Association of Torquetenovirus Viremia with Physical Frailty and Cognitive Impairment in Three Independent European Cohorts

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Abstract

Introduction: Immunosenescence and inflammaging have been implicated in the pathophysiology of frailty. Torquetenovirus (TTV), a single-stranded DNA anellovirus, the major component of the human blood virome, shows an increased replication rate with advancing age. An elevated TTV viremia has been associated with an impaired immune function and an increased risk of mortality in the older population. The objective of this study was to analyze the relation between TTV viremia, physical frailty, and cognitive impairment. ***Methods:*** TTV viremia was measured in 1,131 nonfrail, 45 physically frail, and 113 cognitively impaired older adults recruited in the MARK-AGE study (overall mean age 64.7 ± 5.9 years), and then the results were checked in two other independent cohorts from Spain and Portugal, including 126 frail, 252 prefrail, and 141 nonfrail individuals (overall mean age: 77.5 ± 8.3 years). ***Results:*** TTV viremia ≥4log was associated with physical frailty (OR: 4.69; 95% CI: 2.06–10.67, $p < 0.0001$) and cognitive impairment (OR: 3.49, 95% CI: 2.14–5.69, $p < 0.0001$) in the MARK-AGE population. The association between TTV DNA load and frailty status was confirmed in the Spanish cohort, while a slight association with cognitive impairment was observed (OR: 1.33; 95% CI: 1.000–1.773), only in the unadjusted model. No association between TTV load and frailty or cognitive impairment was found in the Portuguese sample, although a negative association between TTV viremia and MMSE score was observed in Spanish and Portuguese females. ***Conclusions:*** These

findings demonstrate an association between TTV viremia and physical frailty, while the association with cognitive impairment was observed only in the younger population from the MARK-AGE study. Further research is necessary to clarify TTV's clinical relevance in the onset and progression of frailty and cognitive decline in older individuals.

Keywords:
Torquetenovirus, Cognitive impairment, Physical frailty, Aging, Inflammation

Introduction

Torquetenovirus (TTV) is a small, single-stranded circular DNA virus classified into the Anelloviridae family, detectable in various body fluids of healthy individuals [1, 2] and highly prevalent in the general population [3, 4]. TTV is generally considered to be a nonpathogenic endogenous virus, but an increased TTV viremia has been associated with immune suppression [5], decreased natu- ral killer activity, lower CD4+ count, and a higher risk of having a CD4/CD8 ratio <1 [4, 6].

Moreover, TTV load ≥4 log copies/mL has been asso- ciated with an increased risk of mortality in the older population [6]. A direct causal link between TTV infection and specific diseases is lacking, although the virus might aggravate the course of some diseases [7, 8] through increased stimulation of the inflammatory response and by promoting immunosenescence [4, 9].

Longitudinal studies in elderly populations show that other viruses responsible for chronic persistent infections such as cytomegalovirus (CMV) can induce T-cell changes (oligoclonal, memory T cells, reduction in the naïve T cells, and CD4/CD8 ratio), in turn associated with an increased risk of physical frailty and mortality [10, 11]. Other evidence demonstrates an association of viral pathogen burden of herpes simplex virus and CMV with cognitive decline and dementia [12, 13].

Investigations on the implication of TTV in physical frailty and cognitive impairment, however, are lacking. Therefore, the main aim of the present study was to analyze the relation between TTV viremia, physical frailty, and cognitive impairment in a population

recruited in the MARK-AGE project and two other independent cohorts from Spain and Portugal.

