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ARTICLE

Genetic and clinical determinants of telomere length

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Summary

Many epidemiologic studies have identified important relationships between leukocyte telomere length (LTL) with genetics and health. Most of these studies have been significantly limited in scope by focusing predominantly on individual diseases or restricted to GWAS analysis. Using two large patient populations derived from Vanderbilt University and Marshfield Clinic biobanks linked to genomic and phenomic data from medical records, we investigated the inter-relationship between LTL, genomics, and human health. Our GWAS confirmed 11 genetic loci previously associated with LTL and two novel loci in *SCNN1D* and *PITPNM1*. PheWAS of LTL identified 67 distinct clinical phenotypes associated with both short and long LTL. We demonstrated that several diseases associated with LTL were related to one another but were largely independent from LTL genetics. Age of death was correlated with LTL independent of age. Those with very short LTL (<−1.5 standard deviation [SD]) died 10.4 years ($p < 0.0001$) younger than those with average LTL (±0.5 SD; mean age of death = 74.2 years). Likewise, those with very long LTL (>1.5 SD) died 1.9 years ($p = 0.0175$) younger than those with average LTL. This is consistent with the PheWAS results showing diseases associating with both short and long LTL. Finally, we estimated that the genome (12.8%) and age (8.5%) explain the largest proportion of LTL variance, whereas the phenome (1.5%) and sex (0.9%) explained a smaller fraction. In total, 23.7% of LTL variance was explained. These observations provide the rationale for expanded research to understand the multifaceted correlations between TL biology and human health over time, leading to effective LTL usage in medical applications.

Introduction

Telomeres are specialized structures found at the ends of chromosomes. These structures consist of highly repetitive DNA sequences and specific protein complexes. Telomeres acts as protective caps that are integral for genomic stability and cell function. They are maintained by the holoenzyme telomerase, a ribonucleoprotein consisting minimally of the RNA template telomerase RNA component (TERC) and telomerase reverse transcriptase (TERT). Other than germ cells and a few other highly proliferative cell types, most human cells, including leukocytes, do not have sufficient *TERT* expression to support telomerase activity and maintain telomere length. This is influenced by a direct interaction between telomeres and the *TERT* promotor.[1] It is now well accepted that telomeres shorten with each cell division[2] and that this is inversely correlated with age in humans. When a cell reaches the point at which telomeres become excessively short—the "Hayflick limit"—the cell enters replicative senescence.[3] Accumulation of senescence cells in organs over time is one of the driving causes of chronic organ inflammation, organ failure, and diseases of advanced age.[4,5]

Numerous population studies have shown that leukocyte telomere length (LTL) has a normal distribution and varies widely among individuals of the same age. Age and sex account for approximately 2%–10% and <1% of LTL variance, respectively.[6] Numerous epidemiological studies have associated LTL with many human diseases and traits. For example, short LTL has been associated with elevated body mass index, tobacco use,[7] and alcohol use.[8] Short LTL has also been associated with increased risk for diabetes,[9] hypertension,[10] and cardiovascular diseases.[11] Long LTL, in contrast, has been correlated with exercise[12–15] and healthy diets.[15,16] It has been hypothesized that short telomeres in humans reflect poor health and predict early death. However, most of these epidemiologic studies have been limited in scope and do not capture the full complexity of relationships between human diseases and LTL. In fact, only recently has cause and effect between LTL-associated diseases been systematically addressed through Mendelian randomization approaches.[17–22]

Genetics also plays a role in LTL. Rare deleterious variants in genes that maintain telomere function are known to cause telomeropathies such as dyskeratosis congenital (*TERC* [MIM: 127550]). Telomeropathies are Mendelian diseases that are characterized by exceptionally short TL

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with comorbidities including pulmonary fibrosis and bone marrow failure (*TERT* [MIM: 614742]).[23,24] Common genetic variants in these same genes have also been associated with normal variation in LTL. To date, 18 genome-wide association studies (GWASs) have identified over 100 SNPs associated with LTL that reach GWAS significance.[18,25] With the exception of one recently published study by Codd et al., these GWASs have not been able to parse the complex relationships between LTL, genetics, and human disease in a single study.

To better understand the relationships between LTL, genetics, and disease, we conducted both a GWAS and phenome-wide association study (PheWAS) on LTL with 62,271 individuals linked to genetic and electronic health record (EHR) data from two independent biobanks. This approach allowed us to evaluate interactions between LTL, genetics, and disease simultaneously. We find that LTL associates with a large spectrum of diseases and an earlier age of death. In addition, these associations are largely independent of common variants associated with LTL.

Materials and methods

Study populations

All experiments and analyses were conducted in two independent populations: Marshfield Clinic's personalized medicine research project (PMRP)[26] and the biobank of Vanderbilt University Medical Center (BioVU).[27,28] Both biobank cohorts link DNA to EHR data and have been described previously. In short, the participants retained from PMRP consisted of 16,596 consenting adults living in a 19-zip code region surrounding Marshfield, WI. The population was 57.2% female, and with a mean and median age at blood draw of 49.5 and 48.0, respectively. A large percent of PMRP participants receive most of their healthcare within Marshfield Clinic Health System. On average, each participant had 33.4 years of EHR data available for analysis. In comparison, BioVU consists of over 200,000 patients of any age. To make BioVU comparable with PMRP, we limited our analysis to 45,645 white non-Hispanic participants with European ancestry. All BioVU samples were collected after age 19. The mean and median age at blood draw for this subpopulation of BioVU was 53.9 and 54.9, respectively, 57.9% were female, and all had pre-existing genome-wide SNP array data. Unlike PMRP participants who receive most of their care within Marshfield Clinic Health System, BioVU participants had on average 11.6 years of EHR data reflecting patients who may be transient in nature and/or receive more specialty care at Vanderbilt University Medical Center. To reduce the probability of somatic events impacting LTL, individuals with a history of hematologic malignancies were excluded (Table S1A).

