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Long Leukocyte Telomere Length Is Associated with Increased Risks of Soft Tissue Sarcoma: A Mendelian Randomization Study

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Abstract: Background: Leukocyte telomere length (LTL) has been associated with the risks of several cancers in observational studies. Mendelian randomization (MR) studies, using genetic variants as instrumental variables, have also shown associations of genetically predicted LTL with cancer risks. In this study, we performed the first MR analysis on soft tissue sarcoma (STS) to investigate the causal relationship between LTL and the risk of STS. Methods: Genotypes from eleven LTL-associated single nucleotide polymorphisms (SNPs) in 821 STS cases and 851 cancer-free controls were aggregated into a weighted genetic risk score (GRS) to predict LTL. Multivariate logistic regression was used to assess the association of STS risk with individual SNPs and aggregated GRS. Results: Four SNPs displayed evidence for an individual association between long LTL-conferring allele and increased STS risk: rs7675998 (odds ratio (OR) = 1.21, 95% confidence interval (CI) = 1.02–1.43), rs9420907 (OR = 1.31, 95% CI = 1.08–1.59), rs8105767 (OR = 1.18, 95% CI = 1.02–1.37), and rs412658 (OR = 1.18, 95% CI = 1.02–1.36). Moreover, longer genetically predicted LTL, calculated as GRS, was strongly associated with an increased risk of STS (OR = 1.44, 95% CI = 1.18–1.75, $p < 0.001$), and there was a significant dose-response association (p for trend <0.001 in tertile and quartile analyses). The association of longer LTL with higher STS risk was more evident in women than in men. In stratified analyses by major STS subtypes, longer LTL was significantly associated with higher risks of leiomyosarcoma and gastrointestinal stromal tumors. Conclusions: Longer LTL is associated with increased risks of STS.

Keywords: soft tissue sarcoma; cancer risk; leukocyte telomere length; Mendelian randomization

1. Introduction

Soft tissue sarcomas (STS) constitute a heterogeneous combination of malignancy derived from mesenchymal tissue [1–3]. Overall, STS account for 1% of adult malignancies and 15% of pediatric malignancies [4]. STS can arise from any part of the body and are most commonly derived from extremities [1]. The overall 5-year survival rate is about 65%, and the 5-year survival rates for localized, regional, and distant STS were 81.2%, 57.4%, and 15.9%, respectively [5]. Most STS are sporadic, and their etiology and genetic susceptibility are not well understood. STS are an aging-related disease with a median age at diagnosis of 60 years and the median age of death being 66 years [6]. Radiation exposure is a strong risk factor for STS, and viral infection (e.g., HPV and HIV) predisposes to certain

subtypes of STS [2,3]. Other potential risk factors such as occupational exposures to herbicides and chlorophenols need more compelling evidence [2,3]. A few inherited genetic syndromes, such as neurofibromatosis type 1 (NF1), Li–Fraumeni syndrome (LFS), and retinoblastoma (Rb), have been associated with increased risks of STS [2,3]. No common genetic variant has been unequivocally linked to adult STS susceptibility due to the rarity and heterogeneity of this disease [7].

Telomeres are hexameric nucleotide repeats and protein complex capping both ends of eukaryotic chromosome arms [8,9]. Telomeres prevent the termini of linear chromosomes from fusion and degradation [10,11]. The shortening of telomeres is recognized as a 'molecular clock' that curbs organisms' age, which will finally cause chromosomal instability, cellular senescence, cell cycle arrest, and eventually apoptosis [11,12]. Telomerase is activated in 90% of tumors and contributes to tumor cells' immortal growth properties in the presence of shortened telomeres [13].

Telomere length is often measured in readily accessible leukocyte DNA, and leukocyte telomere length (LTL) is highly correlated with the telomere length in other tissues [14]. LTL is under strong genetic control, with an estimated heritability of up to 80% from classic twin studies [15,16]. Although LTL is generally inversely correlated with age, there is a considerable interindividual variation of LTL among people of the same ages [17,18]. In addition to genetic factors, LTL can also be shortened by environmental factors such as smoking and occupational exposure to harmful chemicals [19–21]. The interindividual variation of LTL has been shown to contribute to genetic susceptibility to cancer and other diseases [22–27]. Earlier retrospective case-control studies suggested that short LTL was a risk factor for some cancers, but later large prospective studies and recent Mendelian randomization studies using genetically predicted LTL have increasingly found that long LTL was a risk factor for a number of cancers, such as melanoma, B-cell lymphoma, lung adenocarcinoma, glioma, renal cell carcinoma, and osteosarcoma [24–37].

We previously reported that longer LTL, measured by the standard real-time quantitative PCR (qPCR) method, was associated with a higher risk of STS in a pilot case-control study of 137 pairs of STS cases and controls [38]. However, the assessment of disease association of an intermediate phenotypic biomarker such as LTL in a retrospective case-control study is subjected to several limitations, including reverse causation, environmental confounding, treatment confounding, variability in sample preparation, and variability in technical measurement. Mendelian randomization (MR) is an approach using common genetic variations as instruments to study the causal relations between risk factor/intermediate biomarkers and health outcomes in observational data, which is less affected by confounding, reverse causation, and technical variability [39]. There are three assumptions in MR studies: (1) the selected genetic variants are associated with the studied risk factor/biomarker; (2) the genetic variants are independent of other confounding factors that are associated with the selected risk factor/biomarker and disease; (3) the genetic variants only influence disease risk through their effects on the risk factor/biomarker. Eleven independent single nucleotide polymorphisms (SNPs) have been unequivocally identified to be associated with LTL by large scale genome-wide association studies (GWAS) [40–42]. Numerous MR studies have used these SNPs to assess genetically predicted LTL and risks of diseases, including cancers [27–37,39]. However, no MR study of LTL and STS risk has been reported to date. In this study, we used a large case-control study and applied an MR approach to test the hypothesis that genetically predicted longer LTL is associated with increased risks of STS. The large sample size also allowed us to perform stratified analyses of the major histological subtypes of STS.

