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Cytomegalovirus is associated with reduced telomerase activity in the Whitehall II cohort

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Abstract

Telomere length and telomerase activity have received increased attention as markers of cellular aging, but the determinants of inter-individual variation in these markers are incompletely understood. Cytomegalovirus (CMV) infection may be particularly important for telomere and telomerase dynamics due to its dramatic impact on peripheral blood lymphocyte composition, i.e., increasing the number and proportions of highly differentiated T cells that are characterized by shorter telomere length (TL) and lowered telomerase activity (TA). However, the possible relationship between CMV infection and leukocyte TL and TA has not been well-examined *in vivo*. This study examined the associations of CMV seropositivity and CMV IgG antibodies with leukocyte (TL) and (TA) in a sample of 434 healthy individuals (ages 53–76) from the Whitehall II cohort. Positive CMV serostatus was significantly associated with lower TA among women, and higher CMV IgG antibody levels were associated with lower TA in the overall sample. However, neither CMV seropositivity nor CMV IgG antibody levels (reflecting subclinical reactivation) among the seropositive were significantly associated with TL. These associations were robust to adjustment for age, employment grade, BMI, and smoking status. The results demonstrate that CMV seropositivity and subclinical reactivation predict lower TA. Future longitudinal studies should test whether the association of CMV with lower TA contributes to accelerated telomere shortening over time.

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Keywords

telomeres; telomerase; cytomegalovirus; infections; Whitehall II

1. Introduction

Telomere length (TL) and telomerase activity (TA) have received increasing attention as potential biomarkers of cellular aging (Zglinicki et al., 2005). Both shortened leukocyte TL and altered leukocyte TA are associated with progression of age-related diseases - in particular cardiovascular disease and some cancers - as well as their risk factors including obesity, diabetes, hypertension and smoking (Brouilette et al., 2003; Demissie et al., 2006; Donate and Blasco, 2011; Kroenke et al., 2012; Sanders et al., 2012; Valdes et al.). Several reports have related shorter leukocyte TL to early mortality (Bakaysa et al., 2007; Cawthon et al., 2003; Honig, 2006; Martin-Ruiz et al., 2006). Telomeres are the DNA-protein structures that cap and stabilize the physical ends of chromosomes and can shorten with each round of cell division (Blackburn et al., 2006; Gabriele and Thomas, 2002; Zglinicki et al., 2005). Critically short telomeres lead to cellular senescence, preventing cell replication, and genomic instability, such as chromosomal rearrangement, and thus have been implicated in the loss of tissue homeostasis, organ failure and organismal aging (Armanios and Blackburn, 2012; Donate and Blasco, 2011). One determinant of telomere length (TL) is the cellular enzyme activity of telomerase. In the absence of telomerase, or when this enzyme is expressed at low levels, DNA replication during cell division results in the progressive shortening of telomeric DNA. Telomerase counteracts telomere shortening, thus maintaining length, promoting genomic stability, and ultimately supporting cell longevity (Baird and Kipling, 2004; Donate and Blasco, 2011). Beyond counteracting telomere shortening, TA can protect cells with short telomeres by preventing deleterious chromosome fusions and allowing these cells to continue to proliferate (Blackburn, 2005).

Despite overall telomere shortening and decreased telomerase activity with age, there is large variation in TL and TA across individuals of the same age, with age accounting for only 10% or less of inter-individual telomere length variation (Iwama et al., 1998; Rufer et al., 1999). The mechanisms underlying this inter-individual variation are not well characterized but infection history, particularly with cytomegalovirus (CMV), may play a role (Effros, 2011; Ilmonen et al., 2008; van de Berg et al., 2010). CMV is a ubiquitous herpes virus that is most often asymptomatic. Prevalence increases with age, with most populations reaching over 70% seroprevalence for those over age 60 (Bate et al., 2010; Cannon et al., 2010). Once infected, CMV remains latent in the host for life, with containment of the virus becoming an immune system priority. For example, CMV becomes the primary target of 10–30% of all CD4 T lymphocytes and 50% of all CD8 T lymphocytes in CMV-infected elderly individuals (Pourgheysari et al., 2007; Sylwester et al., 2005).

