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Original Research

Association between 9p21-23 Locus and Frailty in a Community-Dwelling Greek Population: Results from the Hellenic Longitudinal Investigation of Ageing and Diet

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Abstract

BACKGROUND: Frailty is a complex geriatric syndrome arising from a combination of genetic and environmental factors and is associated with adverse health outcomes and mortality. A recent study reported an association between variants of the 9p21-23 locus, associated with a number of age-related disorders, including Alzheimer's disease (AD), and frailty. Frailty has been associated with increased risk of developing AD and it has been proposed that frailty burden may modify AD clinical presentation. In view of the overlapping genetic architecture between the two disorders, it is noteworthy to conduct studies to uncover risk variants that contribute to both AD and frailty. The purpose of this study is to test the reproducibility of the association of 9p21-23 locus with frailty in a population that is ethnically different from previous work and in the context of multidimensional definitions of frailty that will allow us to examine the potential impact to domains pertaining to AD pathology.

METHODS: We operationalized frailty according two definitions and the corresponding instruments, the Frailty Index (FI) and the Tilburg Frailty Indicator (TFI) and we determined genotypes of eight alleles previously identified as risk increasing for frailty in 1172 community-dwelling older participants (57% females) from the HELIAD study with a mean age of 74 years old. We cross-sectionally investigated the association between risk alleles and frailty, as well as with specific components of each definition using linear regression analyses adjusted for age, sex and years of education.

RESULTS: Compared to non-carriers, carriers of rs7038172 C risk allele, were associated with a higher FI Score (β=0.089, p=0.002). Similarly, we found a positive association between the presence of at least one rs7038172 C variant and TFI score (β=0.053, p=0.04). Moreover, the rs7038172 variant was associated, irrespectively of dementia status, with the memory and psychological domain of FI and TFI, respectively.

CONCLUSION: Our study confirms the association of the rs7038172 C allele with the frailty syndrome in a Greek population and in the context of multidimensional definitions of frailty. Furthermore, we report novel associations between this allele and the memory domain of FI and the psychological domain of TFI, that includes memory problems on its components. Given that frailty burden has been shown to modify the AD clinical presentation, it is likely that rs7038172 C allele may accelerate the transition of AD or frailty to dementia Overall, our study corroborates the role of the 9p21-23 region in frailty development and draw potential links with AD pathology.

Key words: Frailty, 9p21-23 locus, Alzheimer, genetics.

Introduction

Frailty is a major geriatric syndrome characterized by the inability to preserve homeostasis and is associated with a number of adverse health outcomes and mortality (1, 2). The etiology of frailty is thought to be multifactorial, arising from a combination of genetic, psychological, lifestyle and environmental factors (3). In an attempt to disentangle the complex nature of frailty, researchers have been investigating the factors contributing to frailty development.

Critically, frailty syndrome and Alzheimer disease (AD) share a common pathophysiological mechanism (4) and frailty has been recently associated with higher risk of AD (5) and AD disease pathology (6). Interestingly, it

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has been proposed that co-occurrence of frailty syndrome may influence the relationship between established AD biomarkers and cognitive status (7). Considering the overlapping genetic architecture of frailty and AD (8), it is of great value to conduct genetic association studies to uncover shared genetic variation between the two syndromes that may underlie the mechanism behind the transition of frailty to AD and with the potential to be used as biomarkers for diagnostic or medical purposes (9).

Numerous definitions for frailty syndrome have been described until now, that can be categorized into two main groups (10). The first category of definitions rely only on the physical aspects of frailty with the most widely used to be the Fried definition (10). In contrast, other definitions adopt a more holistic approach and conceptualize frailty as a count of accumulated deficits and clinical conditions, considering not only biological but also psychological, lifestyle and cognitive parameters (11). Two frequently used instruments, the Frailty Index, FI (12) and the Tilburg Frailty Indicator (TFI) (13, 14) belong to this category.

Twin studies suggest a significant genetic contribution to the development of the frailty syndrome, with an estimated heritability of 43% for physical frailty (15) and between 25% (15) and 45% (3) for multidimensional frailty. Sathyan et al (16) examined the 9p21-23 locus, based on the rationale that genetic variation in this region have been identified in a number of Genome Wide Association Studies (GWASs) of complex disorders, such as cardiovascular diseases (17), atherosclerosis (18), myocardial infarction (19) and amyotrophic lateral sclerosis (20). Similarly, variants at 9p23 locus have been linked to restless leg syndrome (21), obsessive-compulsive disorder (22) and several types of cancer (23), (24). Importantly, the 9p21 locus has been implicated, through genetic association studies, in the development of late-onset Alzheimer disease (25) and vascular dementia (26). Thus, genetic variation in 9p21-23 locus might confer a higher risk for both frailty and AD and mediate the transition from normal status to frail and frail status to demented or vice vers.

