilical cord blood-derived monocytes improve cognitive deficits and reduce amyloid-beta pathology in PSAPP mice. *Cell Transplant*. 2015;24(11):2237–50.
26. Avagyan H, Goldenson B, Tse E, Masoumi A, Porter V, Wiedau-Pazos M, et al. Immune blood biomarkers of Alzheimer disease patients. *J Neuroimmunol*. 2009;210(1–2):67–72.
27. Fiala M, Lin J, Ringman J, Kermani-Arab V, Tsao G, Patel A, et al. Ineffective phagocytosis of amyloid-beta by macrophages of Alzheimer's disease patients. *Journal of Alzheimer's disease: JAD*. 2005;7(3):221–32. discussion 55–62.
28. Gu BJ, Huang X, Ou A, Rembach A, Fowler C, Avula PK, et al. Innate phagocytosis by peripheral blood monocytes is altered in Alzheimer's disease. *Acta Neuropathol*. 2016;132(3):377–89.
29. Richard KL, Filali M, Prefontaine P, Rivest S. Toll-like receptor 2 acts as a natural innate immune receptor to clear amyloid beta 1–42 and delay the cognitive decline in a mouse model of Alzheimer's disease. *The Journal of neuroscience: the official journal of the Society for Neuroscience*. 2008;28(22):5784–93.
30. Maness LM, Banks WA, Podlisny MB, Selkoe DJ, Kastin AJ. Passage of human amyloid beta-protein 1–40 across the murine blood-brain barrier. *Life sciences*. 1994;55(21):1643–50.
31. Roberts KF, Elbert DL, Kasten TP, Patterson BW, Sigurdson WC, Connors RE, et al. Amyloid-beta efflux from the central nervous system into the plasma. *Ann Neurol*. 2014;76(6):837–44.
32. Deane R, Bell RD, Sagare A, Zlokovic BV. Clearance of amyloid-beta peptide across the blood-brain barrier: implication for therapies in Alzheimer's disease. *CNS Neurol Disord Drug Targets*. 2009;8(1):16–30.
33. Wang J, Gu BJ, Masters CL, Wang YJ. A systemic view of Alzheimer disease - insights from amyloid-beta metabolism beyond the brain. *Nat Rev Neurol*. 2017;13(10):612–23.

## Figures



**Figure 1**

Detection of Aβ1-42 uptake-related receptors and Aβ-degrading enzymes in the monocytes of AD patients. (a) Following CD14 positive selection by magnetic activated cell sorting, monocytes were identified by flow cytometry by their forward-side-scatter appearance (n=21 for AD; n=18 for CN). (b-f) the expression of TLR2, TREM2, CD36, CD33 and MSR1 was assessed in monocytes, and the expression levels are depicted in representative histograms; grey curves indicate negative control staining (n=21 for AD; n=18 for CN). (e) The expression level of TLR2 was decreased in AD patients compared with CN subjects (n=21 for AD; n=18 for CN). (g-k) there was no significant difference between AD patients and CN controls in the expression levels of TREM2, CD36, CD33 and MSR1 (n=21 for AD; n=18 for CN). (i-m) there was no significant difference between AD patients and CN controls in the expression levels of Aβ degrading enzymes, including cathepsin D and cathepsin S (n=12 for AD; n=12 for CN).

