Genetically predicted telomere length and Alzheimer’s disease endophenotypes: a Mendelian randomization study

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Abstract

Telomere length (TL) is associated with biological aging, consequently influencing the risk of age-related diseases such as Alzheimer’s disease (AD). We aimed to evaluate the potential causal role of TL in AD endophenotypes (i.e., cognitive performance, N = 2233; brain age and AD-related signatures, N = 1134; and cerebrospinal fluid biomarkers (CSF) of AD and neurodegeneration, N = 304) through a Mendelian randomization (MR) analysis. Our analysis was conducted in the context of the ALFA (ALzheimer and FAmilies) study, a population of cognitively healthy individuals at risk of AD. A total of 20 single nucleotide polymorphisms associated with TL were used to determine the effect of TL on AD endophenotypes. Analyses were adjusted by age, sex, and years of education. Stratified analyses by APOE-ɛ4 status and polygenic risk score of AD were conducted. MR analysis revealed significant associations between genetically predicted longer TL and lower levels of CSF Aβ and higher levels of CSF NfL only in APOE-ɛ4 non-carriers. Moreover, inheriting longer TL was associated with greater cortical thickness in age and AD-related brain signatures and lower levels of CSF p-tau among individuals at a high genetic predisposition to AD. Further observational analyses are warranted to better understand these associations.

Keywords: Alzheimer’s disease, Cerebrospinal fluid biomarkers, Mendelian randomization, Neuroimaging, Polygenic risk score, Telomere length

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Introduction
Telomere length (TL) is a candidate biomarker of biological aging and aging-related outcomes [1, 2]. Telomeres are tandem deoxyribonucleic acid (DNA) repeats of a short sequence unit located at the end of the eukaryotic linear chromosomes that undergo attrition every time a somatic cell divides [3]. Shorter telomeres are associated with mortality [4], increased rates of age-related diseases [5–8], progression to dementia [9], and higher overall risk of developing Alzheimer's disease (AD) [10, 11].

Previous studies have reported associations between TL and cognitive performance [12] as well as cognitive decline in cognitively unimpaired individuals [13]. Furthermore, TL is thought to affect brain structure; shorter TL has been associated with cortical atrophy in healthy subjects [14]. However, both longer and shorter TL have been associated with lower hippocampal volumes in the general population [15–19]. To date, no studies have evaluated the impact of TL in cortical or hippocampus regions differentially affected by AD or aging-related processes. Moreover, the potential interplay between TL and AD pathology remains to be elucidated. TL has been shown to be independent of amyloid-β (Aβ) deposition in AD patients [20, 21]. In contrast, Mahoney et al. [22] described an interaction between longer TL, cognitive decline, and abnormal levels of cerebrospinal fluid (CSF) Aβ and tau in participants of the AD Neuroimaging Initiative.

TL and AD are affected by common lifestyle-related risk factors [23, 24]. Among other reasons, these common factors may be confounders and lead to reverse causation in observational studies, partially explaining the heterogeneity of results found in the literature. The Mendelian randomization (MR) approach was developed to overcome these limitations. Unlike observational studies, the MR approach does not explore the association directly but through genetic instrumental variables (e.g., single nucleotide polymorphisms, SNPs) whose estimation of TL is not influenced by confounding or reverse causation [25, 26]. Previous MR studies have described potential causal effects of genetically predicted TL on the risk of AD [27–30]. However, other MR studies showed inconsistent results regarding TL and cognitive traits [31, 32].

The aim of the present study was to evaluate whether TL may play a causal role in the underlying pathophysiological mechanisms of AD by influencing its endophenotypes measured as cognitive performance, brain structure, and CSF biomarkers of AD and neurodegeneration, in a sample of cognitively unimpaired individuals at increased risk of AD.

