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Apolipoprotein E gene in physiological and pathological aging

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ABSTRACT

Introduction: The genetic background plays a role on longevity. The distribution of the apolipoprotein E gene (*APOE*) variants (ε2, ε3, ε4) may differ across age groups, especially in the oldest old and despite geographical and ethnic specificities. Since the ε4 variant is associated with Alzheimer's disease (AD), it might represent an opportunity for exploring the relationship of *APOE* with physiological and pathological aging.
Aim: To explore the role played by *APOE* genotype/alleles on physiological and pathological brain aging.
Materials and Methods: The study was conducted in a cohort of centenarians (n = 106), and two cohorts of octogenarians (without cognitive decline, n = 351 controls; and with AD, n = 294).
Results: No significant differences in genotype/allele distributions were observed comparing controls to centenarians. The prevalence of ε2/ε3, ε3/ε3, ε3/ε4 and ε4/ε4 genotypes were significantly different in centenarians compared to AD. The prevalence of ε2 and ε3 alleles were significantly higher in centenarians, whereas the ε4 was less frequent. The ε4 allele was positively associated with AD, whereas a negative association was found for ε2 and ε3 alleles.
Conclusions: Our study indicates that ε4 allele is strongly associated with AD. *APOE* significantly affects AD risk, but apparently not longevity.

1. Introduction

The global increase of life expectancy and the rapid aging of the population represent major epidemiological phenomena characterizing the past two centuries. They have major economic implications for public health policies due to the higher complexity of persons referred to clinical services (often affected by multiple age-related diseases, chronic conditions, and disabilities). This has led many researchers at focusing on the biology of aging for better understanding its underlying mechanisms with the final aims of preventing the onset of age-related conditions.

Centenarians represent an extraordinary model for studying successful aging and identifying its biological determinants (Franceschi et al., 2017). The prevention or delayed onset of age-related conditions is the most reasonable way for reaching the considerable age of 100 years of life.

Survival analyses of centenarians and their families as well as research on monozygotic and dizygotic twins have suggested that the longevity phenotype presents a genetic component, accounting for about 25% of the intra-population phenotypic variance (De Benedictis and Franceschi, 2006; Franceschi et al., 2007). Specific genetic patterns have also been associated to successful aging (De Benedictis and Franceschi, 2006; Kulminski et al., 2015; Franceschi and Garagnani, 2016).

In this context, the apolipoprotein E gene (*APOE*) is probably the most thoroughly investigated. *APOE* is a protein involved in the lipid metabolism and the transport of cholesterol to neurons (Riedel et al., 2016; Rosenberg et al., 2016). There are three common alleles of the *APOE* gene (*i.e.*, ε2, ε3, and ε4), which encode three isoforms of the protein (*i.e.*, E2, E3, and E4) (Verghese et al., 2011). *APOE* is recognized as the most important gene associated with longevity in many studies across Europe and in a number of meta-analyses (Deelen et al., 2011; Fortney et al., 2015).

Interestingly, the *APOE* gene variant ε4 is also associated with early and late onset Alzheimer's disease (AD) (Genin et al., 2011; Lescai et al., 2011; Skillback et al., 2018). A long-lasting controversy in the literature is open about the possible antagonistic pleiotropic effects of the ε4 allele of the *APOE* gene, thus conferring to the individual advantages on cognitive tasks early in life, but cognitive and neural disadvantages at more advanced age (Albin, 1993; Han and Bondi, 2008).

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It should be noted that due to a probable genetic drift and natural selection, there are large differences in the distribution of the *APOE* alleles across geographical areas and populations (Hallman et al., 1991; Singh et al., 2006). For example, the prevalence of *APOE* ε4 is relatively high in Northern Europe (15–21%), relatively modest in Central Europe (11–13%), and low in Southern Europe (4–6%) (Lewis and Brunner, 2004). Such differences may explain why a recent meta-analysis showed only a modest pooled effect size for: 1) the negative association between ε4 allele and longevity, and 2) the positive association of ε2 allele and this trait (Revelas et al., 2018). On the other hand, the association between ε4 allele and sporadic AD is so strong to lead researchers at indicating the effect of *APOE* on AD similar to that of a "major gene" rather than a mere "risk gene" (Genin et al., 2011).

In general, the ε4 heterozygosity is associated with a 3- or 4-fold increased risk of developing AD, while homozygosity raises the risk to 10–15 times (compared to the most common genotype ε3/ε3) (Corder et al., 1993; Poirier et al., 1993). Moreover, the role of *APOE* ε4 allele has recently been revised across different forms of dementia in a large cohort of elderly, showing how this allele may influence the cerebrospinal fluid biochemical profile and the life span of AD patients (Skillback et al., 2018).