In the aforementioned study, Sathyan et al (16) identified eight Single-Nucleotide Polymorphisms, SNPs (rs518054, rs571221, rs1855850, rs1324192, rs7019262, rs10511667, rs7034231,rs7038172) residing at the 9p21-23 locus, that conferred a higher risk for frailty status, as defined with Fried definition. The strongest signal was observed for the G allele of rs518054, located at the enhancer region of NIFB gene, a transcription-factor encoding gene, that acts as an epigenetic regulator and it is involved in cell differentiation (16).

The purpose of this study is to test the reproducibility of the genetic association of 9p21-23 locus with frailty, in an independent European Caucasian population of older adults. To this end, we used genotyping data from the HELIAD study, an ethnically homogeneous cohort of unrelated individuals of Greek ethnic background (27), and examined the association of the 9p21-23 risk SNPs with frailty status as defined by FI and TFI definitions. Furthermore, given that the 9p21-23 locus has been linked with AD, we examined the association of significant SNPs with specific domains of multidomain definitions, including cognitive and memory domains that pertain to AD susceptibility.

Methods

Participants

The Hellenic Longitudinal Investigation of Aging and Diet (HELIAD) study is a large-scale, population-based, multidisciplinary study designed to estimate the prevalence and incidence of age-related neuropsychiatric conditions in Greece as well as to explore possible associations with lifestyle factors and diet. Participants, aged over 65 years, were selected through random sampling from the record of two Greek municipalities; Larisa and Marousi, and they all gave their informed consent prior to enrollment. HELIAD participants were recruited in 2011 and they are reevaluated at intervals of approximately 3 years, repeating the baseline examination and consensus diagnosis at each follow-up. Currently, two evaluations per person have been completed.

Qualified neurologists performed detailed medical evaluation to all participants. Next, they administrated questionnaires together with trained neuropsychologists to gather exhaustive information pertaining several domains: demographics, medical history, neurological, psychiatric, and neuropsychological assessment, anthropometry, and lifestyle parameters including nutrition, physical activity, sleep and social life. The duration of the evaluation was about 2–2.5 h per participant. Details on the design and scope of HELIAD study have been extensively described previously (27–29). In brief, the participants' recruitment began in 2011, for baseline assessment, and a follow-up, is currently being conducted. HELIAD study was approved by the ethics committee of the University of Thessaly and the National and Kapodistrian University of Athens.

Frailty Assessment

Frailty was assessed using two instruments, FI (12) and TFI (30, 31), that assess frailty taking into account multiple factors including physical, emotional, cognitive, psychological and social parameters, in accordance to the respective definitions. In the HELIAD study, we have collected information from the HELIAD data to operationalize frailty using Fried's phenotype model (32, 33), in accordance with the Sathyan et al study (16). However, due to the circumscribed criteria of the Fried definition and the fact that HELIAD is a population-

based study and not a target-based study, only 43 of 1172 individuals in the HELIAD sample were identified as frail. Thus, we define frailty using FI and TFI instruments, yielding a higher number of individuals as frail, compared to the Fried model (33), in order to ensure a priori reasonable power, similarly with that of the discovery study that included 206 frail patients (32.5%) (16). Furthermore, operationalizing frailty with multidimensional definitions of frailty that include cognitive measures and not only physical aspects of frailty, gave us the opportunity to examine the association of the 9p21-23 region with cognitive and memory domains of frailty, that pertain to AD pathology.

The Frailty Index was developed by Rockwood et al (12) and considers frailty as a sum of an accumulation of deficits. Frail individuals were defined based on a FI score calculated as a ratio of the number of deficits present in an individual divided by the total number of items evaluated (12). The FI score was constructed using 61 related deficits that were clustered into functional, psychological, cognitive and memory domains. The functional domain included items related to medical conditions or everyday activities, the psychological domain included mood-related problems, the cognitive domain included cognitive measures and the memory domain included problems with memory (Suppl Appendix 1). The presence of each deficit scored one point. FI items. The FI score was considered as a dichotomous variable, using a cut-off point of 0.25 to define frail individuals (12), as well as a continuous variable with higher scores indicating the presence of more deficits and thus a greater degree of frailty.

