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

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Abstract

Background: A significant proportion of individuals with REM sleep behavior disorder (RBD) will progress to dementia with Lewy bodies (DLB). We aimed to examine whether the *APOE* ε4 allele, associated with DLB, is also associated with idiopathic RBD.

Methods: The two SNPs tagging the different *APOE* alleles (rs429358 and rs7412) were genotyped in individuals who were initially diagnosed with RBD (n=480) and in controls (n=823).

Results: *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), and this lack of association remained after adjustment for age and sex. Furthermore, allele frequencies of *APOE* ε4 were similar in those who converted to DLB (0.14) and those who converted to Parkinson's disease (0.12) or multiple system atrophy (0.14, p=1.0).

Conclusions: The *APOE* ε4 allele is neither a risk factor for RBD nor it is associated with conversion from RBD to DLB or other synucleinopathies.

Introduction

Rapid eye movement (REM) sleep behavior disorder (RBD), characterized by lack of atonia and enacting of dreams during REM sleep, is currently the strongest clinical prodromal feature preceding the development of an overt synucleinopathy. In long term follow-up, more than 80% of individuals with idiopathic RBD developed either Parkinson's disease (PD), dementia with Lewy bodies (DLB) or multiple system atrophy (MSA).[1, 2] It was recently suggested that RBD may define a subtype of PD patients,[3] further supported by the association of RBD with cognitive decline,[4] dementia,[5] hallucinations[6] and autonomic dysfunction,[7] as compared to PD patients without RBD. In addition, pathological studies in brains of PD patients with and without RBD that demonstrated a more widespread α-synuclein accumulation associated with RBD.

If indeed RBD represents a subtype of PD, or a subtype of synucleinopathies, it is possible that it has specific genetic background. A preliminary study that examined the association of RBD with several genetic risk factors for PD, identified an association mainly with *MAPT* and *SCARB2*, and marginal or lack of association with other markers.[8] A recent study suggested that RBD is associated with mutations in *GBA* in both idiopathic RBD and PD cohorts.[9] This association was stronger than the association of *GBA* mutations with PD in a similar population,[10] suggesting that *GBA* may be one of the genetic factors that is more specific to RBD. Furthermore, the association of *GBA* mutations with DLB[11] also seems to be stronger than the association with PD.[12] Conversely, mutations in *LRRK2* were not associated with RBD,[13-15] further supporting the hypothesis that RBD has a distinct genetic background.

One of the strongest genetic factors associated with DLB is the *APOE* epsilon4 (ε4) allele,[16] and PD patients who carry this allele may be at increased risk for developing

dementia.[17] Since both RBD and the *APOE* ε4 allele are 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.

Methods

Population

The study population included consecutively recruited, unrelated idiopathic RBD patients (n=480) and controls (n=823) of European ancestry. RBD patients were collected by collaborators from the international RBD study group and were diagnosed using polysomnography according to the ICSD-2 criteria. The control group was composed of 253 elderly controls (age 59.5±9.8 years, matched to the available 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. However, all control groups had nearly identical frequencies of the *APOE* ε4 allele (0.13, 0.13 and 0.14, respectively), suggesting lack of age-effect, 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.

Genotyping

DNA was extracted using a standard salting-out protocol. To determine the *APOE* haplotypes, two tagging single nucleotide polymorphisms (SNPs), rs429358 and rs7412, were genotyped using TaqMan SNP genotyping assays (C___3084793_20 and C____904973_10, respectively, ThermoFisher Scientific Inc.) according to the manufacturer's instructions. Genotypes were called using the QuantStudio™ 7 Flex Real-Time PCR System and Software (v 1.0). Carriers of T in

rs429358 and T in rs7412 were determined as carriers of the ε2 allele, carriers of T in rs429358 and C in rs7412 were determined as carriers of the ε3 allele, and carriers of C in rs429358 and C in rs7412 were determined as carriers of the ε4 allele.

Statistical analysis

Categorical variables are presented as percentage or frequencies, whereas continuous variables are presented as mean ± standard deviation. 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. To further avoid a potential bias due to age, and since sex distribution was different among patients with RBD vs. controls, 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.).

Results

Lack of association between the *APOE* ε4 and RBD risk and age at onset

Table 1 details the different *APOE* alleles in RBD patients and controls. The frequency of the two SNPs defining the *APOE* alleles did not deviate from HWE. 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). 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). Data on age at onset (AAO) of RBD was available for 307 individuals, and 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).

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[b], 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
[b] including individuals without information on conversion

The *APOE* ε4 allele is not associated with conversion to PD, DLB or MSA.

Since most of the patients in our cohort are being followed-up longitudinally, we examined whether the *APOE* ε4 allele is associated with conversion to either PD, dementia/DLB, or MSA. 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 or whose information was not available 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).