Methods
Study participants
The present is a cross-sectional analysis that was conducted in the context of an existing study, the ALFA (ALzheimer and FAmilies) study [33]. Briefly, the ALFA study (Clinicaltrials.gov Identifier: NCT01835717) includes a total of 2573 cognitively unimpaired participants, aged 45–74, many of them kindred of AD patients (86% had at least one parent with dementia regardless of age at onset, 48% of the participants had at least one parent diagnosed with AD before the age of 75).
Participants were characterized at their baseline visit in 2013–2014 at multiple levels (sociodemographic, anthropometric, clinical, epidemiological, cognitive, and neuroimaging). For a subset of the ALFA study participants, we additionally collected blood samples for further genetic analysis. Moreover, a subset of the ALFA study participants (ALFA+ study) underwent lumbar puncture for CSF biomarker analysis. ALFA exclusion criteria were [1] cognitive performance falling outside established cutoffs: Mini-Mental State Examination [34, 35] < 26, Memory Impairment Screen [36, 37] < 6, Time-Orientation subtest of the Barcelona Test II [38] < 68, semantic fluency [39, 40] (animals) < 12, and Clinical Dementia Rating scale [41] > 0; [2] major psychiatric disorders (DSM-IV-TR) or diseases that could affect cognitive abilities; [3] severe auditory and/or visual, neurodevelopmental, and/or psychomotor disorders, significant diseases that could interfere with cognition; [4] neurological disorders; [5] brain injury that could interfere with cognition; and [6] family history of AD with a suspected autosomal dominant pattern. Further details of ALFA participants can be found in Molinuevo et al. [33].

The study was conducted in accordance with the directives of the Spanish Law 14/2007, of 3rd of July, on Biomedical Research (Ley 14/2007 de Investigación Biomédica). All participants accepted the study procedures by signing an informed consent form.

**Genetic data Genotyping**

DNA samples were obtained from whole blood samples by applying a salting-out protocol. DNA was eluted in 800μl of H2O (Milli-Q) and quantified using Quant-iTT dsDNA Assay Kit (Life Technologies) at the Cancer Epigenetics and Biology Program (PEBC; IDIBELL, Barcelona, Spain). The DNA concentration for each sample was additionally normalized. Genome-wide genotyping was performed using the Illumina Infinium NeuroChip [42] backbone version 1.0 and 1.2, based on the genome-wide genotyping array (Infinium HumanCore-24 v1.0). PLINK was used for the quality control (QC) of the genetic data [43]. We applied the following sample QC thresholds: sample missingness rates > 2% and heterozygosity 3 standard deviations (SD). Individuals showing sex discordancess and high percentage of shared alleles (i.e., identity-by-descent (IBD) > 0.185) [44] were also excluded. A total of 2280 individuals remain after the genetic QC procedure.

**Imputation**

Genetic imputation was done using the Michigan imputation server, according to established guidelines (https://imputationserver.sph.umich.edu) and the European ancestry haplotype reference panel (HRC r1.1 2016) [45, 46]. The genetic datasets were phased using EAGLE software v.2.4, and data were rechecked using the default QC in the Michigan imputation server. After imputation, 15,805,054 variants were obtained with an imputation quality of 90% (r-squared parameter > 0.1).

**Genetic variants associated with longer telomere length**

From the final genetic sample of the ALFA study, 20 SNPs independently associated with mean TL (p-val < 5 × 10^-8) in a genome-wide meta-analysis of 78,592 individuals were selected [47]. Proxies for those SNPs were included through LDlink [48] when the genetic information was not available in our sample. Proxies were considered as those SNPs with r^2 ≥ 0.9, except for the genetic variants rs55749605 (r^2 = 0.84) and rs7705526 (r^2 = 0.79). See the list of r^2 in Additional file 1: Supplementary Table 1. Departures from the Hardy-Weinberg equilibrium and allele frequencies were inspected using the compareGroups R package [49]. Effect sizes (i.e., SD change in TL per copy of the effect allele) and standard errors (SE) were obtained from summary statistics from Li et al. [47]. Detailed information of those SNPs is presented in Additional file 1: Supplementary Table 1.

**Apolipoprotein (APOE) genotyping**

The APOE allelic variants were obtained from allelic combinations of the rs429358 and rs7412 polymorphisms [50]. According to the genotypes of these polymorphisms, subjects were classified depending on the status of the ε4 allele (non-carriers, carriers of at least one ε4 allele). For the purpose of the present study, APOE-ε2/ε4 individuals (N = 47) were excluded.

**Polygenic risk score of Alzheimer’s disease**

Genetic variants that passed the post-imputation QC were used to compute the polygenic risk score (PRS) for AD (PRS-AD) using PRSice version 2 [51]. PRSice combined AD GWAS hits (p-value < 5 × 10^-8), including the APOE region, by summing up all the SNP alleles carried by the participants weighted by the SNP allele effect size estimated in a previous GWAS [52]. PRS-AD was Z-standardized. Individuals with a PRS-AD above percentile 75th were classified within the high genetic predisposition group, whereas those with lower PRS were classified within the low genetic predisposition group.

