UNIVERSITY OF ALGARVE

CONSTITUTIVE *OGG1* VARIANT TOGETHER WITH *BRCA* MUTATIONS DISPLAY ACCELERATED TELOMERE SHORTENING

Sofia Maria Morgadinho Ferreira

Dissertation for the Master Degree in Oncobiology

Thesis supervisors

Dr. Ana Osório
Dr. Ana-Teresa Maia (UAlg)

2015

UNIVERSITY OF ALGARVE

CONSTITUTIVE *OGG1* VARIANT TOGETHER WITH *BRCA* MUTATIONS DISPLAY ACCELERATED TELOMERE SHORTENING

Sofia Maria Morgadinho Ferreira

Dissertation for the Master Degree in Oncobiology

Thesis supervisors

Dr. Ana Osório
Dr. Ana-Teresa Maia (UAlg)

2015

UAlg
UNIVERSIDADE DO ALGARVE

Constitutive *OGG1* variant together with *BRCA* mutations display accelerated telomere shortening

Declaração de autoria de trabalho

Setembro de 2015

Declaro ser a autora deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam da listagem de referências incluídas

_____

(Sofia Maria Morgadinho Ferreira)

UAlg
UNIVERSIDADE DO ALGARVE

Copyright

Setembro de 2015

Eu, *Sofia Maria Morgadinho Ferreira*, na qualidade de estudante (nº 42291) do mestrado Oncobiologia da Universidade do Algarve, declaro que não autorizo a divulgação dos dados contidos na Tese intitulada "**Constitutive *OGG1* variant together with *BRCA* mutations display accelarated telomere shortening**".

_____

(Sofia Maria Morgadinho Ferreira)

This thesis, submitted for the degree of Master at the University of Algarve, has been elaborated in the Human Cancer Genetics laboratory at the Spanish National Cancer Research Center (CNIO). The student was supported by the Exchange Programme Scholarship Erasmus +

Erasmus plus Exchange Programme Funding

Human Genetics Group

Human Cancer Genetics Programme

Spanish National Cancer Research Centre – Madrid, Spain

To my dearest and beloved family

Para a minha querida e amada família

Acknowledgements

This thesis would have taken far longer without the contribution of many others. Here, I express my immensurable appreciation and deepest gratitude to the people who contributed somehow for that.

My foremost thanks and gratitude go to my supervisors, **Dr. Ana Osório** and **Dr. Ana-Teresa Maia** for their extraordinary supervision, guidance since very early stages of my stay in CNIO and for their input in this thesis. From them I received the most valuable comments, constructive critics and knowledge that I will bring with me to my future career. My particular acknowledgement to the extreme kindness and very useful advices of **Dr. Ana Osório**, and to **Dr. Ana-Teresa Maia**, who since the very beginning have been a source of inspiration to pursue a scientific, and always found a time in her very thigh schedule to give her assistance.

I gratefully acknowledge **Dr. Javier Benítez**, Director of the Human Cancer Genetics Programme, to have hosted me in his Programme to perform my Master Research Project and for his close supervision of my work outcomes.

Thanks to **Carlos Benítez**, who made a close but relaxed supervision, which easily contributed to a positive work environment.

Thanks to my Spanish laboratory mates, **Ali**, **Javi**, **Bea**, **Nere** and **Carlos** included for their contagious joviality and good disposition, that always contributed to a very light and pleasant environment in my daily work. A particular thank to **Laura** for her kindness, support and active interest in my welfare, to **Oriol** for making my work time even more pleasant with his very bright and tasteful musical suggestions, and to **Maria** whit whom I got precious recommendations.

My thanks to **Irene**, who helped me to deal with the undesirable bureaucracies always with a great smile and good disposition.

A very sincere thanks to my dear **Ale**, for being there in the most crucial times, for her immense support and without her days would have passed more slowly. To **Tere,** for being the first and more compassionate one receiving in the laboratory, and for all much needed lucid and mature advices. To both, for encouraging me to fight for more and better and for providing such rich source of conversation and amusement.

A very special thanks with all my heart to **Sílvia**, *aquela míuda que curto tótil*, for her sensibility and determination, for her advices and her sharing, for her care and concern, without whom the learning curve would be steeper. Also, for making my stay in Madrid far more pleasant and familiar

My sincere thanks to **Martim**, whom is a great classmate and always supported and encouraged me to go abroad. Without his very inspirational words I would never had this opportunity to do my Master thesis project in such a great institute.

A special thanks to my extraordinary friends **Lilocas** and **Marta** for their helpful “informatic” support and obviously to their friendship.

Words fail me to express my deepest appreciation to my **boyfriend**, who made everything worth it and always made me believe, being the greatest source of encouragement, wisdom and love.

And above all, I offer my utmost gratitude to **all my Family**, without whom this thesis would have been possible. I will be eternally indebted to them, for their immeasurable love and endless support.

Abstract

Osorio, Milne et al. (2014) reported a SNP, rs2304277, in the *OGG1* gene, with evidence of potential association with increased ovarian cancer risk in *BRCA1* germline mutation carriers (p=4.8x10^{-3}). The protein OGG1 is a main player in the DNA BER pathway, responsible for recognizing and excising oxidized guanines (8-oxoG).

In the literature, oxidative stress has been well characterized to have a natural site-specific preference for guanines, in which telomeric DNA is enriched due to several TTAGGG repeats. 8-oxoG is highly mutagenic and affects DNA replication, and at the telomere level it can impair the recruitment and affinity of the shelterin complex proteins. This complex caps and protects telomeres from aberrant chromosomal rearrangements. Not only, when not properly corrected, 8-oxoG can give rise to ssDNA breaks. Therefore, telomeres are more susceptible to this kind of damage, and disruption of the normal telomere function and length can result in carcinogenesis, as a consequence of genomic instability. Hence, given the role of OGG1 and telomeres composition, we aimed to explore whether the increase of cancer risk in the carriers of rs2304277 (together with a germline mutation in the *BRCA* gene), might be due to an altered OGG1 function, which could accelerate the telomere shortening, resulting from a weaker response upon oxidative stress harms.

For the functional characterization of the polymorphism, the experimental approach was based on the evaluation of the *OGG1* mRNA expression levels by qPCR and measurement of telomeres length by High Throughput Q-FISH of the different *OGG1* genotypes and *BRCA1/2* mutation status from peripheral blood samples. Very preliminary results, suggest that the variant leads to a decrease in *OGG1* mRNA levels which, and together with a mutation in *BRCA* gene might contribute to an accelerated telomere shortening. These results might explain, in part, the increase of an individual's lifetime risk of developing cancer when harbouring both genetic conditions.

Keywords: OGG1, SNP, BRCA, Ovarian cancer, telomeres shortening, oxidative stress

Resumo

Recentemente, Osorio, Milne et al. (2014) realizaram uma análise compreensiva de dezoito genes que envolvem a via de reparação de *DNA Base Excision Repair* (BER), numa ampla série de portadores de uma mutação na linha germinal em um dos genes de elevada penetrância *BRCA1* ou *BRCA2* provenientes do consórcio CIMBA (Consortium of Investigators of Modifiers of BRCA1 and BRCA2). A escolhida via de reparação de ADN, BER, foi feita com base no conceito de letalidade sintética entre membros da via BER, como PARP1 (Poly ADP ribose polymerase), BRCA1 e BRCA2.

Desses 18 genes escolhidos, foram identificados onze SNPs (polimorfismos de base única), com evidências de associação com o aumento de risco para o desenvolvimento de cancro da mama e/ou dos ovários. Desses onze SNPs, cinco localizam-se em genes de ADN glicosilases, e aqueles com mais fortes evidências de associação ao risco de desenvolver os referidos cancros, localizam-se em dois genes de ADN glicosilases: rs1466785 no gene *NEIL2* (endonuclease VIII-like2) (HR: 1.09, 95% CI (1.03–1.16), p= 2.761023) para o cancro da mama em portadores de uma mutação no *BRCA2*, e rs2304277 no gene *OGG1* (8-guanine DNA glycosylase) (HR: 1.12 95%CI: 1.03–1.21, p= 4.861023) para o cancro dos ovários em portadores de uma mutação no gene *BRCA1*. Este estudo focou-se e contribuiu para a caracterização funcional do polimorfismo rs2304277 no gene *OGG1*, de forma a estabelecer uma possível explicação para a sua associação com o aumento de risco de desenvolver cancro, mais concretamente cancro do ovário em portadores de uma mutação na linha germinal no gene *BRCA1*.

8-oxoguanina DNA glicosilase, OGG1, é uma enzima com um papel fundamental nas etapas primárias de ação da via de reparação BER, sendo responsável por fazer o reconhecimento e excisão de bases quimicamente alteradas que perturbam minimamente a hélix do ADN, no caso concreto desta ADN glicosilase, as guaninas oxidadas: 8-oxodeoxyguanosina (8-oxoG). Na literatura, está muito bem descrita e caracterizada uma tendência natural do stress oxidativo sobre as guaninas, que torna o ADN telomérico mais suscetível e sensível a este tipo de agressão, uma

vez que são estruturas enriquecidas em guaninas, devido às suas várias repetições da sequência “TTAGGG”.

8-oxoG é o produto mais comumente gerado por stress oxidativo e é altamente mutagénico pois possuí uma grande propensão em emparelhar com adeninas, podendo conduzir a transversões GC para TA durante a replicação do ADN. Embora essa transversão possa ser não-sinónima, alterando o produto final da proteína e, num cenário mais drástico, a sua função, a substituição de guaninas por timinas diminui o recrutamento e afinidade das proteínas que cobrem e protegem fisicamente os telómeros. O conjunto destas proteínas forma um complexo denominado de *shelterin*.

As proteínas do complexo shelterin impedem o reconhecimento das extremidades cromossomais, conhecidas por telómeros, como quebras intra-cromossomais formando uma configuração fechada destas estruturas. Desta forma, os mecanismos de reparação de ADN danificado não são ativados e, consequentemente, os telómeros não são sujeitos a fusões cromossómicas aberrantes, o que levaria à instabilidade cromossómica. Na literatura, está muito bem descrito que telómeros criticamente curtos podem funcionar como um promotor da carcinogénese devido à sua subsequente instabilidade cromossómica.

No entanto, este não representa o único problema relativo às guaninas oxidadas. Algumas ADN polimerases, as de maior fidelidade, poderão encontrar dificuldades na replicação de ADN na presença de 8-oxoG, uma vez que guaninas oxidadas conduzem a uma ligeira modificação na cadeia de ADN. Comprometendo assim a replicação normal do ADN e possivelmente a dos telómeros, podendo contribuir ainda para um encurtamento dos telómeros mais acelerado. A disrupção do tamanho normal dos telómeros e da sua normal função pode resultar em carcinogénese, como consequência da instabilidade genómica advinda de telómeros curtos que acabam por perder a proteção do complexo shelterin. Telómeros criticamente curtos têm sido também descritos como promotores de tumores. As quebras de ADN, mais especificamente em apenas em uma das cadeias (ssDNA) podem também acontecer quando a intervenção da via BER não é corretamente executada ou concluída. Os sítios abásicos provocados pela intervenção BER

fragilizam o ADN facilitando a sua quebra. Quebras de ADN de cadeia simples são assim as maiores responsáveis pelo encurtamento acelerado de telómeros, segundo von Zglinicki, Pilger et al. (2000).

ADN oxidado é a maior fonte de danos de ADN, e o stress oxidativo poderá vir tanto de fontes endógenas (espécies reactivas de oxigénio provindas do metabolismo celualr) como exógenas (elementos patogénicos ou radiação ionizante). O que propencia a tumourigénese é o desequilíbrio entre as espécies reativas de oxigénio (ROS) e as defesas antioxidantes, tal como OGG1. Resumidamente, os telómeros são estruturas mais suscetíveis à oxidação, devido ao seu conteúdo rico em guaninas que são entre as bases, as preferencialmente oxidadas. As consequências de 8-oxoG no ADN telomérico podem promover uma disrupção no tamanho e função dos telómeros conduzindo a um encurtamento acelerado e crítico dos telómeros, destabilizando a homeostase cromossómica levando à génese tumoral.

Portanto, dado o papel de OGG1 no reconhecimento e correção das 8-oxoG e a composição enriquecida dos telómeros em guaninas, procurámos explorar se o aumento de risco de desenvolver cancro nos indivíduos portadores do polimorfismo rs2304277 no gene *OGG1* e uma mutação nos genes *BRCA1/2* se deve a uma perturbação na eficácia da ação da enzima OGG1, que poderá favorecer um encurtamento de telómeros mais acelerado, como resultado de uma resposta alterada às consequências provocadas pelo stress oxidativo.

Para a caracterização funcional do polimorfismo, a abordagem experimental foi feita com base na avaliação dos níveis de expressão de mRNA de *OGG1* por qPCR e medição do tamanho dos telómeros (TL) por High Troughput Q-FISH para os diferentes genótipos de OGG1 (wild type ou portadores da variante) e *BRCA* status de amostras de sangue periférico. Resultados muito perliminares, sugerem que a variante possa levar à diminuição dos níveis de mRNA de *OGG1* que, juntamente com uma mutação no gene *BRCA* contribuí a um encurtamento dos telomeros mais acelarado, o que poderá explicar em parte, o aumento de risco de desenvolver cancro em pessoas que abrigam ambas as condições genéticas.

Sendo o cancro uma doença poligénica, são então importantes estudos como este, feitos com base na descoberta e caracterização de variantes genéticas que possam contribuir para o desenvolvimento de cancro, contribuindo para uma melhor avaliação de risco da população saudável.

List of Figures and Tables

FIGURES

TABLES

Table of contents

1. Abbreviations

Abbreviations are displayed in alphabetic order

	8-oxoG	8-oxodeoxyguanosine
	53BP1	53 binding protein 1
A	**A**	Adenine
	AP	Apurinic/apyrimidinic
	APE	Apurinic or apyrimidinic endonuclease
B	**BER**	Base excision repair
	BRCA1	Breast cancer susceptibility gene 1
	BRCA2	Breast cancer susceptibility gene 2
	BRCAX	Breast cancer susceptibility gene X
C	**CIMBA**	Consortium of investigators of modifiers of *BRCA1* and *BRCA2*
D	**DDR**	DNA damage response
	DHPLC	High-performance liquid chromatography
	dsDNA	Double-stranded DNA
F	**F**	Forward
	FBC	Familial breast cancer
	FBOC	Familial breast and ovarian cancer
G	**G**	Guanine
	GWAS	Genome-wide association studies

H	**HR**	Homologous recombination
L	**LD**	Linkage disequilibrium
M	**miRNA**	MicroRNA; micro ribonucleic acid
	MRN	Mre11-Rad50-Nbs1 complex
	mRNA	Messenger ribonucleic acid
N	**NEIL2**	Endonuclease VIII-like2
	NHEJ	Non-homologous end joining
O	**OGG1**	8-oxoguanine DNA glycosylase
P	**PARP1**	Poly ADP ribose polymerase
	PCR	Polymerase Chain Reaction
	POT1	Protection of telomeres 1
Q	**Q-FISH**	Quantitative Fluorescence in situ hybridization
	qPCR	Quantitative Polymerase Chain Reaction
R	**R**	Reverse
	RAP1	Repressor and activator protein 1
	ROS	Reactive oxygen species
	RT-PCR	Reverse Transcription Polymerase Chain Reaction
S	**SNP**	Single-nucleotide polymorphism
	ssDNA	Single-stranded DNA

	TIN2	TRF1-interacting protein 2
	TL	Telomere length
T	**TRF1**	Telomeric repeat-binding factor 1
	TRF2	Telomeric repeat-binding factor 2
	TPP1	POT1- and TIN2 interacting protein
U	**UTR**	Untranslated region
W	**wt**	Wild type

2. Glossary

Concepts are displayed in alphabetic order

C	**Causal SNP**	The SNP which is affecting a trait/phenotype/disease
F	**Fine Mapping**	Pos-genotyping analysis with greater resolution that searches for the causal SNP at a susceptibility locus
	Five-year survival rate	Percentage of patients who are alive at least five years after the diagnose of their cancer
	Five-year relative survival rate	A more accurate prognosis with a percentage of a group of patients with a certain type and stage of cancer, who are alive at least five years after the diagnose of their cancer
H	**Haplotype**	A set of alleles in LD inherited in a dependent way, as a unit
	HapMap Project	Catalogue that aimed to map the haplotypes of the human genome, describing what there variants are and how they are distributed among populations
I	**Incidence**	Number of new cases of a specific disease occurring during a certain period in a population
L	**Linkage Disequilibrium**	Correlation of neighbouring alleles at different loci, that are inherited dependently, reflected in haplotypes. In other words, individuals who carry a specific SNP are often predictably carrying specific nearby alleles that are in LD
M	**Major Allele**	epresents the commonest allele in a given population
	Minor Allele	Represents the least common allele in a given population
P	**Polymorphism**	Genetic variation present in a population

	Polygenic Model	Describes a trait/phenotype/disease which is influenced not by a single gene but for more
	Prevalence	Total number of cases with a specific disease in a population at a certain period
R	**Risk Factors**	Anything that might affect the chance of an individual of getting the disease
S	**Synthetic Lethality**	Combination of perturbations in at least two genes/proteins leads to cellular death
	SNP	DNA sequence variation occurring in at least 1% of the population. Polymorphic site which is diallelic, having a major allele and a minor allele
	Susceptibility	Likelihood to be influenced or harmed by a particular condition
T	**TagSNP**	Representative SNP in a determined region of the genome with high LD ($r^2 \geq 0.8$) with a group of other SNPs: haplotype

3. Introduction

3.1. Telomeres

Derived from the Greek words *telos* and *meros* meaning “end” and “part”, respectively, telomeres are the structures that caps the chromosome ends representing a crucial role in the genome integrity maintenance (Aubert and Lansdorp 2008). Discovered and described for the first time in the early 1930’s by Herman Muller and Barbara McClintock (Mason and Perdigones 2013).

These eukaryotic specialized nucleoproteins structures prevent abnormal chromosomal fusion or rearrangements shielding the natural ends of linear chromosomes that resemble DNA breaks (Rhodes and Giraldo 1995, O'Sullivan and Karlseder 2010). Since linear DNA fragments are toxic to mammalian cells, enzymes that degrade or repair those fragments are activated, and inappropriate repair can lead to genomic instability and, eventually, carcinogenesis (O'Sullivan and Karlseder 2010). Hanahan and Weinberg (2011) include genome instability in the ten cancer hallmarks. Telomeres are therefore vital structures for maintenance of genomic integrity.

Mammalian telomeres are made of long stretches of double-stranded DNA TTAGGG repeats (9-15kb in humans) and at the 3’ end are 50-300 nucleotide single-stranded repeats, the so-called G-overhang (Figure 3.1 and Figure 3.2, left) (O'Sullivan and Karlseder 2010). Telomeric-loop, also referred as T-loop, is the end structure in telomeres that works as a protective cap masking the natural ends of the telomeres from the DNA damage response (DDR) machinery, through a closed configuration (O'Sullivan and Karlseder 2010).

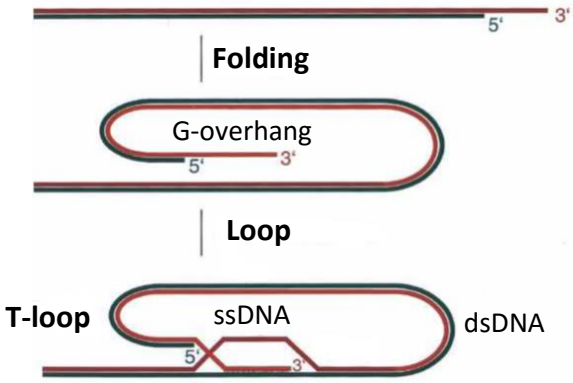


Figure 3.1 T-loop structure. The G-overhang (ssDNA) bends into the double-stranded DNA (dsDNA) telomeric repeat array. Adapted from de Lange (2005)

3.1.1. SHELTERIN COMPLEX

Besides this T-loop configuration, the distinction of telomeres from intra-chromosomal breaks is also ensured by the recruitment of the shelterin complex protein to the TTAGGG telomeric repeats. The shelterin complex has a wide and vital role in the telomere length regulation and protection (Figure 3.2, right) (Liu, O'Connor et al. 2004, de Lange 2005, O'Sullivan and Karlseder 2010). These proteins cap the chromosome ends hiding them from the surveillance of the DDR, avoiding an inappropriate action from the DNA repair pathways, which would process telomeres ends as intra-chromosomal breaks and start fusing them (de Lange 2005). Shelterin proteins also have a part in the intracellular signalling for cell proliferation, DNA repair and recombination regulation (Xin, Liu et al. 2008).

This complex consists of six proteins: telomeric repeat-biding factor 1 and 2 (TRF1 and TRF2); repressor and activator protein 1 (RAP1); TRF1-interacting protein 2 (TIN2); protection of telomeres (POT1) and POT1- and TIN2 interacting protein (TPP1) (Figure 3.2, right) (Xin, Liu et al. 2008, O'Sullivan and Karlseder 2010). TRF1, TRF2 and POT1 recognize and bind to TTAGGG repeats and interrelate with the rest of the shelterin proteins: TIN2 TPP1 and RAP1, allowing cells to differentiate telomeres from DNA damaged sites (de Lange 2005).

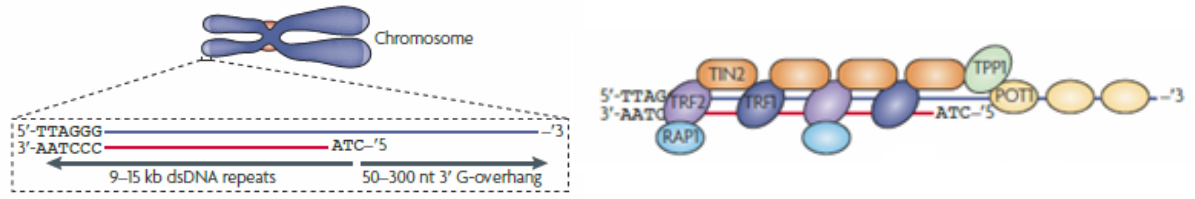


Figure 3.2 Structure of human telomeres consists in many kilobases of TTAGGG repeats that extend in the 3' direction, forming the G-overhang strand (left image). Shelterin complex (TRF1, TRF2, RAP1, TIN2, POT1 and TPP1) covers the double- and single-strand telomeric DNA, protecting these structures (right image). Adapted from O'Sullivan and Karlseder (2010).

3.1.2. TELOMERE SHORTENING

Losing around 100-200 bp in every division (Aubert and Lansdorp 2008), somatic cells can only undergo a limited number of divisions before telomeres become

dysfunctional (< 3Kb) and establish replicative senescence (Figure 3.3). Telomeres are therefore vital to preserve genetic information and integrity in each cell division (Sun, Tan et al. 2015)

Telomerase is a ribonucleoprotein, which binds to the first few nucleotides in the 3' end of telomeres, and adds the six-nucleotide repeating sequence: 5'-TTAGGG-3', using RNA primers as template. Like that, it elongates telomeres reversing their shortening, a normal consequence of cell division (Weinrich, Pruzan et al. 1997, Cong, Wright et al. 2002).

