

The impact of pathogen burden on leukocyte telomere length in the Multi-Ethnic Study of Atherosclerosis

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SUMMARY

Several infections have been linked to telomere shortening and in some cases these associations have varied by sex. We assessed the association between seropositivity to four persistent pathogens (cytomegalovirus (CMV), herpes simplex virus-1, *Helicobacter pylori*, *Chlamydia pneumoniae*), and total pathogen burden on leukocyte telomere length in a diverse US sample. Data came from the Multi-Ethnic Study of Atherosclerosis, a population-based cohort study. We utilized cross-sectional survey data, and biological samples from participants tested for pathogens and telomere length ($N = 163$). Linear regression was used to examine the association between seropositivity for individual pathogens as well as total pathogen burden and telomere length, adjusting for various confounders. CMV seropositivity and increased total pathogen burden level were significantly associated with shorter telomere length among females ($\beta = -0.1204$ (standard error (S.E.) 0.06), $P = 0.044$) and ($\beta = -0.1057$ (S.E. = 0.05), $P = 0.033$), respectively. There was no statistically significant association among males. Our findings suggest that prevention or treatment of persistent pathogens, in particular CMV, may play an important role in reducing telomere shortening over the life course among women. Future research is needed to confirm these novel findings in larger longitudinal samples.

Key words: *Chlamydia pneumoniae*, cytomegalovirus, *Helicobacter pylori*, herpes simplex virus, pathogen burden, telomere.

INTRODUCTION

The mechanisms underlying inter-individual variation in telomere length are not well understood.

Telomeres are the nucleoprotein ends of chromosomes that function to protect chromosome ends from degradation or fusion and they shorten each time a cell divides, while the enzyme telomerase regulates the elongation process [1]. The process of telomere shortening has important health implications as reduced leukocyte telomere length has been linked to many chronic diseases of aging including

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cardiovascular disease and some cancers [2–5], as well as all-cause mortality [6].

Some preliminary studies have suggested that persistent herpesvirus infections, such as cytomegalovirus (CMV), and persistent bacterial infections including *Helicobacter pylori* (*H. pylori*) and *Chlamydia pneumoniae* (*C. pneumoniae*) may influence telomere length [7–9]. The mechanisms implicated are common across these pathogens, including induction of inflammation, generation of reactive oxygen species, and autoimmune changes – all of which may influence cellular machinery and ultimately telomere length [10].

Given that numerous persistent pathogens may contribute to physiologic processes related to telomere shortening, the total number of pathogens to which individuals have been exposed in their lifetime (i.e. total pathogen burden), may also play an important role in predicting telomere length. To our knowledge, no studies have examined the association between total pathogen burden and leukocyte telomere length.

It has been well established that female sex hormones influence telomere dynamics [11, 12]. At the same time, there is evidence that infectious disease susceptibility and immune response differ by sex, which may partly be driven by hormonal differences or gender disparate exposures, such as child care [13, 14]. In addition, previous work has shown an association between CMV infection and lower telomerase, an enzyme that helps maintain telomere length, in women but not in men [9]. Therefore, it is possible that sex (and/or gender) may modify the influence of pathogen burden on telomere length.

To address the gaps in research linking infections to telomere length in humans and potential variability in these associations by sex, we examined the association between seropositivity to four persistent pathogens (CMV, herpes simplex virus (HSV)-1, *H. pylori* and *C. pneumoniae*) as well as total pathogen burden and leukocyte telomere length among a sample of men and women participating in the Multi-Ethnic Study of Atherosclerosis (MESA).

METHODS

Study population

MESA is a longitudinal, US multi-site study of 6814 participants ages 45–84 who were recruited from six US communities, were free of clinical cardiovascular disease at the time of the baseline visit from July 2000 to September 2002, and who identified

themselves as white, black, Hispanic, or Chinese [15]. All individuals were tested for *C. pneumoniae* and a subset of 1000 participants were randomly selected from the baseline cohort for serotesting for CMV, HSV-1 and *H. pylori*, 999 of which had complete information on serostatus for all four pathogens [16]. Telomeres were assessed on a random subsample of approximately 1000 white, black, and Hispanic participants who agreed to participate in an ancillary study examining the effects of stress on cardiovascular outcomes (i.e. The MESA Stress Study) [17]. In total, 163 participants with overlapping data on pathogens and telomere length were included in the present study, which was reviewed by the Institutional Review Board of the University of Michigan and at each MESA site.