2. Results

2.1. Characteristics of the Study Population

The distribution of selected characteristics of the 821 STS patients and 851 age- and gender-matched controls are shown in Table 1. All the participants were Caucasians. The average diagnosis age for STS patients was 56.39 and for controls was 57. There are slightly more females than males in both cases

and controls. The major histological subtypes were leiomyosarcoma (33.1%), gastrointestinal stromal tumors (GIST, 26.8%), liposarcoma (22.0%), and angiosarcoma (7.3%).

Table 1. Selected characteristics of the study population.

Characteristics	Cases n = 821	Controls n = 851	p Value
Age, years (mean, SD)	56.39 (11.58)	57.00 (8.62)	0.22
Gender, n (%)			
Male	388 (47.26)	406 (47.71)	
Female	433 (52.74)	445 (52.29)	0.85
Histology, n (%)			
Leiomyosarcoma	272 (33.1)		
GIST *	220 (26.8)		
Liposarcoma	181 (22.0)		
Angiosarcoma	60 (7.3)		
Other	88 (10.7)		

* GIST: gastrointestinal stromal tumors.

2.2. Association between Individual SNP and STS Risk

The association between each individual LTL-associated SNP and STS risk is shown in Table 2. Among the 11 SNPs, four were nominally significantly associated with STS risk: *NAF1* rs7675998 (OR = 1.21, 95% CI = 1.02–1.43, p = 0.026), *OBFC1* rs9420907 (OR = 1.31, 95% CI = 1.08–1.59, p = 0.007), *ZNF208* rs8105767 (OR = 1.18, 95% CI = 1.02–1.37, p = 0.030), and *ZNF676* rs412658 (OR = 1.18, 95% CI = 1.02–1.36, p = 0.025). In all these 4 SNPs, alleles related to longer LTL were associated with increased risks of STS.

Table 2. Individual leukocyte telomere length (LTL)-associated single nucleotide polymorphisms (SNPs) and soft tissue sarcoma (STS) risk.

SNP ID	Chr.	Position	Gene	Allele *	β *	EAF Case	EAF Control	OR ** (95% CI)	p Value
rs11125529	2	54475866	ACYP2	A/C	0.065	0.131	0.129	1.01 (0.82–1.24)	0.922
rs6772228	3	58376019	PXK	T/A	0.041	0.942	0.948	0.9 (0.67–1.2)	0.472
rs10936599	3	169492101	TERC	C/T	0.1	0.756	0.737	1.11 (0.94–1.3)	0.216
rs7675998	4	164007820	NAF1	G/A	0.048	0.8	0.766	1.21 (1.02–1.43)	0.026
rs2736100	5	1286516	TERT	C/A	0.085	0.487	0.48	1.02 (0.89–1.18)	0.730
rs9420907	10	105676465	OBFC1	C/A	0.142	0.164	0.13	1.31 (1.08–1.59)	0.007
rs3027234	17	8136092	CTC1	C/T	0.103	0.784	0.762	1.14 (0.96–1.34)	0.127
rs8105767	19	22215441	ZNF208	G/A	0.064	0.317	0.283	1.18 (1.02–1.37)	0.030
rs412658	19	22359440	ZNF676	T/C	0.086	0.382	0.344	1.18 (1.02–1.36)	0.025
rs6028466	20	38129002	DHX35	A/G	0.058	0.066	0.059	1.13 (0.85–1.5)	0.393
rs755017	20	62421622	ZBTB46	G/A	0.019	0.134	0.132	1.02 (0.84–1.25)	0.832

* Alleles are short allele/long allele. Short alleles are used as the reference allele and long allele as effect allele. EAF: effect allele frequency; estimates of SNP–LTL association were from published genome-wide association studies (GWAS). ** Adjusted by age and gender.

2.3. Association between GRS and STS Risk

We then constructed a GRS using the 11 SNPs and tested the association of this GRS with STS risk (Table 3). Dichotomized at the median GRS in controls, individuals with higher GRS (longer LTL) were associated with a 1.44-fold increased risk of STS (95% CI = 1.18–1.75, $p = 3.63 \times 10^{-4}$). In the tertile analysis, the ORs for the risk of STS in the medium and longest tertile groups were 1.35 (95% CI = 1.05–1.73) and 1.56 (95% CI = 1.22–1.99, respectively (p for trend 4.36×10^{-4}). In quartile analysis, the ORs for the risk of STS were gradually elevated with the rising of GRS and the ORs in 2nd, 3rd, and 4th (longest) quartile groups were 1.25 (95% CI = 0.93–1.68), 1.43 (95% CI = 1.07–1.92), and 1.79 (95% CI = 1.35–2.38), respectively (p for trend 3.20×10^{-5}).

Table 3. Association of LTL genetic risk score (GRS) with STS risk.