**Cognitive performance evaluation**

During a neuropsychological evaluation at baseline, all participants were administered a cognitive test battery for the detection of early decline in longitudinal follow-up. This battery assesses episodic verbal memory measured by means of the Memory Binding Test (MBT) [53,
cognitive measures from MBT and from WAIS-IV subtests (EM) and EF were calculated by creating Z-scores for the cognitive composites to assess global episodic memory coding, and semantic fluency. Moreover, two additional total paired recall, MBT delayed free recall, WAIS-IV cuneus, angular, supramarginal, superior frontal, and middle frontal [59]. Aging brain signature was calculated as the average of the cortical thickness of specific brain regions known to be affected by aging: calcarine, caudal insula, cuneus, caudal fusiform, dorsomedial frontal, lateral occipital, precentral, and inferior frontal [60]. We used AD and aging brain signatures as the main outcomes to test the association between genetically predicted TL and brain structure. Higher values in these signatures represent a thicker cortex of the areas included in the signature. AD and aging brain signatures values were available for a total of 1134 participants.

**CSF biomarkers: collection and measurement**

CSF biomarkers were acquired from lumbar punctures for a subset of participants. In brief, CSF total-tau (t-tau) and phosphorylated-tau (p-tau) were measured using the electrochemiluminescence immunoassays Elecsys® phospho-tau(181P) CSF and total-tau CSF, respectively, on a fully automated cobas e 601 instrument (Roche Diagnostics International Ltd.). The rest of the CSF biomarkers Aβ42, Aβ40 and neurofilament light (NfL), were measured with in vitro diagnostic assays and robust prototype assays as part of the NeuroToolKit (Roche Diagnostics International Ltd, Rotkreuz, Switzerland) on both cobas e 601 and e 411 instruments. All measurements were performed at the Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden.

The CSF Aβ42/40 ratio was used as a biomarker of Aβ pathology [61]. The aforementioned biomarkers were used as the main outcomes to test the cross-sectional association between genetically predicted TL and core AD and neurodegeneration biomarkers in a total of 304 participants.

**Statistical analysis**

Alleles were harmonized to take as reference the effect allele associated with longer TL. In this study, genetic associations with longer TL were obtained from the summary statistics of a previously computed meta-GWAS [47]. Furthermore, genetic associations with the outcomes (i.e., AD endophenotypes) were assessed in our sample by linear regression models including age, sex, years of education, and APOE-e4 status as covariates (see Additional file 2: Supplementary Tables 1-15; Fig. 1). We used inverse-variance weighted (IVW) [62], maximum
1. **Selection of SNPs associated with telomere length (TL)**
   
   a) Measure TL (X) in leukocytes  
   b) Genome-wide association analysis of TL

![Diagram showing qPCR and association analysis](image)

2. **Mendelian Randomization procedure**

   **Step 1**
   - SNP association with TL: $\beta_{Xj}$
   - Inverse-variance weighted method
     
     $$\beta_{IVW} = \frac{\sum_j \beta_{Xj} \beta_{Yj} SE(\beta_{Yj})^{-2}}{\sum_j \beta_{Xj}^2 SE(\beta_{Yj})^{-2}}$$

   **Step 2**
   - SNP association with outcome: $\beta_{Yj}$

   **Step 3**
   - Causal effect of SNP on outcome: $\beta_{XY}$

Data source:
- Li et al., 2020
- Molinuevo et al., 2016

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**Fig. 1** (See legend on previous page.)
likelihood [63], weighted median [64], and weighted mode [65] methods with summarized data to estimate the causal effect of genetically predicted longer TL on the outcomes of the study in a two-sample MR design.

Leave-one-SNP-out and Cochran Q statistic [66] were used as ad hoc sensitivity analyses for evaluating the robustness of significant results. MR-Pleiotropy RESidual Sum and Outlier (MR-PRESSO) was also used to identify horizontal pleiotropic outliers [67]. MR-Egger regression intercept test was used as ad hoc sensitivity analysis for evaluating directional pleiotropy [68]. Effect sizes were reported in the SD change ($\beta_{IVW}$) per copy of the allele associated with longer TL. Causal associations with $p$-values below 0.05 were considered to be nominal significant. False discovery rate (FDR) corrections for multiple comparisons were performed at a significant level of 0.05 [69, 70].

