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Telomere Length and the Risk of Cutaneous Malignant Melanoma in Melanoma-Prone Families with and without CDKN2A Mutations

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Abstract

Introduction: Recent evidence suggests a link between constitutional telomere length (TL) and cancer risk. Previous studies have suggested that longer telomeres were associated with an increased risk of melanoma and larger size and number of nevi. The goal of this study was to examine whether TL modified the risk of melanoma in melanoma-prone families with and without CDKN2A germline mutations.

Materials and Methods: We measured TL in blood DNA in 119 cutaneous malignant melanoma (CMM) cases and 208 unaffected individuals. We also genotyped 13 tagging SNPs in TERT.

Results: We found that longer telomeres were associated with an increased risk of CMM (adjusted OR = 2.81, 95% CI = 1.02–7.72, P = 0.04). The association of longer TL with CMM risk was seen in CDKN2A- cases but not in CDKN2A+ cases. Among CMM cases, the presence of solar injury was associated with shorter telomeres (P = 0.002). One SNP in TERT, rs2735940, was significantly associated with TL (P = 0.002) after Bonferroni correction.

Discussion: Our findings suggest that TL regulation could be variable by CDKN2A mutation status, sun exposure, and pigmentation phenotype. Therefore, TL measurement alone may not be a good marker for predicting CMM risk.

Introduction

Cutaneous malignant melanoma (CMM) is an etiologically heterogeneous disease with genetic, host, environmental factors, and their interactions contributing to its development. The main environmental risk factor is ultraviolet radiation (UVR), which may influence melanoma risk through multiple mechanisms, such as directly causing DNA damage, influencing the expression of apoptosis-related molecules and inducing immunosuppression. [1] Host phenotypic factors such as having a large number of benign and dysplastic nevi (DN), blond or red hair color, light eye color, freckling, and poor tanning ability have also been associated with increased melanoma risk. [2] Approximately 10% of CMM cases occur in a familial setting. [3] To date, two high-risk melanoma susceptibility genes, CDKN2A on chromosome 9p21 and CDK4 on 12q14, have been identified. Germline mutations of the CDKN2A gene have been described in approximately 20% of familial melanoma kindreds. [4–5] Mutations of CDK4 are rare, and only a few families worldwide have been found to harbor mutations. Although germline CDKN2A mutations are associated with a high risk of CMM, the penetrance of this gene is incomplete and varies by age and geographical location. [6] Additionally, phenotypic manifestations such as age at diagnosis, presence/number of DN, number of melanomas, and cosegregation of pancreatic cancer vary significantly among mutation carriers even within a single

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family. These findings suggest that other factors modify the effect of CDRN2A.

Telomeres are located at the ends of chromosomes, and consist of tandem nucleotide repeats (TTAGGG)n, the telomerase enzyme, the shelterin protein complex, and many other accessory proteins. They maintain genomic stability and chromosomal integrity by protecting chromosome ends from degradation, end-to-end fusion, and atypical recombination. [7] Telomeres shorten with each cell division, due to ineffective replication of the 3' end of DNA. [8] The telomerase enzyme complex consists of the reverse transcriptase, TERT, and additional proteins which are essential to maintain telomere length (TL). Telomerase is upregulated in the majority of cancers [9] and in the immortalization of skin keratinocytes. [10] Previous studies have shown that genetic variation in TERT was associated with melanoma risk.

Recently, a germline mutation in the promoter of TERT was identified in a melanoma-prone family that caused a 2-4 fold increase of TERT transcription. [12] Multiple mutations in the TERT promoter were also found in primary melanoma tissues with high frequency (33%), [12–13] suggesting that the dysregulation of TERT may play an important role in the genesis of melanoma.

TL is influenced by multiple factors, including both genetic and environmental. A twin study indicated 78% heritability for mean TL in blood [14], and subsequent studies mapped several candidate loci for TL using linkage and genome-wide association analyses. [15–20] Telomeres are highly sensitive to damage by oxidative stress, alkylation, and UVR, which can cause telomere shortening without DNA replication by inducing telomeric double-strand breaks at high frequency. [21] Epidemiologic studies examining the association between constitutional TL and cancer risk have generated inconsistent results. Although shortened TL has been associated with increased risk of a number of cancers such as bladder, gastric, and head and neck [22], associations between longer TL and increased risk were reported for other cancer types including Non-Hodgkin Lymphoma [23], Hepatitis B Virus-Related Hepatocellular Carcinoma [24], and melanoma. [25–26] Similarly, a previous study also found that longer telomeres were associated with larger size and number of melanocyte nevi. [27] These findings suggest that the association of TL with cancer risk is complex and cancer-type specific, which can be tumor suppressing or promoting depending on the host or cell type's susceptibility to genetic and environmental exposures. The goal of our study was to examine whether TL modified the cell type's susceptibility to genetic and environmental exposures.