LTL GRS	Control *n* (%)	Case *n* (%)	OR * (95% CI)	*p* Value	*p* for Trend
Dichotomize					
Short	410 (56.47)	316 (43.53)	1 (reference)		
Long	403 (47.30)	449 (52.70)	1.44 (1.18–1.75)	3.63×10^{-4}	
Tertile					
Shortest	280 (58.09)	202 (41.91)	1 (reference)		
Medium	262 (50.58)	256 (49.42)	1.35 (1.05–1.73)	0.019	
Longest	271 (46.89)	307 (53.11)	1.56 (1.22–1.99)	3.85×10^{-4}	4.36×10^{-4}
Quartile					
1 (shortest)	207 (59.31)	142 (40.69)	1 (reference)		
2	203 (53.85)	174 (46.15)	1.25 (0.93–1.68)	0.139	
3	200 (50.38)	197 (49.62)	1.43 (1.07–1.92)	0.015	
4 (longest)	203 (44.62)	252 (55.38)	1.79 (1.35–2.38)	5.25×10^{-5}	3.20×10^{-5}

* Adjusted by age and gender.

2.4. Gender-Specific Association of LTL GRS with STS

We then performed stratified analyses to determine whether the association of LTL GRS with the risk of STS was different in men and women (Table 4). The association was highly significant in women (OR = 1.68, 95% CI = 1.26–2.23, $p = 3.54 \times 10^{-4}$), but not in men (OR = 1.14, 95% CI = 0.85–1.53, $p = 0.39$).

Table 4. Gender-specific association of LTL GRS with the risk of STS.

LTL GRS	Control *n* (%)	Case *n* (%)	OR * (95% CI)	*p* Value
Male				
Short	175 (52.87)	156 (47.13)	1 (reference)	
Long	209 (49.76)	211 (50.24)	1.14 (0.85–1.53)	0.39
Female				
Short	235 (59.49)	160 (40.51)	1 (reference)	
Long	194 (44.91)	238 (55.09)	1.68 (1.26–2.23)	3.54×10^{-4}

* Adjusted by age.

2.5. Association of LTL GRS with Histologic Subtypes

We next performed stratified analyses to analyze the associations of LTL GRS with the risks of major STS subtypes (Table 5). The most significant association was observed in GIST: longer LTL conferred a 2.2-fold increased risk (95% CI = 1.58–3.06, $p = 3.03 \times 10^{-6}$). The association was also significant in leiomyosarcoma (OR = 1.36, 95% CI = 1.02–1.83, $p = 0.038$), but not in liposarcoma (OR = 1.05, 95% CI = 0.75–1.47, $p = 0.772$) or angiosarcoma (OR = 1.19, 95% CI = 0.70–2.02).

Table 5. Association of LTL genetic risk score (GRS) with the risk of major STS subtypes.

LTL GRS	Control *n* (%)	Case *n* (%)	OR * (95% CI)	*p* Value
Leiomyosarcoma				
Short	410 (78.85)	110 (21.15)	1 (reference)	
Long	403 (74.22)	140 (25.78)	1.36 (1.02–1.83)	0.038
GIST				
Short	410 (86.68)	63 (13.32)	1 (reference)	
Long	403 (74.63)	137 (25.37)	2.20 (1.58–3.06)	3.03×10^{-6}
Liposarcoma				
Short	410 (83.33)	82 (16.67)	1 (reference)	
Long	403 (81.91)	89 (18.09)	1.05 (0.75–1.47)	0.772
Angiosarcoma				
Short	410 (93.82)	27 (6.18)	1 (reference)	
Long	403 (92.64)	32 (7.36)	1.19 (0.7–2.02)	0.528

* Adjusted by age and gender.

3. Discussion

In this case-control study, we used a two-sample MR approach to assess the associations between genetically predicted LTL and the risk of STS. We found a strong association between higher GRS (longer LTL) and an increased risk of STS with a dose–response relationship. Stratified analyses found the significant associations were more evident in GIST and leiomyosarcoma. This is the first MR study to show that long LTL predisposes to the development of STS.

There have been numerous epidemiologic investigations that assessed the association of LTL with the risk of different cancers. Earlier small, retrospective case-control studies produced inconsistent results, although most retrospective studies showed that short LTL predisposes to cancer development due to reverse causation [43–50]. Later large prospective studies have provided evidence for cancer-type-specific associations; both short and long LTL can predispose to cancer development [24,26,51,52]. Recent MR studies have further shown that genetically predicted long LTL is associated with increased risks of several cancers, including B-cell lymphoma, melanoma, lung adenocarcinoma, neuroblastoma, glioma, meningioma, and osteosarcoma [27–33]. Together with the results of the previous case-control study that long LTL as measured by qPCR conferred an increased risk of STS [38], our data provide compelling evidence for the association of long LTL with increased risks of STS.

