

1 **TITLE**

2 Genetic Determinants of Telomere Length in African American Youth

3

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39

40 **ABSTRACT**

41 Telomere length (TL) is associated with numerous disease states and is affected by genetic and  
42 environmental factors. However, TL has been mostly studied in adult populations of European or  
43 Asian ancestry. These studies have identified 34 TL-associated genetic variants recently used as  
44 genetic proxies for TL. The generalizability of these associations to pediatric populations and  
45 racially diverse populations, specifically of African ancestry, remains unclear. Furthermore, six  
46 novel variants associated with TL in a population of European children have been identified but  
47 not validated. We measured TL from whole blood samples of 492 healthy African American  
48 youth (children and adolescents between 8 and 20 years old) and performed the first genome-  
49 wide association study of TL in this population. We were unable to replicate neither the 34  
50 reported genetic associations found in adults nor the six genetic associations found in European  
51 children. However, we discovered a novel genome-wide significant association between TL and  
52 rs1483898 on chromosome 14. Our results underscore the importance of examining these genetic  
53 associations with TL in diverse pediatric populations such as African Americans.

54

## 55 INTRODUCTION

56 Telomeres are DNA-protein structures composed of tandem hexamer repeat sequences  
57 (TTAGGG<sub>n</sub>) that cap the ends of each chromosome<sup>1</sup>. Telomeres play a vital role in maintaining  
58 DNA stability and integrity, and are therefore, critical for preserving genomic information<sup>2,3</sup>.  
59 With each mitotic division, a portion of telomeric DNA is lost. The cell enters senescence upon  
60 reaching a critical telomere length (TL) threshold<sup>4</sup>. TL has thus become an important biomarker  
61 of aging and overall health<sup>5-8</sup>. A complex interaction between genetic<sup>9</sup> and non-genetic factors<sup>10</sup>  
62 affects TL. While heritability estimates of TL range from 36% to 82%<sup>11</sup>, much is still unknown  
63 about genetic factors leading to variation in TL<sup>12,13</sup>.

64  
65 Although epidemiological research in pediatric populations has linked TL to early life adversity<sup>14</sup>  
66 and environmental exposures<sup>15,16</sup>, few studies have focused on the genetic determinants of TL in  
67 pediatric populations. In contrast, several genetic studies of TL in European and Asian adults  
68 have identified and replicated 34 genetic variants associated with TL<sup>17-26</sup>. Over 30 studies have  
69 used these variants as genetic proxies for TL through Mendelian randomization approaches to  
70 address reverse causation when examining association between TL and disease in diseased  
71 patients<sup>17,27,28</sup>. However, recent studies in Chinese newborns and European children have failed  
72 to replicate these variants, suggesting that they are not generalizable across age groups<sup>29,30</sup>. One  
73 study, by Stathopoulou *et al.*, reported six novel genetic variants associated with TL in European  
74 children (age 4-18 years) not previously discovered in adult telomere studies<sup>30</sup>. Replication of  
75 these six genetic variants has not yet been attempted. Given that adult TL appears determined  
76 prior to adulthood<sup>31</sup>, further research in diverse pediatric populations is necessary to validate the  
77 existence of genetic effects on TL early in life.

78 Previous genetic studies of TL have been done almost exclusively in populations of European  
79 ancestry<sup>32</sup>, yet there is evidence that TL varies by race/ethnicity<sup>32-34</sup>. African Americans have  
80 been shown to have longer telomeres throughout life<sup>34-36</sup> and a greater rate of telomere attrition  
81 than populations of European ancestry<sup>37</sup>. Population-specific differences in genetic variants have  
82 previously been shown across the genome<sup>38</sup>. Thus, it is possible that population-specific  
83 variation of genetic factors contributing to TL influences the difference in TL observed between  
84 populations of African and European ancestries<sup>33</sup>.

85

86 To further understand the relationship between genetic variants and TL, we performed the first  
87 large-scale genetic study of TL in African American children and adolescents (n=492) from the  
88 Study of African Americans, Asthma, Genes and Environments (SAGE). Herein, we analyze  
89 genome-wide genetic data to attempt validation of previously reported genetic associations with  
90 TL and identify genetic variants influencing TL in African American children and adolescents.

## 91 **RESULTS**

### 92 **Study Population**

93 Demographic information for the study population (n=492) is presented in Table 1. The age of  
94 participants ranged from 8 to 20 with a median age of 15.8 (IQR = 12.4, 18.3; Table 1). Median  
95 African ancestry was 0.81 (IQR = 0.74, 0.85; Table 1) and increased African ancestry was  
96 significantly associated with longer TL ( $\beta = 0.333$ ,  $P = 0.022$ , Figure 1). While individuals with  
97 public health insurance had significantly longer TL than individuals with private health insurance  
98 ( $P = 1.84 \times 10^{-4}$ ), there was no significant association of age or maternal education with TL  
99 (Supplementary Table S1).

100

### 101 **Evaluation of Previous Variants**

102 We evaluated 40 variants, 34 from adult studies (Table 2) and six from a pediatric study (Table  
103 3), for an association with log-transformed TL. None of the variants from either the adult or  
104 pediatric studies were significantly associated with TL in our study population ( $P > 0.05$ ). To  
105 determine whether the combined effect of the six previously discovered pediatric variants was  
106 associated with TL in our study population, we calculated a weighted genetic prediction score  
107 (GPS) by aggregating the allele associated with longer TL in European children weighted by the  
108 published  $\beta$ -coefficient<sup>30</sup>. There was no significant association between the GPS and TL in our  
109 study population of African American children and adolescents ( $\beta = 0.377$ ,  $P = 0.150$ , Figure 2).

110

### 111 **Discovery Genome-Wide Association Study**

112 We performed a discovery GWAS to identify significant and suggestive associations between  
113 common genetic variants and TL in our study population. We identified a novel association

114 between rs1483898 and TL that reached genome-wide significance ( $P = 7.86 \times 10^{-8}$ , Figure 3).  
115 Rs1483898 is an intergenic single nucleotide polymorphism (SNP) located proximal to the  
116 *LRFN5* gene on chromosome 14. An increase in copies of the rs1483898 A allele was  
117 significantly associated with longer TL ( $\beta = 0.148$ ,  $P = 7.86 \times 10^{-8}$ , Figure 4). We also discovered  
118 41 suggestive associations between common variants and TL ( $P < 2.32 \times 10^{-6}$ , Supplementary  
119 Table S2). Of particular note were rs9675924 ( $\beta = -0.171$ ,  $P = 2.27 \times 10^{-6}$ , Supplementary Table  
120 S2) located in *CABLES1* and rs4305653 ( $\beta = 0.167$ ,  $P = 1.81 \times 10^{-6}$ , Supplementary Table S2)  
121 located in *TTC37*. These genes have been previously associated with telomere biology<sup>39,40</sup>.  
122

## 123 **DISCUSSION**

124 In this study, we contribute to the nascent body of research on genetic determinants of TL by  
125 assessing the generalizability of genetic markers of TL to African American children and  
126 adolescents. Our results are consistent with recent studies in pediatric populations<sup>29,30</sup> that did not  
127 replicate variants associated with TL in adults<sup>17-26</sup>, suggesting that these variants may not play a  
128 significant role in the regulation of TL during the first two decades of growth and development.  
129 However, we were also unable to replicate genetic variants associated with TL in a population of  
130 European children<sup>30</sup>, highlighting potential population-specific effects of genetic associations  
131 with TL. Lastly, we identified a genome-wide significant variant, rs1483898, and 41  
132 suggestively associated variants within genes relevant to telomere biology in a GWAS for TL in  
133 African American children and adolescents.

