Genetic predisposition to long telomeres is associated with increased mortality after melanoma: a study of 2101 melanoma patients from hospital clinics and the general population

Hafsa Ismail1,2, Jens Helby1,3, Lisbet R. Hölmich2,4, Annette H.Chakerah2,4, Lars Bastholt5, Helle Klyver6, Pia Sjogren7, Henrik Schmidt8, Liv Schöllhammer4, Børge G. Nordestgaard1,2,9, Stig E. Bojesen1,2,9

1Department of Clinical Biochemistry and The Copenhagen General Population Study, Herlev and Gentofte Hospital, Copenhagen University Hospital, Denmark
2Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark
3Department of Hematology, Herlev and Gentofte Hospital, Copenhagen University Hospital, Denmark
4Department of Plastic Surgery, Herlev and Gentofte Hospital, Copenhagen University Hospital, Denmark
5Department of Oncology, Odense University Hospital, Denmark
6Department of Plastic Surgery, Rigshospitalet, Copenhagen University Hospital, Denmark
7Department of Plastic Surgery, Aarhus University Hospital, Denmark
8Department of Oncology, Aarhus University Hospital, Denmark
9Copenhagen City Heart Study, Frederiksberg Hospital, Copenhagen University Hospital, Denmark

Keywords: Melanoma, Telomere Homeostasis, Biomarkers, Mortality, Survival
Significance

It is unclear if telomere biology impacts mortality of patients with melanoma. Analysis of measured and genetically predicted telomere length in more than 2000 melanoma patients from hospital clinics and the general population showed that genetically predicted long telomeres were associated with increased mortality after melanoma, but telomere length measured in DNA from leukocytes was not. We speculate that heavily mutated melanoma cells, in individuals with a genetic predisposition to long telomeres may have an inherited ability to keep dividing and avoid apoptosis, which may lead to disease progression and ultimately result in increased mortality.

Abstract

Whether there is an association between measured and genetically predicted telomere length and melanoma mortality is unclear. We tested the hypotheses that measured and genetically predicted telomere length are associated with mortality after a melanoma diagnosis. We followed 2101 patients with melanoma from hospital clinics and the general population for risk of death for up to 26 years. All had telomere length measured in DNA from leukocytes and 2052 of these were genotyped for the three single nucleotide polymorphisms rs7726159 (TERT), rs1317082 (TERC) and rs2487999 (OBFC1); all three genotypes are associated with telomere length, and combined into an allele count from 0 to 6. For each telomere-lengthening allele, the hazard ratios (HR) for mortality in the age-adjusted and multivariable adjusted Cox analysis were 1.12 (95% confidence interval: 1.02 – 1.23) and 1.11 (1.01 –
However, for each standard deviation increase in measured telomere length, HR for mortality was 0.97 (0.88 – 1.08).

In conclusion, in more than 2000 melanoma patients from hospital clinics and from the general population, genetically predicted long telomeres were associated with increased mortality, but measured leukocyte telomere length was not.

1. Introduction

Over the past three decades there has been an increase in the worldwide incidence of melanoma (Miller et al., 2020). Despite a decline in mortality rates for many cancer types since the beginning of the 1990s (Berk-Krauss, Stein, Weber, Polsky, & Geller, 2020), in the same time period there has been an overall increase in melanoma mortality across many different populations (Yang, Salciccioli, Marshall, Sheri, & Shalhoub, 2020).

Melanomas constitute only 4% of all skin cancers, but they are responsible for nearly half of all skin cancer-related deaths (Sample & He, 2018). Less advanced melanomas typically have a favorable prognosis, yet they cause more deaths than more advanced melanomas due to their sheer number (Landow, Gjelsvik, & Weinstock, 2017). There is thus a need for clinical tools to identify early stage melanoma patients with increased risk of adverse disease outcome.

