



75-oligomer oligonucleotide at a serial dilution of  $10^{-1}$  to  $10^{-6}$ . The number of repeats in each standard was calculated as follows:  $100 \text{ pg } 36B4 \text{ 75-oligomer standard} = (100 \times 10^{-12} \text{ g}) \div (23,268.1_{[\text{MW}]} / 6.02 \times 10^{23}_{[\text{Avogadro's number}]}) \div 2 = 1.29 \times 10^9$  diploid genome copies in the  $1 \times (100 \text{ pg})$  36B4 standard. Carrier RNA (Qiagen, Inc., Valencia, CA) was added to each standard to maintain a constant 10 ng of total DNA per reaction. Average TL per chromosome was calculated using the following formula:  $(\text{TL} \div \text{copies of diploid genome}) \div 46$ . All samples for TEL and 36B4 reactions, as well as standard curves, were performed in duplicate on the same plates. As part of routine quality control, 10% of the samples were blind duplicate samples. The overall intraplate coefficient of variation was 0.8%, and the interplate coefficients of variation of the telomere and 36B4 assays were 5.7 and 3.4%, respectively.

**SNP genotyping.** We identified genes coding for proteins involved in telomere regulation, including those directly associated with telomere structure (*TRFI*, *TRF2*, *POT1*, *RAP1*, *TIN2*, and *TPP1*) and those involved in TL maintenance (*TERT*, *TERC*, and *TEPI*). As described in details previously (20,21), we chose haplotype-tagging SNPs (tSNPs) using the National Center for Biotechnology Information database SNP supplemented by the HapMap database (22) to capture the majority of common variation in the genetic region covering 30 kb upstream and 30 kb downstream of each telomere-related gene. The initial set of SNPs was selected based on the following criteria: 1) functional priority (nonsynonymous SNPs > splice site SNPs > synonymous SNPs > 5' untranslated region SNPs > 3' untranslated region SNPs > intronic SNPs); 2) minor allele frequency  $\geq 5\%$  in at least one ethnic group; and 3) tSNPs evenly spaced across the gene region (23). In total, we selected 82 SNPs in 9 gene regions for genotyping. Additionally, we included rs2487999 (*OBFC1*), which was associated with leukocyte TL in a recent genome-wide association study of leukocyte TL (24). For these 82 SNPs, genotyping was performed in all samples using TaqMan SNP genotyping assays. Specific primers and probes were custom designed by Applied Biosystems. All samples were genotyped using the 96.96 Dynamic Array on the Biomark system (Fluidigm, San Francisco, CA) according to standard procedures. Two SNPs, rs4092743 of *TRFI* and rs11556639 of *RAP1* (selected because they were tSNPs with nonsynonymous variation), were not polymorphic in our samples and were therefore excluded in the final analysis. The average undetermined genotype rate was <0.5%, and the concordance rate was >99% in the duplicate samples.

**Statistical analysis.** TLs in our samples approximated a normal distribution. We thus conducted analysis with the TL variable treated as either continuous or categorical (quartiles). The distributions of diabetes risk factors were examined by case-control status across race/ethnicity. Generalized linear models (GLMs) were used to conduct tests for trend of continuous variables. Unconditional logistic models were used to test for trend across quartiles of TL. Differences in mean TL (kb) according to ethnic groups and case-control status were examined using GLMs. To account for the correlation within matched case-control sets, we used mixed-effects regression modeling case-control clusters as a random effect.

To examine the prospective association between TL and clinical diabetes risk, conditional logistic regression was used to estimate the odds ratio (OR) and 95% CI for clinical diabetes in each TL quartile using the lowest quartile as the reference category. To test for log-linear trends, we modeled the median TL within each quartile as a continuous variable. Finally, we estimated the OR of developing diabetes risk per 1-kb increase in TL in each race/ethnic group. Our base model used conditional logistic regression to account for matching factors (age, ethnicity, clinical center, duration of follow-up, and time of blood draw). In the full model, we further adjusted for potential confounders, including BMI (modeled as a continuous covariate), smoking (never, past, and current smokers), alcohol intake (never, past, and current drinkers), physical activity (quintiles), and current postmenopausal hormone use (yes or no).

