The effect on melanoma risk of genes previously associated with telomere length

Mark M Iles et al.

A full list of authors appears in the main paper

Supplementary Material
Supplementary Methods

Note on associations of variants in the TERT region with cancer risk

The C allele of rs401681 has been associated with shorter telomere length and increased risk of cancer of the lung, urinary bladder, prostate, cervix and basal cell carcinoma (1,2), but decreased risk of melanoma (3), pancreatic cancer (4) and, less statistically significantly, colorectal cancer (p=8.4x10⁻³) (1). The minor allele (A) of rs7726159, which is associated with increased telomere length, is associated with increased risk of breast and ovarian cancer, but a decreased risk of prostate cancer (5). The G allele of rs2736100 is associated with longer telomere length (6) and confers an increased risk of glioma (7) and lung adenocarcinoma (8,9), but is protective for testis cancer (10) and probably colorectal cancer (p=2.5x10⁻⁵) (11). The minor allele at rs2736108 is associated with longer telomeres and is protective against a subtype of breast cancer while the minor allele at rs7705526 is associated with longer telomeres and higher risk of subtypes of ovarian cancer (12). The
minor alleles of rs10069690 and rs2242652 increase risk of subtypes of breast and ovarian cancer, but have no apparent effect on telomere length (12). Thus we see that variants in *TERT* demonstrate increased risk for basal cell carcinoma and cancer of the lung, urinary bladder, prostate and cervix. The same variants are protective against melanoma, testis cancer, pancreatic cancer and, quite likely, colorectal cancer. The effect on breast and ovarian cancer is less clear and seem to depend on cancer subtype.

**Samples and Genotyping**

*GenoMEL*

Phase 1 of the original GenoMEL GWAS consisted of cases and controls collected from 8 centers across 6 different European countries. Standard quality control (QC) measures were applied to both samples and SNPs, giving a total of 1,353 cases and 3,571 controls. Most GenoMEL Phase 1 samples were genotyped on the Illumina HumanHap300 BeadChip version 2 duo array (with 317k tagging SNPs), with the exception of the French cases, which were genotyped on the Illumina HumanCNV370k array.

Phase 2 of the GenoMEL GWAS consisted of cases and controls from 10 centers (4 not in Phase 1) in 8 different European countries and Israel, supplemented with controls from the Wellcome Trust Case Control Consortium (WTCCC) (13). In both phases cases were preferentially selected to have a family history of melanoma, multiple primary tumours or an early
age of onset. After QC 1,450 cases and 2,668 controls remained (see (3) for
details of QC and samples). The GenoMEL Phase 2 samples were genotyped
on the Illumina 610k array.

UK Data

1,085 cases from Leeds and 1,392 from Cambridge (UK) were matched with
controls from the WTCCC. The Leeds cases were obtained from a population-
based study of incident melanoma cases diagnosed between September
2000 and December 2012 from a geographically defined area of Yorkshire
and the Northern region of the UK (14,15,16). Controls were ascertained by
contacting general practitioners (family doctors) to identify eligible individuals.
These controls were frequency-matched with cases for age and sex from
general practitioners who had also had cases as part of their patient register.
A further 220 controls were sex- and age-matched and from the same primary
care practice as incident cases of colorectal cancer recruiting from hospitals in
Leeds (17). The Cambridge cases were recruited as part of the SEARCH
study (18,19), an ongoing population-based study in Eastern England. Cases
were ascertained through the Eastern Cancer Registry and Information
Centre, and were aged between 18 and 70 years at diagnosis. All cases were
genotyped on the Illumina HumanOmniExpressExome-8 v1.0. Controls are
taken from the WTCCC.

In all the studies included here, written informed consent was obtained from
each subject and the investigations were performed after approval by the
institutional review board for each recruiting center.

*Houston (M.D. Anderson)*

931 cutaneous melanoma (CM) non-Hispanic white patients were recruited together with 1,026 cancer-free controls (friends or acquaintances of patients reporting to other clinics at the M.D. Anderson Cancer Center) frequency matched on age and sex. These were supplemented with an additional 873 individuals presenting for treatment for CM at MD Anderson which did not have BMI recorded. All samples were collected between March 1998 and August 2008.

