BRIEF COMMUNICATION

The Effect on Melanoma Risk of Genes Previously Associated With Telomere Length

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Telomere length has been associated with risk of many cancers, but results are inconsistent. Seven single nucleotide polymorphisms (SNPs) previously associated with mean leukocyte telomere length were either genotyped or well-imputed in 11 108 case patients and 13 933 control patients from Europe, Israel, the United States and Australia, four of the seven SNPs reached a P value under .05 (two-sided). A genetic score that predicts telomere length, derived from these seven SNPs, is strongly associated ($P = 8.92 \times 10^{-9}$, two-sided) with melanoma risk. This demonstrates that the previously observed association between longer telomere length and increased melanoma risk is not attributable to confounding via shared environmental effects (such as ultraviolet exposure) or reverse causality. We provide the first proof that multiple germline genetic determinants of telomere length influence cancer risk.

The ends of chromosomes are protected from instability by tandem nucleotide repeats, known as telomeres. Telomeres shorten both with age and following exposures associated with cancer risk, such as smoking and ultraviolet (UV) irradiation (1,2). Thus, telomere maintenance processes are natural candidates for explaining carcinogenesis. Telomere length has been associated with risk of various age-related diseases, including cancers (3,4). However, with inconsistent results between retrospective and prospective studies (4–7) and methodological concerns (8), conclusions have been at best cautious. The recognition that any reported association might be because of either reverse causation (the cancer itself or therapeutics affecting telomere length) (9) or shared environmental factors affecting both telomere length and cancer risk has meant that the question of a causal relationship remains unresolved.

There has, however, been consistency in studies of melanoma. Longer telomeres have been associated both with increased melanoma risk in a study involving 557 cases (10), and increased nevus number (2,11), a major risk factor for melanoma (12). A prospective study of 47 102 subjects (13) found no association between telomere length and overall cancer risk after adjusting for shared risk factors, although it did not account for potential differences in direction of effect by cancer site (14). However, alleles in the telomerase-coding gene *TERT* that predispose to shorter telomere length, increase the risk of most cancers but are protective for melanoma (Supplementary Materials, available online) (15). Additionally, high penetrance melanoma mutations have been reported in genes encoding components of the Shelterin complex (*POT1*), which is crucial for the maintenance and signaling function of telomeres (16): *POT1* mutations resulted in longer telomeres (17).

The existence of genetic variants influencing both telomere length and cancer susceptibility would argue against either reverse causality or shared environmental effect (the latter affecting even prospective studies), explaining the association between telomere length and cancer risk. A recent meta-analysis (18) identified seven genome-wide statistically significant loci for mean leukocyte telomere length, five (*TERC*, *TERT*, *NAF1*, *OBFC1*, and *RTEL1*) containing known telomere-related genes, and two others (*ZNF208* and *ACYP2*). Of these loci, other than *TERT*, only *TERC* and *RTEL1* have been associated with risk of any disease (18–22). The study investigated the effect of the top SNP at each of the seven loci on risk of coronary artery disease (CAD) but, despite a huge sample size (>22 000 case patients and 64 000 control patients), no SNP was statistically significantly associated. A score based on genotypes at these loci and effect estimates from the telomere meta-analysis showed modest

association with CAD risk (P = .01, associating shorter telomeres with increased risk). Another study of similar design (albeit smaller and more limited coverage) (23), found genome-wide statistical significance for association between mean telomere length and *TERC*, *TERT*, *OBFC1*, a novel locus at 3p14.4, and support for *ACYP2*, *NAF1*, and *RTEL1*. Of these, only *TERT* was associated with risk of breast, ovarian, and prostate cancer, while *OBFC1* was associated with a subtype of ovarian cancer.

Given the potential role of telomere length in melanoma development, we investigated the variants identified by the telomere meta-analysis (18) in a genome-wide association study (GWAS) of melanoma. Our study consisted of 11 108 case patients and 13 933 control patients (Supplementary Table 1, available online) from Europe, Israel, the United States, and Australia. Written informed consent was obtained from each subject, and the investigations were performed after approval by the institutional review board for each recruiting center. As by far the biggest study of germline determinants of telomere length to date, we used the effect estimates for the seven SNPs from the telomere meta-analysis (18).