To determine the associations between genetic variants and TLs, we calculated the mean differences of leukocyte TL according to SNP genotypes by fitting GLMs, treating TL as a dependent variable and SNPs as independent variables. The additive inheritance model was used in the single SNP analysis, resulting in the mean change in TL per each additional copy of the minor allele. Conditional logistic regression models were used to estimate the OR for diabetes risk associated with each additional copy of the minor allele for each SNP. Multivariable models were adjusted for matching factors (age, clinical center, time of blood draw, duration of follow-up, and ethnicity) and other potential confounders, including BMI, physical activity, current postmenopausal hormone use, alcohol consumption, and cigarette smoking. To account for potential false positives due to multiple comparisons, we calculated the false discovery rate by incorporating all *P* values from multiple tests performed for SNPs in the GLM analysis (25). The PROC MULTTEST procedure in SAS 9.2 was used to obtain the multiple testing-adjusted *P* values.

To further evaluate whether the association between TL and risk of clinical diabetes was causal, we conducted instrumental variable analyses using genetic instruments (i.e., Mendelian randomization analysis) (14,26). As described previously (14), SNPs had to satisfy three classical assumptions in order to be considered for use as instrumental variables: 1) robustly associated with TL, 2) independent of diabetes factors, and 3) affected diabetes only through their effects on TL. Instrumental variables were selected separately for each racial/ethnic group to account for potential allelic heterogeneity. In total, we selected six instruments for whites (rs34368910, rs4888444, rs4975605, rs938886, rs2228041, and rs12880583), four for blacks (rs872072, rs938886, rs1713458, and rs4387287), seven for Hispanics (rs35276863, rs729421, rs11972248, rs4635969, rs2853669, rs2736098, and rs2853676), and two for Asians (rs11556640 and rs2297613). The predicted OR of diabetes per kb increase in TL was estimated from the joint contributions of the ethnicity-specific instrumental variables. We subsequently pooled the ethnicity-specific OR via a random-effects model. All other analyses were performed with SAS software, version 9.2 (SAS Institute, Cary, NC).

TABLE 1  
Baseline characteristics according to race/ethnicity and diabetes case-control status in 4,057 postmenopausal women

	White			Black			Hispanic			Asian		
	Cases	Controls	<i>P</i> value	Cases	Controls	<i>P</i> value	Cases	Controls	<i>P</i> value	Cases	Controls	<i>P</i> value
<i>n</i>	1,012	1,023		400	819		162	331		101	207	
Age (years)*	63.96	63.92	0.90	60.97	60.95	0.96	60.15	60.21	0.92	63.37	63.58	0.82
BMI (kg/m <sup>2</sup> )*	32.53	26.46	<0.001	33.32	29.74	<0.001	31.30	27.78	<0.001	26.83	23.96	<0.001
Waist (cm)*	99.50	82.83	<0.001	97.28	87.81	<0.001	93.81	83.80	<0.001	84.64	75.84	<0.001
Blood pressure*												
Systolic	132.97	126.48	<0.001	132.80	129.64	0.002	129.52	124.37	0.002	113.83	129.55	0.06
Diastolic	76.34	74.52	<0.001	78.46	77.91	0.35	75.29	74.63	0.46	79.16	78.07	0.34
Smoking (ever)†	47.72	50.45	0.22	49.20	56.59	0.02	29.27	37.27	0.08	27.18	30.69	0.52
Alcohol (ever)†	86.71	89.72	0.04	80.15	84.22	0.08	74.52	79.20	0.25	62.38	59.90	0.68
Physical activity*‡	9.98	13.86	<0.001	8.74	11.45	0.001	9.96	12.82	0.06	13.38	12.76	0.72
T2D family history†	48.96	29.75	<0.001	62.72	39.38	<0.001	65.84	39.51	<0.001	52.48	36.23	0.04
Hormone therapy (ever)†	51.78	62.95	<0.001	35.34	47.07	<0.001	43.21	55.59	0.01	65.00	66.67	0.77
Telomere length (kb)*	3.87	3.90	0.62	4.11	4.25	0.11	4.29	4.49	0.18	3.88	4.04	0.37

\*Mean. †Percentage. ‡Total METs per week (kcal/week/kg). Energy expenditure from recreational physical activity.

