



Article

Persistence of the *APOE* $\epsilon 4$ Allele Among Sardinian Nonagenarians: Longitudinal Survival Evidence from a Blue Zone Cohort

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Abstract

Background: Genetic variants associated with lifespan are typically identified by comparing long-lived individuals with younger populations. However, factors influencing the attainment of advanced age may differ from those affecting survival once extreme age has already been reached. The Sardinian “Longevity Blue Zone” (LBZ) represents a well-characterized longevity population in which this distinction can be investigated. **Methods:** We conducted a prospective survival study in 150 community-dwelling nonagenarians from the Sardinian LBZ with follow-up for more than seven years. Previously investigated candidate polymorphisms involved in aging-related pathways, including *APOE*, *ACE1*, *IL6*, *TNF α* , *FOXO3A*, *KLOTHO*, and *G6PD*, were reanalyzed using Kaplan–Meier curves and Cox proportional hazards models adjusted for age at recruitment, sex, and comorbidity burden (CIRS score). **Results:** Most polymorphisms showed no association with residual survival after age 90. In unadjusted analyses, carriers of the *APOE* $\epsilon 4$ allele displayed lower mortality than non-carriers (HR 0.49, 95% CI 0.26–0.93). However, after adjustment for age at recruitment, sex, and comorbidity (CIRS score), the association was attenuated and no longer statistically significant (HR 0.49, 95% CI 0.24–1.03). **Conclusions:** In this cohort of Sardinian nonagenarians, candidate longevity-associated polymorphisms did not significantly influence survival beyond age 90. The absence of an independent mortality disadvantage among *APOE* $\epsilon 4$ carriers indicates that the allele is compatible with survival into extreme age rather than conferring a survival advantage. These findings highlight the importance of distinguishing genetic determinants of longevity attainment from factors governing mortality dynamics at extreme ages.

Keywords: human longevity; Blue Zone; single nucleotide polymorphisms; Sardinia



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1. Introduction

Human longevity is a heterogeneous phenotype resulting from the interaction between genetic background, environmental exposures, lifestyle, and stochastic events across the life course [1]. While average life expectancy has increased worldwide, survival to extreme ages remains rare and unevenly distributed across populations. Individuals who reach the tenth decade of life constitute a demographically selected group that has already survived multiple mortality filters operating earlier in adulthood and later in life. Consequently, genetic factors associated with achieving exceptional longevity may differ from those influencing residual survival once extreme age has already been attained.

The Sardinian “Longevity Blue Zone” (LBZ) represents one of the most intensively studied longevity populations [2,3]. This geographically restricted area is characterized by an unusually high prevalence of nonagenarians and centenarians [4] and by a demographic structure shaped by historical isolation and reduced genetic heterogeneity [5]. These features minimize background genetic noise and confounding, providing a compelling natural setting for detecting subtle survival effects that may be obscured in more heterogeneous populations. Because of these characteristics, Sardinia has provided valuable insight into the genetic architecture of human aging. Previous investigations in this kind of population have examined candidate polymorphisms involved in lipid metabolism [6–8], inflammatory pathways [9–12], insulin signaling [13], oxidative stress responses [14,15], and cellular maintenance processes [16,17]. However, most studies, including our previous work, have focused primarily on the probability of reaching advanced age rather than on survival trajectories within cohorts of already long-lived individuals.

The distinction between longevity attainment and late-life survival is particularly relevant in genetic studies. Alleles that increase the risk of cardiovascular disease, neurodegeneration, or metabolic disorders may be progressively removed from the population through demographic selection before extreme ages are reached. As a result, the genetic composition of nonagenarians reflects decades of survival filtering. After approximately 90 years of age, mortality dynamics change, becoming increasingly dominated by frailty heterogeneity and stochastic factors, potentially reducing the detectable influence of many previously identified longevity-associated variants.