Mendelian randomization results

**Cognition outcomes**

No statistically significant associations were identified between genetically predicted longer TL and any of the cognitive outcomes in the entire sample, nor in stratified analyses by $APOE$-ε4 status or by the genetic predisposition to AD based on high/low categories of PRS-AD (Additional file 4: Supplementary Tables 1-5).

**Neuroimaging outcomes**

IVW estimates revealed statistically significant associations between genetically predicted longer TL and greater cortical thickness for both AD and aging signatures only in individuals at high genetic predisposition to AD [AD signature: $\beta_{IVW} = 0.064$, SE = 0.029, $q$-value = 0.032; aging signature: $\beta_{IVW} = 0.062$, SE = 0.029, $q$-value = 0.032] (Fig. 2A, B; Table 1). Maximum likelihood, weighted median, and weighted mode methods suggested similar patterns of effect on cortical thickness. The MR-Egger intercept test did not provide evidence for directional pleiotropy [AD signature: $p$-value = 0.998; Supplementary Tables 1-3. The final sample size was determined by the number of participants with available genotype information ($N = 2233$) and the number of participants with available information for each one of the main outcomes included in the study, that is, cognitive performance ($N = 2233$), neuroimaging signatures ($N = 1134$), and CSF biomarkers ($N = 304$).

### Results

**Descriptive of study participants**

Characteristics of ALFA study participants included in our study can be found in Additional file 3: Supplementary Tables 1-3. The final sample size was determined by the number of participants with available genotype information ($N = 2233$) and the number of participants with available information for each one of the main outcomes included in the study, that is, cognitive performance ($N = 2233$), neuroimaging signatures ($N = 1134$), and CSF biomarkers ($N = 304$).

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aging signature: \( p \)-value = 0.939]. Furthermore, according to both Cochran \( Q \) and MR-PRESSO, there was no evidence of increased heterogeneity (see Table 1). IVW leave-one-out analyses showed MR estimates did not fluctuate after sequentially removing each of the 20 SNPs used as instrumental variables (Additional file 5: Supplementary Figs. 1-2). No other statistically significant associations were found on individuals at low genetic predisposition to AD, as well as in the entire sample, or in stratified analyses by \( APOE \)-ɛ4 status (Additional file 4: Supplementary Tables 1-5).

### Table 1

|                     | High genetic predisposition to AD (\( N = 295 \)) | Aging signature |
|---------------------|-------------------------------------------------|-----------------|
| **Causal inference methods** | | |
| AD signature        | | |
| \( \beta \)          | 0.064                                           | 0.062           |
| SE                  | 0.029                                           | 0.029           |
| \( p \)-value        | 2.89E−02                                        | 3.18E−02        |
| \( q \)-value*       | 3.18E−02                                        | 3.18E−02        |
| \( \delta \)         | 13.267                                          | 2.65E−01        |
| \( SE \)             | 2.604                                           | 2.65E−01        |
| \( p \)-value        | <0.0001                                         | <0.0001         |
| \( q \)-value*       | 2.65E−01                                        | 2.65E−01        |
| **Sensitivity methods** | | |
| Cochran \( Q \), heterogeneity test | 0.995                                           | 0.998           |
| MR-PRESSO, global test | 0.997                                          | 0.998           |
| MR-Egger, intercept test | 0.998                                          | 0.939           |

*\( q \)-value refers to the false discovery rate-adjusted \( p \)-value

### Core AD and neurodegeneration CSF biomarkers

Statistically significant associations were found between genetically predicted longer TL and lower levels of CSF Aβ ratio \( [\beta_{IVW} = −0.007, \ SE = 0.002, \ q \text{-value} = 0.001] \) as well as higher levels of CSF NfL \( [\beta_{IVW} = 13.267, \ SE = 2.604, \ p \text{-value} < 0.0001] \) only in \( APOE \)-e4 non-carriers (Fig. 3A, B; Table 2). Robust MR methods suggested significantly analogous patterns of effect on both CSF Aβ ratio and NfL. There was no evidence of unbalanced directional pleiotropy according to the MR-Egger intercept test [CSF Aβ ratio: \( p \)-value

![Fig. 3](image-url)
Cochran Q and MR-PRESSO also indicated the paucity of heterogeneity in MR estimates (see Table 2). Nevertheless, the leave-one-out analysis showed IVW estimates lost significance when excluding rs35640778 from both MR analyses (Additional file 5: Supplementary Figs. 3-4).

