APOE ε4 is also required in *TREM2 R47H* variant carriers for Alzheimer's disease to develop.

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In late-onset Alzheimer's disease (AD), the ε4 allele of the apolipoprotein E gene (APOE) is the major known genetic risk factor [1]. In 2013 two research groups reported the *R47H* variant of triggering receptor expressed on myeloid cells 2 (TREM2), is associated with AD by almost as much as *APOE ε4* [2,3]. A loss-of-function *R47H* mutation in *TREM2* is also one of the strongest single allele genetic risk factors for AD [2,3], providing a link between microglia dysfunction and AD pathogenesis. *TREM2* encodes a single-pass type I membrane protein that forms a receptor-signaling complex with the TYRO protein tyrosine kinase-binding protein (TYROBP) triggering immune responses in certain macrophages and dendritic cells.

At Queen Square Brain Bank for Neurological disorders (Institute of Neurology, UCL) and London Neurodegenerative Diseases Brain Bank (Institute of Psychiatry, Psychology and Neuroscience, KCL) we have identified 16 *TREM2* variant cases, 11 cases with neuropathological confirmation of AD and 5 cases identified as normal controls with no underlying AD pathology at the time of death (Figure 1). The cohort includes 5 AD cases with *R47H* variant (cases 6-10) that also carry an *APOE ε4* allele; an AD case carrying an *R47H* variant with no *APOE ε4* allele (case 5) and the remaining 5 AD cases carrying different *TREM2* variants described previously to be associated with AD pathogenesis (cases 1-4) or an additional *PS1* mutation (case 11). Two normal controls carry the *R47H* variant and do not carry an *APOE ε4* allele (cases 15 and 16), two controls have a different *TREM2* mutation and the remaining *R47H* control case died at young age (cases 12-14). This is a small cohort of pathologically confirmed cases that potentially link the *R47H TREM2* variant and *APOE ε4* allele with a diagnosis of AD. In our cohort three other *TREM2* variants *(T96K, Q22X, D87N)* are present with an *APOE ε4* allele, suggesting that *APOE ε4* allele may also be the driving factor rather than then *TREM2* variant. Where the presence of the *R47H TREM2* variant is found in the absence of *APOE ε4* AD does not manifest (Figure 1). The single AD case in this cohort (case 5) with a *R47H* variant which lacked an *APOE ε4* allele, pathologically had an additional diagnosis of frontotemporal lobar degeneration with TDP-43 inclusions (FTLD-TDP subtype A). This case had a much later age of onset compared to the other cases and the additional diagnosis was more than typically observed when a secondary TDP-43 pathology is seen in elderly patients with AD. These findings are supported by pathologically confirmed cases reported in the literature. Korvatska et al reported a large late onset family in which the *R47H* variant co-segregated with 75% of cases [4]. The *R47H* variant was confirmed in 11 individuals affected by AD, and all 11 cases also carried an *APOE ε4* allele. Three unaffected individuals were also shown to carry the *R47H* variant: two died before the typical age of late onset AD and one died at age 87, was both cognitively normal and an *APOE ε3ε3* carrier [4]. Yuan et al included ten *R47H* variant pathologically confirmed AD cases all of which carried an *APOE ε4* allele [5]. Krasemann et al reported the *R47H* variant cases used in their study

also carried an *APOE ε4* allele [6]. Studies that have been unable to show a significant correlation between carrying both the *R47H* variant and an *APOE ε4* allele [3,7]. Including GWAS studies from clinical samples without pathological confirmation and or including other *TREM2* variants in the analysis and not just the *R47H* variant or correlated *APOE ε4* status in AD cases without a *R47H* variant [3,7].