134  
135 Genetic determinants of TL are critical to understanding inter-individual variation in TL.  
136 However, most studies of TL have been performed in adults, after the developmental time  
137 window when age-dependent telomere shortening may have already occurred<sup>32</sup>. Studies  
138 conducted among adults have identified and replicated 34 variants that have been used in recent  
139 years as proxies of TL in studies of disease risk<sup>17,27</sup>. We did not replicate these genetic  
140 associations in our study population of African American children and adolescents. Pediatric  
141 studies of TL by other groups<sup>29,30</sup> have also been unable to replicate associations found among  
142 adults, suggesting that the genetic components influencing TL may differ between adult and  
143 pediatric populations. It is possible that variants identified in adults relate to telomere  
144 maintenance in adulthood but do not regulate TL during earlier developmental windows. For  
145 example, resistance to telomere shortening during childhood may be influenced by genetic



146 factors impacting telomerase, a critical enzyme in telomere elongation<sup>41</sup> that is influenced by  
147 genetic loci<sup>42</sup> and shows age-related reduction in activity<sup>43</sup>. TL is determined prior to adulthood  
148 dependent on the TL setting at birth and the rates of shortening and elongating during the first  
149 two decades of life<sup>10</sup>. These factors have genetic influences that have yet to be fully  
150 characterized<sup>1,10</sup>.

151  
152 We attempted replication of six TL-associated genetic variants discovered in healthy children of  
153 European ancestry<sup>30</sup>. We found no significant association with TL among the six variants  
154 independently or in a weighted GPS, which tests cumulative variation at multiple genetic loci.  
155 Heritability estimates of TL range from 36% to 82%<sup>11</sup>, yet has only been reported in populations  
156 of European ancestry and may not be generalizable to other populations. Similarly, genetic  
157 determinants of TL have primarily been studied in populations of European or Asian descent.  
158 Recent studies attempting to replicate and/or identify genetic associations with TL in non-  
159 European populations, including Punjabi Sikh<sup>24</sup>, Han Chinese<sup>26,44</sup> and Bangladeshi<sup>45</sup>, have had  
160 mixed success. Among the limited set of studies assessing TL-associated genetic variants in  
161 populations of African ancestry, all have been performed in adult populations. One study  
162 discovered genetic variants associated with TL in adults of European ancestry that were not  
163 associated in an adult population of African Americans<sup>22</sup>. Another study in adult African  
164 Americans was only able to replicate the effects of variants in *TERC*, the gene encoding the  
165 enzyme telomerase, that had been identified in populations of European ancestry<sup>46</sup>. We found a  
166 significant positive association between the proportion of African ancestry and TL in African  
167 American children and adolescents, which is consistent with research among adults<sup>34</sup>.  
168 Considering TL dynamics vary by race/ethnicity<sup>32-34</sup>, our study augments the current literature

169 by demonstrating that TL-associated genetic variants differ between ancestral populations in the  
170 pediatric age range. Ancestry-specific genetic associations with a phenotypic trait have been  
171 demonstrated previously<sup>47</sup>, thus, the difference we observed may result from population-specific  
172 effects impacting genetic regulation of TL. It is worth noting regional variation in environmental  
173 and social exposures between SAGE's urban San Francisco Bay Area and the more rural Nancy,  
174 France of the Stathopoulou *et al.* study<sup>48</sup> as potential factors effecting the association between  
175 genetic variants and TL and possibly precluding replication of the Stathopoulou *et al.* study.

176  
177 We identified associations with TL in biologically relevant pathways relating to apoptosis, cell  
178 senescence and telomere replication. The most significant association, reaching genome-wide  
179 significance, was for rs1483898. Rs1483898 is located on the q21.1 arm of chromosome 14 with  
180 the closest gene, *LRFN5*, encoding a neuronal transmembrane protein. In our population of  
181 African American children and adolescents, increasing copies of the A allele associated with  
182 longer TL. The A allele of rs1483898 has allele frequencies of 0.74, 0.86 and 0.45 in the African,  
183 European and East Asian populations of 1000Genomes, respectively<sup>49</sup>.

184  
185 We identified 41 variants that were suggestively associated with TL. The A allele of rs9675924,  
186 located within cell cycle regulator *CABLES1* on chromosome 18, associated with shorter TL.  
187 *CABLES1* is co-expressed with protein kinase *CDK5*, a known contributor to apoptosis in certain  
188 neuronal diseases<sup>39</sup>. *CABLES1* has also been shown to inhibit cell proliferation and induce cell  
189 senescence in umbilical endothelial cells<sup>40</sup>. The C allele of rs4305653 associated with longer  
190 telomeres; the variant is located on chromosome 5 within *TTC37*, a component of the SKI  
191 complex which mediates protein-protein interactions. *TTC37* is co-expressed with apoptosis-

192 promoting protein *APAF1* and with *TEPI*, a protein that binds to *TERC* and is essential for  
193 telomere replication<sup>39</sup>. Ultimately, co-expression is only a proxy for co-regulation<sup>50</sup>; replication  
194 and further investigation of our results are needed to better characterize relevant associations  
195 between these genetic loci and telomere biology.

196  
197 Our study lacked an independent replication cohort to assess the reproducibility of our genetic  
198 associations due to the unique characteristics of our study population (African American children  
199 and adolescents with genetic and TL data). Measurement of TL in our study population provided  
200 a snapshot of TL at a specific point in the life course. Longitudinal studies of TL are required to  
201 understand changes in TL over the life course. Our inability to replicate the reported variants  
202 may also be explained by limited statistical power to discover weak or moderate genetic effects  
203 on TL. The major advantages of our study are that (1) it is the first large-scale study to  
204 investigate the genetic determinants of TL in a population of minority children and adolescents,  
205 and (2) our depth of phenotype data allowed us to adjust for social, environmental and genetic  
206 covariates (Supplementary Table S1).

207  
208 In summary, the paucity of research on factors affecting TL in pediatric and non-European  
209 populations creates a knowledge gap in the scientific understanding of gene-environment  
210 interactions regulating telomeres. Epidemiological studies reporting associations between TL and  
211 disease risk are potentially biased by the disease itself or exposures relating to treatment. Genetic  
212 proxies for TL have recently been employed to overcome these and other potential biases, such  
213 as social and environmental exposures. A critical assumption when using genetic proxies for TL  
214 is that they are generalizable across age and racial/ethnic groups. However, we were unable to

215 replicate previous findings of TL-associated variants in our study population. We also identified  
216 novel genetic associations with TL that have not been identified in previous studies in pediatric  
217 or adult populations. Further telomere research in pediatric populations from diverse ancestral  
218 backgrounds is required to fully understand the impacts of age- and population-effects on the  
219 genetic regulation of TL.

## 220 **METHODS**

### 221 **Ethics statement**

222 This study has been approved by the institutional review boards of University of California San  
223 Francisco and all participant centers. Written informed consent was obtained from all subjects or  
224 from their appropriate surrogates for participants under 18 years old.

225

### 226 **Study population**

227 Our study included 492 healthy controls from the Study of African Americans, Asthma, Genes  
228 and Environments (SAGE). SAGE is one of the largest ongoing gene-environment interaction  
229 studies of asthma in African American children and adolescents in the USA. SAGE includes  
230 detailed clinical, social, and environmental data on asthma and asthma-related conditions. Full  
231 details of the SAGE study protocols have been described in detail elsewhere<sup>51-53</sup>. Briefly, SAGE  
232 was initiated in 2006 and recruited participants with and without asthma through a combination  
233 of clinic- and community-based recruitment centers in the San Francisco Bay Area. Recruitment  
234 for SAGE ended in 2015. All participants in SAGE self-identified as African American and self-  
235 reported that all four grandparents were African American.

236

237 After all quality control procedures relating to TL measurement, TL was computed for 596  
238 healthy controls in SAGE from whole blood. There were 495 healthy controls with complete sex,  
239 age, African ancestry, maternal educational attainment and health insurance information. Three  
240 individuals showed extreme outlier measurements for TL (three times the interquartile range)  
241 and were thus removed.