The disproportionate impact of CMV on the T cell lymphocyte pool may be important for understanding telomere and telomerase dynamics. The chronic antigenic stimulation associated with immune system control of CMV results in dramatic changes in the cellular composition of peripheral blood, including a large increase in the number of so-called ‘effector-memory’ T lymphocytes (Effros, 2011; Pawelec et al., 2004). Significantly, upon repeated stimulation, these T lymphocytes have been shown *ex vivo* to lose the ability to induce telomerase, resulting in telomere erosion and eventual replicative sensescence (Hodes et al., 2002; Valenzuela and Effros, 2002). While these changes in the circulating lymphocyte pool were initially seen as an immunological signature of aging, it is now clear that, independent of age, CMV infection is one “driving force” behind these alterations (Pawelec et al., 2009).

Significantly, population studies show a nearly linear CMV seroconversion rate that ranges from a 0.55%–2% increase per year in non-Hispanic White populations in Western Europe and the US (Colugnati et al., 2007; Hecker et al., 2004). Thus there is an age-related increase in CMV prevalence, paralleled by an accumulation of effector-memory T cells that are known to have low TL and TA, which, taken together, might partially account for the age effects on TL and TA. CMV infection may likewise be a potential explanation for the large inter-individual variation in these parameters. Assuming that the association of CMV with T cell telomere length would contribute to average leukocyte telomere length and telomerase activity (van de Berg et al., 2010), we hypothesized that CMV seropositivity would be associated with lower telomerase activity and shorter leukocyte telomere length in our sample. Here we report that first, contrary to these expectations, CMV seropositivity was found to be associated only with lower TA in women.

A parameter that is important to consider in parallel with CMV serostatus is the plasma level of CMV-specific antibodies. CMV IgG antibody titers have been found to increase linearly with CMV viral load (measured as CMV DNA in leukocytes), suggesting they are a good indicator of host immune response to viral replication (Kuo et al., 2008; van Zanten et al., 1995). Increased anti-CMV IgG antibodies have been associated with higher levels of the inflammatory cytokines TNF-α and IL-6 along with reduced immune response to influenza vaccination among both young and older individuals (Trzonkowski et al., 2003; Turner, 2012). In epidemiological studies, CMV antibodies have been linked to inflammatory processes, cardiovascular disease, frailty, cognitive decline, and mortality (Aiello et al., 2006; Pawelec et al.; Roberts et al., 2010; Schmaltz et al., 2005; Sorlie et al., 2000; Strandberg et al., 2004). We hypothesized that among those individuals who are CMV seropositive, higher CMV IgG antibody levels representing increased chronic antigenic stimulation would be associated with lower telomerase activity and shorter telomere length. Here we report, first, contrary to these expectations, a hypothesis that was supported for TA but not for TL in this sample.

2. Materials and Measures

2.1 Sample

Participants were from the Heart Scan sub-sample of the Whitehall II epidemiological cohort, recruited during 2006 to 2008 to investigate the psychosocial, demographic and biological risk factors for coronary artery calcification (Steptoe et al., 2010). The sample of 543 included 294 men and 249 women, aged 53–76 years. All participants were screened to ensure that they had no history or objective signs of coronary heart disease, and no previous diagnosis or treatment for hypertension, diabetes, inflammatory diseases or allergies. Volunteers were of white European origin and 56.5% were in full-time employment. Socioeconomic status was defined by current (or most recent) grade of employment within the British civil service, and selection into the study was stratified to include adequate representation of higher, intermediate and lower grade employment groups. Measurement of TL did not commence at the beginning of the study; therefore, only 434 participants have TL data and were included in TL analyses. The final sample used in the TA analyses was 416 given that there was also some loss of TA data due to sample non-viability. All participants gave written consent, and the study was approved by the University College London Hospital (UCLH) Committee on the Ethics of Human Research.

2.2 Isolation of Peripheral Blood Mononuclear Cells

Peripheral blood mononuclear cells (PBMC) were isolated from whole blood samples (20ml) by density gradient centrifugation on Ficoll Paque Plus (Amersham Pharmacia

Biotech) then stored at −80°C in RPMI-1640 with 10% DMSO and 20% fetal bovine serum, until the time of analysis.