Measures

Exposures

Serum IgG antibodies to CMV, HSV-1, and *H. pylori* were detected using commercially available kits, employing an indirect enzyme immunoassay (DiaMedex Corp., Miami, Florida, USA). The sensitivity and specificity of the tests ranged from 94% to 100% (DiaMedex Corp). IgG antibodies to *C. pneumoniae* were detected using a microimmunofluorescent antibody assay (Focus Technologies, Cypress, California, USA). Serum IgG antibodies for each pathogen were treated as continuous or dichotomized according to the below cutoff values. Individuals were classified as CMV seronegative, equivocal, or seropositive if values were <8.0, between 8.0 and 9.0, or 10.0+ ELISA Units (EU)/ml, respectively. Cutoff values for being classified as HSV-1 seronegative, equivocal, and seropositive were <16.0, 16.0–19.9, and 20.0+ EU/ml, respectively. For *H. pylori*, EU/ml values of <0.90 were classified as negative, 0.90–1.09 as equivocal, and ≥ 1.10 as positive. All individuals with equivocal values (CMV; $N=0$, HSV-1; $N=0$, *H. pylori*; $N=6$, *C. pneumoniae*; $N=0$) were categorized as seropositive. Total pathogen burden level was constructed by summing the number of pathogens for which individuals were seropositive and then dichotomizing individuals into low/reference group (seropositive for 0–2 pathogens) and high (seropositive for 3–4 pathogens) total pathogen burden level. These cut points were based on prior literature and the need to ensure adequate sample size among the reference group (low category) [18, 19].

Outcome

Leukocyte telomere length was measured by quantitative polymerase chain reaction (Q-PCR) performed using DNA isolated from purified leukocytes [20]. A four-point standard curve (twofold serial dilutions from 10 to 1.25 ng DNA) was used to transform cycle threshold into nanograms of DNA. Baseline background subtraction was performed by aligning amplification plots to a baseline height of 2% in the first five cycles. The cycle threshold was set at 20% of maximum product. All samples were run in triplicate, and the median was used for calculations. The amount of telomeric DNA (T) was divided by the amount of single-copy control gene (36B4) DNA (S), producing a normalized measurement of leukocyte telomere length (T/S ratio). Two control samples were run in each experiment to allow for normalization between experiments, and periodical reproducibility experiments were performed to guarantee correct measurements. The intra-assay and inter-assay coefficient of variability for Q-PCR was 6% and 7%, respectively. Leukocyte telomere length was treated as continuous.

Covariates

Socio-demographic, behavioral, and clinical information was collected via questionnaire at baseline. Demographic covariates for which data were collected and were hypothesized to be potential confounders of interest included age, sex, race/ethnicity, and socioeconomic status. Race/ethnicity was self-reported as non-Hispanic black, Hispanic, and non-Hispanic white. Annual family income was categorized as <US \$25 000, \$25 000–\$50 000, and >\$50 000 and education level was categorized as high school or less; some college (including Associate's Degree or technical school); or bachelor's or graduate degree. In addition, hypothesized behavioral and clinical confounders were pack-years of smoking (the number of packs of cigarettes smoked per day times the number of years the person reporting smoking), body mass index (BMI) (kg/m^2) calculated from measured weight and height, and diabetes history. Diabetes history was assessed according to the 2003 American Diabetes Association criteria and categorized as normal, impaired fasting glucose (IFG), untreated diabetes, or treated diabetes [21, 22].

Statistical analysis

We first examined the association between seropositivity for individual pathogens as well as total pathogen

burden and covariates of interest. For continuous, normally distributed characteristics (i.e. leukocyte telomere length and BMI), t tests were used to detect statistically significant differences for mean values between groups. For continuous, but not normally distributed characteristics (i.e. age and pack-years of smoking) Wilcoxon rank-sum tests were performed and medians and interquartile ranges (IQRs) were estimated for each group. Fisher's exact tests were used to test for significant differences in proportions for categorical characteristics to accommodate small cell sizes.