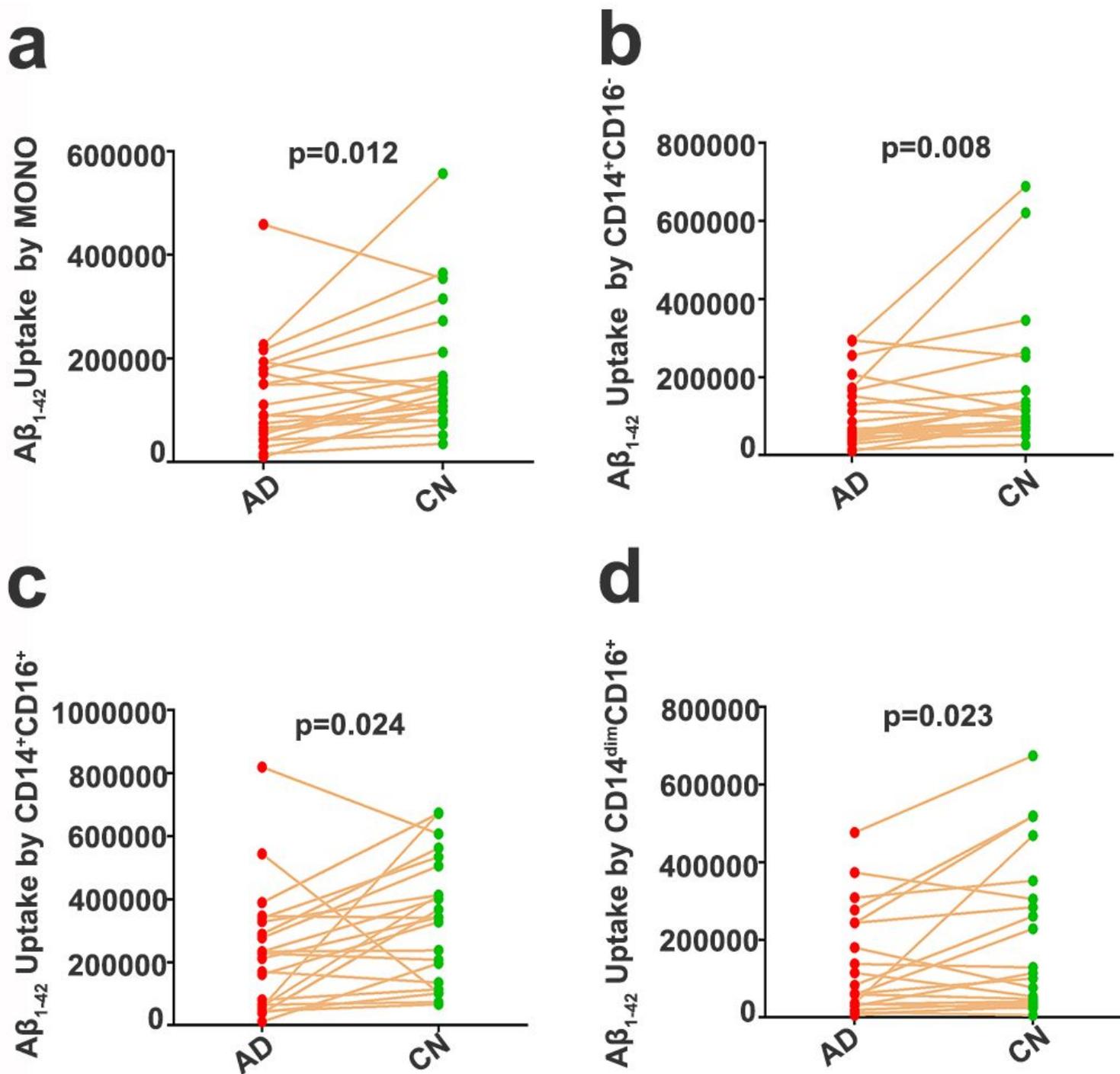


Figure 2

Comparison of Aβ<sub>1-42</sub> uptake by monocyte subsets between AD patients and CN subjects. Compared with CN controls (n=22), AD patients (n=22) had decreased Aβ<sub>1-42</sub> uptake by total monocytes (a), the CD14<sup>+</sup>CD16<sup>-</sup> subset (b), the CD14<sup>+</sup>CD16<sup>+</sup> subset (c) and the CD14<sup>dim</sup>CD16<sup>-</sup> subset (d). For abbreviations, see list