The TFI was developed by Gobbens et al (30) and assesses frailty status through self-report data pertaining to physical, psychological and social domains. More specifically, the physical domain is evaluated by questions about physical health, weight loss, walking, balance, hearing, vision, hand strength, and fatigue. The psychological part includes questions about memory problems, anxiety and mood disorders whereas the social part consists of questions about living alone, missing people and receiving adequate support.

TFI is distinguished from FI as it is based on self-report data and it does not assess disability or diseases because it considers frailty as a predisability state (30). We used a slightly modified TFI, we excluded 2 of the 15 original criteria, due to the lack of data regarding subjective decreased hand strength and ability to cope with problems. Participants who met at least 5 criteria were considered as frail (30).

Genotyping and imputation

Genome-wide genotyping was performed for 1,446 individuals at the facilities of the "centre national de recherche en génétique humaine" (Evry, France) using the Illumina Infinium Global Screening Array (GSA, GSAsharedCUSTOM_24+v1.0), as part of the European Alzheimer DNA biobank (EADB) project. A detailed description of the EADB genotyping, QC and imputation can be found elsewhere (34).

In summary, variants included in the marker list for removal, provided by Illumina, or variants not uniquely aligned in GRCh37 genome were excluded for further analysis. Moreover, variant intensity quality control (QC), was conducted for all autosomal variants, according to established thresholds (35).

Next, we performed sample quality control using PLINK v1.9 software (36–38). Specifically, samples with missingness > 0.05, sex inconsistencies or with heterozygosity rate that deviated more than ± 6 SD (Standard Deviation) from the mean, were excluded. To identify population outliers, we run Principal Component Analysis (PCA), using as reference dataset the population of 1000 Genome (phase 3) and we projected the combined dataset (1000GP3 samples and the EADB samples) onto two dimensions, using the flashPCA2 software (39). To control for cryptic relatedness, we excluded one individual from each pair of samples with a kinship coefficient more than 0.125 (cut-off for third-degree relatives), yielding a final sample size of 1251 unrelated individuals.

Regarding quality controls of variants, we excluded variants showing a missingness > 0.05 in at least one genotyping center or having a differential missingness test P<10-10. The Hardy-Weinberg equilibrium test (p<10–6) was performed only in controls.

Imputation

To improve the accuracy of imputation, we compared the frequencies of variants (chi-square test) against two reference panels, the population of the Haplotype Reference Consortium r1.1 (HRC) (40) excluding samples from 1000genomes as well as the Finnish and the non-Finnish population of Genome Aggregation Database v3 (gnomAD) (41)). Variants showing a x2 >3,000 in both HRC and gnomAD or a x2>3,000 in one reference panel and not present in the other were excluded. Finally, GWASs were performed between controls across genotyping centers to assess frequency differences between genotyping centers, using the software SNPTEST (42), under an additive model and adjusting for Principal Components (PCs). Variants having a Likelihood Ratio Test of p < 10-5 were excluded. Furthermore, we removed ambiguous variants with Minor Allele Frequency (MAF) > 0.4 and we kept only one copy of any duplicated variants, prioritizing the one with the lowest missingness.

All samples and variants, passing the above QC metrics were imputed o Michigan Imputation Server (v1.2.4) (43), using the TOPMed Freeze 5 reference panel. Phasing and imputation were performed using EAGLE v2.4 (44) and Minimac4 v4-1.0.2 software, respectively.

Statistical Analysis

All statistical analyses were performed using SPSS 23 (SPSS, Chicago, IL, USA). Baseline characteristics between the two groups were compared through analysis of variance for continuous variables and Pearson's x2 for categorical variables.

Associations between each SNP and frailty status, as determined according to FI and TFI instruments, were tested using linear regression analysis with frailty status as the outcome and SNP genotype as the predictor.

The number of minor homozygotes for the majority of SNPs was too small to provide sufficient statistical power, thus we performed all analyses under the dominant genetic model. More specifically, the genotype of each SNP was dichotomized to carriers and non-carriers and we included in the model as covariates age (years), sex and years of education. We evaluated the Hardy–Weinberg equilibrium for each SNP through Pearson's x2. As evidence of statistical significance and given that our goal was to replicate results of the discovery study, we used the uncorrected $p < 0.05$ (16). A post-hoc power analysis was also performed using the R package "genpwr" for the SNPs (45) that showed significant associations. We estimated that given our sample size, we had > 80% power to detect a statistically significant SNP association with FI outcome at a significance level set at alpha=0.05 and under a dominant genetic model. Accordingly, the estimated power was 71% to detect significant association with TFI outcome as the available sample size for analysis was slightly diminished due to missing values.