Materials and Methods

Study Population

The details of this family study have been previously described. [28–29] In brief, US families with at least two living first degree relatives with a history of invasive melanoma were ascertained through health care professionals or self-referrals. All family members willing to participate in the study underwent a full-body skin examination for phenotypes (type and total number of nevi, extent of freckling, skin complexion, evidence for solar injury, and hair and eye color) and completed risk factor questionnaires for sun-related exposures such as tanning ability. All diagnostic of melanoma were confirmed by histologic review of pathologic material, pathology reports, or death certificates for deceased CMM cases. The study was approved by the National Cancer Institute Clinical Center Institutional Review Board and conduct-

ed according to the Declaration of Helsinki. Informed consent was obtained from all participants.

The current study was based on 53 families (23 families segregating CDRN2A mutations [CDKN2A+] and 30 families without known mutations [CDKN2A-]). All study participants were Caucasian. Two controls were selected for each case. The study population for genotyping was comprised of 183 CMM cases and 379 unaffected individuals. TL data was available from a subset of individuals (119 CMM cases and 208 unaffected individuals). The unaffected individuals included 144 unaffected family members and 64 genetically unrelated spouses. Demographic and CMM risk factors did not differ significantly among subjects who were included and not included in the TL analysis (data not shown).

Telomere Length Measurement

DNA was extracted from whole blood (N = 267) whenever available and from EBV-transformed lymphocytes (N = 60) when whole blood DNA was not available. Quantitative PCR was used to measure telomere length. The average, relative TL was estimates from the ratio of the telomere (T) repeat copy number to a single gene copy number (36B4 gene; S), expressed as the T/S ratio for each sample using standard curves. All samples for both the telomerases and single-copy gene reactions were performed in triplicate. Three blind replicate samples were interspersed with the samples to assess inter-plate variability. The coefficients of variation (CVs) within triplicates of the telomere assay, single-gene assay, and T/S ratio were 0.87%, 0.65%, and 6.67% respectively. The inter-assay CVs were 0.98%, 1.62%, and 7.92% respectively.

SNP Genotyping

13 tag SNPs in TERT were genotyped at the NCBCore Genotyping Facility (Advanced Technology Center, Gaithersburg, MD; http://snp500cancer.nci.nih.gov) using a custom-designed iSelect Infinium assay (Illumina, www.illumina.com), which included a total of 27,904 tag SNPs that were selected for a variety of cancers. Tag SNPs were selected using a minimum minor allele frequency (MAF) criterion of MAF $ > 5%$ based upon HapMap data for Caucasian (CEU) and Yoruban (YRI) samples using Tagzilla, software that implements a tagging algorithm based on pairwise linkage disequilibrium. [30] SNPs within the region spanning 20 kb 5' of the start of transcription (exon 1) to 10 kb 3' of the end of the last exon were grouped using a binning threshold of r2 > 0.8 to define a gene/region. When there were multiple transcripts available for genes, only the primary transcript was assessed. SNPs with low completion (<90%) and low concordance (<95%) were excluded. Among 586 genotyped samples, 20 were excluded due to either low completion (<90%, n = 12) or Mendelian inconsistencies (n = 8). Four individuals were further removed from all analyses due to missing CMM status.