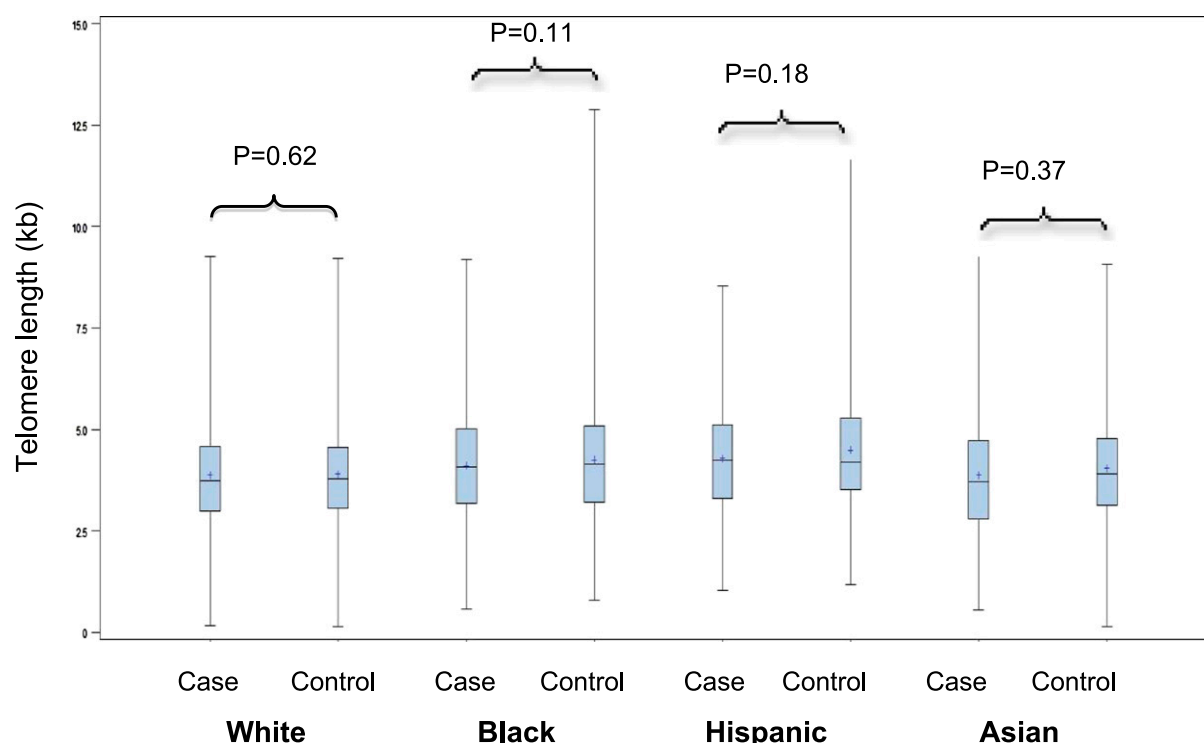


FIG. 1. TL (kb) by clinical diabetes status and race/ethnicity. The boxplots show the mean (+), median, and interquartile range. The vertical line indicates the minimum and maximum value of TL in each group. (A high-quality color representation of this figure is available in the online issue.)

**RESULTS**

**Baseline characteristics according to diabetes case status.** The mean TL was 3.97 kb (SD = 1.37 kb) among diabetic case participants and 4.12 kb (SD = 1.40 kb) among control participants who remained free of the disease. As

expected, case participants generally had higher BMI, larger waist circumference, higher systolic blood pressure, higher percentage of current or former smokers, less physical activity, higher proportion with a family history of diabetes, and lower proportion with past use of hormone therapy

TABLE 2  
Baseline characteristics according to leukocyte TL among 2,382 (control participants) postmenopausal women