Next, we grouped FI and TFI items into major domains (46-48) and tested the association of variants that reached a statistical significance of $p < 0.05$, with each frailty domain, separately. Regarding the memory domain of FI and the psychological domain of TFI, that includes memory features on its components and as such the results could be confounded by the presence of dementia, we repeated the analyses after excluding patients with dementia.

Results

From the entire sample of the 1984 HELIAD study individuals, we included only those aged older than 65 years old, with available genotype data and who were unrelated to others in the sample, yielding a final sample size of 1172 individuals of Greek ethnicity. Regarding FI and TFI instruments, 1172 and 1161 individuals, respectively had completed information for the scoring, and thus were eligible for analysis. Demographic characteristics are summarized in Table 1. When we defined frailty based on FI instrument, the mean age of study participants was 73.89 (±5.27) years and females accounted for 57% of the study cohort. The mean number of years of education was 6.8 (±4.5). Demographic characteristics were similar for the participants (n=1161) assessed with TFI instrument (Table 1).

Table 1. Demographic characteristics of study participants

	FRAILTY INDEX		
	Frail (n=236)	**Non-frail (n=936)**	**All (n=1172)**
Sex, n (%)			
Male	80 (33.9)	423 (45.2)	503 (42.9)
Female	156 (66.1)	513 (54.8)	669 (57.1)
Age (mean ± SD) (years)	75.59 (±5.34)	73.46 (±5.17)	73.89 (±5.27)
Edu (mean ± SD) (years)	5.66 (±4.17)	7.11 (±4.52)	6.81 (±4.49)
BMI (mean ± SD)	29.95 ((±5.22)	29.04 (±4.55)	29.21 (±4.70)

The observed allele frequencies of all SNPs did not differ from the expected frequencies under the Hardy-Weinberg equilibrium (Table S1).

Details about chromosomal location, population-based frequency, linkage disequilibrium patterns and putative regulatory functions of the 8 SNPs were studied (Table 2).

For each SNP examined, we categorized the participants into carriers (heterozygotes and homozygotes for minor allele) and non-carriers (homozygotes for major allele) and we estimated the prevalence of frailty by each definition, separately. The results are displayed in Table 3.

Frailty Index

Linear regression analysis revealed a positive relationship between rs7038172 C allele and FI score, inserted as a continuous variable (β=0.089, SE=0.008, p=0.002) (Table 4).

No significant association between rs518054, rs10511667, rs1855850, rs571221, rs7019262, rs7034231, rs1324192 and FI score was observed.

Tilburg Frailty Indicator

Similar to FI, we observed a significant relationship between the rs7038172 C allele and TFI score. More specifically, carriers of the rs7038172 C allele exhibited higher TFI scores compared to non-carriers in the adjusted linear regression model (β=0.053, SE=0.139, p=0.04) (Table 4).

The analysis revealed no significant associations of rs518054, rs10511667, rs1855850, rs571221, rs7019262, rs7034231, and rs1324192 with TFI scores.

Association of risk SNPs with specific domains of Frailty Index and Tilburg Definitions

The results showed that the C allele of rs7038172 was significantly associated with the FI memory domain and the TFI psychological domain. In particular, rs7038172 C carriers were marginally associated with a higher score in the FI memory domain (OR=1.39, p=0.05) (Table S2).

Table 2. Information about genomic location, population-based frequency (gnomAD), Linkage disequilibrium (LD) patterns and regulatory features of the 8 SNPs studied