Materials and Methods

Study Population

In the present study, we measured TTV viremia in peripheral blood from 1,289 RASIG (Randomly recruited Age-Stratified Individuals from the General population) subjects (mean age: 64.7 ± 5.9 years) who comprised 1,131 nonfrail, 45 physically frail, and 113 cognitively impaired subjects. Participants were recruited during the MARK-AGE cross-sectional study [14, 15], and they came from six different European countries (Italy, Germany, Poland, Greece, Austria, Belgium). Seropositivity for human immunodeficiency virus (HIV), hepatitis B virus (HBV) (except seropositivity by vaccination), and hepatitis C virus (HCV) represented exclusion criteria. Details of the recruitment procedures and the collection of anthropometric, clinical, and demographic data, as well as of laboratory parameters assays have already been reported [16–18].

Fresh whole blood was collected after overnight fasting. Samples of plasma, serum, peripheral blood mononuclear cells, and whole blood from the various recruitment centers were shipped to the MARK-AGE Biobank located at the University of Hohenheim, Stuttgart, Germany. From the Biobank, coded samples were subsequently sent to the Scientific and Technological Pole of INRCA of Ancona, Italy, on dry ice, where they were stored at −80°C until use [16]. To further investigate the association between TTV vire- ognitive status, two other independent cohorts Spain and Portugal were analyzed.

The Spanish cohort was composed of 256 older adults (aged 65 and over) recruited from nursing homes and senior associations located in the region of Galicia (NW of Spain). Exclusion criteria included a history of cancer, or any chronic infection (e.g., human immunodeficiency virus, hepatitis B virus, hepatitis C virus), or taking medications that could affect the immune system. Ethical approval was obtained from the University of A Coruña Ethics Committee (CE 18/2014). All participants, or their relatives in case of inability, gave written informed consent and completed a questionnaire with information about lifestyle, demographics, and medical issues. Qualified staff with wide experience

in the gerontology field conducted the clinical evaluation of the individuals [19]. In order to maximize adherence to the criteria, the staff to be involved received standardized training prior to the start of the study. Whole blood was obtained by venipuncture into vacutainer tubes containing ethylenediaminetetraacetic acid as an anticoagulant. Samples were collected early in the morning and coded to ensure a blinded study. They were immediately transported to the laboratory, where they were aliquoted and stored at −80°C. Later, samples were sent on dry ice to the Scientific and Technological Pole of IN- RCA of Ancona, Italy, where they were stored at −80°C until use.

The Portuguese study population comprised 260 older adults aged 65 years or older recruited from community-dwelling and care centers (nursing homes and day care centers) located in the northern region of Portugal (metropolitan area of Porto and Cávado subregion). All participants were informed of the goals of the study, nature of participation risks and benefits, and asked to sign an informed consent form before being included in the study. Exclusion criterio included severe dementia and/or cognitive impairment, lack of ability to communicate, severe impairment of sight and hearing, and receiving palliative care. Ethical approval was obtained from the Ethics Committee of the Institute of Public Health of the University of Porto (ISPUP) (No. CE17081). Whole blood samples were obtained in the morning by venipuncture and collected into vacutainer tubes containing ethylenediaminetetraacetic acid as an anticoagulant; samples were coded to ensure blinded analysis. Samples were immediately transported in a cooler (4°C, transport within 40 min maximum) to the laboratory, aliquoted, and stored at −80°C. Then, the samples were shipped on dry ice to the Scientific and Technological Pole of INRCA of Ancona, Italy, where they were stored at −80°C until use.

Frailty and Cognitive Status

Physical frailty was defined according to the Frailty Phenotype [20]. In the MARK-AGE population, participants were considered physically frail if they fulfilled at least 2 out of 4 frailty criteria described by Fried: unintentional weight loss, exhaustion, low physical activity, and reduced handgrip strength. Since gait speed was not available, 4 criteria were used instead of 5, adjusting the cutoff as previously reported [21]. Participants were considered cognitively impaired when scoring below the 10th percentile on global cognitive functioning. Global cognitive functioning was based on the scores of

participants on the 15-Picture Word Learning test (to evaluate immediate and delayed memory function), the Stroop test, and the Digit Symbol Substitution Test. These scores were transformed into z-scores and combined into one global cognitive functioning score that was adjusted for the level of education [21].