Among APOE-ε4 carriers, MR-PRESSO indicated the presence of increased heterogeneity due to the inclusion of the outlier rs228595 in the analyses (Additional file 4: Supplementary Table 2). However, the MR estimate remained null after correction of pleiotropy via outlier removal.

In stratified analysis by PRS-AD, a nominal statistically significant association was found between SNPs predicting longer TL and lower levels of CSF p-tau only in individuals classified as high genetic predisposition to AD [βIVW = −9.186, SE = 4.534, p-value = 0.043] (Fig. 4; Table 2). No violations of MR assumptions were found according to the MR-Egger intercept test, Cochran Q, and MR-PRESSO. Furthermore, MR showed similar estimates when sequentially leaving out genetic variants from the analyses (Additional file 5: Supplementary Fig. 5). No other statistically significant associations were identified in the entire sample in relation to genetically predicted TL and CSF biomarkers.

Discussion

In this study, we explored the potential causal role of genetically predicted longer TL on AD endophenotypes measured as cognitive performance, brain structure (i.e.,

| Causal inference methods | Aβ ratio | NFL | High genetic AD risk (N= 106) |
|--------------------------|----------|-----|-----------------------------|
|                          | β        | SE  | p-value                     |
| Inverse-variance weighted| −0.007   | 0.002 | 3.26E−04  | 6.52E−04  | 13.267 | 2.604 | 3.50E−06 | 1.40E−06 | −9.186 | 4.534 | 4.28E−02 | 1.48E−01 |
| Maximum likelihood       | −0.007   | 0.002 | 2.19E−03  | 4.37E−03  | 13.044 | 3.312 | 8.22E−05 | 3.29E−04 | −8.932 | 4.563 | 5.03E−02 | 1.68E−01 |
| Weighted median          | −0.007   | 0.002 | 4.41E−03  | 8.83E−03  | 13.627 | 3.568 | 1.34E−04 | 5.37E−04 | −8.571 | 6.224 | 1.69E−01 | 3.07E−01 |
| Weighted mode            | −0.007   | 0.002 | 2.62E−03  | 5.24E−03  | 13.327 | 3.259 | 4.33E−05 | 1.73E−04 | −7.867 | 7.917 | 3.20E−01 | 5.38E−01 |

Sensitivity methods

| p-value       | p-value       | p-value       |
|---------------|---------------|---------------|
| Cohran Q, heterogeneity test | 0.412 | 0.254 | 0.921 |
| MR-PRESSO, global test | 0.602 | 0.505 | 0.911 |
| MR-Egger, intercept test | 0.170 | 0.742 | 0.543 |

Aβ amyloid-β, AD Alzheimer’s disease, CSF cerebrospinal fluid, NFL neurofilament light, PRESSO Pleiotropy RESidual Sum and Outlier, SE standard error

*p-value refers to the false discovery rate-adjusted p-value

Table 2 Statistically significant results reflecting the effect of genetically predicted longer telomere length on CSF biomarkers

Fig. 4 Scatter plot representing the significant causal effect of genetically predicted longer TL on CSF p-tau among individuals at high genetic predisposition to AD. Each dot represents the genetic association of one given SNP with TL in the X-axis and the genetic association of this SNP with an aging signature in the Y-axis. The slope of the line represents the inverse-variance weighted causal estimate
AD signature and aging signature), and CSF biomarkers of AD and neurodegeneration, in cognitively unimpaired individuals from the ALFA study. We performed stratified analysis by APOE-ε4 status and the estimated genetic predisposition of developing AD (i.e., PRS-AD). We observed statistically significant associations between genetically predicted longer TL, lower levels of CSF Aβ ratio, and higher levels of CSF NfL only in APOE-ε4 non-carriers. Additionally, stratified analysis by the PRS-AD revealed statistically significant associations between SNPs predicting longer TL, greater cortical thickness in AD and aging signatures and lower levels of p-tau only among individuals at high genetic predisposition of developing the disease.