During normal cell division, somatic cells do not express or have low expression of telomerase and therefore, have progressive telomere shortening (Hodes 1999), but is strongly active in germ, stem and most of the tumour cells, in order to prevent telomere attrition (Mason and Perdigones 2013).

Critically short telomeres can lose their shelterin complex bond domains, and as mentioned above, activate DDR mechanisms. To avoid recognition of critically short telomeric ends as double-stranded (dsDNA) breaks and inappropriate end-to-end DNA fusions, the cell gets into a terminal arrest, known as replicative senescence (Aubert and Lansdorp 2008, Munoz-Espin and Serrano 2014, Sun, Tan et al. 2015). This occurs when a cell reaches its limit of replicative lifespan being a major tumour suppressor mechanism together with apoptosis (Collado, Blasco et al. 2007, Munoz-Espin and Serrano 2014).

Cells in which this tumour suppressor mechanism, replicative senescence, does not work properly, might go through inappropriate DNA fusions and rearrangements, which can favour consequently, translocations, aneuploidy and amplifications/deletions (Figure 3.4) (Artandi and DePinho 2010). These phenomena of aberrant rearrangements lead to chromosomal instability, which is one of the two most important cancer hallmark (Hanahan and Weinberg 2011).

There are several retrospective case-control and longitudinal studies suggesting that short telomeres may predispose to cancer (Wu, Amos et al. 2003, Shao, Wood et al. 2007, Wentzensen, Mirabello et al. 2011, Zhang, Chen et al. 2015)

T

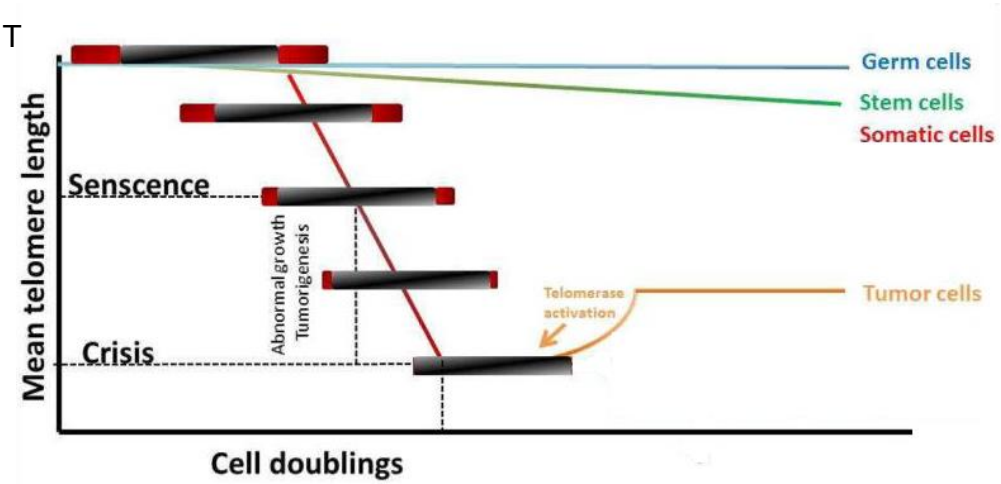
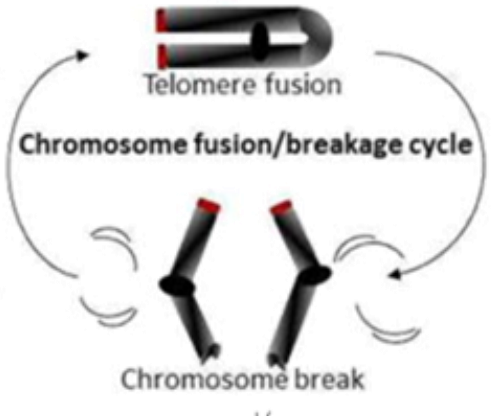


Figure 3.3 Telomere Length dynamics in germ, stem, somatic and tumour cells. Adapted from Mason and Perdigones (2013).



and/or translocations, aneuploidy,
amplifications or deletions

Figure 3.4 Critically short telomeres can undergo into aberrant fusions, promoting chromosome breaks, translocations, aneuploidies, amplifications and deletions. Adapted from Mason and Perdigones (2013).

3.1.2.1. OXIDATIVE STRESS AND ITS CONTRIBUTE TO TELOMERE CRISIS

Besides the normal cell division and aging, oxidative stress is also a contributor to telomere shortening/crisis due to its natural site-specific preference to oxidize guanines, for which telomeric DNA is enriched (TTAGGG repeats) (Oikawa and Kawanishi 1999)**.**

Oxidized guanines, also called 8-Oxodeoxyguanosines (8-oxoG), are the most common product generated by oxidative stress, and are highly mutagenic because of

their propensity to mismatch with Adenine promoting GC-to-TA transversions during DNA replication (von Zglinicki 2002). Despite the fact that those transversions can be non-synonymous, modifying the final product of a protein and its function, the loss of guanines to thymines, at telomeres level, decreases the recruitment and affinity of the shelterin complex, possibly leading to aberrant and inappropriate end-to-end fusions, since the shelterin complex is no longer able to mask the telomeric ends from the DDR (Figure 5) (von Zglinicki 2002, Klaunig, Kamendulis et al. 2010, Wang, Rhee et al. 2010, Georgakilas 2012). Cellular processes such as apoptosis, ageing and chromosomal stability maintenance are affected by the loss of the shelterin complex, insofar as mediators of the telomeres signalling (Figure 3.5) (Oikawa and Kawanishi 1999, von Zglinicki 2002, Coluzzi, Colamartino et al. 2014).

Additionally, 8-oxoG can contribute to disturb telomere homeostasis also by affecting the DNA replication, as they can lead to change in the normal DNA molecule conformation, which can hinder the binding of the high-fidelity DNA polymerases during normal replication, and possibly disrupting telomere length and its function (Figure 3.5) (Oikawa and Kawanishi 1999, von Zglinicki 2002, Wang, Rhee et al. 2010).

Several studies have shown higher levels of 8-oxoG in various human cancers: Miyake, Hara et al. (2004), Weiss, Goode et al. (2005), Diakowska, Lewandowski et al. (2007) and Tanaka, Fujita et al. (2008).

Oxidative stress also gives rise to DNA breaks, mostly single-stranded DNA (ssDNA) breaks, either generated as a consequence of the disintegration of the oxidized sugar or the intervention of DNA Base Excision Repair (BER) pathway over the oxidized bases that create abasic sites (Oikawa and Kawanishi 1999, von Zglinicki, Pilger et al. 2000, von Zglinicki 2002, Klaunig, Kamendulis et al. 2010, Georgakilas 2012). A study (von Zglinicki, Pilger et al. 2000) has shown that the accumulation of ssDNA breaks is the major responsible for telomere shortening in human fibroblasts.

Oxidized DNA is the major source of DNA damage in living organisms, and both endogenous and exogenous sources of oxidative stress can contribute to carcinogenesis when the load of Reactive Oxygen Species (ROS) is not counteracted by the antioxidant defences (Figure 3.6) (Klaunig, Kamendulis et al. 2010). In

conclusion, since telomeric DNA is a highly protected structure its repair is not that efficient, making it more vulnerable to aggressions, e.g. oxidative stress, as the proteins that cover it hamper the DNA repair machinery access (von Zglinicki 2002, Coluzzi, Colamartino et al. 2014).

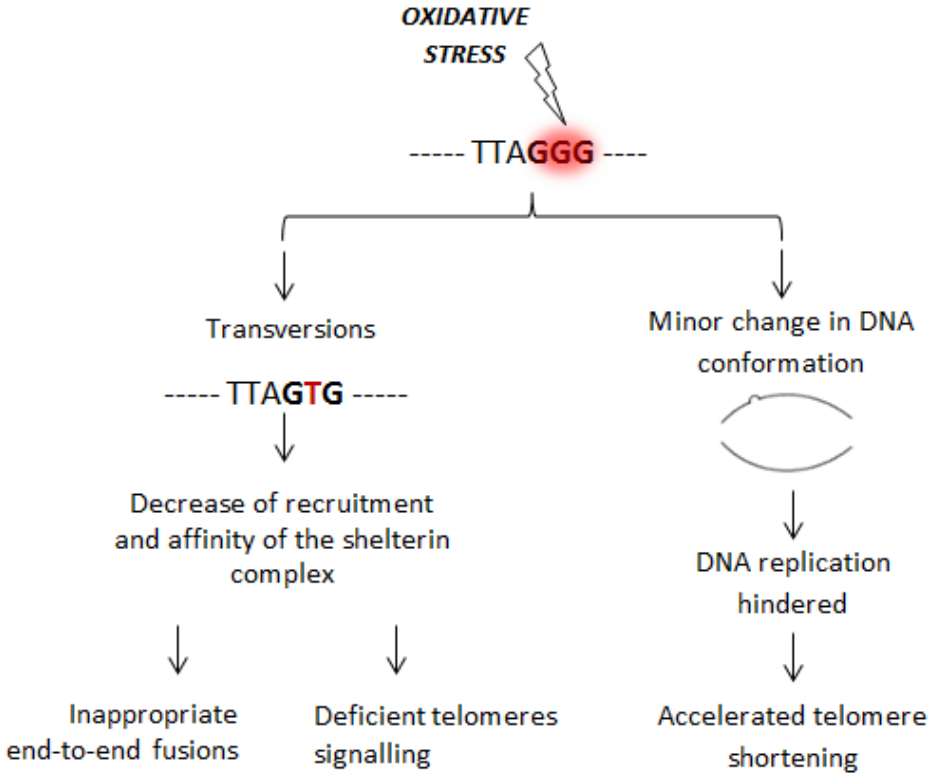


Figure 3.5 **Oxidative stress effects over telomeres.**

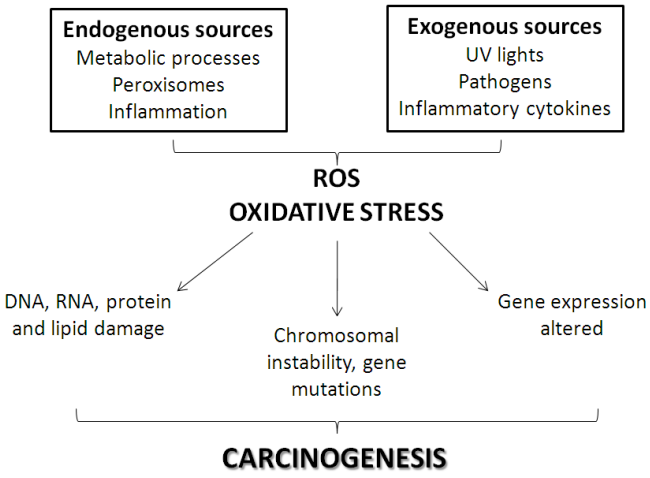


Figure 3.6 **ROS effect in cells and its role in cancer development**

3.2. Base Excision Repair pathway

The BER pathway is the principal responsible for the correction of oxidative lesions in the DNA, being the unique process specialized in the single-base lesions reparation with only four core proteins: a DNA glycosylase, an apurinic or apyrimidinic endonuclease (APE), a DNA polymerase and a DNA ligase (Kow 1994, Kim and Wilson 2012). BER action mode is the following: the damaged base is excised by the DNA glycosylase creating an apurinic/apyrimidinic (AP) site; the AP endonuclease cleaves the AP site generating a 3'OH and 5' deoxyribose phosphate terminus, which is forward filled by the DNA polymerase and sealed by the DNA ligase (Figure 3.7) (Kow 1994, Mendelsohn 2008, Kim and Wilson 2012). Additionally, BER pathway also corrects damaged bases by alkylation (Sedgwick, Bates et al. 2007) and deamination (Kavli, Otterlei et al. 2007). All BER repair processes restore the appropriate DNA base with high accuracy, maintaining the template function of the DNA and avoiding the decay of genetic information (Jacobs and Schar 2012, Kim and Wilson 2012).

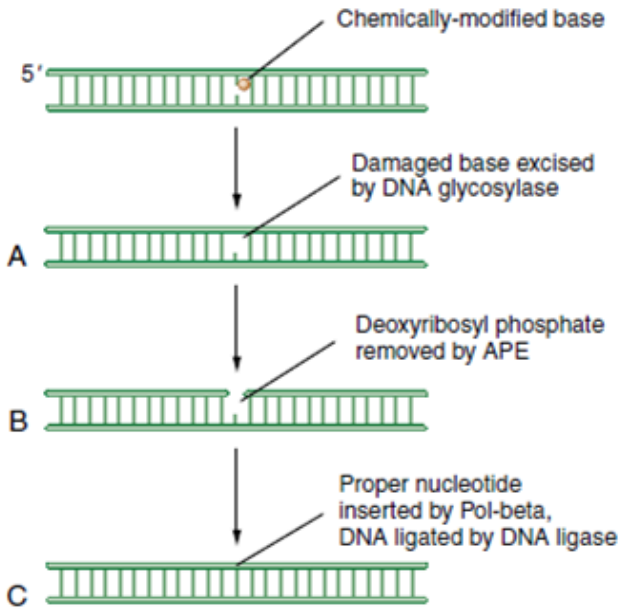


Figure 3.7 Scheme of Base excision Repair pathway action mode. Small lesions in DNA are often recognized and corrected by BER mechanisms repair. At first the lesion is recognized and flipped out by DNA glycosylase**(A)**, and then the phosphodiester backbone of the DNA is cleaved by APE **(B)**. An error-free DNA polymerase (Pol-β) fills the gap with the appropriated nucleotide ending with DNA ligase sealing the base to the double-stranded DNA restoring the normal sequence**(C)**. Adapted from (Mendelsohn 2008)

3.2.1. 8-oxoguanine DNA glycosylase, OGG1

OGG1, 8-oxoguanine DNA glycosylase, is an enzyme that acts in the early steps of the mentioned pathway. First found in yeast (Nash, Bruner et al. 1996) and later in humans (Radicella, Dherin et al. 1997), OGG1 is an enzyme which plays a key role in the repair of such lesions, making the recognition and excision of the chemically modified bases causing minor perturbations in the DNA helix, cleaving the glycosidic bond (Jacobs and Schar 2012, Kim and Wilson 2012). Single-base lesions are then flipped out generating an abasic- AP-site, being further processed by the subsequent enzymes, in order to restore the original DNA sequence before the DNA polymerase gets the opportunity to miss-insert an adenine, preventing a transversion mutation (Jacobs and Schar 2012).

Up till now, eleven different DNA glycosylases have been identified in mammals which can be divided into distinct superfamilies (Jacobs and Schar 2012). OGG1 is a helix-hairpin-helix glycosylase (Figure 3.8) whose respective motif is able to recognize both single-strand and double-strand DNA, by hydrogen bond-mediated interactions with the DNA-phosphate backbone (Shinmura and Yokota 2001). Such interactions are fundamental for DNA glycosylases function.

The *OGG1* gene is localized in the human chromosome 3p25.3 and consists of 11 exons, covering about 38.275 bp of DNA. Even though with 18 different possible transcripts, its principal protein product has 424 amino acids residues with a theoretical molecular weight of 4.95kDa (Ensembl 2015).

Ablation of *ogg1* in *Saccharomyces cerevisiae* leads to the accumulation of G->T transversion mutations (Thomas, Scot et al. 1997), and results in telomere base damage and length alteration (Lu and Liu 2010). Also, the *Ogg1* null mice exhibit abnormal level of chromosomal 8-oxoG but are still viable (Klungland, Rosewell et al. 1999). In humans, there is evidence that non-synonymous polymorphisms in the *OGG1* gene might associate with cancer (Zhou, Li et al. 2015), such as non-small cell lung cancer (Duan, Hua et al. 2012) and esophageal cancer (Wang, Gan et al. 2013). Moreover, Osorio, Milne et al. (2014) showed an association between a polymorphism

in the 3'-untranslated region (UTR) in the *OGG1* gene and cancer risk in Breast Cancer Susceptibility Genes 1 and 2 (*BRCA1* and *BRCA2*) germline mutation carriers.

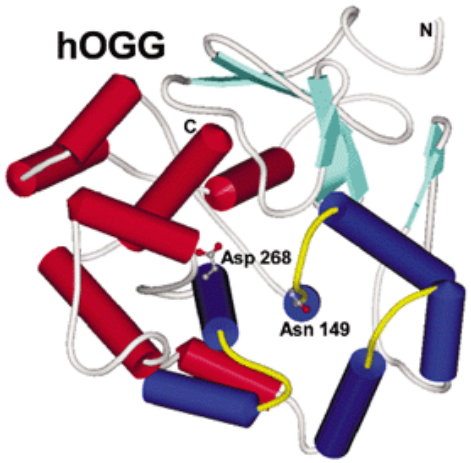


Figure 3.8 Structural representation of hOGG1. The six helices are coloured in dark blue and the three DNA binding loops in yellow. Adapted from (Drohat, Kwon et al. 2002)

3.3. Cancer

The denomination of cancer was initially given by the "Father of Medicine", Hippocrates (460 – 370 BC) as *karkinos* and *karkinoma* to describe non-ulcer and ulcer forming tumours, respectively. This Greek designation for crab was most likely applied because of the finger-like projections, which remind the crab legs shape. Later the "cancer" domination was given by the roman physician, Celsus (28 – 50 BC), which translated the previous Greek words into Latin (ACS 2014).

Cancer is a common designation for many different diseases (Britannica 2015). Although, there are many kinds of cancer, they share this designation because different cancers start the same way: an abnormal cell growth out of control behaving like autonomous cells (Mendelsohn 2008, NCI 2015). All body cells can effectively become into tumour/cancer, but each type has its own characteristics (UK 2015).

Carcinogenesis is a multistage disease in which not one, but several mutations are required. At least three to six mutations seem to be the necessary to reach this development (Vogelstein and Kinzler 1993). These mutations have to be acquired by

oncogenes and/or tumour suppressor genes, in a way that affects the net rate of cell division. According to Land, Parada et al. (1983), at least one mutation in two cooperating oncogenes are necessary for a tumourigenic transformation. Not only a multistage disease, cancer is also multifactorial, combining genetic and environmental factors (Hanahan and Weinberg 2011).

Before reaching the malignant stage, denominated cancer, tumour cells undergo through a series of stages, which in the majority of cases include: a hyperplasia (characterized by an increased number of cells in a tissue), dysplasia (where the tissue looks abnormal and disorganized but still not invasive, and cells look less differentiated). Cancer stage is achieved when tumour cells acquire invasive properties (Mendelsohn 2008).

Hanahan and Weinberg (2011) defined the necessary functional competences that a cell needs to contribute to genesis of malignancies: the hallmarks of cancer, which allow cancer cells to survive, proliferate and disseminate. Those hallmarks include: resistance to cell death; genome instability and mutation; sustenance of proliferative signalling; replicative immortality; escape from immune system surveillance; evasion of growth suppressors; deregulation of cellular energy; angiogenesis induction; inflammation promoted by tumour and activation of invasion and metastasis properties (Figure 3.9).

The acquisition of this set of malignant competences is mainly regulated by genomic instability and premalignant inflammation. The first one involves processes that are involved in major reprogramming processes of cell growth/proliferation and metabolism, the second allows cancer cells to escape from immune system surveillance and antagonistically operates for an enhancement of tumour progression through the immune system (Hanahan and Weinberg 2011).

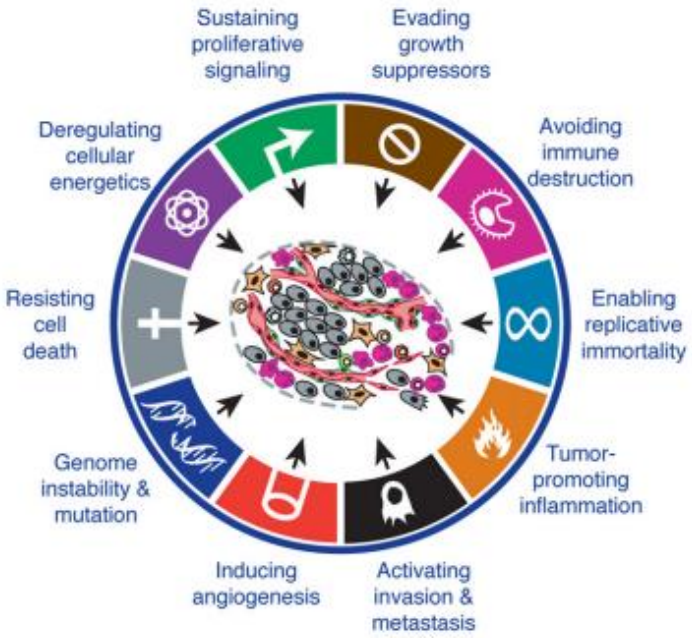


Figure 3.9 Hallmarks of cancer that gives the necessary functional competences a cell need to contribute to carcinogenesis. Adapted from Hanahan and Weinberg (2011)

3.4. Breast cancer and Ovarian Cancer

3.4.1. Epidemiology

Breast:

According to GLOBOCAN (2012), female breast cancer is by far the most frequent cancer, the most common cause of death (Figure 3.10) and the most frequently diagnosed cancer among women worldwide, accounting for 23% of all women's malignant cancers. In 2012, 1,67 million new breast cancer cases were diagnosed representing 25% of all diagnosed cancers (GLOBOCAN, 2012).

The lifetime risk for breast cancer, in the United States, is approximately 1 in 8, and 12.3% of worldwide women will be diagnosed worldwide with breast cancer at some point during their lifetime (Ghoussaini, Pharoah et al. 2013).

With respect to prevalence, in 2011, 2,899,726 women were living with breast cancer. The 5-year survival rate is 89.2%. Such a high percentage is influenced by the stage at the time of the diagnosis, referring to the extent of cancer, where 61% is detected in the localized state (Figure 3.11) (NCI 2015).

Ovary:

As to the incidence in 2012, around 239,000 new cases of ovarian cancer were reported, representing nearly 4% of the new cases of women cancer. Ovarian cancer is the seventh most common cancer affecting women worldwide, and the 18th overall (Ferlay, Soerjomataram et al. 2015).

Among all cancers of the female reproductive system, ovarian cancer is the one that causes more deaths, being the eighth most common cause of cancer death in women worldwide. 14 030 deaths were estimated for 2013. The five-year relative survival rate is 44%, considering all the stages of diagnosis (Ferlay, Soerjomataram et al. 2015). In 2012, 192,446 women were living in the United States with ovarian cancer, and approximately 1.3% of women will be diagnosed at some point of their lifetime (GLOBOCAN 2012).

This type of cancer is usually fatal because most of the cases (60%) are diagnosed in an advanced stage, when the cancer is already metastasized. The five-year relative survival rate for this stage is very low, being 28.3% (Figure 3.12) (Cronin, Ries et al. 2014).