Telomeres are located at the ends of chromosomes and consist of proteins and tandem nucleotide repeats of the sequence TTAGGG. During DNA replication the telomeres shorten by each normal cell division because of the end-replication problem (de Lange, 2009). Reaching a critically short length of the telomeres, the cell eventually enters senescence or apoptosis. This process limits uncontrolled cellular division and can be protective against cancer (Hanahan & Weinberg, 2011).

Telomere attrition can be counteracted by the telomerase complex, and its reactivation by mutations within the TERT promoter (Srinivas, Rachakonda, & Kumar, 2020) occurs in up to 80% in melanomas (Heidenreich & Kumar, 2017). Moreover, in a study of 26 cancer types in more than 47,000 individuals from the Danish general population, short telomere length in DNA from peripheral blood leukocytes was associated with
increased mortality in 177 individuals who were diagnosed with melanoma after blood sampling (Weischer et al., 2013). However, another study with 1019 patients having American Joint Committee on Cancer (AJCC) stage I-II melanoma could not confirm this finding, when adjusting for potential confounders (Rachakonda et al., 2018). Thus, there is no conclusive evidence that short telomeres are associated with increased risk of mortality in melanoma patients.

Telomere length is a complex heritable trait with an estimated heritability ranging from 36% to 82% in twin studies. The two potential causes of heritability are variability in telomere length per se and genetic variations that influence telomere maintenance (Srinivas et al., 2020). In genome-wide association studies (GWAS) many telomere length-associated genetic variants have been identified, and genetic variance is generally not associated with any potential confounders. Thus, by performing analyses based on telomere length-associated single nucleotide polymorphisms (SNPs) (Codd et al., 2010; Levy et al., 2010; Pooley et al., 2013) it is possible to study the isolated effect of genetically predicted telomere length on mortality after a melanoma diagnosis.

The purpose of this study was to test the hypothesis that measured and genetically predicted telomere length is associated with mortality after a diagnosis of melanoma. To assess this, we used telomere length measured in DNA from leukocytes and genotypes from three previously described telomere length-associated SNPs (rs7726159, rs1317082 and rs2487999) located in three genes (TERT, TERC and OBFC1) (Rode, Nordestgaard, & Bojesen, 2016) involving 2101 melanoma patients ascertained from hospital clinics (n =847) and from the general population (n=1205).

2. Materials and methods

Individuals

We included patients from the hospital melanoma cohort with blood samples from February 1997 to November 2009 at the Department of Oncology at Odense University Hospital and three plastic surgery departments at Aarhus University Hospital, Herlev and Gentofte Hospital and Rigshospitalet, Copenhagen. Additionally, individuals diagnosed with melanoma were included from two population-based studies: the 1991-1994 examination of the Copenhagen City Heart Study (CCHS) and the Copenhagen General Population Study (CGPS) in which individuals were recruited from 2003 to 2013 (Rode et al., 2016). Further details can be found in the suppl. material.

Verification of diagnoses and vital status

Hospital melanoma cohort

Melanoma diagnoses were defined according to International Classification of Diseases edition ten (ICD-10). We obtained information on tumour characteristics, tumour stage at diagnosis and disease recurrence from the national Danish Melanoma Database (Hölmich et al., 2016; Pedersen et al., 2018). Melanoma classification according to the AJCC staging system requires histopathological information, which was
retrieved from the Danish Melanoma Database and the national Danish Pathology Register (Bjerregaard & Larsen, 2011). Disease staging was performed according to the 7th edition of the AJCC guidelines. The national Danish Civil Registration System provided information on vital status and emigration status until 19th Apr 2018.

**General population melanoma cohort**

From the national Danish Cancer Registry, we identified patients diagnosed with melanoma and the diagnoses were defined according to ICD-7 (until 31st Dec 1977) and ICD-10 (from 1st Jan 1978 and onwards). Codes are listed in the suppl. material. The national Danish Civil Registration System provided information on vital status and emigration status until 19th Apr 2018.

