

# Glymphatic Efficiency Is a Critical Factor for Using Abnormal Tau in Peripheral Tissues as Biomarker for Alzheimer's Disease

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### Abstract

Alzheimer's Disease or other dementias are characterized by the accumulation of abnormal tau and amyloid  $\beta$  peptides in brains. Therefore, abnormal tau and amyloid peptides in peripheral tissues or blood have been explored as diagnostic biomarkers. On the other hand, recent studies have revealed glymphatics a special drainage system for brain's wastes. We aimed to investigate whether effectiveness of glymphatic system affects the quantity of abnormal tau in the peripheral tissues. We have previously shown that aged IL33 KO ( $Il33^{-/-}$ ) mice develop Alzheimer's like disease. Despite a large quantity of abnormal tau in brains,  $Il33^{-/-}$  mice showed a much lower amount of abnormal tau drained to the peripheral tissues kidneys than in wild type mice. Our further study showed that it was caused by defective glymphatic drainage since IL33 KO impaired glymphatics. Thus, it is necessary to identify biomarkers, which can evaluate efficiency of glymphatic drainage. Simultaneous measurement of these biomarkers and abnormal tau in peripheral tissues or blood may be critical for accurate diagnosis of Alzheimer's disease.

**Keywords:** Alzheimer's Disease; Biomarkers; Glymphatics; Kidneys; Tau

### Abbreviations

AD	:	Alzheimer's Disease
AQP4	:	Aquaporin4
PHF	:	Paired Helical Fragment Tau
WT	:	Wild Type

### Introduction

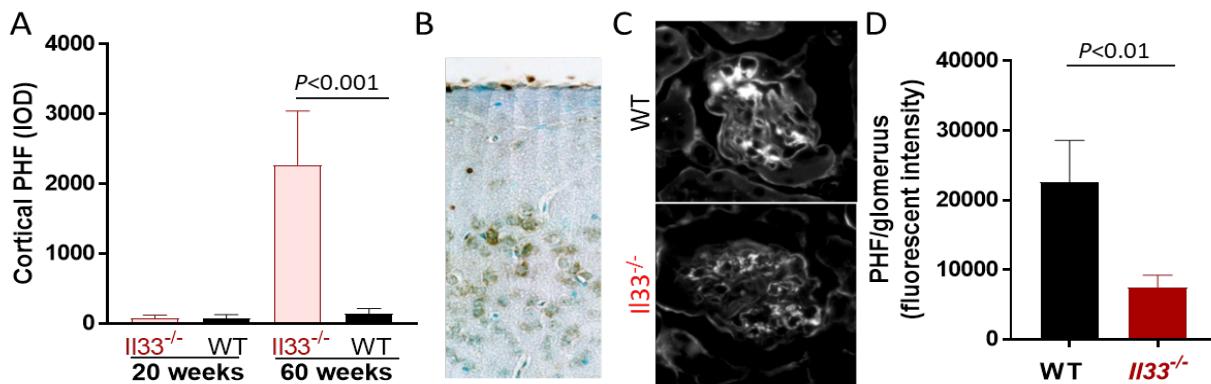
Due to slow development over a long period of time with little or no symptoms, sporadic Alzheimer's disease (AD) is often diagnosed after irreversible neurodegeneration in brains. Thus, early diagnosis of this disease has been a medical priority [1]. This will give opportunities for possible interventions before irreversible neurodegeneration. Two strategies, i.e. special imaging technology of brains and biomarkers, have been under development for early diagnosis [2]. Using biomarkers for early diagnosis obviously has many advantages. It is simple, financially friendly and suitable for large-scale screening. Alzheimer's disease is characterized

by accumulation of amyloid plaques and neurofibrillary tangles, which are composed of amyloid  $\beta$  peptides and abnormal tau proteins, respectively.