Samples were genotyped using the Illumina HumanOmni1-Quad_v1-0_B array and called using the BeadStudio algorithm. No adjustment was made for ethnicity as the genomic inflation factor was 1.02. Data were analysed by regressing case-control status on genotype (coded according to an additive model). This study has been previously published (20) and a more detailed description of the QC procedures applied to these data can be found there.

*Australia*

Cases were genotyped on Illumina Omni1-Quad or HumanHap610 while controls were genotyped on Illumina Omni1-Quad or HumanHap610 or HumanHap670 (21). A more detailed description of the QC procedures applied to these data can be found in a previous publication (21).
AMFS: 549 cases collected by the Australian Melanoma Family Study (AMFS) (22), whose recruitment occurred from 2001-2005 and case probands identified from population-based state cancer registries. 430 control probands were selected from the electoral roll and frequency-matched to cases by city, age and sex. Blood was requested from all probands. A 20ml blood sample was collected in EDTA tubes by local pathology services and transported to a central laboratory in Sydney within 48h of collection. White blood cells were separated on a Ficoll gradient and plasma obtained by centrifugation, and stored at -70°C. Guthrie Spots were obtained from 1ml blood. Remaining blood was used for DNA extraction. Buccal swabs were collected from participants who did not wish to give blood. All AMFS case probands and population control probands were aged between 18-39 years inclusive.

QMEGA: 1,617 Australian melanoma cases of European descent were collected through the Queensland study of Melanoma: Environment and Genetic Associations (Q-MEGA) (23) The combined 4342 controls represent 3 control sets: 1,799 unrelated individuals from the Brisbane Adolescent Twin Study (21,24), 2,155 endometriosis patients recruited by QIMR Berghofer Medical Research Institute (QIMR) from 1995 to 2002 (25); and 553 healthy controls from the Study of Digestive Health (SDH) recruited through the Genomics Research Centre (Griffith University, Queensland, Australia) (26).

Western Australia: 1,253 melanoma cases of European descent recruited through the Western Australian Melanoma Health Study (WAMHS) (27) were
combined with 898 controls, who were Australian Caucasian participants from a Inflammatory Bowel disease study (593 controls, 96 mild ulcerative colitis, 176 with severe ulcerative colitis, 33 with Ulcerative Colitis NOS) recruited from the IBD Clinical and Research Programme at the Royal Brisbane & Women’s Hospital (RBWH), Brisbane, Queensland (28).

French Data

535 French melanoma cases and 856 French controls were genotyped using the Illumina Human660W-Quad array at the Centre National de Genotypage (CNG, Evry, France). The melanoma patients and controls came from the same collections as those included in Genomel, the French MELARISK collection for the patients (29) and the Supplementation in Vitamins and Mineral Antioxidants (SU.VI.MAX) study for the controls (30). Quality control procedures applied to these data were similar to those previously described (3).

Imputation

Imputation was conducted genome-wide on the GenoMEL Phase 1 samples, excluding SNPs with MAF<0.03 (or in some studies MAF<0.01), HWE p-value<10^{-4} (in controls) and missingness >0.03. IMPUTEv2 (31,32) was used and the reference panel used was 1000 Genomes Phase 1 integrated variant set (March 2012 release, excluding SNPs with MAF<0.001 in the CEU European samples). rs10936599 (TERC) was genotyped in all samples, while
the remaining 6 SNPs were imputed in at least some samples, with INFO>0.97 in all studies. We note that the telomere GWAS data (33) are imputed from a much smaller reference panel (HapMap2: 3.1M SNPs in 270 individuals, 30 of them European trios) than the melanoma reference panel (1000 Genomes Phase 1 integrated variant set: 38M SNPs, 1092 individuals, 500 of them European) and the authors themselves note particular problems with imputation in the TERT region. Of the three regions (TERT, OBFC1 and RTEL1) where a much more statistically significant melanoma hit is found close to the telomere hit, the top melanoma hit is not present in the HapMap2 reference panel. The LD between the most statistically significant telomere SNP and the most statistically significant melanoma SNP is quite high in TERC ($r^2=0.77$), OBFC1 ($r^2=0.66$) and RTEL1 ($r^2=0.003$, but $D'=0.47$, suggesting that the LD is reasonably high given the difference in allele frequencies). LD is weaker in TERT ($r^2=0.02$, $D'=0.16$), but then LD is generally low across the TERT region and there is good evidence that multiple causal loci may exist (12). In other words, the level of LD between the telomere and melanoma hits and the absence of the most statistically significant melanoma SNPs in HapMap2 suggest that the telomere length meta analysis and the melanoma study may be identifying the same underlying signal in each region.