In the Spanish and Portuguese populations, subjects included in 77.5 ± 8.3 years) were classified as frail (n = 126), prefrail (n = 252), or nonfrail (n = 141) according to the five Fried's criteria [20]. Individuals with positive for three or more criteria were classified as frail; those with positive for one or two criteria were classified as prefrail, while those with no positive items were classified as nonfrail [22, 23].

In the Spanish cohort, cognitive status was assessed using the Mini-Mental State Examination scale (Spanish version by Blesa et al.) [24]. Scores of this questionnaire range from 0 to 30 and are adjusted for age and education level. Cognitive impairment was defined for those participants who scored 24 or less.

The cognitive status in the Portuguese study population was also evaluated via the Mini-Mental State Examination scale (Portuguese adaptation by Morgado et al.) [25]. The maximum score is 30, adjusted according to literacy, and the cutoff values for the senior Portuguese population, indicative of cognitive impairment, are ≤22 (0–2 years of literacy), ≤24 (3–6 years of literacy), and ≤27 (more than 6 years of literacy).

TTV DNA Detection and Quantification

Viral DNA was extracted from whole blood samples using QIAamp DNA Blood mini kit (Qiagen GmbH, Germany) according to the manufacturer's instructions. Presence and load of TTV DNA were determined in a single-step in-house TaqMan PCR assay as described elsewhere [26]. This assay uses forward and reverse primers designed on a highly conserved segment of the untranslated region of the viral genome and has therefore the capacity to detect all currently known species of TTV. The lower limit of detection was 10 copies of TTV DNA per ml of blood. The procedures used to quantitate the copy numbers and assess specificity, sensitivity, intra- and inter-assay precision, and reproducibility have previously been described [26].

Statistical Analysis

Subject characteristics were reported as mean ± standard error of the mean for continuous variables and percentages were used for categorical variables. The normality of the included variables was assessed using the Kolmogorov-Smirnov test. Differences among groups were checked by the one-way analysis of variance (ANOVA) or Kruskal-Wallis test for continuous variables and Pearson's χ^2 test for categorical variables.

We used multinomial logistic regression to assess the impact of variables on physical frailty and cognitive impairment; the physically nonfrail and the cognitively unimpaired groups were used as the reference groups, respectively. The parameters that were significantly associated with frailty or cognitive impairment in the univariate analysis (*p* value <0.15) were considered potential confounders and included in the multinomial logistic regression models. Each model included age, sex, and country as fixed covariates. The level of statistical significance was set at $\alpha \leq 0.05$. All the analyses were performed using the SPSS/Win program (version 22.0; SPSS Inc., Chicago, IL).

Results

General Characteristics of Participants

Demographic and clinical characteristics of MARK- AGE participants are shown in Table 1. Physically frail and cognitively impaired subjects were older than non- frail/cognitively healthy individuals. No difference in sex distribution, education level, BMI, blood pressure, smoking habit, history of falls, C-reactive protein, triglycerides, and fasting glucose serum levels were observed between groups. Frequency of widowhood, percentage of subjects with poor/fair self-reported health, Charlson Comorbidity Index, and number of medications were higher among physically frail and cognitively impaired subjects as compared to nonfrail and cognitively healthy individuals. The frequency of hospitalization in the last year was increased in physically frail with respect to nonfrail and cognitively impaired subjects. Decreased total cholesterol, high-density lipoproteins (HDL), and low-density lipoproteins were found in subjects with cognitive impairment as compared to nonfrail, while physically frail showed only a reduction in high-density lipoproteins as compared to nonfrail individuals. Demographic and clinical characteristics

of Spanish and Portuguese participants are reported in online supplementary Tables S1–S3 (for all online suppl. material, see www.karger.com/doi/10.1159/000528169).