All 7 SNPs were either genotyped or well-imputed (Supplementary Materials, available online) in all melanoma GWAS samples; we tested for association between each SNP genotype and melanoma risk using SNPTEST2 (Supplementary Methods, available online) (24). Four of the seven SNPs reached nominal statistical significance, P values lower than .05 (rs10936599 in *TERC*, P = .0003; rs2736100 in *TERT*, P = .02; rs7675998 in *NAF1*, P = .03; rs9420907 in *OBFC1*, P = .001) (Table 1). The telomere-associated SNPs in *TERC*, *TERT*, *OBFC1*, and *RTEL1* are near (8-150kb from) SNPs strongly associated with melanoma risk (rs12696304 in *TERC*, P = .0001; rs455433 in *TERT*, P = 2.26x10^{-16}; rs2995264 in *OBFC1*, P = 7.10x10^{-6}; rs75691080 in *RTEL1*, P = 1.02x10^{-6}) (Supplementary Figure 1, available online). Further analysis suggests the two studies may be identifying the same underlying signal in each region (Supplementary Materials, available online).

The estimated effect of these seven SNPs on telomere length (18) and their estimated effect on melanoma risk are surprisingly well correlated (Pearson's correlation = 0.92,

P = .002, two-sided) (Table 1; Supplementary Figure 2, available online). For all but the least statistically significant telomere SNP (*ACYP2*), the allele associated with decreased telomere length is more frequent in control patients than melanoma case patients, consistent with a protective role for shorter telomeres in melanoma.

For each sample in our study, we constructed a genetic score predicting telomere length by calculating a weighted mean of genotype dosage across the seven telomere length–associated SNPs. The weights for each SNP were the age- and sex-adjusted effect estimates (log odds ratios) from the telomere meta-analysis (18). We then used this score in a logistic regression of melanoma risk (Supplementary Materials, available online).

We found a strong association between increased telomere score and increased risk of melanoma (P = 8.92×10^{-9}) that was consistent across geographic regions (Figure 1). Categorizing telomere score into quartiles, we observed a linear effect on melanoma risk; those in the highest quartile are estimated to be at 1.29 times the risk of melanoma of those in the lowest quartile (Supplementary Figure 3, available online).

Thus, several previously-identified telomere-associated SNPs, as well as a score based on their combined effect, are associated with melanoma risk. The fact that the telomere-associated SNP is often far less statistically significant than the strongest melanoma-associated SNP at several loci is likely in part because the telomere GWAS data are imputed from a reference panel with far fewer SNPs (Supplementary Materials, available online), so the effect of these telomere loci on melanoma risk will be underestimated here. Indeed, given the large number of genetic variants that are not able to be imputed and the possibility that several genetic variants could be responsible for the signal at a single locus, it is unlikely that the top SNP identified is a functional variant, and so the effect of the locus on both melanoma risk and telomere length is likely underestimated.

Previous studies have found at best a weak association between telomere-associated loci and disease risk. This highly statistically significant association confirms the hypothesis that the genetic factors underlying telomere length have an especially strong influence on melanoma risk and that, unusually, longer telomere length

Table 1. Results for each telomere length-associated SNP, including effect on telomere length, melanoma risk and *P* value for melanoma association*

SNP	Chromosome	Position	Related gene	Minor allele	MAF	Telomere length beta	Melanoma beta	Melanoma *P* value†
rs10936599	3	169492101	TERC	T	0.252	−0.097	−0.079	.0003
rs2736100	5	1286516	TERT	C	0.486	0.078	0.078	.02
rs7675998	4	164007820	NAF1	A	0.217	−0.074	−0.063	.03
rs9420907	10	105676465	OBFC1	C	0.135	0.069	0.083	.001
rs8105767	19	22215441	ZNF208	G	0.291	0.048	0.028	.16
rs755017	20	62421622	RTEL1	G	0.131	0.062	0.026	.35
rs11125529	2	54475866	ACYP2	A	0.142	0.056	−0.004	.86

* Telomere association information and minor allele frequency taken from telomere length genome-wide association study (18). MAF = minor allele frequency; SNP = single nucleotide polymorphism.