242

## 243 **Covariates**

244 Maternal educational attainment and health insurance type were used as proxies of SES<sup>54–56</sup>.

245 Maternal educational attainment was dichotomized based on whether a participant's mother had

246 pursued education beyond high school (i.e.,  $\leq 12$  versus  $> 12$  years of education). Health

247 insurance type was defined as private versus public insurance. The genetic ancestry of each

248 participant was determined using the ADMIXTURE software package<sup>57</sup> with the supervised

249 learning mode assuming two ancestral populations (African and European) using HapMap Phase

250 III data from the YRI and CEU populations as references<sup>58</sup>.

251

## 252 **Variant selection and genotyping**

253 TL associated variants were selected for replication using the following criteria set *a priori*: i)

254 published association reaches genome-wide significance ( $P \leq 5 \times 10^{-8}$ ) on NHGRI-EBI GWAS

255 Catalog by October 26, 2017; ii) variant used as genetic proxy of TL in at least one study; iii)

256 variant reaches suggestive genome-wide significance ( $P \leq 5 \times 10^{-5}$ ) in a novel GWAS of TL in

257 children; iv) variant has a minor allele frequency (MAF) of at least 1% in the SAGE study

258 population. We identified variants from 11 studies<sup>17–26,30</sup>. Ten of the 11 studies were performed

259 in adult populations and nine of the 11 studies were performed in populations of European

260 descent, with the remaining two performed in Punjab Sikh<sup>24</sup> and Han Chinese<sup>26</sup> populations. In

261 total, we identified 40 variants from the literature, of which 12 were genotyped and 28 were

262 imputed. The 28 imputed SNPs had  $r^2$  (squared correlation between imputed and expected

263 genotypes) ranging from 0.88 to 1.00.

264

265 DNA was isolated from whole blood collected from SAGE participants at the time of study  
266 enrollment using the Wizard® Genomic DNA Purification kits (Promega, Fitchburg, WI).  
267 Samples were genotyped with the Affymetrix Axiom® LAT1 array (World Array 4, Affymetrix,  
268 Santa Clara, CA), which covers 817,810 SNPs. This array was optimized to capture genetic  
269 variation in African-descent populations such as African Americans and Latinos<sup>59</sup>. Genotype call  
270 accuracy and Axiom array-specific quality control metrics were assessed and applied according  
271 to the protocol described in further detail in Online Resource 1. Data was submitted to the  
272 Michigan Imputation Server and phased using EAGLE v2.3 and imputed from the Haplotype  
273 Reference Consortium r1.1 reference panel using Minimac3<sup>60</sup>. Imputed SNPs were included if  
274 they had an  $r^2$  higher than 0.3. Quality control inclusion criteria consisted of individual  
275 genotyping efficiency > 95%, Hardy-Weinberg Equilibrium (HWE)  $P > 10^{-4}$ , and MAF > 5%.  
276 Cryptic relatedness was also assessed to ensure that samples were effectively unrelated. Samples  
277 with an estimate of genetic relatedness greater than 0.025 were excluded. After quality control  
278 procedures, 7,519,176 imputed and genotyped SNPs were available for analysis.

279

## 280 **Telomere length measurement**

### 281 *DNA isolation and quantification*

282 Genomic DNA was isolated from whole blood according to manufacturer's recommendation  
283 using Wizard® Genomic DNA Purification Kits (Promega, Fitchburg, WI). A NanoDrop® ND-  
284 1000 spectrophotometer (Thermo Scientific) was used to assess DNA quality and quantity. All  
285 samples assayed had absorbance ratios (260/280) between 1.8 and 2.0.

286

### 287 *Determination of Relative Telomere Length*

288 Relative TL for each sample was determined using the quantitative real time PCR (qPCR)  
289 method first described by Cawthon *et al.*, which quantified TL in terms of telomere/single copy  
290 gene (T/S) expression ratios<sup>61</sup>. This protocol was modified with regard to data processing and  
291 control samples as previously published by O’Callaghan *et al.* and described in further detail in  
292 Supplemental Methods<sup>62</sup>. In brief, relative TL for each sample was calculated using the delta-  
293 delta  $C_T$  ( $2^{-\Delta\Delta C_T}$ ) formula<sup>61</sup>. Using this formula, the TL computed for each SAGE sample is  
294 proportional to the T/S ratio of that sample normalized to the T/S ratio of the PCR plate positive  
295 DNA control sample<sup>61,63,64</sup>. Inter- and intra-experimental coefficients of variation for our internal  
296 control (1301 cell line DNA) were 3% and 4.25%, respectively. Average amplification efficiency  
297 across plates was  $\geq 90\%$  for telomere and 36B4 assays. As TL was not normally distributed in  
298 our study population, we performed all parametric tests on a log-transformation of TL.

299

### 300 **Replication analysis**

301 Genotypes for all 40 previously published SNP’s in adults and children were tested for  
302 association with log-transformed TL in a multivariable linear regression analysis. Regression  
303 analyses were run separately for each SNP under an additive model to calculate the individual  
304 effect of the SNP on TL. Each regression analysis was adjusted for biological, environmental and  
305 social factors that may impact TL including sex, age, African ancestry, maternal education, and  
306 health insurance type. We adjusted for qPCR plate ID in all regression analyses to ensure that  
307 our results were not impacted by qPCR batch effects. To ensure direct comparison of results  
308 between previous studies and our current study we coded the effect alleles in our analysis to be  
309 the same as those used in previous studies.

310



## 311 **Genetic Prediction Score construction**

312 Recent research suggests the cumulative effect of multiple genetic markers may be a stronger  
313 predictor of a quantitative phenotype than the individual markers<sup>65,66</sup>. We therefore constructed a  
314 weighted GPS based on the six variants from Stathopoulou *et al.* to test their cumulative effect  
315 on TL<sup>30</sup>. We calculated each subject's weighted GPS by summing the number of alleles (0, 1 or  
316 2) associated with longer telomeres after weighting the allele count by the reported  $\beta$ -coefficient  
317 from the literature. We assumed that an effect allele having a positive  $\beta$ -coefficient meant that  
318 each additional copy of that allele was positively associated with TL. We used the GPS as a  
319 predictor in a linear regression against log-transformed TL controlling for sex, age, genetic  
320 ancestry, maternal educational attainment, health insurance type and batch effects. We were  
321 unable to calculate a weighted GPS based on the 34 variants in adult studies because the effect  
322 size could not be standardized across the studies.

323

## 324 **Calculation of population-specific genome-wide significance threshold**

325 The standard GWAS threshold for statistical significance is  $5 \times 10^{-8}$ . This number was derived  
326 by applying a Bonferroni correction for multiple testing to a dataset of one million independent  
327 markers/SNPs. However, in many cases, this threshold is overly conservative and can be  
328 inappropriate when (1) a smaller number of markers is genotyped, and (2) the assumption of  
329 independence of tests is violated.

330

331 In order to adjust the Bonferroni correction based on the actual number of independent test  
332 performed on our dataset, we determined the number of independent tests using the protocol  
333 published by Sobota *et al.*<sup>67</sup>. This method estimates the effective number of independent tests in

334 a genetic dataset after accounting for linkage disequilibrium (LD) between SNPs using the LD  
335 pruning function in the PLINK 1.9 software package<sup>68</sup>. The following parameters were used in  
336 PLINK 1.9 as advised by the authors: 100 SNP sliding window, step size of 5 base pairs, and a  
337 variance inflation factor of 1.25. Applying this method on 7,519,176 genotyped and imputed  
338 SNPs yielded 431,896 independent tests, which was then used to calculate the genome-wide  
339 significance threshold (Bonferroni correction  $0.05/431,896=1.2 \times 10^{-7}$ ). A suggestive threshold  
340 was set at  $2.3 \times 10^{-6}$  for association results based on the widely used formula:  $1/(\text{effective}$   
341  $\text{number of tests})^{69}$ .

342

### 343 **Discovery Genome-Wide Association Study**

344 We performed a genome-wide association study (GWAS) using 7,519,176 genotyped and  
345 imputed SNPs to assess the relationship between SNP genotype and log-transformed TL. The  
346 GWAS linear regression model adjusted for sex, age, African ancestry, maternal educational  
347 attainment, health insurance type and batch effects. All testing was performed using PLINK1.9<sup>68</sup>.  
348 Manhattan plots (Figure 3a, 3b) were generated using the qqman package<sup>70</sup> in the R statistical  
349 software environment (R Development Core Team 2010) and LocusZoom<sup>71</sup>. Curated protein-  
350 protein interactions were extracted using the STRING database<sup>39</sup>. An integrated confidence score  
351 for the interaction ranges from 0.5 (medium confidence) to 1 (high confidence).