Variant	Ref	Alt	Genomic coordinates (hg19)	Cytoband	gnomAD NFE Freq	SNPs within LD (r^2), EUR	Gene, Location	Chromatin state	Interacting gene	Proteins bound
rs518054	T	G	9:13689066-13689067	9p23	0.21	rs571221 (0.98) rs522221 (0.96)	non coding	Enhancer	NFIB	CTCF
rs7019262	G	A	9:13614384-13614385	9p23	0.62	rs10961173 (0.96) rs12380076 (0.89) rs1324192 (0.88) rs1408321 (0.97)	non coding	Enhancer	-	P300
rs571221	T	C	9:13690235-13690236	9p23	0.21	rs518054 (0.89) rs522221 (0.96)	non coding	-	-	-
rs10511667	A	G	9:18989696-18989697	9p22.2	0.12	rs4130083 (0.84)	SAXO1, intron1	Enhancer	-	-
rs7034231	G	T	9:28119512-28119513	9p21.2	0.85	-	LINGO2,			
intron 6	Enhancer	-	-							
rs7038172	T	C	9:16708269-16708270	9p22.2	0.05	rs16934924 (1.0) rs2297175 (1.0)	BNC2,			
intron 3	Enhancer	BNC2	GATA3 PORL2A							
rs1855850	T	C	9:10480030-10480031	9p23	0.67	rs1853231 (0.92)	PTPRD	-	-	-
rs1324192	A	T	9:13612345-13612346	9p23	0.65	rs10961173 (0.92) rs12380076 (0.99) rs1408321 (0.89) rs7019262 (0.88)	non coding	-	-	-

NFE: Non-Finnish European, LD: linkage disequilibrum, EUR: Europeans; Data is derived from rVarBase (http://rv.psych.ac.cn/index.do) and gnomAD database (https://gnomad.broadinstitute.org/).

Table 3. Results of the association between SNPs genotype status and frailty, stratified by definition

Variant	Risk Allele	Frailty Index Definition			Tilburg Definition		
		Non-frail (n=936)	Frail (n=236)	p-value	Non-frail (n=742)	Frail (n=419)	p-value
rs518054	G			0.17			0.30
non-carriers (n=681)		537 (57.4%)	144 (61%)		428 (57.7%)	249 (59.4%)	
carriers (n=491)		399 (42.6%)	92 (39%)		314 (42.3%)	170 (40.6%)	
rs10511667	G			0.93			0.39
non-carriers (n=916)		732 (78.2%)	184 (78 %)		578 (77.9%)	330 (78.8%)	
carriers (n=256)		204 (21.8%)	52 (22%)		164 (22.1%)	89 (21.2%)	
rs1855850	C			0.15			0.12
non-carriers (n=489)		383 (40.9%)	106 (44.2%)		299 (40.3%)	184 (43.9%)	
carriers (n=683)		553 (59.1%)	130 (55.1%)		443 (59.7%)	235 (56.1%)	
rs571221	C			0.25			0.41
non-carriers (n=690)		546 (58.3%)	144 (61%)		436 (58.8%)	250 (59.7%)	
carriers (n=482)		390 (41.7%)	92 (39%)		306 (41.2%)	169 (40.3%)	
rs7019262	A			0.28			0.11
non-carriers (n=364)		295 (31.5%)	69 (29.2%)		222 (29.9%)	141 (33.7%)	
carriers (n=808)		641 (68.5%)	167 (70.8%)		520 (70.1%)	278 (66.3%)	
rs7034231	T			0.27			0.38
non-carriers (n=831)		668 (71.4%)	163 (69.1%)		528 (71.2%)	294 (70.2%)	
carriers (n=341)		268 (28.6%)	73 (30.9%)		214 (28.8%)	125 (29.8%)	
rs1324192	T			0.43			0.10
non-carriers (n=391)		314 (33.5%)	77 (32.6%)		239 (32.2%)	151 (36%)	
carriers (n=781)		622 (66.5%)	159 (67.4%)		503 (67.8%)	268 (64%)	
rs7038172	C			0.02			0.15
non-carriers (n=999)		809 (86.4%)	190 (80.5%)		641 (86.4%)	352 (84%)	
carriers (n=173)		127 (13.6%)	46 (19.5%)		101 (13.6%)	67 (16%)	

*p value from exact Fisher test

Table 4. Linear regression association results of rs7038172 with frailty status as defined by Frailty Index, adjusted for age, sex and years of education

Variant	FRAILTY INDEX (236 frail vs 936 non-frail)		TILBURG DEFINITION (419 frail vs 742 non-frail)	
	β (S.E)	P value	β (S.E)	P value
rs7038172 (C)				
Non-carriers (n=999)	1		1	
Carriers (n=173)	0.089 (±0.008)	0.002	0.053 (±0.139)	0.04

Regarding TFI, the presence of rs7038172 C allele was associated with a higher score in the TFI psychological domain (OR=1.51, p=0.02) (Table S3). The results for the association rs7038172 C allele with other domains of FI and TFI did not reach statistical significance.