Among candidate genes investigated in aging research, the *APOE* locus has consistently shown one of the strongest relationships with lifespan in multiple populations [18–20]. The *APOE* $\epsilon 4$ allele is typically associated with increased mortality risk and is usually underrepresented among centenarians [8,21]. Nevertheless, a previous study in the Sardinia LBZ has reported weaker $\epsilon 4$ depletion than in other populations [22], suggesting that its demographic impact may vary across genetic backgrounds or environmental contexts. Whether this allele continues to influence mortality risk after individuals have already reached extreme age remains unclear.

In a previous case–control study in this population, we analyzed the distribution of several aging-related polymorphisms in Sardinian nonagenarians using a cross-sectional approach [15]. The cross-sectional design could not determine whether these variants affected survival among the oldest-old. Longitudinal analyses are required to determine whether genetic variants continue to exert measurable selective effects after advanced age. Survival analysis can evaluate whether specific genotypes influence residual lifespan after age 90, rather than the probability of reaching old age per se [23]. This strategy reduces cohort heterogeneity, minimizes birth-cohort effects, and aligns more closely with the biological mechanisms of late-life survival.

Therefore, in the present study, we extended our previous work by applying a prospective survival approach to these candidate polymorphisms. Our aim was to evaluate whether these previously studied longevity-associated candidate variants influence residual survival after age 90, with a specific focus on whether *APOE* $\epsilon 4$ frequency, observed in unadjusted survival comparisons, persisted after adjustment for key clinical and demographic covariates.

2. Materials and Methods

2.1. Study Population

The geographic, demographic, and socio-cultural characteristics of the study population have been described in detail previously [15]. Briefly, the population consisted of nonagenarians residing in six mountainous villages in the central-eastern region of Sardinia

(the LBZ). These villages constitute a historically semi-isolated community characterized by long-standing endogamy, traditional dietary habits, and physically active lifestyles that persist into old age [24]. A total of 150 unrelated individuals were enrolled between 2012 and 2014. Eligibility criteria were: (i) age ≥ 90 years at baseline assessment, (ii) lifelong residence in the LBZ area (born in the study area and residing there for most of life), and (iii) provision of written informed consent. Exclusion criteria were non-native ancestry, inability to provide consent without a legal representative, and missing baseline covariate data required for survival models. The baseline cohort included 61 men and 89 women, with an age of range 90–101 years.

Demographic data were collected through structured home interviews, and venous blood samples were obtained for genomic DNA extraction.

2.2. Follow-Up and Survival Ascertainment

Participants were followed prospectively from baseline enrollment (2012–2014) until death or censoring at last confirmed contact, with survival status ascertained via municipal registry offices, family contacts, and local health records. The primary endpoint was all-cause mortality. Time-to-event was calculated as the interval between the baseline assessment and the date of death. Participants known to be alive at the end of follow-up were censored at their last confirmed contact date.

2.3. Common Genetic Variants Associated with Human Longevity

The panel of variants genotyped in this study was selected on the basis of our previous work [15] and convergent evidence implicating these loci in biological pathways relevant to aging and survival. Table 1 summarizes the candidate variants investigated; below, we briefly outline the rationale for their inclusion.

Table 1. Genetic longevity markers selected in this study.

Gene Name and Abbreviation	Variant Locus	References
Apolipoprotein E (<i>APOE</i>)	<i>rs429358</i> (T>C, p.C112R) and <i>rs7412</i> (C>T, p.R158C)	[6–8]
Apolipoprotein E promoter	<i>rs449647</i> , <i>rs769446</i> , <i>rs405509</i>	This study
Angiotensin-converting enzyme (<i>ACE1</i>)	<i>rs1799752</i> : intron variant: <i>Alu</i> sequence	[8,13,25]
Interleukin 6 (<i>IL6</i>)	<i>rs1800795</i> (–174G/C in the promoter region)	[9,10]
Tumor necrosis factor α	<i>rs1800629</i> (–308G/A in the promoter region)	[10,26,27]
Forkhead box O3A (<i>FOXO3A</i>)	<i>rs2802292</i> (intron T>C)	[13]
Klotho	<i>rs9536314</i> (KL-VS variant)	[16,17]
<i>G6PD</i> ¹	<i>rs5030868</i> in Xq28 chromosome	[14]

¹ Glucose-6-phosphate dehydrogenase.