Nowadays, most studies exploring the association of a gene locus with longevity have been conducted in cognitively normal individuals, in most cases nonagenarians (Deelen et al., 2011; Beekman et al., 2013; Deelen et al., 2014; Fortney et al., 2015). In the present study, we analyse the *APOE* gene profile in a cohort selectively composed of centenarians and semi-supercentenarians from Northern Italy, comparing it to 1) octogenarians without cognitive decline (i.e., controls), and 2) well-characterized patients with AD from the same geographical region. Our approach allows to maximize the eventual differences between subjects with extreme longevity (i.e., centenarians) and the comparison groups (i.e., controls and AD patients) for discriminating the role played by the *APOE* alleles in the physiological and pathological aging of the brain.

2. Materials and methods

2.1. Study design and participants

To identify the potential participants for the study, we contacted 46 registry offices in Northern Italy for collecting contact information and dates of birth of living people aged 100 years and older at the time of enrolment. A letter explaining the methods and goals of the study was sent to each eligible individual. We applied stringent demographic criteria to select the eligible subjects for this study (Gentilini et al., 2013).

Briefly, we recruited 106 subjects born in Northern Italy between the 1899 and 1908. A trained multidisciplinary staff went to each centenarian's house or nursing home to administer to all subjects a standardized structured questionnaire focused on their health status, cognitive and physical function, pharmacological treatments, clinical history, and lifestyle habits (Skytthe et al., 2011). Clinical documentation was also retrieved for recording data about past and current diseases. The cognitive status was assessed by the Italian version of the Mini-Mental State Examination (MMSE) test (Ravaglia et al., 1999).

In the present study, we also take advantage of two other existing cohorts. The first one was composed of 294 patients with AD referred outpatients to the Geriatric Unit of the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico (Milan, Italy). The second cohort (i.e., the control group) was constituted by 351 age-matched octogenarians without cognitive decline (previously described in Guerini et al., 2014; Gussago et al., 2016). The diagnosis of AD was made according to current recommendations (Dubois et al., 2014). AD diagnosis was supported by brain imaging (MRI and FDG-PET) and, when the subjects consented to lumbar puncture, also by the assessment of β-amyloid, tau, and p-tau levels.

At the recruitment, blood samples from all participants were collected between 8 and 9 a.m., following at least 6 h of fasting. The study protocol received approval from the local Ethical Committee. All subjects gave their informed consent to participate in the study.

2.2. DNA extraction and APOE genotyping

Genomic DNA was extracted using a previously described salting-out method (Miller et al., 1988) and its concentration and purity were determined by the means of spectrophotometric analysis. *APOE* genotyping was performed using PCR restriction fragment length polymorphism (PCR-RFLP) as previously described (Arosio et al., 2004). In brief, a 244 bp *APOE* fragment was amplified by PCR (step 1: 96 °C for 5 min; step 2 for 30 cycles: 95 °C for 1 min, 60 °C for 1.10 min, and 70 °C for 2 min; step 3: 70 °C for 10 min) with the primer pairs: 5′-GATCAA GCTTCCAATCACAGGCAGGAAG-3′ and 5′-GATCCGGCCGCACACGTCC TCCATG-3′. The amplified fragment was digested by using the HhaI enzyme and the products were visualized on agarose gel.

2.3. Statistical analyses

Statistical analyses were performed using the SPSS statistical package (SPSS version 24, Chicago, IL, USA). Age of the groups was expressed as mean values ± standard deviation (SD). Hardy–Weinberg equilibrium (HWE) was tested with the X^2 test. Genotype/allele frequencies were compared between centenarians and AD patients, between AD patients and controls and between centenarians and controls by using the Fisher's exact test. A binary logistic regression analysis adjusted by sex was performed to test if a given allele/genotype was associated to the risk to develop AD and the chance to become a centenarian.

A p value < 0.05 was considered as threshold for statistical significance.

3. Results

Centenarians, AD patients, and controls had a mean age (± standard deviation [SD]) of 102.48 ± 2.95, 79.56 ± 5.62, and 79.40 ± 6.47 years, respectively. The results of genotype/allele frequency distributions and the data of binary logistic regression are shown in Table 1. The genotype distributions were in HWE in all groups considered. There were no centenarians carrying the ε2/ε2 and ε4/ε4 genotypes and only one of them carried the ε2/ε4 genotype.

No significant differences in the genotype and allele distributions were observed comparing the cohort of controls to centenarians (Table 1A). A p value close to statistical significance was only found for the ε4 allele distribution (Table 1A).