Statistical Analysis

The Wilcoxon-Mann-Whitney test was used to assess whether TL differed significantly between spouse controls and unaffected family members. TL was not significantly different in the two control groups (P = 0.63), therefore we combined all controls in the analyses. The Wilcoxon-Mann-Whitney test was used to assess whether TL differed significantly between DNA from whole blood and DNA from EBV-transformed lymphocytes. TL did not differ significantly by DNA source in unaffected individuals (P = 0.47) or in CMM cases (P = 0.09), however we still adjusted for DNA source in all regression models. We also performed a sensitivity analysis by restricting the evaluation to individuals whose DNA was extracted from whole blood. Spearman correlation was used.
Table 1. Distribution of age, gender, CDKN2A, pigmentation phenotype, and sun exposure variables in 53 melanoma-prone families by CMM status.

|                        | Unaffected Individuals (n = 208) | CMM Cases (n = 119) | p1    |
|------------------------|----------------------------------|--------------------|-------|
|                        | N      | %     | N      | %     |       |
| **Age at Blood Draw**  |        |       |        |       |       |
| ≤30                    | 41     | 19.7  | 16     | 13.4  |       |
| 30–40                  | 49     | 23.6  | 25     | 21    |       |
| 40–50                  | 46     | 22.1  | 34     | 28.6  |       |
| 50–60                  | 40     | 19.2  | 22     | 18.5  |       |
| 60+ 6+                 | 32     | 15.4  | 22     | 18.5  | 0.45  |
| **Gender**             |        |       |        |       |       |
| Female                 | 121    | 58.2  | 59     | 49.6  |       |
| Male                   | 87     | 41.8  | 60     | 50.4  | 0.13  |
| **CDKN2A**             |        |       |        |       |       |
| Non-Carrier            | 178    | 85.6  | 59     | 49.6  |       |
| Carrier                | 30     | 14.4  | 60     | 50.4  | <.0001|
| **Moles**              |        |       |        |       |       |
| 0–24                   | 58     | 29.4  | 7      | 6.4   |       |
| 25–49                  | 37     | 18.8  | 14     | 12.8  |       |
| 50–99                  | 53     | 26.9  | 17     | 15.6  |       |
| 100+                   | 49     | 24.9  | 71     | 65.1  | <.0001|
| **Solar injury**       |        |       |        |       |       |
| None/mild              | 133    | 67.2  | 54     | 50.5  |       |
| Moderate               | 42     | 21.2  | 31     | 29    |       |
| Severe                 | 23     | 11.6  | 22     | 20.6  | 0.01  |
| **MC1R**               |        |       |        |       |       |
| Wild type              | 35     | 24.8  | 7      | 7.2   |       |
| 1 nonsynonymous variant| 63     | 44.7  | 46     | 47.4  |       |
| 2 nonsynonymous variants| 43    | 30.5  | 44     | 45.4  | 0.001 |
| **Tanning ability**    |        |       |        |       |       |
| Tan/Little burn         | 94     | 51.9  | 46     | 46.9  |       |
| Burn/Little tan         | 87     | 48.1  | 52     | 53.1  | 0.43  |
| **Skin type**          |        |       |        |       |       |
| Dark/medium            | 64     | 32.7  | 18     | 16.8  |       |
| Pale/fair              | 132    | 67.3  | 89     | 83.2  | 0.003 |
| **Eye color**          |        |       |        |       |       |
| Black/brown            | 54     | 27.8  | 25     | 22.9  |       |
| Hazel                  | 44     | 22.7  | 28     | 25.7  |       |
| Green/gray             | 16     | 8.2   | 11     | 10.1  |       |
| Blue                   | 80     | 41.2  | 45     | 41.3  | 0.76  |
| **Hair color**         |        |       |        |       |       |
| Black/brown            | 93     | 47.4  | 50     | 45.9  |       |
| Blond brown/light brown| 56     | 28.6  | 32     | 29.4  |       |
| Blond                  | 28     | 14.3  | 11     | 10.1  |       |
| Red                    | 19     | 9.7   | 16     | 14.7  | 0.47  |
| **Freckles**           |        |       |        |       |       |
| None/few               | 67     | 40.6  | 18     | 20    |       |
| Moderate               | 40     | 24.2  | 29     | 32.2  |       |
| Many                   | 58     | 35.2  | 43     | 47.8  | 0.004 |

1P-values were obtained by comparing CMM cases to unaffected individuals using the chi-square test.
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to evaluate the correlation between TL and age at blood draw, both as continuous variables.