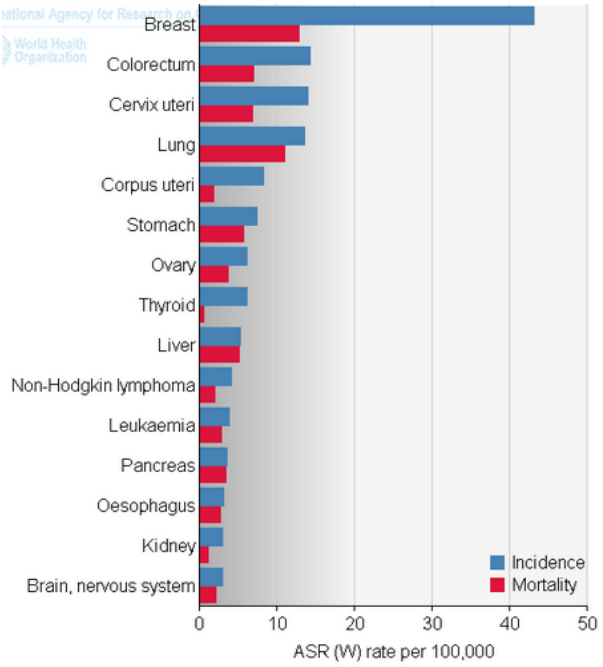


Figure 3.10 Estimate incidence and mortality rates of the different cancers per 100 000 women per year (ASR: age-standardised rate). Adapted from GLOBOCAN (2012)

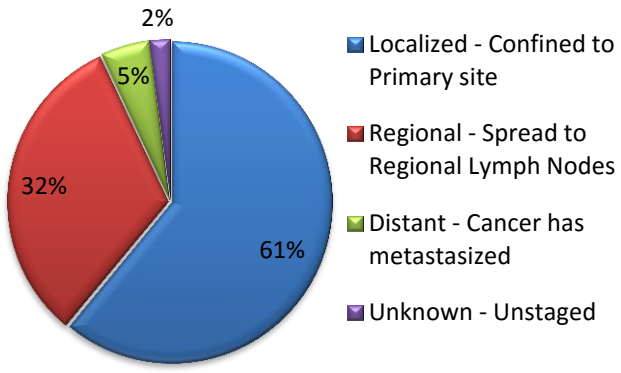


Figure 3.11 Pie with the representative percentages of the different stages of breast cancer at the time of diagnosis. Adapted from

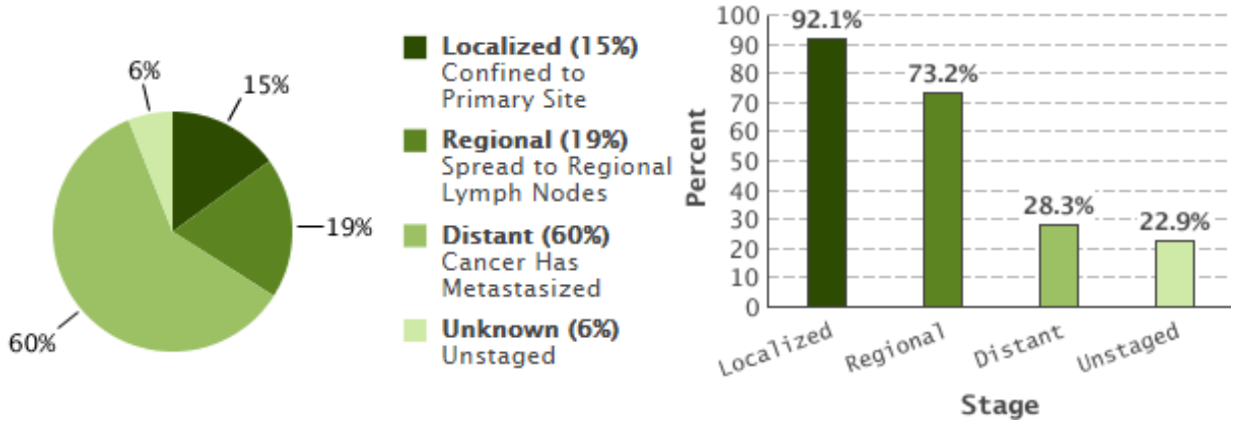


Figure 3.12 **Percentage of ovarian cancer diagnosed** (left) **and 5-year relative survival by stage** (right)**.** Adapted from Cronin, Ries et al. (2014)

3.4.2. Risk factors

The risk factor concept involves anything that might affect the chance of an individual of getting the disease (WHO 2015). A risk factor can be environmental or even genetic. Evidently, different cancers have different risk factors and different load of the relative risk.

Breast:

Together with gender, i.e. being a woman, the highest risk factor for breast cancer is the age, with the incidence rate higher among older women, although, the risk increases across all ages until 80 years-old (UK 2015).

Another risk factor that substantially increases the risk of developing breast cancer is personal history of the disease (Figures 2013). The risk is highly correlated with the number of affected first-degree relatives: 1.8 times higher for women with one first-degree relative affected, 3 times higher with two, and nearly 4 times higher with 3 or more affected relatives (Epidemiology 2015). The younger the age of onset of the affected relative, the greater the risk for a woman to develop the disease (Figures 2013, Epidemiology 2015).

Up to date, have been identified high-, moderate- and low-susceptibility genes that modifies the risk for breast cancer. For instances, mutations in the *BRCA1* (Hall,

Lee et al. 1990) and *BRCA2* (Wooster, Neuhausen et al. 1994) high-susceptibility genes increase the risk for breast cancer, but also to ovarian, and other cancers. Deleterious mutations in the *BRCA1* gene confer around 65% increase of cumulative risk for breast cancer, while deleterious mutations in the BRCA2 gene confers around 39% (Antoniou, Pharoah et al. 2003, Chen, Iversen et al. 2006, Chen and Parmigiani 2007, Milne, Osorio et al. 2008).

It is believed that differences in worldwide incidence rates are due to the variability in reproductive patterns, hormonal factors, lifestyle and environmental factors. All these factors are partially responsible for modulating the probability of breast cancer development throughout a woman's lifetime. Table 1 briefly introduces some of the known risk factors with their relative risk values (Figures 2013, Epidemiology 2015, UK 2015).

Ovary:

Like breast cancer and most other cancers, the risk of developing ovarian cancer increases with age. Most of the ovarian cancers are developed after menopause. Women who had a first full-term pregnancy after 35 years-old or who never carried a pregnancy to term show higher risk. Also, breastfeeding may lower the risk (UK 2015).

Long-term use of hormonal contraceptives, on the contrary of breast cancer risk, lowers ovarian cancer risk, and the longer the use the lower the risk. Curiously, risk continues lowering for years after stoppage of use. Oestrogen/hormone therapies after menopause show an increased risk of developing ovarian cancer (UK 2015).

A strong family history of breast and/or ovarian cancer is a very important risk factor, where about 1 in 10 ovarian cancers, 10%, are caused by an inherited faulty gene, like *BRCA1* and *BRCA2*. The risk increases the number of affected relatives. Familial history of other cancers such as breast cancer is also linked to an increased risk. Women who have a personal history of breast cancer have an increase risk, as well (UK 2015). A germline deleterious mutation in the *BRCA1* gene confers approximately 39% increase of cumulative risk, while in the *BRCA2* gene conferes

around 11% (Antoniou, Pharoah et al. 2003, Chen, Iversen et al. 2006, Chen and Parmigiani 2007, Milne, Osorio et al. 2008).

As to lifestyle, a low-fat diet for at least 4 years showed a reduced risk and being overweight during pre-menopause increases the risk. While smoking shows a slight increase, drinking alcoholic beverages does not seem to increase the risk of ovarian cancer overall (UK 2015).

Table 1 Risk factor and its relative risk for breast cancer in women. Relative risk is a comparison of the absolute risk of disease among people with a particular risk factor with the risk among people without that risk factor. Relative risk higher than 1.0 means the risk is higher among people with the particular risk factor vs. the people without. Adapted from (Figures 2013).

Relative risk	***Risk factor***
>4.0	Age (65 or older vs. <65 years-old)
	Certain inherited genetic mutations for breast cancer (*BRCA1* and/or *BRCA2*)
	Mammographically dense breasts
	Personal history of early breast cancer onset (<40 years-old)
	Two or more first-degree relatives with early onset age
2.1-4.0	Personal history of breast cancer (40 or older years-old)
	High endogenous estrogen or testosterone levels (postmenopausal)
	High-dose radiation to chest
	One first-degree relative with breast cancer
1.1-2.0	Alcohol consumption
	Ashkenazi Jewish heritage
	Early menarche (<12 years-old)
	Height (tall vs. short)
	Late age at first full term pregnancy (>30 years-old)

Late menopause (>55 years-old)
Never breastfed
No full term pregnancies
Obesity
Personal history of endometrium, ovary or colon cancer
Recent oral contraceptive use
Recent and long-term use of menopausal hormonal therapy

3.4.3. Familial breast and ovarian cancer (FBOC) syndrome

Cancer is a sporadic disease that occurs due to the accumulation of genetic changes, but a small percentage of the cases are considered hereditary. Familial cancer is characterized by having a higher number of cancer cases within a family than statistically expected, which have a genetic component. While hereditary cancer shows a clear pattern of inheritance, clustering of early-onset age and multiple primary cancer cases in an individual (Berliner, Fay et al. 2007, Berliner, Fay et al. 2013).

The first detailed and significantly description about hereditary breast cancer was published in 1866 by Paul Broca (1824 – 1880) (van der Groep, van der Wall et al. 2011). Broca made a pedigree of his wife's family who suffered from early onset of breast cancer, and he suggested that breast cancer could be inherited because four generations had breast cancer (Figure 3.13) (van der Groep, van der Wall et al. 2011).

Up to now high-, moderate- and low-susceptibility genes that increase the risk in Familial breast and ovarian cancer (FBOC) have been identified. FBOC syndrome is mostly caused by a germline mutation in high-susceptibility genes *BRCA1* (chromosome 17q21) (Hall, Lee et al. 1990) and *BRCA2* (chromosome 13q12-13) (Wooster, Neuhausen et al. 1994) (Figure 3.14). These mutations not only increase the risk for breast and ovarian cancer, but also prostate and pancreatic cancer (van der

Groep, van der Wall et al. 2011). The overall prevalence of these mutations is 1 in 400 for *BRCA1* and 1 in 800 to *BRCA2* (Petrucelli, Daly et al. 2010).

The penetrance is the most significant clinical aspect in FBOC syndrome, being breast and ovarian cancer the predominant phenotypes. The penetrance of these mutations vary within families therefore, there is no exact estimate risk for individuals harbouring germline *BRCA1* and *BRCA2* mutations (Petrucelli, Daly et al. 2010). Those differences can be due to the type and/or position of the mutation and other genetic and/or environment factors (Petrucelli, Daly et al. 2010).

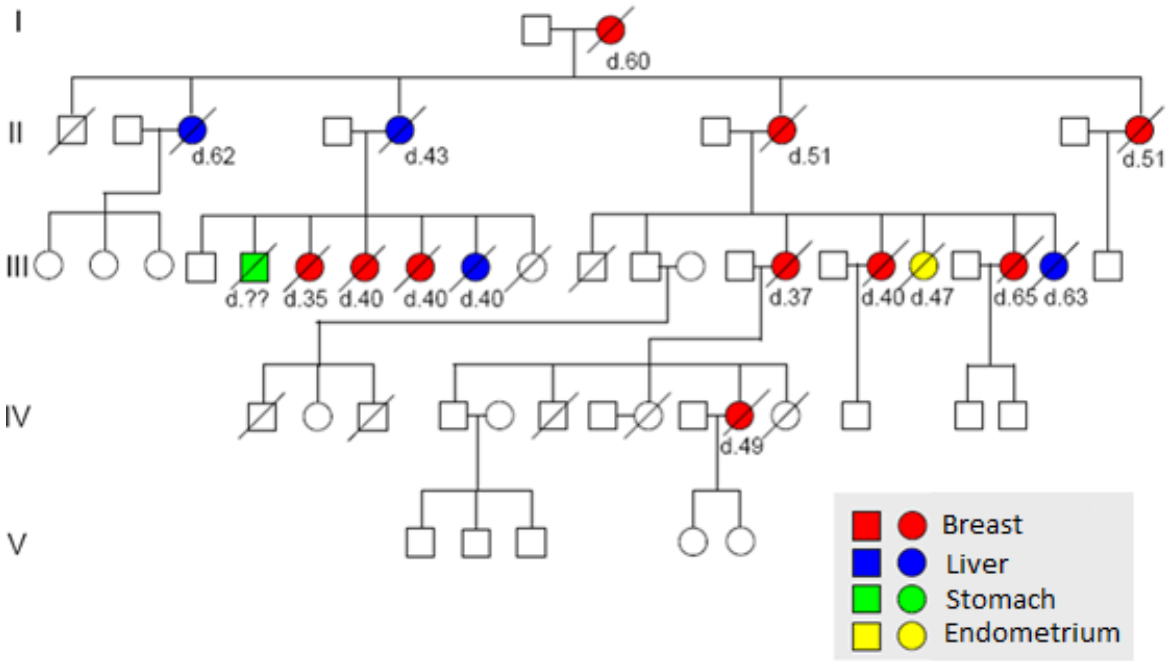


Figure 3.13 Genealogic tree of Paulo Broca's wife's family (1788-1856). Adapted from Gómez (2011)

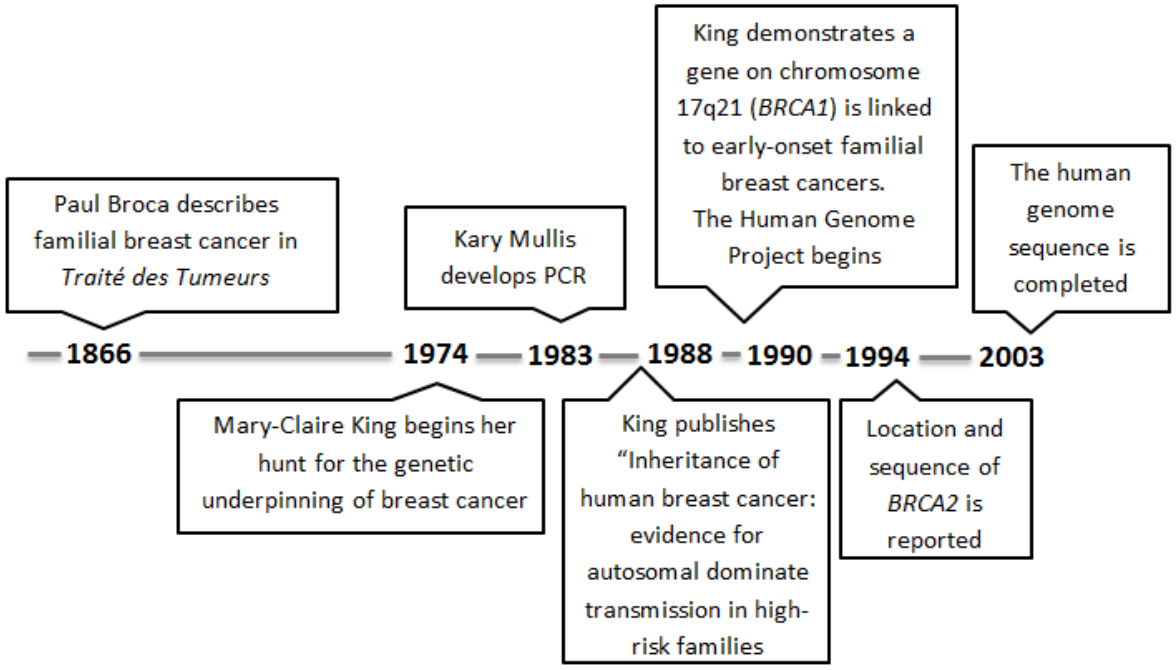


Figure 3.14 Timeline of hunt for the genetic underpinnings of the familial breast cancer. Adapted from Hurst (2014)

3.4.3.1. Breast cancer susceptibility genes

Germline mutations in *BRCA1* and *BRCA2* genes, which predisposes to breast, ovarian and even other cancers, explain around 25% of familial breast cancers (FBC). There are considerable differences in the manifestation of the disease among all the carriers of an inherited mutation in *BRCA*. This differences, in addition to the fact that not all the individuals that inherited a *BRCA* mutation will develop cancer, suggest the existence of other genetic and/or environmental factors modifying the risk of developing cancer (Couch, Nathanson et al. 2014).

Approximately 5% of the FBC cases are due to mutations in high-susceptibility genes involved in other familial syndromes such as *TP53, PTEN, STK11 and CDH11* (Borresen, Andersen et al. 1992, Chen, Lindblom et al. 1998, Pharoah, Guilford et al. 2001, Leggett, Young et al. 2003).

Moderate penetrance genes include the *RAD51C,* ATM and CHEK2 genes, involved in fanconi and non-fanconi anemias (e.g. (Meindl, Hellebrand et al. 2010)) (Meijers-Heijboer, van den Ouweland et al. 2002, Thompson, Duedal et al. 2005)), representing 5% of the FBC.

Recently, 41 low susceptibility polymorphisms were associated to FBC (Michailidou, Hall et al. 2013), which together with the 26 polymorphisms previously identified explain around 14% of the FBC cases (Ghoussaini, Pharoah et al. 2013)

Nevertheless, 51% of FBC cases do not have mutations or the alleles described above and are thus categorized as BRCAX families. These cases can be explained by a polygenic model or by some gene that remains to be identified (Melchor and Benitez 2013). Figure 3.15 shows the distribution of FBC patients according with their mutations/variants in low-, moderate-, high-susceptibility genes/loci.

Established in 2006, The Consortium of Investigators of Modifiers of *BRCA1* and *BRCA2* (CIMBA), provides currently the largest sample size for the study of common genetic modifiers of breast and ovarian cancer risk. So far, 94 single-nucleotide polymorphisms (SNPs) have been associated with low susceptibility to breast cancer by Genome-Wide Association Studies (GWAS). (Cox, Dunning et al. 2007, Easton, Pooley et al. 2007, Stacey, Manolescu et al. 2007, Ahmed, Thomas et al. 2009, Milne, Benitez et al. 2009, Thomas, Jacobs et al. 2009, Turnbull, Ahmed et al. 2010, Broeks, Schmidt et al. 2011, Figueroa, Garcia-Closas et al. 2011, Michailidou, Hall et al. 2013, Michailidou, Beesley et al. 2015).

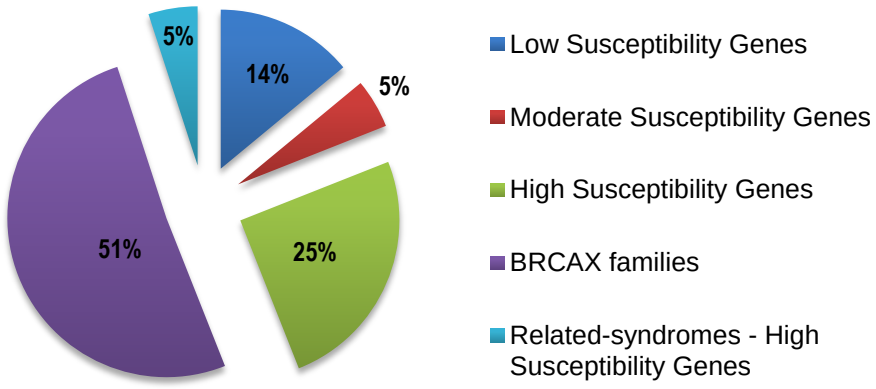


Figure 3.15 Pie with the representative percentages of familial breast cancer mutations in low-, moderate-, high-penetrance genes, *BRCAX* and related syndromes up to date described that predispose to breast cancer. Adapted from (Melchor and Benitez 2013)

3.5. Breast Cancer Susceptibility Genes, BRCAs

3.5.1. Function as Homologous Recombination DNA repair members

BRCA proteins play a very important role in genome and chromosome integrity and maintenance, during cell division. It is notable how BRCA-deficient cells accumulate espontaneously aberrations in chromosomes' structure and number, due to impairment in Homologous DNA Recombination (HR) pathway, (Venkitaraman 2002, Venkitaraman 2014). Both BRCA1 and BRCA2 are individually crucial for an efficient HR, a mechanism of DNA repair error-free using as template an intact homologous sequence, such as sister chromatid (Venkitaraman 2014).

DsDNA breaks may be create during chromosome duplication thus, BRCA-deficiency is typically accompanied by dsDNA breaks or aberrant chromosomes structure and number since their absent or normal performance leads the cells to reroute the DNA reparation to an error-prone mechanism: nonhomologous end joining (NHEJ) (Venkitaraman 2014). BRCAs are therefore classified as tumour suppressor proteins, with fundamental functions in the genomic stability maintenance.

The *BRCA* genes follow the Knudson's "two-hit" hypothesis (Knudson 1971) which postulates that mutation or loss in one of the alleles of a tumour suppressor gene is not enough to trigger cancer, being necessary the occurrence of a second "hit" to completely inactivate the protein. However, it is known that for *BRCA* genes just one impaired allele can actually affect BRCA normal performance due to haploinsufficiency (Cousineau and Belmaaza 2007, Konishi, Mohseni et al. 2011, Nisman, Kadouri et al. 2013). That phenomenon might increase the susceptibility of the carrier and accelerate the loss of the second allele.

BRCA1 and BRCA2 have many and distinct functions although, in a simplified perspective BRCA1 acts in the early steps of HR reparation, whereas BRCA2 stabilizes and supports the replication-associated lesions structures. BRCA2 not only plays as HR

member but also works in the surveillance of the mitotic spindle assembly checkpoint. In Figure 3.16 is possible to see BRCA1 and BRCA2 HR-mediated functions.