TL (kb)	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P value for trend
<i>n</i>	591	590	589	591	
Median	2.65	3.57	4.35	5.07	
Range§	2.21–2.91	3.36–3.78	4.17–4.58	5.18–6.34	
Age (years)*	63.00	63.00	62.00	60.00	<0.0001
Ethnicity†					
White	47.55	48.64	42.69	32.66	<0.0001
Black	33.50	28.64	34.69	41.29	
Hispanic	9.98	13.05	14.63	17.60	
Asian	8.97	9.66	7.99	8.46	
BMI (kg/m <sup>2</sup> )*	26.74	26.23	26.58	26.54	0.14
Waist (cm)*	82.40	82.00	82.50	81.80	0.82
Blood pressure (mmHg)*					
Systolic	126.00	126.00	125.00	127.00	0.73
Diastolic	75.00	75.00	76.00	76.50	0.20
Fasting insulin (μIU/mL)*	6.53	6.28	6.56	6.56	0.70
Fasting glucose (mmol/mL)*	5.11	5.06	5.11	5.06	0.93
HOMA-IR*	1.50	1.43	1.44	1.47	0.81
Smoking (ever)†	46.14	44.94	40.65	43.66	0.20
Alcohol (ever)†	83.76	85.01	82.36	83.79	0.82
Physical activity*,‡	8.00	9.50	8.33	7.50	0.55
T2D family history†	33.45	32.26	34.47	39.42	0.05
Hormone therapy (ever)†	57.36	56.95	55.69	57.29	0.87

HOMA-IR, homeostasis model assessment of insulin resistance. \*Median. †Percentage. ‡Total METs per week (kcal/week/kg). Energy expenditure from recreational physical activity. §Interquartile range.

(Table 1). Mean TL also varied across ethnic groups in both case participants and control participants (Fig. 1). Within each ethnic group, diabetes case participants appeared to have consistently shorter baseline TL (30–200 base pairs [bp]) than control participants, but this difference was modest. Black and Hispanic participants had relatively longer TLs compared with whites and Asians, even after adjusting for age.

#### Baseline characteristics according to TL quartiles.

Table 2 shows the distribution of diabetes-related characteristics by TL quartiles in controls. As expected, there were significant TL differences by age in that older women had shorter TLs than their younger counterparts ( $P < 0.0001$  for linear trend). TLs also appeared to differ by ethnicity but not by other traditional diabetes risk factors such as BMI, waist circumference, fasting glucose, fasting insulin, homeostasis model assessment of insulin resistance, alcohol, smoking, hormone therapy, or physical activity level.

#### Prospective association between TL and clinical diabetes risk.

In pooled analysis combining samples from all ethnicities assuming a linear relation (Table 3), every 1-kb increase in the TL was associated with a decreased risk of clinical diabetes (OR 0.94 [95% CI 0.89–0.99]) after adjusting for matching variables (age, ethnicity, date of blood collection, duration of follow-up, and clinical center). The relation between TL and diabetes risk was attenuated in multivariable analysis after further adjustment for known diabetes risk factors. Moreover, there was no significant relation between TL and diabetes in stratified analysis by

race/ethnicity. ORs for diabetes risk (95% CI) per 1-kb increment in TL were 1.00 (0.90–1.11) in whites, 0.95 (0.85–1.06) in blacks, 0.96 (0.79–1.17) in Hispanics, and 0.88 (0.70–1.10) in Asians.

#### Associations between SNPs, TL, and risk of clinical diabetes.

Of the 80 SNPs genotyped, 29 SNPs were associated with leukocyte TL levels in pooled samples combining all ethnic groups (Supplementary Table 1). For 16 of 29 SNPs, each additional copy of the minor allele was associated with decreased TL (the mean per-allele decrease ranged from 0.07 to 0.33 kb; all  $P < 0.05$ ). Eight SNPs (rs2981084, rs2975852, rs6979, rs1865493, rs1760904, rs1760897, rs1713458, and rs3772190) remained significantly associated with decreased TL after accounting for multiple comparisons. In contrast, the minor alleles of the remaining 13 SNPs were associated with increased TL, with the mean per-allele increase ranging from 0.07 to 2.04 kb (all  $P < 0.05$ ). Six SNPs (rs34368910, rs10244817, rs938886, rs1288583, rs1711188, and rs4387287) remained significantly associated with increased TL. The minor allele frequencies of the SNPs that were associated with TLs also appeared to vary by ethnicity. However, no significant associations were observed between these 29 SNPs and the risk of developing clinical diabetes, except rs4387287 on *OBFC1* (OR 0.88 [95% CI 0.78–1.00]) (Supplementary Table 2).