**Ethics**

The study was conducted according to the Declaration of Helsinki. All participants gave written informed consent, and Danish ethics committees approved the studies (KA-02152, KF-100.2039/91 and KF-01-144/01).

**Telomere length measurements**

We measured telomere length in peripheral blood leukocyte DNA isolated by using the Qiagen blood kit (Scherczinger, Bourke, Ladd, & Lee, 1997). A modified monochrome multiplex quantitative polymerase chain reaction (qPCR) method (Cawthon, 2009) was used to measure the relative telomere length. Telomere length was expressed as the relative telomere (T) to single-copy gene (S) ratio (T/S ratio). In each plate DNA from the cell line NTERA-2 was included as internal control. During measurements of the hospital and the general population melanoma cohorts the coefficients of variation varied between 6.7% and 16.8%. Further details can be found in the suppl. material.

**SNP genotyping**

Many SNPs associated with telomere length have been identified (Codd et al., 2010; Levy et al., 2010; Pooley et al., 2013), but inclusion of them all would likely create a potential for pleiotropic effects and weak instrument bias. Therefore, we chose the three SNPs which had the strongest association with telomere length measured in DNA from leukocytes in a GWAS (Pooley et al., 2013), but within genes with established biological relevance for telomere biology: rs7726159 at 5p15.3 in the **TERT** gene, rs1317082 at 3q26.2 in the **TERC** gene and rs2487999 at 10q24.3 in the **OBFC1** gene. **TERT** and **TERC** are important for telomere elongation (Pooley et al., 2013). **OBFC1** encodes for part of the CST complex, which has a role in the regulation of telomerase activity (Chen, Redon, & Lingner, 2012).
We used a Taqman assay (Applied Biosystems, Life Technologies, Carlsbad, CA) on the ABI PRISM 7900 HT Sequence Detection System for the SNP genotyping. The three genotypes were combined to the number of telomere-lengthening alleles per individual. Thus, individuals with an allele count of zero have a genetic predisposition to the shortest telomeres.

In addition, we constructed a polygenic score based on the three SNPs. This is a weighted allele score, constructed by assigning each SNP a weight per allele according to the SNP's average per allele effect size on measured T/S ratio.

**Covariates**

In our multivariable adjusted analyses we included potentially confounding variables: age (< vs. ≥ 70 years), sex, cohort (hospital vs. general population), disease stage (AJCC I vs. AJCC II-IV vs. unknown), time of blood sampling (before vs. on/after diagnosis) and Charlson comorbidity index (none vs. any comorbidity). Further details can be found in the suppl. material.

**Statistical analyses**

We used STATA version 13 (Stata Corp LP, College Station, TX). Level of significance was a P-value of less than 0.05 and all P-values were two-sided.

Analyses of associations between covariates and telomere length allele count were estimated using Cuzick’s extension of the Wilcoxon rank sum test for all variables. Hardy-Weinberg equilibria were evaluated with Pearson’s Chi square test. Test for interaction were performed with a likelihood ratio test comparing the main effect model with a model including a two-factor interaction term.

Overall mortality was the primary endpoint. The Kaplan-Meier method was used to compute the unadjusted mortality probability as a function of time. The Cox proportional hazards model was used to compute hazard ratios and corresponding 95% confidence intervals for death with age or multivariable adjustment for the covariates mentioned above.

To examine the robustness of the associations found in these analyses, we also performed Cox analyses stratified according to the covariates included in the multivariable models, and these analyses were multivariable adjusted.

As we adjusted for whether the blood samples were obtained before or on/after the diagnosis using delayed entry, the Cox analyses were performed using left-truncated time since diagnosis. To prevent immortal time bias follow-up began at the date of blood sampling for patients with blood sampling performed after their diagnosis. Patients with blood sampling performed before their diagnosis were included at the time of diagnosis, accounting for the preceding time since blood sampling.

Follow-up ended at death, emigration from Denmark (n = 7) or April 19th, 2018, whichever came first.
In the analysis of measured leukocyte telomere length, we assessed risk per standard deviation increase in telomere length.

