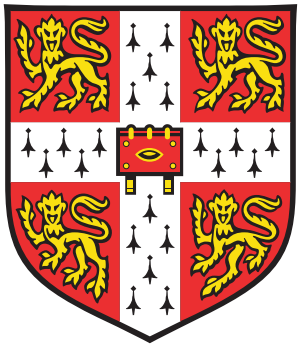
Drivers of melanoma susceptibility



Carla Daniela Robles Espinoza

Wellcome Trust Sanger Institute

University of Cambridge

This dissertation is submitted for the degree of

Doctor of Philosophy

Christ's College

September 2014

Para quienes siempre estuvieron allí:
María Elena, Gabriel, Gabrielito, José y Daniel.

Declaration

I hereby declare that except where specific reference is made to the work of others, the contents of this dissertation are original and have not been submitted in whole or in part for consideration for any other degree or qualification in this, or any other University. This dissertation is the result of my own work and includes nothing which is the outcome of work done in collaboration, except where specifically indicated in the text. This dissertation contains less than 60,000 words as per the requirements of the Degree Committee for the Faculty of Biology.

Carla Daniela Robles Espinoza
September 2014

Acknowledgements

First and foremost, I would like to thank my supervisor Dave Adams for welcoming me into his lab after a rather "interesting" first meeting, and for his kindness and patience over these last few years. I have learnt from him not only things related to my project and science but also important lessons in life. He is a true mentor to me.

I would like to thank all members of the Experimental Cancer Genetics Team at the Sanger Institute for filling every working day with fun and interesting lunch-time discussions, and for their disposition to help whenever I asked: James, Alistair, Mamun, Martín, Clara, Stefan, Marco, Chi, Louise, Rebecca, Marcela, Mia, Gabi, Theo and Richard, and to Ximena who is practically a member of the team as well. I also thank Thomas and Sendu, from the Vertebrate Resequencing team, for their patience and help with analysis pipelines.

Also, none of this work would have been possible without my wonderful collaborators: Julia, Tim and Mark, from the University of Leeds, Nelleke, from the Leiden University Medical Centre, and Nick, Lauren and Antonia, from QIMR Berghofer. I thank them for trusting me with the analysis of their melanoma families, for patiently answering all questions I asked, and for very valuable advice regarding this project. I would also like to thank Andrew, Víctor and Carlos, from the University of Oviedo, for their expertise in protein analyses (and for answering e-mails at strange times during the night!).

I would like to acknowledge Moyra, Yip and Lucy, whom I met the first week I arrived in Cambridge and who have become some of my closest friends. I would like to thank them for keeping me sane, making sure I ate at adequate times and generally taking care of me during these last years. Equally, I owe much of my present happiness to Abi, Rachael and Sarah, whom I met on my first day at Sanger. I thank them for their care, for listening, and for the fun weekend trips we had during these past four years. I would like to thank Carlos, Mariana and Claudia, for their everlasting friendship and support across different time zones.

I would like to acknowledge the Wellcome Trust and the Consejo Nacional de Ciencia y Tecnología of Mexico, my funding bodies, for making sure I was not homeless and

hungry during these last years, and for trusting in me. This work would not have been possible without their generous support.

I would like to thank, from the bottom of my heart, those who stayed back home and have not forgotten me: My childhood friends, María and Linda, for awaiting me every time I go back to Mexico, for their life advice, help and understanding. I would like to thank my parents, María Elena and Gabriel, and my little brothers, Gabrielito and José, for their love, their neverending support and enthusiasm.

Finally, with all my love, I would like to thank Daniel. Above all, for understanding my constant travelling and moving to different countries. I will be forever thankful to him for filling every day with laughter, and for his love, support and patience.

Abstract

Cutaneous melanoma is a cancer of melanocytes, the pigment-producing cells in our skin. It is one of the most aggressive human malignancies, constituting only about 2% of all dermatological cancers but being responsible for over 75% of all deaths from skin cancer. It has recently become a major public health problem, as it is now the fifth most common cancer in the United Kingdom after its incidence more than quadrupled in the last three decades. For these reasons, understanding the biological processes that are involved in its development is of great importance for devising novel treatments and for the management of patients in the clinic.

The study of the genetic factors that influence melanoma risk can uncover mechanisms that are relevant in the transition from a benign melanocyte to a malignant melanoma. Approximately 10% of all melanoma cases are familial, and about half of these familial cases can be explained by pathogenetic variants in genes such as cyclin-dependent kinase inhibitor 2A (*CDKN2A*), cyclin-dependent kinase 4 (*CDK4*), breast cancer 2 (*BRCA2*), BRCA1-associated protein-1 (*BAP1*) and in the promoter of the telomerase reverse transcriptase (*TERT*). However, about 50% of all familial melanoma cannot be explained by mutations in known genes. In this dissertation, I detail the methodology I followed in an effort to uncover additional high-penetrance melanoma susceptibility genes.

I analysed exome and genome sequence data from a total of 184 individuals that belong to 105 melanoma-prone families from the United Kingdom, The Netherlands and Australia that did not have any pathogenetic variants in known susceptibility genes. I applied different gene prioritisation strategies and developed novel software tools in order to devise a list of plausible melanoma susceptibility candidate genes; these analyses suggested that genes regulating telomere function could be influencing melanoma risk. After performing functional experimental analyses, our research team was able to determine that carriers of rare variants in the protection of telomeres (*POT1*) gene, a member of the shelterin complex that safeguards telomere integrity, are at high risk for developing melanoma. We successfully described the mechanism by which this

happens, showing that the variants identified either disrupt *POT1* mRNA splicing or abolish the ability of POT1 to bind to telomeres, and lead to increased telomere length in carriers when compared to melanoma cases with wild-type *POT1*.

The main finding of the work described in this dissertation is the identification of telomere dysfunction as an important contributor to the risk of developing melanoma, and possibly other cancers. Our analyses suggest that *POT1* is the second most commonly mutated high-penetrance melanoma susceptibility gene reported thus far, and moreover, that rare variants in this gene constitute the first hereditary mechanism for telomere lengthening in humans.

Contents

List of Figures

List of Tables

Nomenclature

Roman Symbols

ACD Adrenocortical dysplasia homolog

BAP1 BRCA1 associated protein-1

BRCA2 Breast cancer 2, early onset

CDK4 Cyclin-dependent kinase 4

CDKN2A Cyclin-dependent kinase inhibitor 2A

CLL Chronic lymphocytic leukaemia

GWAS Genome-wide association studies

IARC International Agency for Research on Cancer

MC1R Melanocortin 1 receptor

MITF Microphthalmia-associated transcription factor

MPM Multiple primary melanomas

NGS Next-generation sequencing

OB Oligonucleotide/oligosaccharide-binding

POT1 Protection of telomeres

QFMP Queensland Familial Melanoma Project

RB1 Retinoblastoma protein 1

SMG1 SMG1 phosphatidylinositol 3-kinase-related kinase

TERT Telomerase reverse transcriptase

WES Whole-exome sequencing

WGS Whole-genome sequencing