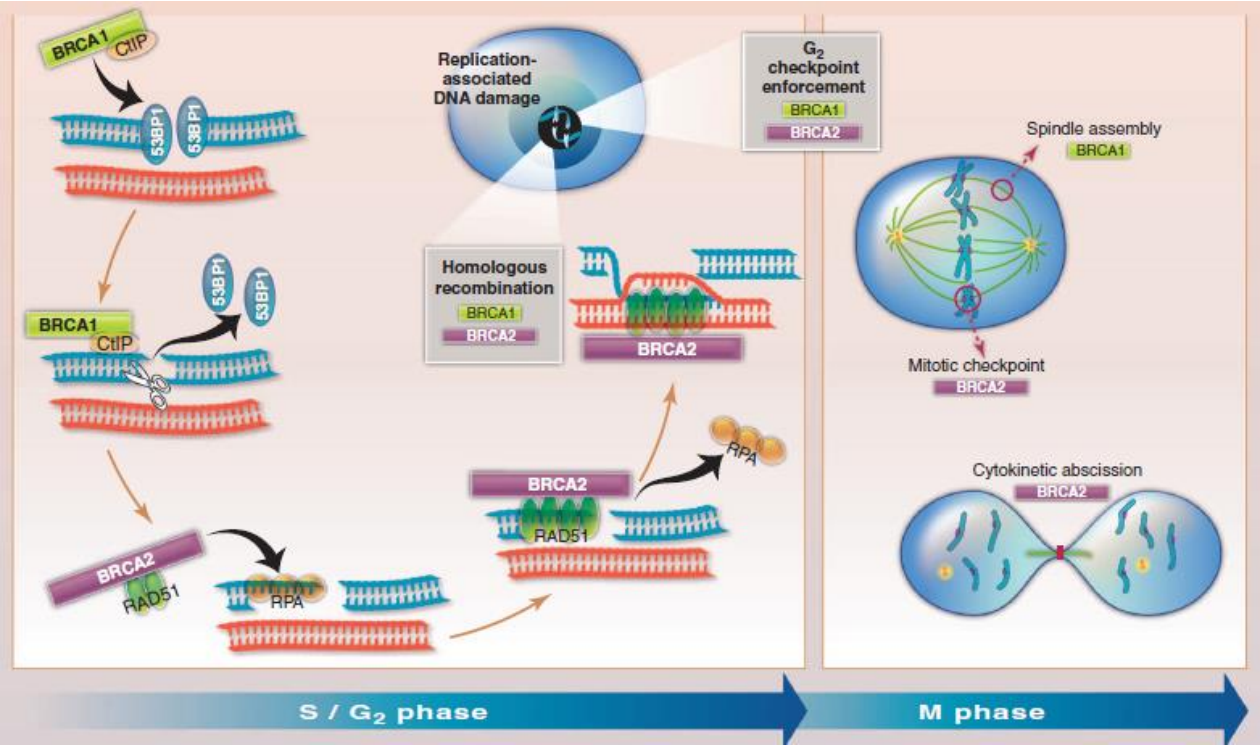


Figure 3.16 Some BRCA1 and BRCA2 HR-mediated functions at different stages of cell division. Both BRCA1 and BRCA2 participate in the G2 checkpoint: BRCA1 helps in the initiation of HR moving 53 Bindingprotein 1 (53BP1) and triggers the end resection (represented by the scissors). Thus, BRCA2 binds to the ssDNA and dsDNA junctions at the lesion, moving RPA away. During mitosis, BRCA1contributes to mitotic spindle assembly, and BRCA2 watches for mitotic checkpoint and participates in the abscission step of cytokinesis. Adapted from Venkitaraman (2014)

3.5.2. Functional involvement in telomere maintenance

It has been described that BRCA1/2 have a functional role in the telomere maintenance. For instance, previous reports (Xiong, Fan et al. 2003, Ballal, Saha et al. 2009) suggest that BRCA1 can regulate both telomere length and stability due to its interaction with Mre11-Rad50-Nbs1 (MRN) complex (complex that binds to shelterin proteins) and telomerase. Regarding BRCA2, Badie, Escandell et al. (2010) have shown BRCA2 is with telomeres during the S and G2 cell cycle phases, in order to facilitate the loading of RAD51 into telomeres. *BRCA2* conditional deletion leads to shorter and fragmented telomeres representing telomere fragility (Badie, Escandell et al. 2010).

Together BRCA1 and BRCA2, despite mediating HR also contribute to telomere integrity and length.

3.6. Genome-wide association studies, GWAS

Genome-wide association studies (GWAS) look for genetic variation, such as SNPs, that are more frequently present in a group of people with the disease than controls. The search for variants/SNPs/polymorphisms can be made without a priori information about its genetic function or mechanism, or can be restricted to candidate genes. The HapMap project made possible the existence of comprehensive studies such as GWAS, where describes common patterns of human DNA sequence variation displayed as a haplotype map (International HapMap 2005). GWASs are a good approach to identify common alleles with low penetrance in the disease (Figure 3.17), of common multifactorial and polygenic diseases (Easton and Eeles 2008). GWAS are used to mapping genomic areas associated with a disease/phenotype (International HapMap 2005).

Systematic studies of common genetic variants are simplified by the linkage disequilibrium (LD) phenomenon, because it is possible to obtain a large amount of information on genomic variation without complete resequencing and to get an efficient selection of tag SNPs, optimizing the association analyses (International HapMap 2005).

GWAS are widely used in cancer research, particularly in the commonest cancer types such as breast, prostate, colorectal, lung and melanoma (Easton and Eeles 2008).

3.6.1. Tag SNPs for association studies

A “tag SNP” is a representative SNP in a determined region of the genome with high LD ($r^2 \geq 0.8$) with a group of other SNPs: haplotype. Like that, it is possible to search for an association with a phenotype based on the genotypes: a unique SNP or a

combination of various SNPs. LD phenomenon saves the entire genotyping of every SNP in a chromosomal region (International HapMap 2005).

The tag SNPs selection looks for maximum efficiency with the least loss of information. Hence, some methods need a single SNP serving as a proxy for others, while others use the combination of alleles, or haplotypes, to serve as proxies. The causal SNP for a disease is rarely genotyped but a good set of tag SNPs will provide association between a genotype and phenotype, which the pinpointed SNP might be in LD with the causal one. For the identification of the causal SNP, is necessary a greater resolution in the selection of haplotypes blocks as a Fine mapping approach.

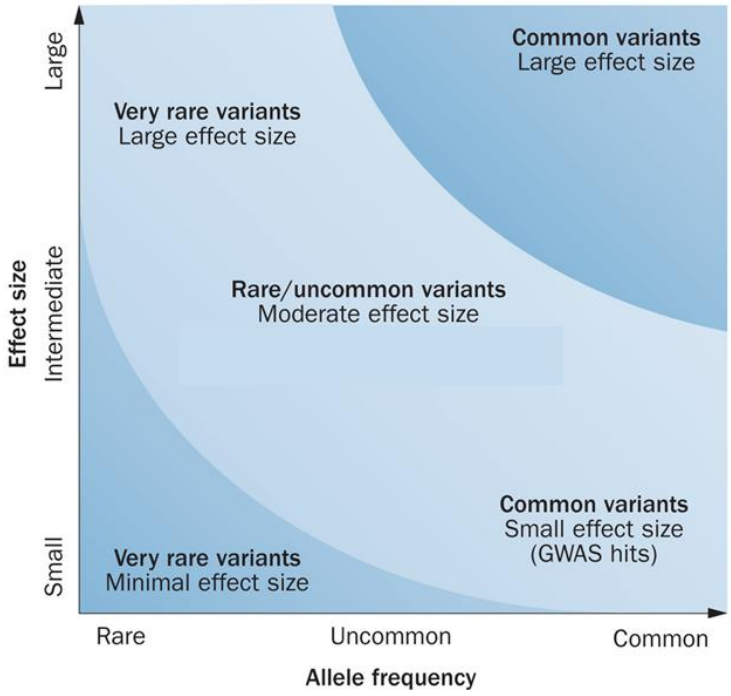


Figure 3.17 Genome-wide association studies are effective in detecting common alleles with low penetrance in a disease. Adapted from Eeles, Goh et al. (2014)

3.6.2. SNPs in DNA glycosylases genes involved in the BER pathway

Given the synthetic lethality between members of BER pathway: Poly ADP ribose polymerase (PARP1) and BRCA1 and BRCA2, Osorio, Milne et al. (2014) performed a comprehensive analysis using a tagging SNP method of 18 genes involved in BER pathway in a large series of *BRCA1* and *BRCA2* mutation carriers from CIMBA consortium. 144 tagSNPs were analysed in a sample of 23,463 individuals.

Evidence of association with breast and/or ovarian cancer was obtained for eleven SNPs, of which, five located in DNA gycosylases genes. The strongest evidences of was for rs1466785 in the *NEIL2* gene (endonuclease VIII-like2) (HR: 1.09, 95% CI (1.03–1.16), p = 2.761023): associated with breast cancer risk in *BRCA2* mutation carriers, and for rs2304277 in the *OGG1* gene (HR: 1.12 95%CI: 1.03–1.21, p = 4.861023), associated with ovarian cancer risk in *BRCA1* mutation carriers (Osorio, Milne et al. 2014).

These loci should be extensively studied, considering these results.

3.6.2.1. SNP in OGG1 associated with ovarian cancer risk

An impaired Homologous Recombination DNA repair mechanism, due to a *BRCA* mutation, makes cells critically dependent on other DNA repair machineries such as BER pathway. As mentioned above, Osorio, Milne et al. (2014) reported the rs2304277 variant in the *OGG1* gene with strong evidences of association with ovarian cancer in *BRCA1* mutation carriers. Knowing, this SNP was identified by a tagging SNP approach, it is possible this is not the causal SNP, but only a SNP in LD with the causal one.

This SNP has two alleles, an Adenine (A) and a Guanine (G), whit the ancestral and minor allele being the A allele, present in 35% of the population, according to the 1000 Genomes project (dbSNP 2015). This polymorphism is located in a 3'UTR, a regulatory region that can in many aspects, influence the mRNA molecule in its stability or translation. At 3'URT regions, contain specific binding sites for both regulatory proteins and microRNAs. Hence, the stability of the mRNA molecule or its translation efficiency can be altered. A polymorphism at 3'UTR can, for instance, decrease or increase the binding affinity of those microRNA (miRNA) and regulatory proteins, thus changing the final product of mRNA and respective translated protein (Barrett, Fletcher et al. 2012, Pichon, Wilson et al. 2012).

OGG1 is a key player in BER pathway, involved in the repair of oxidized guanines, bases in which telomeres are enriched. Given the natural preference of oxidative stress over guanines, OGG1 role, and telomeres composition, this study

followed this line of reasoning to understand and answer whether the increased cancer risk is due to a less effective OGG1 response upon oxidative stress, leading to accelerate telomere shortening.

4. Objectives

We hypothesized that the association with cancer risk in the carriers of the rs2304277 variant might be due to an altered OGG1 function, that could accelerate the telomere shortening, resulting from a weaker response upon oxidative stress consequences.

In order to answer the biological question the aims of this study were:

First aim:

- To investigate whether the rs2304277 variant, located in the 3' UTR region of *OGG1* can modify the expression of its messenger ribonucleic acid (mRNA), leading to an altered response upon oxidative stress.

Second aim:

- To investigate whether the presence of the rs2304277 variant in *OGG1* alone, or in conjunction with the *BRCA* mutation can modify telomere length, given the role of OGG1 in the telomere homeostasis.

5. Materials & Methods

5.1. Samples

The samples mentioned below and used for this study were collected through individuals that attended the genetic counselling consultancy for familial cancer in the Fuenlabrada Hospital University – Madrid, Spain from 2011 to 2014. Informed consent was obtained from all individuals involved in this research project.

We have followed a cross-sectional approach in a cohort of familiar breast and ovarian cancer cases (FBOC) from 101 families, harbouring deleterious mutations in *BRCA1* and *BRCA2* and *BRCAX*. With *BRCAX* it is mean cases with familiar history for hereditary breast cancer but that do not harbour mutations in any of the identified genes for FBC, as reported above in the "Breast Cancer Susceptibility Genes" section. Individuals from these families met high risk criteria (Milne, Osorio et al. 2008) and had been previously screened for mutations in *BRCA1* and *BRCA2* by a combination of denaturing high-performance liquid chromatography (DHPLC) and direct sequencing as previously reported (Diez, Osorio et al. 2003)

Peripheral blood samples from 223 individuals were used, which 68 (31%) of the samples corresponded to controls: healthy women and men with no personal or familiar antecedent of cancer and age range from 18 to 84 years old. 45 (20%) and 49 (22%) individuals harboured a mutation in *BRCA1* and *BRCA2*, respectively, while 61 samples (27%) a mutation in *BRCAX*. In the Table 4 it is possible to see the mentioned numbers and its relative frequencies within the sample.

5.2. Genotyping

The genotyping for the polymorphism rs2304277 present in *OGG1* gene was made in order to know the alleles of which sample included in this study: homozygote for the common allele (G/G), heterozygous (GA) or homozygote for the minor allele (A/A).

5.2.1. DNA extraction

DNA was extracted from peripheral blood cells of the cited samples using MagNAPure LC 2.0 (Roche Diagnostics, Indianapolis, Indiana) manufacturer's conditions. DNA quantification and quality was assessed by NanoDrop® (ND-1000 V3.7.1).

5.2.2. PCR - DNA amplification

The PCR reagents: HotMaster Taq Buffer with Mg^{2+} (5PRIME, 1x); dNTPs (Thermoscientific, 1.25nU); *OGG1* DNA primers (F/R) (SIGMA, 10mM) (Table 2) and HotMaster Taq DNA Polymerase (5PRIME, 0,4U/µl) were loaded to 1µl of DNA at 25ng/µl. The amplification ran at the following conditions: 94ºC for 5 minutes followed by 35 cycles at 94ºC for 30 seconds; 65ºC for 45 seconds; 72ºC for 45 seconds and 72ºC for 5 minutes.

To check the PCR quality, the products were run in 2% agarose gels to confirm a single band (~200bp) and if there was any contamination in the negative controls (H_2O instead of DNA).

Table 2 Oligonucleotide primers used for PCR

OGG1 DNA	Forward	5' GACCTTTCTCGGACCCCATA 3'
	Reverse	5' ACTCCTCCCCATCCCTACC 3'

5.2.3. Genotyping

Cleaning up of PCR products for subsequent sequencing was made by ExoSAP-IT® (Affymetrix): 2µl of ExoSAP-IT to each 5µl PCR DNA product (diluted into 1:2) at the following conditions: 37ºC for 15 minutes and 80ºc for 15 minutes. The sequencing was performed by the Sanger method in the Genomics Unit at CNIO.

5.3. RNA expression analysis

In line with the hypothesis of this work, this step was made to evaluate whether there were different levels of *OGG1* mRNA concerning their different genotypes: homozygote for the common allele (G/G), heterozygous (GA) or homozygote for the minor allele (A/A).

5.3.1. RNA extraction

Using TRIzol Reagent (Ambion®, Life Techonogies) according to manufacturer's instructions, RNA was extracted from peripheral blood cells. Both RNA quantity and quality were assessed by NanoDrop® (ND-1000 V3.7.1).

5.3.2. Reverse transcription PCR (RT-PCR)

RNA at concentration of 1000 ng/µl was reverse transcribed using High Capacity cDNA Reverse Transcription kit according to Applied Biosystems (Life Technologies) manufacturer's instructions. Briefly, the mix components: RT buffer (1x); dNTPs (4mM); random primers (1x); RNAsin (1U/µl) and finally the MultiScribe Rtase (2.5U/µl) were added to the RNA. The thermal cycling conditions started with at 25ºC for 10 minutes; 37ºC for 120 minutes and a last step at 85ºC for 5 minutes.

5.3.3. Quantitative PCR (qPCR)

50ng/µl of cDNA obtained in the previous step was loaded with GoTaq® qPCR MasterMix (1x); *OGG1* cDNA primers (Forward (F)/Reverse (R)) (Table 3) and *GAPDH* cDNA primers (F/R) (SIGMA, 10mM) and CXR as a reference dye (Promega). All the mentioned reagents were used following the GoTaq® qPCR Master Mix (Promega) following manufacture's conditions. *OGG1*-specif primers that did not distinguish between the genotypes of the polymorphism were used, and normalization of the

samples was carried out with the internal control – *GAPDH.* The relative expression was measured using a comparative Ct method.

Table 3 Oligonucleotide primers used for qPCR

OGG1 cDNA	Forward	5’ CTCCACTCCTGCCCTGTG 3’
	Reverse	5’ AGAGAAAAGGCATTCGATGG 3’
GAPDH cDNA	Forward	5’ CCTGCACCACCAACTGCTTA 3’
	Reverse	5’ CCATCACGCCACAGTTTCC 3’

5.4. Telomere Length Measurement

5.4.1. High Throughput Quantitative Fluorescence in situ Hybridization(Q-FISH)

Telomere length (TL) was measured by High Throughput Q-FISH, also known by quantitative fluorescence *in situ* hybridization, as described in (Canela, Vera et al. 2007). Mononuclear cells from peripheral blood were cultured in clear-bottom black-walled 96-well plates (Greiner, Longwood, FL) precoated with 0.001% (wt/vol) (poly)L-lysine solution (Sigma-Aldrich, St. Louis, MO) for 30 minutes at 37ºC. (Poly)L-lysine was removed before the addition of cells (30 000 – 90 000 cells/well). When the subconfluency of adherent cells was reached, cells were washed three times with PBS and fixed with methanol/acetic acid (3/1, vol/vol) for 1hour at room temperature. After the fixation step, cells were hybridized with PNA-tel Cy3-labeled probe together with DAPI, and their signals were obtained simultaneously captured at 100x magnification using a COHU CCD camera on a Leica DMRA microscope (Leica, Heidelberg, Germany).

Signal from telomere fluorescence was quantified by TFL-TELO program (Peter Lansdorp, Vancouver, Canada) and converted into Kb by external calibration with lymphocyte cell lines (L5178Y-S and L5178Y-R) with known TL of 10.2 and 79.7 kb, respectively. TL values were normalized by the fluorescence intensity of centromere repeats in each nuclei.

5.6. Statistical analysis

TL (Kb) was adjusted to the age, using the best fit line for male and female controls, distinctly. Then, the difference between the actual and the predicted value was calculated for each sample. The Kb obtained by High Throughput Q-FISH were adjusted following this method: y=-0.0301*(Male age)+11.011; or y=-0.0941*(Female age)+13.963 (Annex 1).

Kolmogorov-Smirnov test was used to evaluate if the data were normally distributed or not. The statistical significance of the values given by different comparative analysis performed (qPCR and High Throughput Q-FISH) in the present study was either measured by Mann-Whitney U test or Unpaired Student t test according with their distribution. Where the first mentioned test, Mann-Whitney U test, was used for comparative analysis that involved variables not following non-normally distributed variants and the second test, Unpaired Student t test, for normally distributed variants.

Test for equality between two regression slopes was calculated using “R” according with a predesigned script, used to calculate the statistical significance in the analysis of the Figure 10. Statistical calculations were done using SPSS version 18 (SPSSI« Inc, Chicago, Illinois), the R Project for Statistical Computing, GraphPad Prim 5.03 (San Diego, California), and graphics were performed by GraphPad and Microsoft Office© Excell 2007.

P-values lower than 0.05 were considered as statistically significant.

6. Results

6.1. Different genotypes for rs2304277 in the population

Genotyping results allowed the determination of the estimate relative frequencies of the different genotypes in the population analysed. The Table 4 and Annex 2 represents all the mentioned values and percentages obtained by genotyping.

With a sample of 223, 146 were homozygote for the common allele (G/G), 71 heterozygous (G/A) and only 6 homozygote for the minor allele (A/A). The minor allele is present in 34.5% of the population. These results meet what was reported in Osorio, Milne et al. (2014) and dbSNP (2010).

Within the control group (no mutations in the *BRCA* genes), wherein 26 were heterozygous (G/A) and 4 homozygote for the minor allele (A/A) 31% of the sample corresponded to controls, the minor allele is present in

From those 223 samples, 45 and 49 harboured a mutation in *BRCA1* and *BRCA2*, respectively. From the *BRCA1* mutation group 14 were heterozygous (G/A), representing 31% of all *BRCA1* mutation carriers, and from the *BRCA2* mutation group 17, representing 35% of all BRCA2 mutation carriers.

The 61 samples categorized as *BRCAX*, 16 had the minor allele representing 26.6% of the *BRCAX* group. Wherein 14 were heterozygous (G/A) and 2 homozygote for the minor allele (A/A).

6.2. Levels of OGG1 mRNA is reduced in minor allele carriers for the rs2304277 variant

Taking into consideration the first aim of the work, *OGG1* mRNA was quantified by qPCR in the different groups of the sample.

Primarily, concerning their *BRCA* status and its rs2304277 genotype the analysis of the *OGG1* mRNA expression levels showed that the group of individuals that carrier the minor allele (heterozygous or homozygote for the minor allele) seem to have

lower mean levels of *OGG1* mRNA, independently of their status or type of *BRCA* mutation (Figure 6.18, Annex 2). Statistical analysis obtained by using unpaired *t*-test, revealed significant differences in the expression between the carriers and non-carriers of the minor allele (A) in the Controls and mutated *BRCA2* group (p=0.025 and p=0.04, respectively) (Figure 6.18). Since all samples with a minor allele presented lower mean of *OGG1* mRNA independently of their status or *BRCA* gene mutated, we can can either assume *BRCA* mutated or not, does not influence the *OGG1* mRNA expression.

Such curious results leaded us to analyse and compare the OGG1 transcript levels regardless their status or *BRCA* mutated gene and only looking into their rs2304277 genotype (Figure 6.19). Interestingly, we found that the group of the carriers of the minor allele (A) have significant lower mean *OGG1* transcript levels compared with the non-carriers, as we could predict by the previous analysis. By using Mann-Whitney *U* test, it was possible to see that the difference of the mRNA expression between the carriers and the non-carriers of the minor allele (A) were statistically significant: p= 0.013 (Figure 6.19).

We can infer the presence of the minor allele per se has a modifier effect at the *OGG1* transcript levels, being possible to identify a constant pattern of lower *OGG1* mRNA levels when compared with the non-carriers of the minor allele, regardless their status or *BRCA* gene mutated.

Table 4 **Number and relative frequencies of the different series within the sample (*n*=223).** K%%over the total population" represents their relative frequencies over the sample (n=223) and the "% within its group" represents their relative frequencies within the groups which are inserted according with their *BRCA* mutation status or controls.

	n	**% over the total population**	**% within its group**
Total	223	100	
Homozygote for the common allele (G/G)	146	65.5	
Heterozygous (G/A)	71	31.8	
Homozygote for the minor allele (A/A)	6	2.7	
Controls	**68**	**30.5**	
Homozygote for the common allele (G/G)	38	17.0	55.9
Heterozygous (G/A)	26	11.7	38.2
Homozygote for the minor allele (A/A)	4	1.8	5.9
BRCA1	**45**	**20.2**	
Homozygote for the common allele (G/G)	31	13.9	68.9
Heterozygous (G/A)	14	6.3	31.1
BRCA2	**49**	**22.0**	
Homozygote for the common allele (G/G)	32	14.3	65.3
Heterozygous (G/A)	17	7.6	34.7
BRCAX	**61**	**27.4**	
Homozygote for the common allele (G/G)	45	20.2	73.8
Heterozygous (G/A)	14	6.3	23
Homozygote for the minor allele (A/A)	2	0.9	3.3

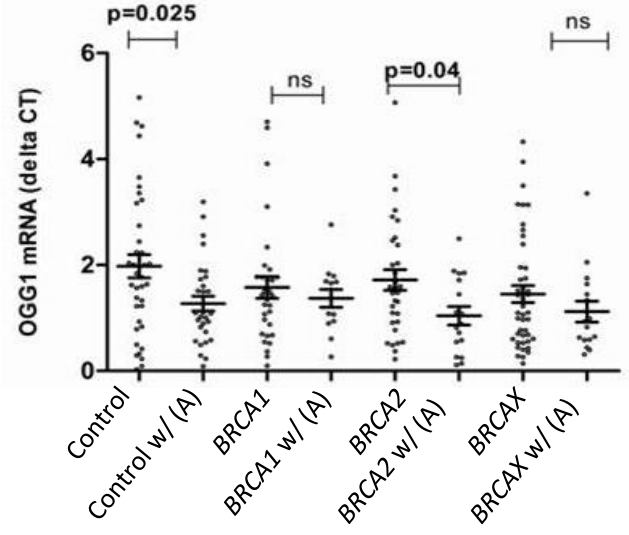


Figure 6.18 Carriers of the rs2304277 minor allele (A) show decreased *OGG1* mRNA expression levels in all groups. Relative *OGG1* mRNA expression was significantly different between the non- and carriers of the minor allele (A) in the Controls and *BRCA2* mutated group (p=0.025 and p=0.04, respectively), where the carriers the show less *OGG1* mRNA levels. Even without statistical significance, it is possible to see a decrease in the relative *OGG1* mRNA expression in the carriers of the minor Allele (A) compared with the non-carriers of the *BRCA1* and *BRCAX* groups.