Genomic risk score (GRS) based on SNPs identified was directly associated with TL, indicating a strong instrument (Fig. 2). Genetic instruments were selected independently

TABLE 3

Adjusted OR (95% CI) of diabetes risk according to leukocyte TL in 4,057 postmenopausal women

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P value for trend	Continuous (per 1 kb)
<b>All*</b>						
Median (range)§	2.63 (2.21–2.91)	3.58 (3.36–3.78)	4.36 (4.17–4.58)	5.61 (5.18–6.34)		
n (case/control)	469/591	422/590	395/589	382/591		
Base	1	0.89 (0.73–1.07)	0.85 (0.70–1.03)	0.87 (0.71–1.07)	0.16	0.94 (0.89–0.99)
Base + matching†	1	0.89 (0.74–1.08)	0.85 (0.70–1.33)	0.88 (0.71–1.08)	0.17	0.94 (0.89–0.99)
Full model‡	1	0.90 (0.71–1.13)	0.84 (0.67–1.07)	0.93 (0.72–1.19)	0.44	0.96 (0.90–1.02)
<b>White</b>						
Median (range)§	2.59 (2.20–2.82)	3.42 (3.26–3.62)	4.16 (3.98–4.34)	5.27 (4.86–6.02)		
n (case/control)	275/253	246/253	229/253	258/253		
Base	1	0.90 (0.68–1.19)	0.82 (0.63–1.07)	0.90 (0.68–1.19)	0.37	0.98 (0.91–1.06)
Base + matching†	1	0.89 (0.69–1.15)	0.82 (0.62–1.06)	0.90 (0.68–1.18)	0.35	0.98 (0.91–1.06)
Full model‡	1	0.86 (0.60–1.22)	0.87 (0.61–1.26)	0.97 (0.66–1.42)	0.90	1.00 (0.90–1.11)
<b>Black</b>						
Median (range)§	2.62 (2.20–2.93)	3.70 (3.46–3.94)	4.56 (4.45–4.80)	5.77 (5.37–6.54)		
n (case/control)	101/203	110/205	95/203	92/204		
Base	1	1.07 (0.75–1.53)	0.92 (0.64–1.32)	0.90 (0.62–1.32)	0.44	0.92 (0.84–1.02)
Base + matching†	1	1.07 (0.75–1.52)	0.91 (0.63–1.31)	0.89 (0.61–1.31)	0.40	0.92 (0.84–1.01)
Full model‡	1	1.12 (0.75–1.69)	0.92 (0.61–1.40)	0.96 (0.61–1.49)	0.60	0.95 (0.85–1.06)
<b>Hispanic</b>						
Median (range)§	2.91 (2.39–3.19)	3.87 (3.74–4.00)	4.68 (4.40–4.91)	6.27 (5.68–7.26)		
n (case/control)	49/81	29/82	50/81	33/82		
Base	1	0.57 (0.32–1.01)	0.97 (0.56–1.68)	0.60 (0.31–1.14)	0.35	0.87 (0.74–1.02)
Base + matching†	1	0.54 (0.30–0.97)	0.96 (0.55–1.66)	0.61 (0.32–1.18)	0.40	0.87 (0.74–1.32)
Full model‡	1	0.56 (0.27–1.14)	1.06 (0.55–2.07)	0.85 (0.39–1.84)	0.94	0.96 (0.79–1.17)
<b>Asian</b>						
Median (range)§	2.51 (2.10–2.85)	3.48 (3.37–3.68)	4.35 (4.10–4.51)	5.61 (5.18–6.44)		
n (case/control)	33/51	23/53	22/51	23/52		
Base	1	0.63 (0.31–1.29)	0.64 (0.30–1.39)	0.57 (0.25–1.27)	0.19	0.89 (0.73–1.09)
Base + matching†	1	0.64 (0.29–1.33)	0.63 (0.29–1.36)	0.54 (0.24–1.23)	0.16	0.87 (0.71–1.29)
Full model‡	1	0.53 (0.23–1.21)	0.65 (0.28–1.52)	0.53 (0.21–1.32)	0.23	0.88 (0.70–1.10)

\*Further adjusted for ethnicity. †Adjusted for age, date of blood collection, and clinical center. ‡Adjusted for age, date of blood collection, clinical center, BMI, physical activity, hormone therapy, alcohol consumption, and smoking. §Interquartile range.