For both biobanks, DNA was extracted from blood using the QIAGEN Gentra Autopure (Minneapolis, MN) extraction protocol conducted at each institution. DNA samples were subsequently stored at −70°C. All samples were genotyped using different but comparable Illumina whole-genome SNP arrays. For PMRP, three batches were genotyped with one of two beadchips including HumanCoreExome_Goncalo_15038949 and Human660w-quad_v1_h-b37.Ilmn chip. BioVU samples were all genotyped on the Illumina Infinium Expanded Multi-Ethnic Genotyping Array plus custom content on a rolling base. All genotyping batches were controlled for during follow-up analysis. Genomes were imputed as outlined in Stanaway et al.[29] and aligned to the GRCh37/hg19_feb2009 reference genome. Genetic variants with minor allele frequencies of less than 1% and those with imputation R-squared quality metrics less than 0.3 were removed. Only variants that were observed in both cohorts were considered, leaving 7,407,505 SNPs for GWAS analysis.

This study was approved by institutional review boards at Marshfield Clinic and Vanderbilt University.

Relative average LTL measurements and adjustments

Residual DNA samples from whole-genome SNP array experiments described above were used to measure "relative average" LTL. All LTL experiments were conducted at Marshfield Clinic Research Institute using a modified qPCR technique as described in Blauwkamp et al.[30] This technique expresses LTL as a ratio of telomere repeat number relative to a single copy gene (i.e., RNAseP). All LTL assays were run in quadruplicate and standardized by quantifying against a standard curve present on each assay plate. For all analyses, LTL was adjusted for age, sex, genotyping batch, and three top principal components based on genomic data. Residuals from this adjustment were then *Z* scored to normalize data between the two cohorts. These *Z* scored adjusted LTL measurements were used for all analyses unless otherwise described. Quality controls and LTL calculation equations are defined in Table S1B.

Statistical methods

Because of data access restrictions, all analyses were run independently at each institution. All results were combined via meta-analyses using a fixed effects model with inverse variance weighting given there were only two cohorts evaluated. METAL[31] was used for GWAS and metasens R package (R package version 1.0-1) was used for all other meta-analyses.

GWAS

GWASs were run using linear regression (PMRP-RVTest[32] and BioVU-PLINK2[33]). SNP genotypes were associated with *Z* score adjusted LTL. Age, sex, three genetic principal components, and genotyping batches were used as covariates. Secondary genomic analyses included fine mapping studies and a phenome-adjusted GWAS. For fine mapping, the top SNP at each loci was included as a covariate in the original model to identify additional SNPs that were independently associated with LTL. Phenome adjusted GWAS was conducted by pre-adjusting the LTL (phenome-adjusted LTL) prior to *Z* score adjustment with the 67 sentinel phenotypes identified in the PheWAS. This was done using a forward-sequential adjustment method using a linear model approach. After each adjustment, the residuals of LTL were *Z* scored and carried forward in the subsequent phecode LTL adjustment. Residuals from the final adjustment were *Z* scored and used as the dependent variable in the GWAS as described above.

PheWAS

PheWASs were run using a logistic regression with the PheWAS R package.[34] *Z* scored adjusted LTL was associated with phecodes. Sex and age at last visit were used as covariates. Disease status was defined by International Classification of Disease codes mapped to phecodes (version 1.2).[35] Individuals coded two or more times at distinct dates were considered a case; those without any phecode were considered controls. Individuals with a single phecode were excluded. Only phenotypes for which at least 50 cases were available were considered for PheWAS. To limit likely redundant phenotypes that fall in the same code class, we manually curated the top

associations (Table S8), which we called sentinel phenotypes. This reduced the number of associations to 67 from 115. These sentinel phenotypes were used to create the phenome-adjusted LTL measurement described in the GWAS method section.

Secondary analyses included genome-adjusted PheWAS, an LTL dependency network, and association testing between phecodes and a weighted polygenic risk score (GRS). Genome-adjusted PheWAS was conducted by pre-adjusting LTL with all genome-wide significant variants (13 variants, Table 1) and used as an independent variable in the PheWAS as described above. In the LTL dependency network, sentinel phecodes were added as independent covariates in the logistic model. Co-dependent phecodes were defined when dependent phecode was no longer associated ($p < 0.05$) with LTL after adjusting for independent phecode. GRS analyses was limited to 13 independent GWAS SNPs and associate with sentinel phenotypes.

LTL association with death

There were 3,099 and 2,870 participants who had died following enrollment in PMRP and BioVU, respectively. To first evaluate if age of death was associated with LTL, we correlated *Z* scored adjusted LTL with age of death using linear regression via the LM function in R base. Because it has been shown that cellular senescence occurs only when telomeres reach a threshold of short TL, and that extremely short TL associates with telomeropathies that can lead to early death,[23,24] we categorized LTL into five bins (very short < -1.5 SD, short $= -1.5$ to -0.5 SD, average $= -0.5$ to 0.5, long $= 0.5$ to 1.5, and very long >1.5). Age of death for participants in each bin was associated across bins using an unpaired two-tailed t test. To determine if phenotypes identified by PheWAS contributed to early death, this binning process was repeated using the phenome-adjusted LTL.