The biological mechanisms of the association between longer LTL and higher cancer risks are not well understood. Recent studies have suggested that telomere dysfunction may have a binary effect on carcinogenesis [13]: both short and long telomeres can facilitate cancer development. Extremely shortened telomere length could result in increased chromosome end-to-end joining, thereby cause genome instability and malignant transformation. On the other hand, very long telomeres could increase cancer risk by allowing continued cellular proliferation and delaying cellular senescence and apoptosis, hence providing an environment that factors the accumulation of genetic lesions. Another potential biological explanation for the strong association between long LTL and high STS risk may be related to the distinct tissue origination of sarcomas. Unlike carcinomas, which originate from epithelial cells, sarcomas are malignant tumors derived from mesenchyme. Adult human tumors are predominantly epithelial carcinomas, whereas pediatric human tumors and murine tumors are primarily sarcomas and lymphomas. Longer telomere length in the pediatric population and mice than in adult human beings may partially explain this differential distribution of carcinoma and sarcoma [53]. Consistently, long LTL have been associated with increased risks of lymphoma, osteosarcoma, and STS [30,33,38,54]. Furthermore, Yan et al. previously reported long telomeres in tumor tissues of

liposarcoma, leiomyosarcoma, and high-grade STS [55], in contrast to the generally shorter telomeres in epithelial tumors compared to adjacent normal tissues.

In this study, we found that the association between long LTL and increased STS risk is highly significant in women but not in men. Interestingly, long LTL has been consistently associated with increased risks of most female cancers, including breast cancer [24,56,57], ovarian cancer [24,27], and endometrial cancer [24,27,58], suggesting that the estrogen regulation of telomeres may be linked to these female-specific associations. Estrogen can activate telomerase through direct binding to the promoter region of hTERT and prevent telomere shortening [59–61]. There was a positive correlation of the circulating estradiol with LTL in women [62], and LTL was longer in women who had a history of long-term hormone replacement therapy (HRT) than those without HRT [63]. The positive association between estrogen exposure and LTL may partially explain the association of longer LTL with increased risks of female STS.

The major strength of this study is the large sample size of histologically confirmed STS cases. Published prospective studies and MR studies of LTL and cancer risks have not included STS due to the rarity of the disease. We selected 11 SNPs that have been unequivocally associated with LTL through large scale GWAS. These SNPs are believed to meet the assumptions of MR and have been widely used in MR studies of LTL and disease risks. There are also a couple of limitations. First, the current GRS only explains approximately 2% of LTL variations. More powerful genetic instruments will increase statistical power and minimize confounding issues. Additional SNPs associated with LTL need to be identified to produce stronger GRS. Second, we could only perform stratified analyses on the major subtypes. STS represents a heterogeneous group containing more than 50 different histologic subtypes. Future large studies are needed to assess other STS histologic subtypes.

4. Materials and Methods

4.1. Study Population and Data Collection

STS patients were newly registered, histologically confirmed patients recruited from The University of Texas MD Anderson Cancer Center. The demographic and basic epidemiological information, including smoking, alcohol drinking, occupation, family history, and medical history, were obtained from the patient history database that all new patients filled when they registered into MD Anderson Cancer Center. Controls were healthy individuals with no cancer history who came to Kelsey-Seybold, one of the largest multispecialty physician groups in the Houston metropolitan area, for annual health checkups. Controls were frequency-matched to the cases by age, sex, and ethnicity. Demographic and epidemiological information of controls were collected by in-person interviews using standardized questionnaires. This study was approved by the institutional review board of MD Anderson Cancer Center on 7 April 2003 (ethic code: Lab03-0320), and all patients signed an informed consent form.

4.2. Genotyping

Genomic DNA was isolated from peripheral blood using the QIAamp blood DNA extraction kit (Qiagen, Valencia, CA, USA). All the genotyping was done in the Genotyping Core of MD Anderson Cancer Center using Illumina's Infinium OncoArray-500K Beadchip. Genome Studio software (Illumina, San Diego, CA, USA) was utilized to analyze the genotyping data. The mean concordance rate of 2% replicated samples was 99.2%. We removed nonconcordant SNPs for analyses. All the samples had an overall SNP call rate >95%. Individual SNPs with minor allele frequency (MAF) <1% and call rate <90% were excluded for analysis. Imputation was performed using the Michigan Imputation Server (https://imputationserver.sph.umich.edu/), an online server that generates phased and imputed genotypes using the Haplotype Reference Consortium (HRC Version r1.1) reference panels [64]. Eleven independent SNPs were associated with LTL by large scale GWAS [40–42] and were used to construct a genetic risk score (GRS). Among these SNPs, four SNPs (rs10936599, rs2736100,

rs9420907, and rs755017) were directly genotyped on OncoArray-500K, and the other seven were imputed with a high imputation accuracy (mean R^2) of 0.96.

4.3. Mendelian Randomization (MR) Analysis and GRS Construction

A two-sample MR design was used to assess the association between genetically predicted LTL and the risk of STS. The SNP–LTL effects (estimate for each SNP) were derived from published GWAS [40–42]. A GRS was calculated using 11 LTL-associated SNPs according to the following formula:

$$GRSi = \sum_{j=1}^{11} WjXij$$

where GRS_i is the risk score for individual i. x_{ij} (x_{ij} = 0, 1, or 2) is the number of telomere length increasing alleles for the j-th SNP, and w_j is the weight or effect coefficient (β estimate) for each SNP. A higher GRS value for an individual represents longer genetically inferred LTL. Weighted GRS counted the number of alleles associated with longer LTL that an individual carried across all 11 LTL-associated SNPs, with the addition of w_j for each SNP. Weighted GRS produces higher specificity than unweighted GRS by assigning more weight to SNPs with stronger effects.