Factors Associated with Physical Frailty and Cognitive Impairment

Tables 2 and 3 show the results of the association between the participants' sociodemographic and clinical characteristics and physical frailty or cognitive impairment, respectively, in adjusted multinomial regression models, with nonfrail and cognitively healthy participants as a reference group. The main predictors of physical frailty were age ≥65 years, BMI, TTV viremia ≥4log, depressive symptoms, and smoking habit. The main predictors of cognitive impairment were age ≥50 years, TTV viremia ≥4 log, depressive symptoms, and Charlson Comorbidity Index ≥3. To prevent the possible confounding effect of CMV infection, multinomial regression models have been performed adding CMV antibody levels among the independent variables (online suppl. Tables S4, S5). The association of TTV viremia ≥4log with physical frail- ty (OR: 4.17, 95% CI: 1.58–10.99) and cognitive impair- ment (OR: 2.93, 95% CI: 1.63–5.27) is still confirmed in the MARK-AGE population.

Table 4 shows the associations among TTV viremia, physical frailty, and cognitive status in unadjusted and adjusted multinomial regression performed in Spain and Portugal cohorts (descriptive TTV viremia data in these cohorts can be seen in online suppl. Table S6). In Spain, TTV DNA load was associated with frailty status (frail vs. nonfrail) in both the unadjusted and adjusted models after correction for age, sex, depression, and years of education. Interestingly, TTV viremia was associated with low physical activity and slow walking time, but not with low grip strength by multinomial logistic regression considering the whole sample (Spain and Portugal) (online suppl. Tables S7–S9).

A slight association between TTV viremia and cognitive impairment was observed in participants from Spain, not confirmed in the adjusted model. No association between TTV load and frailty or cognitive impairment was observed for the Portuguese cohort.

However, a linear regression analysis showed a negative association between TTV viremia and MMSE score in females in the whole sample (Spain and Portugal) (β coefficient = −0.136; $p < 0.05$; online suppl. Table S10). Similarly, TTV viral load was negatively associated with the global cognitive functioning score in the MARK-AGE sample both in males and in females (online suppl. Table S11). TTV viremia was

significantly higher in physical frailty as compared to nonfrail subjects in all cohorts, while TTV DNA load was increased in cognitive impairment as compared o nonfrail only in the MARK-AGE population (Fig. 1).

Discussion

Epidemiological evidence demonstrates that frailty may increase the risk of future cognitive decline and vice versa, suggesting that cognition and frailty may mutually interact in advancing aging [27, 28]. The search for biomarkers of physical and cognitive frailty that provide additional information to that obtained from clinical data, improving its prognostic power, is becoming increasingly important [29]. Immunosenescence, inflammaging [30], and immune risk profile [31] are considered among the causes of increased susceptibility to frailty and death in older subjects [32]. TTV is a major component of the human blood virome that causes chronic infection with unknown clinical consequences. TTV viral load increases with age and is associated with decreased NK cell activity, reduced CD4/CD8 T cell ratio, and has been suggested as a possible marker of immunosenescence. Moreover, TTV viremia ≥4 log copies/mL has been associated with all- cause mortality in older population [6].

In this study, we found a strong association of TTV viremia ≥4log with physical frailty (OR: 4.69, 95% CI: 2.06–10.67) and cognitive impairment (OR: 3.49, 95% CI: 2.14–5.69) in the MARK-AGE population, and these results were confirmed also after correction for CMV sero- prevalence. Then, we attempted to validate these results in other two independent cohorts from Spain and Portugal. Because of lower TTV viremia levels in the sample from Spain compared to that from Portugal (3.36 log cop- ies/mL, 95% CI: 3.20–3.53 vs. 4.35 log copies/mL, 95% CI: 4.20–4.49), it was not possible to apply the same TTV cut- off ≥4 log copies/mL as used in the MARK-AGE cohort. Therefore, TTV viremia was treated as a continuous variable in all subsequent statistical analyses. Differences in TTV prevalence and viral load in relation to geographical origin have previously been shown in the MARK-AGE sample. The reasons for these differences could depend on environmental, lifestyle, immunological factors, and presence of pathologies [4, 33, 34].

