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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* ($P=2.7\times10^{-105}$) and *TREM2*, particularly for p.R47H ($P=5.4\times10^{-24}$) and p.R62H ($P=1.6\times10^{-14}$). 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 autopsy-confirmed) 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

R.G., T.O., A.C.N., A.B.K., G.D.S., J.B.: analysis and interpretation of data; R.G., T.O., A.C.N., A.B.K., G.D.S., J.B.: critical revision of manuscript for intellectual content; A.C.N., A.B.K., G.D.S.: acquisition of data; R.G., J.B.: study concept and design; R.G., J.B.: writing of first manuscript draft; R.G., J.B.: study supervision.

Conflicts of interest

The authors have no conflicts of interest.

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