4.4. Statistical Analysis

We used χ^2 test or Fisher's exact test to compare allele frequencies of each individual SNP between cases and controls. We then analyzed the association between each SNP and the risk of STS using a multivariate logistic regression model adjusting for age and gender. To analyze the association between GRS and the risk of STS, we dichotomized GRS at the median value or categorized into three and four groups based on the tertile and quartile distribution in controls and used a multivariate logistic regression model to calculate odds ratio (OR) and corresponding 95% confidence interval (95% CI). We also collected smoking, BMI, and medical history data from both cases and controls. Adjusting for smoking, BMI, and chronic diseases such as diabetes and hypertension in multivariate logistic regression did not attenuate the risk estimate. Since these variables are not risk factors for STS, we did not include them in our final multivariate model. All data were analyzed using R software (v3.4.1) or STATA (v13, STATA Corp., College Station, TX, USA). The glm() function in R Package was used for unconditional logistic regression analysis. All p values were two-sided with $p < 0.05$ considered statistically significant.

5. Conclusions

This is the first MR study to evaluate the association of LTL and the risk of STS. Our data demonstrated that genetically predicted long LTL is strongly associated with an increased risk of STS, and the association was more evident in women than in men.

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References

1. Clark, M.A.; Fisher, C.; Judson, I.; Thomas, J.M. Soft-tissue sarcomas in adults. *N. Engl. J. Med.* **2005**, *353*, 701–711. [CrossRef] [PubMed]
2. Burningham, Z.; Hashibe, M.; Spector, L.; Schiffman, J.D. The epidemiology of sarcoma. *Clin. Sarcoma Res.* **2012**, *2*, 14. [CrossRef] [PubMed]

3. Hui, J.Y. Epidemiology and etiology of sarcomas. *Surg. Clin. N. Am.* **2016**, *96*, 901–914. [CrossRef] [PubMed]
4. Siegel, R.L.; Miller, K.D.; Jemal, A. Cancer statistics, 2019. *CA Cancer J. Clin.* **2019**, *69*, 7–34. [CrossRef] [PubMed]
5. Howlader, N.; Noone, A.M.; Krapcho, M.; Miller, D.; Brest, A.; Yu, M.; Ruhl, J.; Tatalovich, Z.; Mariotto, A.; Lewis, D.R.; et al. (Eds.) *SEER Cancer Statistics Review, 1975–2016*; National Cancer Institute: Bethesda, MD, USA, 2018.
6. Lessler, J.; Chaisson, L.H.; Kucirka, L.M.; Bi, Q.; Grantz, K.; Salje, H.; Carcelen, A.C.; Ott, C.T.; Sheffield, J.S.; Ferguson, N.M.; et al. Assessing the global threat from Zika virus. *Science* **2016**, *353*, aaf8160. [CrossRef]
7. Benna, C.; Simioni, A.; Pasquali, S.; De Boni, D.; Rajendran, S.; Spiro, G.; Colombo, C.; Virgone, C.; DuBois, S.G.; Gronchi, A.; et al. Genetic susceptibility to bone and soft tissue sarcomas: A field synopsis and meta-analysis. *Oncotarget* **2018**, *9*, 18607–18626. [CrossRef]
8. Shammas, M.A. Telomeres, lifestyle, cancer, and aging. *Curr. Opin. Clin. Nutr. Metab. Care* **2011**, *14*, 28–34. [CrossRef]
9. Blackburn, E.H.; Greider, C.W.; Szostak, J.W. Telomeres and telomerase: The path from maize, Tetrahymena and yeast to human cancer and aging. *Nat. Med.* **2006**, *12*, 1133–1138. [CrossRef]