352 **REFERENCES**

- 353
- 354 1. Blackburn EH, Epel ES, Lin J. Human telomere biology: A contributory and interactive  
355 factor in aging, disease risks, and protection. *Science*. 2015;350(6265):1193-1198.
- 356 2. Armanios M. Telomeres and age-related disease: how telomere biology informs clinical  
357 paradigms. *J Clin Invest*. 2013;123(3):996-1002.
- 358 3. Calado RT, Young NS. Telomere Diseases. *N Engl J Med*. 2009;361(24):2353-2365.
- 359 4. Tümpel S, Rudolph KL. The role of telomere shortening in somatic stem cells and tissue  
360 aging: lessons from telomerase model systems. *Ann N Y Acad Sci*. 2012;1266(1):28-39.
- 361 5. Bekaert S, Meyer TD, Oostveldt PV. Telomere Attrition as Ageing Biomarker. *Anticancer*  
362 *Res*. 2005;25(4):3011-3021.
- 363 6. Mather KA, Jorm AF, Parslow RA, Christensen H. Is Telomere Length a Biomarker of  
364 Aging? A Review. *J Gerontol Ser A*. 2011;66A(2):202-213.
- 365 7. Monaghan P. Telomeres and life histories: the long and the short of it. *Ann N Y Acad Sci*.  
366 2010;1206(1):130-142.
- 367 8. Heidinger BJ, Blount JD, Boner W, Griffiths K, Metcalfe NB, Monaghan P. Telomere length  
368 in early life predicts lifespan. *Proc Natl Acad Sci*. 2012;109(5):1743-1748.
- 369 9. Slagboom PE, Droog S, Boomsma DI. Genetic determination of telomere size in humans: a  
370 twin study of three age groups. *Am J Hum Genet*. 1994;55(5):876-882.
- 371 10. Dugdale HL, Richardson DS. Heritability of telomere variation: it is all about the  
372 environment! *Phil Trans R Soc B*. 2018;373(1741):20160450.
- 373 11. Hjelmborg JB, Dalgård C, Möller S, et al. The heritability of leucocyte telomere length  
374 dynamics. *J Med Genet*. 2015;52(5):297-302.
- 375 12. Monaghan P, Eisenberg DTA, Harrington L, Nussey D. Understanding diversity in telomere  
376 dynamics. *Phil Trans R Soc B*. 2018;373(1741):20160435.
- 377 13. Lin J, Epel E, Blackburn E. Telomeres and lifestyle factors: Roles in cellular aging. *Mutat*  
378 *Res Mol Mech Mutagen*. 2012;730(1):85-89.
- 379 14. Coimbra BM, Carvalho CM, Moretti PN, Mello MF, Belangero SI. Stress-related telomere  
380 length in children: A systematic review. *J Psychiatr Res*. 2017;92:47-54.
- 381 15. Martens DS, Cox B, Janssen BG, et al. Prenatal Air Pollution and Newborns' Predisposition  
382 to Accelerated Biological Aging. *JAMA Pediatr*. 2017;171(12):1160-1167.

- 383 16. Lee EY, Lin J, Noth EM, et al. Traffic-Related Air Pollution and Telomere Length in  
384 Children and Adolescents Living in Fresno, CA: A Pilot Study. *J Occup Environ Med.*  
385 2017;59(5):446-452.
- 386 17. Codd V, Nelson CP, Albrecht E, et al. Identification of seven loci affecting mean telomere  
387 length and their association with disease. *Nat Genet.* 2013;45(4):422-427e2.
- 388 18. Pooley KA, Bojesen SE, Weischer M, et al. A genome-wide association scan (GWAS) for  
389 mean telomere length within the COGS project: identified loci show little association with  
390 hormone-related cancer risk. *Hum Mol Genet.* 2013;22(24):5056-5064.
- 391 19. Mangino M, Hwang S-J, Spector TD, et al. Genome-wide meta-analysis points to CTC1 and  
392 ZNF676 as genes regulating telomere homeostasis in humans. *Hum Mol Genet.*  
393 2012;21(24):5385-5394.
- 394 20. Mangino M, Richards JB, Soranzo N, et al. A genome-wide association study identifies a  
395 novel locus on chromosome 18q12.2 influencing white cell telomere length. *J Med Genet.*  
396 2009;46(7):451-454.
- 397 21. Prescott J, Kraft P, Chasman DI, et al. Genome-Wide Association Study of Relative  
398 Telomere Length. *PLOS ONE.* 2011;6(5):e19635.
- 399 22. Levy D, Neuhausen SL, Hunt SC, et al. Genome-wide association identifies OBFC1 as a  
400 locus involved in human leukocyte telomere biology. *Proc Natl Acad Sci.*  
401 2010;107(20):9293-9298.
- 402 23. Lee JH, Cheng R, Honig LS, et al. Genome wide association and linkage analyses identified  
403 three loci—4q25, 17q23.2, and 10q11.21—associated with variation in leukocyte telomere  
404 length: the Long Life Family Study. *Front Genet.* 2014;4.
- 405 24. Saxena R, Bjonnes A, Prescott J, et al. Genome-Wide Association Study Identifies Variants  
406 in Casein Kinase II (CSNK2A2) to be Associated With Leukocyte Telomere Length in a  
407 Punjabi Sikh Diabetic CohortCLINICAL PERSPECTIVE. *Circ Genomic Precis Med.*  
408 2014;7(3):287-295.
- 409 25. Gu J, Chen M, Shete S, et al. A Genome-Wide Association Study Identifies a Locus on  
410 Chromosome 14q21 as a Predictor of Leukocyte Telomere Length and as a Marker of  
411 Susceptibility for Bladder Cancer. *Cancer Prev Res (Phila Pa).* 2011;4(4):514-521.
- 412 26. Liu Y, Cao L, Li Z, et al. A Genome-Wide Association Study Identifies a Locus on TERT  
413 for Mean Telomere Length in Han Chinese. *PLOS ONE.* 2014;9(1):e85043.
- 414 27. Machiela MJ, Lan Q, Slager SL, et al. Genetically predicted longer telomere length is  
415 associated with increased risk of B-cell lymphoma subtypes. *Hum Mol Genet.*  
416 2016;25(8):1663-1676.
- 417 28. Davey Smith G, Hemani G. Mendelian randomization: genetic anchors for causal inference  
418 in epidemiological studies. *Hum Mol Genet.* 2014;23(R1):R89-98.

- 419 29. Weng Q, Du J, Yu F, et al. The known genetic loci for telomere length may be involved in  
420 the modification of telomeres length after birth. *Sci Rep.* 2016;6:38729.
- 421 30. Stathopoulou MG, Petrelis AM, Buxton JL, Froguel P, Blakemore AIF, Visvikis-Siest S.  
422 Genetic Determinants of Leucocyte Telomere Length in Children: a Neglected and  
423 Challenging Field. *Paediatr Perinat Epidemiol.* 2015;29(2):146-150.
- 424 31. Benetos A, Kark JD, Susser E, et al. Tracking and fixed ranking of leukocyte telomere length  
425 across the adult life course. *Aging Cell.* 2013;12(4):615-621.
- 426 32. Aviv A, Shay JW. Reflections on telomere dynamics and ageing-related diseases in humans.  
427 *Philos Trans R Soc B Biol Sci.* 2018;373(1741).
- 428 33. Hansen MEB, Hunt SC, Stone RC, et al. Shorter telomere length in Europeans than in  
429 Africans due to polygenetic adaptation. *Hum Mol Genet.* 2016;25(11):2324-2330.
- 430 34. Hunt SC, Chen W, Gardner JP, et al. Leukocyte telomeres are longer in African Americans  
431 than in whites: the National Heart, Lung, and Blood Institute Family Heart Study and the  
432 Bogalusa Heart Study. *Aging Cell.* 2008;7(4):451-458.
- 433 35. Drury SS, Esteves K, Hatch V, et al. Setting the Trajectory: Racial Disparities in Newborn  
434 Telomere Length. *J Pediatr.* 2015;166(5):1181-1186.
- 435 36. Rewak M, Buka S, Prescott J, et al. Race-Related Health Disparities and Biological Aging:  
436 Does Rate of Telomere Shortening Differ Across Blacks and Whites? *Biol Psychol.*  
437 2014;99:92-99.
- 438 37. Aviv A, Chen W, Gardner JP, et al. Leukocyte Telomere Dynamics: Longitudinal Findings  
439 Among Young Adults in the Bogalusa Heart Study. *Am J Epidemiol.* 2009;169(3):323-329.
- 440 38. Jorgenson E, Tang H, Gadde M, et al. Ethnicity and Human Genetic Linkage Maps. *Am J*  
441 *Hum Genet.* 2005;76(2):276-290.
- 442 39. von Mering C, Huynen M, Jaeggi D, Schmidt S, Bork P, Snel B. STRING: a database of  
443 predicted functional associations between proteins. *Nucleic Acids Res.* 2003;31(1):258-261.
- 444 40. Pu Z, Wang Y, Liu X, et al. Cables1 Inhibits Proliferation and Induces Senescence by  
445 Angiotensin II via a p21-Dependent Pathway in Human Umbilical Vein Endothelial Cells. *J*  
446 *Vasc Res.* 2017;54(1):13-21.
- 447 41. Blackburn EH, Greider CW, Szostak JW. Telomeres and telomerase: the path from maize,  
448 *Tetrahymena* and yeast to human cancer and aging. *Nat Med.* October 2006.
- 449 42. Atzmon G, Cho M, Cawthon RM, et al. Genetic variation in human telomerase is associated  
450 with telomere length in Ashkenazi centenarians. *Proc Natl Acad Sci.* 2010;107(suppl  
451 1):1710-1717.