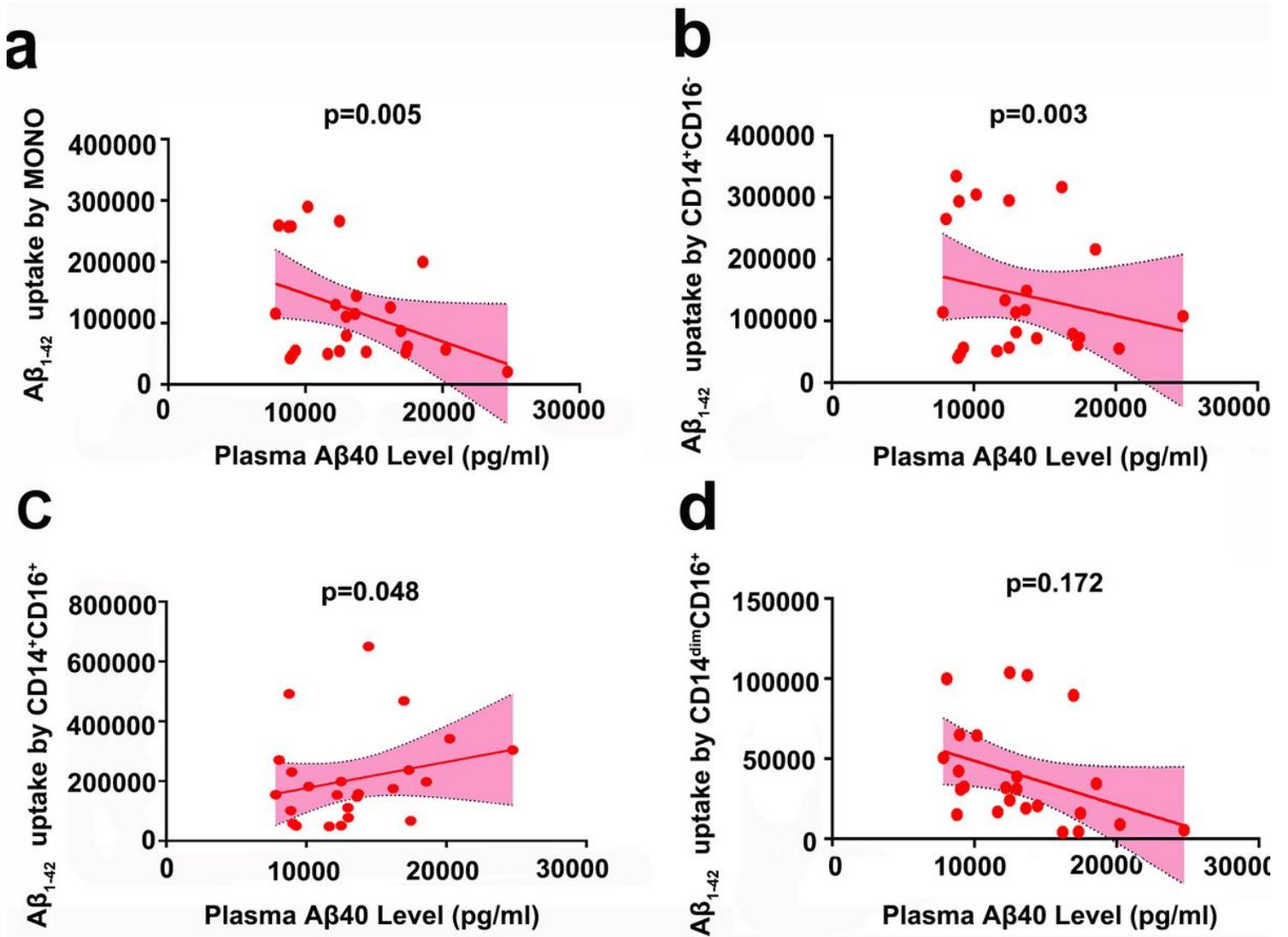


Figure 3

Association of monocyte  $A\beta_{1-42}$  uptake with plasma  $A\beta_{1-40}$  levels in CN subjects. Correlations between plasma  $A\beta_{1-42}$  and the uptake of  $A\beta_{1-42}$  by all monocytes (a), by the  $CD14^+CD16^-$  subset (b), by the  $CD14^+CD16^+$  subset (c), and by the  $CD14^{dim}CD16^-$  subset (d).  $n=25$ . For abbreviations, see list