2.3 Measurement of leukocyte telomere length

Genomic DNA was extracted from PBMC in a QIAcube workstation using the QIAamp DNA blood mini kit (Qiagen, Crawley, UK) according to manufacturer's instructions and stored in 10mM Tris-HCl, 0.5mM EDTA, pH 9.0 at −20°C. Relative mean telomere length was measured in triplicate by a monochrome multiplex Quantitative Real-Time PCR (Q-PCR) assay using a Bio-Rad CFX96TM Real-Time PCR Detection System (Bio-Rad, Hemel Hempstead, UK), as previously described (Cawthon, 2009). PCR reactions were carried out in a final volume of 25μl containing approximately 20 ng of sample DNA diluted in 4μl of pure water, 12.5μl of QuantiFast SYBR Green master mix (Quiagen, Crawley, UK), the telomere primers telg and telc, each at a final concentration of 900 nM and the human beta-globin primers hbgu and hbgc, each at a concentration of 500 nM. Primer sequences were: telg, ACACTAAGGTTTGGGTTTGGGTTTGGGTTTGGGTTAGTGT; telc, TGTTAGGTATCCCTATCCCTATCCCTATCCCTATCCCTAACA; hbgu, CGGCGGCGGGCGGCGCGGGCTGGGCGGCTTCATCCACGTTCACCTTG and hbgd, GCCCGGCCCGCCGCGCCCGTCCCGCCGGAGGAGAAGTCTGCCGTT. The thermal cycling conditions were as follows: Stage 1: 15 min at 95°C; Stage 2: 2 cycles of 15s at 94°C, 15 s at 49°C; and Stage 3: 32 cycles of 15 s at 94°C, 10 s at 62°C, 15 s at 73°C with signal acquisition (providing Ct values for the amplification of the telomere template), 10 s at 84°C, 15 s at 87°C with signal acquisition (providing Ct values for the amplification of the hbg template). Reactions containing serial dilutions of a reference DNA standard were included in each PCR plate to generate the telomere (T) and beta-globin gene (S) standard curves required for quantitation. Relative mean telomere length, expressed as a T/S ratio, was derived as previously described (Cawthon, 2009).

2.4 Measurement of leukocyte telomerase activity

Leukocyte telomerase activity was measured by the Telomerase Repeat Amplification Protocol (TRAP) using a commercial assay (TRAPeze, Telomerase Detection Kit, Upstate/CHEMICON, Temecula, CA) as described previously (Epel et al., 2010). PBMCs were thawed at 37°C and washed twice with cold DPBS (PBS without Mg2+ and Ca2+; Invitrogen, Carlsbad, CA, USA). Cells were pelleted and resuspended in 1ml of DPBS. Live cells were counted using a hemocytometer (Bright-Line hemocytometer, Reichert, Buffalo, NY, USA) using Trypan blue (Invitrogen). One million live cells were pelleted and lysed with 1 x CHAPS buffer according to the TRAPeze kit manufacturer instructions. An extract corresponding to 5000 cells/μl was prepared for each PBMC sample and two concentrations corresponding to 5000 and 10000 cells were assayed for each sample to ensure that the assay was in the linear range. The reaction was performed according to the TRAPeze kit manufacturer instructions and radioactive products fractionated by 10% polyacrylamide-8M urea sequencing gel electrophoresis. The gel was exposed to a phosphoimager plate overnight and scanned on STORM 860 (GE Healthcare). As positive control standards, 293T human cancer cells were used and telomerase activity was expressed as the equivalent of number of 293T cells per 10,000 PBMCs. Telomerase activity was quantified using ImageQuant 5.2 software (GE Healthcare) as described previously (Epel et al., 2010).

2.5 Measurement of CMV

CMV IgG antibody titers were measured from thawed serum samples using a solid-phase enzyme immunoassay system as described previously(Buka et al., 2001). Briefly, diluted aliquots of serum were reacted with CMV antigen bound to a solid-phase surface. Following the addition of enzyme-linked anti-human IgG and enzyme substrate, quantification of CMV

IgG antibody titers was determined by the amount of color generated from the reaction of antigen-bound enzyme and soluble substrate and measured as optical densities read by a spectrophotometer. A sample was categorized as seropositive if the optical density ratio was 1.0 or greater. All CMV assays were carried out by the Stanley Neurovirology Laboratory of the Johns Hopkins University School of Medicine.