10. Palm, W.; de Lange, T. How shelterin protects mammalian telomeres. *Annu. Rev. Genet.* **2008**, *42*, 301–334. [CrossRef]
11. Gasser, S.M. A sense of the end. *Science* **2000**, *288*, 1377–1379. [CrossRef]
12. Blasco, M.A. Telomeres and human disease: Ageing, cancer and beyond. *Nat. Rev. Genet.* **2005**, *6*, 611–622. [CrossRef] [PubMed]
13. Hackett, J.A.; Greider, C.W. Balancing instability: Dual roles for telomerase and telomere dysfunction in tumorigenesis. *Oncogene* **2002**, *21*, 619–626. [CrossRef] [PubMed]
14. Daniali, L.; Benetos, A.; Susser, E.; Kark, J.D.; Labat, C.; Kimura, M.; Desai, K.; Granick, M.; Aviv, A. Telomeres shorten at equivalent rates in somatic tissues of adults. *Nat. Commun.* **2013**, *4*, 1597. [CrossRef] [PubMed]
15. Jeanclos, E.; Schork, N.J.; Kyvik, K.O.; Kimura, M.; Skurnick, J.H.; Aviv, A. Telomere length inversely correlates with pulse pressure and is highly familial. *Hypertension* **2000**, *36*, 195–200. [CrossRef]
16. Slagboom, P.E.; Droog, S.; Boomsma, D.I. Genetic determination of telomere size in humans: A twin study of three age groups. *Am. J. Hum. Genet.* **1994**, *55*, 876–882.
17. Aubert, G.; Lansdorp, P.M. Telomeres and aging. *Physiol. Rev.* **2008**, *88*, 557–579. [CrossRef]
18. Lansdorp, P.M.; Verwoerd, N.P.; van de Rijke, F.M.; Dragowska, V.; Little, M.T.; Dirks, R.W.; Raap, A.K.; Tanke, H.J. Heterogeneity in telomere length of human chromosomes. *Hum. Mol. Genet.* **1996**, *5*, 685–691. [CrossRef]
19. Huda, N.; Tanaka, H.; Herbert, B.S.; Reed, T.; Gilley, D. Shared environmental factors associated with telomere length maintenance in elderly male twins. *Aging Cell* **2007**, *6*, 709–713. [CrossRef]
20. Wu, Y.; Liu, Y.; Ni, N.; Bao, B.; Zhang, C.; Lu, L. High lead exposure is associated with telomere length shortening in Chinese battery manufacturing plant workers. *Occup. Environ. Med.* **2012**, *69*, 557–563. [CrossRef]
21. Starkweather, A.R.; Alhaeeri, A.A.; Montpetit, A.; Brumelle, J.; Filler, K.; Montpetit, M.; Mohanraj, L.; Lyon, D.E.; Jackson-Cook, C.K. An integrative review of factors associated with telomere length and implications for biobehavioral research. *Nurs. Res.* **2014**, *63*, 36–50. [CrossRef]
22. Fasching, C.L. Telomere length measurement as a clinical biomarker of aging and disease. *Crit. Rev. Clin. Lab. Sci.* **2018**, *55*, 443–465. [CrossRef] [PubMed]
23. Mons, U.; Muezzinler, A.; Schottker, B.; Dieffenbach, A.K.; Butterbach, K.; Schick, M.; Peasey, A.; De Vivo, I.; Trichopoulou, A.; Boffetta, P.; et al. Leukocyte telomere length and all-cause, cardiovascular disease, and cancer mortality: Results from individual-participant-data meta-analysis of 2 large prospective cohort studies. *Am. J. Epidemiol.* **2017**, *185*, 1317–1326. [CrossRef] [PubMed]
24. Weischer, M.; Nordestgaard, B.G.; Cawthon, R.M.; Freiberg, J.J.; Tybjaerg-Hansen, A.; Bojesen, S.E. Short telomere length, cancer survival, and cancer risk in 47,102 individuals. *J. Natl. Cancer Inst.* **2013**, *105*, 459–468. [CrossRef] [PubMed]
25. Rode, L.; Nordestgaard, B.G.; Bojesen, S.E. Long telomeres and cancer risk among 95,568 individuals from the general population. *Int. J. Epidemiol.* **2016**, *45*, 1634–1643. [CrossRef] [PubMed]