2.3.1. Apolipoprotein E (*APOE*)

APOE is a 3 kb gene located on chromosome 19q13.32. Two missense variants in exon 4, *rs429358* (T>C, p.C112R) and *rs7412* (C>T, p.R158C), define the three major *APOE* haplotypes: $\epsilon 3$ (T/C), $\epsilon 2$ (T/T), and $\epsilon 4$ (C/C). The markedly low frequency of the $\epsilon 4$ allele reported in French centenarians by Schächter et al. in 1994 [8] stimulated extensive replication efforts in long-lived populations (reviewed in [6]).

2.3.2. Angiotensin-Converting Enzyme 1 (*ACE1*)

ACE1 is a 21 kb gene on chromosome 17q23.3. An insertion/deletion polymorphism in intron 16 (*rs1799752*), caused by an *Alu* repeat, modulates circulating ACE levels: the

Del/Del genotype is associated with higher enzyme activity than Ins/Ins [28]. An increased frequency of the Del allele was observed among centenarians in the original report by Schächter et al. [8].

2.3.3. Interleukin 6 (IL-6)

IL6, located on chromosome 7p15.3, encodes a pleiotropic cytokine central to inflammatory regulation [29]. The promoter variant rs1800795 (−174G>C) influences IL-6 expression: the −174C allele is associated with lower circulating IL-6 concentrations than the −174G allele [29]. A higher frequency of the −174C allele has been reported in Italian centenarians [30].

2.3.4. Tumor Necrosis Factor Alpha (TNF-α)

TNFA spans 2762 bp at 6p21.33 and encodes a key pro-inflammatory cytokine involved in immune regulation and cellular homeostasis [31]. Although the promoter polymorphism −308G>A has been extensively studied, investigations in Italian [26] and Finnish oldest-old cohorts [27] and a meta-analysis [11] did not confirm a robust association with longevity.

2.3.5. Forkhead Box O3A (FOXO3A)

FOXO3, located on chromosome 6q21, encodes a forkhead transcription factor with a conserved DNA-binding domain [32]. It regulates oxidative stress responses, DNA repair, apoptosis, immune function, and stem-cell maintenance [33]. Associations between *FOXO3* variants and human longevity have been consistently reported across populations [13,34]. Functional homologs, including *daf-16* in *Caenorhabditis elegans* [35] and *dFOXO* in *Drosophila melanogaster* [36], further support its evolutionary role in lifespan regulation.

2.3.6. KLOTHO

The *KL* gene on chromosome 13q13.1 encodes the transmembrane protein Klotho, which modulates insulin sensitivity and mineral metabolism [37]. High expression is observed in the choroid plexus [38] and renal distal tubules [39]. Klotho overexpression extends lifespan in mice [40], whereas deficiency induces a syndrome resembling accelerated aging [14]. In humans, heterozygous carriers of the *KL-VS* variant show an increased likelihood of surviving to advanced age [41], with meta-analytic evidence indicating a modest but significant effect in males [42].

2.3.7. Glucose-6-Phosphate Dehydrogenase (G6PD)

G6PD, located on Xq28, encodes a key enzyme of the pentose phosphate pathway (EC 1.1.1.49), generating NADPH, which is required for antioxidant defense and nucleotide synthesis [37]. Loss-of-function variants cause G6PD deficiency, characterized by reduced glutathione availability. Notably, a higher prevalence of G6PD deficiency, particularly in hemizygous males, has been reported among Sardinian centenarians [14,38].