2.6 Additional covariates

Employment grade was used as an indicator of socioeconomic status (SES); participants were classified according to their current or most recent civil service grade into lower (administrative assistant, administrative officer and executive office), intermediate (higher executive office and senior executive office) and higher (grades 7 to 1) SES. Current smoking status was assessed by questionnaire and categorized as current, former, or never. Height and weight were measured by a research nurse using standardized methods, and used to calculate body mass index (BMI) as weight in kilograms divided by height in meters squared. Physical activity was measured as reporting moderate or vigorous exercise <1 hour, 1–4 hours, 5–7 hours, and >7 hours per week.

3. Statistical Analysis

Leukocyte telomere length was normally distributed, but telomerase activity was right skewed, and hence was natural log transformed prior to analyses. Among all respondents who were CMV positive, CMV IgG antibodies were normally distributed and analyzed continuously in regression models, and divided into tertiles based on all seropositive respondents (n=253) for the calculations in Figure 1 and 2. The relationships of leukocyte TL and TA with CMV seropositivity status and continuous IgG antibody levels were analyzed by multiple regression analysis. Age and sex were included as covariates in all models. Other demographic and control variables were included in preliminary models but were not significant in fully adjusted models and thus excluded for parsimony.

4. Results

Table 1 shows descriptive characteristics of the sample. Leukocyte TL was inversely correlated with BMI ($r = -0.102$, $p = 0.034$) and smoking ($r = -0.090$, $p = 0.060$, trend) but was not statistically significantly related to age, sex, physical activity, or employment grade. Leukocyte TA was significantly higher in women (11.65 ± 7.40) than in men (9.66 ± 6.66) ($p < 0.001$) but was not related to age, BMI, smoking status, physical activity or employment grade. There was no significant relationship between leukocyte TL and TA in the overall sample ($r = 0.012$, $p = 0.83$). However, stratifying analyses by sex showed that there was a near significant association in women ($r = 0.14$, $p = 0.070$) but not in men ($r = -0.10$, $p = 0.19$).

Results from multivariate regression models examining the association of CMV with TL and TA are shown in Table 2. Neither CMV seropositivity ($\beta = -0.003$, $p = .674$) nor CMV IgG ($\beta = -0.005$, $p = 0.45$) was significantly associated with leukocyte TL in the full sample, but results for TA showed consistent associations. Specifically, CMV seropositivity was significantly associated with lower TA among women ($\beta = -0.177$, $p = 0.060$) but not men ($\beta = -0.031$, $p = 0.733$) in sex-stratified models, with a marginally significant association in the combined sample ($\beta = -0.095$, $p = 0.145$). Among the total 191 CMV positive respondents, CMV IgG antibody response was significantly associated with lower telomerase ($\beta = -0.163$, $p = .008$). Looking separately by sex, the association of CMV IgG with telomerase was stronger for women ($\beta = -.256$, $p = 0.005$) compared with men ($\beta = -.107$, $p = 0.197$). Figures 1 and 2 illustrate the associations of CMV IgG antibody levels with TL and TA, respectively. Although the age range in our sample is relatively small, we tested the

interaction of age with CMV seropositivity and CMV IgG antibody level on TL and TA, since CMV specific T-cell proliferation towards senescent cells with shorter telomeres may increase with age. None of the age interactions were significant.

5. Discussion

The determinants of leukocyte TL and TA, while correlated with many important aging-related outcomes, are not well understood. T-cell replicative senescence due to latent viral infections such as CMV may be an important determinant of TL and TA but has not been extensively studied *in vivo* (Effros, 2011; van de Berg et al., 2010). Our study is the first to test the association of the highly prevalent persistent CMV infection with leukocyte TL and TA in humans. Consistent with our hypothesis, we found that higher CMV IgG among CMV seropositive individuals was associated with lower TA, while CMV infection per se was only associated with lower TA in women. TL was not associated with either CMV seropositivity or CMV IgG antibody levels among seropositives, contrary to our hypothesis.