Those abnormal neuronal proteins have been detected in peripheral tissues, the circulation and cerebra-spinal fluid [3-6]. Therefore, numerous studies have explored whether abnormal tau and amyloid peptides in the peripheral tissue or blood could be diagnostic biomarkers [3-7]. Despite many years' intense studies, it remains unclear about accuracy of those biomarkers in peripheral tissues or blood for early diagnosis [6-9]. IL33 is cytokine, which is widely expressed in astrocytes [10]. We have demonstrated its critical role in regulation of anti-aging or anti-oxidative mechanisms in several organs [11-13]. Importantly, mice lacking IL33 ( $Il33^{-/-}$ ) gene develop dementia at old age with similar symptoms to Alzheimer's [13]. More importantly, those mice also showed accumulation of abnormal tau in their brains [13].

Recently, we further investigated the relationship between tau in peripheral tissues and glymphatic drainage, by comparison of those between WT and  $Il33^{-/-}$  mice. We have shown that three anti-aging mechanisms, i.e. repair of DNA double-strand-break, autophagy of damaged molecules and glymphatics in neurons or

brains in  $\text{Il33}^{-/-}$  mice are defected [12,13]. Immunohistochemistry detected PHF in the cortical and hippocampal neurons in  $\text{Il33}^{-/-}$  mice after 60 weeks but not in WT mice (Figure 1A). However, we did detect a trace amount of abnormal tau, Paired Helical Fragments (PHF), in brains of WT at 60 weeks by western blot on the cortical tissues (Figure 1B), suggesting generation of abnormal tau in WT mice.

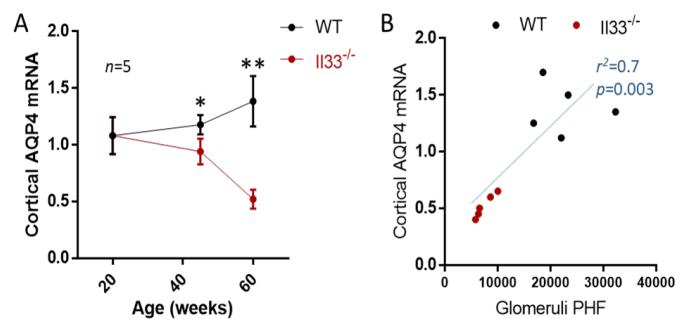


**Figure 1:** Abnormal tau PHF in the cortical tissue and glomeruli in wild type and  $\text{Il33}^{-/-}$  mice.

**A.** Western blot detection of PHF in the cortical tissues in various groups of mice as indicated. PHF was quantified by density of PHF bands;  $n=5$ . **B.** Immunohistochemistry reveals accumulation of PHF in cortical neurons (brown). **C.** Immunofluorescence shows PHF in glomeruli of mice at 60 weeks. **D.** Summary of PHF in glomeruli based on fluorescent intensity (see C) in mice;  $n=5$ .

Since glymphatic system is able to drain abnormal neuronal proteins and wastes to the circulation, we investigated whether brain PHF tau in WT mice was drained into peripheral tissues. Glomeruli are critical for excretion of body's wastes. We decided to examine the presence of PHF in kidneys of old WT mice with both immunofluorescence and western blot. Despite a trace amount of abnormal tau in their brains, old WT mice (60 weeks) showed a significant quantity of phosphate tau AT8 and PHF tau in the glomeruli (Figure 1C and D). We next examined abnormal tau in glomeruli of age-matched  $\text{Il33}^{-/-}$  mice, which showed a robust accumulation of abnormal tau, i.e. AT8, PHF and insoluble MC1 in their neurons in the cortical and hippocampal regions [13] (Figure 1A). Unexpectedly, abnormal tau in glomeruli of these  $\text{Il33}^{-/-}$  mice was nearly 3 folds lower than WT mice (Figure 1C and D). Thus, quantity of abnormal tau in peripheral tissue or blood did not correlate to their accumulation in the brains, at least in the case of  $\text{Il33}^{-/-}$  mice (Figure 1C). Glymphatics play a critical role in drainage of brain wastes [14,15]. We next asked whether deficiency of glymphatics was responsible for the reduction of abnormal tau in peripheral tissues. Aquaporin4 (AQP4) is a critical molecule for generation convective flow to drive wastes to para-venous space of glymphatics [16,17]. We compared AQP4 expression in brains between WT and  $\text{Il33}^{-/-}$  mice. RT-PCR revealed a compatible

amount of mRNA of brain AQP4 in young  $\text{Il33}^{-/-}$  and WT mice (Figure 2A).