Considering that the associated domains of FI and TFI include memory and cognitive measures, and, as such, the result could be confounded by the presence of dementia, we performed an exploratory analysis excluding the patients with dementia (n=58). Notably, there was a marginally significant trend towards higher score in the FI memory domain (OR=1.41, p=0.05) among rs7038172 carriers whereas rs7038172 carriers were associated with a higher score in the TFI psychological domain (OR=1.59, p=0.01) (Tables S2 and S3).

Discussion

In the present study, we examined the relationship between common variants at the 9p21-23 locus, previously associated with frailty (16), and frailty syndrome in a large population of unrelated to each other Greek individuals from the HELIAD study. The rs7038172 C allele, located in the 9p21-23 region, was associated with frailty syndrome, as defined with FI and TFI, a finding that replicates the results of the Sathyan et al (16) study, in a ethnically different population. Moreover, the rs7038172 risk allele was linked with a higher score in the psychological domain of TFI as well as with a higher marginal score of FI memory domain, irrespectively of dementia status.

We sought to replicate the results from the Sathyan et al [16] study, that investigated the role of 9p21-23 region on frailty development, in an Ashkenazi Jewish cohort. The 9p21-23 region has been implicated in the pathogenesis of several age-related disorders, including cardiovascular diseases and myocardial infarction (49), and it has also been associated with longevity (50). Critically, 9p21-23 region has been found to be a risk locus for late-onset Alzheimer disease (25) and vascular dementia (26).

It is of critical clinical importance to assess the relationship of 9p21-23 locus with frailty development because this region has been suggested as a potential therapeutic target for a number of conditions and as such it could provide information for novel treatment options for frailty (51). In this line, it is noteworthy to identify the causative variants that drive the signal seen in GWAs studies. Given that 9p21-23 locus has been identified as a risk locus for AD development and that AD and frailty pathology are closely connected, risk alleles residing in this region may contribute to the interplay between transition from frailty to AD and vice versa. This is extremely prominent, considering that frailty has been shown to modify the association between AD pathology and AD clinical manifestation (52). Therefore, risk variants shared between the two disorders may explain the discrepancies between established AD biomarkers and AD clinical presentation that is mediated by frailty syndrome (52).

In contrast to the Sathyan et al (16) study, which used the Fried definition to determine frailty, in the HELIAD cohort frailty syndrome was operationalized with two other instruments, the Frailty Index (FI) (12) and Tilburg Frailty Indicator (30, 31). By employing the multidomain definitions of frailty, we acquired a sample size of frail individuals, similar to that of the discovery study (16), thus ensuring adequate statistical power to make meaningful comparisons. Furthermore, given the lack of consensus on the definition of frailty, it is worthwhile to investigate the associations between the 9p21-23 locus and frailty in case of other definitions as well as in populations with different ethnic backgrounds. The operationalization of frailty with multidomain definitions that include cognitive measures and not only physical aspects of frailty, gave us the opportunity to examine the association of the 9p21-23 region with cognitive and memory domains of frailty, that pertain to AD pathology. By performing additional analysis, we unraveled novel associations of 9p21-23 risk alleles with memory components of FI and TFI definitions not previously reported, providing a mechanistic insight into this association that may be also relevant to the established connection between frailty syndrome and AD.

In line with the Sathyan et al (16) study, the rs7038172 C allele was associated with a higher risk for frailty, as defined by both FI and TFI instruments. Critically, functional in-silico analysis revealed that the rs7038172 variant is located in the enhancer region of Basonuclin 2, BNC2 gene (Table 2). Aberrant expression of the BNC2 gene has been shown to contribute to tumor progression in several studies (53, 54) and it has been proposed that BNC2 may function as a tumor suppressor gene (55–57). Furthermore, in the study of Chen et al (58), cell lines overexpressing 4R tau isoforms (contain exon 10), known to be more abundant in the brain of Alzheimer

patients compared to 3R tau isoforms (lack exon 10), were characterized by under expression of the BNC2 gene compared to cell lines overexpressing 3R tau isoforms. Thus, it is likely that the rs7038172 C allele may contribute to frailty development by altering BNC2 expression, leading to aberrant BCN2 levels and through this mechanism may affect health or cognitive domains, included in FI and TFI definitions.