The observed link between CMV and TA adds novel data to a larger literature showing a driving role of CMV in immunosenescence, i.e., the decline of immunity with increasing age. In the earlier literature CMV was viewed as a contributing factor — rather than a direct cause — of age-related immunosenescence. However, there is now accumulating evidence indicating that CMV is more likely to be a primary cause, independent of age (Dock, 2011; Pawelec et al., 2005). During the course of chronic CMV infection, CMV-specific T cells are intermittently stimulated, whereby they proliferate, differentiate and develop features of senescence, which include downregulation of telomerase and telomere shortening (Dock, 2011). CMV appears to drive age-impairment of the immune system by causing differentiation-related cellular dysfunction, such as loss of proliferative capacity, as well as by steady accumulation of CMV-specific cells, due in part to apoptosis resistance, which displace more functional cells that are specific for other antigens (Moss, 2010). The result is a reduced overall T cell repertoire and immune competence. On the basis of this literature one could logically predict an inverse association between CMV and TA, and the current study is one of the first to confirm this prediction using a population-based epidemiological design.

Our findings of an association of CMV with TA but not TL may point to a dynamic process by which the immune response to CMV is not strongly related to a variable characteristic, TL, in a cross-sectional study, but reduces telomerase activity. Beyond its role in counteracting telomere attrition, telomerase activity also synthesizes the correct telomeric DNA binding sites for the sequence-specific protective telomeric proteins, as well as acting to prevent chromosome fusions (Blackburn, 2005). Upregulation of telomerase is thus seen as a potential mechanism for overcoming replicative senescence, a process that may be inhibited in those with (subclinically) reactivating CMV.

Additionally or alternatively, the immune response to CMV may cause the leukocyte cell populations in the blood draw samples used in this study to contain a higher fraction of those leukocyte cells that characteristically have lower telomerase than other leukocyte subtypes (Lin et al., 2010). TL and TA measured in leukocytes reflect sums or averages over cells with potentially very different replicative histories(Weng, 2012). As described previously, the various leukocyte and lymphocyte subtypes differ in telomerase activity levels, with CD8+CD28− cells having notably low TL and TA levels (Lin et al., 2010).

A recent study of 159 healthy volunteers aged 20–95 in the U.K. found that CMV seropositivity accelerated telomere attrition in T-cell populations, though the effects were small and found only in lymphocytes of those aged 60 and over. This effect was due to the shift in T cell pool composition away from naïve T cells that have longer telomeres and

towards an increase in highly differentiated T cells with decreased telomeres (van de Berg et al., 2010). In the same paper, primary CMV infection among 19 renal transplant recipients was also found to coincide with a steep drop in lymphocyte TL. This drop in lymphocyte TL was related to the change in the T cell subset distribution away from naïve CD8+ T cells and towards differentiated T cells in both CD4 T cells and CD8 cells (van de Berg et al., 2010). Thus, the TL decreases associated with CMV infection appear in part driven by a change in the cellular composition of the peripheral blood, the main cellular source of TL determination, and not a direct cellular effect of telomere attrition. TA was not measured in this study. Based on these findings, changes in blood white blood cell composition due to CMV are a potential source of bias and complexity in TL studies. The results of the present study suggest, however, that the robust effects of CMV on peripheral blood leukocyte heterogeneity, and in particular on the expansion of late-differentiated T cells, do not have a major implication for research using overall leukocyte TL, but may bias TA findings.

There are several possible explanations for the inverse association between CMV antibody levels and TA. Research has identified a number of possible pathways whereby control of viral latency and lower TA intersect. For example, the immunosuppressive hormone cortisol inhibits TA in CD4 and CD8 T cells (Choi et al., 2008), whereas its analogue hydrocortisone promotes viral replication in latently infected cells (Lathey and Spector, 1991). Significantly, research has shown that CMV can directly increase cortisol production by its ability to infect and replicate in human adrenocortical cells, where it causes steroidogenesis (Trevisan et al., 2009). In conjunction, this group has previously reported an aberrant cortisol release in healthy CMV-positive individuals involving a flattened circadian decline (Steptoe et al., 2009), which implies a more constant level of exposure to the hormone. Such a flattened secretion pattern has repeatedly been associated with impaired immunity and enhanced inflammatory activity, both in healthy and clinical populations (Edwards et al., 2010; Matthews et al., 2006; Miller et al., 2008), which, in turn, may further promote elevated viral reactivation.