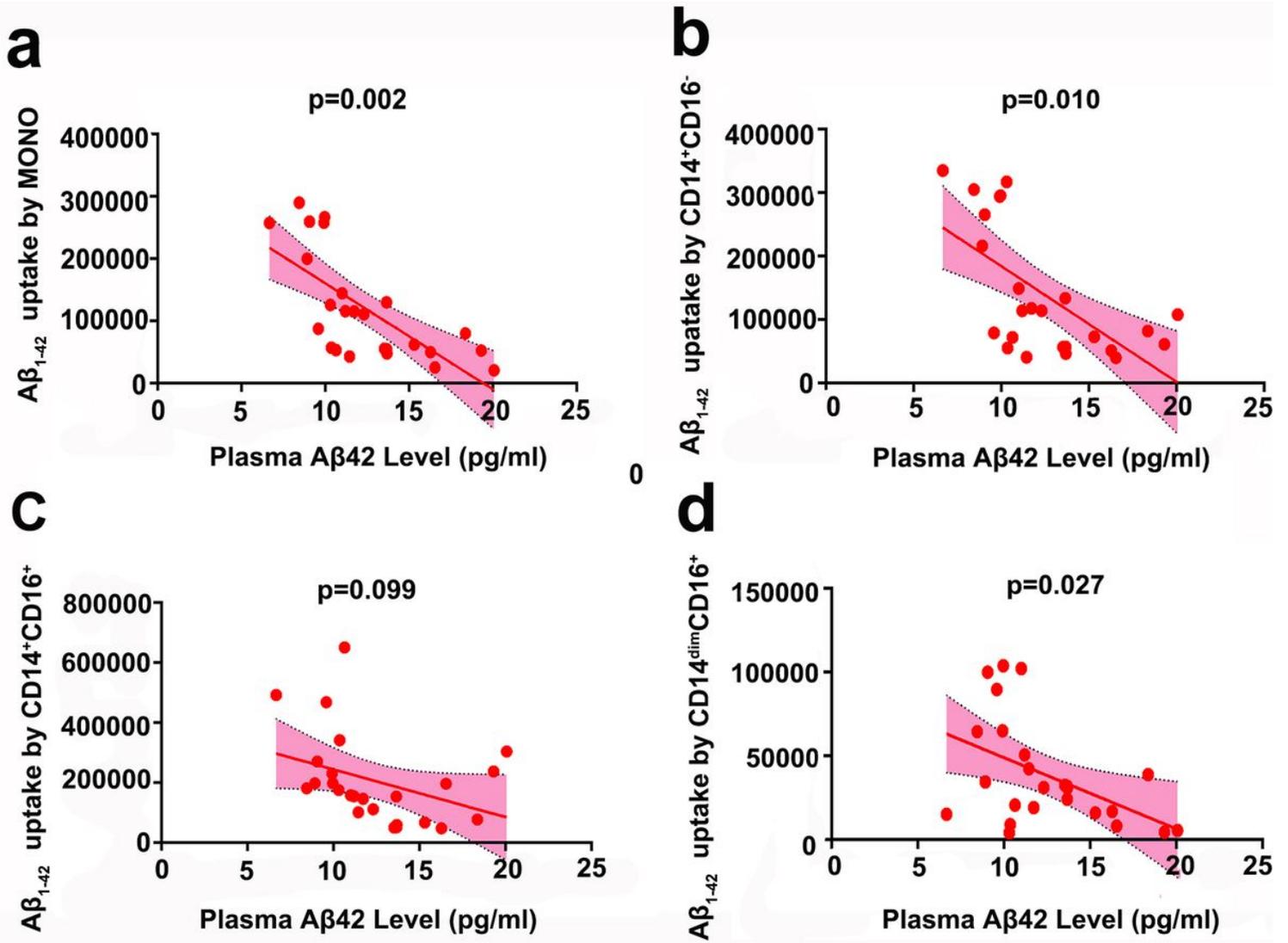


Figure 4

Association of monocyte  $A\beta_{1-42}$  uptake with plasma  $A\beta_{1-42}$  levels in CN subjects. Correlations between plasma  $A\beta_{1-42}$  and the uptake of  $A\beta_{1-42}$  by all monocytes (a), by the  $CD14^+CD16^-$  subset (b), by the  $CD14^+CD16^+$  subset (c), and by the  $CD14^{dim}CD16^-$  subset (d).  $n=25$ . For abbreviations, see list