Variance in LTL

To estimate the variance of LTL explained by age and sex, we used the coefficient of determination (r^2) derived from a linear model of LTL ~ age and LTL ~ sex. Variance explained by the genome was measured with the GCTA program (version 1.92.4[36]) using the restricted maximum likelihood option. GCTA evaluated all genetic variants used in GWAS or independent GWAS significant SNPs. To estimate the LTL variance explained by the phenome, omic-data-based complex trait analysis (OSCA) program (version 1.92.4[37]) was used by applying the restricted maximum likelihood option. OSCA was conducted on all phenotypes evaluated during PheWAS or limited to sentinel phenotypes. Final variance explained by the genome and phenome were extrapolated by considering the contribution of age and sex already adjusted for in the LTL measurements during GCTA and OSCA analyses.

Results

LTL in PMRP and BioVU

We measured LTL on 62,271 participants: 16,596 from PMRP and 45,675 from BioVU. As a biological control, raw unadjusted LTL values were inversely correlated with age at blood draw and were nearly identical in both populations (Pearson's coefficient, PMRP $r = -0.294$, BioVU $r = -0.292$, meta-analysis $r = 0.294$, p (probability value) $< 2E{-}16$, and Table S2. As reported previously,[38] females had longer age-adjusted LTL measurements than males (Pearson's coefficient in meta-analysis, $r = 0.0910$, $p < 2E{-}16$).

GWAS of LTL identifies two novel loci

We conducted GWAS analysis on both cohorts and combined via meta-analysis. There were 11 loci associated with LTL that passed genome-wide significance ($p < 5E{-}8$) (Figure 1A, QQ plots Figure S1). Of these 11 loci, 9 have previously been identified and 8 have been linked to genes well defined in telomere biology (variants in or near *TERT* [MIM: 187270], *TERC* [MIM: 602322], *STN1/OBFC1* [MIM: 613128], *RTEL1* [MIM: 608833], *MPHOSPH6* [MIM: 605500], *NAF1* [MIM: 617868], *POT1* [MIM: 606478], *CTC1* [MIM: 613129]) (Table 1). To determine if there were any additional SNPs independently associated with LTL beyond the top SNPs for each locus, we performed fine mapping analyses. Two additional variants at the *RTEL* locus were identified (Table 1, Figure S2). In total, there were 13 SNPs (referred to as sentinel SNPs) independently contributing to LTL variance from the 11 loci that reached GWAS significance. When considering a suggestive p value threshold of $p < 1E{-}5$, there were 45 loci associated with LTL including 13 novel signals (Tables S4 and S5). Table S4 further describes our results in the context of previously reported GWAS findings.

There were two novel SNPs associated with LTL that reached GWAS significance (rs144939807, *PITPNM1* [MIM: 608794] and rs13306651, *SCNN1D* [MIM: 601328]). Both novel SNPs were associated in BioVU and not in PMRP ($p > 0.05$), although the direction of effect were consistent in both populations. The SNP's low allele frequency and sample size differences for each cohort may have impacted power. Interestingly, both SNPs were genotyped directly (not imputed) and were not in linkage disequilibrium with other SNPs potentially explaining why other GWASs may not have identified these loci previously. After manual inspection, both SNPs were confirmed to have good genotype quality data and were in Hardy-Weinberg equilibrium.

Genetic variants associated with LTL are not influenced by LTL-related phenotypes

There were 67 clinically significant sentinel phenotypes associated with LTL as defined in the subsequent PheWAS and described in the following paragraph. Because we had both genomic and phenomic data in the same population, we could evaluate if any of the diseases influenced GWAS results. When comparing the original GWAS on LTL to the phenome-adjusted LTL, all original loci remained significant, no new loci reached genome-wide significance (Figure S3), and no significant differences in SNP effect sizes were observed (Figure S4 and Table S6). This suggested that clinical phenotypes associated with LTL in our populations did not influence GWAS findings.

PheWAS of LTL identifies 67 phenotypes linked to LTL

We conducted a PheWAS consisting of 1,279 clinical phenotypes on 62,271 individuals. Overall, the PheWAS detected 115 phenotypes that reached phenome-wide significance ($p < 3.9E{-}5$ assuming alpha < 0.05 and 1,279

Table 1. GWAS summary statistics and discovery

SNP_RSid	SNP_function	Gene (closest)	Chr:position	Telomere biology	Test	Other	New locus	Effect size[a]	SE	p value	TAF
rs35446936	intronic	ACTRT3; TERC	3: 169486508	telomerase	A	G	no	−0.0958	0.0068	1.28E−44	0.2433
rs7705526	intronic	TERT	5: 1285974	telomerase	A	C	no	0.0767	0.0065	7.92E−32	0.3324
rs1265164	intronic	STN1/OBFC1	10: 105674854	CST complex	A	G	no	0.0849	0.0085	2.17E−23	0.1416
rs71325458	intergenic	MHENCR; STMN3; RTEL1	20: 62268333	helicase	A	G	no	0.1677	0.0203	1.31E−16	0.9778
rs2086240	intergenic	NAF1; NPY1R	4: 164098317	dyskerin complex	T	G	no	−0.0485	0.0071	7.20E−12	0.231
rs3787089 (CA)	ncRNA_intronic	RTEL1-TNFRSF6B	20: 62316630	helicase	T	C	no	−0.0435	0.0065	1.98E−11	0.6839
rs61753459 (CA)	exonic-synonymous	RTEL1	20: 62321189	helicase	T	C	no	0.0678	0.0107	2.86E−10	0.0849
rs144939807[b]	exonic_nonsynonymous	PITPNM1	11: 67262411	unknown	T	C	yes	0.1491	0.0243	8.50E−10	0.0151
rs7248898	intronic	ZNF257	19: 22245354	undefined TF[c]	A	G	no	0.0394	0.0066	2.24E−09	0.2818
rs6503089	intergenic	VAMP2; TMEM107; CTC1	17: 8073296	CST complex	T	C	no	−0.0361	0.0063	1.03E−08	0.3423
rs113394869	intronic	POT1	7: 124518172	shelterin	C	G	no	−0.0371	0.0065	1.13E−08	0.2831
rs2967355	intronic	MPHOSPH6	16: 82200103	nucleotide metabolism	A	C	no	0.0386	0.007	4.02E−08	0.2283
rs13306651[b]	Exonic_nonsynonymous	SCNN1D	1: 1226063	unknown	A	G	yes	0.128	0.0235	4.99E−08	0.0168

[a]Effect size in same direction same in both cohort.
[b]SNP genotyped in BioVU cohort.
[c]TF, Transcription factor proposed to regulated genes involved in telomere biology.