- 452 43. Iwama H, Ohyashiki K, Ohyashiki JH, et al. Telomeric length and telomerase activity vary  
453 with age in peripheral blood cells obtained from normal individuals. *Hum Genet.*  
454 1998;102(4):397-402.
- 455 44. Ding H, Yan F, Zhou L-L, et al. Association between previously identified loci affecting  
456 telomere length and coronary heart disease (CHD) in Han Chinese population. *Clin Interv*  
457 *Aging.* May 2014.
- 458 45. Delgado DA, Zhang C, Chen LS, et al. Genome-wide association study of telomere length  
459 among South Asians identifies a second RTEL1 association signal. *J Med Genet.*  
460 2018;55(1):64-71.
- 461 46. Hamad R, Tuljapurkar S, Rehkopf DH. Racial and Socioeconomic Variation in Genetic  
462 Markers of Telomere Length: A Cross-Sectional Study of U.S. Older Adults. *EBioMedicine.*  
463 2016;11:296-301.
- 464 47. Torgerson DG, Ampleford EJ, Chiu GY, et al. Meta-analysis of Genome-wide Association  
465 Studies of Asthma In Ethnically Diverse North American Populations. *Nat Genet.*  
466 2011;43(9):887-892.
- 467 48. Billon S, Lluch A, Guéguen R, Berthier AM, Siest G, Herbeth B. Family resemblance in  
468 breakfast energy intake: the Stanislas Family Study. *Eur J Clin Nutr.* 2002;56(10):1011-  
469 1019.
- 470 49. 1000 Genomes Project Consortium, Auton A, Brooks LD, et al. A global reference for  
471 human genetic variation. *Nature.* 2015;526(7571):68-74.
- 472 50. Eisen MB, Spellman PT, Brown PO, Botstein D. Cluster analysis and display of genome-  
473 wide expression patterns. *Proc Natl Acad Sci.* 1998;95(25):14863-14868.
- 474 51. Borrell LN, Nguyen EA, Roth LA, et al. Childhood Obesity and Asthma Control in the  
475 GALA II and SAGE II Studies. *Am J Respir Crit Care Med.* 2013;187(7):697-702.
- 476 52. Nishimura KK, Galanter JM, Roth LA, et al. Early-Life Air Pollution and Asthma Risk in  
477 Minority Children. The GALA II and SAGE II Studies. *Am J Respir Crit Care Med.*  
478 2013;188(3):309-318.
- 479 53. Thakur N, Oh SS, Nguyen EA, et al. Socioeconomic Status and Childhood Asthma in Urban  
480 Minority Youths. The GALA II and SAGE II Studies. *Am J Respir Crit Care Med.*  
481 2013;188(10):1202-1209.
- 482 54. Drury S, Theall K, Gleason M, et al. Telomere length and early severe social deprivation:  
483 linking early adversity and cellular aging. *Mol Psychiatry.* 2012;17(7):719-727.
- 484 55. Mitchell C, Hobcraft J, McLanahan SS, et al. Social disadvantage, genetic sensitivity, and  
485 children's telomere length. *Proc Natl Acad Sci.* 2014;111(16):5944-5949.

- 486 56. Needham BL, Fernandez JR, Lin J, Epel ES, Blackburn EH. Socioeconomic status and cell  
487 aging in children. *Soc Sci Med.* 2012;74(12):1948-1951.
- 488 57. Alexander DH, Novembre J, Lange K. Fast model-based estimation of ancestry in unrelated  
489 individuals. *Genome Res.* 2009;19(9):1655-1664.
- 490 58. The International HapMap 3 Consortium. Integrating common and rare genetic variation in  
491 diverse human populations. *Nature.* 2010;467(7311):52-58.
- 492 59. Hoffmann TJ, Zhan Y, Kvale MN, et al. Design and coverage of high throughput genotyping  
493 arrays optimized for individuals of East Asian, African American, and Latino race/ethnicity  
494 using imputation and a novel hybrid SNP selection algorithm. *Genomics.* 2011;98(6):422-  
495 430.
- 496 60. Loh P-R, Danecek P, Palamara PF, et al. Reference-based phasing using the Haplotype  
497 Reference Consortium panel. *Nat Genet.* 2016;48(11):1443-1448.
- 498 61. Cawthon RM. Telomere measurement by quantitative PCR. *Nucleic Acids Res.*  
499 2002;30(10):e47.
- 500 62. O'Callaghan NJ, Fenech M. A quantitative PCR method for measuring absolute telomere  
501 length. *Biol Proced Online.* 2011;13:3.
- 502 63. Raschenberger J, Kollerits B, Hammerer-Lercher A, et al. The association of relative  
503 telomere length with symptomatic peripheral arterial disease: Results from the CAVASIC  
504 study. *Atherosclerosis.* 2013;229(2):469-474.
- 505 64. Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR.  
506 *Nucleic Acids Res.* 2001;29(9):e45.
- 507 65. Dudbridge F. Power and Predictive Accuracy of Polygenic Risk Scores. *PLoS Genet.*  
508 2013;9(3).
- 509 66. Talmud PJ, Hingorani AD, Cooper JA, et al. Utility of genetic and non-genetic risk factors in  
510 prediction of type 2 diabetes: Whitehall II prospective cohort study. *BMJ.* 2010;340:b4838.
- 511 67. Sobota RS, Shriner D, Kodaman N, et al. Addressing Population-Specific Multiple Testing  
512 Burdens in Genetic Association Studies. *Ann Hum Genet.* 2015;79(2):136-147.
- 513 68. Chang CC, Chow CC, Tellier LC, Vattikuti S, Purcell SM, Lee JJ. Second-generation  
514 PLINK: rising to the challenge of larger and richer datasets. *GigaScience.* 2015;4:7.
- 515 69. Duggal P, Gillanders EM, Holmes TN, Bailey-Wilson JE. Establishing an adjusted p-value  
516 threshold to control the family-wide type 1 error in genome wide association studies. *BMC*  
517 *Genomics.* 2008;9:516.
- 518 70. Turner SD. qqman: an R package for visualizing GWAS results using Q-Q and manhattan  
519 plots. *bioRxiv.* May 2014:005165.

520 71. Pruim RJ, Welch RP, Sanna S, et al. LocusZoom: regional visualization of genome-wide  
521 association scan results. *Bioinforma Oxf Engl*. 2010;26(18):2336-2337.

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541

542 **AUTHOR CONTRIBUTIONS**

543 A.M.Z, M.J.W, J.W., S.S.O, and E.G.B. were involved in the conception and design of the study.  
544 A.M.Z., M.J.W, S.S.O., E.Y.L., J.W., P.C.G., J.R.L., A.C.Y.M., C.E., D.H., S.H., M.G.C.,  
545 L.A.S., K.L.K., O.R.A., J.M., and E.G.B were involved in the analysis and interpretation of data.

546 S.S., C.E., A.D., K.M., E.B.B., M.A.L., H.J.F., K.B.D., L.N.B., and E.G.B. planned and  
547 supervised the collection of data. C.E., O.R.A., M.G.C., and M.J.W. generated telomere data. All  
548 authors provided revisions and approval of the final manuscript.

