Is APOE ε4 required for Alzheimer’s disease to develop in TREM2 p.R47H variant carriers?

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Sir,

Murray and colleagues [1] reported the occurrence of pathologically-confirmed Alzheimer’s disease (AD) in cases harbouring the TREM2 p.R47H variant only when APOE ε4 alleles were also present. They suggested that the presence of an ε4 APOE allele is necessary for the development of AD pathology in TREM2 p.R47H carriers. This is an important hypothesis as it can have significant impact on individual risk assessment and on understanding the mechanisms of disease. However, these observations were done in a small cohort of 16 subjects harbouring TREM2 variants. These 16 subjects included 11 AD patients with neuropathological confirmation and 5 controls with no underlying AD pathology at the time of death. The authors reported that 5 of the AD cases with the TREM2 p.R47H variant also carried an APOE ε4 allele; 1 AD case carried the p.R47H variant with no APOE ε4 allele; and the remaining 5 AD cases carried different TREM2 variants. The AD case in the cohort harbouring the TREM2 p.R47H without the APOE ε4 allele had an additional pathological diagnosis of frontotemporal lobar degeneration with TDP-43 inclusions.

Based on these findings, the authors proposed that without an APOE ε4 allele AD would not develop in TREM2 p.R47H variant carriers. They supported this speculation with three main additional pieces of evidence from the literature: 1) Korvatska et al. reported a large late-onset AD family in which the TREM2 p.R47H variant co-segregated with 75% of cases, and all the 11 AD TREM2 p.R47H carriers also presented at least one APOE ε4 allele [2]; 2) other pathologically-confirmed cases in the literature presented both variants [3]; and 3) the functional binding of TREM-2 to ApoE, which is reduced in the presence of p.R47H [4].

Even though AD-associated TREM2 variants are rare, the ε4 allele of APOE is not an uncommon haplotype. Since both are strong genetic risk factors for AD, it is expected that these will be seen co-occurring in many AD patients, particularly if the cohort studied is small.

To further test this hypothesis in a larger cohort, we have analysed the whole exome sequencing (WES) data from the Alzheimer’s Disease Sequencing Project (ADSP) which includes both clinically- and pathologically-diagnosed AD patients and controls. Data was obtained from dbGaP phs000572.v7.p4 [5]. Briefly, all cases met NINCDS-ADRDA criteria for AD [6], and had information on age-at-onset or age-at-death, information on the occurrence of autopsy assessment, and APOE ε2/ε3/ε4 alleles genotype data. Data regarding Phenotype (case/control); TREM2 variants; APOE genotype; Autopsy; Braak stage; and Exclusion Criteria were assessed. Recently published findings in this dataset confirm associations with APOE (P=1.7×10⁻⁸⁴), and TREM2 variants, chiefly p.R47H (P=4.8×10⁻¹²) [7].
This project included WES of 369 samples classified as cases (n=266) or controls (n=103) with an AD-related TREM2 variant (p.R47H, p.R62H or p.Q33X) in addition to information available on APOE genotyping (APOE ε2/ε3/ε4). In this larger sample, 45.1% of cases had a TREM2 variant and one or more APOE ε4 alleles, while the remaining 54.9% harboured a TREM2 variant without any APOE ε4 alleles.

When restricting this analysis to pathologically-confirmed AD cases with Braak stages of 5 or 6 (n=81), 36 (44.4%) patients carried TREM2 variants and at least one APOE ε4 allele and, notably, 45 (55.6%) AD cases did not carry any APOE ε4 allele. The same is found when analysing only AD patients carriers of TREM2 p.R47H (n=35): 16 (45.7%) had at least one APOE ε4 allele while 19 (54.3%) had no APOE ε4 alleles. The cohort also included 15 pathologically-assessed controls (Braak=0) harbouring TREM2 variants (p.R47H or p.R62H), 3 of these also had at least one APOE ε4 allele and 12 did not carry any APOE ε4 alleles. However, the selection of samples for the ADSP project was partly based on genetic risk, with cases being preferentially selected to have low known genetic risk (i.e., the sample is enriched for cases without APOE ε4), which means that the frequency of ε4 alleles in this dataset is skewed [5].

To overcome this potential bias, we performed a similar analysis on the ADGC dataset, which used the Illumina Exome Array (v1.1) for genotyping [8]. Genotyped subjects (n=12,794), were sampled randomly from among ADGC datasets with available DNA, and some of these subjects also had WES in the ADSP; this sample set had a distribution of APOE genotypes representative of most AD case-control datasets and findings reported in the study included strong associations at both APOE (P=2.7×10^{-105}) and TREM2, particularly for p.R47H (P=5.4×10^{-24}) and p.R62H (P=1.6×10^{-14}). Of these, 7,285 were AD cases and 342 carried at least one of the TREM2 p.R47H or p.R62H variants. APOE genotypes were available for 274 of these individuals. In this set of samples, 176 cases (64.2%) had a TREM2 variant and at least one APOE ε4 allele, while the remaining 98 cases (35.8%) presented a TREM2 variant without any APOE ε4 alleles.

From these 274 individuals, 155 were pathologically-confirmed to have AD. When restricting the analysis to these individuals, 97 (62.6%) carried TREM2 variants and at least one APOE ε4 allele, while 58 (37.4%) cases did not carry any APOE ε4 allele. More specifically for TREM2 p.R47H, 25 of these carriers did not harbour any APOE ε4 allele.

Murray and colleagues’ conclusions are three-fold and we address these individually below:

1. **The APOE ε4 allele may be the driving factor rather than the TREM2 variant.** This hypothesis was tested in one of the original papers describing TREM2 variants in AD, where the authors assessed the association of TREM2 p.R47H with AD in APOE ε4 negative individuals (see Table S3 from [9]). The association was highly significant, conclusively showing that the TREM2 association is independent of APOE ε4.

2. **Pathologically confirmed AD cases carrying the p.R47H variant also carry an APOE ε4 allele and without an APOE ε4 allele AD does not develop.** As shown above, in larger cohorts of pathologically-confirmed AD cases, there are 19 and 25 cases (ADSP and
ADGC cohorts, respectively) with TREM2 p.R47H and without APOE ε4, which conclusively refutes this hypothesis.

3. **An individual is unlikely to develop AD without having an APOE ε4 allele if they are TREM2 p.R47H positive.** This can, again, be refuted by the work of Jonsson and colleagues [9] showing independent association of TREM2 with AD. Additionally, we report on 274 cases of the ADGC cohort (155 of which are autopsyConfirmed) with TREM2 variants and without APOE ε4, showing that AD does indeed develop in TREM2-positive, APOE ε4 negative individuals.

In summary, by studying a larger cohort including a substantial number of neuropathologically-confirmed AD cases, we show that Alzheimer’s disease pathology exists in a significant number of cases carrying only the TREM2 p.R47H, without the presence of APOE ε4 alleles.

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**Author contributions**


**Conflicts of interest**

The authors have no conflicts of interest.

**References**