In the Spanish participants, TTV DNA load was associated with frailty status in both the unadjusted (OR: 1.76; 95% CI: 1.12–2.75) and adjusted models (OR: 2.42; 95% CI: 1.09–5.34). Moreover, TTV viremia was also associated with low physical activity and slow walking time, but not with low grip strength in the whole sample (Spain and Portugal). With regard to cognitive impairment, a slight association between TTV viremia was observed only in the unadjusted model in Spain (OR: 1.33; 95% CI: 1.00– 1.77).

By contrast, in Portugal the association between TTV load and frailty or cognitive impairment was not confirmed using the multinomial regression model, probably due to the small sample size of individuals with physical frailty and cognitive decline (38 and 20 subjects, respectively). However, we found a negative association between TTV viremia and MMSE score in females from Spain and Portugal, while in the MARK-AGE population a negative association between TTV DNA load and global cognitive functioning score was observed in both sexes. TTV has been demonstrated to be related to the immune status of the host. In particular, studies on healthy individuals and older people have demonstrated that low TTV viremia reflects strong immune responses, while high TTV DNA load reflects an impaired immune function [3, 4]. TTV can activate TLR9, inducing immune cells to produce proinflammatory cytokines [9]. During aging, the host's immune control of TTV replication de- creases [35], which contributes to the induction of chronic inflammation.

Proinflammatory markers such as C-reactive protein and interleukin 6 are elevated in frail compared to non- frail older adults, which supports the role of inflammation in the pathophysiology of frailty [36]. Similarly, patients with cognitive frailty present high levels of proinflammatory cytokines such as interleukin-6 and TNF-α [37]. Therefore, the increased TTV DNA load might be an (noncausal) indicator of progressive frailty and cognitive decline, signaling weaning immune aspects of these phenotypes.

Additionally, the inversion of the CD4/CD8 ratio and changes in T cell senescence are implicated with cognitive decline and frailty [38, 39]. Mice deficient in T cells are cognitively impaired, and repopulation with T cells can reverse this defect [40]. Consistent with this evidence, previous findings in the MARK-AGE population demonstrate a negative association between CD4+ T cells and TTV DNA load, and a relation between TTV viremia ≥4 log copies/mL and CD4/CD8 ratio <1 in males [4]. Therefore, the increased TTV replication rate, in addition to being a biomarker of

immunosenescence, could provide useful information for the early identification of physical frailty and cognitive impairment. The strengths of this study include its relatively large sample size, with the inclusion of two additional independent cohorts.

This study has also some limitations, such as the cross- sectional nature of the data, the lack of gait speed in the MARK-AGE study with the resulting use of four criteria instead of the five physical frailty criteria originally proposed by Fried [20], and the different mean age of Spanish and Portuguese cohorts compared to the MARK-AGE population.

In conclusion, these findings provide additional support for theories linking immunosenescence and TTV to physical frailty and cognitive impairment and provide insight into the potential roles of TTV in the development of the two conditions. Furthermore, the inclusion of TTV viremia in the diagnosis of physical frailty and cognitive impairment could help identify these pathological conditions at an early stage, and its monitoring would enable to evaluate the progression of frailty and the response to the interventions applied. Further studies are necessary to clarify the possible involvement of TTV in the pathogenesis of physical frailty and cognitive decline, as well as longitudinal studies should be conducted to understand if TTV DNA load may represent a useful biomarker for the prediction of both types of decline and its coexistence.

Acknowledgments

The authors thank the subjects who participated in the MARK- AGE study and Dr. Elisabetta Farnocchia for her excellent technical assistance.