549

#### 550 **COMPETING INTERESTS**

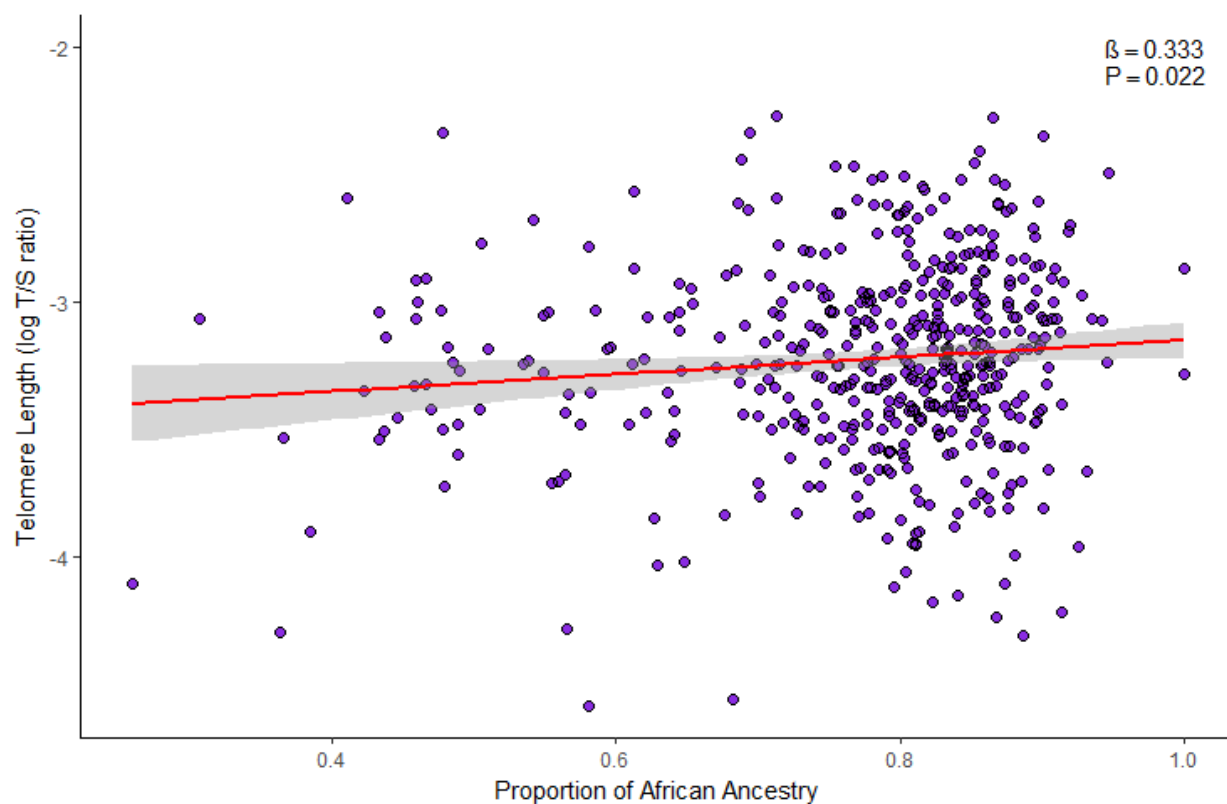
551 The authors declare no competing financial interests.

552 **Table 1. Demographic characteristics of healthy African American children and**  
553 **adolescents (n=492) in SAGE: San Francisco Bay Area, 2006-2015**  
554

Variable	N (%)
Median age [IQR]	15.8 [12.4, 18.3]
Sex (% Female)	270 (55.3)
Median relative telomere length [IQR] (T/S ratio)	0.0393 [0.0319, 0.0506]
Median African ancestry [IQR]	0.81 [0.74, 0.85]
<i>Maternal education attainment</i>	
≤ High school	185 (37.6)
>High school	307 (62.4)
<i>Insurance</i>	
Public	260 (52.8)
Private	232 (47.2)

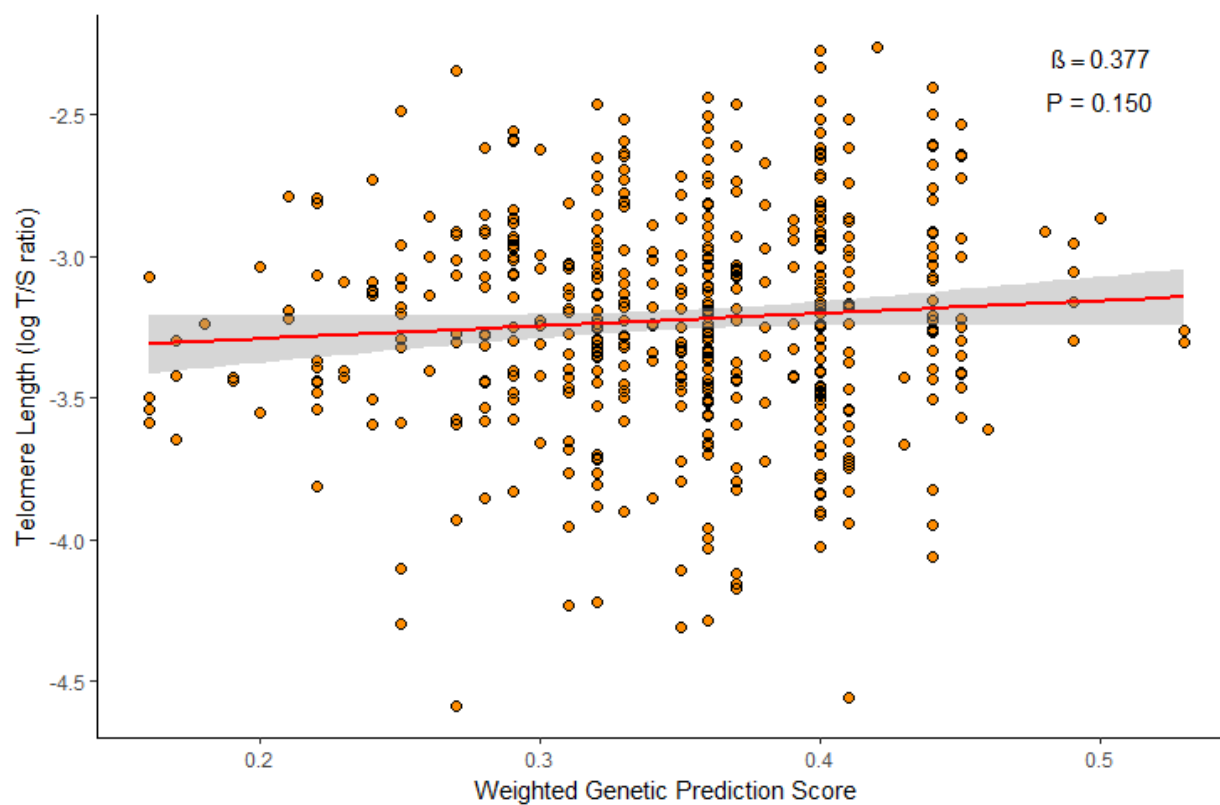
555

556 **Figure 1. Association between log-transformed TL and African ancestry in healthy African**  
557 **American children and adolescents in SAGE: San Francisco Bay Area, 2006-2015**  
558



559

560 **Figure 2. Adjusted association between log-transformed TL and GPS in healthy African**  
561 **American children and adolescents in SAGE: San Francisco Bay Area, 2006-2015.**  
562 Regression association adjusted for sex, age, genetic ancestry, maternal educational attainment,  
563 health insurance type and batch effects.  
564



565

566 **Table 2. Adjusted analysis of log-transformed TL using 34 SNPs found in adult studies in healthy African American children**  
567 **and adolescents in SAGE: San Francisco Bay Area, 2006-2015.** Regression adjusted for sex, age, genetic ancestry, maternal  
568 educational attainment, health insurance type and batch effects. <sup>a</sup>Reported effect allele. <sup>b</sup>Additive Linear regression  $\beta$  coefficient.  
569 <sup>c</sup>EAF: Effect Allele Frequency in previous studies and current study. <sup>d</sup>T-test test statistic reported instead of  $\beta$  coefficient. <sup>e</sup>NR = Not  
570 Reported.  
571