We used linear regression to first estimate the association between seropositivity for each individual pathogen and telomere length adjusting for age, sex, race/ethnicity, and education level. Next, we additionally adjusted for pack-years of smoking, BMI (kg/m^2), and diabetes history. Analogous models were run for the association between total pathogen burden and telomere length. We repeated all models stratified by sex and also ran models including an interaction term between sex and the primary exposure (i.e. pathogen seropositivity or total pathogen burden). Analyses were performed using PROC MIXED in SAS to adjust for clustering by MESA sites (SAS Institute Inc., Cary, North Carolina, USA).

RESULTS

Table 1 shows the demographic and health characteristics of our study population by pathogen burden level. A higher proportion of blacks, participants with lower education, pack-years of smoking, and hormone use were associated with higher pathogen burden. The unadjusted associations between participant characteristics and telomere length are shown in Table 2. In the full sample, individuals seropositive to CMV, HSV-1, and *C. pneumonia* as well as those with high pathogen burden had shorter mean telomere lengths than those who were seronegative to these pathogens or had low total pathogen burden, respectively, but no statistically significant associations were observed. Of the other covariates of interest, only increased age was statistically significantly associated with shorter telomere length ($\beta = -0.0029$ (standard error, s.e. = 0.0014), P -value 0.0400). Among females, increased pack-years of smoking was statistically significantly associated with shorter telomere length ($\beta = -0.0041$ (s.e. = 0.0018), P -value 0.025), and there was a marginally statistically significant association between increasing BMI (kg/m^2) ($\beta = -0.0045$

Table 1. Selected characteristics of the Multi-Ethnic Study of Atherosclerosis (MESA) study sample by pathogen burden level

	Mean (s.e.) ^a , median (IQR) ^b , or <i>N</i> (%) ^c		<i>P</i> -value
	Low pathogen burden (<i>N</i> = 34)	High pathogen burden (<i>N</i> = 129)	
Telomere length ^a	0.917 (0.159)	0.900 (0.166)	0.6
Age in years ^b	58 (50–66)	55 (51–63)	0.472
Sex ^c			
Female	17 (50)	83 (64.3)	0.127
Male	17 (50)	46 (35.7)	
Race/ethnicity ^c			
White	17 (50)	18 (14)	<0.001
Black	3 (8.8)	38 (29.5)	
Hispanic	14 (41.2)	73 (56.6)	
Education ^c			
Complete HS/GED or less	5 (14.7)	68 (52.7)	<0.001
Some college	14 (41.2)	39 (30.2)	
Bachelor's Degree or more	15 (44.1)	22 (17.1)	
Income ^c			
<\$5000–\$24 999	7 (20.6)	40 (32.8)	0.063
\$25 000–\$49 999	10 (29.4)	47 (38.5)	
\$50 000–\$100 000+	17 (50)	35 (28.7)	
Pack-years of smoking ^b	4.3 (0–15.0)	0 (0–3.8)	0.005
BMI (kg/m ²) ^a	28.1 (5.8)	29.1 (5.5)	0.351
Diabetes mellitus ^c			
Normal	28 (82.4)	104 (80.6)	0.717
IFG	2 (5.9)	13 (10.1)	
Untreated/treated DM	4 (11.8)	12 (9.3)	
Hormone medication use ^c			
No	23 (67.7)	106 (82.8)	0.051
Yes	11 (32.4)	22 (17.2)	

^a *T* test (mean and s.e. displayed).

^b Wilcoxon rank-sum test (median and IQR displayed).

^c Fisher's exact test (*n* and % displayed).

(s.e. = 0.0025), *P*-value = 0.068), CMV seropositivity ($\beta = -0.0998$ (s.e. = 0.0548), *P*-value 0.0720) and shorter telomere length. Among males, the only covariate that was statistically significantly associated with shorter telomere length was education level, with individuals with some college education having statistically significantly shorter telomere length than those that completed high school/GED or less ($\beta = -0.1282$ (s.e. = 0.0496), *P*-value = 0.012).