We defined tertiles of TL distribution using cut-points based on the distribution among all unaffected individuals (Short: <0.53; Medium: 0.53–0.72; Long: >0.72) and evaluated the associations between TL and CMM risk factors using a generalized estimating equation approach that accounts for familial correlation in the variance computation, age at blood draw, gender, and DNA source. Conditional logistic regression was used to obtain the odds ratios (OR) and 95% confidence intervals (CIs) for the association between CMM risk and TL, with the shortest telomere tertile used as the reference group. We included age at blood draw, gender, and DNA source in the basic model, and further adjusted for germline CDKN2A mutation status, number of nevi, solar injury, and MC1R (as a surrogate for pigmentation characteristics) [28] in the final model. Conditioning on families was used to account for family ascertainment and differences in disease prevalence among families. While this approach ignores residual familial correlations among family members, it gives estimates that are attenuated toward the null and is thus conservative [31].

We used conditional logistic regression models to estimate the trend p-value for the association between CMM and each TERT SNP, using codominant coding for genotypes (0,1,2) with the homozygote of the common allele as the reference group, and adjusted for age at exam/diagnosis and gender. The associations between TL and genotypes were assessed using a generalized estimating equations (GEE) approach to account for correlation among family members [32], adjusted for age at blood draw, gender, and CMM. ORs and 95% CIs were computed using cumulative logistic regression for ordinal outcomes (PROC GENMOD, SAS 9.1). The working correlation matrix was the independent correlation matrix. We used a Bonferroni correction to account for the number of SNPs and outcomes (CMM and TL) tested, and thus used \( P < 0.05/26 \) (0.002) to define statistical significance. All statistical tests were two-sided and data was analyzed using SAS version 9.1 (SAS Institute, Cary, NC).

**Results**

In total, there were 119 CMM cases and 208 unaffected individuals included in the TL analysis. As expected, CDKN2A mutations, pale or fair skin type, increased number of nevi, increased number of freckles, solar injury, and MC1R variants were significantly associated with CMM risk in these families (Table 1).

As expected, TL was negatively correlated with age at blood draw among both unaffected individuals (\( r = -0.14 \)) and CMM cases (\( r = -0.12 \)) (Figure 1). Among unaffected individuals, TL did not differ significantly by any CMM risk factor examined (Table 2). Among CMM cases, shorter telomeres were significantly associated with the presence of moderate or severe solar injury (\( P = 0.017 \)) after adjusting for age at blood draw, gender, and DNA source (whole blood or EBV-transformed lymphocytes). Longer telomeres appeared to be associated with increased number of moles in both unaffected and CMM individuals, however, the association was not significant in either phenotype group after the covariate adjustment.

Although solar injury was more common among CMM cases than unaffected individuals, CMM cases had longer telomeres (36.1% in the longest tertile) compared to unaffected individuals (33.2%) (Table 3). After adjustment for age at blood draw, gender, DNA source, CDKN2A carrier status, number of nevi, solar injury, and MC1R, individuals in the longest TL category had close to a 3-fold increase in CMM risk compared to individuals in the shortest TL category (OR = 2.81, 95% CI = 1.02–7.72, \( P = 0.04 \)) (Table 3). When CDKN2A+ and CDKN2A- cases were separately compared to unaffected individuals, we found that the association of longer TL with CMM risk was seen in CDKN2A- cases (OR = 3.34, 95% CI = 1.12–10.00, \( P = 0.03 \); comparing longest to shortest TL) but not in CDKN2A+ cases (OR = 1.00, 95% CI = 0.42–2.38, \( P = 0.99 \)) (Table 4).

The association between CMM and TL did not change significantly when we restricted the analysis to individuals whose DNA was drawn from whole blood (age and gender adjusted OR = 1.83, 95% CI = 0.85–3.90, \( P = 0.12 \)). Similarly, results did not vary significantly by age at blood draw (\(<50 \text{ vs. } >50 \text{ years} \)), time of blood draw in relation to CMM diagnosis (before vs. after CMM diagnosis), age at CMM diagnosis (<40 vs. >40 years), or number of melanomas (single vs. multiple) (data not shown).

We examined whether genetic variants in TERT were associated with CMM risk and TL in blood. This analysis included 562 individuals (183 CMM cases and 379 unaffected individuals). Among the 13 tag SNPs genotyped in this analysis, one SNP, rs2735940, was significantly associated with TL (\( P = 0.002 \)) after Bonferroni correction. Another SNP,
rs10078761, showed suggestive association with CMM status \( (P = 0.003) \).