Reference	SNP	Chr. Position (GRCh38.p7)	Associated Gene	Effect Allele <sup>a</sup>	Previously Reported			SAGE (n=492)		
					EAF <sup>c</sup>	$\beta$	P	EAF	$\beta^b$	P
Codd <i>et al.</i> <sup>17</sup>	rs11125529	2:54475866	<i>ACYP2</i>	C	0.86	-0.056	4.48E-8	0.82	-0.0525	0.090
	rs2736100	5:1286516	<i>TERT</i>	A	0.51	-0.078	4.38E-19	0.50	-0.0341	0.145
	rs7675998	4:164007820	<i>NAF1</i>	A	0.28	-0.074	4.35E-16	0.28	-0.0318	0.275
	rs8105767	19:22215441	<i>ZNF208</i>	A	0.71	-0.048	1.11E-9	0.55	0.0086	0.721
	rs10936599	3:169492101	<i>TERC</i>	T	0.25	-0.097	2.54E-31	0.08	0.0119	0.789
	rs9420907	10:105676465	<i>OBFC1</i>	A	0.87	-0.069	6.90E-11	0.51	-0.00484	0.847
	rs755017	20:62421622	<i>RTEL1</i>	A	0.87	-0.062	6.71E-9	0.73	-0.00021	0.994
Pooley <i>et al.</i> <sup>18</sup>	rs6772228	3:58376019	<i>PXK</i>	A	0.05	0.120	4.67E-17	0.01	-0.2023	0.083
Mangino <i>et al.</i> <sup>19</sup>	rs10936601	3:169528449	<i>TERC</i>	C	0.27	4.45E-4	4.00E-15	0.48	-0.00066	0.978
	rs3027234	17:8136092	<i>CTC1</i>	T	0.23	-0.057	2.29E-8	0.07	0.0223	0.644
	rs1317082	3:169497585	<i>TERC</i>	G	0.29	0.068	1.00E-8	0.08	0.0119	0.789
	rs412658	19:22359440	<i>ZNF676</i>	T	0.35	0.056	1.00E-8	0.57	-0.0067	0.791
Mangino <i>et al.</i> <sup>20</sup>	rs9419958	10:105675946	<i>OBFC1</i>	T	0.14	0.083	9.00E-11	0.50	0.00484	0.847
	rs2162440	18:35214006	<i>BRUNOLA, PIKC3C</i>	G	NR <sup>e</sup>	-1.06	3.00E-6	0.58	0.0319	0.212
Prescott <i>et al.</i> <sup>21</sup>	rs12696304	3:169481271	<i>TERC</i>	G	0.27	-0.03	2.00E-14	0.53	-0.0021	0.929
Levy <i>et al.</i> <sup>22</sup>	rs4452212	2:137015991	<i>CXCR4</i>	A	0.65	-0.08	2.00E-6	0.14	-0.0465	0.180
	rs2736428	6:31843924	<i>SLC44A4</i>	T	0.29	0.08	3.00E-6	0.10	0.0499	0.227
	rs1975174	19:22515251	<i>ZNF676</i>	T	0.47	0.07	2.00E-6	0.67	0.0097	0.702
	rs4387287	10:105677897	<i>OBFC1</i>	A	0.08	0.12	2.00E-11	0.61	0.0022	0.934
Lee <i>et al.</i> <sup>23</sup>	rs10466239	10:43849827	<i>FXYD4, RASGEF1A</i>	T	0.07	4.51 <sup>d</sup>	7.00E-6	0.11	-0.0298	0.452
	rs34596385	6:141926004	<i>AK097143</i>	T	0.05	-4.53 <sup>d</sup>	6.00E-6	0.01	-0.0891	0.513
	rs11787341	8:19102564	<i>LOC100128993</i>	A	0.06	4.91 <sup>d</sup>	9.00E-7	0.07	0.0320	0.520
	rs10904887	10:17188641	<i>TRDMT1</i>	T	0.47	4.61 <sup>d</sup>	4.00E-6	0.81	-0.0130	0.697
	rs16859140	3:111792594	<i>TMPRSS7</i>	C	0.28	4.58 <sup>d</sup>	5.00E-6	0.11	-0.0151	0.697

	rs73394838	22:30225973	<i>ASCC2</i>	G	0.06	4.44 <sup>d</sup>	9.00E-6	0.27	0.00541	0.839
	rs4902100	14:62549819	<i>SYT16</i>	G	0.28	4.64 <sup>d</sup>	4.00E-6	0.23	-0.0011	0.968
	rs7680468	4:108304199	<i>DKK2, PAPSS1</i>	T	0.03	-5.47 <sup>d</sup>	5.00E-8	0.02	-0.0025	0.978
Saxena <i>et al.</i> <sup>24</sup>	rs2098713	5:37144574	<i>C5orf42</i>	T	0.47	-0.25	3.00E-6	0.76	-0.0543	0.058
	rs74019828	16:58209274	<i>CSNK2A2</i>	A	0.16	-0.38	5.00E-8	0.05	-0.0074	0.899
Gu <i>et al.</i> <sup>25</sup>	rs6028466	20:38129002	<i>DHX35</i>	A	NR <sup>e</sup>	0.192	3.00E-7	0.25	-0.0473	0.103
	rs654128	6:117086378	<i>KPNA5</i>	T	NR <sup>e</sup>	0.122	3.00E-6	0.09	-0.0588	0.151
	rs621559	1:43645411	<i>WDR65</i>	A	NR <sup>e</sup>	0.16	2.00E-6	0.32	-0.0344	0.196
	rs398652	14:56525569	<i>PELI2</i>	A	NR <sup>e</sup>	0.12	2.00E-6	0.35	-0.0115	0.647
Liu <i>et al.</i> <sup>26</sup>	rs17653722	12:52587518	<i>KRT80</i>	T	NR <sup>e</sup>	0.122	7.00E-6	0.06	0.0085	0.871