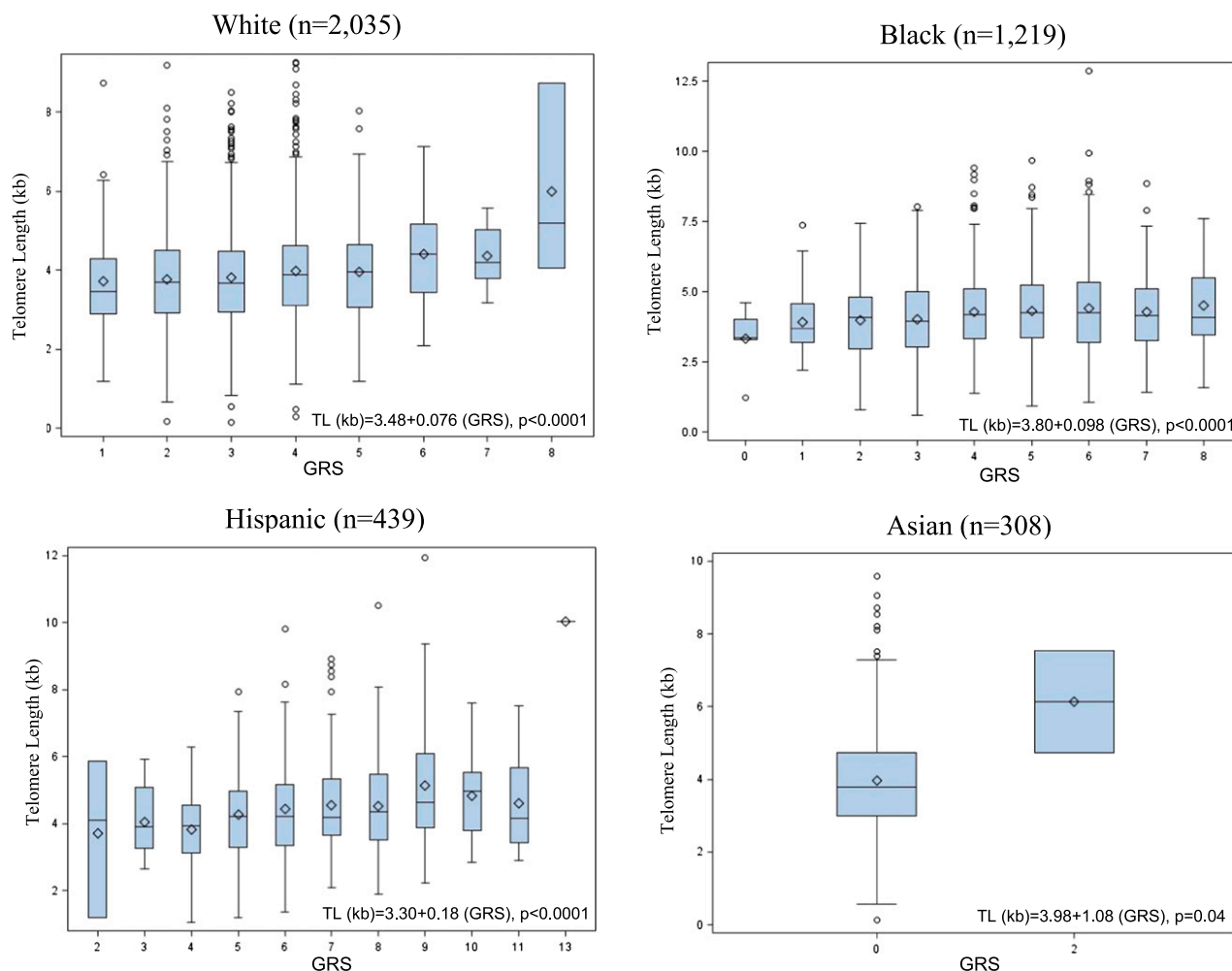
across each ethnic group. The pooled results from the ethnicity-specific Mendelian randomization analysis did not support a causal role of TL in the development of clinical diabetes, using either multiple instruments in a joint model ( $P = 0.82$ ) or as a GRS ( $P = 0.52$ ) (Table 4).

## DISCUSSION

In this large multiethnic cohort of postmenopausal women followed for an average of 6 years, leukocyte TL was modestly associated with risk of clinical diabetes. However, the modest association between TL and diabetes risk could be explained by traditional diabetes risk factors. Mendelian randomization analysis showed no significant association between genetically elevated TL levels and risk of clinical diabetes.