Genetically predicted TL and cognitive performance
We did not observe any statistically significant association between genetically predicted longer TL and cognitive performance outcomes. Previous literature showed inconsistent findings regarding TL effect on cognitive performance. Similar to our approach, Hägg et al. [31] reported an association between genetically predicted longer TL and better scores on EF, but not for global cognition or EM, in a large cohort of cognitively healthy individuals (N=17,052). Most recently, Demanelis et al. [32] found genetically predicted TL to be associated with several aging-related traits but not cognitive function among participants of the UK Biobank (N=337,522).

Regarding observational studies, Yaffe et al. [73] reported an association between longer TL and better performance at baseline on the EF domain among community-dwelling elders. In contrast, longer TL at baseline has also been associated with a decline in EF only among cognitively healthy APOE-ε4 carriers [22]. Albeit, Takata et al. [74] did not find any association between TL and cognitive decline. In the Rotterdam study (N=1961) a U-shaped association between TL and risk of AD was found, meaning that both, extremely shorter TL as well as extremely longer TL, were associated with an increased risk of AD, with stronger associations seen for APOE-ε4 carriers [75]. Alternatively, shorter TL has been shown to predict AD incidence only among APOE-ε4 non-carriers [76].

In the present study, the lack of statistically significant associations may be due to its cross-sectional nature as well as the idiosyncrasies of the ALFA cohort, which is composed of middle-aged (i.e., mean age of 56.3 years) and cognitively unimpaired individuals.

Genetically predicted TL and brain structure
Our results showed a significant association between genetically predicted longer TL and greater cortical thickness in both the AD and aging brain signatures, only among individuals at high genetic predisposition of AD. To our knowledge, no previous MR analyses have evaluated the causal effect of TL on brain structure, nor in regions primarily affected by AD or normal aging-related atrophy. Longer TL has been previously associated with larger hippocampal volumes in non-demented individuals in observational cross-sectional studies [15, 17]. Furthermore, a population-based study (i.e., Dallas Heart Study) found TL to be associated with total cerebral volume and specific subsegmental regions of the cortex [14].

Mechanisms explaining how TL could affect brain structure during the life-course are not completely understood. TL is known to play a crucial role in brain development with implications in embryonic and adult neurogenesis [77]. However, there is still a scientific debate over the existence of telomere attrition in neurons due to its post-mitotic nature. Even so, telomerase reactivation (i.e., the enzyme that maintains TL in the cell) reversed brain tissue degeneration among aged telomerase-deficient mice [78]. Therefore, inherited longer TL might protect brain structure through multiple mechanisms, either in regions primarily affected by AD-related processes or aging itself.

Stratified analyses did not show significant differences in the association between genetically predicted TL and brain structure in relation to APOE-ε4, nor PRS-AD subgroups. Similarly, King et al. [14] described that associations between TL and cortical volumes remained significant after adjusting by APOE-ε4 status.

Genetically predicted TL and biomarkers of AD and neurodegeneration
Our results showed significant associations between SNPs predicting longer TL, lower levels of CSF Aβ ratio, and higher levels of CSF NfL in APOE-ε4 non-carriers. These results suggested an unexpected adverse effect of longer TL on CSF biomarkers related to both core AD pathology and neurodegeneration. However, stratified analysis by PRS-AD suggested a protective effect of inherited longer TL on core AD pathology, with longer TL nominally significant associated with lower levels of p-tau among individuals at high genetic predisposition of AD.

To our knowledge, no previous MR studies have tested the effect of genetically predicted TL on AD biomarkers and observational studies are scarce. TL has been previously associated with AD pathology with implications on cognitive function in participants of the AD Neuroimaging Initiative [22]. Moreover, Flanary et al. [79] showed that senescence of isolated human microglia related to telomere shortening enhanced Aβ accumulation and induced AD progression. However, underlying biological mechanisms explaining the association are not fully understood yet.

Given the complexity of microglia homeostasis [80], we might speculate that unexplored roles of TL on microglia
activation could lead to both beneficial or detrimental effects on AD pathology and neurodegeneration processes, which would partially explain the aforementioned disparity of results. However, it remains unclear how APOE-ε4 carriership and the genetic predisposition of AD would affect this putative association. Interestingly, some of the genetic variants which were used as instrumental variables to estimate genetically predicted telomere length in the present study were primarily related to the risk of cardiovascular disease [81], and they were later found to be causally associated with AD risk [27]. Cardiovascular risk factors comprise important targets for preventing AD especially during midlife [24]. Indeed, midlife vascular risk factors have been previously associated with elevated amyloid burden [82], tau pathology, and cognition [83], supporting their role on the etiology of AD. Thus, the TL effect on cardiovascular health outcome could be mediating or contributing to the causal associations described in our cohort. Nevertheless, further studies with larger sample sizes are required to better understand these results since a variety of undisclosed pathological mechanisms could be responsible for this complex interplay.