In the analyses of genetically predicted telomere length, we assessed risk according to allele count as a continuous variable, per standard deviation increase in the allele count, and as a categorical variable with three groups; zero to two, three, and four to six telomere-lengthening alleles, respectively. To facilitate comparison with the analyses on risk assessment per telomere-lengthening allele, we assessed risk for a genetic predisposition (polygenic score) to a 0.1 higher T/S ratio.

As a sensitivity analysis, we examined mortality at five-year follow-up according to allele count as a continuous variable. 3. Results

A total of 2101 patients from hospital clinics and the general population had leukocyte telomere length measured, and 2052 patients from hospital clinics and the general population were genotyped for the three single nucleotide polymorphisms (Suppl. Fig. 1). All genotypes were in Hardy-Weinberg equilibrium.

The telomere length allele count was associated with increasing telomere length, as expected, but not with any of the potential confounders (Table 1). Measured telomere length in quintiles was associated with all the confounding variables (Suppl. Table 1).

The median follow-up time after diagnosis was ten years (range zero to 26 years).

**Genetic predisposition to shorter telomere length and melanoma mortality**

Compared to patients with a genetic predisposition to shorter telomeres (allele count zero-two), those disposed to longest telomeres (allele count four-six) had a hazard ratio (HR) for mortality of 1.42 (95% confidence interval (CI)= 1.09 – 1.84) in the age-adjusted and of 1.39 (95% CI= 1.06 – 1.81) in the multivariable adjusted model (Fig. 1). In both models, we observed a stepwise mortality increase by increasing number of telomere-lengthening alleles.

Similarly, the HR for mortality per telomere-lengthening allele was 1.12 (95% CI= 1.02– 1.23) in the age-adjusted and 1.11 (95% CI= 1.01 – 1.23) in the multivariable adjusted Cox proportional hazards model (Fig. 2).

After stratifying according to the covariates included in the multivariable model, the association between a genetically predicted longer telomere length and increased mortality remained largely stable across subgroups of each covariate (Fig. 3).

In a sensitivity analysis, the polygenic score was associated with telomere length (Suppl. Fig. 2), and the HR for mortality per 0.1 unit increase of polygenic score was 2.41 (95% CI= 1.17 – 4.94) in the age-adjusted and 2.25 (95% CI= 1.07 – 4.71) in the multivariable adjusted Cox proportional hazards model (Suppl. Fig. 3).

Finally, the three genotypes individually were similarly associated with mortality, when orientated according to their effect on telomere length (Suppl. Table 2).

This article is protected by copyright. All rights reserved
**Measured and genetically predicted telomere length and melanoma mortality**

In the analysis of measured leukocyte telomere length, the HR for mortality was 0.97 (95% CI= 0.88 – 1.08) per standard deviation longer telomere length (Fig. 4). When only including individuals from whom the blood was sampled one year prior to diagnosis or later (n =1523), estimates were similar (data not shown). When assessing mortality per standard deviation increase in the allele count, the HR was 1.12 (95% CI= 1.01 – 1.23) (Fig. 4).

**Sensitivity analysis**

When truncating follow-up five years after diagnosis, estimates per telomere-lengthening allele remained similar to the estimates with maximum follow-up, however no longer significant with the number of deaths reduced by more than half (Suppl. Fig. 4).

4. Discussion

The most important finding from this study of more than 2000 melanoma patients from hospital clinics and the general population was that genetic determinants of long telomere length were associated with increased mortality. This is a novel finding.

Telomere length measured in DNA from peripheral blood leukocytes was, however, not associated with risk of death after multivariable adjustment. This apparent inconsistency versus genetic findings is interesting, and has several possible explanations, which might work alone or in combination: 1) less suited measurement; telomere length was measured in DNA from a mixture of leukocyte subpopulations from peripheral blood and not from melanoma cells, 2) low statistical power; leukocyte telomere length could be a very weak indicator of melanoma telomere biology, and 3) the telomere genotypes affect prognosis through different mechanisms than through leukocyte telomere length.