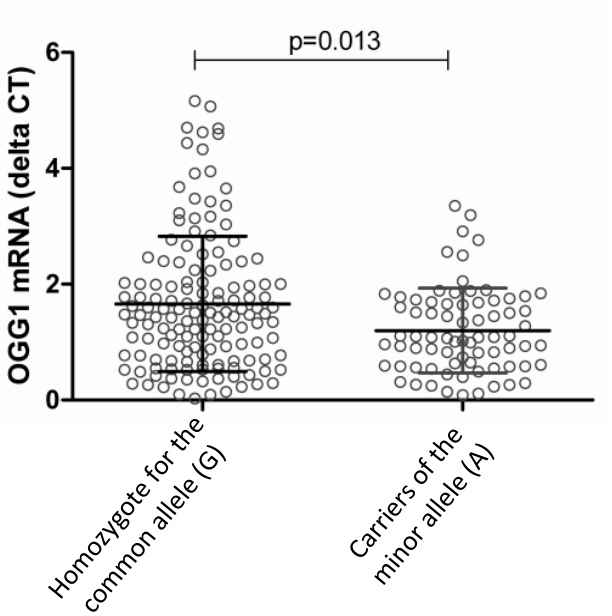


Figure 6.19 Carriers of the rs2304277 minor allele (A) show lower relative *OGG1* mRNA expression compared with the non-carriers. Relative *OGG1* mRNA expression between 146 individuals non-carriers and 77 individuals carriers of the minor allele is statistically different (p=0.013). The mean expression in the carriers of the minor allele is lower.

6.3. Accelerated telomere shortening in individuals who carry the minor allele and a BRCA1/2 mutation

Knowing the role of OGG1 in the maintenance of the telomere homeostasis and its length (Lu and Liu 2010, Jacobs and Schar 2012), we measured the length of the telomeres in the leucocytes by high throughput Q-FISH to check whether they were shorter in the presence of the minor allele (A), since they also showed less *OGG1* mRNA expression levels.

Initially, we have evaluated the telomere length distribution of 40 women (Annex 1, left picture) and 28 healthy men (Annex 1, right picture) as a function of age, in order to obtain a regression line to adjust the TL (Kb) of the FBOC individuals analysed. As expected, we found a decrease in the TL with age (Annex 1). Test for the equality

between the two regression slopes was calculated using “R” according with predesigned scrip.

We have analysed the possible effect of the minor allele on the TL and interestingly, as we predicted, the analysis revealed a contribution of the presence of the allele (A) in TL. In the Figure 6.20, we can see the rate of TL decay with age between the different groups, and individuals that harbour *BRCA1/2* mutations together with the minor allele (A) displayed a significantly accelerated telomere shortening during lifetime compared with the controls and the group of individual that harbour the condition (G/G) and a BRCA1/2 mutation ($p<0.0001$).

Thus, we aimed to test whether a faster telomere shortening would also drag to a shorter telomere length phenotype. Our results confirmed that indeed happened, in which TL was significantly shorter in the individuals that harboured the minor allele (A) together with a *BRCA* mutation compared to the Controls ($p=0.034$) and even not statistically significant, to the group of individuals that harbour the condition (G/G) and a *BRCA1/2* mutation (Figure 6.21).

Thus, we tested whether the displayed faster telomere shortening in the individuals harbouring *BRCA1/2* mutation plus the minor allele (A) could lead to a shorter telomere length phenotype. Interestingly, our results confirmed this accelerated telomere shortening also that leaded to a short telomere length phenotype, which was statistically significant compared to the Controls ($p=0.034$) and, even if not statistically different, with the *BRCA1/2* mutation carriers homozygote for the common allele (G/G), we found therefore a trend.

Hence, the high throughput Q-FISH results come across with the hypothesis of this work, sustaining the concept of an accelerated telomere shortening in the individuals that carrier the minor allele (A) plus a *BRCA1/2* mutation. Possibly, this may explain how the variant rs2304277 showed an association with an increased cancer risk together with a *BRCA1/2* mutation reported in Osorio, Milne et al. (2014).

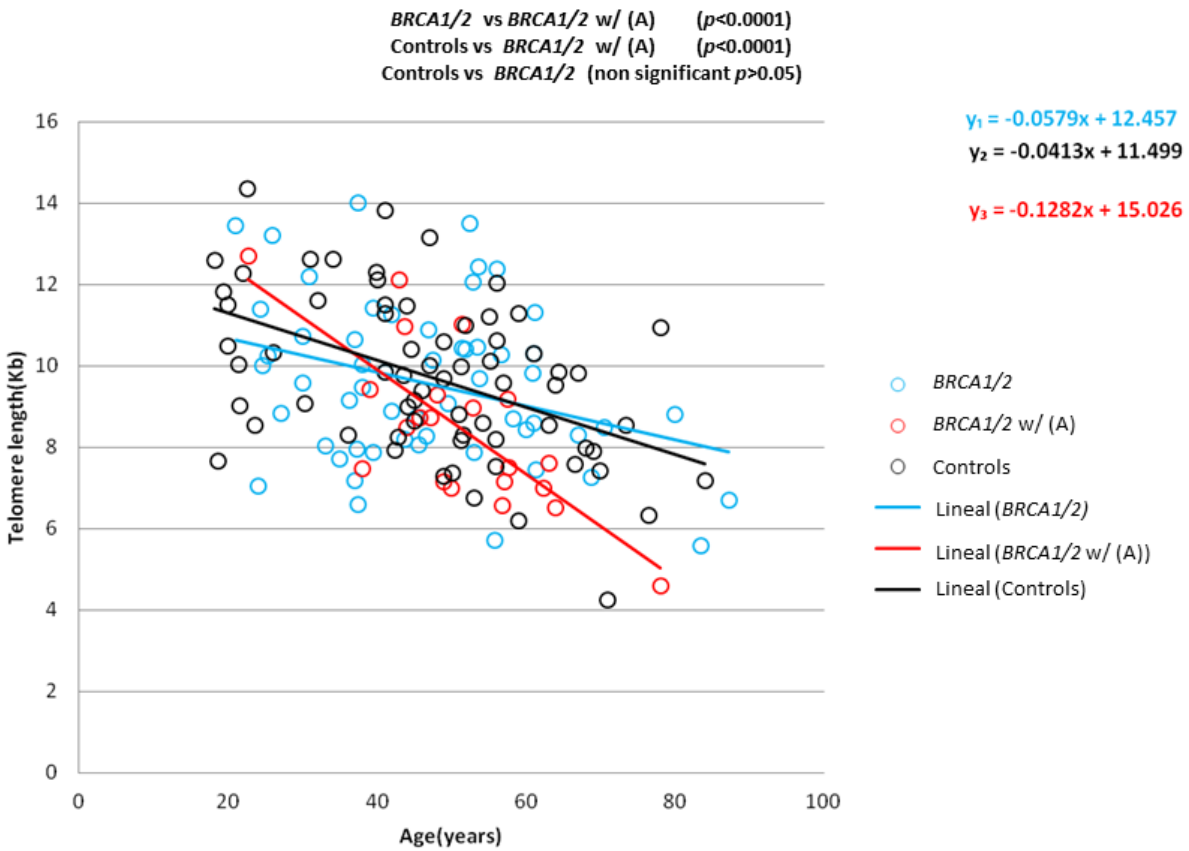


Figure 6.20 Telomere shortening rate during lifetime in FBOC series and Controls. Distribution of leukocyte telomere length as a function of the age from: *BRCA1/2* (homozygote for the common allele (G/G), blue), *BRCA1/2* carriers of the rs2304277 minor allele (A) (red) and Controls in black. Individuals that harbour *BRCA1/2* mutations together with the rs2304277 minor allele (A) displayed a significantly accelerated telomere shortening during lifetime compared with the controls and the group of individual that harbour the (G/G) and a *BRCA1/2* mutation (p<0.0001).

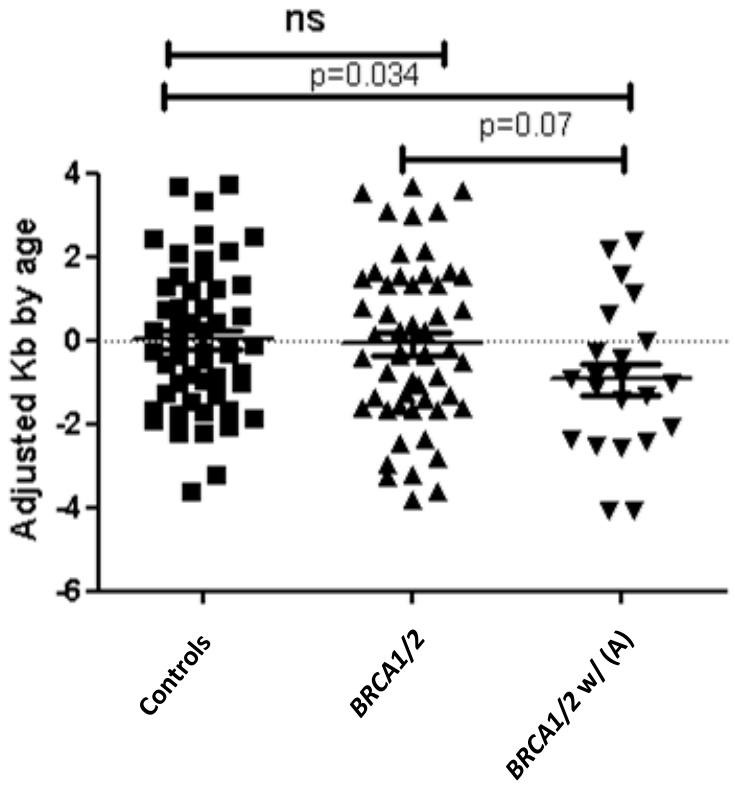


Figure 6.21 Carriers of the minor allele (A) harbouring a mutation in the *BRCA1/2* gene show shorter telomeres phenotype. The given telomere length after the adjustment to age showed significantly shortened telomeres (*p*=0.034) in the carriers of the rs2304277 minor allele that also harbour a mutation in the *BRCA1/2* gene compared to controls.

7. Discussion

The starting point of this study aimed to understand why the SNP in *OGG1* seemed to increase the risk of developing cancer as reported in Osorio, Milne et al. (2014). We have checked whether the reason for this risk modification could involve the telomere length. Knowing the *OGG1* role and its importance to regulate the oxidation consequences among guanines (Thomas, Scot et al. 1997, Wang, Rhee et al. 2010), it is reasonable to associate that any impairment in this enzyme may affect the telomeres integrity due to their elevate amount of guanines. Dysfunctional telomeres can accumulate structural aberrations and abnormal chromosome end-to-end fusions, which might initiate or promote tumourigenesis as a consequence of chromosomal instability (Cong, Wright et al. 2002, O'Sullivan and Karlseder 2010).

A polymorphism at 3'UTR can, for instances, influence the mRNA molecule by different ways altering the final product of transcript and/or respective translated protein (Barrett, Fletcher et al. 2012, Pichon, Wilson et al. 2012). Thus, the experimental approach for this work followed this line of reasoning evaluating the *OGG1* mRNA levels given their genotype for *OGG1*, to understand if somehow this variant in 3'UTR would show differences in *OGG1* mRNA levels.

7.1. OGG1 mRNA expression levels

Indeed, by qPCR it was possible to see changes in the *OGG1* mRNA levels between the homozygote for the rs2304277common allele (G/G) and the carriers of the minor allele (A). Considering their status: controls or a *BRCA* gene mutated, we could see a pattern of lower expression in the carriers of the minor allele (A) along the different groups compared with the respective homozygote for the common allele (G/G). This shows the *OGG1* transcript levels are not dependent/influenced by their status (Controls or a *BRCA* gene mutated) (Figure 6.18). This analysis allowed us to discard any influence that BRCA status might have in *OGG1* transcription.

Such curious results leaded us to analyse and compare the *OGG1* transcript levels between all the individuals that were homozygote for the common allele (G/G) and the individuals with the minor allele (G/A) or (A/A), not taking into consideration

their status (Controls or a *BRCA* gene mutated). It is worth to note that we found that the group of the carriers the minor allele (A) had significant lower mean *OGG1* transcript levels compared with the non-carriers (p= 0.013) (Figure 6.19). On top of all these findings, this approach allowed us to make another observation, despite a lower mean *OGG1* mRNA amount among the carriers of the minor allele (A) they also seem to not be able to overexpress it. The Figure 6.19 shows that the homozygotes for the common allele (G/G) cohort has a wide range of different levels of *OGG1* mRNA expression in contrast to the carriers of the minor allele (A), who seem to have lower and not much more of its expression. Is not possible to make that inference with this study, but is something that should be addressed as future work.

Overall, we can assume that the presence of the minor allele (A) per se has a modifier effect at the *OGG1* transcript levels, leading to their decrease. This might be a post-transcriptional effect due to a greater affinity with regulatory proteins or higher instability of the mRNA molecule, which could be addressed as future work.

7.2. Telomere length

Insofar as *OGG1* mRNA seems impaired in individuals who carry the minor allele (A) and regarding the coding enzyme function, the measurement of the TL of the same sample group used above was certainly a reasonable approach to meet our hypothesis and to answer the biological question we were working on.

Interestingly, the given results by High Throughput Q-FISH showed the presence of the minor allele (A) can indeed accelerate the telomere shortening, sustaining our hypothesis. Although, the minor allele (A) per se is not sufficient, requiring a *BRCA* mutation but, also a *BRCA* mutation alone is not sufficient to drag an accelerated shortening. In other words, an accelerated telomere shortening is observable in individuals who have both a *BRCA* mutation and carry the minor allele (A) of the variant rs2304277 (Figure 6.20). Not only have they showed an accelerated telomere shortening but also shorter telomere phenotype (Figure 6.21). The impairment

of the *OGG1* mRNA and consequently its translation is not sufficient for an accelerated telomere shortening or shorter telomere phenotype, being necessary an impaired *BRCA* gene.

This phenomenon can be explained by the fact that an impaired HR DNA repair mechanism due to a *BRCA* mutation can make the individuals more dependent on other members of the DNA repair machinery as BER, in this specific case, OGG1. Also, oxidative stress and its effect over the DNA/telomeres is well-described in the literature as a promoter of genomic instability when not properly corrected (Oikawa and Kawanishi 1999, von Zglinicki 2002, Klaunig, Kamendulis et al. 2010). The individuals who show less expression of *OGG1* mRNA - the carriers of the minor allele (A) - may have a less efficient manner to correct the damage caused by the oxidative stress over G-enriched structures as telomeres. Therefore under oxidative stress conditions they can have a higher contribution to an accelerated telomeres shortening, when other DNA repair mechanism is impaired, as HR. This can lead to chromosomal instability and later to carcinogenesis, and like that meeting the premise described in Osorio, Milne et al. (2014): rs2304277 shows an association with cancer risk in patients with BRCA mutations.

Knowing BRCA1 and BRCA2 have a role in the telomere homeostasis (Ballal, Saha et al. 2009, Badie, Escandell et al. 2010), their deficiency might affect telomeres length, making this effect more evident in the presence of a variant in *OGG1* that appears to affect its mRNA expression levels is also present.

7.3. Advantages and disadvantages of using peripheral blood

Collection of peripheral blood is a very cheap and non-traumatic procedure. That procedure is made for diagnosis purposes and what is left can be also used for research purposes. Since we are looking forward to making a functional characterization of a germinal polymorphism, we can easily evaluate phenotypic

differences between the different genotypes (G/G) (G/A) (A/A) just using peripheral blood.

However, peripheral blood collection also faces some disadvantages regarding what we want to investigate. Since we are exploring a possible effect of the variant in the risk of developing cancer, it would put this study closer from its hypothesis if we had tumour samples from carriers of the minor allele (A) to check whether the OGG1 mRNA was down-regulated or not, and if those samples showed more chromosomal instability/aberrant genomic fusions when compared with cancers that were homozygote for the common allele (G/G).

Concerning the balance between the advantages and disadvantages of this study, we can claim that the advantages are greater providing us a successful experiment with very interesting outcomes. It will be obviously necessary more work to prove our hypothesis with more accuracy.

8. Conclusions

Carrying an inherited mutation in BRCA genes predisposes to breast, ovarian and even other cancers, however there are considerable differences in the manifestation of the disease. The fact is, that not all the individuals that inherited a BRCA mutation develop cancer, suggesting the existence of other genetic or environmental factors modifying the risk of develop cancer (Couch, Nathanson et al. 2014).

As we have foreseen, we found by the genotyping results, the minor allele ((G/A) or (A/A)) of the OGG1 variant rs2304277 is present in 34.5% of the population, as reported in Osorio, Milne et al. (2014) e dbSNP (2010). With respect to the OGG1 mRNA expression levels, diminished levels were observed in the carriers of the minor allele (A) when compared to the non-carriers (homozygote for the common allele (G/G)), indicating a possible influence of the variant in the regulation of the mRNA molecule since it is located in a regulatory region: 3'UTR. Thus, we aimed to analyse the possible influence of the polymorphism over the TL, since there is an impairment in the OGG1 mRNA levels.

The TL analysis showed that individuals that harbour a BRCA mutation plus rs2304277 minor allele (A) have a significant accelerated telomere shortening ($p<0.0001$) and not only, they also show shorter TL when compared with the Controls ($p=0.034$) and BRCA1/2 mutation group. Hence, we are facing a synergetic effect between both genetic conditions over the TL, promoting its shortening. The impairment of the OGG1 mRNA and consequently its translation is not sufficient for an accelerated telomere shortening or shorter telomere phenotype, being necessary an impaired BRCA gene. That said, we can assume the presence of the minor allele (A) makes this effect more evident in carriers of a BRCA gene mutation.

It is worth noting that our findings may explain in some means how the variant rs2304277, more specifically the presence of the minor allele (A), can modify the risk of developing cancer and perhaps influence the manifestation of the disease among the individuals that harbour a BRCA mutation. Considering the key role of the OGG1 enzyme in the repair of oxidized DNA (Radicella, Dherin et al. 1997) and the critical role of BRCA1 and BRCA2 in various cellular processes, as the maintenance of the

telomere homeostasis (Ballal, Saha et al. 2009, Badie, Escandell et al. 2010), it Is reasonable to consider that individuals with both systems impaired are more prone to develop cancer as a consequence of genomic instability from critically shorter telomeres.

Due to such small amount of individuals homozygotes for the minor allele (A/A) (6individuals) included in the sample used, we cannot confirmed if being homozygote for the minor allele gives a higher risk of developing cancer compared to the individuals that are heterozygous. Our experiments did not explored that aspect either.

Overall, such results meet our hypothesis, where the increase of cancer risk might be do is due to a less effective OGG1 action over the oxidative stress consequences due to a diminished OGG1 mRNA levels. A weaker response upon the oxidative stress aggression over the DNA, more specifically structures enriched in guanines as telomeres, might favour an accelerated telomere shortening.

Nonetheless, there is future work to address to find if indeed this is the causal variant. The association of this variant with an increased risk to develop ovarian cancer in BRCA1 mutation carriers was obtained by a tagSNP approach. As known, this approach uses a set of representative SNP in a determined region of the genome with high LD with a group of other SNPs. It will be necessary to do an approach with a greater resolution as Fine Mapping, to find if the causal variant is the one mentioned in this study or any other in LD with rs2304277.

More experiments are underway towards our hypothesis. Using patient-derived lymphoblastoid cells, we are using a concentration time-course of H2O2 treatments to understand how OGG1 mRNA is regulated according with the different conditions of concentration and time exposure, depending on the BRCA mutation status and presence or not of the variant. H2O2 is very commonly used in research for oxidative stress studies and in that way we can easily manipulate the conditions in cell culture. As well as the OGG1 mRNA expression levels evaluation, the TL and 8-oxoG levels will be considered. We do believe these experiments will enrich considerably our conclusions towards what we aim to answer.

9. Bibliography

ACS. (2014). "The history of cancer." Retrieved September, 2015, from http://www.cancer.org/acs/groups/cid/documents/webcontent/002048-pdf.pdf.

Ahmed, S., G. Thomas, M. Ghoussaini, C. S. Healey, M. K. Humphreys, R. Platte, J. Morrison, M. Maranian, K. A. Pooley, R. Luben, D. Eccles, D. G. Evans, O. Fletcher, N. Johnson, I. dos Santos Silva, J. Peto, M. R. Stratton, N. Rahman, K. Jacobs, R. Prentice, G. L. Anderson, A. Rajkovic, J. D. Curb, R. G. Ziegler, C. D. Berg, S. S. Buys, C. A. McCarty, H. S. Feigelson, E. E. Calle, M. J. Thun, W. R. Diver, S. Bojesen, B. G. Nordestgaard, H. Flyger, T. Dork, P. Schurmann, P. Hillemanns, J. H. Karstens, N. V. Bogdanova, N. N. Antonenkova, I. V. Zalutsky, M. Bermisheva, S. Fedorova, E. Khusnutdinova, Search, D. Kang, K. Y. Yoo, D. Y. Noh, S. H. Ahn, P. Devilee, C. J. van Asperen, R. A. Tollenaar, C. Seynaeve, M. Garcia-Closas, J. Lissowska, L. Brinton, B. Peplonska, H. Nevanlinna, T. Heikkinen, K. Aittomaki, C. Blomqvist, J. L. Hopper, M. C. Southey, L. Smith, A. B. Spurdle, M. K. Schmidt, A. Broeks, R. R. van Hien, S. Cornelissen, R. L. Milne, G. Ribas, A. Gonzalez-Neira, J. Benitez, R. K. Schmutzler, B. Burwinkel, C. R. Bartram, A. Meindl, H. Brauch, C. Justenhoven, U. Hamann, G. Consortium, J. Chang-Claude, R. Hein, S. Wang-Gohrke, A. Lindblom, S. Margolin, A. Mannermaa, V. M. Kosma, V. Kataja, J. E. Olson, X. Wang, Z. Fredericksen, G. G. Giles, G. Severi, L. Baglietto, D. R. English, S. E. Hankinson, D. G. Cox, P. Kraft, L. J. Vatten, K. Hveem, M. Kumle, A. Sigurdson, M. Doody, P. Bhatti, B. H. Alexander, M. J. Hooning, A. M. van den Ouweland, R. A. Oldenburg, M. Schutte, P. Hall, K. Czene, J. Liu, Y. Li, A. Cox, G. Elliott, I. Brock, M. W. Reed, C. Y. Shen, J. C. Yu, G. C. Hsu, S. T. Chen, H. Anton-Culver, A. Ziogas, I. L. Andrulis, J. A. Knight, kConFab, G. Australian Ovarian Cancer Study, J. Beesley, E. L. Goode, F. Couch, G. Chenevix-Trench, R. N. Hoover, B. A. Ponder, D. J. Hunter, P. D. Pharoah, A. M. Dunning, S. J. Chanock and D. F. Easton (2009). "Newly discovered breast cancer susceptibility loci on 3p24 and 17q23.2." Nat Genet **41**(5): 585-590.