**Statistical Analysis**

For the single SNP analysis, imputed genotypes were analysed as expected genotype counts based on the posterior probabilities (gene dosage) using
SNPTEST2 (34) assuming an additive model (two-sided). For the telomere score analysis, melanoma case-control status was regressed in a logistic regression on the telomere score (two-sided significance reported). In both analyses the European samples were analysed as four separate datasets (GenoMEL Phase 1, Phase 2, UK (Leeds and Cambridge) data, French data), the US data as a single dataset and the Australian data as four separate datasets (AMFS, QMEGA-610k, QMEGA-Omni, WAMHS). For the analysis of GenoMEL Phase 1 and 2 data, geographical region was included as a covariate as has been done previously (14). In addition adjustment for principal components was applied to all datasets but made little difference to the results (the French data were slightly less statistically significant and the US and Australian datasets slightly more statistically significant), so were not used in the final analysis. All datasets were combined using an inverse weighted fixed effects meta analysis, with the exception of rs2736100 and rs7675998 which showed evidence of heterogeneity ($I^2=0.63$ and 0.34, respectively). These two were combined using the method of Dersimonian and Laird (35) to estimate the between-studies variance, $\hat{\tau}^2$. An overall random effects estimate was then calculated using the weights $1/(\nu_i + \hat{\tau}^2)$ where $\nu_i$ is the variance of the estimated effect.

**GenoMEL membership:**

*Barcelona:* Paula Aguilera, Celia Badenas, Cristina Carrera, Francisco Cuellar, Daniel Gabriel, Estefania Martinez, Melinda Gonzalez, Pablo Iglesias, Josep Malvehy, Rosa Marti-Laborda, Montse Mila, Zighe Ogbah, Joan-Anton Puig
Butille, Susana Puig and Other members of the Melanoma Unit: Llúcia Alós, Ana Arance, Pedro Arguíis, Antonio Campo, Teresa Castel, Carlos Conill, Jose Palou, Ramon Rull, Marcelo Sánchez, Sergi Vidal-Sicart, Antonio Vilalta, Ramon Vilella.

Brisbane: The Queensland study of Melanoma: Environmental and Genetic Associations (Q-MEGA) Principal Investigators are: Nicholas G. Martin, Grant W. Montgomery, David Duffy, David Whiteman, Stuart MacGregor, Nicholas K. Hayward. The Australian Cancer Study (ACS) Principal Investigators are: David Whiteman, Penny Webb, Adele Green, Peter Parsons, David Purdie, Nicholas Hayward.

Emilia-Romagna: Maria Teresa Landi, Donato Calista, Giorgio Landi, Paola Minghetti, Fabio Arcangeli, Pier Alberto Bertazzi.

Genoa: Department of Internal Medicine and Medical Specialties, Laboratory of Genetics of Rare Hereditary Cancers, University of Genoa/San Martino-IST Research Hospital: Giovanna Bianchi Scarrà, Paola Ghiorzo, Lorenza Pastorino, William Bruno, Sabina Nasti, Linda Battistuzzi, Paola Origone, Virginia Andreotti. Medical Oncology Unit, San Martino-IST Research Hospital: Paola Queirolo.

Glasgow: Rona Mackie, Julie Lang.

Leeds: Julia A Newton Bishop, Paul Affleck, Jennifer H Barrett, D Timothy
Bishop, Jane Harrison, Mark M Iles, Juliette Randerson-Moor, Mark Harland, John C Taylor, Linda Whittaker, Kairen Kukalizch, Susan Leake, Birute Karpavicius, Sue Haynes, Tricia Mack, May Chan, Yvonne Taylor, John Davies, Paul King.


Nielsen, Anita Schmidt Casslén.

*Norway: Oslo University Hospital:* Per Helsing, Per Arne Andresen, Helge Rootwelt. *University of Bergen:* Lars A. Akslen, Anders Molven.