Many *TREM2* variants have been identified which can impact TREM2 localization within the cell. The *R47H* variant is found on the extracellular portion of the protein and impacts ligand binding [8]; the expression and protein levels remain unaltered [7]. Unlike other variants, TREM2 containing the *R47H* variant is mostly localized to the trans-Golgi network rather than the endoplasmic reticulum (ER), comparable to the wild-type receptor [9,10]. Studies employing a TREM2 R47H-Fc chimeric protein revealed the *R47H* variant significantly reduces TREM2 binding to cells [8] and the three isoforms of *APOE* [11]. Although binding seems to occur independently of *APOE* isoforms [11,12], several studies demonstrate that TREM2- *APOE* binding is not dependent on lipid loading [11]. However, others have found that lipidation was necessary to drive TREM2 binding [12] and lipid association was reported to be necessary for TREM2 binding to *APOE* from cynomolgus macaque CSF and serum [11]. *APOE* binding to TREM2 was found to induce TREM2 signaling in reporter cell lines; though how its binding to TREM2 would alter signaling in vivo remains to be determined. As *APOE* can bind to apoptotic cells and amyloid plaques [11], it has been proposed that an interaction between TREM2 and *APOE* may indirectly allow it to mediate recognition and phagocytosis of these substrates. A study by Krasemann et al. shows the mechanism controlling the transition from homeostatic to neurodegenerative microglia (MGnD) is dependent on *APOE*. They also show that the removal of TREM2 locks microglia into a homeostatic state blocking the formation of MGnD microglia, similarly to the effects of *APOE* deficiency. Pathway analysis identified *APOE* as a major upstream inducer of the MGnD microglia phenotype, and the authors turned to TREM2 because it has high affinity for anionic phospholipids in complex with *APOE* on the surface of apoptotic neurons or in lipoproteins. Krasemann et al also found that acquisition of MGnD microglia is dependent on *APOE* and mediated through TREM2 signaling [6].

We propose that pathologically confirmed AD cases carrying the *R47H* variant also carry an *APOE ε4* allele and without an *APOE ε4* allele AD does not develop. As both genetic variants have been confirmed to increase the risk of AD the likelihood of receiving donated brains with both variants is also increased. Our observations from cases donated and published studies suggest that *APOE ε4* allele moderates AD risk in TREM2 *R47H* variants; therefore you are unlikely to develop AD without having an *APOE ε4* allele if you are *TREM2 R47H* positive. No pathological studies have confirmed the lack of underlying AD pathology in *R47H* variant cases without an *APOE ε4* allele and

there is a greater need to obtain pathological confirmation in these cases to validate a connection between the two genetic risk factors. The identification of the *TREM2* locus as a risk factor for AD is important to understand the mechanism by which it influences disease risk. Evidence based on pathologically confirmed cases highlights the association of the *R47H* variant and *APOE ε4* allele in AD although further investigations are needed to determine the effect of *APOE* on *TREM2*. The link between innate immunity and AD pathogenesis, highlighted by genetics studies, emphasizes the importance of exploring *APOE* function in microglia.

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Conflict of interests

The authors declare no conflict of interest in relation to this work.

Author contributions

CT, AH, AK provided the cases from the London Neurodegenerative Disease Brain Bank and detailed pathological and genetic data. TL and CM conceived the study performed the data collection and immunohistochemical staining, prepared the figures and wrote the manuscript. All authors read and approved the final manuscript

Figure 1: Case demographics and comparison of pathological hallmarks in a *TREM*$^{+}$ *APOE ε4*$^{-}$ control case (case 16; panels a and b) and *TREM*$^{+}$*APOE ε4*$^{+}$ Alzheimer's disease case (case 9; panels c-f). The table details the case demographics of the *TREM2* variant cases identified at Queen Square Brain Bank and Institute of Psychiatry, Psychology and Neuroscience. Immunohistochemical analysis of *R47H* variant carriers shows no Aβ deposition in *TREM2 R47H*$^{+}$ *APOE ε4*$^{-}$ (a and b) compared to the characteristic Alzheimer's disease pathology observed in the *TREM2 R47H*$^{+}$ *APOE ε4*$^{+}$ cases: Aβ plaques observed in the hippocampus (c) and frontal cortex (d), along with tau immunohistochemistry in the hippocampus (e) and occipital cortex (f) Scale bar in a represents 500μm in a, c and f; 30μm in b and d: 100 μm in e.

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