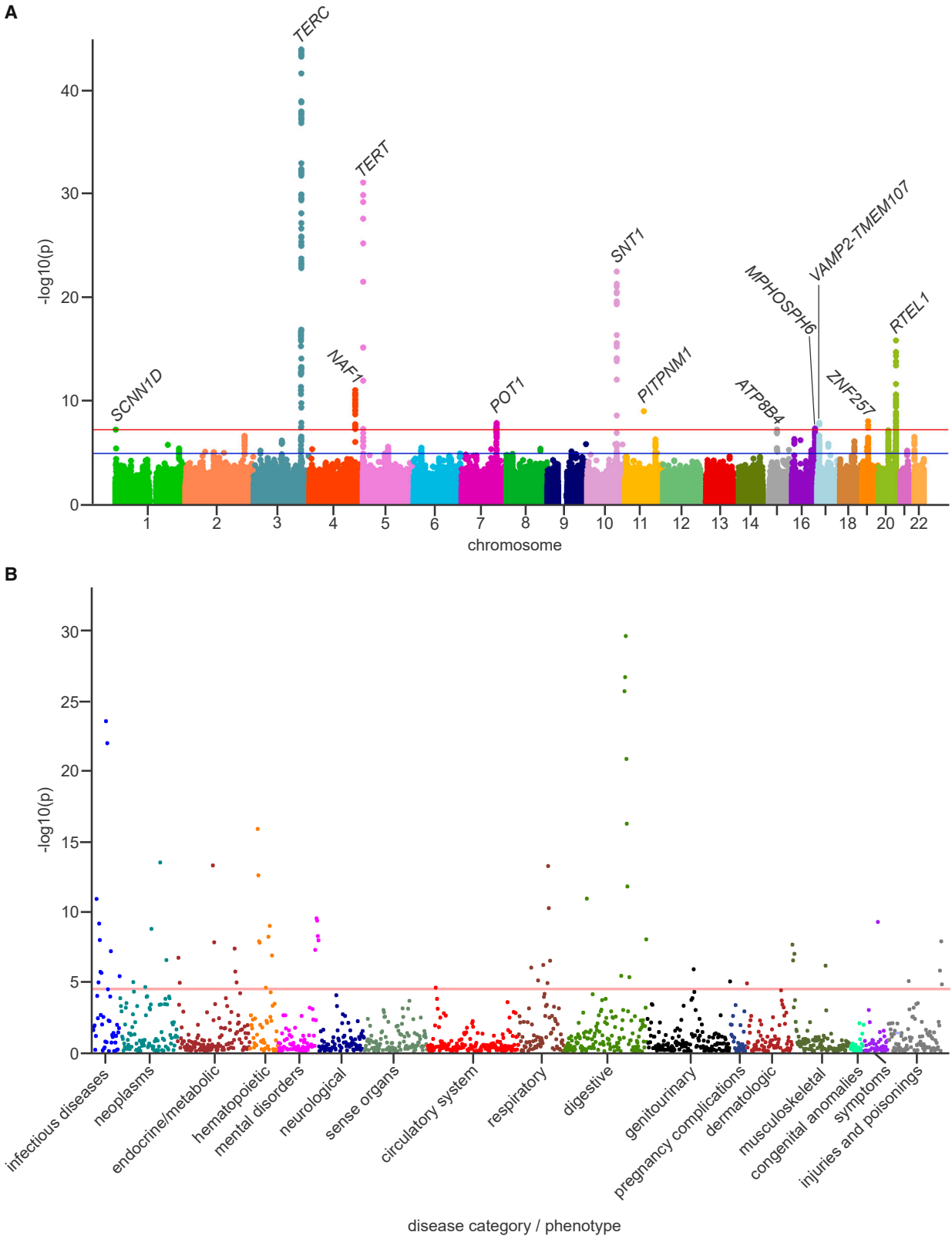

Figure 1. Visual summary of GWAS and PheWAS results
Manhattan plot of (A) GWAS and (B) PheWAS results. Red lines show genome-wide (p = 1E−08) and phenome-wide (p = 3.9E−05) significance. Blue line designates suggestive significance (p = 1E−06). We refer to p as the probability (p) value.

