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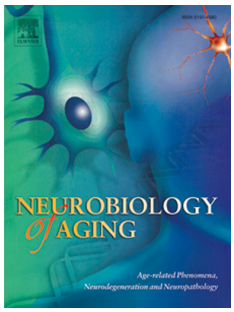


The dementia-associated *APOE* ε4 allele is not associated with REM sleep behavior disorder

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# The dementia-associated *APOE* ε4 allele is not associated with REM sleep behavior disorder

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**Abstract:** The current study aimed to examine whether the *APOE* ε4 allele, associated with dementia with Lewy bodies (DLB), and possibly with dementia in Parkinson's disease (PD), is also associated with idiopathic REM sleep behavior disorder (RBD). Two SNPs, rs429358 and rs7412, were genotyped in RBD patients (n=480) and in controls (n=823). *APOE* ε4 allele frequency was 0.14 among RBD patients and 0.13 among controls (OR=1.11, 95% CI 0.88-1.40, *p*=0.41). *APOE* ε4 allele frequencies were similar in those who converted to DLB (0.14) and those who converted to PD (0.12) or multiple system atrophy (0.14, *p*=1.0). The *APOE* ε4 allele is neither a risk factor for RBD nor it is associated with conversion from RBD to DLB or other synucleinopathies.

**1. Introduction:** Rapid eye movement (REM) sleep behavior disorder (RBD) is currently the strongest clinical prodromal feature preceding the development of an overt synucleinopathy, including Parkinson's disease (PD), dementia with Lewy bodies (DLB) or multiple system atrophy (MSA) (Iranzo, et al., 2014). One of the strongest genetic factors associated with DLB is the *APOE* epsilon4 (ε4) allele (Pickering-Brown, et al., 1994), and PD patients who carry this allele may be at increased risk for developing dementia. Since both RBD and the *APOE* ε4 allele are possibly associated with DLB, and with dementia in PD patients, we aimed to examine whether the *APOE* ε4 allele is associated with RBD and conversion to DLB. See Supplementary file for detailed introduction and full list of references.

**2. Methods:** The study population included idiopathic RBD patients (n=480) and controls (n=823) of European ancestry. RBD patients were diagnosed using clinical interview and polysomnography according to the ICSD-2 (International Classification of Sleep Disorders, version 2) criteria. The control group was composed of 253 elderly controls (age 59.5±9.8 years, matched to the available age at onset (AAO) of RBD, n=307, age 59.2±11.5), 510 young controls (age 34.0±6.5 years), and additional 60 controls with no available data on age. All control groups had nearly identical frequencies of the *APOE* ε4 allele (0.13, 0.13 and 0.14, respectively), which allowed us to analyze all controls combined. All

individuals signed informed consent forms at enrollment, and the study protocols were approved by the respective institutional review boards. DNA was extracted using a standard salting-out protocol. Two single nucleotide polymorphisms (SNPs), rs429358 and rs7412, were genotyped using TaqMan SNP genotyping assays. Genotypes were called using the QuantStudio™ 7 Flex Real-Time PCR System and Software (v 1.0). Goodness of fit test with one degree of freedom was applied to look for deviation from the Hardy-Weinberg equilibrium (HWE) among the controls. Differences in *APOE* allele or carriage frequencies were analyzed using the Fisher's exact test, and differences in continuous variables were analyzed using t-test. A logistic regression model with age and sex as covariates was also applied. All statistical analysis was done using SPSS statistics V.23 (IBM Inc.). Detailed methods can be found in the supplementary file.

**3. Results:** The allele frequency of *APOE* ε4 was 0.14 among RBD patients and 0.13 among controls (OR=1.11, 95% CI 0.88-1.40, $p$=0.41). Overall, 25.8% of RBD patients carried at least one *APOE* ε4 compared to 23.0% among controls ($p$=0.25, Fisher's exact test), and there were more homozygous carriers of the *APOE* ε4 allele among controls (3.2%) as compared to RBD patients (2.7%). Logistic regression model adjusted for age and sex also demonstrated lack of association between *APOE* ε4 allele carriage and risk for RBD (OR = 1.25, 95% CI 0.87-1.79, $p$=0.23). There was no difference in AAO when comparing carriers (n=88) and non-carriers (n=219) of the *APOE* ε4 allele (59.1 ± 8.4 vs. 59.3 ± 12.6 years, respectively, $p$=0.92, t-test). A total of 140 RBD patients (29.2%) were reported to have converted to either PD (n=98, 70% of the converters), dementia/DLB (n=28, 20%) or MSA (n=14, 10%). The carrier frequencies of one or more *APOE* ε4 in these groups were similar; 23.5%, 25.0% and 28.6%, respectively ($p$=0.91), and the allele frequencies were 0.12, 0.14 and 0.14 ($p$=1.0). The *APOE* ε4 allele frequency among those that did not convert was slightly higher, 0.15 (Table 1), with a total of 26.5% carriers of at least one *APOE* ε4 allele, compared to 24.3% among those who converted ($p$=0.65). More detailed results can be found in the supplementary file.