Limitations and strengths

Our study is not free of limitations. Potential limitations could be due to MR assumptions. For instance, IVW estimates could be biased in the presence of pleiotropy. To ensure estimates were reliable, sensitivity analyses through the MR-Egger method were performed. Nevertheless, we cannot completely ensure whether other unexplored factors are confounding these effects [62]. In addition, our sample size is small when compared to other MR studies, which can result in a relative loss of significance when exploring multiple MR methods with different assumptions. However, we ensured the inclusion of strong instruments by applying a two-sample MR approach in which the estimates of the genetic associations with the exposure of interest (i.e., longer TL) were calculated in a previous genome-wide meta-analysis of 78,592 individuals.

It is noteworthy that our cohort is rather selected and composed of middle-aged cognitively unimpaired individuals. This could entail a recruitment bias, particularly in individuals at high genetic predisposition of AD. Individuals at high genetic predisposition might have already shown signs of cognitive decline at recruitment, which was an exclusion criterion of the present study. Furthermore, this also implies that our participants showed a low prevalence of other common comorbidities and that our results might not be generalizable to individuals at more advanced stages in the AD continuum. Indeed, given its cross-sectional nature, endophenotypes included in the present study might reflect a transient stage within the AD pathological process. Finally, those associations that did not survive FDR multiple comparisons correction should be interpreted with caution.

Despite the aforementioned limitations, the strengths of our study include the robust and well-characterized cohort of cognitively unimpaired middle-aged individuals. This included the extensive characterization of cognitive outcomes, the use of high-resolution brain scans, the quantification of AD biomarkers and neurodegeneration biomarkers in CSF, and the genome-wide genotyping. Another important strength is also the higher proportion of APOE-ε4 carriers in the ALFA participants compared to the general population (19% vs. 14% in the general population; \( p < 0.001 \)) [84] and the available PRS of AD. Although carriership of the APOE-ε4 allele is the largest genetic risk factor of AD, PRS of AD has been also associated with cognitive decline [85], hippocampal function [86], and core AD CSF biomarkers [87]. In this sense, this study also has evidenced the benefits of using PRS-AD to characterize individuals at high genetic predisposition of AD (besides APOE) in population-based cohort studies and elucidate specific biological patterns associated with telomere length homeostasis.

To our knowledge, no prior MR studies have explored the potential causal association between TL and these AD endophenotypes (i.e., cognitive performance, AD and neurodegeneration. This result opens new avenues to study the role of telomeres on AD progression.

Conclusions

In conclusion, the results of this cross-sectional study show that genetically predicted longer TL may protect brain structure in cognitively unimpaired individuals. In addition, APOE alleles and the genetic predisposition to AD could modulate TL effects on CSF biomarkers of AD and neurodegeneration. This result opens new avenues to study the role of telomeres on AD progression.

Abbreviations

Aβ: Amyloid-β; AD: Alzheimer’s disease; APOE: Apolipoprotein E; CSF: Cerebrospinal fluid; DNA: Deoxyribonucleic acid; EF: Executive function; EM: Episodic memory; GWAS: Genome-wide association study; IVW: Inverse-variance weighted; MBT: Memory Binding Test; MCI: Mild cognitive impairment; MR: Mendelian randomization; MRI: Magnetic resonance imaging; NTL: Neurofilament light; PACC: Preclinical Alzheimer Cognitive Composite; PRESSO: Pleiotropy RESidual Sum and Outlier; PRS: Polygenic risk score; QC: Quality control; SE: Standard error; SD: Standard deviation; SNP: Single nucleotide polymorphism; TL: Telomere length; WAIS: Wechsler Adult Intelligence Scale.
Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13195-022-01101-9.

