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Genetically inferred telomere length and testicular germ cell tumor risk

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Abstract

Background: Studies evaluating the association between peripheral blood leukocyte telomere length and testicular germ cell tumor (TGCT) risk have produced conflicting results.

Methods: Using available genotype data from the Testicular Cancer Consortium (TECAC), polygenic risk score (PRS) and mendelian randomization (MR) analyses of genetic variants previously associated with leukocyte telomere length were used to assess potential etiologic associations between telomere length and TGCT risk.

Results: Genetically inferred telomere length was not associated with TGCT risk among 2,049 cases and 6,921 controls with individual-level genotype data (odds ratio (OR)=1.02, 95% confidence interval (CI)= 0.97–1.07). MR analyses using summary statistic data further indicated no evidence for an association between telomere length and TGCT risk among all available TECAC consortium participants (3,558 cases; 13,971 controls).

Conclusions: Our analyses in the largest molecular genetic testicular cancer study to date provide no evidence for an association between genetically inferred peripheral blood leukocyte telomere length and TGCT risk.

Impact: The lack of evidence for an overall association indicates that peripheral blood leukocyte telomere length is likely not a strong biomarker for TGCT risk.

Keywords

Testicular germ cell tumor; telomere length; genetic variants; Mendelian randomization; TECAC

Introduction

Telomeres are AGGGTT nucleotide repeats that protect chromosomes from degradation (1). Excessively long telomere length (TL), with upregulated telomerase activity, may result in immortalized cells with unlimited potential for growth and proliferation, promoting tumorigenesis (2). Several recent studies have identified peripheral leukocyte TL (LTL) as a biomarker correlated with tissue-specific telomere length and associated with solid tumor risk (1,3–5).

Recent genome-wide association studies (GWAS) have identified genetic variants associated with peripheral LTL that have utility as a surrogate genetic measure in deciphering associations with cancer risk (1,4–6). As the association between LTL and testicular germ cell tumor (TGCT) risk is poorly understood (1,6), we used a polygenic risk score (PRS) of

telomere length-associated variants and Mendelian randomization (MR) approaches to examine if genetically-inferred LTL is associated with TGCT risk.

Material and Methods

The Testicular Cancer Consortium (TECAC) was utilized to investigate the relationship between inferred leukocyte telomere length and TGCT risk (7). In total, TECAC includes imputed individual-level data from the NCI, UPENN, and UK cohorts (2,049 cases; 6,921 controls), as well as additional GWAS meta-analysis summary statistics from all 17,529 consortium participants (3,558 cases; 13,971 controls). Data used in this analysis are from individuals with European ancestry and are available through direct application to TECAC (www.tecac.org) and in dbGAP phs001349.v1.p1.

LTL was genetically-inferred for participants with available genotype data (NCI, UPENN, and UK cohorts), using a PRS (1,5) containing 22 germline variants associated with LTL (Supplementary Table 1) (8):

$$PRS_i = \sum_{j=1}^{22} w_j x_{ij}$$

where PRS_i is the polygenic risk score for individual i, w_j is the estimated LTL-associated variant weight in base pairs per LTL length-increasing allele, and x_{ij} is the number of LTL length-increasing alleles for the i^{th} individual and the j^{th} LTL variant. The PRS was standardized to have mean 0 and standard deviation of 1 and association tests were conducted separately for the NCI, UPENN, and UK cohorts using logistic regression. Results were combined using fixed effects meta-analysis.

Using available GWAS meta-analysis summary statistics from TECAC (7), we extracted associations between LTL-associated variants and TGCT risk. Summary statistics-based MR analyses were conducted merging LTL-associated variants into a genetic instrument across all available TECAC participants (1,5,6). As standard errors were not available for the original LTL-associated variants (8), other published variants with detailed summary statistics were used (6). MR-Egger regression was utilized to evaluate heterogeneity and potential pleiotropy of included LTL variants (1,6).

All statistical analyses were performed in R version 3.6.3 with two-sided significance levels ($P< 0.05$).

Results

Of 17 available LTL-associated variants, four were found to be nominally associated with TGCT risk ($P<0.05$) with only one (rs28616016) demonstrating a positive association (Table 1, Supplemental Table 2, Supplemental Figure 1). There was no overall association between LTL-associated variants and TGCT risk (P=0.72, Figure 1) and no association between the LTL PRS and TGCT risk (odds ratio (OR)=1.02, 95% confidence interval (CI)=0.97–1.07, P=0.45, P_{het}=0.34; Supplemental Figure 2). Stratified analyses by TGCT histologic group

(seminoma=824 cases, non-seminoma germ cell=1,046 cases) also suggested no association between the LTL PRS and TGCT risk (seminoma: OR=1.04, 95% CI=0.96–1.12, P=0.33; non-seminoma germ cell: OR=1.01, 95% CI=0.95–1.08, P=0.71; Supplemental Figure 3). Likewise, MR analyses indicated no directionality between the LTL-associated variants and TGCT risk (maximum-likelihood method OR=0.63, 95% CI=0.22–1.80, P=0.39; Supplemental Tables 3–4, and Supplemental Figure 4). The MR-Egger regression intercept was not statistically significant (P=0.56), indicating no pleiotropic effects (Supplemental Table 4).