Table 3 shows the covariate-adjusted associations between pathogen seropositivity as well as total pathogen burden level and telomere length among the total sample and stratified by sex. Among the full sample, there were no statistically significant associations between seropositivity for individual pathogens or total pathogen burden level and telomere length. In stratified models, however, there was a statistically

significant association between CMV seropositivity and telomere length among women after adjusting for age, race/ethnicity, education level, pack-years of smoking, BMI (kg/m²), and diabetes history ($\beta = -0.1204$ (s.e. = 0.06), *P*-value = 0.044). Higher total pathogen burden level was also statistically significantly associated with shorter telomere length among women only ($\beta = -0.1057$ (s.e. = 0.05), *P*-value = 0.033) in the fully adjusted model. There was a marginally statistically significant interaction between CMV seropositivity and sex (*P* = 0.08) and between total pathogen burden level and sex (*P* = 0.11).

DISCUSSION

To our knowledge, this is the first study to examine the association between seropositivity for a wide

Table 2. Mean difference in leukocyte telomere length for selected characteristics of the Multi-Ethnic Study of Atherosclerosis (MESA) study sample, overall and stratified by gender

	Total sample (n = 163)		Female (n = 100)		Male (n = 63)	
	Mean diff. (s.e.)	P-value	Mean diff. (s.e.)	P-value	Mean diff. (s.e.)	P-value
Age in years	-0.0029 (0.0014)	0.04	-0.0030 (0.0018)	0.094	-0.0028 (0.0023)	0.23
Sex						
Male	REF					
Female	0.0118 (0.0265)	0.656				
Race/ethnicity						
White	REF		REF		REF	
Black	-0.0167 (0.0381)	0.662	-0.0350 (0.0482)	0.47	0.0270 (0.0622)	0.666
Hispanic	-0.0123 (0.0331)	0.712	0.0234 (0.0430)	0.587	-0.0673 (0.0517)	0.198
Education						
Complete HS/GED or less	REF		REF		REF	
Some college	-0.0450 (0.0297)	0.131	0.0056 (0.0368)	0.878	-0.1282 (0.0496)	0.012
Bachelor's Degree or more	-0.0213 (0.0332)	0.521	0.0108 (0.0425)	0.8	-0.0754 (0.0533)	0.162
Income						
<\$5000-\$24 999	0.0233 (0.0334)	0.486	-0.0087 (0.0426)	0.838	0.0611 (0.0597)	0.311
\$25 000-\$49 999	0.0030 (0.0319)	0.926	-0.0290 (0.0417)	0.488	0.0370 (0.0529)	0.488
\$50 000-\$100 000+	REF		REF		REF	
Pack-years of smoking	-0.0009 (0.0011)	0.387	-0.0041 (0.0018)	0.025	0.0007 (0.0014)	0.605
BMI (kg/m ²)	-0.0030 (0.0023)	0.196	-0.0045 (0.0025)	0.068	0.0029 (0.0059)	0.619
Diabetes mellitus						
Normal	REF		REF		REF	
IFG	-0.0125 (0.0450)	0.781	-0.0138 (0.0593)	0.816	-0.0066 (0.0701)	0.926
Untreated/treated DM	0.0396 (0.0437)	0.366	-0.0066 (0.0537)	0.902	0.1164 (0.0751)	0.127
Hormone medication use						
No	REF		REF		REF	
Yes	0.0195 (0.0322)	0.547	0.0249 (0.0342)	0.469	-0.1853 (0.1757)	0.296
Pathogen burden						
Low (0-2)	REF		REF		REF	
High (3-4)	-0.0167 (0.0318)	0.6	-0.0567 (0.0421)	0.181	0.0245 (0.0498)	0.624
CMV serostatus						
Negative	REF		REF		REF	
Positive	-0.0267 (0.0352)	0.449	-0.0998 (0.0548)	0.072	0.0121 (0.0499)	0.809
HSV serostatus						
Negative	REF		REF		REF	
Positive	-0.0095 (0.0461)	0.836	-0.1058 (0.0807)	0.193	0.0273 (0.0605)	0.654
<i>Helicobacter pylori</i> serostatus						
Negative	REF		REF		REF	
Positive	0.0087 (0.0269)	0.747	0.0139 (0.0334)	0.679	-0.0002 (0.0456)	0.997
<i>Chlamydia pneumoniae</i> serostatus						
Negative	REF		REF		REF	
Positive	-0.0393 (0.0285)	0.17	-0.0635 (0.0330)	0.058	0.0199 (0.0564)	0.725

s.e., standard error.

array of persistent pathogens as well as total pathogen burden and telomere length. We identified a stronger association between CMV seropositivity as well as higher total pathogen burden level and telomere length among females compared with males, suggesting a sex-specific association of persistent pathogens on telomere shortening. Taken together, seropositivity for persistent pathogens

may be a particularly salient risk factor for cellular aging among women.