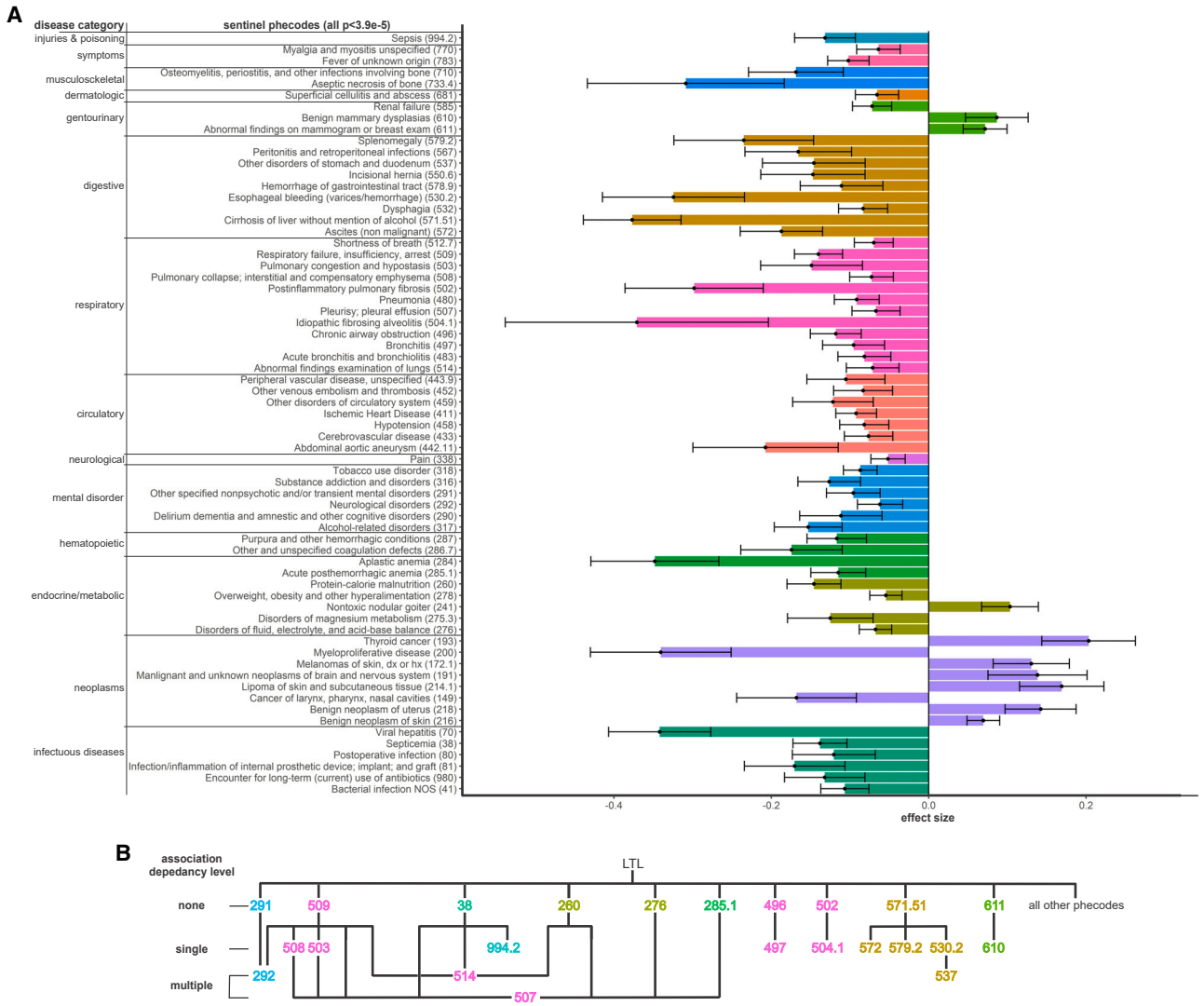

Figure 2. Analysis of sentinel phenotypes

(A) Forest plot showing effect size and 95% confidence intervals of the 67 identified sentinel phenotypes.

(B) Dependency network among sentinel phenotypes. First row phenotypes are associated with LTL independently from other phenotypes. Second and third row phenotypes are associated with LTL dependent on above phenotypes.

association tests) (Figure 1B, Table S7). This high association rate is consistent with large number of diseases associated with LTL reported to date. Like a GWAS where many SNPs in a locus are associated with LTL due to linkage disequilibrium, correlations in diseases also existed due to the hierarchical structure of the phenotypes. To simplify this, we manually identified within each group of phecodes those with the strongest association by p value. As an example, multiple phecodes defining "chronic liver disease and cirrhosis" (phecode 571) were all significant, while "cirrhosis of liver without mention of alcohol" (phecode 571.51) was the most significant and defined as the sentinel phecode for this grouping. This simplification process resulted in 67 sentinel phenotypes across diverse disease groups (Table 2) and the selection structure is shown in (Table S8). Of the 67 sentinel phenotypes, 58 associated with short LTL while 8 phenotypes associated with long LTL (Figure 2A). Interestingly, the latter 8 phenotypes were all neoplastic in nature.

It was expected that additional correlations in the phenotypes existed beyond the hierarchical structure of the phenome due to disease comorbidities. To identify diseases dependently associated with LTL, we developed an LTL dependency network by conducting a conditional analysis where each sentinel phenotype was individually used as a covariate in the logistic model. Like SNPs in LD as observed in LocusZoom plots, pairs of diseases that explain similar variance in LTL will result in loss of association due to confounding effects. Of the 67 sentinel phenotypes evaluated, 12 were dependent on 9 phenotypes for their association with LTL (Figures 2B and S5, Table S9). For example, the association between LTL and "other disorders of the stomach and duodenum" was dependent on "esophageal bleeding," whereas the association between

Table 2. Counts of TL associated phecodes per disease group

Disease group	Counts
Circulatory system	7
Dermatologic	1
Digestive	9
Endocrine/metabolic	5
Genitourinary	3
Hematopoietic	4
Infectious diseases	6
Injuries and poisonings	1
Mental disorders	6
Musculoskeletal	2
Neoplasms	8
Neurological	1
Respiratory	12
Symptoms	2
Grand total	67

esophageal bleeding and LTL was not dependent on "cirrhosis of the liver without mention of alcohol." In addition to the nine phenotypes that had large confounding effects, there were multiple others that partially influenced the association between LTL and sentinel phenotype (Figure S5, Table S9).

Codd et al. also performed a similar PheWAS as ours although limited to 123 disease phenotypes. We manually compared disease descriptions. Of these 123 diseases evaluated by Codd et al., 93 were tested in our PheWAS. Of the 93 diseases, 33 were significant as defined by Codd et al., whereas 16 were significant in our PheWAS. This included 11 that were significant in both (Table S7.1).