*Paris:* Florence Demenais, Marie-Françoise Avril, Brigitte Bressac-de Paillerets, Valérie Chaudru, Nicolas Chateigner, Eve Corda, Patricia Jeannin, Fabienne Lesueur, Mahaut de Lichy, Eve Maubec, Hamida Mohamdi and the French Family Study Group including the following Oncogeneticists and Dermatologists: Pascale Andry-Benzaquen, Bertrand Bachollet, Frédéric Bérard, Pascaline Berthet, Françoise Boitier, Valérie Bonadona, Jean-Louis Bonafé, Jean-Marie Bonnetblanc, Frédéric Cambazard, Olivier Caron, Frédéric Caux, Jacqueline Chevrant-Breton, Agnès Chompret (deceased), Stéphane Dalle, Liliane Demange, Olivier Dereure, Martin-Xavier Doré, Marie-Sylvie Doutre, Catherine Dugast, Laurence Faivre, Florent Grange, Philippe Humbert, Pascal Joly, Delphine Kerob, Christine Lasset, Marie Thérèse Leccia, Gilbert Lenoir, Dominique Leroux, Julien Levang, Dan Lipsker, Sandrine Mansard, Ludovic Martin, Tanguy Martin-Denavit, Christine Mateus, Jean-Loïc Michel, Patrice Morel, Laurence Olivier-Faivre, Jean-Luc Perrot, Caroline Robert, Sandra Ronger-Savle, Bruno Sassolas, Pierre Souteyrand, Dominique Stoppa-Lyonnet, Luc Thomas, Pierre Vabres, Eva Wierzbicka.

*Philadelphia:* David Elder, Peter Kanetsky, Jillian Knorr, Michael Ming, Nandita Mitra, Althea Ruffin, Patricia Van Belle
Poland: Tadeusz Dębiak, Jan Lubiński, Aneta Mirecka, Sławomir Ertmański.

Slovenia: Srdjan Novakovic, Marko Hocevar, Barbara Peric, Petra Cerkovnik.

Stockholm: Veronica Höiom, Johan Hansson.

Sydney: Graham J. Mann, Richard F. Kefford, Helen Schmid, Elizabeth A. Holland

**AMFS Investigators**

Anne E Cust¹, Elizabeth A Holland², Helen Schmid³, Bruce K Armstrong¹,
Richard F Kefford³, Joanne F Aitken³, Graham G Giles⁴,⁵, John L Hopper⁵,
Graham J Mann³, Mark A Jenkins⁴

1 Cancer Epidemiology and Services Research, Sydney School of Public
Health, The University of Sydney, Australia

2 Westmead Institute for Cancer Research, University of Sydney at
Westmead Millennium Institute and Melanoma Institute Australia, Sydney,
Australia

3 Viertel Centre for Research in Cancer Control, Cancer Council Queensland,
Spring Hill, Brisbane, Australia

4 Centre for Molecular, Environmental, Genetic and Analytic (MEGA)
Epidemiology, Melbourne School of Population Health, The University of
Melbourne, Australia

5 Cancer Epidemiology Centre, Cancer Council Victoria, Melbourne, Australia

**Q-MEGA and QTWIN investigators:**

Jimmy Z. Liu¹, Zhen Zhen Zhao¹, Joanne F Aitken², Anjali K.Henders¹,
Mitchell Stark¹, David L. Duffy¹, Jodie N. Painter¹

1 QIMR Berghofer, Brisbane, QLD 4029, Australia
Thanks A. Baxter, M. de Nooyer, I. Gardner, D. Statham, B. Haddon, M.J. Wright, J. Palmer, J. Symmons, B. Castellano, L. Bardsley, S. Smith, D. Smyth, L. Wallace, M.J. Campbell, A. Caracella, M. Kvaskoff, O. Zheng, B. Chapman and H. Beeby for their input in project management, sample processing and database development. We are grateful to the many research assistants and interviewers for assistance with the studies contributing to the QMEGA and QTWIN collections.

We acknowledge with appreciation all the participants in the endometriosis studies. We thank Endometriosis Associations for supporting study recruitment and S. Nicolaides and the Queensland Medical Laboratory for the pro bono collection of blood samples and other pathology services for assistance with blood collection.