**Figure 2:** Reduction in cortical AQP4 expression in  $\text{Il33}^{-/-}$  mice positively correlates to decreased PHF tau in their glomeruli. **A.** Quantitative RT-PCR shows the time course of cortical AQP4 mRNA in mice as indicated. Average quantity of AQP4 mRNA at 20 weeks of WT mice is taken as 1.00 for comparison. **B.** Chart shows plot of glomerular PHF versus cortical mRNA for each mouse. Linear regression reveals a positive regression between glomerular PHF and cortical AQP4; also note a low  $r^2$ , suggesting potential involvement of other factors beyond AQP4.

A significant increase in brain AQP4 mRNA was then observed in WT mice between 45 and 60 weeks. In contrast,  $\text{Il33}^{-/-}$  mice showed a sharp reduction in their brain AQP4 mRNA after 45 weeks, and thus, it led to a 2.5-fold lower of AQP4 mRNA than that in WT at 60 weeks. Although it remains unclear about quantitative relationship between AQP4 and efficacy of glymphatic, a positive correlation between kidney PHF and cortical AQP4 mRNA clearly suggest a dramatic reduction of glymphatic capacity in  $\text{Il33}^{-/-}$  mice (Figure 2B). We have previously demonstrated a defect in completion of autophagic digestion in neurons in  $\text{Il33}^{-/-}$  mice after

45 weeks [13]. Critical role of autophagy in neurons for elimination of neuronal damaged or old proteins has been well known, and its deficiency has been linked to neurodegeneration and Alzheimer's disease [18-21]. Taking all these together, we hypothesize that quantity of blood/peripheral abnormal tau depends on not only generation rate of abnormal tau, but also their elimination speed by autophagy and drainage by glymphatics. It can be briefly expressed by the following mathematic formula: Tau in neurons, i.e.  $Tau_{neuron} = (Tau_{generation} - Tau_{autophagy})$ . Thus, Tau in blood, i.e.  $Tau_{blood} = Tau_{neuron} \times glymphatics_{efficacy}$ . This formula may explain why peripheral abnormal tau in WT mice is more than  $\text{I}1\text{3}3^{-/-}$  mice.  $Tau_{neuron}$  could be much higher in  $\text{I}1\text{3}3^{-/-}$  mice, which is well demonstrated by accumulation of abnormal tau due to its increased generation and decreased autophagic digestion [13]. However, a robustly smaller coefficient for glymphatics efficacy (i.e.  $glymphatics_{efficacy}$ ) in  $\text{I}1\text{3}3^{-/-}$  mice after 45 weeks will be sufficient to bring  $Tau_{blood}$  down to the level, which is much lower than WT mice, despite accumulation of a large quantity of abnormal tau in neurons in  $\text{I}1\text{3}3^{-/-}$  mice. In fact, defective or low glymphatic drainage efficacy has been blamed for tauopathy [15-17].

## Conclusion

Abnormal Tau drained to the peripheral tissues or blood has been considered a potential biomarker for diagnosis of dementias such as Alzheimer's disease. However, abnormal tau concentration in the peripheral tissue or blood was determined not only by its concentration in the diseased brains or generation speed, but also by neuronal autophagy activity and effectiveness of glymphatic drainage. In fact, defective autophagy and glymphatics are critical contribute to Alzheimer's disease. Thus, it is necessary to identify biomarkers for activity of autophagy and glymphatics in peripheral blood. Only combination of these biomarkers with abnormal tau in peripheral tissues or the circulation may more precisely reflect the status of pathological changes in the brains.

## Conflict of Interest

All authors declare no conflict of interests.

## Acknowledgments

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