Antoniou, A., P. D. Pharoah, S. Narod, H. A. Risch, J. E. Eyfjord, J. L. Hopper, N. Loman, H. Olsson, O. Johannsson, A. Borg, B. Pasini, P. Radice, S. Manoukian, D. M. Eccles, N. Tang, E. Olah, H. Anton-Culver, E. Warner, J. Lubinski, J. Gronwald, B. Gorski, H. Tulinius, S. Thorlacius, H. Eerola, H. Nevanlinna, K. Syrjakoski, O. P. Kallioniemi, D. Thompson, C. Evans, J. Peto, F. Lalloo, D. G. Evans and D. F. Easton (2003). "Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case Series unselected for family history: a combined analysis of 22 studies." Am J Hum Genet **72**(5): 1117-1130.

Artandi, S. E. and R. A. DePinho (2010). "Telomeres and telomerase in cancer." Carcinogenesis **31**(1): 9-18.

Aubert, G. and P. M. Lansdorp (2008). "Telomeres and aging." Physiol Rev **88**(2): 557-579.

Badie, S., J. M. Escandell, P. Bouwman, A. R. Carlos, M. Thanasoula, M. M. Gallardo, A. Suram, I. Jaco, J. Benitez, U. Herbig, M. A. Blasco, J. Jonkers and M. Tarsounas (2010). "BRCA2 acts as a RAD51 loader to facilitate telomere replication and capping." Nat Struct Mol Biol **17**(12): 1461-1469.

Ballal, R. D., T. Saha, S. Fan, B. R. Haddad and E. M. Rosen (2009). "BRCA1 localization to the telomere and its loss from the telomere in response to DNA damage." J Biol Chem **284**(52): 36083-36098.

Barrett, L. W., S. Fletcher and S. D. Wilton (2012). "Regulation of eukaryotic gene expression by the untranslated gene regions and other non-coding elements." Cell Mol Life Sci **69**(21): 3613-3634.

Berliner, J. L., A. M. Fay, S. A. Cummings, B. Burnett and T. Tillmanns (2013). "NSGC practice guideline: risk assessment and genetic counseling for hereditary breast and ovarian cancer." J Genet Couns **22**(2): 155-163.

Berliner, J. L., A. M. Fay and G. Practice Issues Subcommittee of the National Society of Genetic Counselors' Familial Cancer Risk Counseling Special Interest (2007). "Risk assessment and genetic counseling for hereditary breast and ovarian cancer: recommendations of the National Society of Genetic Counselors." J Genet Couns **16**(3): 241-260.

Borresen, A. L., T. I. Andersen, J. Garber, N. Barbier-Piraux, S. Thorlacius, J. Eyfjord, L. Ottestad, B. Smith-Sorensen, E. Hovig, D. Malkin and et al. (1992). "Screening for germ line TP53 mutations in breast cancer patients." Cancer Res **52**(11): 3234-3236.

Britannica, E. (2015). "Cancer." Retrieved September, 2015, from http://www.britannica.com/science/cancer-disease.

Broeks, A., M. K. Schmidt, M. E. Sherman, F. J. Couch, J. L. Hopper, G. S. Dite, C. Apicella, L. D. Smith, F. Hammet, M. C. Southey, L. J. Van 't Veer, R. de Groot, V. T. Smit, P. A. Fasching, M. W. Beckmann, S. Jud, A. B. Ekici, A. Hartmann, A. Hein, R. Schulz-Wendtland, B. Burwinkel, F. Marme, A. Schneeweiss, H. P. Sinn, C. Sohn, S. Tchatchou, S. E. Bojesen, B. G. Nordestgaard, H. Flyger, D. D. Orsted, D. Kaur-Knudsen, R. L. Milne, J. I. Perez, P. Zamora, P. M. Rodriguez, J. Benitez, H. Brauch, C. Justenhoven, Y. D. Ko, N. Genica, U. Hamann, H. P. Fischer, T. Bruning, B. Pesch, J. Chang-Claude, S. Wang-Gohrke, M. Bremer, J. H. Karstens, P. Hillemanns, T. Dork, H. A. Nevanlinna, T. Heikkinen, P. Heikkila, C. Blomqvist, K. Aittomaki, K. Aaltonen, A. Lindblom, S. Margolin, A. Mannermaa, V. M. Kosma, J. M. Kauppinen, V. Kataja, P. Auvinen, M. Eskelinen, Y. Soini, G. Chenevix-Trench, A. B. Spurdle, J. Beesley, X. Chen, H. Holland, kConFab, Aocs, D. Lambrechts, B. Claes, T. Vandorpe, P. Neven, H. Wildiers, D. Flesch-Janys, R. Hein, T. Loning, M. Kosel, Z. S. Fredericksen, X. Wang, G. G. Giles, L. Baglietto, G. Severi, C. McLean, C. A. Haiman, B. E. Henderson, L. Le Marchand, L. N. Kolonel, G. G. Alnaes, V. Kristensen, A. L. Borresen-Dale, D. J. Hunter, S. E. Hankinson, I. L. Andrulis, A. M. Mulligan, F. P. O'Malley, P. Devilee, P. E. Huijts, R. A. Tollenaar, C. J. Van Asperen, C. S. Seynaeve, S. J. Chanock, J. Lissowska, L. Brinton, B. Peplonska, J. Figueroa, X. R. Yang, M. J. Hooning, A. Hollestelle, R. A. Oldenburg, A. Jager, M. Kriege, B. Ozturk, G. J. van Leenders, P. Hall, K. Czene, K. Humphreys, J. Liu, A. Cox, D. Connley, H. E. Cramp, S. S. Cross, S. P. Balasubramanian, M. W. Reed, A. M. Dunning, D. F. Easton, M. K. Humphreys, C. Caldas, F. Blows, K. Driver, E. Provenzano, J. Lubinski, A. Jakubowska, T. Huzarski, T. Byrski, C. Cybulski, B. Gorski, J. Gronwald, P. Brennan, S. Sangrajrang, V. Gaborieau, C. Y. Shen, C. N. Hsiung, J. C. Yu, S. T. Chen, G. C. Hsu, M. F. Hou, C. S. Huang, H. Anton-Culver, A. Ziogas, P. D. Pharoah and M. Garcia-Closas (2011). "Low penetrance breast cancer susceptibility loci are associated with specific breast tumor subtypes: findings from the Breast Cancer Association Consortium." Hum Mol Genet **20**(16): 3289-3303.

Canela, A., E. Vera, P. Klatt and M. A. Blasco (2007). "High-throughput telomere length quantification by FISH and its application to human population studies." Proc Natl Acad Sci U S A **104**(13): 5300-5305.

Chen, J., P. Lindblom and A. Lindblom (1998). "A study of the PTEN/MMAC1 gene in 136 breast cancer families." Hum Genet **102**(1): 124-125.

Chen, S., E. S. Iversen, T. Friebel, D. Finkelstein, B. L. Weber, A. Eisen, L. E. Peterson, J. M. Schildkraut, C. Isaacs, B. N. Peshkin, C. Corio, L. Leondaridis, G. Tomlinson, D. Dutson, R. Kerber, C. I. Amos, L. C. Strong, D. A. Berry, D. M. Euhus and G. Parmigiani (2006). "Characterization of BRCA1 and BRCA2 mutations in a large United States sample." J Clin Oncol **24**(6): 863-871.

Chen, S. and G. Parmigiani (2007). "Meta-analysis of BRCA1 and BRCA2 penetrance." J Clin Oncol **25**(11): 1329-1333.

Collado, M., M. A. Blasco and M. Serrano (2007). "Cellular senescence in cancer and aging." Cell **130**(2): 223-233.

Coluzzi, E., M. Colamartino, R. Cozzi, S. Leone, C. Meneghini, N. O'Callaghan and A. Sgura (2014). "Oxidative stress induces persistent telomeric DNA damage responsible for nuclear morphology change in mammalian cells." PLoS One **9**(10): e110963.

Cong, Y. S., W. E. Wright and J. W. Shay (2002). "Human telomerase and its regulation." Microbiol Mol Biol Rev **66**(3): 407-425, table of contents.

Couch, F. J., K. L. Nathanson and K. Offit (2014). "Two decades after BRCA: setting paradigms in personalized cancer care and prevention." Science **343**(6178): 1466-1470.

Cousineau, I. and A. Belmaaza (2007). "BRCA1 haploinsufficiency, but not heterozygosity for a BRCA1-truncating mutation, deregulates homologous recombination." Cell Cycle **6**(8): 962-971.

Cox, A., A. M. Dunning, M. Garcia-Closas, S. Balasubramanian, M. W. Reed, K. A. Pooley, S. Scollen, C. Baynes, B. A. Ponder, S. Chanock, J. Lissowska, L. Brinton, B. Peplonska, M. C. Southey, J. L. Hopper, M. R. McCredie, G. G. Giles, O. Fletcher, N. Johnson, I. dos Santos Silva, L. Gibson, S. E. Bojesen, B. G. Nordestgaard, C. K. Axelsson, D. Torres, U. Hamann, C. Justenhoven, H. Brauch, J. Chang-Claude, S. Kropp, A. Risch, S. Wang-Gohrke, P. Schurmann, N. Bogdanova, T. Dork, R. Fagerholm, K. Aaltonen, C. Blomqvist, H. Nevanlinna, S. Seal, A. Renwick, M. R. Stratton, N. Rahman, S. Sangrajrang, D. Hughes, F. Odefrey, P. Brennan, A. B. Spurdle, G. Chenevix-Trench, C. Kathleen Cunningham Foundation Consortium for Research into Familial Breast, J. Beesley, A. Mannermaa, J. Hartikainen, V. Kataja, V. M. Kosma, F. J. Couch, J. E. Olson, E. L. Goode, A. Broeks, M. K. Schmidt, F. B. Hogervorst, L. J. Van't Veer, D. Kang, K. Y. Yoo, D. Y. Noh, S. H. Ahn, S. Wedren, P. Hall, Y. L. Low, J. Liu, R. L. Milne, G. Ribas, A. Gonzalez-Neira, J. Benitez, A. J. Sigurdson, D. L. Stredrick, B. H. Alexander, J. P. Struewing, P. D. Pharoah, D. F. Easton and C. Breast Cancer Association (2007). "A common coding variant in CASP8 is associated with breast cancer risk." Nat Genet **39**(3): 352-358.

Cronin, K. A., L. A. Ries and B. K. Edwards (2014). "The Surveillance, Epidemiology, and End Results (SEER) Program of the National Cancer Institute." Cancer **120 Suppl 23**: 3755-3757.

dbSNP. (2010). "NCBI dbSNP Short Genetic Variations." Retrieved September, 2015, from http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=2304277.

dbSNP. (2015). "rs2304277." Retrieved September, 2015, from http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=2304277.

de Lange, T. (2005). "Shelterin: the protein complex that shapes and safeguards human telomeres." Genes Dev **19**(18): 2100-2110.

Diakowska, D., A. Lewandowski, W. Kopec, W. Diakowski and T. Chrzanowska (2007). "Oxidative DNA damage and total antioxidant status in serum of patients with esophageal squamous cell carcinoma." Hepatogastroenterology **54**(78): 1701-1704.

Diez, O., A. Osorio, M. Duran, J. I. Martinez-Ferrandis, M. de la Hoya, R. Salazar, A. Vega, B. Campos, R. Rodriguez-Lopez, E. Velasco, J. Chaves, E. Diaz-Rubio, J. Jesus Cruz, M. Torres, E. Esteban, A. Cervantes, C. Alonso, J. M. San Roman, R. Gonzalez-Sarmiento, C. Miner, A. Carracedo, M. Eugenia Armengod, T. Caldes, J. Benitez and M. Baiget (2003). "Analysis of BRCA1 and BRCA2 genes in Spanish breast/ovarian cancer patients: a high proportion of mutations unique to Spain and evidence of founder effects." Hum Mutat **22**(4): 301-312.

Drohat, A. C., K. Kwon, D. J. Krosky and J. T. Stivers (2002). "3-Methyladenine DNA glycosylase I is an unexpected helix-hairpin-helix superfamily member." Nat Struct Biol **9**(9): 659-664.

Duan, W. X., R. X. Hua, W. Yi, L. J. Shen, Z. X. Jin, Y. H. Zhao, D. H. Yi, W. S. Chen and S. Q. Yu (2012). "The association between OGG1 Ser326Cys polymorphism and lung cancer susceptibility: a meta-analysis of 27 studies." PLoS One **7**(4): e35970.

Easton, D. F. and R. A. Eeles (2008). "Genome-wide association studies in cancer." Hum Mol Genet **17**(R2): R109-115.

Easton, D. F., K. A. Pooley, A. M. Dunning, P. D. Pharoah, D. Thompson, D. G. Ballinger, J. P. Struewing, J. Morrison, H. Field, R. Luben, N. Wareham, S. Ahmed, C. S. Healey, R. Bowman, S. collaborators, K. B. Meyer, C. A. Haiman, L. K. Kolonel, B. E. Henderson, L. Le Marchand, P. Brennan, S. Sangrajrang, V. Gaborieau, F. Odefrey, C. Y. Shen, P. E. Wu, H. C. Wang, D. Eccles, D. G. Evans, J. Peto, O. Fletcher, N. Johnson, S. Seal, M. R. Stratton, N. Rahman, G. Chenevix-Trench, S. E. Bojesen, B. G. Nordestgaard, C. K. Axelsson, M. Garcia-Closas, L. Brinton, S. Chanock, J.

Lissowska, B. Peplonska, H. Nevanlinna, R. Fagerholm, H. Eerola, D. Kang, K. Y. Yoo, D. Y. Noh, S. H. Ahn, D. J. Hunter, S. E. Hankinson, D. G. Cox, P. Hall, S. Wedren, J. Liu, Y. L. Low, N. Bogdanova, P. Schurmann, T. Dork, R. A. Tollenaar, C. E. Jacobi, P. Devilee, J. G. Klijn, A. J. Sigurdson, M. M. Doody, B. H. Alexander, J. Zhang, A. Cox, I. W. Brock, G. MacPherson, M. W. Reed, F. J. Couch, E. L. Goode, J. E. Olson, H. Meijers-Heijboer, A. van den Ouweland, A. Uitterlinden, F. Rivadeneira, R. L. Milne, G. Ribas, A. Gonzalez-Neira, J. Benitez, J. L. Hopper, M. McCredie, M. Southey, G. G. Giles, C. Schroen, C. Justenhoven, H. Brauch, U. Hamann, Y. D. Ko, A. B. Spurdle, J. Beesley, X. Chen, kConFab, A. M. Group, A. Mannermaa, V. M. Kosma, V. Kataja, J. Hartikainen, N. E. Day, D. R. Cox and B. A. Ponder (2007). "Genome-wide association study identifies novel breast cancer susceptibility loci." Nature **447**(7148): 1087-1093.

Eeles, R., C. Goh, E. Castro, E. Bancroft, M. Guy, A. A. Al Olama, D. Easton and Z. Kote-Jarai (2014). "The genetic epidemiology of prostate cancer and its clinical implications." Nat Rev Urol **11**(1): 18-31.

Ensembl. (2015). "Gene: 4968." Retrieved 17th of March, 2015, from http://www.ensembl.org/Homo_sapiens/Gene/Summary?db=otherfeatures;g=4968;r=3:9749944-9788246.

Epidemiology, C. (2015). "Collaborative Group on Hormonal Factors in Breast Cancer." Retrieved 28th of March, 2015, from http://www.ceu.ox.ac.uk/research/29/collaborative-group-on-hormonal-factors-in-breast-cancer.

Ferlay, J., I. Soerjomataram, R. Dikshit, S. Eser, C. Mathers, M. Rebelo, D. M. Parkin, D. Forman and F. Bray (2015). "Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012." Int J Cancer **136**(5): E359-386.

Figueroa, J. D., M. Garcia-Closas, M. Humphreys, R. Platte, J. L. Hopper, M. C. Southey, C. Apicella, F. Hammet, M. K. Schmidt, A. Broeks, R. A. Tollenaar, L. J. Van't Veer, P. A. Fasching, M. W. Beckmann, A. B. Ekici, R. Strick, J. Peto, I. dos Santos Silva, O. Fletcher, N. Johnson, E. Sawyer, I. Tomlinson, M. Kerin, B. Burwinkel, F. Marme, A. Schneeweiss, C. Sohn, S. Bojesen, H. Flyger, B. G. Nordestgaard, J. Benitez, R. L. Milne, J. Ignacio Arias, M. P. Zamora, H. Brenner, H. Muller, V. Arndt, N. Rahman, C. Turnbull, S. Seal, A. Renwick, H. Brauch, C. Justenhoven, T. Bruning, G. Network, J. Chang-Claude, R. Hein, S. Wang-Gohrke, T. Dork, P. Schurmann, M. Bremer, P. Hillemanns, H. Nevanlinna, T. Heikkinen, K. Aittomaki, C. Blomqvist, N. Bogdanova, N. Antonenkova, Y. I. Rogov, J. H. Karstens, M. Bermisheva, D. Prokofieva, S. H. Gantcev, E. Khusnutdinova, A. Lindblom, S. Margolin, G. Chenevix-Trench, J. Beesley, X. Chen, A. M. G. kConFab, A. Mannermaa, V. M. Kosma, Y. Soini, V. Kataja, D. Lambrechts, B. T. Yesilyurt, M. R. Chrisiaens, S. Peeters, P. Radice, P. Peterlongo, S. Manoukian, M. Barile, F. Couch, A. M. Lee, R. Diasio, X. Wang, G. G. Giles, G. Severi, L. Baglietto, C. Maclean, K. Offit, M. Robson, V. Joseph, M. Gaudet, E. M. John, R. Winqvist, K. Pylkas, A. Jukkola-Vuorinen, M. Grip, I. Andrulis, J. A. Knight, A. M. Mulligan, F. P. O'Malley, L. A. Brinton, M. E. Sherman, J. Lissowska, S. J. Chanock, M. Hooning, J. W. Martens, A. M. van den Ouweland, J. M. Collee, P. Hall, K. Czene, A. Cox, I. W. Brock, M. W. Reed, S. S. Cross, P. Pharoah, A. M. Dunning, D. Kang, K. Y. Yoo, D. Y. Noh, S. H. Ahn, A. Jakubowska, J. Lubinski, K. Jaworska, K. Durda, S. Sangrajrang, V. Gaborieau, P. Brennan, J. McKay, C. Y. Shen, S. L. Ding, H. M. Hsu, J. C. Yu, H. Anton-Culver, A. Ziogas, A. Ashworth, A. Swerdlow, M. Jones, N. Orr, A. Trentham-Dietz, K. Egan, P. Newcomb, L. Titus-Ernstoff, D. Easton and A. B. Spurdle (2011). "Associations of common variants at 1p11.2 and 14q24.1 (RAD51L1) with breast cancer risk and heterogeneity by tumor subtype: findings from the Breast Cancer Association Consortium." Hum Mol Genet **20**(23): 4693-4706.

Figures, B. C. F. (2013). "American Cancer Society " Atlanta: American Cancer Society, Inc.

Georgakilas, A. G. (2012). "Oxidative stress, DNA damage and repair in carcinogenesis: have we established a connection?" Cancer Lett **327**(1-2): 3-4.

Ghoussaini, M., P. D. Pharoah and D. F. Easton (2013). "Inherited genetic susceptibility to breast cancer: the beginning of the end or the end of the beginning?" Am J Pathol **183**(4): 1038-1051.

GLOBOCAN. (2012). "GLOBOCAN 2012: Estimated Cancer Incidence, Mortality and Pervalence in 2012." Retrieved 19th of March, 2015, from http://globocan.iarc.fr/Pages/fact_sheets_population.aspx.

Gómez, Á. (2011). "Feed back Ciencia." Retrieved September, 2015, from http://www.feedbackciencia.com/jose-luis-soto-el-peligro-esta-al-acecho-pero-podemos-defendernos/.

Hall, J. M., M. K. Lee, B. Newman, J. E. Morrow, L. A. Anderson, B. Huey and M. C. King (1990). "Linkage of early-onset familial breast cancer to chromosome 17q21." Science **250**(4988): 1684-1689.

Hanahan, D. and R. A. Weinberg (2011). "Hallmarks of cancer: the next generation." Cell **144**(5): 646-674.

Hodes, R. J. (1999). "Telomere length, aging, and somatic cell turnover." J Exp Med **190**(2): 153-156.

Hurst, J. H. (2014). "Pioneering geneticist Mary-Claire King receives the 2014 Lasker~Koshland Special Achievement Award in Medical Science." J Clin Invest **124**(10): 4148-4151.

International HapMap, C. (2005). "A haplotype map of the human genome." Nature **437**(7063): 1299-1320.

Jacobs, A. L. and P. Schar (2012). "DNA glycosylases: in DNA repair and beyond." Chromosoma **121**(1): 1-20.

Kavli, B., M. Otterlei, G. Slupphaug and H. E. Krokan (2007). "Uracil in DNA--general mutagen, but normal intermediate in acquired immunity." DNA Repair (Amst) **6**(4): 505-516.

Kim, Y. J. and D. M. Wilson, 3rd (2012). "Overview of base excision repair biochemistry." Curr Mol Pharmacol **5**(1): 3-13.

Klaunig, J. E., L. M. Kamendulis and B. A. Hocevar (2010). "Oxidative stress and oxidative damage in carcinogenesis." Toxicol Pathol **38**(1): 96-109.

Klungland, A., I. Rosewell, S. Hollenbach, E. Larsen, G. Daly, B. Epe, E. Seeberg, T. Lindahl and D. E. Barnes (1999). "Accumulation of premutagenic DNA lesions in mice defective in removal of oxidative base damage." Proc Natl Acad Sci U S A **96**(23): 13300-13305.

Knudson, A. G., Jr. (1971). "Mutation and cancer: statistical study of retinoblastoma." Proc Natl Acad Sci U S A **68**(4): 820-823.

Konishi, H., M. Mohseni, A. Tamaki, J. P. Garay, S. Croessmann, S. Karnan, A. Ota, H. Y. Wong, Y. Konishi, B. Karakas, K. Tahir, A. M. Abukhdeir, J. P. Gustin, J. Cidado, G. M. Wang, D. Cosgrove, R. Cochran, D. Jelovac, M. J. Higgins, S. Arena, L. Hawkins, J. Lauring, A. L. Gross, C. M. Heaphy, Y. Hosokawa, E. Gabrielson, A. K. Meeker, K. Visvanathan, P. Argani, K. E. Bachman and B. H. Park (2011). "Mutation of a single allele of the cancer susceptibility gene BRCA1 leads to genomic instability in human breast epithelial cells." Proc Natl Acad Sci U S A **108**(43): 17773-17778.