Phenotypes associated with LTL are not influenced by LTL-related genetics

Similar to our previous phenome-adjusted GWAS that accounted for sentinel phenotypes, we evaluated if the sentinel SNPs identified in our GWAS of LTL impacted PheWAS. When including the 13 sentinel SNPs as covariates, all 67 sentinel phenotypes remained significant (Table S10) with no meaningful change in effect size (Figure S6). Likewise, sentinel phenotypes were not significantly associated with LTL GRS ($p > 0.030$) (Table S11). These results taken together with phenome-adjusted GWAS results, suggest that LTL-associated phenotypes and SNPs are largely independent of each other.

Short and long LTL correlates with age of early death

In our populations, 5,969 individuals (3,099 for PMRP and 2,870 for BioVU) are deceased since enrollment. We first associated LTL and age of death using a linear model. The Pearson's coefficient was $r = 0.172$ ($p = 1.62E{-}9$). One form of cellular senescence or cell death occurs only when telomeres reach a shortened threshold.[39–41] This is exemplified in individuals affected by telomeropathies, where TL are extremely short, and often cause diseases that trigger early death.[17] Therefore, we hypothesized that a linear model may not be an appropriate analysis method. We categorized LTL into five bins by standard deviation: very short (<−1.5 SD), short (−1.5 to −0.5 SD), average (−0.5 to 0.5 SD), long (0.5 to 1.5 SD), and very long (>1.5 SD). Mean age of death in an average bin is 74.2 years of age. Very short, short, and very long categories were significantly associated with early death by 10.4 ($p < 0.0001$), 2.8 ($p < 0.0001$), and 1.9 ($p = 0.0175$) years, respectively, when compared with average LTL (Figure 3, Table S12). This is consistent with our PheWAS results showing that clinical phenotypes are associated with both short and long LTL.

We next investigated whether early cause of death could be attributed to sentinel phenotypes identified in the PheWAS. When adjusting out the effects of the 67 sentinel phenotypes on LTL, age of death remained largely unchanged (Figure 3). This is not surprising given that most individuals did not change bins after adjusting for the sentinel phenotypes (data not shown). Therefore, age of death is likely driven by other factors independent of the sentinel phenotypes.

LTL variance explained

Because so many different variables were associated with LTL, and we could measure these effects in the same populations, we sought to quantify the amount of LTL variance that could be explained by the genome, phenome, age at blood draw (corresponding to age at LTL assay), and sex (Figure 4, Table S13). Not surprisingly, one of the largest contributors to LTL variance was age (8.5%, $p < 1E{-}300$). An even larger contributor to LTL variance was use of all SNPs with 12.8% ($p = 1.1E{-}60$), of which 0.5% was explained by GWAS significant SNPs. Even though there were many phenotypes associated with LTL, sentinel phenotypes did not contribute significantly to LTL variance (0.067%, $p = 0.36$), whereas the entire phenome accounted for 1.5% ($p = 1.07E{-}13$). Finally, sex accounted for 0.86% ($p < 1E{-}300$). Based on these analyses, 76.3% of LTL variance remained unexplained.

Discussion

The goal of this study was to conduct a comprehensive evaluation of the inter-relationships between LTL, genomics, and clinical phenotypes presented in medical records. We rediscovered multiple associations and characterized many novel findings that may lead to new biological insights in telomere biology.

The GWAS rediscovered 11 SNPS associated with LTL and 2 novel nonsynonymous variants in *PITPNM1* (rs144939807) and *SCNN1D* (rs13306651). Both genes