26. Zhang, X.; Zhao, Q.; Zhu, W.; Liu, T.; Xie, S.H.; Zhong, L.X.; Cai, Y.Y.; Li, X.N.; Liang, M.; Chen, W.; et al. The association of telomere length in peripheral blood cells with cancer risk: A systematic review and meta-analysis of prospective studies. *Cancer Epidemiol. Biomark. Prev.* **2017**, *26*, 1381–1390. [CrossRef] [PubMed]
27. Telomeres Mendelian Randomization, C.; Haycock, P.C.; Burgess, S.; Nounu, A.; Zheng, J.; Okoli, G.N.; Bowden, J.; Wade, K.H.; Timpson, N.J.; Evans, D.M.; et al. Association between telomere length and risk of cancer and non-neoplastic diseases: A mendelian randomization study. *JAMA Oncol.* **2017**, *3*, 636–651. [CrossRef]
28. Walsh, K.M.; Codd, V.; Rice, T.; Nelson, C.P.; Smirnov, I.V.; McCoy, L.S.; Hansen, H.M.; Elhauge, E.; Ojha, J.; Francis, S.S.; et al. Longer genotypically-estimated leukocyte telomere length is associated with increased adult glioma risk. *Oncotarget* **2015**, *6*, 42468–42477. [CrossRef]
29. Walsh, K.M.; Whitehead, T.P.; de Smith, A.J.; Smirnov, I.V.; Park, M.; Endicott, A.A.; Francis, S.S.; Codd, V.; Group, E.C.T.; Samani, N.J.; et al. Common genetic variants associated with telomere length confer risk for neuroblastoma and other childhood cancers. *Carcinogenesis* **2016**, *37*, 576–582. [CrossRef]
30. Machiela, M.J.; Lan, Q.; Slager, S.L.; Vermeulen, R.C.; Teras, L.R.; Camp, N.J.; Cerhan, J.R.; Spinelli, J.J.; Wang, S.S.; Nieters, A.; et al. Genetically predicted longer telomere length is associated with increased risk of B-cell lymphoma subtypes. *Hum. Mol. Genet.* **2016**, *25*, 1663–1676. [CrossRef]
31. Kuo, C.L.; Pilling, L.C.; Kuchel, G.A.; Ferrucci, L.; Melzer, D. Telomere length and aging-related outcomes in humans: A Mendelian randomization study in 261,000 older participants. *Aging Cell* **2019**, *18*, e13017. [CrossRef]
32. Muskens, I.S.; Hansen, H.M.; Smirnov, I.V.; Molinaro, A.M.; Bondy, M.L.; Schildkraut, J.M.; Wrensch, M.; Wiemels, J.L.; Claus, E.B. Longer genotypically-estimated leukocyte telomere length is associated with increased meningioma risk. *J. Neurooncol.* **2019**, *142*, 479–487. [CrossRef] [PubMed]
33. Zhang, C.; Hansen, H.M.; Semmes, E.C.; Gonzalez-Maya, J.; Morimoto, L.; Wei, Q.; Eward, W.C.; DeWitt, S.B.; Hurst, J.H.; Metayer, C.; et al. Common genetic variation and risk of osteosarcoma in a multi-ethnic pediatric and adolescent population. *Bone* **2020**, *130*, 115070. [CrossRef] [PubMed]
34. Zhang, C.; Doherty, J.A.; Burgess, S.; Hung, R.J.; Lindstrom, S.; Kraft, P.; Gong, J.; Amos, C.I.; Sellers, T.A.; Monteiro, A.N.; et al. Genetic determinants of telomere length and risk of common cancers: A Mendelian randomization study. *Hum. Mol. Genet.* **2015**, *24*, 5356–5366. [CrossRef] [PubMed]
35. Pierce, B.L.; Kraft, P.; Zhang, C. Mendelian randomization studies of cancer risk: A literature review. *Curr. Epidemiol. Rep.* **2018**, *5*, 184–196. [CrossRef] [PubMed]
36. Kachuri, L.; Saarela, O.; Bojesen, S.E.; Davey Smith, G.; Liu, G.; Landi, M.T.; Caporaso, N.E.; Christiani, D.C.; Johansson, M.; Panico, S.; et al. Mendelian Randomization and mediation analysis of leukocyte telomere length and risk of lung and head and neck cancers. *Int. J. Epidemiol.* **2018**. [CrossRef] [PubMed]
37. Machiela, M.J.; Hofmann, J.N.; Carreras-Torres, R.; Brown, K.M.; Johansson, M.; Wang, Z.; Foll, M.; Li, P.; Rothman, N.; Savage, S.A.; et al. Genetic variants related to longer telomere length are associated with increased risk of renal cell carcinoma. *Eur. Urol.* **2017**, *72*, 747–754. [CrossRef]
38. Xie, H.; Wu, X.; Wang, S.; Chang, D.; Pollock, R.E.; Lev, D.; Gu, J. Long telomeres in peripheral blood leukocytes are associated with an increased risk of soft tissue sarcoma. *Cancer* **2013**, *119*, 1885–1891. [CrossRef]
39. Davies, N.M.; Holmes, M.V.; Davey Smith, G. Reading mendelian randomisation studies: A guide, glossary, and checklist for clinicians. *BMJ* **2018**, *362*, k601. [CrossRef]
40. Pooley, K.A.; Bojesen, S.E.; Weischer, M.; Nielsen, S.F.; Thompson, D.; Amin Al Olama, A.; Michailidou, K.; Tyrer, J.P.; Benlloch, S.; Brown, J.; et al. A genome-wide association scan (GWAS) for mean telomere length within the COGS project: Identified loci show little association with hormone-related cancer risk. *Hum. Mol. Genet.* **2013**, *22*, 5056–5064. [CrossRef]
41. Mangino, M.; Hwang, S.J.; Spector, T.D.; Hunt, S.C.; Kimura, M.; Fitzpatrick, A.L.; Christiansen, L.; Petersen, I.; Elbers, C.C.; Harris, T.; et al. Genome-wide meta-analysis points to CTC1 and ZNF676 as genes regulating telomere homeostasis in humans. *Hum. Mol. Genet.* **2012**, *21*, 5385–5394. [CrossRef]
42. Codd, V.; Nelson, C.P.; Albrecht, E.; Mangino, M.; Deelen, J.; Buxton, J.L.; Hottenga, J.J.; Fischer, K.; Esko, T.; Surakka, I.; et al. Identification of seven loci affecting mean telomere length and their association with disease. *Nat. Genet.* **2013**, *45*, 422–427. [CrossRef] [PubMed]