Another clue for a shared pathway may be provided by the observation of a stronger inverse association between CMV antibody levels and telomerase in women. Such gender specificity is evocative in light of the well-studied effects of sex steroids, such as progesterone and estradiol, on telomere dynamics (Gladych et al., 2011; Lee et al., 2005; Wang et al., 2000). While similar involvement of sex hormones in CMV replication has been less intensively studied, in vitro studies shown that estrogens inhibit CMV replication (Speir et al., 2000), while enhancing telomerase expression (Imanishi, 2005), and that viral shedding varies along the menstrual cycle in the context of immune impairment (Mostad et al., 2000). In addition to such shared physiological mechanisms, it is also possible the inverse association is a direct consequence of non-clinical CMV reactivation, as evidenced by elevated antibody titers, which is causing TA downregulation in CMV specific cells as a result of recurrent antigenic stimulation (Akbar and Henson, 2011).

There are several limitations to our study. First, our sample was screened for good health at baseline. This sample selection could have biased effects between CMV and TL and TA toward the null, since those with chronic disease at baseline who were excluded were likely those who had already experienced greater telomere shortening. The fact that age was not associated with telomere length in our sample, as it has been found to be in many other studies, suggests this healthy sample selection may be important. While there was no statistically significant association between CMV and TL, the direction of the association for both CMV seropositivity and CMV IgG antibodies was in the hypothesized direction (negative). We explored whether the association between CMV and TL might be non-linear. Investigations of categorical TL found stronger but still non-significant associations between CMV seropositivity and the odds of being in the highest quartile of TL compared to the

other three quartiles (OR=.76, 95% CI (.48–1.20)). For continuous CMV IgG antibody levels, the association was in the same direction but not statistically significant (OR=.89, 95% CI .70–1.04). Future replication of these analyses in larger population representative samples will clarify the importance of this downward bias due to healthy sample selection.

Relatedly, down-regulation of telomerase due to CMV may not have yet translated into observable telomere shortening for this healthy sample. Follow-up studies of telomere shortening over time will be able to assess whether the rate of change of TL is different for those who are CMV positive or have higher CMV IgG. Future work should test whether CMV plays a role, at least in part, in the association between telomerase activity and the onset of chronic disease, especially vascular disease. Several studies have demonstrated a positive association between the quantity of CMV antibodies and different manifestations of cardiovascular disease (CVD) (Blum et al., 2003; Gattone et al., 2001; Nieto, 1998). Additional studies should also test the association of a broader range of infections and overall infectious burden on TL and TA. Given their role in promoting systemic inflammation, chronic infections may play a role in telomere shortening (Ilmonen et al., 2008; Valenzuela and Effros, 2002). Increased understanding of the infectious determinants of cellular aging could improve primary prevention and treatment of this important risk factor for chronic disease and accelerated aging.

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Highlights

- Inter-individual variation in telomere length and telomerase activity is poorly understand
- Chronic infections such as cytomegalovirus (CMV) may play a role.
- Higher CMV antibody levels were associated lower telomerase activity
- No associations were found for leukocyte telomere length
- CMV infection may contribute to downregulation of telomerase activity