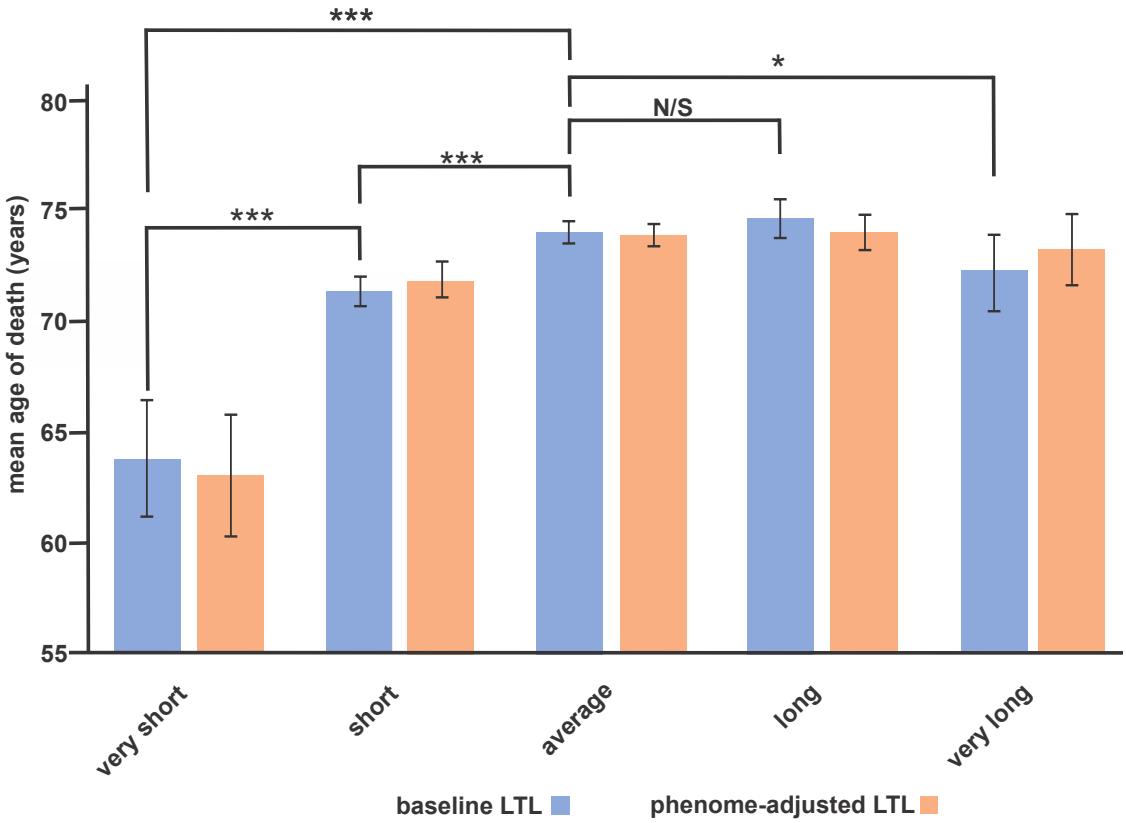

Figure 3. Age of death is associated with telomere length

Mean age of death with 95% confidence intervals for baseline age-adjusted LTL (blue) and phenome-adjusted age-adjusted LTL (orange). LTL values were binned according to SD (very short: SD < -1.5, short: $-1.5 <$ SD < -0.5, average: $-0.5 <$ SD < 0.5, long: $0.5 <$ SD < 1.5, very long: SD > 1.5). Significance between baseline bins is shown (N/S, not significant; * $p < 0.05$, *** $p < 0.0001$). We refer to p as the probability (p) value.

have unknown relationships with telomere biology but deserve additional follow-up. SNP rs13306651, a missense variant with a minor allele frequencies of 1.7% on chromosome 1, causes an Ala636Thr substitution (non-polar to polar amino acid substitution) in the cytoplasmic C-terminal tail of the transmembrane protein SCNN1D. SCNN1D is a subunit of the epithelial sodium channel that controls the transport of Na^+ ions into epithelial cells. Its primary physiological role is to conserve sodium and water homeostasis.[42] Hereditary disorders resulting from mutations in EnaC subunits includes multi-system pseudohypoaldosteronism, Liddle syndrome, and cystic fibrosis-like diseases.[42] SNP rs144939807 is a missense variants with a minor allele frequencies of 1.5% on chromosome 11 and causes an Arg883Gln substitution (positively charged to polar amino acid substitution) in a non-structured region of the phosphatidylinositol transfer protein membrane-associated 1 (PITPNM1) protein. PITPNM1 is a cytoplasmic protein that functions in the transfer of phosphatidylinositol between the endoplasmic reticulum with both the Golgi apparatus and plasma membrane.[43] No hereditary disorders resulting from mutations in *PITPNM1* have been reported but may have a role in retinal degeneration based on animal studies.[62]

Via PheWAS, 67 sentinel phenotypes were associated with LTL that reached a conservative experiment-wise Bonferroni threshold ($p < 3.91E{-}5$). Short LTL was associated with increased disease risk for most conditions; neoplastic disease was the exception (Figure 2A). Although many epidemiologic studies have linked long LTL to increase risk for malignant disease,[44] we show similar trends for benign neoplasms suggesting shared biological mechanisms in tumorigenesis. A hypothesis that may explain this observation includes the simple assumption that transformed cells with shorter TL have less replicative potential than those with longer TL. Transformed cells with replicative potential are capable of forming cell masses and thus accumulating somatic mutations that may circumvent TL-dependent bottlenecks. Of potential relevance, gain of function promoter mutations in *TERT* are commonly observed across different tumor types and considered early drivers in tumorigenesis.[44–46] Of course, this hypothesis is likely an oversimplification given that those with rare loss of function germline variants in components of telomerase (e.g., *TERT*) are more prone to genomic instability and increased risk for hematologic cancers.[47] These observations are important to consider given continued development of therapeutics that aim to

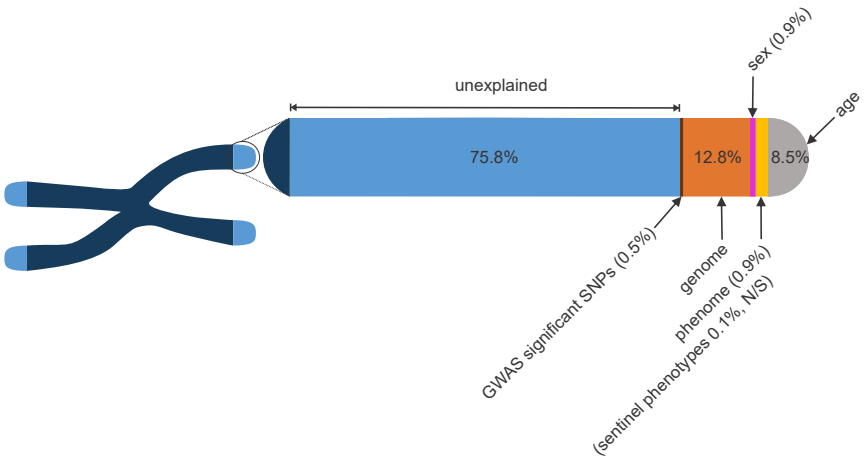

Figure 4. LTL variance explained by age, sex, phenome, and genome
Shown is the variance explained by GWAS and PheWAS results.

shorten TL for cancer treatment[48] or increase TL for longevity.[49,50]

Even though there were 67 sentinel phenotypes associated with LTL, some associations were dependent on each other (Figure 2B). An intuitive example included the association between "benign mammary dysplasia" and LTL, along with its dependency on "abnormal findings on mammograms or breast exams." Less intuitive was an observation of dependency between "pleurisy" (inflammation of the pleural cavity), "protein-calorie malnutrition," "septicemia," and LTL. These may represent critically ill individuals with compromised immune systems. In total, 55 phenotypes were distinctively associated with LTL while 12 were dependent on other phenotypes. These types of analyses can help interpret PheWAS results when defining possible comorbidities that are dependent on other traits. A challenge of all association-based studies is determining causation. Although we can infer that "benign mammary dysplasia" likely causes an "abnormal finding on mammogram," we cannot determine with confidence if LTL causes disease, disease causes variation in LTL, or if there is an intermediary factor that influences both. This is important given the multitude of epidemiological studies that have conclusively shown that variation in LTL is associated with many factors. Although causation is difficult to determine, we had an opportunity to quantify how much variation in LTL can be attributed to multiple variables, including gender, age, genetics, and diseases.