Study of Digestive Health (SDH) Team:

Chief Investigators: David Whiteman, Adele Green, Nicholas Hayward, Peter Parsons, Sandra Pavey, David Purdie, Penny Webb (Queensland Institute of Medical Research); David Gotley, Mark Smithers (University of Queensland / Princess Alexandra Hospital); Paul Drew, Glyn Jamieson (University of Adelaide); Paul Drew, David Watson (Flinders University of South Australia); Andrew Clouston (Mayne Pathology).
Research Staff: D. Nancarrow, D. Hussey, E. Smith, G. Mayne

Project Manager: S. O'Brien (QIMR)

Data Manager: T. Sadkowsky (QIMR)

Research Nurses: A. McMurtrie, L. Terry, M. Connard, L. Jackman, S. Perry, M. Davis (QLD); D. Roffe, M. Martin, L. Smith (SA)

Clinical Collaborators: I. Brown (S&N Pathology, QLD), N. Walker (QML Pathology, QLD); Justin Bessell (Flinders Medical Centre, SA)

William Tam (Royal Adelaide Hospital, SA), Andrew Ruskowicz (Institute of Medical and Veterinary Science, SA).

We gratefully acknowledge the cooperation of the following institutions:

Sullivan and Nicolaides Pathology (Brisbane); Queensland Medical Laboratory (Brisbane); Queensland Health Pathology Services (Brisbane); Institute of Medical and Veterinary Science (Adelaide); SouthPath (Adelaide).

We also acknowledge the contribution of the study nurses and research assistants and would like to thank all of the people who participated in the study.
IBD investigators

Lisa Simms\textsuperscript{1}, Grant W. Montgomery\textsuperscript{2}, Peter Visscher\textsuperscript{3}

1. Inflammatory Bowel Diseases Laboratory, QIMR Berghofer Medical Research Institute, Brisbane, Australia
2. Molecular Epidemiology, QIMR Berghofer Medical Research Institute, Brisbane, Australia
3. The Queensland Brain Institute, The University of Queensland, QBI Building, St Lucia, Queensland 4071, Australia

The authors express their thanks to Sullivan and Nicolaides Pathology, Queensland Medical Laboratories and the Queensland Health Pathology Service for identifying participants for this study. They are also grateful to Peter Schultz, Lauren Aoude, Loralie Parsonson, Stephen Walsh, Mitchell Stark, John Cardinal and Herlina Handoko for technical support.
Supplementary Tables

Supplementary Table 1 Numbers of cases and controls from each study

<table>
<thead>
<tr>
<th></th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>GenoMEL Phase 1</td>
<td>1,354</td>
<td>2,341</td>
</tr>
<tr>
<td>GenoMEL Phase 2</td>
<td>1,450</td>
<td>1,399</td>
</tr>
<tr>
<td>UK data</td>
<td>2,570</td>
<td>2,682</td>
</tr>
<tr>
<td>Houston</td>
<td>1,804</td>
<td>1,026</td>
</tr>
<tr>
<td>AMFS</td>
<td>549</td>
<td>430</td>
</tr>
<tr>
<td>QMEGA</td>
<td>1,617</td>
<td>4,342</td>
</tr>
<tr>
<td>Western Australia</td>
<td>1,253</td>
<td>898</td>
</tr>
<tr>
<td>France</td>
<td>511</td>
<td>815</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>11,108</strong></td>
<td><strong>13,933</strong></td>
</tr>
</tbody>
</table>
Supplementary Figures
Supplementary Figure 1. Manhattan plots of association with melanoma risk 1 megabase (Mb) either side of the strongest SNP at each telomere-associated locus. SNPs that were genotyped in the all studies in the melanoma GWAS are indicated by black circles, those that were imputed in all studies by red circles and those that were genotyped in some and imputed in others by green circles. The location of the telomere-associated SNP is indicated by a vertical line. The SNP itself is marked with an asterisk if it reached p<0.05 (otherwise it is absent). The y-axis ranges from 0 (p=1) to 7 (p=10^{-7}) except for the TERT plot where SNPs in the region reached far higher statistical significances. Results based on meta-analysis of two-sided results from SNPTEST2 (34) using gene dosage and assuming an additive model. Chromosome (Chr) indicated on the x-axis of each plot.
Supplementary Figure 2. Estimated effect of each telomere-associated SNP on telomere length (beta from linear regression) plotted against estimated effect on melanoma risk (beta from logistic regression).
Supplementary Figure 3. Plot showing effect of telomere score on melanoma risk. Here the telomere score is divided into quartiles and melanoma case-control status regressed on the resulting categorical variable with the lowest quartile (by telomere length) as the baseline. This analysis does not include the Australian data. 95% confidence interval is indicated by horizontal bars, relative sample size of each group is indicated by the size of the squares. Exact odds ratios (ORs) are given in the right hand column.


in China.