To test this hypothesis, we performed additional analyses investigating the association of the rs7038172 C variant with each domain of the FI and TFI frailty criteria. Notably, the results revealed a trend toward a higher score in the FI memory domain among carriers of the rs7038172 C variant and a significant association of rs7038172 SNP with the TFI psychological domain, that includes memory problems among its components. These results were independent of dementia status. Intriguingly, the rs7038172 C allele was not associated with the cognitive domain of FI and TFI , perhaps because the most impaired cognitive domain of frail patients relates to memory performance and, thus this association, is likely more evident and more robust to be captured (59). A recent study reported that individuals with even a low level of AD pathology might be more prone to AD dementia if they have high amounts of frailty compared to individuals with lower burden of frailty (52). Thus, the rs7038172 C allele might be part of the genetic mechanism that contributes to the transition of AD or frailty to dementia, in a time and context dependent manner. Given the link between rs7038172 C allele and memory performance, it is likely that rs7038172 C allele might also accelerate the progression from AD to dementia and thus partially explain the key role of frailty syndrome in the natural history of AD (52).

On the other hand, the SNP that showed the strongest association with frailty, in the discovery study of Sathyan et al (16), was the rs518054. This SNP is located in the enhancer region of the NFIB gene, a major transcription factor that promotes cell survival (60), regulates cell differentiation (61) and mediates epigenetic modifications (62). The reason our study failed to replicate this association may pertain to the different definitions used. It is likely that the rs518054 C allele has an effect on physical aspects of frailty that dominate the Fried definition (63) and that the association signal may be diluted using multidimensional definitions, that assess other factors to identify frail individuals (12, 13).

The current study has several limitations. First, the lack of functional studies allowed us only to draw hypotheses regarding the biological mechanism underlying the associations identified. It is likely that the effect of these variants may be exerted in conjunction with other unknown genetic variants or/and environmental factors that may also contribute to frailty susceptibility. Furthermore, longitudinal studies need to be conducted to examine if frail carriers of rs7038172 C allele, run a higher risk of AD dementia over time compared to frail non-carriers.

The present study also has several strengths. To the best of our knowledge, this is first study that replicated the finding that the rs7038172 C allele contributes to frailty status, as reported by Sathyan et al (16). This is extremely prominent, considering the fact that the current analysis was performed in a larger and completely different ethnic cohort, in Greek individuals (n=1330), instead of Ashkenazi Jewish adults (n=637), as in the Sathyan et al (16) study. Critically, the analysis was carried out by employing frailty definitions other than that of Fried and colleagues, which was used in the discovery study (16). This allowed us not only to replicate associations between variants at the 9p21-23 locus and frailty but also to assess the relevance of these observations in the context of other frailty definitions. Following the multidimensional approach to define frailty allowed us to perform further analyses investigating the mechanism behind the association between rs7038172 C allele and frailty development and these analyses unraveled a potential link with AD pathology. This study has the merit of having a large and well-characterized representative sample of older Greek men and women for whom relevant clinical and genetic information were available. The availability of comprehensive assessments permitted the investigation of two definitions, through which we uncovered novel relationships between candidate SNPs and frailty status and we provided insights into the causal mechanism and the nature of the allele's effect on frailty.

Conclusion

In conclusion, we confirmed the association between the rs7038172 C allele and the frailty syndrome, in a Greek population of older adults and in the context of multidimensional definitions of frailty. Furthermore, we reported novel associations of the rs7038172 allele with the memory of FI and psychological domain of TFI, that include memory on its components. Given that frailty burden has been shown to modify the AD clinical presentation, it is likely that rs7038172 C allele may accelerate the transition of AD or frailty to dementia. The lead SNP rs518054 of the initial study was not associated with the multidimensional concept of frailty, suggesting that it exerts its impact on physical aspects. Overall, the present study corroborates the role of the 9p21-23 region in frailty development and draw potential links with AD pathology. Cross-sectional, long-term prospective and functional studies need to be conducted to confirm this finding and to delineate the potential causal mechanisms behind these associations. The present study substantially contributes to the existing knowledge regarding the genetic underpinnings of frailty development one of the most important geriatric syndromes.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Boards of the University of Thessaly, Larisa, Greece, and the National and Kapodistrian University of Athens, Greece, Ethics Committees.

Data Availability Statement: We carefully documented data, methods, and materials used to conduct the research in the article. We will share anonymized data at the request of other quali-fied investigators for purposes of replicating procedures and results.

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