**Discussion**

In this exploratory analysis, we evaluated TL in blood in relation to CMM, *CDKN2A* germline mutation status, and CMM risk factors in 53 melanoma-prone families with and without *CDKN2A* mutations. Consistent with a previous report that found that longer telomeres were associated with the development of sporadic CMM [26], we found that longer telomeres were also associated with increased CMM risk in melanoma families, although the association was only seen in cases without *CDKN2A* mutations.

Although extremely short telomeres cause genomic instability and therefore increase cancer risk, senescence induced by telomere

| Table 2. Distribution of age, gender, *CDKN2A*, pigmentation phenotype, and sun exposure variables in 53 melanoma-prone families by telomere length, stratified by CMM status. |
|---|
| **Unaffected Individuals** | **CMM Cases** |
| | Short (n = 70) | Medium (n = 69) | Long (n = 69) | Short (n = 34) | Medium (n = 42) | Long (n = 43) |
| **Age at blood draw** | | | | | | |
| ≤50 | 42 | 30.9 | 50 | 36.8 | 44 | 32.4 | 16 | 21.3 | 26 | 34.7 | 33 | 44 |
| >50 | 28 | 38.9 | 19 | 26.4 | 25 | 34.7 | 0.009 | 18 | 40.9 | 16 | 36.4 | 10 | 22.7 | 0.03 |
| **Gender** | | | | | | |
| Female | 38 | 31.4 | 45 | 37.2 | 38 | 31.4 | 14 | 23.7 | 21 | 35.6 | 24 | 40.7 |
| Male | 32 | 36.8 | 24 | 27.6 | 31 | 35.6 | 0.77 | 20 | 33.3 | 21 | 35 | 19 | 31.7 | 0.2 |
| **CDKN2A** | | | | | | |
| Non-Carrier | 60 | 33.7 | 58 | 32.6 | 60 | 33.7 | 16 | 27.1 | 19 | 32.2 | 24 | 40.7 |
| Carrier | 10 | 33.3 | 11 | 36.7 | 9 | 30 | 0.37 | 18 | 30 | 23 | 38.3 | 19 | 31.7 | 0.09 |
| **Moles** | | | | | | |
| 0–49 | 34 | 35.8 | 34 | 35.8 | 27 | 28.4 | 6 | 28.6 | 8 | 38.1 | 7 | 33.3 |
| >50 | 31 | 30.4 | 33 | 32.4 | 38 | 37.3 | 0.83 | 22 | 25 | 31 | 35.2 | 35 | 39.8 | 0.61 |
| **Solar injury** | | | | | | |
| None/mild | 43 | 32.3 | 47 | 35.3 | 43 | 32.3 | 6 | 11.1 | 22 | 40.7 | 26 | 48.1 |
| Moderate/Severe | 22 | 33.8 | 20 | 30.8 | 23 | 35.4 | 0.19 | 22 | 41.5 | 16 | 30.2 | 15 | 28.3 | 0.017 |
| **MC1R** | | | | | | |
| Wild type/1 variant | 30 | 30.6 | 38 | 38.8 | 30 | 30.6 | 11 | 20.8 | 21 | 39.6 | 21 | 39.6 |
| 2 variants | 16 | 37.2 | 8 | 18.6 | 19 | 44.2 | 0.61 | 14 | 31.8 | 15 | 34.1 | 15 | 34.1 | 0.51 |
| **Tanning ability** | | | | | | |
| Tan/little burn | 30 | 31.9 | 37 | 39.4 | 27 | 28.7 | 10 | 21.7 | 15 | 32.6 | 21 | 45.7 |
| Burn/little tan | 28 | 32.2 | 28 | 32.2 | 31 | 35.6 | 0.73 | 17 | 32.7 | 19 | 36.5 | 16 | 30.8 | 0.08 |
| **Skin type** | | | | | | |
| Dark/medium | 23 | 35.9 | 26 | 40.6 | 15 | 23.4 | 5 | 27.8 | 6 | 33.3 | 7 | 38.9 |
| Pale/fair | 40 | 30.3 | 41 | 31.1 | 51 | 38.6 | 0.15 | 23 | 25.8 | 32 | 36 | 34 | 38.2 | 0.97 |
| **Eye color** | | | | | | |
| Black/brown | 15 | 27.8 | 24 | 44.4 | 15 | 27.8 | 9 | 36 | 4 | 16 | 12 | 48 |
| Hazel/green/gray/blue | 49 | 35 | 42 | 30 | 49 | 35 | 0.89 | 19 | 22.6 | 35 | 41.7 | 30 | 35.7 | 0.65 |
| **Hair color** | | | | | | |
| Black/brown | 27 | 29 | 35 | 37.6 | 31 | 33.3 | 11 | 22 | 14 | 28 | 25 | 50 |
| Blond brown/light brown/blond | 28 | 33.3 | 29 | 34.5 | 27 | 32.1 | 0.58 | 11 | 25.6 | 18 | 41.9 | 14 | 32.6 | 0.26 |
| Red | 9 | 47.4 | 3 | 15.8 | 7 | 36.8 | 0.5 | 6 | 37.5 | 7 | 43.8 | 3 | 18.8 | 0.09 |
| **Freckles** | | | | | | |
| None/few | 21 | 31.3 | 29 | 43.3 | 17 | 25.4 | 3 | 16.7 | 5 | 27.8 | 10 | 55.6 |
| Moderate/many | 33 | 33.7 | 27 | 27.6 | 38 | 38.8 | 0.06 | 18 | 25 | 28 | 38.9 | 26 | 36.1 | 0.3 |