2.4. DNA Extraction and Genotyping

Genomic DNA was extracted from peripheral blood leukocytes using a standard salting-out procedure, as described previously [15]. All samples were processed under identical conditions by the same operator to minimize technical variability. Genotyping methods, PCR conditions, and quality control procedures were as reported in our previous study. Genotyping quality control included duplicated samples (concordance 100%), and consistency checks against expected allele frequencies. Hardy–Weinberg equilibrium was evaluated for each autosomal variant in the overall sample using an exact test (*p*-value threshold < 0.05), and none showed evidence of major deviation after accounting for small genotype strata.

2.5. Statistical Analysis

Baseline characteristics were summarized using descriptive statistics. The study targeted a small set of candidate gene variants selected a priori on biological grounds and previously examined in this cohort; therefore, analyses were conducted within a hypothesis-driven framework rather than a genome-wide discovery paradigm. No formal multiple-testing adjustment was applied because the study evaluates a limited, pre-specified set of candidate variants rather than a discovery-scale screen. Accordingly, findings were interpreted conservatively as exploratory and hypothesis-generating, emphasizing effect sizes, confidence intervals, and consistency across analyses. This approach preserves statistical power in a small sample of extreme-age individuals while limiting overinterpretation of isolated significant associations.

Survival probabilities were estimated using the Kaplan–Meier method, and differences between genotype groups were summarized as median (interquartile range) survival times and compared with the log-rank test. These unadjusted Kaplan–Meier analyses provided an initial descriptive comparison of mortality risk across genotypes and identified any variants warranting further investigation in multivariable models. Cox proportional hazards regression was used to evaluate the association between genetic polymorphisms and mortality risk, with survival time as the scale and age, sex, and comorbidity (CIRS score) as covariates. Hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated to quantify relative mortality risk. The proportional hazards assumption was assessed using Schoenfeld residuals and log-minus-log survival plots. When minor violations of proportionality were detected, sensitivity analyses were performed to check the robustness of results. A two-sided p -value < 0.05 was considered statistically significant.

2.6. Follow-Up Procedures and Determination of Vital Status

Participants were followed prospectively for over seven years from baseline. For deceased individuals, the date of death was confirmed via official documents. Participants still alive at the end of the follow-up period were censored at their last confirmed contact date. Time-to-event was calculated in years from baseline interview to death or censoring. Continuous follow-up and complete ascertainment of outcomes enhanced the reliability of mortality estimates.

2.7. Ethics Statement

The study complied with the Declaration of Helsinki and was approved by the Institutional Ethics Committee of the *Azienda Ospedaliero-Universitaria* di Sassari (Prot. No. 136/CE, 9 February 2012). All participants provided written informed consent.

3. Results

Of the 150 participants recruited at baseline, 14 individuals (8 males and 6 females, 9%) were still alive as of November 2025. The genotypes and allele frequencies of all analyzed markers were reported in our previous paper [15]. For *APOE* genotypes, only $\epsilon 3/3$, $\epsilon 2/3$, and $\epsilon 3/4$ were observed with appreciable frequencies; only one participant was a carrier of the homozygous $\epsilon 2/2$ genotype.

Table 2 summarizes the observed survival durations (in years) of study participants by genotype.

No significant differences in survival were observed for 7 of the 10 polymorphisms examined. Given the number of variants tested and the limited sample size, the results should be interpreted as exploratory and require replication in independent cohorts. However, in the case of *APOE*, participants carrying the $\epsilon 3/4$ genotype had significantly longer unadjusted median survival than those with the more common $\epsilon 3/3$ genotype ($p = 0.024$).

(Table 2), whereas no significant difference was detected between carriers of the $\epsilon 2/3$ and $\epsilon 3/3$ genotypes. A similar survival advantage was seen for carriers of the AT genotype of the $-491A/T$ variant, and the GT genotype of the $-219G/T$ variant in the *APOE* promoter, consistent with linkage disequilibrium between those promoter alleles and the $\epsilon 4$ defining haplotype (rs429358C-rs7412C-rs449647A-rs769446T-rs405509T) [15].