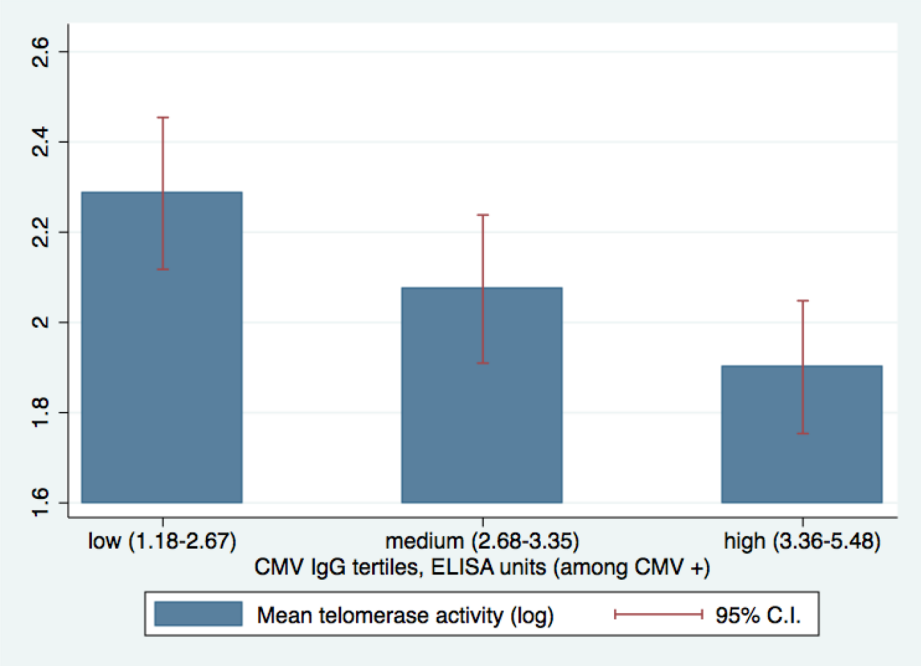

Figure 1.
CMV IgG antibody levels and telomerase activity (TA). Average leukocyte asTA(logged) is shown for the overall sample in three tertiles of CMV IgG levels among CMV + (low n=63, med n=60, high n=68). Error bars indicate 95% confidence intervals.

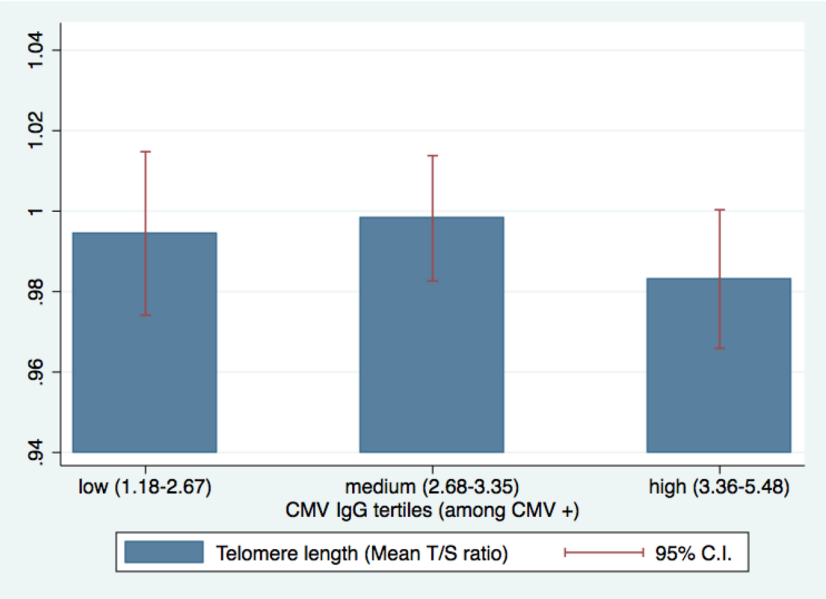

Figure 2.

CMV IgG antibody levels and telomere length (TL). Average leukocyte TL (T/S ratio) is shown for the overall sample in three tertiles of CMV IgG levels among CMV + (low n=65, med n=66, high n=68). Error bars indicate 95% confidence intervals.

Table 1

Descriptive Statistics: Whitehall Heart Scan Sample (n=434)

	Mean	S.D	range
Age	63.30	5.60	54–76
Male	45.86%		
Telomere length (T/S ratio)	0.99	0.07	0.79–1.24
Telomerase activity (per 1000 live cells) (n=416)	10.42	7.08	1.23–39.98
Ln(telomerase activity) (n=416)	2.13	0.68	0.20–3.69
CMV seropositive	47.95%		
CMV IgG if positive (ELISA units) (n=253)	3.00	0.78	1.02–5.46

Table 2

Association of CMV with Telomere length and Telomerase Activity

Variable	Telomere length		Telomerase Activity	
	β	*p-value*	β	*p-value*
Overall Sample				
CMV +	−0.003	*0.674*	−0.095	*0.145*
CMV IgG (among + only)	−0.005	*0.45*	*−0.163*	****0.008*
Men				
CMV +	−0.011	*0.318*	−0.031	*0.733*
CMV IgG (among + only)	−0.006	*0.557*	−0.107	*0.197*
Women				
CMV +	0.004	*0.665*	−0.177	**0.06*
CMV IgG (among + only)	−0.005	*0.59*	−0.256	****0.005*

Models adjusted for age, gender

* p<.10

** p<.05

*** p<.01