† Two-sided *P* values from meta-analysis of results from SNPTEST2 (24) using gene dosage and assuming an additive model.
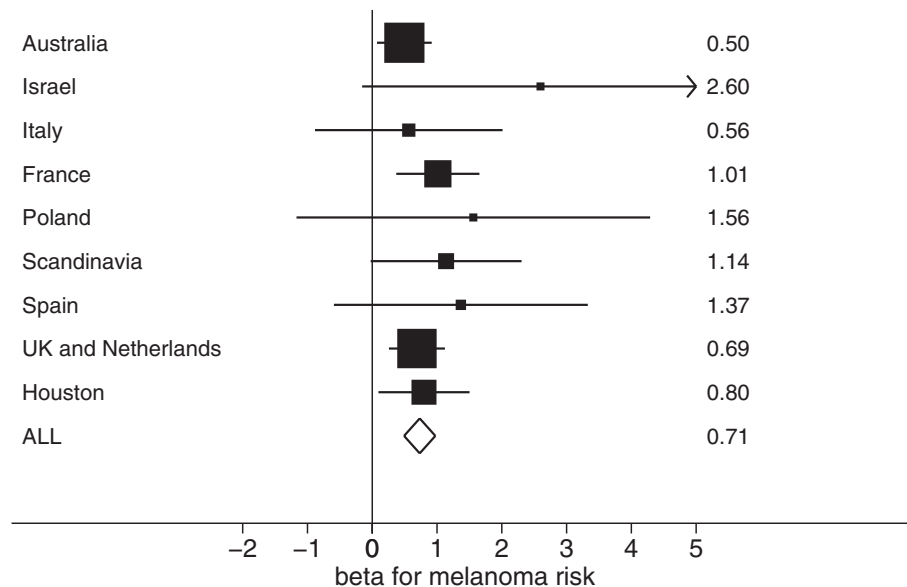
Figure 1. Forest plot of estimated effect size (with a 95% confidence interval indicated by **horizontal bars**) for telomere score on melanoma risk in nine geographic regions (and combined result). The relative sample size of each group is indicated by the size of the squares. Exact effect sizes (betas from SNPTEST2) are given in the right hand column.

predisposes an individual to melanoma. These seven loci explained in total only 1.2% of the variation in telomere length [18], and the combined score presented here explains only 0.14% of the variation in melanoma risk (McFadden's pseudo-r^2). This order of magnitude is unsurprising for such a score. To put these values in context, the most statistically significant single SNP for melanoma risk in the Leeds data set is rs258322, near *MC1R* (the red hair gene); it explains 5.7% of the variation in pigmentation but only 1.29% of the variation in melanoma risk.

The biggest limitation of the present study is that it only considers the effect on melanoma risk of the seven most statistically significant loci from the telomere length GWAS, as these are the only ones for which results are publicly available. Ideally we would have included a larger number of potentially telomere-associated SNPs, rather than just those reaching genome-wide statistical significance.

Our findings do not imply that telomere length acts directly on cancer risk and could reflect pleiotropic effects of telomere-length loci (such as the ease with which telomerase is reactivated in a melanocytic nevus). However, a mechanism for melanoma has been proposed, namely that longer telomeres increase the duration of proliferation of cells in a melanocytic nevus [11]. If senescence is delayed in melanocytes, this could allow further mutations to occur, increasing the chance of malignancy [10]. This is the first time that a strong association between multiple telomere-associated loci and any disease risk has been established.