43. Wu, X.; Amos, C.I.; Zhu, Y.; Zhao, H.; Grossman, B.H.; Shay, J.W.; Luo, S.; Hong, W.K.; Spitz, M.R. Telomere dysfunction: A potential cancer predisposition factor. *J. Natl. Cancer Inst.* **2003**, *95*, 1211–1218. [CrossRef] [PubMed]
44. Ma, H.; Zhou, Z.; Wei, S.; Liu, Z.; Pooley, K.A.; Dunning, A.M.; Svenson, U.; Roos, G.; Hosgood, H.D., III; Shen, M.; et al. Shortened telomere length is associated with increased risk of cancer: A meta-analysis. *PLoS ONE* **2011**, *6*, e20466. [CrossRef] [PubMed]
45. Wentzensen, I.M.; Mirabello, L.; Pfeiffer, R.M.; Savage, S.A. The association of telomere length and cancer: A meta-analysis. *Cancer Epidemiol. Biomark. Prev.* **2011**, *20*, 1238–1250. [CrossRef] [PubMed]
46. Hou, L.; Zhang, X.; Gawron, A.J.; Liu, J. Surrogate tissue telomere length and cancer risk: Shorter or longer? *Cancer Lett.* **2012**, *319*, 130–135. [CrossRef]
47. Gu, J.; Chen, M.; Shete, S.; Amos, C.I.; Kamat, A.; Ye, Y.; Lin, J.; Dinney, C.P.; Wu, X. A genome-wide association study identifies a locus on chromosome 14q21 as a predictor of leukocyte telomere length and as a marker of susceptibility for bladder cancer. *Cancer Prev. Res. (Phila)* **2011**, *4*, 514–521. [CrossRef]
48. Bau, D.T.; Lippman, S.M.; Xu, E.; Gong, Y.; Lee, J.J.; Wu, X.; Gu, J. Short telomere lengths in peripheral blood leukocytes are associated with an increased risk of oral premalignant lesion and oral squamous cell carcinoma. *Cancer* **2013**, *119*, 4277–4283. [CrossRef]
49. Sanchez-Espiridion, B.; Chen, M.; Chang, J.Y.; Lu, C.; Chang, D.W.; Roth, J.A.; Wu, X.; Gu, J. Telomere length in peripheral blood leukocytes and lung cancer risk: A large case-control study in Caucasians. *Cancer Res.* **2014**, *74*, 2476–2486. [CrossRef]
50. Pooley, K.A.; Sandhu, M.S.; Tyrer, J.; Shah, M.; Driver, K.E.; Luben, R.N.; Bingham, S.A.; Ponder, B.A.; Pharoah, P.D.; Khaw, K.T.; et al. Telomere length in prospective and retrospective cancer case-control studies. *Cancer Res.* **2010**, *70*, 3170–3176. [CrossRef]
51. Gu, J.; Wu, X. Re: Short telomere length, cancer survival, and cancer risk in 47,102 individuals. *J. Natl. Cancer Inst.* **2013**, *105*, 1157. [CrossRef]
52. Seow, W.J.; Cawthon, R.M.; Purdue, M.P.; Hu, W.; Gao, Y.T.; Huang, W.Y.; Weinstein, S.J.; Ji, B.T.; Virtamo, J.; Hosgood, H.D., III; et al. Telomere length in white blood cell DNA and lung cancer: A pooled analysis of three prospective cohorts. *Cancer Res.* **2014**, *74*, 4090–4098. [CrossRef] [PubMed]
53. DePinho, R.A. The age of cancer. *Nature* **2000**, *408*, 248–254. [CrossRef]
54. Hosnijeh, F.S.; Matullo, G.; Russo, A.; Guarrera, S.; Modica, F.; Nieters, A.; Overvad, K.; Guldberg, P.; Tjonneland, A.; Canzian, F.; et al. Prediagnostic telomere length and risk of B-cell lymphoma-results from the EPIC cohort study. *Int. J. Cancer* **2014**, *135*, 2910–2917. [CrossRef] [PubMed]
55. Yan, P.; Benhattar, J.; Coindre, J.M.; Guillou, L. Telomerase activity and hTERT mRNA expression can be heterogeneous and does not correlate with telomere length in soft tissue sarcomas. *Int. J. Cancer* **2002**, *98*, 851–856. [CrossRef]
56. Campa, D.; Barrdahl, M.; Santoro, A.; Severi, G.; Baglietto, L.; Omichessan, H.; Tumino, R.; Bueno-de-Mesquita, H.B.A.; Peeters, P.H.; Weiderpass, E.; et al. Mitochondrial DNA copy number variation, leukocyte telomere length, and breast cancer risk in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. *Breast Cancer Res.* **2018**, *20*, 29. [CrossRef]
57. Samavat, H.; Xun, X.; Jin, A.; Wang, R.; Koh, W.P.; Yuan, J.M. Association between prediagnostic leukocyte telomere length and breast cancer risk: The Singapore Chinese health study. *Breast Cancer Res.* **2019**, *21*, 50. [CrossRef] [PubMed]
58. Sun, Y.; Zhang, L.; Zhao, L.; Wu, X.; Gu, J. Association of leukocyte telomere length in peripheral blood leukocytes with endometrial cancer risk in Caucasian Americans. *Carcinogenesis* **2015**, *36*, 1327–1332. [CrossRef] [PubMed]
59. Kyo, S.; Takakura, M.; Kanaya, T.; Zhuo, W.; Fujimoto, K.; Nishio, Y.; Orimo, A.; Inoue, M. Estrogen activates telomerase. *Cancer Res.* **1999**, *59*, 5917–5921.
60. Misiti, S.; Nanni, S.; Fontemaggi, G.; Cong, Y.S.; Wen, J.; Hirte, H.W.; Piaggio, G.; Sacchi, A.; Pontecorvi, A.; Bacchetti, S.; et al. Induction of hTERT expression and telomerase activity by estrogens in human ovary epithelium cells. *Mol. Cell. Biol.* **2000**, *20*, 3764–3771. [CrossRef]
61. Boggess, J.F.; Zhou, C.; Bae-Jump, V.L.; Gehrig, P.A.; Whang, Y.E. Estrogen-receptor-dependent regulation of telomerase activity in human endometrial cancer cell lines. *Gynecol. Oncol.* **2006**, *103*, 417–424. [CrossRef]

62. Hapangama, D.K.; Turner, M.A.; Drury, J.A.; Quenby, S.; Saretzki, G.; Martin-Ruiz, C.; Von Zglinicki, T. Endometriosis is associated with aberrant endometrial expression of telomerase and increased telomere length. *Hum. Reprod.* **2008**, *23*, 1511–1519. [CrossRef] [PubMed]
63. Lee, D.C.; Im, J.A.; Kim, J.H.; Lee, H.R.; Shim, J.Y. Effect of long-term hormone therapy on telomere length in postmenopausal women. *Yonsei Med. J.* **2005**, *46*, 471–479. [CrossRef] [PubMed]
64. Das, S.; Forer, L.; Schonherr, S.; Sidore, C.; Locke, A.E.; Kwong, A.; Vrieze, S.I.; Chew, E.Y.; Levy, S.; McGue, M.; et al. Next-generation genotype imputation service and methods. *Nat. Genet.* **2016**, *48*, 1284–1287. [CrossRef] [PubMed]