As two included LTL-associated variants (rs7705526 *TERT* and rs28616016 *RFWD3*) are in linkage disequilibrium with previously-identified TGCT GWAS variants (rs2736100 *TERT*, rs4888262 *RFWD3*; R^2_{EUR}= 0.51, 0.69; respectively) (7), we conducted sensitivity analyses removing these variants to investigate any potential influence of these variants on the overall LTL PRS association with TGCT risk (Supplemental Figures 5–6). These sensitivity analyses with TGCT published variants removed indicated suggestive evidence for an association between the remaining LTL-associated variants and TGCT risk (*TERT* removed P=0.12; *TERT* and *RFWD3* removed P=0.03; Supplemental Figures 5a and 6a) and between the variant removed LTL PRS and TGCT risk (*TERT* removed P=0.005, P_{het}=0.42; *TERT* and *RFWD3* removed P=0.049, P_{het}=0.29; Supplemental Figures 5b and 6b).

Discussion

Our study of 3,558 TGCT cases and 13,971 controls provides little overall evidence for an association between LTL-associated variants and TGCT risk. This lack of association was consistently observed among individual telomere length-associated variants, as well as within PRS and MR analyses. The *TERT* and *RFWD3* variants, in linkage disequilibrium with previously-identified TGCT GWAS variants, were the only variants to demonstrate evidence suggesting an association with LTL (indirect and direct association, respectively). Sensitivity analyses removing the *TERT* and *RFWD3* variants suggested a marginal association between LTL and TGCT risk, indicating the relationship between LTL and TGCT may be complex.

Studies with comparable sample sizes to our investigation detected associations between inferred LTL and cancer risk (1,4). Our genetic approach does not contain the biases typically associated with studies of measured LTL (e.g., differences in LTL by DNA extraction approach) (5) and only utilized weights from a single large LTL GWAS (8) ensuring improved PRS weighting and more accurate downstream analyses. Some LTL variants were not included into our analysis due to unavailable data (rs547680822 and rs4027719) or low-quality imputation (rs188891454, rs144510686, and rs28372734). It is not likely these exclusions significantly affected our overall findings as these variants cumulatively explain ~0.2% of the variation in measured LTL (8). Current LTL variants identified by GWAS (N=75,000 individuals) explain a small percentage of the variance in measured telomere length (6,8), suggesting GWAS in larger samples may discover additional LTL variants that could better explain telomere length in testicular tissue or capture relevant aspects of telomere length more important for TGCT risk.

Our study finds no compelling evidence for an overall relationship between current LTL variants and TGCT risk suggesting LTL is likely not a strong biomarker of TGCT risk. However, some components of LTL, specifically when removing *TERT* and *RFWD3* variants, do demonstrate evidence for a relationship with TGCT risk, suggesting specific genetic elements of LTL may be relevant for TCGT risk.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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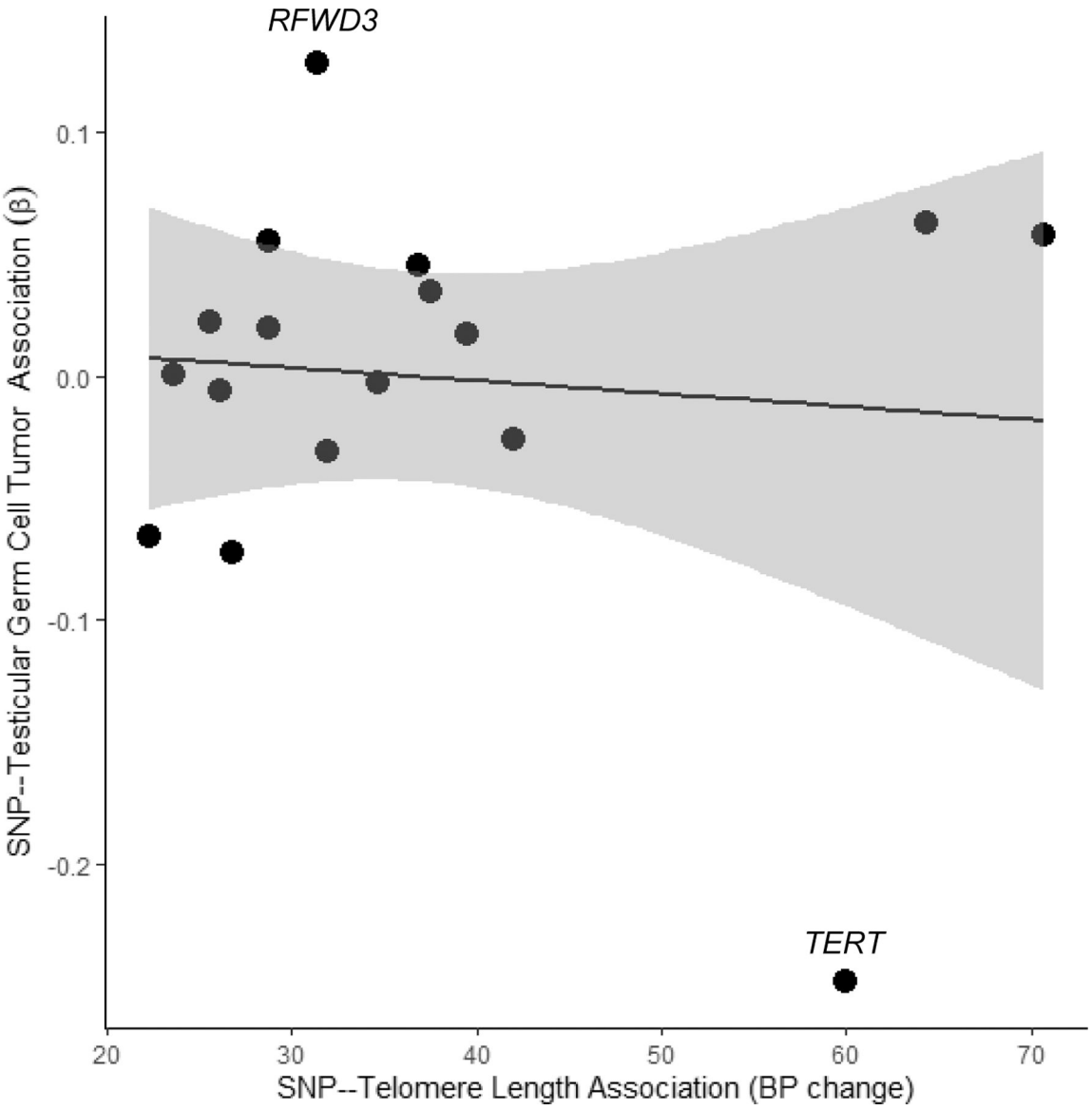