To date, six proteins, including telomeric repeat binding factor 1 and 2 (TRF1 and TRF2), human TRF2-interacting telomeric protein (hRap1), TRF1-interacting nuclear factor 2 (TIN2), TIN2- and POT1-organizing protein (TPP1), and protection of telomeres 1 (POT1), have been identified, forming the so-called shelterin complex, a constitutive

component of telomeres (27). Mutations in these telomere-regulating proteins may disrupt telomere structure, leading to chromosome instability (28). Previous studies have identified the crucial role of TRF2 in protecting chromosomal ends in that TRF1 appears to serve as a negative regulator of TL (29,30). In addition, POT1 is known to bind specifically to telomeric overhangs and regulates the access of telomerase to the repeats that affect telomerase activity (31–34). Thus, POT1 is thought to be the major regulator of TL (35,36). More recently, TPP1, a newly identified telomeric protein, was identified as a regulator of POT1 for telomeric targeting (36). However, the exact mechanistic functions by which these proteins interact in telomere homeostasis remain unknown, though mutually reinforcing mechanisms seem likely to involve both telomere-associated proteins and telomerase activity (37). In a meta-analysis of genome-wide association studies (24), the region containing *OBFC1* (rs4387287) was identified to show significant associations with leukocyte TL, which was confirmed by a subsequent study that also identified *TERC* (rs3772190) to be significantly associated with TL (38). Consistent with these previous reports, we observed that each copy of the A allele (rs4387287 in *OBFC1*) was



**FIG. 2.** TL by GRS according to race/ethnicity. The boxplots show the mean ( $\diamond$ ), median, and interquartile range. The vertical line indicates the minimum and maximum value  $>1.5$  times the upper quartile of TL in each group. Outliers ( $\circ$ ) are displayed when the individual TL is  $>1.5$  times the interquartile range of the upper quartile value. GRS represents the unweighted sum of alleles from the selected SNPs. The selected SNPs for each of the ethnic groups are as follows: 1) whites, rs34368910, rs4888444, rs4975605, rs938886, and rs12880583; 2) blacks, rs872072, rs938886, rs1713458, and rs4387287; 3) Hispanics, rs35276863, rs729421, rs11972248, rs4635969, rs2853669, rs2736098, and rs2853676; and 4) Asians, rs11556640 and rs2297613. (A high-quality color representation of this figure is available in the online issue.)

TABLE 4

Evaluation of potential casual relation between TL and diabetes risk via Mendelian randomization analysis in 4,057 postmenopausal women

	Relative risk (95% CI) (per 1-kb increment in TL)				
	White	Black	Hispanic	Asian	Combined#
Conventional multivariable analyses					
Simple model 1*	0.98 (0.92–1.06)	0.92 (0.84–1.02)	0.87 (0.74–1.02)	0.88 (0.72–1.08)	0.94 (0.89–0.99)
Multivariable model†	0.98 (0.91–1.06)	0.92 (0.84–1.01)	0.87 (0.74–1.02)	0.87 (0.71–1.07)	0.94 (0.89–0.99)
Full model‡	1.00 (0.90–1.11)	0.95 (0.85–1.06)	0.96 (0.79–1.17)	0.88 (0.70–1.10)	0.96 (0.90–1.03)
Mendelian randomization analyses					
Mendelian instrument: GRS§	1.26 (0.67–2.40)	0.53 (0.25–1.11)	0.81 (0.45–1.45)	1.40 (0.38–5.08)	0.88 (0.59–1.31)
Mendelian instrument: SNP	1.60 (1.00–2.56)	0.54 (0.26–1.13)	1.01 (0.60–1.69)	1.40 (0.38–5.08)	1.06 (0.65–1.71)

\*Simple model 1 adjusted for age. †Multivariable model adjusted for age, ethnicity, date of blood collection, duration of follow-up, and clinical center. ‡Full model adjusted for age, date of blood collection, clinical center, BMI, physical activity, hormone therapy, alcohol consumption, and smoking. §GRS represents the unweighted sum of alleles from the selected SNPs. The selected SNPs for each of the ethnic groups are as follows: 1) whites, rs34368910, rs4888444, rs4975605, rs938886, rs2228041, and rs12880583; 2) blacks, rs872072, rs938886, rs1713458, and rs4387287; 3) Hispanics, rs35276863, rs729421, rs11972248, rs4635969, rs2853669, rs2736098, and rs2853676; and 4) Asians, rs11556640 and rs2297613. ||SNPs used in GRS were included as separate covariates in the joint multivariable instrumental variables (IV) model. #Combined ethnicity-specific estimates using a meta-analytic random effects model.