Table 2. Survival of study participants according to genotypes.

Genotypes	No. of Participants	Median Survival (IQR) (yrs)	<i>p</i> -Value [#]
<i>APOE</i>			
$\epsilon 2/2$	1		
$\epsilon 2/3$	13	3.10 (3.2)	0.461
$\epsilon 2/4$	0		
$\epsilon 3/3$	121	3.10 (2.4)	Ref.
$\epsilon 3/4$	15	4.30 (2.4)	0.024
$\epsilon 4/4$	0		
<i>APOE -491 A/T</i>			
A/A	78	2.95 (2.6)	
A/T	56	4.15 (2.7)	0.028
T/T	16	3.25 (1.8)	
<i>APOE -427 C/T</i>			
C/C	2	3.10	
T/C	27	3.90 (2.8)	0.848
T/T	121	3.30 (2.8)	
<i>APOE -219 G/T</i>			
G/G	45	2.50 (2.7)	
G/T	80	3.95 (2.6)	0.004
T/T	25	3.70 (2.8)	
<i>ACE1 Ins/Del</i>			
Del/Del	78	2.96 (2.4)	
Ins/Del	56	3.50 (2.8)	0.912
Ins/Ins	16	2.90 (2.8)	
<i>IL6 -174G/C</i>			
G/G	78	3.00 (2.6)	
G/C	50	3.90 (2.7)	0.129
C/C	22	3.80 (3.7)	
<i>TNFα -308 G/A</i>			
G/G	80	3.05 (2.9)	
G/A	52	3.65 (2.2)	0.991
A/A	18	3.45 (2.7)	
<i>FOXO3A</i>			
T/T	78	3.05 (2.8)	
T/G	56	3.90 (2.2)	0.233
G/G	16	3.25 (3.8)	
<i>KLOTHO</i>			
wt/wt	115	3.50 (2.8)	
wt/VS	31	3.00 (2.7)	0.312
VS/VS	4	2.65 (4.6)	
<i>G6PD</i> *			
B	146	3.45 (2.4)	0.216
Mut	4	1.85 (3.6)	

* Glucose-6-phosphate dehydrogenase; [#] Log rank *p* values are derived from unadjusted Kaplan–Meier comparisons; covariate-adjusted estimates for *APOE* are reported in Table 3.

Survival Analysis

Kaplan–Meier survival curves were constructed for all candidate variants (Figure 1). The largest unadjusted separation was observed for *APOE* genotypes (Figure 2), with longer survival in $\epsilon 3/4$ compared with $\epsilon 3/3$ (log rank $p = 0.024$). This unadjusted pattern did not persist as a statistically significant association after covariate adjustment in multivariate analysis (Table 3). When Kaplan–Meier curves were constructed separately for the two sexes, no statistically significant differences were found (log-rank test: males 0.23; females 0.11), despite a tendency towards a greater survival for carriers of the $\epsilon 4$ allele that could be visually noticed (Supplementary Figure S1). Instead, a previous study in one LBZ village found that the frequency of the *APOE* $\epsilon 4$ allele was substantially higher in males than in females (12.2% vs. 2.4%) [22], suggesting sex-specific and early-life selection effects.

