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 e3e3 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 \textit{APOE} $\epsilon 4$ allele [6]. Studies that have been unable to show a significant correlation between carrying both the $R47H$ variant and an \textit{APOE} $\epsilon 4$ allele [3,7]. Including GWAS studies from clinical samples without pathological confirmation and or including other \textit{TREM2} variants in the analysis and not just the $R47H$ variant or correlated \textit{APOE} $\epsilon 4$ status in AD cases without a $R47H$ variant [3,7].

Many \textit{TREM2} variants have been identified which can impact \textit{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, \textit{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 \textit{TREM2} R47H-Fc chimeric protein revealed the $R47H$ variant significantly reduces \textit{TREM2} binding to cells [8] and the three isoforms of \textit{APOE} [11]. Although binding seems to occur independently of \textit{APOE} isoforms [11,12], several studies demonstrate that \textit{TREM2- APOE} binding is not dependent on lipid loading [11]. However, others have found that lipidation was necessary to drive \textit{TREM2} binding [12] and lipid association was reported to be necessary for \textit{TREM2} binding to \textit{APOE} from cynomolgus macaque CSF and serum [11]. \textit{APOE} binding to \textit{TREM2} was found to induce \textit{TREM2} signaling in reporter cell lines; though how its binding to \textit{TREM2} would alter signaling in vivo remains to be determined. As \textit{APOE} can bind to apoptotic cells and amyloid plaques [11], it has been proposed that an interaction between \textit{TREM2} and \textit{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 \textit{APOE}. They also show that the removal of \textit{TREM2} locks microglia into a homeostatic state blocking the formation of MGNd microglia, similarly to the effects of \textit{APOE} deficiency. Pathway analysis identified \textit{APOE} as a major upstream inducer of the MGNd microglia phenotype, and the authors turned to \textit{TREM2} because it has high affinity for anionic phospholipids in complex with \textit{APOE} on the surface of apoptotic neurons or in lipoproteins. Krasemann et al also found that acquisition of MGNd microglia is dependent on \textit{APOE} and mediated through \textit{TREM2} signaling [6].

We propose that pathologically confirmed AD cases carrying the $R47H$ variant also carry an \textit{APOE} $\epsilon 4$ allele and without an \textit{APOE} $\epsilon 4$ 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 \textit{APOE} $\epsilon 4$ allele moderates AD risk in \textit{TREM2} $R47H$ variants; therefore you are unlikely to develop AD without having an \textit{APOE} $\epsilon 4$ allele if you are \textit{TREM2} $R47H$ positive. No pathological studies have confirmed the lack of underlying AD pathology in $R47H$ variant cases without an \textit{APOE} $\epsilon 4$ 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 TREM2 APOE ε4 control case (case 16; panels a and b) and TREM2 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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