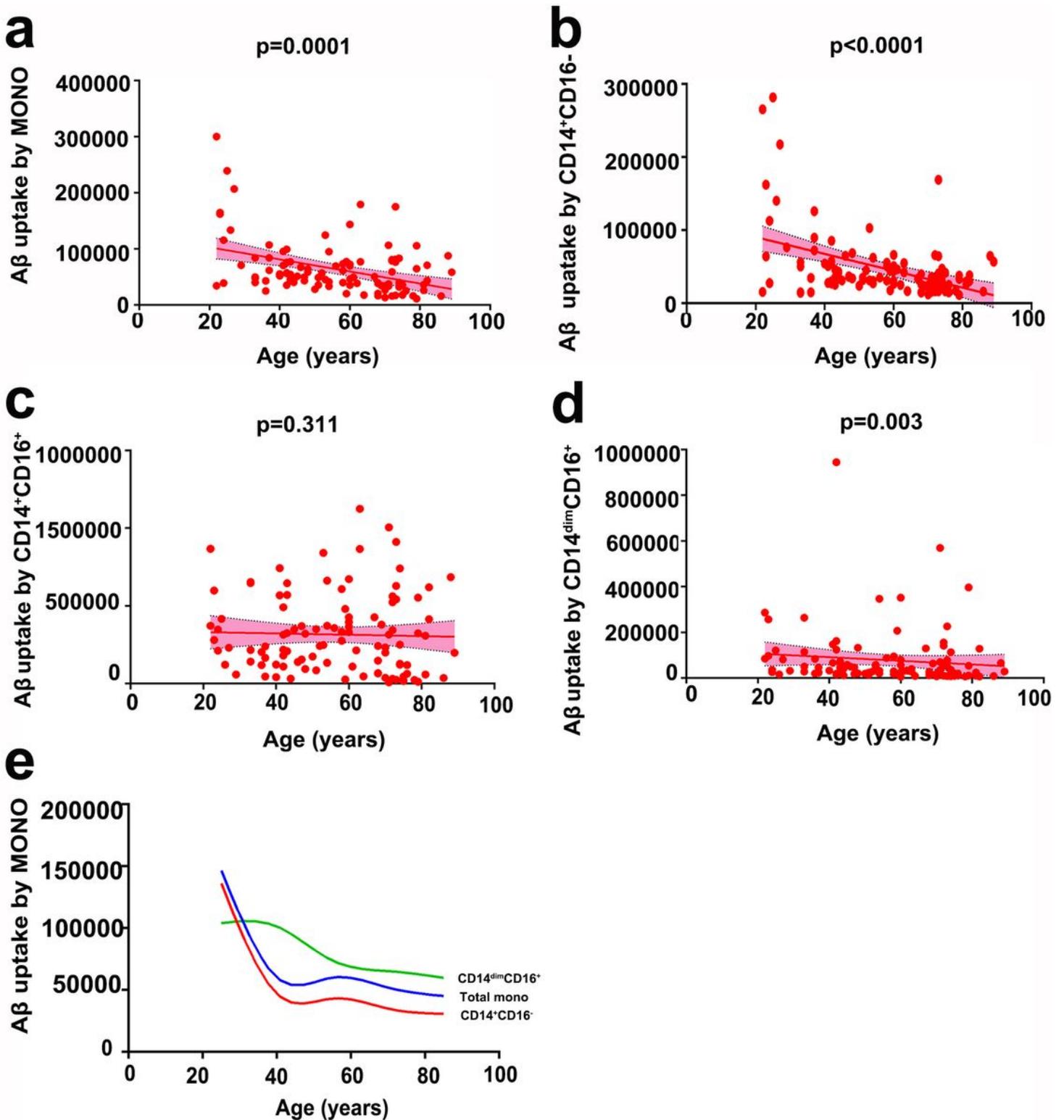
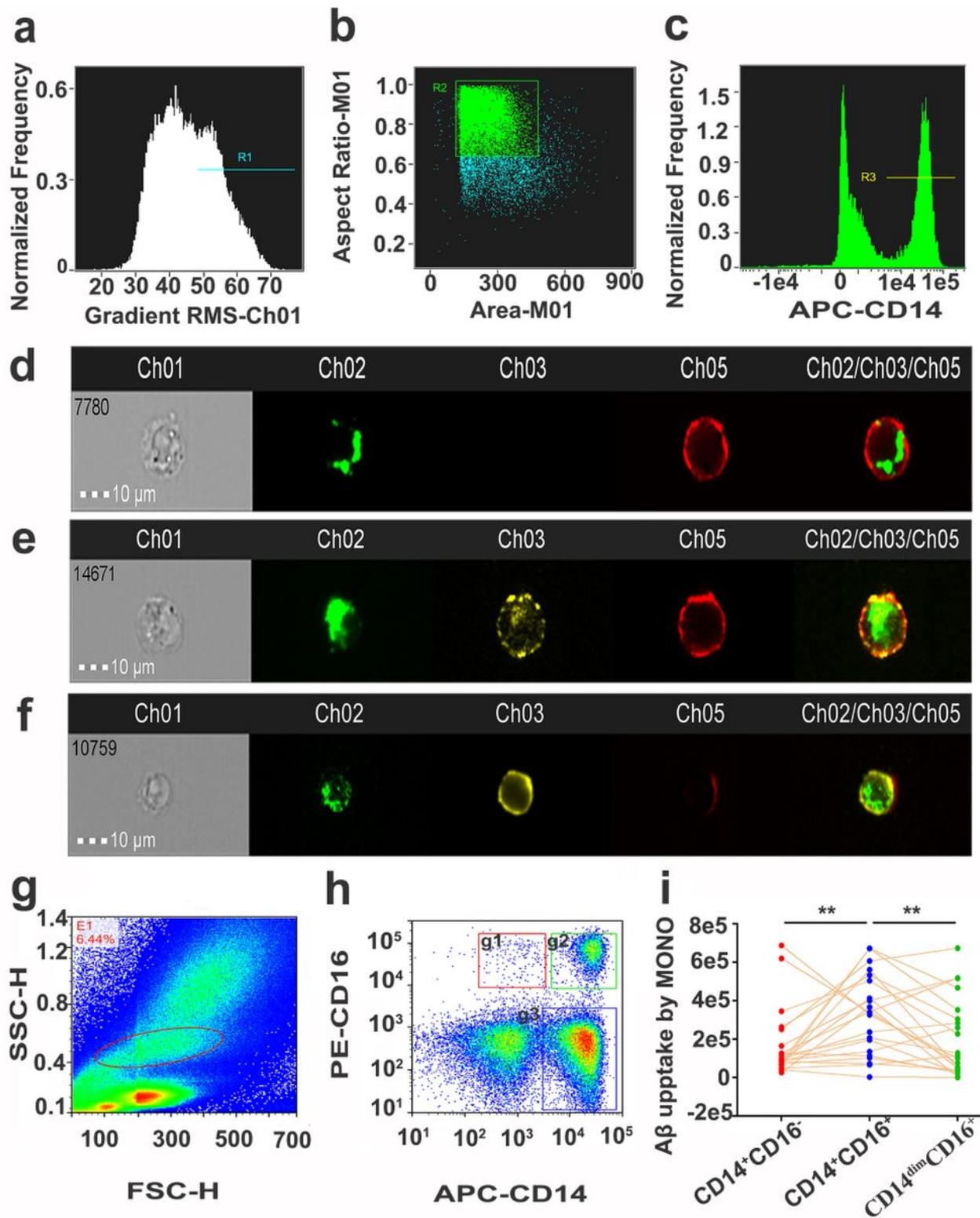