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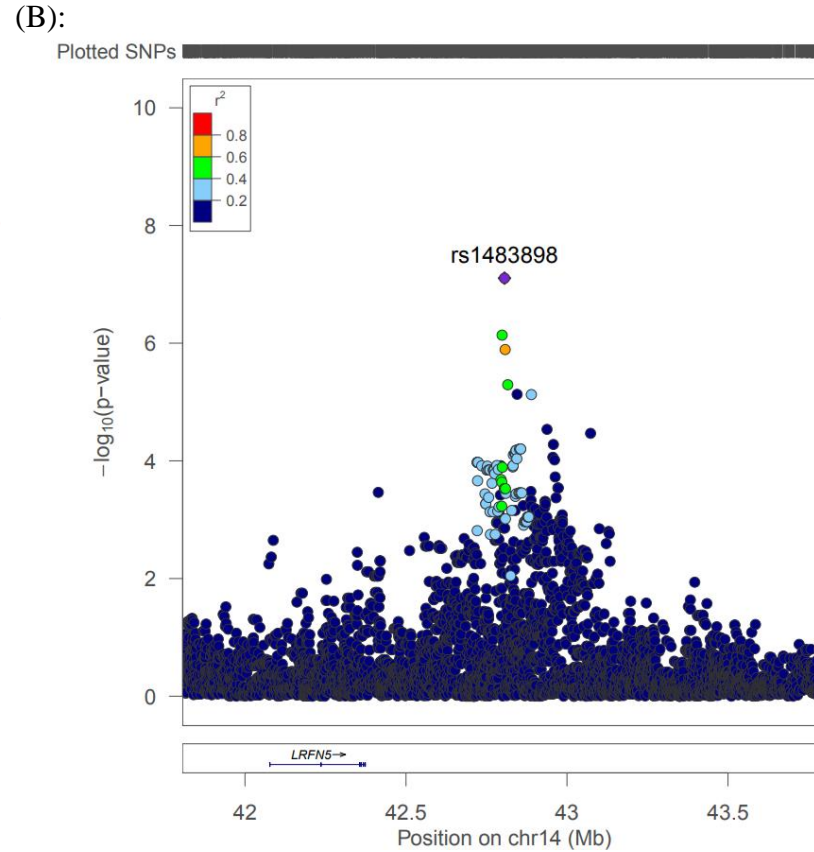
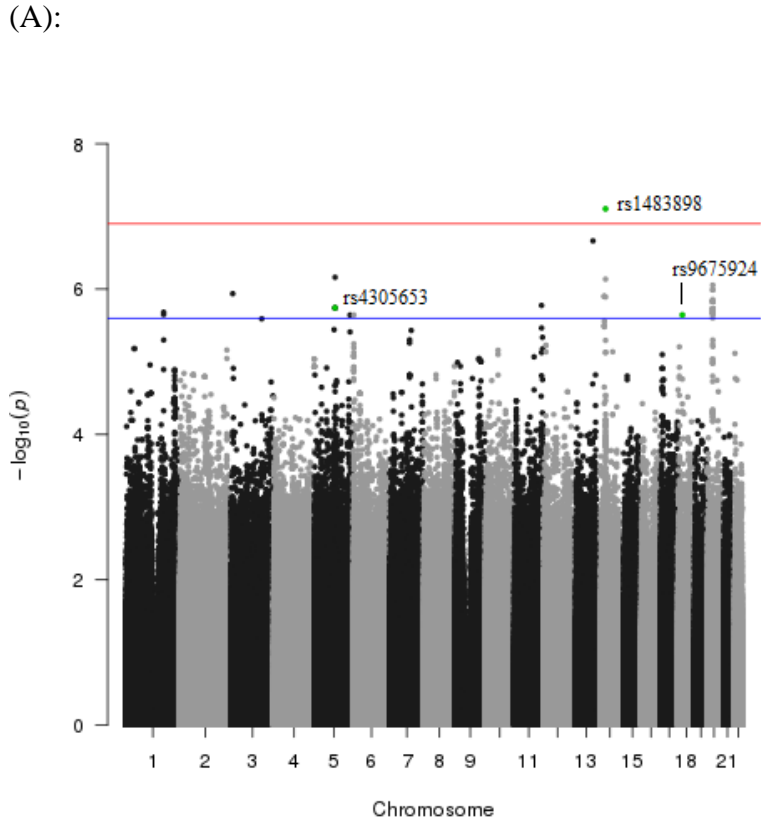
573 **Table 3. Adjusted analysis of log-transformed TL using six SNP's found in pediatric study healthy African American children**  
574 **and adolescents in SAGE: San Francisco Bay Area, 2006-2015.** Regression adjusted for sex, age, genetic ancestry, maternal  
575 educational attainment, health insurance type and batch effects. <sup>a</sup>Effect Allele. <sup>b</sup>Additive Linear Regression  $\beta$  coefficient. <sup>c</sup>EAF: Effect  
576 Allele Frequency. <sup>d</sup>T-Test value used instead of  $\beta$  coefficient.  
577

SNP	Chr. Position	Associated Gene	EA <sup>a</sup>	Previously Reported			SAGE (n=492)		
				EAF <sup>c</sup>	$\beta$ (SE)	P	EAF <sup>c</sup>	$\beta^b$ (SE)	P
rs594119	6:124940213	<i>NKAIN2</i>	T	0.19	-0.05 (0.01)	2.19E-5	0.15	-6.1E-2 (0.03)	0.055
rs11703393	22:44512124	<i>PARVB</i>	G	0.27	-0.04 (0.01)	1.69E-4	0.43	-3.4E-2 (0.02)	0.166
rs2300383	21:35250086	<i>ITSN1</i>	G	0.47	-0.04 (0.01)	7.42E-6	0.73	2.2E-2 (0.03)	0.438
rs528983	4:115976690	<i>NDST4</i>	G	0.11	-0.07 (0.01)	7.88E-6	0.15	-2.1E-2 (0.03)	0.523
rs12678295	8:2748377	<i>MYOM2, CSMD1</i>	G	0.40	-0.04 (0.01)	1.92E-4	0.22	-4.7E-3 (0.03)	0.870
rs10496920	2:142996517	<i>LRP1B, LOC100129955</i>	G	0.18	0.05 (0.01)	4.60E-4	0.15	-4.4E-3 (0.03)	0.895

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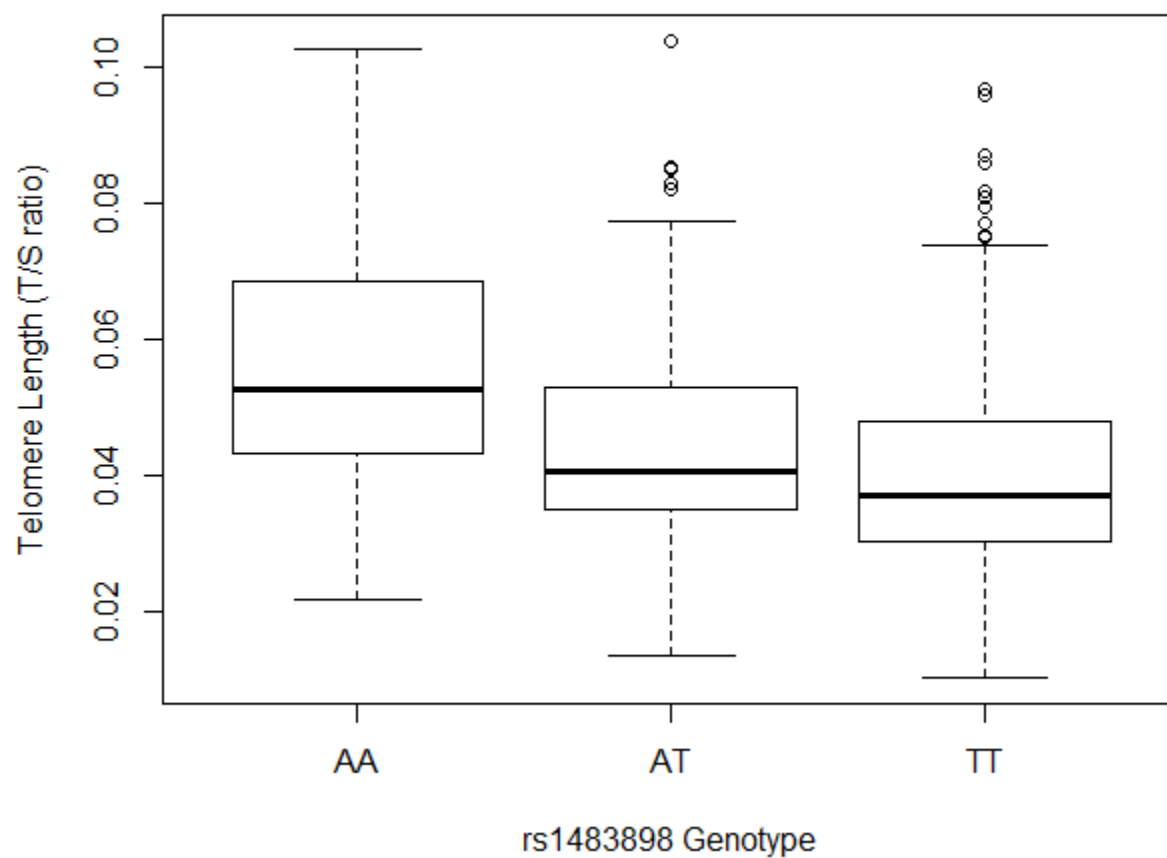


579 **Figure 3: Results of GWAS for TL in healthy African American children and adolescents in SAGE: San Francisco Bay Area,**  
 580 **2006-2015.** (A) Manhattan plot of the GWAS of TL with three SNPs relevant to telomere biology highlighted. Genome-wide  
 581 significance threshold is indicated as red line ( $P = 1.2 \times 10^{-7}$ ) and suggestive significance threshold is indicated as blue line ( $P = 2.3 \times$   
 582  $10^{-6}$ ). (B) Expansion of 1 Mb flanking region around the top hit (rs1483898) with surrounding SNPs colored by amount of linkage  
 583 disequilibrium with the top SNP, indicated by pairwise  $r^2$  values from hg19/November 2014 1000 Genomes AFR.  
 584  
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587 **Figure 4: Comparison of mean TL between rs1483898 genotypes in healthy African**  
588 **American children and adolescents in SAGE: San Francisco Bay Area, 2006-2015**



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