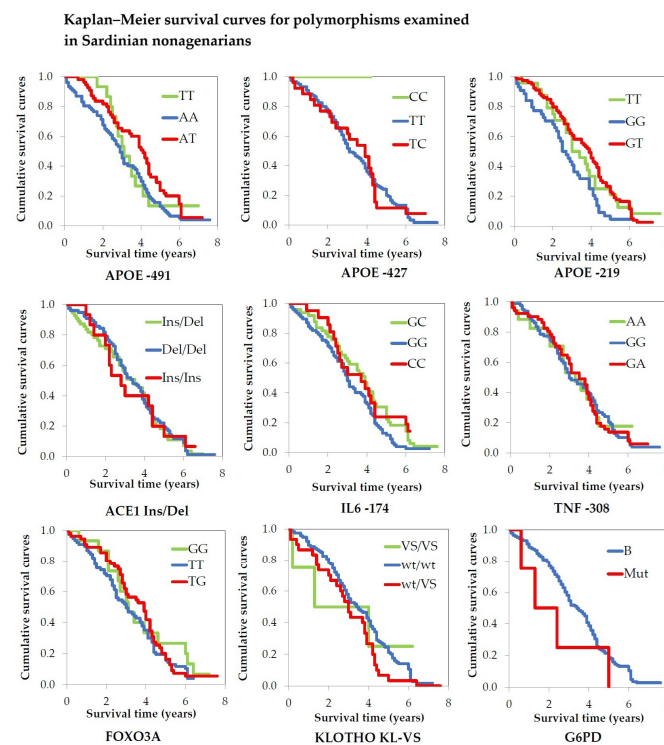


Figure 1. Survival analysis among study participants according to all gene variants except *APOE* $\epsilon 2/3/4$.

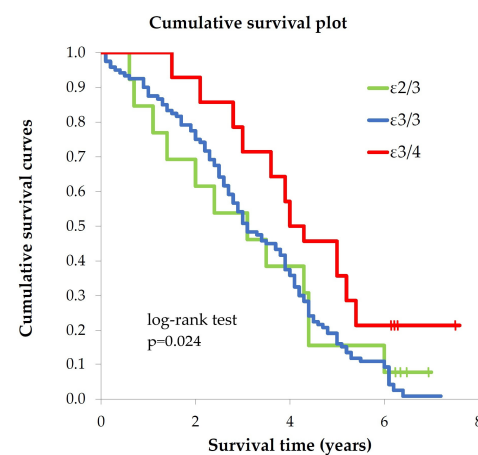


Figure 2. Kaplan–Meier survival curves by *APOE* $\epsilon 2/3/4$ genotype (unadjusted). The unadjusted log rank test indicates longer survival in $\epsilon 3/4$ versus $\epsilon 3/3$; adjusted Cox estimates are reported in Table 3.

We further investigated the impact of APOE variants on survival using multivariable Cox regression, adjusting for potential confounders. Table 3 summarizes the results of the Cox proportional-hazards analysis.

Table 3. Cox regression analysis with survival as the outcome and APOE genotypes as the exposure. Covariates: age at recruitment, female sex, and comorbidity (CIRS Score).

Covariates	Unadjusted (HR ¹ and 95% CI)	Adjusted (HR ¹ and 95% CI)
APOE (ϵ 4 vs. ϵ 3)	0.489 (0.256–0.933) *	0.491 (0.235–1.028)
Female Sex	1.562 (1.106–2.204) *	1.458 (1.017–2.091) *
Age at recruitment	1.061 (1.002–1.123) *	1.059 (1.004–1.117) *
CIRS score	1.211 (1.044–1.406) *	1.199 (1.034–1.390) *

¹ Hazard Ratio; * $p < 0.05$; The APOE ϵ 4 association is statistically significant in unadjusted analysis but not in the adjusted model (95% CI crosses 1.0).

In unadjusted analyses, carriers of the APOE ϵ 4 allele had lower mortality than non-carriers (HR 0.489, 95% CI 0.256–0.933). However, after adjustment for age at recruitment, female sex, and comorbidity burden (CIRS score), the association was attenuated and no longer statistically significant (HR 0.491, 95% CI 0.235–1.028). In contrast, age, sex, and comorbidity remained independent predictors of mortality in the fully adjusted model.

4. Discussion

In this prospective study of Sardinian nonagenarians followed for more than seven years, no consistent effects of most candidate polymorphisms on residual survival after age 90 were detected. Rather than indicating genetic determinants of longevity attainment, these findings suggest that, once extreme age has been reached, mortality risk may be influenced less by many previously proposed aging-related variants and more by factors not captured by the candidate polymorphisms examined.