**4. Discussion:** Although RBD is a strong risk factor for developing DLB, and although DLB was reported to be associated with the *APOE* ε4 allele, our results demonstrate lack of association between the *APOE* ε4 allele and RBD or its age at onset. These and previous results further suggest that RBD may have a distinct genetic background; it is associated with *GBA* mutations (Gan-Or, et al., 2015b), but unlike PD it is not associated with *LRRK2* mutations (Fernandez-Santiago, et al., 2016), and unlike DLB it is not associated with the *APOE* ε4 allele. Thus far, *GBA*, *SCARB2*, and potentially *SNCA* (Gan-Or, et al., 2015a) overlap between RBD, PD and DLB (Supplementary Figure 1, see Supplementary file). Whether RBD has additional, unique genetic factors that were not identified in PD or DLB cohorts is still to be determined. Our current study identified similar frequencies of *APOE* ε4 allele in those who progressed to PD, DLB and MSA, suggesting that *APOE* alleles do not affect the type of subsequent synucleinopathy. Our study has some limitations, and a more detailed discussion including full list of references can be found in the supplementary file. Our results support a distinct genetic background for RBD-associated neurodegeneration, probably suggesting a specific genetic association with synucleinopathy rather than tauopathy/amyloidopathy.

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**Disclosure statement**

ZGO received consultation fees from Sanofi/Genzyme. JYM reports grants from Merck, GlaxoSmithKline, received speaking honoraria from Valeant Pharmaceutical, and Otsuka Pharmaceutical, serves on the advisory boards of Sanofi-Aventis, Servier, Merck, Jazz Pharma, Valeant Pharma, Impax Laboratories, Glaxo-SmithKline, UCB Canada, received consultancy fees from Otsuka Pharma, and Valeant Pharma. JPR reports no conflict of interests. JP reports no conflict of interests. SCW received honoraria from Pfizer, Bristol-Myers Squibb, SmithKline Beecham and Eli Lilly. IA received speaker honoraria form UCB Pharma. SS reports no conflict of interests. YD is on the advisory board and received travel and consultancy fees from UCB Phrma, bioprojet, and Jazz Pharma. CSL reports no conflict of interests. MTH reports no conflict of interests. BH received grant from UCB, speaker honoraria from UCB, Otsuka, Abbvie, Lundbeck, Lilly, Mundipharma. Serving on advisory boards or consulting for Mundipharma, Axovant. Received travel support from Habel Medizintechnik, Vivisol. AS reports no conflict of interests. CCM received fees for serving on advisory board of UCB pharma, lecture fees from UCB Pharma, Orkyn. VCD received funding from Orkyn, LVL medical, Teva and UCB. MB reports no conflict of interests. LFS reports no conflict of interests. GP served on the advisory board of UCB pharma, Jazz pharmaceuticals and Bioproject. EA reports no conflict of interests. PY received honoraria for speakers bureaus by Sanofi Genzyme, Biomarin, UCB pharma, Medice, ResMed and Heinen und Loewenstein. Member of advisory boards for Sanofi Genzyme, Biomarin, Vanda and Medice. AH received travel support Habel Medizintechnik, received lecture honoraria from UCB, Heinen und Löwenstein. TRB reports no conflict of interests. SGE reports no conflict of interests. MR reports no conflict of interests. PAD reports no conflict of interests. AD received research grants from Novartis pharma, Jazz Pharmaceuticals, Biron soins du sommeil. Received speaker honoraria from UCB and Paladin labs.

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**Table 1. *APOE* haplotypes in individuals with RBD and controls**

| ***APOE*** | **ε2/ε2 n, (%)** | **ε2/ε3 n, (%)** | **ε3/ε3 n, (%)** | **ε2/ε4 n, (%)** | **ε3/ε4 n, (%)** | **ε4/ε4 n, (%)** | **Total carriers of ε4, n (%)** | **ε4 allele frequency** |
|---|---|---|---|---|---|---|---|---|
| **RBD patients, n=480** | 4 (0.8) | 51 (10.6) | 301 (62.7) | 4 (0.8) | 107 (22.3) | 13 (2.7) | 124 (25.8) | 0.14 |
| **RBD converted to synucleinopathy[a], n=140** | 3 (2.1) | 12 (8.6) | 91 (65.0) | 1 (0.7) | 32 (22.9) | 1 (0.7) | 34 (24.3) | 0.13 |
| **RBD not converted to synucleinopathy, n=340** | 1 (0.3) | 39 (11.5) | 210 (61.8) | 3 (0.9) | 75 (22.1) | 12 (3.5) | 90 (26.5) | 0.15 |
| **Controls, n=823** | 5 (0.6) | 111 (13.5) | 518 (62.9) | 14 (1.7) | 149 (18.1) | 26 (3.2) | 189 (23.0) | 0.13 |

n, number; RBD, REM sleep behavior disorder

[a] PD, dementia/DLB or MSA