References

1. Valdes AM, Andrew T, Gardner JP, et al. Obesity, cigarette smoking, and telomere length in women. *Lancet*. 2005;366(9486):662–664.
2. Han J, Qureshi AA, Prescott J, et al. A Prospective Study of Telomere Length and the Risk of Skin Cancer. *J Invest Dermatol*. 2009;129(2):415–421.
3. Ma H, Zhou Z, Wei S, et al. Shortened Telomere Length Is Associated with Increased Risk of Cancer: A Meta-Analysis. *PLoS ONE*. 2011;6(6):e20466.
4. Wentzensen IM, Mirabello L, Pfeiffer RM, Savage SA. The association of telomere length and cancer: a meta-analysis. *Cancer Epidemiol Biomarkers Prev*. 2011;20(6):1238–1250.
5. Pooley KA, Sandhu MS, Tyrer J, et al. Telomere length in prospective and retrospective cancer case-control studies. *Cancer Res*. 2010;70(8):3170–3176.
6. Shen M, Cawthon R, Rothman N, et al. A prospective study of telomere length measured by monochrome multiplex quantitative PCR and risk of lung cancer. *Lung Cancer*. 2011;73(2):133–137.
7. Lan Q, Cawthon R, Gao Y, et al. Longer telomere length in peripheral white blood cells is associated with risk of lung cancer and the rs2736100 (CLPTM1L-TERT) polymorphism in a prospective cohort study among women in China. *PLoS One*. 2013;8(3):e59230.
8. Cunningham JM, Johnson RA, Litzelman K, et al. Telomere length varies by DNA extraction method: implications for epidemiologic research. *Cancer Epidemiol. Biomarkers Prev*. 2013;22(11):2047–2054.
9. Savage SA, Gadalla SM, Chanock SJ. The long and short of telomeres and cancer association studies. *J Natl Cancer Inst*. 2013;105(7):448–449.
10. Nan H, Du M, De Vivo I, et al. Shorter telomeres associate with a reduced risk of melanoma development. *Cancer Res*. 2011;71(21):6758–6763.
11. Bataille V, Kato BS, Falchi M, et al. Nevus size and number are associated with telomere length and represent potential markers of a decreased senescence in vivo. *Cancer Epidemiol Biomarkers Prev*. 2007;16(7):1499–1502.
12. Chang YM, Newton-Bishop JA, Bishop DT, et al. A pooled analysis of melanocytic nevus phenotype and the risk of cutaneous melanoma at different latitudes. *Int J Cancer*. 2009;124(2):420–428.
13. Weischer M, Nordestgaard BG, Cawthon RM, Freiberg JJ, Tybjærg-Hansen A, Bojesen SE. Short telomere length, cancer survival, and cancer risk in 47102 individuals. *J Natl Cancer Inst*. 2013;105(7):459–468.
14. Gu J, Wu X. Re: short telomere length, cancer survival, and cancer risk in 47102 individuals. *J Natl Cancer Inst*. 2013;105(15):1157.
15. Rafnar T, Sulem P, Stacey SN, et al. Sequence variants at the TERT-CLPTM1L locus associate with many cancer types. *Nat Genet*. 2009;41(2):221–227.
16. Horn S, Figl A, Rachakonda PS, et al. TERT promoter mutations in familial and sporadic melanoma. *Science*. 2013;339(6122):959–961.
17. Robles-Espinoza CD, Harland M, Ramsay AJ, et al. POT1 loss-of-function variants predispose to familial melanoma. *Nat Genet*. 2014;46(5):478–481.
18. Codd V, Nelson CP, Albrecht E, et al. Identification of seven loci affecting mean

telomere length and their association with disease. *Nat Genet.* 2013;45(4):422–427.

19. Dubois PC, Trynka G, Franke L, et al. Multiple common variants for celiac disease influencing immune gene expression. *Nat Genet.* 2010;42(4):295–302.

20. Houlston RS, Cheadle J, Dobbins SE, et al. Meta-analysis of three genome-wide association studies identifies susceptibility loci for colorectal cancer at 1q41, 3q26.2, 12q13.13 and 20q13.33. *Nat Genet.* 2010;42(11):973–977.

21. Sawcer S, Hellenthal G, Pirinen M, et al. Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. *Nature.* 2011;476(7359):214–219.

22. Wrensch M, Jenkins RB, Chang JS, et al. Variants in the CDKN2B and RTEL1 regions are associated with high-grade glioma susceptibility. *Nat Genet.* 2009;41(8):905–908.

23. Pooley KA, Bojesen SE, Weischer M, 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(24):5056–5064.

24. Marchini J, Howie B, Myers S, McVean G, Donnelly P. A new multipoint method for genome-wide association studies by imputation of genotypes. *Nat Genet.* 2007;39(7):906–913.

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