Figure 1.

The effect of each variant on genetically-predicted telomere length and testicular germ cell tumor risk. Estimates for the single nucleotide polymorphism (SNP)--telomere and SNP--testicular germ cell tumor associations are presented in Table 1. A trend line and 95% confidence interval are plotted using a linear model (P= 0.7150).

Table 1.

Associations of telomere length-associated variants from Taub et al. (2019) with testicular germ cell tumor risk

								Telomere Length Association[a]	Association with TGCT status[b]	
Nearby gene	**CHR**	**Position (hg37)**	**rsID**	**Ref**	**Alt**	**Controls**	**Cases**	**BP**	**β**	**p-value**
ACYP2	2	54495222	rs7579722	G	C	13,968	3,556	37.5	0.03	0.38792
RPN1	3	128422176	rs60092972	A	T	13,968	3,555	23.6	0.00	0.99844
TERC	3	169482335	rs2293607	C	T	13,968	3,555	70.7	0.06	0.08411
NAF1	4	164048199	rs4691895	G	C	13,970	3,558	39.5	0.02	0.62386
TERT	5	1285974	rs7705526	C	A	13,968	3,555	60.0	−0.25	9.85×10^{-14}
POT1	7	124494861	rs10246424	G	A	13,970	3,556	28.8	0.06	0.08427
TERF1	8	73950559	rs12679652	A	G	13,969	3,557	28.8	0.02	0.52630
SH3PXD2A	10	105679341	rs2488002	T	C	13,968	3,556	64.3	0.06	0.11589
DCAF4	14	73432100	rs78517833	A	T	13,970	3,558	36.8	0.04	0.35244
TCL1A	14	96180685	rs11846938	T	G	13,968	3,555	25.6	0.02	0.52844
TERF2	16	69391714	rs9925619	C	G	13,970	3,556	26.8	−0.07	0.02568
RFWD3	16	74676964	rs28616016	C	T	13,971	3,557	31.4	0.13	1.11×10^{-5}
ZNF676	19	22424997	rs281173	G	A	13,969	3,557	22.3	−0.07	0.02835
SAMHD1	20	35578680	rs4810362	G	A	13,968	3,558	34.7	0.00	0.94697
LINC01429	20	50453984	rs6091385	C	T	13,968	3,556	31.9	−0.03	0.48316
RTEL1	20	62336258	rs6062497	T	C	13,968	3,555	42.0	−0.03	0.40639
CHKB	22	51034870	rs131742	G	A	13,968	3,555	26.1	−0.01	0.84410

[a] Positive change in number of base pairs

[b] β estimate for each variant from GWAS meta-analysis of all five TECAC cohorts

Two variants were not identified in any of the study cohorts: rs547680822 (TOPMed AAF = 0.00), rs4027719 (TOPMed AAF= 0.43)

Three variants were not included due to low imputation score: rs188891454, rs144510686, rs28372734

TGCT= Testicular germ cell tumor

BP= Base pairs

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