1P-values were obtained by comparing individuals in the telomere tertiles using a generalized estimating equation accounting for familial correlation in the variance and adjusting for age at blood draw, gender, and DNA source.

Short: <0.53; Medium: 0.53–0.72; Long: >0.72.

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shortening places a limit on cell proliferation and is believed to provide a barrier for cancer growth [33]. On the other hand, longer telomeres, which may result from upregulated telomerase when cells reach a critically short TL but do not undergo senescence or apoptosis, may be associated with increased proliferative potential and cancer susceptibility. Constitutive telomerase expression in TERT-deficient transgenic mouse models resulted in increased incidence of epidermal tumors and skin wound healing [34]. Therefore, longer telomeres, in combination with decreased cell senescence, may greatly increase the proliferative potential of melanocytes, which leads to an increased susceptibility for nevi and melanoma. Consistent with a previous study which found that longer telomeres were associated with larger size and number of nevi [27], we found that longer telomeres were associated with an increased number of moles.

Interestingly, the association between longer TL and CMM risk was seen in CDKN2A+ cases but not in CDKN2A− cases. One possible reason is that melanomas in individuals with CDKN2A mutations may develop from telomere-independent mechanisms. Alternatively, the uncontrolled cell proliferation coupled with impaired DNA repair caused by CDKN2A mutations may lead to genomic instability and telomere shortening. CDKN2A-deficient mice exhibited increased levels of intracellular reactive oxygen species (ROS) in response to UVR [35] and demonstrated reduced ability to process UVR-induced DNA damage [36]. In proliferating cells, telomere DNA can be lost due to the inability of the DNA replication machinery to duplicate the linear DNA ends. Consistent with this hypothesis, a recent study demonstrated that telomere length shortening was significantly associated with hypermethylation of CDKN2A promoters in breast cancer [37].

Due to a high content of guanines, telomeres are especially sensitive to damage by oxidative stress [38]. Although the exact mechanism is unknown, telomere shortening is likely caused by oxidative DNA damage and deficiency in DNA repair in telomeric regions [39]. Our observation that moderate or severe solar injury was associated with shorter TL is in line with these findings. It is not clear why the association only occurred among CMM cases but not in unaffected individuals. One possibility is that solar injury reflected the interaction of sun exposure and sun sensitivity, and CMM cases are more likely to have sensitive skin types and be deficient in DNA repair capacities.

Some, but not all of our findings are consistent with those of Bodelon et al. based on a Mediterranean population [40]. In that study TL was also significantly associated with age but not with other CMM risk factors among unaffected individuals [40], but in contrast to our results, TL was not associated with CMM risk. The inconsistency is likely due to the differences in the populations, sun exposure, pigmentation characteristics, family history of melanoma, and melanoma phenotypes (usually diagnosed at early-stage in US because of more frequent screening) between the Mediterranean and American populations.