Ad 1): DNA was isolated from peripheral blood with different subpopulations of leukocytes. The relative distribution of subtypes of peripheral blood leukocytes varies from day-to-day (Winkel, Statland, Saunders, Osborn, & Kupperman, 1981) and different subtypes possess different telomere lengths (Lin et al., 2015). Also, blood cell telomere length can be a highly dynamic feature which might fluctuate during a life time and exhibit considerable length changes over comparatively short periods of time in individual cases (Svenson et al., 2011). Thus, the telomere length in leukocytes is probably highly variable and a less suited indicator of the telomere biology state in melanoma cells, hence not necessarily predictive of survival. Additionally, (i) melanoma survival is impacted by immune response (Sacdalan, Lucero, & Sacdalan, 2018), (ii) melanomas with a predominantly immune gene expression profile are more likely to be low-grade and have a better prognosis (Nsengimana et al., 2015), and (iii) the interplay between the tumor and immune cells impact tumor progression and metastasis (Tucci et al., 2019), and how such an immune response is reflected in the average telomere length from a mixture of leukocyte subpopulations in the peripheral blood is not clear.
Ad 2): Although there was an association between telomere length and genotypes, these subtle interplays could lead to leukocyte telomere length being highly variable, and in combination with an analytical imprecision higher than for average routine blood tests, could simply mean that the number of melanoma patients necessary to detect an association between telomere length and prognosis should be much higher than the included 2000 patients.

Ad 3): Somatic mutations of TERT affect melanoma prognosis, likely through upregulation of telomerase activity (Gandini et al., 2021), telomerase activity increases from normal skin to benign melanocytic lesions and finally to melanoma (Carvalho et al., 2006), and telomeres are elongated through other mechanisms than telomerase in some melanosomas (Durant, 2012). Somatic TERT mutations are acquired early in melanoma (Shain et al., 2018; Shain et al., 2015), and long telomeres may give pre-malignant melanocytes time to progressively accumulate oncogenic mutations (Viceconte et al., 2017). Also, induced expression of TERT maintains telomere length and causes a marked lifespan extension of human melanocytes in culture (Bandyopadhyay et al., 2001). Finally, in support of our findings, Nsengiamana (Nsengimana et al., 2015) found an association between disease subtype and genetically predicted telomere length. All these findings point to telomere elongation or maintenance to be very important for survival and proliferation of melanoma cells. This way to escape cell death might be particularly important for melanoma, which has one of the highest mutational burdens of all cancers ("Pan-cancer analysis of whole genomes," 2020), probably due to the constant exposure to mutagenic UV-light. If not counterbalanced by other mechanisms, this would normally reduce proliferation. We therefore speculate, that regardless of the lack of association with leukocyte telomere length, ultimately a genetic predisposition to long telomere length may be a driving factor for increased mortality in melanoma, as detected in this study, and discussed previously (Cleal, Norris, & Baird, 2018).

Among the strengths of our study is the large sample size, the prospective observational design with up to 26 years of nearly complete follow-up, the accuracy of the information on date of death as well as the possibility for us to study both measured and genetically predicted telomere length.

One limitation of our study is that not all patients had the blood sampled on the date of their diagnosis or immediately thereafter. However, we adjusted for whether the blood samples were obtained before or on/after the diagnosis, and only our genetic analysis showed an association between telomere length and melanoma mortality. Since genetic variation is constant throughout life and insensitive to a diagnosis of melanoma, it seems implausible that our genetic estimates would be influenced by the timing of the blood sampling.