Few studies have examined the association between individual persistent pathogens and leukocyte telomere length [4, 23-25]. Van de Berg *et al.* showed that CMV seropositivity was associated with T-cell telomere shortening in a cohort of 159 healthy volunteers 20-95 years of age and attributed this finding to

Table 3. Covariate-adjusted association between pathogen serostatus, pathogen burden, and leukocyte telomere length, overall and stratified by gender

	Total sample (N = 163)					Women (N = 100) ^c					Men (N = 63) ^c				
	Model 1 ^a			Model 2 ^b		Model 1 ^a			Model 2 ^b		Model 1 ^a			Model 2 ^b	
	N	β (S.E.)	P-value	β (S.E.)	P-value	N	β (S.E.)	P-value	β (S.E.)	P-value	N	β (S.E.)	P-value	β (S.E.)	P-value
CMV															
Negative	26	REF		REF		9	REF		REF		17	REF		REF	
Positive	137	-0.0400 (0.04)	0.291	-0.0377 (0.04)	0.323	91	-0.1209 (0.06)	0.042	-0.1204 (0.06)	0.044	46	-0.0039 (0.05)	0.940	-0.0112 (0.05)	0.827
HSV-1															
Negative	14	REF		REF		4	REF		REF		10	REF		REF	
Positive	149	-0.0038 (0.05)	0.938	0.0008 (0.05) (0.05)	0.987	96	-0.1034 (0.0820)	0.211	-0.1057 (0.08)	0.211	53	0.0389 (0.06)	0.537	0.0498 (0.06)	0.416
Helicobacter pylori															
Negative	59	REF		REF		35	REF		REF		24	REF		REF	
Positive	104	-0.0039 (0.03)	0.890	-0.0108 (0.03)	0.706	65	0.0042 (0.04)	0.909	-0.0156 (0.04)	0.683	39	0.0064 (0.04)	0.883	-0.0132 (0.04)	0.761
Chlamydia pneumonia															
Negative	46	REF		REF		34	REF		REF		12	REF		REF	
Positive	117	-0.0470 (0.03)	0.121	-0.0412 (0.03)	0.182	66	-0.0469 (0.04)	0.190	-0.0495 (0.04)	0.178	51	-0.0271 (0.05)	0.617	0.0011 (0.05)	0.984
Pathogen burden															
0-2	34	REF		REF		17	REF		REF		17	REF		REF	
3-4	129	-0.0354 (0.04)	0.314	-0.0367 (0.0354)	0.301	83	-0.0648 (0.05)	0.163	-0.1057 (0.05)	0.033	46	0.0095 (0.05)	0.858	0.0091 (0.05)	0.859

^a Adjusted for age, race/ethnicity, sex, education level.

^b Adjusted for age, race/ethnicity, sex, education level, pack-years of smoking, BMI (kg/m²), and diabetes history.

^c Sex was not included in the sex-stratified model.

CMV seropositive individuals having increased proportions of highly differentiated CD4+ and CD8+ T cells [26]. A similar study by Dowd *et al.* found no association between CMV seropositivity or IgG antibody level and telomere length among 434 adult men and women in the Whitehall II cohort [9]. However, similar to our findings, Dowd *et al.* observed a statistically significant inverse association between CMV seropositivity as well as elevated CMV IgG antibody level and telomerase activity among females compared with males [9]. We are aware of only one study conducted among a clinical population that has examined the association between *H. pylori* and leukocyte telomere length [4]. In a case-control study of gastric cancer conducted among 300 cases and 416 age- and sex-matched controls in Poland, Hou *et al.* found among controls that *H. pylori* seropositivity was statistically significantly associated with shorter telomere length in peripheral leukocyte DNA [4]. In another study Aslan *et al.* assessed change in gastric mucosal tissue telomere length and telomerase activity before and after *H. pylori* eradication treatment among 21 *H. pylori*-infected individuals, finding a statistically significant increase in telomere length in gastric mucosa after treatment [27]. While *H. pylori* seropositivity was associated with shorter leukocyte telomere length in our study, the association did not reach statistical significance possibly due to limited statistical power in our sample.