associated with a TL increment of 0.08 kb. Further, we found that each additional copy of the T allele for rs3772190 (*TERC*) was associated with a 0.09-kb decrease in TL. Despite ethnic differences, several common genetic variants in the telomere and telomerase regulating proteins were robustly associated with TL. However, these SNPs were not associated with risk of clinical diabetes in these women.

Findings from previous studies directly relating TL to diabetes risk have been mixed and limited by their cross-sectional design. In a study of 74 patients with type 2 diabetes, Jeanclos et al. (39) found that age-adjusted leukocyte TLs were not significantly different from age-matched nondiabetic controls ( $P = 0.1$ ). In contrast, five other cross-sectional studies (7–9,40,41) reported that diabetes case participants had shorter TLs than control participants. Notably, telomere shortening is accelerated in human premature-aging syndromes (i.e., Werner syndrome, ataxia telangiectasia, and dyskeratosis congenita) (37) and positively associated with mitochondrial DNA content (41). It is estimated that humans on average lose 20–60 bp of telomere DNA per year (5,42,43). However, such estimates for rates of shortening are not precise as they were not done prospectively but rather in cross-sectional studies where TLs were simply compared in different age-groups. For example, among 383 adults (291 men and 92 women) from 173 families comprising 258 sibling pairs, Vasa-Nicotera et al. (4) found a mean TL shortening of  $29.9 \pm 5.6$  bp per year in men and  $16.8 \pm 9.9$  bp per year in women (mean age,  $65.8 \pm 6.4$  years; range, 47–82 years). In this prospective cohort, we observed an average TL decrease of  $24.6 \pm 4.25$  bp per year. However, there were no statistically significant differences in TL between case participants and control participants after adjustment of traditional risk factors. One possible limitation may be that the follow-up time was not long enough to detect a potential inverse relation between TL and diabetes risk. As such, we cannot completely exclude the possibility that the magnitude of effects on diabetes risk due to TL, if any, may be greater if more case participants were identified during a longer period of follow-up.

Nevertheless, to further examine whether any modest TL-diabetes association previously observed was due to residual confounding and/or reverse causation, we used genetic variants that were associated with changes in TL and

tested whether the effect of TL on diabetes risk is causal. Previous simulation work has shown that combining multiple genetic variants into a single instrument can improve the strength and power of the instrument (44). Thus, we assessed both allele counts (GRS) and multiple variables as separate covariates in the same regression model as instruments in the Mendelian randomization analysis. However, our instrumental analysis did not detect any significant association between genetically determined TLs and diabetes risk.

In summary, although baseline leukocyte TL was weakly associated with diabetes risk in this large multiethnic cohort of postmenopausal women followed for 6 years, this association was not independent of known risk factors. These prospective data do not support the utility of TL in risk stratification for clinical diabetes among postmenopausal women.

#### ACKNOWLEDGMENTS

The Women's Health Initiative program is funded by the National Heart, Lung, and Blood Institute, National Institutes of Health, U.S. Department of Health and Human Services, through contracts N01-WH-22110, -24152, -32100-2, -32105-6, -32108-9, -32111-13, -32115, -32118, -32119, -32122, -42107-26, -42129-32, and -44221. This ancillary study was supported by a grant from the National Institute of Diabetes and Digestive and Kidney Diseases (R21-DK-084452).

No potential conflicts of interest relevant to this article were reported.

N.-c.Y.Y. and S.L. wrote the manuscript and analyzed research data. B.H.C., Y.S., X.L., and Y.C. analyzed research data. J.E.M., M.K., B.V.H., K.L.M., J.D.C., L.S.P., M.L.S., and L.F.T. contributed to discussion and reviewed and edited the manuscript. N.-c.Y.Y. and S.L. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

The authors thank the staff and investigators from all WHI centers as well as WHI participants; without their effort, this study would not be possible.

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