Statement of Ethics

This study was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. The Local Research Ethics Committees of the respective recruiting centers provided ethical approval for the MARK-AGE project, which was registered retrospectively at the German Clinical Trials Register (DRKS00007713; Ethics Committee No. 2008-075-f, Ethik- Kommission bei der Landesärztekammer Baden-Württemberg). The Ethics Committees of the University of A Coruña (approval number CE 18/2014) and of the Institute of Public Health of the University of Porto (ISPUP) (approval number CE17081) approved the study protocol of Spanish and Portuguese

recruitment centers, respectively. All participants of the study gave written informed consent. Moreover, written informed consent from parents/guard- ian/next of kin of all vulnerable participants was obtained.

Conflict of Interest Statement

The authors declare no competing financial interests.

Funding Source

This work was supported by the European Commission (Project Full Name: European Study to Establish Biomarkers of Human Ageing; Project Acronym: MARK-AGE; Project No. 200880) and in part by the Project: SInergie di RIcerca della Rete Aging (SIRI) by Italian Health Ministry. Support was also obtained from the Spanish Ministry of Science and Innovation: MCIN/ AEI/10.13039/501100011033 (Grant PID2020-113788RB-I00), Xunta de Galicia (ED431B 2022/16, ED431F 2017/09), the Ministry of Education, Culture and Sport (BEAGAL18/00142 to V.V.), and the Spanish Ministry of Economy and Competitiveness, co-financed by the European Social Fund (RYC-2015-18394 to L.L-L). Armanda Gomes is supported by Fundação para a Ciência e Tecnologia (FCT-MCTES), and the European Social Fund, through Programa Operacional Capital Humano (POCH), under the Grant SFRH/BD/121802/2016.

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Data Availability Statement

All data generated or analyzed during this study are included in this article and its online supplementary material. Further enquiries can be directed to the corresponding author.

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Table 1. Demographic and clinical characteristics of MARK-AGE participants

Variables	Nonfrail and cognitively healthy (*n* = 1,131)	Physically frail (*n* = 45)	Cognitively impaired (*n* = 113)	*p* value
Females, %	50.7% (573)	46.6% (21)	40.7% (46)	NS
Age, years[b]	54.7±11.2	64.1±9.9[a]	64.2±9.1[a]	<0.0001
Low level of education, % (*n*)	19.5% (220)	26.6% (12)	25.7% (29)	NS
Marital status widowhood, % (*n*)	5.3% (60)	13.3% (6)[a]	12.4% (14)[a]	<0.01
BMI	26.0±0.13	26.9±0.83	25.6±0.55	NS
Systolic blood pressure	132.6±0.5	137.5±3.2	132.3±2.1	NS
Diastolic blood pressure	80.7±0.3	81.4±2.0	80.4±1.3	NS
Current smoker, % (*n*)	18.1% (205)	31.1% (14)	17.7% (20)	NS
Poor/fair self-reported health, % (*n*)	6.6% (75)	48.9% (22)[a]	23.4% (27)[a]	<0.0001
CCI, median (range)[c]	0 (0–3)	0.5 (0–6)[a]	1 (1–4)[*/a]	<0.0001
Falls, % (*n*)	8.8 (100)	13.3 (6)	7.9 (9)	NS
Hospitalization, % (*n*)	12.1 (137)	31.1 (14)[a]	12.4 (14)[*]	<0.0001
Number of drugs	1.1±0.05	2.7±0.25[a]	2.1±0.15[a]	<0.0001
CRP, µg/L	2.02±0.09	3.30±0.79	2.13±0.33	NS
TC, mmol/L	5.58±0.03	5.37±0.18	5.25±0.10[b]	<0.01
HDL, mmol/L	1.53±0.01	1.34±0.06[a]	1.42±0.04[*]	<0.01
LDL, mmol/L	3.34±0.03	3.27±0.16	3.13±0.08[b]	<0.05
TG, mmol/L	1.25±0.02	1.61±0.13	1.32±0.08	NS
FG, mmol/L	5.15±0.03	6.13±0.42	5.42±0.11	NS