It is also a limitation that we did not have information on disease stage in the majority of the patients. Among the patients with known stage, however, there was no association with allele count, so a more complete adjustment for stage might not alter the mortality estimates for the genetically predicted telomere length.
Another limitation of our study is the assumption of no pleiotropy, which means that we assume the genotypes only relate to mortality through telomere length regulation, and not through extra-telomeric effects. Clearly, pleiotropy is impossible to rule out completely, but individually, the associations of each of the three genotypes with mortality were largely similar, and in the main analyses they were combined to an allele count. This makes it less likely that any potential biases would affect our estimates to the same extent and in the same direction, implying that the chances of a pleiotropic effect on our estimates is low.

In conclusion, in more than 2000 melanoma patients from hospital clinics and from the general population, genetically predicted long telomeres were associated with increased mortality, but measured leukocyte telomere length was not. We speculate that melanoma cells, in individuals with a genetic predisposition to long telomeres, may have an inherited ability to keep dividing and to avoid apoptosis, which may lead to disease progression and ultimately result in increased mortality.

References


This article is protected by copyright. All rights reserved


This article is protected by copyright. All rights reserved


**Funding**

We thank the Chief Physician Johan Boserup and Lise Boserup’s Foundation, the Research Council at Herlev and Gentofte Hospital, the Research Foundation for Health Research in the Capital Region of Denmark and Sawmill Owner Jeppe Juhl and Wife Ovita Juhl’s Memorial Fund for financial assistance. The funders had no influence on any parts of this work nor in the preparation of the paper.

**Conflicts of interest**

None.

**Data sharing**

Research data regarding technical details, statistical code and derivative data are available from the corresponding author at stig.egl.bojesen@regionh.dk. Data access for further analyses is possible through direct collaborative agreement or through locally managed access arranged through the corresponding author.

**Figure/table legends**

- **Table 1**: The count of telomere-lengthening alleles is calculated per individual as the sum of telomere-lengthening alleles for each of the three single nucleotide polymorphisms rs7726159 (TERT), rs1317082 (TERC) and rs2487999 (OBFC1). N/A: not applicable. IQR: interquartile range. %*: percent per allele count. AJCC stage**: American Joint Committee on Cancer (AJCC) stage. We lacked information on disease stage on $n=1520$ patients. ‡according to the Charlson comorbidity index. $P_{trend}$ was estimated using Cuzick’s extension of Wilcoxon rank sum test.

- **Figure 1**: Survival and mortality as a function of telomere-lengthening allele count as a categorical variable. The multivariable Cox regression model was adjusted for age at blood sampling ($<$ vs $\geq$ 70
years), sex, cohort (hospital vs general population melanoma cohort), disease stage (AJCC I vs AJCC II-IV vs unknown), time of blood sampling (before vs on/after diagnosis) and Charlson comorbidity index (none vs any comorbidity). $P_{\text{trend}}$ was estimated by modeling the allele count as a continuous variable.

- **Figure 2**: Hazard ratio for mortality per telomere-lengthening allele. The multivariable model was adjusted for age at blood sampling (< vs $\geq$ 70 years), sex, cohort (hospital vs general population melanoma cohort), disease stage (AJCC I vs AJCC II-IV vs unknown), time of blood sampling (before vs on/after diagnosis) and Charlson comorbidity index (none vs any comorbidity).

- **Figure 3**: Hazard ratio for mortality per telomere-lengthening allele stratified according to potentially confounding variables. The multivariable models were adjusted for age at blood sampling (< vs $\geq$ 70 years), sex, cohort (hospital vs general population melanoma cohort), disease stage (AJCC I vs AJCC II-IV vs unknown), time of blood sampling (before vs on/after diagnosis) and Charlson comorbidity index (none vs any comorbidity), except for the stratification variable in question. $P_{\text{interaction}}$ was estimated with a likelihood ratio test comparing the main effect model with a model including a two-factor interaction term.