Figure 5

Correlation and trajectory of Aβ<sub>1-42</sub> uptake by monocyte subsets relative to age. a-d Spearman correlation analysis was utilized to investigate the correlation between ageing and the uptake of Aβ<sub>1-42</sub> by all monocytes (a), by the CD14<sup>+</sup>CD16<sup>-</sup> subset (b), by the CD14<sup>+</sup>CD16<sup>+</sup> subset (c), and by the CD14<sup>dim</sup>CD16<sup>+</sup> subset (d). The trajectory of Aβ<sub>1-42</sub> uptake by total monocytes and the CD14<sup>+</sup>CD16<sup>-</sup> subset relative to age was modelled using Fit spline/ Lowess (cubic) spline. n=104. For abbreviations, see list



**Figure 6**

For imaging flow cytometry, single cells were selected from debris by gating on cells in focus (a) followed by gating on the area and aspect ratio of the brightfield image (b). Cells with high-intensity labelling of CD14 were chosen as monocytes (c). Monocytes were stained with APC-conjugated anti-CD14 mAb (red) and PE-conjugated anti-CD16 mAb (yellow), whereas FITC-conjugated A $\beta$ 1-42 is shown in green. Images of FITC-labelled A $\beta$ 1-42 uptake by the classic CD14<sup>+</sup>CD16<sup>-</sup> monocyte subset (d), intermediate

CD14+CD16+ monocyte subset (e) and non-classical CD14dimCD16+ monocyte subset were collected (f). (h) For the measurement of A $\beta$ 1-42 uptake by monocyte subsets, monocytes were gated based on FSC-H and SSC-H. Three monocyte subsets, including classical monocyte subset CD14+CD16- (g1), intermediate monocyte subset CD14+CD16+ (g2) and non-classical monocyte subset CD14dimCD16+ (g3), were gated based on the expression of CD14 and CD16. (i) A paired t test was utilized to compare A $\beta$ 1-42 uptake between the three monocyte subsets (n=44). For abbreviations, see list

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementalinformationV1.docx](#)