The genome explained the largest proportion of variance in LTL compared with other measured variables (12.8%). After considering the genome, phenome, age, and sex, 23.7% of the LTL variance was explained representing the most comprehensive assessment to date (Figure 4). This analysis assumed all variables were independent. Given that our GWAS and PheWAS results were not largely influenced by each other, the phenome only contributed a small proportion to LTL variance (1.5%), and very few LTL GWAS hits mapped to previously reported disease-associated variants,[25] our assumptions of independence was likely accurate. Even though 23.7% of LTL variance could be quantified, 76.3% remained unexplained. We speculate that a percent of unexplained LTL variance may be attributed to environmental exposures and lifestyle factors not well captured in our study. For example, tobacco use, a phenotype identified in the PheWAS ($p = 1.71E{-}15$), is well known to be associated with LTL.[51–53] However, the amount and duration of tobacco exposure are known to differentially effect LTL.[53] Quantifiable tobacco exposure data is not readily available from an EHR and may result in an underestimate of its effect for our analyses. This may apply to other environmental exposures and phenotypes where severity and duration affects LTL differentially. Improved modeling by incorporating quantifiable exposures and severity data may explain more LTL variance.

Another variable that likely had an unappreciated and potentially large influence on LTL is experimental error. Even though we applied a well-vetted high-throughput qPCR assay and ran all experiments in quadruplicate in a single laboratory with internal controls, high-throughput LTL assays are influenced by multiple factors difficult to control.[54–58] There are more precise methods for measuring TL but have significantly less throughput capacity.[59] Caution should be made when comparing effect sizes across different populations using different methods. Conversely, it was encouraging that LTL in PMRP and BioVU were both correlated with age by nearly identical amounts (8.63% and 8.54%, respectively) thus providing a good biological control when combining cohorts.

It has been suggested that LTL associates with disease burden and early death but this has been difficult to demonstrate in humans. Recently, this has been addressed directly where the investigators found that short LTL at the age of 40 years is, on average, associated with a decrease in life expectancy of ~2.5 years.[18] We find that this relationship is not linear (Figure 3). Instead, we find that early death was associated with both short and long telomeres. We hypothesize that increased risk for neoplasms associated with long LTL could contribute to this observation. Conversely, accounting for sentinel phenotypes associated with long and short LTL did not impact our findings. There may be additional diseases associated with LTL that contribute to age of death but did not reach our stringent PheWAS significance level. Furthermore, under-reported environmental exposures and lifestyles that impact health as mentioned previously (e.g., smoking) may also influence results.

This study was not without its limitations. Similar to previous epidemiologic studies of TL, our study was limited by

TL data derived solely from leukocyte DNA. Although LTL has been shown to be an adequate proxy for TL across most tissue types,[6] TL tissue-specific regulation exists. For example, cell types that regulate telomerase activity differently from leukocytes (e.g., germ cells[6,60]) may not be representative in out GWAS and PheWAS results. Also similar to many other epidemiological studies of TL, our study was limited by its cross-sectional nature. LTL attrition is established to be dynamic over time, as are health trajectories. We limited our study scope to lifetime disease risk even though past, current, and future factors that occur from the perspective of time of blood draw may impact findings and interpretations. Large population cohorts that capture bio-specimens, EHR data, lifestyle, diet, and environmental exposure over time, and including different ethnicities and cultures, such as the *All of Us* Research Program,[61] may further help parse the complex relationships between TL, lifestyle, genetics, and human health. In addition, developing a cohort that seeks to capture individual LTL longitudinal data will serve to confirm sectional studies and provide personalized variability that could be critical in evaluating associations over time. However, these will be logistically difficult to execute. LTL attrition rates are slow (~25–50 bp year[63]) and to measure LTL attrition rates at the individual level using high-throughput techniques may require follow-up measurements over decades.

In conclusion, we provide a catalog of phenotypes associated with LTL and find that these associations appear largely independent of LTL genetics. We also provide evidence that early death, as a phenotype, correlates with short LTL, and to a lesser extent long LTL, suggesting a more nuanced relationship. These observations provide the rationale for expanded research to understand the multifaceted correlations between TL biology and human health over time, leading to the potential for clinical LTL applications.

Data and code availability

The telomere length and genetic datasets generated during this study will be available at dbGaP (study ID: 51209). The PheWAS of telomere length dataset is deposited in the PheWAS catalog (https://phewascatalog.org/).

Supplemental information

Supplemental information can be found online at https://doi.org/10.1016/j.xhgg.2023.100201.

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Declaration of interests

The authors declare no competing interests.

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Web resources

Phecode definition (version 1.2), https://phewascatalog.org/phecodes

metasens R package version 1.0-1, https://CRAN.R-project.org/package=metasens

Human disease database, MalaCards, https://www.malacards.org/

Online Mendelian Inheritance in Man (OMIM), http://www.omim.org

PheWAS catalog, $https://phewascatalog.org/telomere

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