Notably, carriers of the APOE ϵ 4 allele did not show a late-life mortality disadvantage in this cohort. The persistence of APOE ϵ 4 among very old individuals in this population remains noteworthy because ϵ 4 is typically depleted among long-lived individuals in many settings [39]. Our findings do not support a protective role of the ϵ 4 allele, but instead suggest that its presence is compatible with survival into extreme age. In contrast to the marked depletion of ϵ 4 typically observed among long-lived individuals in many populations, our results suggest that the major selective pressures associated with this locus may operate earlier in life, while its influence becomes attenuated among individuals who have already survived to very old age.

These results support the conceptual distinction between genetic determinants of reaching exceptional age and genetic influences on within-cohort survival. Demographic selection before the tenth decade likely reduces the variability in mortality-related genetic risk, thereby limiting the detectable effects of many candidate variants in late-life survival analyses. Consequently, the present findings should be interpreted as describing survival dynamics after longevity has already been achieved, rather than identifying genes that promote longevity itself. Notably, regarding the G6PD gene, none of the carriers of activity-reducing variants survived beyond 5 years of follow-up, indicating that these mutations are not associated with increased longevity (as previously hypothesized) [14,38] and may even reduce residual life expectancy. However, the number of G6PD-deficient individuals was very small, precluding firm conclusions.

Recent evidence indicates that the effect of APOE ϵ 4 on survival can depend on environmental context. In a rural Ghana cohort with high pathogen exposure, ϵ 4 carriers did not have reduced survival compared to ϵ 3/3 individuals [40]. In fact, ϵ 4 was associated

with higher fertility in that setting: women with one $\epsilon 4$ allele had on average one additional child, and those with two $\epsilon 4$ alleles had about 3.5 more children than $\epsilon 4$ -negative women ($p = 0.018$) [40]. These observations imply that in high-infection environments, $\epsilon 4$ may not reduce survival and could confer a reproductive advantage. Notably, Sardinia's population faced heavy infectious burdens (endemic malaria, tuberculosis, and trachoma) until the mid-20th century [41,42], conditions that might have favored genetic variants like $\epsilon 4$ that reduce childhood infection mortality.

Until the mid-1900s, *Plasmodium falciparum* malaria was highly prevalent in Sardinia and a leading cause of early mortality [42]. In such a context, even modest genetic resistance to infection could markedly improve survival. If $\epsilon 4$ provided partial protection against malaria or other childhood infections, this early-life benefit could outweigh its late-life disadvantages. One possible mechanism is that *P. falciparum* requires host phospholipids for its growth [43], and $\epsilon 4$ carriers transport these lipids less efficiently. Consistently, Fujioka et al. found experimentally that *APOE* $\epsilon 4$ inhibits malaria parasite growth [44], suggesting a plausible biological basis for reduced infection risk in $\epsilon 4$ carriers from malaria-endemic regions.

One might reasonably expect that in adulthood, carriers of the *APOE* $\epsilon 4$ allele, classically associated with increased circulating cholesterol levels [45], would be at greater risk of cardiovascular disease and thus have reduced survival. However, we previously demonstrated that, in the Sardinian population, the impact of *APOE* $\epsilon 4$ on cholesterol level is considerably smaller than in other populations [46], indicating that the canonical lipid-mediated risk of this allele does not apply in this context. Moreover, in a recent investigation in the same LBZ community, we showed that nonagenarians with mild hypercholesterolemia paradoxically experienced longer survival than those with normal cholesterolemia [47]. Taken together, these findings strongly suggest that, in this population, the effect of *APOE* $\epsilon 4$ is not mediated by conventional cardiovascular risk pathways and may be fully compatible with the attainment of advanced ages, thereby providing a coherent biological framework for our results.