CCI, Charlson Comorbidity Index; BMI, body mass index; CRP, C-reactive protein; TC, total cholesterol; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; TG, triglycerides; FG, fasting glucose; NS, not significant. * $p < 0.05$ as compared to physically frail. [a] $p < 0.01$ as compared to nonfrail. [b] $p < 0.05$ as compared to nonfrail, Data are reported as mean ± standard error of the mean (SEM) or SD. [c] Nonparametric significance test (Kruskal-Wallis with Dunn posttest)

Table 2. Multinomial logistic regression of physical frailty in MARK-AGE population

Variables	Categories	Nonfrail versus physically frail OR (95% CI)	*p* value
Age, years	35–49	1 (referent)	
	50–64	2.28 (0.64–8.09)	0.200
	≥65	2.72 (1.13–6.51)	0.025
Sex	Females	1.29 (0.61–2.69)	0.504
	Males	1 (referent)	
Countries		1.21 (0.97–1.51)	0.084
Education, years		1.07 (0.66–1.75)	0.772
Hospitalization	Yes	1.97 (0.89–4.37)	0.095
	No	1 (referent)	
History of falls	Yes	0.37 (0.11–1.26)	0.113
	No	1 (referent)	
BMI, kg/m^2		0.90 (0.84–0.97)	0.005
TTV viremia, copies/mL	<4 log	1 (referent)	
	≥4 log	4.69 (2.06–10.67)	<0.0001
Number of medications		0.85 (0.67–1.09)	0.196
Depressive symptoms	Yes	6.48 (2.81–14.93)	0.0001
	No	1 (referent)	
Hypertension	Yes	1.78 (0.76–4.15)	0.181
	No	1 (referent)	
Smoking	Current	2.96 (1.17–7.49)	0.022
	Former	2.75 (1.06–7.11)	0.037
	No	1 (referent)	
Charlson Comorbidity Index	≥3	1.47 (0.57–3.76)	0.421
	0–2	1 (referent)	

Table 3. Multinomial logistic regression of cognitive impairment in MARK-AGE population

Variables	Categories	Cognitively healthy versus cognitively impaired OR (95% CI)	*p* value
Age, years	35–49	1 (referent)	
	50–64	4.98 (2.31–10.74)	<0.0001
	≥65	2.20 (1.32–3.66)	0.0002
Sex	Females	1.33 (0.83–2.14)	0.240
	Males	1 (referent)	
Countries		1.07 (0.95–1.21)	0.245
Education, years		0.79 (0.58–1.09)	0.152
Hospitalization	Yes	0.61 (0.30–1.22)	0.159
	No	1 (referent)	
History of falls	Yes	0.58 (0.25–1.34)	0.203
	No	1 (referent)	
BMI, kg/m^2		0.97 (0.92–1.03)	0.345
TTV viremia	<4 log	1 (referent)	
	≥4 log	3.49 (2.14–5.69)	<0.0001
Number of medications		1.01 (0.86–1.19)	0.870
Depressive symptoms	Yes	2.24 (1.14–4.42)	0.020
	No	1 (referent)	
Hypertension	Yes	1.27 (0.73–2.23)	0.397
	No	1 (referent)	
Smoking	Current	0.95 (0.51–1.77)	0.881
	Former	0.73 (0.31–1.68)	0.461
	No	1 (referent)	
Charlson Comorbidity Index	≥3	6.28 (3.50–11.28)	0.0001
	0–2	1 (referent)	

Table 4. Association among TTV viremia, physical frailty, and cognitive status in adjusted and unadjusted multinomial regression in Spain and Portugal cohorts

Country	OR uncorrected (95% CI)	*p* value	OR corrected (95% CI)*	*p* value
Spain				
Frailty	1.76 (1.12–2.75)	**0.014**	2.42 (1.09–5.34)	**0.029**
Cognitive impairment	1.33 (1.00–1.77)	0.050	1.27 (0.88–1.84)	0.198
Portugal				
Frailty	1.22 (0.79–1.87)	0.368	1.22 (0.79–1.87)	0.368
Cognitive impairment	0.89 (0.575–1.387)	0.601	0.94 (0.56–1.56)	0.807

* The model used was adjusted for age, sex, depression, and years of education. The comparison for frailty status was carried out between frail versus nonfrail (prefrail subjects were excluded).