To our knowledge, no other studies have examined the relationship between HSV-1, *C. pneumoniae* seropositivity nor pathogen burden and leukocyte telomere length. While we did not observe an association between these less well-studied individual pathogens and telomere shortening in the total sample, we did find that increased total pathogen burden level was statistically significantly associated with shorter telomere length among women. Given the association between pathogen burden and telomere length identified here, our findings suggest that there may be a cumulative impact of increased total pathogen burden on telomere shortening, particularly among women. Further research assessing the mechanisms by which pathogen burden may influence telomere shortening and differences in these associations by sex is warranted.

Interestingly, while the severity and prevalence of most viruses is higher among males, *Herpesviridae* family viruses, including CMV, Human Herpes Virus Type 6 (HHV-6), Human Herpes Virus Type 7 (HHV-7), Varicella Zoster Virus (VZV) and

HSV-2 appear to be exceptions [28, 29]. Some have suggested that sex hormones and chromosomes underlie these differences. For example, the female sex hormone estrogen serves to enhance T-cell-mediated immune processes, potentially via the presence of the estrogen response element in the promoters of many of the upregulated inflammation genes [30]. *In vitro* studies suggest that estradiol may trigger the reactivation of CMV and cervical shedding of CMV has been shown to increase during the luteal phase of the menstrual cycle and in later stages of pregnancy – both of which are dominated by progesterone production [31–33]. Together, these hormone-driven differences in immunity and maintenance of latency may be responsible for the observations in population-based studies that women are not only more likely to be seropositive for CMV but to also have elevated levels of circulating CMV IgG antibodies, compared with men [34, 35]. Given the well-established effects of female sex hormones on telomere dynamics, further research examining this shared pathway is warranted [11, 12]. Furthermore, future studies should aim to elucidate the biological mechanisms by which exposure to multiple persistent pathogens over the life course may be particularly detrimental for telomere shortening among women.

There are several reasons why CMV, more so than other pathogens, may contribute to telomere shortening. CMV contributes to oligoclonal T-cell expansion resulting in the accumulation of highly differentiated late-stage CD8+ T cells specific for CMV [36]. For this reason, CMV in particular, may be an important driver of replicative senescence within the T-cell compartment, particularly among those who undergo more frequent subclinical reactivation over time [37]. The pathogen-specific interaction of CMV with the stress hormone cortisol – shown to inhibit telomerase activity in CD4 and CD8 T cells [38] – may also explain why CMV, more so than other pathogens, contributes to telomere shortening. For example, CMV can infect and replicate in human adrenocortical cells, thereby triggering steroidogenesis [39]. Moreover, there are many CMV strains and individuals can be re-infected throughout the course of their lifetime [40–42]. However, much of the incidence of infection occurs early in life and therefore it is unlikely that new CMV infection fully explains our results [43].

This study has several limitations. Given the lack of overlap of those tested for both telomere length and pathogen seropositivity among participants in MESA, our sample size was limited ($n = 163$), and

may have reduced our ability to detect statistically significant associations and sex-specific interactions. Given that coefficients were consistently in the expected direction in the overall sample with pathogen seropositivity and higher total pathogen burden predicting shorter leukocyte telomere length, further analysis with larger samples are warranted. The cross-sectional nature of our study did not permit assessment of the timing of initial infection nor the effect of pathogen seropositivity and total pathogen burden on changes in telomere length over time. Additionally, measurement of the shortest telomere and telomere uncapping have been suggested as more accurate measures of senescence, but these methods have not been widely adopted in epidemiological studies because of the expense related to conducting these assays on a large scale [3, 44]. Nonetheless, to our knowledge, our study represents the largest study to date to examine the association between multiple persistent pathogens and leukocyte telomere length.

Overall, our study confirms earlier findings, which suggest that CMV seropositivity may play an important role in telomere shortening, particularly among women and moreover, some evidence that there may be a cumulative effect of exposure to CMV in the presence of other persistent pathogens on telomere length. Our results suggest that if persistent pathogens are causally related to telomere shortening, treatments to manage reactivation or prevent infection through the development of vaccinations may serve to decrease telomere attrition across the life course. Future research corroborating these findings in larger population-based studies is therefore warranted.

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DISCLAIMERS

None.

DECLARATION OF INTEREST

None.

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