Additional file 1: Supplementary Table 1. Characteristics of Single Nucleotide Polymorphisms (SNPs) associated with longer telomere length. The effect allele is refered to the allele that is associated with longer telomere length. Chromosomal position of the SNPs (genome assembly GRCh37 (hg19)) according to the public archive for genetic variation within and across different species developed and hosted by the National Center for Biotechnology Information (NCBI) in collaboration with the National Human Genome Research Institute (NHGRI) (dbSNP).

Additional file 2: Supplementary Table 1. Linear regression estimates for cognition outcomes in the entire sample. All models are adjusted for covariates: age, sex, education, and APOE status. Supplementary Table 2. Linear regression estimates for neuroimaging outcomes (i.e., Alzheimer’s disease and aging signatures) in the entire sample. All models are adjusted for covariates: age, sex, education, and APOE status. Supplementary Table 3. Linear regression estimates for CSF biomarkers outcomes in the entire sample. All models are adjusted for covariates: age, sex, and APOE status.

Additional file 3: Supplementary Table 1. Characteristics of the study participants with information for cognition outcomes. Mean and SD are shown for continuous variables. Supplementary Table 2. Characteristics of the study participants with information for neuroimaging outcomes. Mean and SD are shown for continuous variables. Supplementary Table 3. Characteristics of the study participants with information for CSF biomarkers. Mean and SD are shown for continuous variables.

Additional file 4: Supplementary Table 1. Results of the effect of genetically predicted longer telomere length on AD endophenotypes among APOE-ε4 carriers. Supplementary Table 2. Results of the effect of genetically predicted longer telomere length on AD endophenotypes among APOE-ε4 carriers. Supplementary Table 3. Results of the effect of genetically predicted longer telomere length on AD endophenotypes among APOE-ε4 carriers.

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Authors’ contributions
B-RF contributed to sample preparation and harmonization, analyzed the data, interpreted the data, and took the lead in writing the manuscript and generated data visualizations. NV-T supervised the statistical analysis. G.S-R, M.S-C, G.O, C.M, K.F, G.K, I.S, M.C.dM, D.P, M.E, K.B, H.Z, and IdV contributed in the acquisition and preparation of the data, provided critical feedback, and helped shape the manuscript. EM.A-U, J.L.M, A.N, J.D.G, and A.S-V provided critical feedback and helped shape the research, analysis, and manuscript. NV-T and M.C-B conceived the presented idea, developed the theoretical formalism, designed the models, supervised the project and the versions of the manuscript, provided critical feedback, and contributed and supervised manuscript writing. All authors read and approved the final version of the manuscript.
manuscript. All authors have agreed both to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature.

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Availability of data and materials

The data that support the findings of this study are available from the corresponding authors (NVT, MCB), upon reasonable request.

Declarations

Ethics approval

The study was conducted in accordance with the directives of the Spanish Law 14/2007, of 3rd of July, on Biomedical Research (Ley 14/2007 de Investigación Biomédica). The ALFA and ALFA+ study protocols have been approved by the Independent Ethics Committee “Parc de Salut Mar” Barcelona and registered at ClinicalTrials.gov (ALFA Identifier: NCT01835717; ALFA+ Identifier: NCT02485730). All participants accepted the study procedures by signing an informed consent form.

Consent for publication

Not applicable.

Competing interests

GK is a full-time employee of Roche Diagnostics GmbH, IS is a full-time employee and shareholder of Roche Diagnostics International Ltd, HZ has served at scientific advisory boards for Denali, Roche Diagnostics, Wave, Santana, and CogRx, has given lectures in symposia sponsored by Fujirebio, Alzecure, and Biogen; and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program. KB has served as a consultant, at advisory boards, or data monitoring committees for Abcam, Axon, Biogen, JOYMOD/Shimadzu, Julius Clinical, Lilly, MagQu, Novartis, Roche Diagnostics, and Siemens Healthineers, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program. JLM is currently a full-time employee of Lundbeck and priorly has served as a consultant or at advisory boards for the following for-profit companies, or has given lectures in symposia sponsored by the following for-profit companies: Roche Diagnostics, Genentech, Novartis, Lundbeck, Oryzon, Biogen, Lilly, Janssen, Green Valley, MSD, Eisai, Alector, BioCioss, GE Healthcare, and ProMIS Neurosciences. MSC has served as a consultant and at advisory boards for Roche Diagnostics International Ltd and has given lectures in symposia sponsored by Roche Diagnostics, S.L.U, and Roche Farma, S.A. The other co-authors declare that they have no competing interests.

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