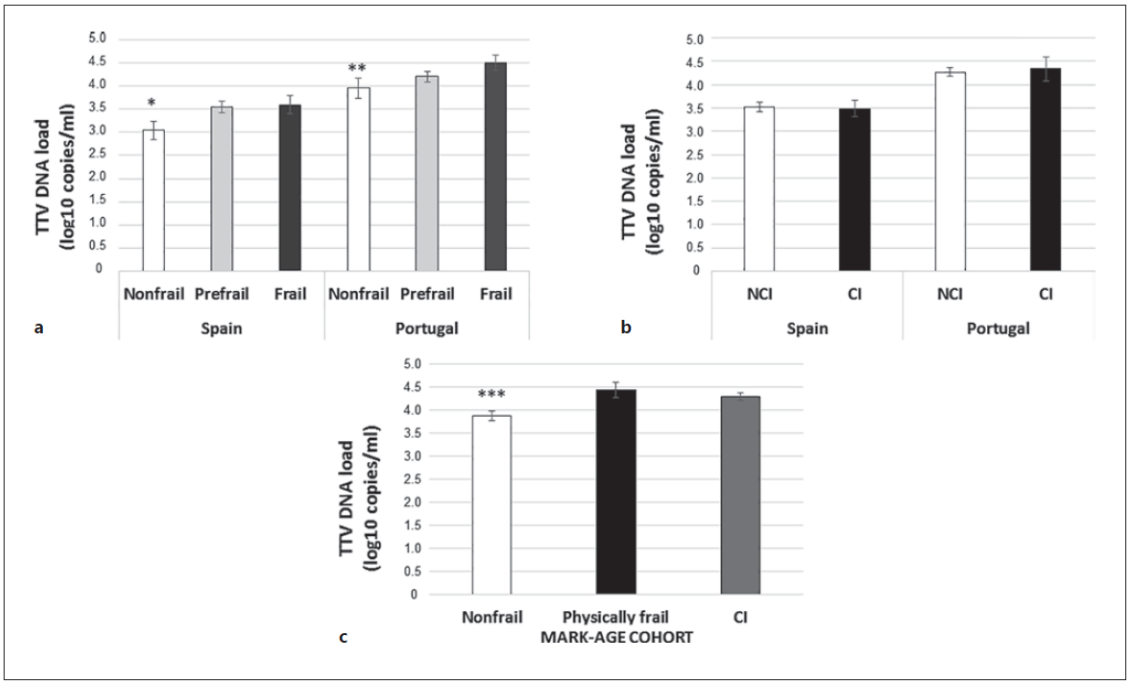

Fig. 1. TTV DNA loads in peripheral blood from MARK-AGE, Spanish, and Portuguese participants. **a** Frail subjects showed significantly higher TTV DNA loads as compared to nonfrail subjects both in Spain and in Portugal. **b** No difference was observed in relation to cognitive status. **c** TTV DNA load was significantly higher in physically frail and cognitively impaired subjects than in nonfrail ones in MARK-AGE population. NCI, no cognitive impairment; CI, cognitive impairment; ANCOVA analysis correcting for age and sex was applied; data are reported from the model adjusted mean ± standard error of the mean (SEM); *$p < 0.05$ as compared to prefrail and frail subjects in Spain, **$p < 0.05$ as com- pared to frail subjects in Portugal, and ***$p < 0.01$ as compared to physically fra ired subjects in the MARK-AGE cohort.