In addition, although the *APOE* $\epsilon 4$ allele is often associated with increased inflammatory reactivity under immune challenge [48], several population studies report lower basal circulating C-reactive protein (CRP) levels in $\epsilon 4$ carriers [49,50]. This suggests a genotype-dependent modulation of systemic inflammatory tone that could be relevant to late-life survival.

Finally, diet is a key environmental factor that modulates the effect of $\epsilon 4$ on survival [51]. It has been suggested that lifestyle factors (e.g., Mediterranean-type diets and physical activity) can mitigate or even reverse *APOE* $\epsilon 4$'s deleterious effect. Supporting this, the diet common in Sardinia and other longevity zones may particularly benefit $\epsilon 4$ carriers by reducing their Alzheimer's and cardiovascular risk to levels comparable to those of $\epsilon 3$ carriers.

Overall, these findings support the emerging view that *APOE* $\epsilon 4$ exerts highly context-dependent effects on survival, conferring advantages under conditions of high infectious burden or limited caloric availability, but becoming detrimental in modern, low-infection, high-calorie environments—an effect consistent with the “environmental mismatch” hypothesis [52]. In favorable ecological contexts, such as traditional societies or diets rich in unsaturated fats, $\epsilon 4$ carriers may experience improved survival, whereas in Western lifestyles its adverse effects predominate, underscoring the importance of gene–environment interactions in longevity. Importantly, *APOE* $\epsilon 4$'s impact on survival is heterogeneous and modulated by competing risks: recent analyses have shown reduced mortality among $\epsilon 4$ carriers with low Alzheimer-type neuropathology [53], alongside evidence of lower cancer incidence in $\epsilon 4$ carriers [54,55]. These opposing influences help explain why some

$\epsilon 4$ carriers achieve exceptional longevity with preserved cognitive function. Mechanistically, *APOE* $\epsilon 4$ affects lipid metabolism and immune responses in ways that may be adaptive in pathogen-rich environments but maladaptive in modern settings, suggesting that environmental modifications (e.g., lifestyle or medical interventions) might mitigate its late-life risks.

This study has several limitations. Given the exploratory nature of the survival analysis, results were interpreted cautiously without correction for multiple comparisons. The relatively small number of nonagenarians reflects the rarity of extreme longevity, even in the Sardinian LBZ. Moreover, survival was assessed only from age 90 onward, which precludes evaluation of genetic influences acting earlier in life. Finally, we did not examine potential interactions between *APOE* $\epsilon 4$ and lifestyle factors (such as diet and physical activity), which should be addressed in future longitudinal studies.

5. Conclusions

In this cohort of Sardinian nonagenarians, most candidate longevity-associated polymorphisms did not influence residual lifespan after age 90. Although unadjusted analyses suggested longer survival among *APOE* $\epsilon 4$ carriers, the association was attenuated and no longer statistically significant after adjustment for age, sex, and comorbidity. These findings indicate that *APOE* $\epsilon 4$ is compatible with survival into extreme age in this population rather than conferring an independent late-life survival advantage, and they highlight the importance of distinguishing longevity attainment from survival dynamics within the oldest old.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/jal6010026/s1>, Figure S1: Kaplan–Meier survival curves for *APOE* in Sardinian nonagenarians stratified by sex.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Ethics Committee of the Azienda Ospedaliero-Universitaria di Sassari (Prot. No. 136/CE, 9 February 2012).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The original contributions presented in this study are included in the article and Supplementary Materials. The data are available from the corresponding author on reasonable request.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

ACE1	Angiotensin-Converting Enzyme
APOE	Apolipoprotein E
CIRS	Cumulative Illness Rating Scale
CRP	C-reactive Protein
FOXO3A	Forkhead Transcription Factor O Subfamily Member 3a

G6PD	Glucose-6-Phosphate Dehydrogenase
LBZ	Longevity Blue Zone
NADPH	Nicotinamide adenine dinucleotide phosphate
SNP	Single-nucleotide polymorphism
TNF	Tumor Necrosis Factor

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