When we compared centenarians to AD patients, the frequencies of ε2/ε3, ε3/ε3, ε3/ε4 and ε4/ε4 genotypes were significantly different. The frequencies of ε2 and ε3 alleles were significantly higher in centenarians, whereas the ε4 allele had a lower prevalence among the long-lived subjects (Table 1B).

Similarly, comparing controls to AD patients, the frequencies of ε2/ε3, ε3/ε3, ε3/ε4 and ε4/ε4 genotypes were significantly different. The frequencies of ε2 and ε3 alleles were significantly higher in controls, whereas the ε4 allele was less frequent in the subjects without cognitive decline (Table 1C).

In the binary logistic regression analyses between centenarians and controls, none allele was associated with the chance of being a centenarian (Table 1A). When analyses were performed comparing AD patients versus centenarians and controls, it was documented that the ε4 allele was positively associated with AD, whereas the ε2 and ε3 alleles were negatively associated (Table 1B and C).

The prevalence of centenarians affected by cognitive impairment was 48.8%. No difference was observed in the prevalence of centenarian carriers of ε4 allele with cognitive impairment compared to

Table 1
Genotype and allele distributions of the apolipoprotein E (*APOE*) gene and binary logistic regression in centenarians (CENT), octogenarians without cognitive decline (CT) and Alzheimer's disease patients (AD). Significant p values and associations are in bold. 95%CI, 95% confidence interval; OR, odds ratio (adjusted by sex).

A	CENT		CT		CENT vs CT Fisher's test	CENT vs CT binary logistic regression	
	n	%	n	%	p value	OR (95%)	p value
Genotype							
ε2/ε2	–	–	10	2.8	0.07	–	–
ε2/ε3	12	11.4	26	7.4	0.14	1.44 (0.68 - 3.05)	0.34
ε2/ε4	1	0.9	2	0.6	0.55	1.73 (0.15 - 19.7)	0.66
ε3/ε3	79	74.5	243	69.3	0.18	1.27 (0.77 - 2.10)	0.35
ε3/ε4	14	13.2	66	18.8	0.12	0.71 (0.38 - 1.33)	0.29
ε4/ε4	–	–	4	1.1	0.35	–	–
Allele							
ε2	13	6.1	48	6.8	0.43	0.85 (0.44 - 1.63)	0.62
ε3	184	86.8	578	82.3	0.07	1.36 (0.87 - 2.15)	0.18
ε4	15	7.1	76	10.9	0.06	0.67 (0.37 - 1.19)	0.17

B	AD		CENT		AD vs CENT Fisher's test	AD vs CENT binary logistic regression	
	n	%	n	%	p value	OR (95%)	p value
Genotype							
ε2/ε2	4	1.4	–	–	0.29	–	–
ε2/ε3	6	2.0	12	11.4	< **0.001**	0.18 (0.06 - 0.49)	< **0.001**
ε2/ε4	3	1.0	1	0.9	0.71	1.02 (0.10 - 10.13)	0.99
ε3/ε3	149	50.7	79	74.5	< **0.001**	0.34 (0.21 - 0.56)	< **0.001**
ε3/ε4	112	38.1	14	13.2	< **0.001**	3.98 (2.16 - 7.33)	< **0.001**
ε4/ε4	20	6.8	–	–	**0.02**	–	–
Allele							
ε2	17	2.9	13	6.1	**0.03**	0.48 (0.22 - 1.03)	**0.05**
ε3	416	70.7	184	86.8	< **0.001**	0.86 (0.23 - 0.56)	< **0.001**
ε4	155	26.4	15	7.1	< **0.001**	4.63 (2.65 - 8.08)	< **0.001**

C	AD		CT		AD vs CT Fisher's test	AD vs CT binary logistic regression	
	n	%	n	%	p value	OR (95%)	p value
Genotype							
ε2/ε2	4	1.4	10	2.8	0.15	0.55 (0.17 - 1.78)	0.32
ε2/ε3	6	2.0	26	7.4	< **0.001**	0.25 (0.10 - 0.62)	0.25
ε2/ε4	3	1.0	2	0.6	0.42	1.95 (0.32 - 11.94)	0.47
ε3/ε3	149	50.7	243	69.3	< **0.001**	0.44 (0.32 - 0.61)	< **0.001**
ε3/ε4	112	38.1	66	18.8	< **0.001**	2.73 (1.90 - 3.92)	< **0.001**
ε4/ε4	20	6.8	4	1.1	< **0.001**	6.56 (2.20 - 19.56)	< **0.001**
Allele							
ε2	17	2.9	48	6.8	< **0.001**	0.43 (0.24 - 0.76)	< **0.001**
ε3	416	70.7	578	82.3	< **0.001**	0.50 (0.38 - 0.65)	< **0.001**
ε4	155	26.4	76	10.9	< **0.001**	3.05 (2.25 - 4.14)	< **0.001**

those without (17.1% *versus* 16.3%; p = 0.576).