- **Figure 4**: Multivariable adjusted hazard ratio for mortality per standard deviation increase in measured telomere length or telomere-lengthening allele count. The adjustment included age at blood sampling (< vs $\geq$ 70 years), sex, cohort (hospital vs general population melanoma cohort), disease stage (AJCC I vs AJCC II-IV vs unknown), time of blood sampling (before vs on/after diagnosis) and Charlson comorbidity index (zero vs >zero).
Figure 1

Survival (%)

<table>
<thead>
<tr>
<th>Adjustment</th>
<th>Allele count</th>
<th>Patients, n</th>
<th>Hazard ratio (95% confidence interval)</th>
<th>P_&lt;sub&gt;meta&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age alone</td>
<td>0-2</td>
<td>1018</td>
<td>1 (reference)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>722</td>
<td>1.16 (0.94 - 1.43)</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>4-8</td>
<td>312</td>
<td>1.42 (1.09 - 1.84)</td>
<td></td>
</tr>
<tr>
<td>Multivariable</td>
<td>0-2</td>
<td>1018</td>
<td>1 (reference)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>722</td>
<td>1.13 (0.91 - 1.49)</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>4-8</td>
<td>312</td>
<td>1.39 (1.06 - 1.81)</td>
<td></td>
</tr>
</tbody>
</table>

Genetically predisposed telomeres

- Shortest
- Intermediate
- Longest

Time since diagnosis of melanoma (years)
### Table 1: Comparison of Age and Multivariable Adjustments

<table>
<thead>
<tr>
<th>Adjustment</th>
<th>Patients</th>
<th>Deaths</th>
<th>Hazard Ratio (95% Confidence Interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age alone</td>
<td>2062</td>
<td>434</td>
<td>1.12 (1.02 to 1.23)</td>
</tr>
<tr>
<td>Multivariable</td>
<td>2052</td>
<td>434</td>
<td>1.11 (1.01 to 1.23)</td>
</tr>
</tbody>
</table>

**Figure 2**

Hazard ratio (95% confidence interval) for mortality per eomere-lengthening allele.
<table>
<thead>
<tr>
<th>Covariate</th>
<th>Disease-free survival</th>
<th>Patients</th>
<th>Deaths</th>
<th>Hazard ratio (95% confidence interval)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td>&lt;70 years</td>
<td>431</td>
<td>0.95</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≥70 years</td>
<td>1621</td>
<td>236</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>Female</td>
<td>1126</td>
<td>130</td>
<td>0.68 (0.94 to 2.25)</td>
<td>0.48</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>832</td>
<td>207</td>
<td>1.15 (1.01 to 3.11)</td>
<td></td>
</tr>
<tr>
<td>Connect</td>
<td>Hospital population</td>
<td>1238</td>
<td>200</td>
<td>1.10 (0.97 to 1.24)</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td>Hospital</td>
<td>847</td>
<td>174</td>
<td>0.94 (0.98 to 1.54)</td>
<td></td>
</tr>
<tr>
<td>Disease stage</td>
<td>ADCC</td>
<td>416</td>
<td>42</td>
<td>1.27 (1.16 to 1.96)</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td>ADCC UN</td>
<td>122</td>
<td>56</td>
<td>0.66 (0.57 to 0.73)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Unknown</td>
<td>1520</td>
<td>355</td>
<td>1.10 (0.99 to 1.23)</td>
<td></td>
</tr>
<tr>
<td>Date of blood taking</td>
<td>Before diagnosis</td>
<td>628</td>
<td>146</td>
<td>1.05 (0.89 to 1.24)</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td>On or after diagnosis</td>
<td>1429</td>
<td>395</td>
<td>1.15 (1.02 to 1.30)</td>
<td></td>
</tr>
<tr>
<td>Comorbidity</td>
<td>None</td>
<td>1481</td>
<td>227</td>
<td>1.15 (1.01 to 1.31)</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>More</td>
<td>986</td>
<td>207</td>
<td>1.07 (0.93 to 1.23)</td>
<td></td>
</tr>
</tbody>
</table>

Figure 3

Hazard ratio (95% confidence interval) for mortality per women-lengthening variable.

Image: pcmr_12971_f3.jpg
Figure 4

(pc当地_12971_f4.jpg)