Kow, Y. W. (1994). "Base excision repair in E. coli--an overview." Ann N Y Acad Sci **726**: 178-180.

Land, H., L. F. Parada and R. A. Weinberg (1983). "Tumorigenic conversion of primary embryo fibroblasts requires at least two cooperating oncogenes." Nature **304**(5927): 596-602.

Leggett, B. A., J. P. Young and M. Barker (2003). "Peutz-Jeghers syndrome: genetic screening." Expert Rev Anticancer Ther **3**(4): 518-524.

Liu, D., M. S. O'Connor, J. Qin and Z. Songyang (2004). "Telosome, a mammalian telomere-associated complex formed by multiple telomeric proteins." J Biol Chem **279**(49): 51338-51342.

Lu, J. and Y. Liu (2010). "Deletion of Ogg1 DNA glycosylase results in telomere base damage and length alteration in yeast." EMBO J **29**(2): 398-409.

Mason, P. J. and N. Perdigones (2013). "Telomere biology and translational research." Transl Res **162**(6): 333-342.

Meijers-Heijboer, H., A. van den Ouweland, J. Klijn, M. Wasielewski, A. de Snoo, R. Oldenburg, A. Hollestelle, M. Houben, E. Crepin, M. van Veghel-Plandsoen, F. Elstrodt, C. van Duijn, C. Bartels, C. Meijers, M. Schutte, L. McGuffog, D. Thompson, D. Easton, N. Sodha, S. Seal, R. Barfoot, J. Mangion, J. Chang-Claude, D. Eccles, R. Eeles, D. G. Evans, R. Houlston, V. Murday, S. Narod, T. Peretz, J. Peto, C. Phelan, H. X. Zhang, C. Szabo, P. Devilee, D. Goldgar, P. A. Futreal, K. L. Nathanson, B. Weber, N. Rahman and M. R. Stratton (2002). "Low-penetrance susceptibility to breast cancer due to CHEK2(*)1100delC in noncarriers of BRCA1 or BRCA2 mutations." Nat Genet **31**(1): 55-59.

Meindl, A., H. Hellebrand, C. Wiek, V. Erven, B. Wappenschmidt, D. Niederacher, M. Freund, P. Lichtner, L. Hartmann, H. Schaal, J. Ramser, E. Honisch, C. Kubisch, H. E. Wichmann, K. Kast, H. Deissler, C. Engel, B. Muller-Myhsok, K. Neveling, M. Kiechle, C. G. Mathew, D. Schindler, R. K. Schmutzler and H. Hanenberg (2010). "Germline mutations in breast and ovarian cancer pedigrees establish RAD51C as a human cancer susceptibility gene." Nat Genet **42**(5): 410-414.

Melchor, L. and J. Benitez (2013). "The complex genetic landscape of familial breast cancer." Hum Genet **132**(8): 845-863.

Mendelsohn, J. (2008). The molecular basis of cancer. Philadelphia, PA, Saunders/Elsevier.

Michailidou, K., J. Beesley, S. Lindstrom, S. Canisius, J. Dennis, M. J. Lush, M. J. Maranian, M. K. Bolla, Q. Wang, M. Shah, B. J. Perkins, K. Czene, M. Eriksson, H. Darabi, J. S. Brand, S. E. Bojesen, B. G. Nordestgaard, H. Flyger, S. F. Nielsen, N. Rahman, C. Turnbull, Bocs, O. Fletcher, J. Peto, L. Gibson, I. dos-Santos-Silva, J. Chang-Claude, D. Flesch-Janys, A. Rudolph, U. Eilber, S. Behrens, H. Nevanlinna, T. A. Muranen, K. Aittomaki, C. Blomqvist, S. Khan, K. Aaltonen, H. Ahsan, M. G. Kibriya, A. S. Whittemore, E. M. John, K. E. Malone, M. D. Gammon, R. M. Santella, G. Ursin, E. Makalic, D. F. Schmidt, G. Casey, D. J. Hunter, S. M. Gapstur, M. M. Gaudet, W. R. Diver, C. A. Haiman, F. Schumacher, B. E. Henderson, L. Le Marchand, C. D. Berg, S. J. Chanock, J. Figueroa, R. N. Hoover, D. Lambrechts, P. Neven, H. Wildiers, E. van Limbergen, M. K. Schmidt, A. Broeks, S. Verhoef, S. Cornelissen, F. J. Couch, J. E. Olson, E. Hallberg, C. Vachon, Q. Waisfisz, H. Meijers-Heijboer, M. A. Adank, R. B. van der Luijt, J. Li, J. Liu, K. Humphreys, D. Kang, J. Y. Choi, S. K. Park, K. Y. Yoo, K. Matsuo, H. Ito, H. Iwata, K. Tajima, P. Guenel, T. Truong, C. Mulot, M. Sanchez, B. Burwinkel, F. Marme, H. Surowy, C. Sohn, A. H. Wu, C. C. Tseng, D. Van Den Berg, D. O. Stram, A. Gonzalez-Neira, J. Benitez, M. P. Zamora, J. I. Perez, X. O. Shu, W. Lu, Y. T. Gao, H. Cai, A. Cox, S. S. Cross, M. W. Reed, I. L. Andrulis, J. A. Knight, G. Glendon, A. M. Mulligan, E. J. Sawyer, I. Tomlinson, M. J. Kerin, N. Miller, I. kConFab, A. Group, A. Lindblom, S. Margolin, S. H. Teo, C. H. Yip, N. A. Taib, G. H. Tan, M. J. Hooning, A. Hollestelle, J. W. Martens, J. M. Collee, W. Blot, L. B. Signorello, Q. Cai, J. L. Hopper, M. C. Southey, H. Tsimiklis, C. Apicella, C. Y. Shen, C. N. Hsiung, P. E. Wu, M. F. Hou, V. N. Kristensen, S. Nord, G. I. Alnaes, Nbcs, G. G. Giles, R. L. Milne, C. McLean, F. Canzian, D. Trichopoulos, P. Peeters, E. Lund, M. Sund, K. T. Khaw, M. J. Gunter, D. Palli, L. M. Mortensen, L. Dossus, J. M. Huerta, A. Meindl, R. K. Schmutzler, C. Sutter, R. Yang, K. Muir, A. Lophatananon, S. Stewart-Brown, P. Siriwanarangsan, M. Hartman, H. Miao, K. S. Chia, C. W. Chan, P. A. Fasching, A. Hein, M. W. Beckmann, L. Haeberle, H. Brenner, A. K. Dieffenbach, V. Arndt, C. Stegmaier, A. Ashworth, N. Orr, M. J. Schoemaker, A. J. Swerdlow, L. Brinton, M. Garcia-Closas, W. Zheng, S. L. Halverson, M. Shrubsole, J. Long, M. S. Goldberg, F. Labreche, M. Dumont, R. Winqvist, K. Pylkas, A. Jukkola-Vuorinen, M. Grip, H. Brauch, U. Hamann, T. Bruning, G. Network, P. Radice, P. Peterlongo, S. Manoukian, L. Bernard, N. V. Bogdanova, T. Dork, A. Mannermaa, V. Kataja, V. M. Kosma, J. M. Hartikainen, P. Devilee, R. A. Tollenaar, C. Seynaeve, C. J. Van Asperen, A. Jakubowska, J. Lubinski, K. Jaworska, T. Huzarski, S. Sangrajrang, V. Gaborieau, P. Brennan, J. McKay, S. Slager, A. E. Toland, C. B. Ambrosone, D. Yannoukakos, M. Kabisch, D. Torres, S. L. Neuhausen, H. Anton-Culver, C. Luccarini, C. Baynes, S. Ahmed, C. S. Healey, D. C. Tessier, D. Vincent, F. Bacot, G. Pita, M. R. Alonso, N. Alvarez, D. Herrero, J. Simard,

P. P. Pharoah, P. Kraft, A. M. Dunning, G. Chenevix-Trench, P. Hall and D. F. Easton (2015). "Genome-wide association analysis of more than 120,000 individuals identifies 15 new susceptibility loci for breast cancer." Nat Genet **47**(4): 373-380.

Michailidou, K., P. Hall, A. Gonzalez-Neira, M. Ghoussaini, J. Dennis, R. L. Milne, M. K. Schmidt, J. Chang-Claude, S. E. Bojesen, M. K. Bolla, Q. Wang, E. Dicks, A. Lee, C. Turnbull, N. Rahman, Breast, C. Ovarian Cancer Susceptibility, O. Fletcher, J. Peto, L. Gibson, I. Dos Santos Silva, H. Nevanlinna, T. A. Muranen, K. Aittomaki, C. Blomqvist, K. Czene, A. Irwanto, J. Liu, Q. Waisfisz, H. Meijers-Heijboer, M. Adank, B. Hereditary, N. Ovarian Cancer Research Group, R. B. van der Luijt, R. Hein, N. Dahmen, L. Beckman, A. Meindl, R. K. Schmutzler, B. Muller-Myhsok, P. Lichtner, J. L. Hopper, M. C. Southey, E. Makalic, D. F. Schmidt, A. G. Uitterlinden, A. Hofman, D. J. Hunter, S. J. Chanock, D. Vincent, F. Bacot, D. C. Tessier, S. Canisius, L. F. Wessels, C. A. Haiman, M. Shah, R. Luben, J. Brown, C. Luccarini, N. Schoof, K. Humphreys, J. Li, B. G. Nordestgaard, S. F. Nielsen, H. Flyger, F. J. Couch, X. Wang, C. Vachon, K. N. Stevens, D. Lambrechts, M. Moisse, R. Paridaens, M. R. Christiaens, A. Rudolph, S. Nickels, D. Flesch-Janys, N. Johnson, Z. Aitken, K. Aaltonen, T. Heikkinen, A. Broeks, L. J. Veer, C. E. van der Schoot, P. Guenel, T. Truong, P. Laurent-Puig, F. Menegaux, F. Marme, A. Schneeweiss, C. Sohn, B. Burwinkel, M. P. Zamora, J. I. Perez, G. Pita, M. R. Alonso, A. Cox, I. W. Brock, S. S. Cross, M. W. Reed, E. J. Sawyer, I. Tomlinson, M. J. Kerin, N. Miller, B. E. Henderson, F. Schumacher, L. Le Marchand, I. L. Andrulis, J. A. Knight, G. Glendon, A. M. Mulligan, I. kConFab, G. Australian Ovarian Cancer Study, A. Lindblom, S. Margolin, M. J. Hooning, A. Hollestelle, A. M. van den Ouweland, A. Jager, Q. M. Bui, J. Stone, G. S. Dite, C. Apicella, H. Tsimiklis, G. G. Giles, G. Severi, L. Baglietto, P. A. Fasching, L. Haeberle, A. B. Ekici, M. W. Beckmann, H. Brenner, H. Muller, V. Arndt, C. Stegmaier, A. Swerdlow, A. Ashworth, N. Orr, M. Jones, J. Figueroa, J. Lissowska, L. Brinton, M. S. Goldberg, F. Labreche, M. Dumont, R. Winqvist, K. Pylkas, A. Jukkola-Vuorinen, M. Grip, H. Brauch, U. Hamann, T. Bruning, G. Network, P. Radice, P. Peterlongo, S. Manoukian, B. Bonanni, P. Devilee, R. A. Tollenaar, C. Seynaeve, C. J. van Asperen, A. Jakubowska, J. Lubinski, K. Jaworska, K. Durda, A. Mannermaa, V. Kataja, V. M. Kosma, J. M. Hartikainen, N. V. Bogdanova, N. N. Antonenkova, T. Dork, V. N. Kristensen, H. Anton-Culver, S. Slager, A. E. Toland, S. Edge, F. Fostira, D. Kang, K. Y. Yoo, D. Y. Noh, K. Matsuo, H. Ito, H. Iwata, A. Sueta, A. H. Wu, C. C. Tseng, D. Van Den Berg, D. O. Stram, X. O. Shu, W. Lu, Y. T. Gao, H. Cai, S. H. Teo, C. H. Yip, S. Y. Phuah, B. K. Cornes, M. Hartman, H. Miao, W. Y. Lim, J. H. Sng, K. Muir, A. Lophatananon, S. Stewart-Brown, P. Siriwanarangsan, C. Y. Shen, C. N. Hsiung, P. E. Wu, S. L. Ding, S. Sangrajrang, V. Gaborieau, P. Brennan, J. McKay, W. J. Blot, L. B. Signorello, Q. Cai, W. Zheng, S. Deming-Halverson, M. Shrubsole, J. Long, J. Simard, M. Garcia-Closas, P. D. Pharoah, G. Chenevix-Trench, A. M. Dunning, J. Benitez and D. F. Easton (2013). "Large-scale genotyping identifies 41 new loci associated with breast cancer risk." Nat Genet **45**(4): 353-361, 361e351-352.

Milne, R. L., J. Benitez, H. Nevanlinna, T. Heikkinen, K. Aittomaki, C. Blomqvist, J. I. Arias, M. P. Zamora, B. Burwinkel, C. R. Bartram, A. Meindl, R. K. Schmutzler, A. Cox, I. Brock, G. Elliott, M. W. Reed, M. C. Southey, L. Smith, A. B. Spurdle, J. L. Hopper, F. J. Couch, J. E. Olson, X. Wang, Z. Fredericksen, P. Schurmann, M. Bremer, P. Hillemanns, T. Dork, P. Devilee, C. J. van Asperen, R. A. Tollenaar, C. Seynaeve, P. Hall, K. Czene, J. Liu, Y. Li, S. Ahmed, A. M. Dunning, M. Maranian, P. D. Pharoah, G. Chenevix-Trench, J. Beesley, I. kConFab, A. Group, N. V. Bogdanova, N. N. Antonenkova, I. V. Zalutsky, H. Anton-Culver, A. Ziogas, H. Brauch, C. Justenhoven, Y. D. Ko, S. Haas, P. A. Fasching, R. Strick, A. B. Ekici, M. W. Beckmann, G. G. Giles, G. Severi, L. Baglietto, D. R. English, O. Fletcher, N. Johnson, I. dos Santos Silva, J. Peto, C. Turnbull, S. Hines, A. Renwick, N. Rahman, B. G. Nordestgaard, S. E. Bojesen, H. Flyger, D. Kang, K. Y. Yoo, D. Y. Noh, A. Mannermaa, V. Kataja, V. M. Kosma, M. Garcia-Closas, S. Chanock, J. Lissowska, L. A. Brinton, J. Chang-Claude, S. Wang-Gohrke, C. Y. Shen, H. C. Wang, J. C. Yu, S. T. Chen, M. Bermisheva, T.

Nikolaeva, E. Khusnutdinova, M. K. Humphreys, J. Morrison, R. Platte, D. F. Easton and C. Breast Cancer Association (2009). "Risk of estrogen receptor-positive and -negative breast cancer and single-nucleotide polymorphism 2q35-rs13387042." J Natl Cancer Inst **101**(14): 1012-1018.

Milne, R. L., A. Osorio, T. R. Cajal, A. Vega, G. Llort, M. de la Hoya, O. Diez, M. C. Alonso, C. Lazaro, I. Blanco, A. Sanchez-de-Abajo, T. Caldes, A. Blanco, B. Grana, M. Duran, E. Velasco, I. Chirivella, E. E. Cardenosa, M. I. Tejada, E. Beristain, M. D. Miramar, M. T. Calvo, E. Martinez, C. Guillen, R. Salazar, C. San Roman, A. C. Antoniou, M. Urioste and J. Benitez (2008). "The average cumulative risks of breast and ovarian cancer for carriers of mutations in BRCA1 and BRCA2 attending genetic counseling units in Spain." Clin Cancer Res **14**(9): 2861-2869.

Miyake, H., I. Hara, S. Kamidono and H. Eto (2004). "Oxidative DNA damage in patients with prostate cancer and its response to treatment." J Urol **171**(4): 1533-1536.

Munoz-Espin, D. and M. Serrano (2014). "Cellular senescence: from physiology to pathology." Nat Rev Mol Cell Biol **15**(7): 482-496.

Nash, H. M., S. D. Bruner, O. D. Scharer, T. Kawate, T. A. Addona, E. Spooner, W. S. Lane and G. L. Verdine (1996). "Cloning of a yeast 8-oxoguanine DNA glycosylase reveals the existence of a base-excision DNA-repair protein superfamily." Curr Biol **6**(8): 968-980.

NCI. (2015). "SEER Stat Fact Sheets: Breast Cancer." Retrieved 26th of March, 2015, from http://seer.cancer.gov/statfacts/html/breast.html.

NCI. (2015). "What is Cancer." Retrieved September, 2015, from http://www.cancer.gov/about-cancer/what-is-cancer.

Nisman, B., L. Kadouri, T. Allweis, B. Maly, T. Hamburger, S. Gronowitz and T. Peretz (2013). "Increased proliferative background in healthy women with BRCA1/2 haploinsufficiency is associated with high risk for breast cancer." Cancer Epidemiol Biomarkers Prev **22**(11): 2110-2115.

O'Sullivan, R. J. and J. Karlseder (2010). "Telomeres: protecting chromosomes against genome instability." Nat Rev Mol Cell Biol **11**(3): 171-181.

Oikawa, S. and S. Kawanishi (1999). "Site-specific DNA damage at GGG sequence by oxidative stress may accelerate telomere shortening." FEBS Lett **453**(3): 365-368.

Osorio, A., R. L. Milne, K. Kuchenbaecker, T. Vaclova, G. Pita, R. Alonso, P. Peterlongo, I. Blanco, M. de la Hoya, M. Duran, O. Diez, Y. C. T. Ramon, I. Konstantopoulou, C. Martinez-Bouzas, R. Andres Conejero, P. Soucy, L. McGuffog, D. Barrowdale, A. Lee, B. Swe, B. Arver, J. Rantala, N. Loman, H. Ehrencrona, O. I. Olopade, M. S. Beattie, S. M. Domchek, K. Nathanson, T. R. Rebbeck, B. K. Arun, B. Y. Karlan, C. Walsh, J. Lester, E. M. John, A. S. Whittemore, M. B. Daly, M. Southey, J. Hopper, M. B. Terry, S. S. Buys, R. Janavicius, C. M. Dorfling, E. J. van Rensburg, L. Steele, S. L. Neuhausen, Y. C. Ding, T. V. Hansen, L. Jonson, B. Ejlertsen, A. M. Gerdes, M. Infante, B. Herraez, L. T. Moreno, J. N. Weitzel, J. Herzog, K. Weeman, S. Manoukian, B. Peissel, D. Zaffaroni, G. Scuvera, B. Bonanni, F. Mariette, S. Volorio, A. Viel, L. Varesco, L. Papi, L. Ottini, M. G. Tibiletti, P. Radice, D. Yannoukakos, J. Garber, S. Ellis, D. Frost, R. Platte, E. Fineberg, G. Evans, F. Lalloo, L. Izatt, R. Eeles, J. Adlard, R. Davidson, T. Cole, D. Eccles, J. Cook, S. Hodgson, C. Brewer, M. Tischkowitz, F. Douglas, M. Porteous, L. Side, L. Walker, P. Morrison, A. Donaldson, J. Kennedy, C. Foo, A. K. Godwin, R. K. Schmutzler, B. Wappenschmidt, K. Rhiem, C. Engel, A. Meindl, N. Ditsch, N. Arnold, H. J. Plendl, D. Niederacher, C. Sutter, S. Wang-Gohrke, D. Steinemann, S. Preisler-Adams, K. Kast, R. Varon-Mateeva, A. Gehrig, D. Stoppa-Lyonnet, O. M. Sinilnikova, S. Mazoyer, F. Damiola, B. Poppe, K. Claes, M. Piedmonte, K. Tucker, F. Backes, G. Rodriguez, W. Brewster, K. Wakeley, T. Rutherford, T. Caldes, H. Nevanlinna, K. Aittomaki, M. A. Rookus, T. A. van Os, L. van der Kolk, J. L. de Lange, H. E. Meijers-Heijboer, A. H. van der Hout, C. J. van Asperen, E. B. Gomez Garcia, N. Hoogerbrugge, J. M. Collee, C. H. van Deurzen, R. B. van der Luijt, P. Devilee, Hebon, E. Olah, C. Lazaro, A. Teule, M. Menendez, A. Jakubowska, C. Cybulski, J. Gronwald, J. Lubinski, K. Durda, K. Jaworska-Bieniek, O. T. Johannsson, C. Maugard, M.

Montagna, S. Tognazzo, M. R. Teixeira, S. Healey, K. Investigators, C. Olswold, L. Guidugli, N. Lindor, S. Slager, C. I. Szabo, J. Vijai, M. Robson, N. Kauff, L. Zhang, R. Rau-Murthy, A. Fink-Retter, C. F. Singer, C. Rappaport, D. Geschwantler Kaulich, G. Pfeiler, M. K. Tea, A. Berger, C. M. Phelan, M. H. Greene, P. L. Mai, F. Lejbkowicz, I. Andrulis, A. M. Mulligan, G. Glendon, A. E. Toland, A. Bojesen, I. S. Pedersen, L. Sunde, M. Thomassen, T. A. Kruse, U. B. Jensen, E. Friedman, Y. Laitman, S. P. Shimon, J. Simard, D. F. Easton, K. Offit, F. J. Couch, G. Chenevix-Trench, A. C. Antoniou and J. Benitez (2014). "DNA glycosylases involved in base excision repair may be associated with cancer risk in BRCA1 and BRCA2 mutation carriers." PLoS Genet **10**(4): e1004256.

Petrucelli, N., M. B. Daly and G. L. Feldman (2010). "Hereditary breast and ovarian cancer due to mutations in BRCA1 and BRCA2." Genet Med **12**(5): 245-259.

Pharoah, P. D., P. Guilford and C. Caldas (2001). "Incidence of gastric cancer and breast cancer in CDH1 (E-cadherin) mutation carriers from hereditary diffuse gastric cancer families." Gastroenterology **121**(6): 1348-1353.

Pichon, X., L. A. Wilson, M. Stoneley, A. Bastide, H. A. King, J. Somers and A. E. Willis (2012). "RNA binding protein/RNA element interactions and the control of translation." Curr Protein Pept Sci **13**(4): 294-304.

Radicella, J. P., C. Dherin, C. Desmaze, M. S. Fox and S. Boiteux (1997). "Cloning and characterization of hOGG1, a human homolog of the OGG1 gene of Saccharomyces cerevisiae." Proc Natl Acad Sci U S A **94**(15): 8010-8015.

Rhodes, D. and R. Giraldo (1995). "Telomere structure and function." Curr Opin Struct Biol **5**(3): 311-322.

Sedgwick, B., P. A. Bates, J. Paik, S. C. Jacobs and T. Lindahl (2007). "Repair of alkylated DNA: recent advances." DNA Repair (Amst) **6**(4): 429-442.

Shao, L., C. G. Wood, D. Zhang, N. M. Tannir, S. Matin, C. P. Dinney and X. Wu (2007). "Telomere dysfunction in peripheral lymphocytes as a potential predisposition factor for renal cancer." J Urol **178**(4 Pt 1): 1492-1496.

Shinmura, K. and J. Yokota (2001). "The OGG1 gene encodes a repair enzyme for oxidatively damaged DNA and is involved in human carcinogenesis." Antioxid Redox Signal **3**(4): 597-609.