4. Discussion

In our study, the *APOE* genotype and allele distributions were significantly different across centenarians, AD patients, and controls; specific genetic/allelic profiles were associated with AD.

APOE represents one of the most studied genes linked to longevity, especially since a French cohort of centenarians was described as depleted of ε4 and enriched of ε2 alleles (Schachter et al., 1994). Nevertheless, whereas there is no doubt that the ε4 allele increases the risk for AD and other aging-related diseases (e.g. cardiovascular diseases) (Liehn et al., 2018) and there is a strong association of certain SNPs at the *APOE* locus with extreme longevity (Sebastiani et al., 2017), the evidence about the role of the ε2 allele remains an open question (Louhija et al., 2001; Garatachea et al., 2014; Sebastiani et al., 2017).

Our centenarians displayed a similar *APOE* gene profiling compared to what we previously described in a cohort of healthy octogenarians without neurodegenerative diseases (Arosio et al., 2007), and data from another study enrolling subjects aged from 18 to 93 years from the same geographical region (Singh et al., 2006). Therefore, in our study the *APOE* gene seems to influence the risk of developing AD, but not the chance to become a centenarian.

Genetic determinants of longevity are dynamic and historically dependent (Govindaraju, 2015; Yashin et al., 2015; Giuliani et al., 2017; Kaplanis et al., 2018). Thus, the *APOE* gene distribution may show wide geographical and ethnic variations (Singh et al., 2006). In particular, the relationship between *APOE* gene and successful aging in the Italian population has been showing controversial results. In the European project on the Genetics of Healthy Ageing (GEHA), which includes a large group of Italian nonagenarian sibling pairs, the variants of *APOE* gene locus was significantly associated with longevity (Kulminski et al., 2015). Conversely, the *APOE* gene did not show a significant association with the longevity trait in data generated by the means of a genome-wide analysis of 333 centenarians and 773 geographically-matched healthy individuals (Giuliani et al., 2018). The absence of difference in *APOE* genotype distribution between centenarians and normal-lived subjects has been also reported in another cohort from Southern Europe (Garatachea et al., 2014). These results suggest the importance to pay special attention to the origin of the

studied samples.

Exceptional longevity determines a heterogeneous phenotype and population studies of extremely old people can be biased by the selection of only relatively healthy survivors who do not draw a real picture of this segment of population. There is, in fact, the risk of a selection bias by choosing only the healthiest centenarians. We tried to minimize such issue by using an unselected cohort of persons aged 100 years and older, and reducing them the burden of the assessment by directly evaluating them at home or the nursing home where they lived.

Another gap in studies focused on exceptional longevity is the relative few data raised from the comparison between long-lived phenotype and age-diseased phenotype. For this reason, in our study we have proposed extreme phenotypes: the centenarians, octogenarians with AD (carefully selected by means of clinical and biomarker evaluations), and healthy octogenarians without cognitive impairment.

Our centenarians were heterogeneous in term of physical and cognitive status, showing a high prevalence of cerebrovascular disease, myocardial infarction, heart failure, arrhythmia, angina, hypertension, COPD, arthrosis as well as dementia (Tedone et al., 2014; Gussago et al., 2016). It is well known that dementia prevalence increases with age, and our findings demonstrate that approximately half of centenarians were affected by severe cognitive impairment in line with the previously published data (Arosio et al., 2017). Despite methodological difficulties in diagnosing dementia in the oldest old, accumulating evidence suggests that vascular dementia is more prevalent in centenarians than AD, although the two forms of dementia may often overlap (Prohovnik et al., 2006). In our centenarians, there were no statistically significant differences in the *APOE* genotype and allele distributions according to the presence of cognitive decline. In particular, our study confirms that the *APOE* ε4 allele is strongly associated with AD, whereas ε2 and ε3 are negatively associated with this disease. We have observed only a trend in the ε4 allele distribution in centenarians compared to controls.

The main strength of this study is the presence of a selected cohort consisting of a substantial number of centenarians compared to well-characterized controls and AD patients, all from the same geographical area. Several limitations of our study should be mentioned: 1) the presence of a cross-sectional design not allowing to draw any cause-effect conclusion, and 2) the absence of analyses of potential genes adjacent to and in linkage disequilibrium with *APOE* gene potentially influencing the impact of its genotype.

In conclusion, our study stresses the role of *APOE* alleles in the pathological aging of the brain characteristic of AD. However, this trait does not appear to be particularly related to the advancing age, thus suggesting a limited positive effect on longevity.

Conflict of interest

The authors have no conflict of interest.

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