Discussion

Although RBD is a strong risk factor for developing DLB,[1, 18] and although DLB was reported to be associated with the *APOE* ε4 allele[16, 19, 20], our results demonstrate lack of association between the *APOE* ε4 allele and RBD or its age at onset. These and previous results[8, 9, 13, 15] further suggest that RBD may have a distinct genetic background; it is associated with *GBA* mutations, [9] but unlike PD it is not associated with *LRRK2* mutations,[13, 15] and unlike DLB it is not associated with the *APOE* ε4 allele. Thus far, *GBA*, *SCARB2,* and potentially *SNCA* overlap between RBD, PD and DLB (Figure 1).[8, 9, 21] Whether RBD has additional, unique genetic factors that was not identified in PD or DLB cohorts is still to be determined.

Since PD patients with RBD are likely to develop dementia and hallucinations,[5, 6] eventually presenting a phenotype similar to DLB, and based on the current and previous genetic and post-mortem results,[22] we hypothesize that RBD-associated synucleinopathy (the central common area in Figure 1) is the same clinical-pathological entity, whether it is defined as parkinsonism first with subsequent dementia and hallucinations, or whether it is defined as DLB with subsequent parkinsonism. In that sense, RBD can be considered as a marker for diffuse synucleinopathy, which may be a better description of the disease than DLB or PD with dementia. Neuropathological data from *GBA* mutations carriers also demonstrated a more diffuse synucleinopathy,[23-25] further supporting this notion. It is possible that stochastic events, or other genetic or environmental factors, determine whether α-synuclein will first be deposited in brain

areas associated with dementia and later in the areas associated with parkinsonism, or vice versa. The observation that α-synuclein can progress in the brain in a prion-like fashion[26-28] may support a stochastic progression hypothesis, however additional neuro-pathological studies are needed to examine this possibility.

The association of RBD with the more devastating synucleinopathy, MSA, also necessitates more studies. Whether unique genetic or environmental factors affect the risk to progress from RBD to MSA is still unknown. Our current study identified similar frequencies of *APOE* ε4 allele in those who progressed to PD, DLB and MSA, suggesting that *APOE* is not one of these factors. Interestingly, a recent study suggested that *GBA* mutations are associated with MSA as well,[29] however this observation is awaiting replications in additional cohorts.

Our study has a few limitations. The control population was younger than the RBD and PD groups. To tackle this limitation, we took two approaches. First, we demonstrated that the frequencies of the *APOE* ε4 allele were similar across generations (i.e. in the elderly and young control groups), which rules out a potential bias. Furthermore, we also performed a logistic regression model with adjustment for age, which further demonstrated lack of association between the *APOE* ε4 allele and RBD. Another potential limitations stems from RBD patients being recruited in multiple centers, which may lead to a potential population dependent bias, in case one or more of these RBD cohorts are enriched in *APOE* ε4 allele carriers. However, since the frequencies of the *APOE* ε4 allele were similar across centers, this possibility was ruled out.

To conclude, our results support a distinct genetic background for RBD-associated neurodegeneration, probably suggesting association with isolated synucleinopathy rather than tauopathy. To examine the hypotheses raised by the current and previous work, larger studies are

necessary, including genome wide association and next-generation sequencing studies focusing on RBD, and comparing them to results from PD, DLB and MSA.

Acknowledgments

Authors' Roles

1) Research project: A. Conception, B. Organization, C. Execution;

2) Statistical Analysis: A. Design, B. Execution, C. Review and Critique;

3) Manuscript: A. Writing of the first draft, B. Review and Critique.

ZGO – 1A, 1B, 1C, 2A, 2B, 3A
JYM – 1A, 1B, 1C, 2C, 3B
JPR – 1C, 2B, 2C, 3B
SCW – 1A, 1B, 1C, 3B
SS – 1B, 1C, 3B
YD – 1B, 1C, 3B
CSL – 1B, 2C, 3B
IA – 1B, 1C, 3B
MTH – 1B, 1C, 3B
BH – 1B, 1C, 3B
AS – 1B, 1C, 3B
CM – 1B, 1C, 3B
VCD – 1B, 1C, 3B
MB – 1B, 1C, 3B
EA – 1B, 1C, 3B
AH – 1B, 1C, 3B
AD – 1B, 1C, 3B
JFG – 1B, 1C, 3B
ND – 1B, 1C, 3B
RBP – 1B, 1C, 3B
PAD – 1A, 1B, 3B
GAR – 1A, 1B, 1C, 2C, 3B

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Tables and Figures.

Figure 1. Venn diagram of the genetic overlap between PD, DLB and RBD.

While *GBA* mutations, *SCARB2* and possible *SNCA* variants are associated with all three conditions, other genetic variants such as *APOE* ε4 in DLB and *LRRK2* mutations in PD are distinctively associated with each condition but not with RBD. It is therefore likely that RBD-associated neurodegeneration (the overlapping area of PD, DLB and RBD) may have distinct genetic background. While thus far no genetic variants that are uniquely associated with RBD were discovered, it is possible that such genetic risk factors do exist, and that they were not discovered in PD/DLB studies since RBD cases are diluted within the cohorts used to study these diseases.