Stacey, S. N., A. Manolescu, P. Sulem, T. Rafnar, J. Gudmundsson, S. A. Gudjonsson, G. Masson, M. Jakobsdottir, S. Thorlacius, A. Helgason, K. K. Aben, L. J. Strobbe, M. T. Albers-Akkers, D. W. Swinkels, B. E. Henderson, L. N. Kolonel, L. Le Marchand, E. Millastre, R. Andres, J. Godino, M. D. Garcia-Prats, E. Polo, A. Tres, M. Mouy, J. Saemundsdottir, V. M. Backman, L. Gudmundsson, K. Kristjansson, J. T. Bergthorsson, J. Kostic, M. L. Frigge, F. Geller, D. Gudbjartsson, H. Sigurdsson, T. Jonsdottir, J. Hrafnkelsson, J. Johannsson, T. Sveinsson, G. Myrdal, H. N. Grimsson, T. Jonsson, S. von Holst, B. Werelius, S. Margolin, A. Lindblom, J. I. Mayordomo, C. A. Haiman, L. A. Kiemeney, O. T. Johannsson, J. R. Gulcher, U. Thorsteinsdottir, A. Kong and K. Stefansson (2007). "Common variants on chromosomes 2q35 and 16q12 confer susceptibility to estrogen receptor-positive breast cancer." Nat Genet **39**(7): 865-869.

Sun, L., R. Tan, J. Xu, J. LaFace, Y. Gao, Y. Xiao, M. Attar, C. Neumann, G. M. Li, B. Su, Y. Liu, S. Nakajima, A. S. Levine and L. Lan (2015). "Targeted DNA damage at individual telomeres disrupts their integrity and triggers cell death." Nucleic Acids Res **43**(13): 6334-6347.

Tanaka, H., N. Fujita, R. Sugimoto, N. Urawa, S. Horiike, Y. Kobayashi, M. Iwasa, N. Ma, S. Kawanishi, S. Watanabe, M. Kaito and Y. Takei (2008). "Hepatic oxidative DNA damage is associated with increased risk for hepatocellular carcinoma in chronic hepatitis C." Br J Cancer **98**(3): 580-586.

Thomas, D., A. D. Scot, R. Barbey, M. Padula and S. Boiteux (1997). "Inactivation of OGG1 increases the incidence of G . C-->T . A transversions in Saccharomyces cerevisiae: evidence for endogenous oxidative damage to DNA in eukaryotic cells." Mol Gen Genet **254**(2): 171-178.

Thomas, G., K. B. Jacobs, P. Kraft, M. Yeager, S. Wacholder, D. G. Cox, S. E. Hankinson, A. Hutchinson, Z. Wang, K. Yu, N. Chatterjee, M. Garcia-Closas, J. Gonzalez-Bosquet, L. Prokunina-Olsson, N. Orr, W. C. Willett, G. A. Colditz, R. G. Ziegler, C. D. Berg, S. S. Buys, C. A. McCarty, H. S. Feigelson, E. E. Calle, M. J. Thun, R. Diver, R. Prentice, R. Jackson, C. Kooperberg, R. Chlebowski, J. Lissowska, B. Peplonska, L. A. Brinton, A. Sigurdson, M. Doody, P. Bhatti, B. H. Alexander, J. Buring, I. M. Lee, L. J. Vatten, K. Hveem, M. Kumle, R. B. Hayes, M. Tucker, D. S. Gerhard, J. F. Fraumeni, Jr., R. N. Hoover, S. J. Chanock and D. J. Hunter (2009). "A multistage genome-wide association study in breast cancer identifies two new risk alleles at 1p11.2 and 14q24.1 (RAD51L1)." Nat Genet **41**(5): 579-584.

Thompson, D., S. Duedal, J. Kirner, L. McGuffog, J. Last, A. Reiman, P. Byrd, M. Taylor and D. F. Easton (2005). "Cancer risks and mortality in heterozygous ATM mutation carriers." J Natl Cancer Inst **97**(11): 813-822.

Turnbull, C., S. Ahmed, J. Morrison, D. Pernet, A. Renwick, M. Maranian, S. Seal, M. Ghoussaini, S. Hines, C. S. Healey, D. Hughes, M. Warren-Perry, W. Tapper, D. Eccles, D. G. Evans, C. Breast Cancer Susceptibility, M. Hooning, M. Schutte, A. van den Ouweland, R. Houlston, G. Ross, C. Langford, P. D. Pharoah, M. R. Stratton, A. M. Dunning, N. Rahman and D. F. Easton (2010). "Genome-wide association study identifies five new breast cancer susceptibility loci." Nat Genet **42**(6): 504-507.

UK, C. R. (2015). "Breast Cancer Risk Factors." Retrieved 21st of March, 2015, from http://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/breast-cancer/risk-factors.

UK, C. R. (2015). "How cancer starts." Retrieved September, 2015, from http://www.cancerresearchuk.org/about-cancer/what-is-cancer/how-cancer-starts.

UK, C. R. (2015). "Ovarian cancer risks and causes." Retrieved September, 2015, from http://www.cancerresearchuk.org/about-cancer/type/ovarian-cancer/about/ovarian-cancer-risks-and-causes.

van der Groep, P., E. van der Wall and P. J. van Diest (2011). "Pathology of hereditary breast cancer." Cell Oncol (Dordr) **34**(2): 71-88.

Venkitaraman, A. R. (2002). "Cancer susceptibility and the functions of BRCA1 and BRCA2." Cell **108**(2): 171-182.

Venkitaraman, A. R. (2014). "Cancer suppression by the chromosome custodians, BRCA1 and BRCA2." Science **343**(6178): 1470-1475.

Vogelstein, B. and K. W. Kinzler (1993). "The multistep nature of cancer." Trends Genet **9**(4): 138-141.

von Zglinicki, T. (2002). "Oxidative stress shortens telomeres." Trends Biochem Sci **27**(7): 339-344.

von Zglinicki, T., R. Pilger and N. Sitte (2000). "Accumulation of single-strand breaks is the major cause of telomere shortening in human fibroblasts." Free Radic Biol Med **28**(1): 64-74.

Wang, Z., L. Gan, W. Nie and Y. Geng (2013). "The OGG1 Ser326Cys polymorphism and the risk of esophageal cancer: a meta-analysis." Genet Test Mol Biomarkers **17**(10): 780-785.

Wang, Z., D. B. Rhee, J. Lu, C. T. Bohr, F. Zhou, H. Vallabhaneni, N. C. de Souza-Pinto and Y. Liu (2010). "Characterization of oxidative guanine damage and repair in mammalian telomeres." PLoS Genet **6**(5): e1000951.

Weinrich, S. L., R. Pruzan, L. Ma, M. Ouellette, V. M. Tesmer, S. E. Holt, A. G. Bodnar, S. Lichtsteiner, N. W. Kim, J. B. Trager, R. D. Taylor, R. Carlos, W. H. Andrews, W. E. Wright, J. W. Shay, C. B. Harley and G. B. Morin (1997). "Reconstitution of human telomerase with the template RNA component hTR and the catalytic protein subunit hTRT." Nat Genet **17**(4): 498-502.

Weiss, J. M., E. L. Goode, W. C. Ladiges and C. M. Ulrich (2005). "Polymorphic variation in hOGG1 and risk of cancer: a review of the functional and epidemiologic literature." Mol Carcinog **42**(3): 127-141.

Wentzensen, I. M., L. Mirabello, R. M. Pfeiffer and S. A. Savage (2011). "The association of telomere length and cancer: a meta-analysis." Cancer Epidemiol Biomarkers Prev **20**(6): 1238-1250.

WHO. (2015). "World Health Organization: risk factors." Retrieved 17th of March, 2015, from http://www.who.int/topics/risk_factors/en/.

Wooster, R., S. L. Neuhausen, J. Mangion, Y. Quirk, D. Ford, N. Collins, K. Nguyen, S. Seal, T. Tran, D. Averill and et al. (1994). "Localization of a breast cancer susceptibility gene, BRCA2, to chromosome 13q12-13." Science **265**(5181): 2088-2090.

Wu, X., C. I. Amos, Y. Zhu, H. Zhao, B. H. Grossman, J. W. Shay, S. Luo, W. K. Hong and M. R. Spitz (2003). "Telomere dysfunction: a potential cancer predisposition factor." J Natl Cancer Inst **95**(16): 1211-1218.

Xin, H., D. Liu and Z. Songyang (2008). "The telosome/shelterin complex and its functions." Genome Biol **9**(9): 232.

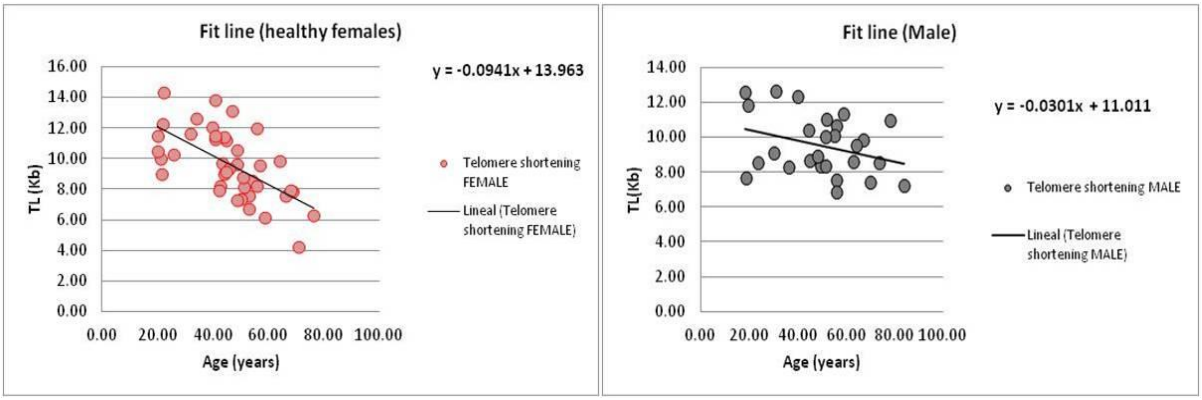
Xiong, J., S. Fan, Q. Meng, L. Schramm, C. Wang, B. Bouzahza, J. Zhou, B. Zafonte, I. D. Goldberg, B. R. Haddad, R. G. Pestell and E. M. Rosen (2003). "BRCA1 inhibition of telomerase activity in cultured cells." Mol Cell Biol **23**(23): 8668-8690.

Zhang, C., X. Chen, L. Li, Y. Zhou, C. Wang and S. Hou (2015). "The Association between Telomere Length and Cancer Prognosis: Evidence from a Meta-Analysis." PLoS One **10**(7): e0133174.

Zhou, P. T., B. Li, J. Ji, M. M. Wang and C. F. Gao (2015). "A systematic review and meta-analysis of the association between OGG1 Ser326Cys polymorphism and cancers." Med Oncol **32**(2): 472.

10. Annexes

Annexe 1 **TL distribution in peripheral blood mononuclear cells as a function of age** for healthy female (*n*=40) and healthy men (*n*=28).



Annexe 2 **Table with the results obtained by genotyping and qPCR.** In blue are represented all the samples that harbour a mutation in *BRAC1*, in green a mutation in *BRCA2*, in red a mutation in *BRCAX* and finally in black the controls. The individuals indicated as "common allele" are homozygote for the common allele (G/G); the "heterozygous" are the ones that carry both allele (G/A) and "minor allele" (highlighted in bold) are homozygote for (A/A).

Sample ID	Genotype	BRCA status	Mean ΔCt
11s1134	Common Allele	BRCA1	2.33539192
11s1272	Common Allele	BRCA1	1.99251687
11s1385	Common Allele	BRCA1	1.49312070
12s1023	Common Allele	BRCA1	1.12142309
12s1058	Common Allele	BRCA1	1.42053089
12s1059	Common Allele	BRCA1	1.71387709
12s108	Common Allele	BRCA1	0.52497891
12s1262	Common Allele	BRCA1	0.54841164
12s1263	Common Allele	BRCA1	1.34956208
12s150	Common Allele	BRCA1	1.77439774
12s23	Common Allele	BRCA1	0.67939905
14S333	Common Allele	BRCA1	1.50901106
12s251	Common Allele	BRCA1	0.69861468
12s745	Common Allele	BRCA1	0.66421933
12s816	Common Allele	BRCA1	1.91799326
12s969	Common Allele	BRCA1	1.77071384
12s970	Common Allele	BRCA1	4.59146755
12s991	Common Allele	BRCA1	4.70371819
13s113	Common Allele	BRCA1	3.10143717
13s114	Common Allele	BRCA1	1.07350652
13s261	Common Allele	BRCA1	0.09508312
13s397	Common Allele	BRCA1	1.25427348
13s424	Common Allele	BRCA1	0.27174598
13s426	Common Allele	BRCA1	0.36941936

13s539	Common Allele	BRCA1	3.91278141
13s541	Common Allele	BRCA1	1.71276919
14s154	Common Allele	BRCA1	0.87794465
14s250	Common Allele	BRCA1	0.96998341
14S528	Common Allele	BRCA1	1.43197670
14s561	Common Allele	BRCA1	1.2369724
14S934	Common Allele	BRCA1	1.77536388
11s1111	Common Allele	BRCA2	0.77150054
11s1276	Common Allele	BRCA2	1.220381604
11s1277	Common Allele	BRCA2	0.372762849
12s1055	Common Allele	BRCA2	2.002941487
12s1056	Common Allele	BRCA2	0.522437879
12s1057	Common Allele	BRCA2	2.462853681
12s1064	Common Allele	BRCA2	3.425514661
12s1065	Common Allele	BRCA2	1.596171034
12s1069	Common Allele	BRCA2	3.032130955
12s1167	Common Allele	BRCA2	2.910627349
12s1193	Common Allele	BRCA2	1.574976674
12s1259	Common Allele	BRCA2	5.066271927
12s152	Common Allele	BRCA2	1.098345474
12s153	Common Allele	BRCA2	3.676492481
12s2	Common Allele	BRCA2	0.543869328
12s646	Common Allele	BRCA2	1.477846294
12s649	Common Allele	BRCA2	1.081754305
12s746	Common Allele	BRCA2	1.668807186
12s748	Common Allele	BRCA2	1.849750659
13S1031	Common Allele	BRCA2	2.033109372
13s305	Common Allele	BRCA2	2.377053665
13s388	Common Allele	BRCA2	0.224656676
13s392	Common Allele	BRCA2	0.928910283
13s431	Common Allele	BRCA2	2.84421434
13s538	Common Allele	BRCA2	1.588130638
13s726	Common Allele	BRCA2	0.486882605
14s117	Common Allele	BRCA2	0.524670037
14s119	Common Allele	BRCA2	1.468010234
14s225	Common Allele	BRCA2	0.914177508
14s252	Common Allele	BRCA2	1.325840854
14s476	Common Allele	BRCA2	2.518780926
14s988	Common Allele	BRCA2	1.308879397
11s1015	Common Allele	BRCAX	0.14174123

11s1131	Common Allele	BRCAX	1.09984043
11s1176	Common Allele	BRCAX	1.10447845
11s1194	Common Allele	BRCAX	1.22086701
11s1271	Common Allele	BRCAX	0.43203806
12s1192	Common Allele	BRCAX	1.43170145
12s253	Common Allele	BRCAX	1.44033509
12s27	Common Allele	BRCAX	0.77103242
12s29	Common Allele	BRCAX	0.55893833
12s30	Common Allele	BRCAX	1.50930494
12s360	Common Allele	BRCAX	0.97670586
12s708	Common Allele	BRCAX	2.39217400
12s747	Common Allele	BRCAX	1.51525873
12s926	Common Allele	BRCAX	1.36463603
12s927	Common Allele	BRCAX	1.06018393
12s928	Common Allele	BRCAX	1.57491122
13s1175	Common Allele	BRCAX	2.76553840
13s1177	Common Allele	BRCAX	0.60352408
13S1180	Common Allele	BRCAX	0.99083308
13S1257	Common Allele	BRCAX	0.35142794
13s1258	Common Allele	BRCAX	1.74974958
13s1262	Common Allele	BRCAX	0.67087918
13s1316	Common Allele	BRCAX	0.68446840
13s1347	Common Allele	BRCAX	0.60528449
13s243	Common Allele	BRCAX	3.94549954
13S260	Common Allele	BRCAX	4.32757604
13s512	Common Allele	BRCAX	1.94075545
13s52	Common Allele	BRCAX	1.95944397
13s535	Common Allele	BRCAX	2.55409849
13S729	Common Allele	BRCAX	0.28399218
13s789	Common Allele	BRCAX	0.52634869
13s793	Common Allele	BRCAX	1.47096656
13s794	Common Allele	BRCAX	0.76773454
13S882	Common Allele	BRCAX	0.40219534
13S884	Common Allele	BRCAX	0.70214493
13s967	Common Allele	BRCAX	0.26118926
14S221	Common Allele	BRCAX	2.66179957
14s473	Common Allele	BRCAX	0.53297049
14s813	Common Allele	BRCAX	1.72250349
14s224	Common Allele	BRCAX	0.70482430
14s563	Common Allele	BRCAX	3.49529638

14S587	Common Allele	BRCAX	0.44137412
14S588	Common Allele	BRCAX	0.96954516
14s812	Common Allele	BRCAX	3.14692931
14s869	Common Allele	BRCAX	3.13880444
11s1011	Common Allele	control	0.029462372
11s1274	Common Allele	control	1.62007555
11s1275	Common Allele	control	0.092611118
12s1022	Common Allele	control	2.230861326
12s1024	Common Allele	control	1.974027882
12s1060	Common Allele	control	2.021366706
12s1067	Common Allele	control	1.599778591
12s1068	Common Allele	control	1.843349643
12S1260	Common Allele	control	4.688377147
12S1261	Common Allele	control	2.24348156
12s171	Common Allele	control	0.931953897
12s252	Common Allele	control	1.332190896
12s650	Common Allele	control	1.639372193
12s651	Common Allele	control	1.768316902
12s742	Common Allele	control	1.99752429
13S1033	Common Allele	control	1.227781715
13S1179	Common Allele	control	2.742431962
13s1317	Common Allele	control	1.22072597
13s1344	Common Allele	control	0.839552595
13S258	Common Allele	control	2.444016632
13s302	Common Allele	control	3.168116844
13s303	Common Allele	control	3.226806952
13S304	Common Allele	control	4.622182366
13s425	Common Allele	control	2.003149844
13s654	Common Allele	control	0.322451636
13S731	Common Allele	control	0.418561679
13S791	Common Allele	control	0.29132392
13s792	Common Allele	control	0.789637514
13s838	Common Allele	control	5.161315747
13S883	Common Allele	control	0.228180791
13s98	Common Allele	control	3.356649929
14S155	Common Allele	control	0.498695202
14s222	Common Allele	control	1.384121345
14s226	Common Allele	control	3.65289493
14s770	Common Allele	control	1.56624771
14S809	Common Allele	control	4.439767932

14s814	Common Allele	control	2.037915032
14s815	Common Allele	control	3.475344028
12s148	Heterozygous	BRCA1	1.79474165
12s149	Heterozygous	BRCA1	0.94039116
12s169	Heterozygous	BRCA1	0.89878916
12s568	Heterozygous	BRCA1	1.68053840
12s569	Heterozygous	BRCA1	1.37513726
13s1072	Heterozygous	BRCA1	1.65752494
13s112	Heterozygous	BRCA1	1.83157798
13s1178	Heterozygous	BRCA1	0.60902285
13S559	Heterozygous	BRCA1	0.26513026
13s67	Heterozygous	BRCA1	1.06682991
13s68	Heterozygous	BRCA1	2.76303584
14S220	Heterozygous	BRCA1	1.53568083
14s562	Heterozygous	BRCA1	1.69006798
13s430	Heterozygous	BRCA1 + BRCA2	1.08370106
11s1268	Heterozygous	BRCA2	1.850284539
14s472	Heterozygous	BRCA2	0.721208789
11s1269	Heterozygous	BRCA2	0.549553317
11s1270	Heterozygous	BRCA2	0.245044307
12s1	Heterozygous	BRCA2	0.141510646
12s1052	Heterozygous	BRCA2	1.447907917
12s1054	Heterozygous	BRCA2	0.81728245
12s1061	Heterozygous	BRCA2	2.495505228
12s3	Heterozygous	BRCA2	0.576475662
13s1181	Heterozygous	BRCA2	0.110584261
13s1346	Heterozygous	BRCA2	1.108603293
13s393	Heterozygous	BRCA2	1.841930932
13s655	Heterozygous	BRCA2	0.880391061
13S837	Heterozygous	BRCA2	1.886430723
13s430	Heterozygous	BRCA1 + BRCA2	1.083701065
14S811	Heterozygous	BRCA2	1.715163862
14s838	Heterozygous	BRCA2	0.264540853
11s1012	Heterozygous	BRCAX	1.64102007
11s1195	Heterozygous	BRCAX	0.99323995
12s1132	Heterozygous	BRCAX	1.75176798
12s645	**Minor Allele**	BRCAX	1.44880366
12s990	Heterozygous	BRCAX	2.05000028

13s1314	Heterozygous	BRCAX	0.59041695
13S1319	Heterozygous	BRCAX	0.64822599
13s1345	Heterozygous	BRCAX	0.44303309
13s1382	Heterozygous	BRCAX	0.63417147
13s447	**Minor Allele**	BRCAX	1.33932134
13S612	Heterozygous	BRCAX	0.31138108
13S728	Heterozygous	BRCAX	0.93235758
13S730	Heterozygous	BRCAX	0.58027720
14S114	Heterozygous	BRCAX	3.35080395
14s531	Heterozygous	BRCAX	0.39809928
14s532	Heterozygous	BRCAX	0.82721233
11s1193	Heterozygous	control	0.538708
12s10	Heterozygous	control	1.009283
12s1050	**Minor Allele**	control	2.395505
12s1051	**Minor Allele**	control	1.106145
12s1062	Heterozygous	control	1.604241
12s1066	Heterozygous	control	2.910631
12s170	Heterozygous	control	1.096323
12s8	**Minor Allele**	control	1.275292
13S1032	Heterozygous	control	1.085633
13s1073	Heterozygous	control	0.293654
13s1261	**Minor Allele**	control	1.897319
13s299	Heterozygous	control	0.827024
13S300	Heterozygous	control	1.510594
13s301	Heterozygous	control	1.498766
13s443	Heterozygous	control	0.491523
13s446	Heterozygous	control	0.232666
13S836	Heterozygous	control	1.77724
13s880	Heterozygous	control	0.0866
13s99	Heterozygous	control	0.960815
14s120	Heterozygous	control	0.898681
14s121	Heterozygous	control	1.020974
14s122	Heterozygous	control	0.584356
14s253	Heterozygous	control	1.730081
14s474	Heterozygous	control	3.192469
14s526	Heterozygous	control	0.73501
14s533	Heterozygous	control	0.564623
14s534	Heterozygous	control	0.931532
14s839	Heterozygous	control	1.880791
14s840	Heterozygous	control	2.558008

14s868	Heterozygous	control	1.504775