In our study, one SNP in TERT (rs2735940) was significantly associated with TL (P = 0.002). Another SNP (rs4635969) in TERT−CLPTM1L, a region that was previously associated with multiple cancers including melanoma [41], showed a suggestive association with TL (P = 0.005). A common polymorphism (rs2853669) in TERT, which was in complete allelic linkage with the recently identified germline mutation in the promoter of TERT, was unfortunately not genotyped in our study and not in LD with any of our genotyped SNPs.

Our study was exploratory due to the limited number of melanoma cases analyzed. In addition, in a small subset of individuals DNA was extracted from EBV-transformed lymphocytes, which could potentially cause bias in TL measurement.

### Table 3. Association of telomere length with melanoma in 53 melanoma-prone families.

| Telomere       | Unaffected (n = 208) | CMM (n = 119) | Model 1<sup>2</sup> | Model 2<sup>3</sup> | Model 3<sup>4</sup> |
|----------------|----------------------|---------------|---------------------|---------------------|---------------------|
|                | N %                  | N %           | OR 95% CI P         | OR 95% CI P         | OR 95% CI P         |
| 1st (short)    | 70 33.7              | 34 28.6       | Ref                 | Ref                 | Ref                 |
| 2nd (medium)   | 69 33.2              | 42 35.3       | 1.39 0.74-2.62 0.31 | 2.42 1.02-5.76 0.05 | 2.03 0.76-5.44 0.16 |
| 3rd (long)     | 69 33.2              | 43 36.1       | 1.38 0.73-2.63 0.33 | 2.89 1.20-6.94 0.02 | 2.81 1.02-7.72 0.04 |

<sup>1</sup>ORs and P-values were obtained from conditional logistic regression with melanoma as the outcome variable.

<sup>2</sup>Telomere tertile: Short: <0.53; Medium: 0.53-0.72; Long: >0.72.

<sup>3</sup>Model 1: age at blood draw, gender, and DNA source adjustment.

<sup>4</sup>Model 2: age at blood draw, gender, DNA source, CDKN2A, and solar injury adjustment.

### Table 4. Association of telomere length with melanoma in 53 melanoma-prone families, stratified by CDKN2A status among cases.

| Telomere       | Unaffected (n = 208) | CDKN2A+ CMM Cases (n = 60) | CDKN2A− CMM Cases (n = 59) |
|----------------|----------------------|-----------------------------|-----------------------------|
|                | N %                  | N % OR 95% CI P             | N % OR 95% CI P             |
| 1st (short)    | 70 33.7              | 18 30 Ref                   | 16 27.1 Ref                 |
| 2nd (medium)   | 69 33.2              | 23 38.3 1.4 0.58-3.36 0.46  | 19 32.2 2.45 0.81-7.44 0.11  |
| 3rd (long)     | 69 33.2              | 19 31.7 1 0.42-2.38 0.99    | 24 40.7 3.34 1.12-10.00 0.03 |

<sup>1</sup>ORs and P-values were obtained from conditional logistic regression with melanoma as the outcome variable. Age at blood draw, gender, DNA source, and solar injury adjustment.

<sup>2</sup>Telomere tertile: Short: <0.53; Medium: 0.53-0.72; Long: >0.72.

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However, we adjusted the analysis for DNA source in our logistic regression models. We also restricted the analysis to individuals with DNA extracted from whole blood and the results showed similar patterns. Another limitation was that our families were ascertained primarily through self- or physician-referral, and thus findings may not apply to other familial melanoma sample sets or to sporadic melanoma patients. The strengths of our study include a rich collection of genetic, exposure, clinical, and pigmentation data in melanoma-prone families with and without known CDK4/V2A mutations. We confirmed results from previous studies which found that longer telomeres were associated with CMM risk. Furthermore, our findings suggest that TL in CMM cases might be influenced by multiple mechanisms with opposing directions. Genetic background associated with proliferation potential and at-risk pigmentation phenotypes may predispose CMM cases to longer TL, whereas CDK4/V2A mutations and sun exposure may cause telomere shortening in these individuals.

Therefore, using TL alone as a potential biomarker to predict CMM risk may oversimplify the complex role and regulation of telomeres.

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Author Contributions

Conceived and designed the experiments: XRY. Performed the experiments: LSB JP LB MY ID AMG XRY. Analyzed the data: LSB XRY. Contributed reagents/materials/analysis tools: PLH RMP WW LM SAS SC MAT. Wrote the paper: LSB XRY.

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