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Original Research

Long telomere length and a *TERT-CLPTM1 locus* polymorphism association with melanoma risk

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Abstract Telomere length has been associated with the development of cancer. Studies have shown that shorter telomere length may be related to a decreased risk of cutaneous melanoma. Furthermore, deregulation of the telomere-maintaining gene complexes, has been related to this oncogenic process. Some variants in these genes seem to be correlated with a change in telomerase expression. We examined the effect of 10 single nucleotide polymorphisms (SNPs) in the *TERT* gene (encoding telomerase), one SNP in the related *TERT-CLPTM1L locus* and one SNP in the *TRF1* gene with telomere length, and its influence on melanoma risk in 970 Spanish cases and 733 Spanish controls.

Genotypes were determined using KASP technology, and telomere length was measured by quantitative polymerase chain reaction (PCR) on DNA extracted from peripheral blood leucocytes.

Our results demonstrate that shorter telomere length is associated with a decreased risk of melanoma in our population (global *p*-value, 2.69×10^{-11}), which may be caused by a diminution of proliferative potential of nevi (melanoma precursor cells). We also obtained significant results when we tested the association between rs401681 variant (*TERT-CLPTM1L locus*) with melanoma risk (Odds ratio, OR; 95% confidence interval, CI = 1.24 (1.08–1.43); *p*-value, 3×10^{-3}).

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This is the largest telomere-related study undertaken in a Spanish population to date. Furthermore, this study represents a comprehensive analysis of some of the most relevant telomere pathway genes in relation to cutaneous melanoma susceptibility.

1. Introduction

Telomeres are structures located at the end of eukaryotic chromosomes and consist of long hexameric nucleotide repetitive sequences (TTAGGG)n. These structures and their associated proteins play the important role of protecting the end of chromosomes from breakage, loss of nucleotides, end-to-end fusion and atypical recombination. Thus, telomeric regions prevent the degradation of genes close to the end of chromosomes from the shortening that occurs in 3′ in each round of DNA replication by the DNA polymerase.

Telomere length is maintained mainly by telomerase, a ribonucleoprotein that forms part of a protein complex that allows the addition of repetitive sequences to the 3′ chain during DNA replication as a protective action against chromosome erosion [1,2]. A large amount of genes and gene products are involved in this process. Specifically, the *TERT* gene encodes for the telomerase enzyme, the *TRF1* gene gives a protein that acts as a negative-modulator of *TERT* activity [3,4] and the *CLPTM1L* gene product seems to participate in *TERT* regulation through some regions in the 3′ end, called the *TERT-CLPTM1L locus* [5].

Genetic variants on this telomere-maintaining gene complex have been related to different kinds of cancers [5–11]. Furthermore, it has been shown that telomere length may be related with cancer risk and with genetic variants in genes encoding telomerase protein complex components, respectively [12–20].

Cutaneous Malignant Melanoma is caused by the malignant transformation of melanocytes, pigment producer cells located within the epidermal basal layer. The incidence of melanoma has been increasing at higher rates than any other malignant tumour in recent decades, and it causes the greatest number of skin-cancer-related deaths worldwide due to its high ability to metastasise [21–24].

Long telomere length in melanocytes with previous oncogenic mutations may delay senescence, favoring malignant transformation by the acquisition of mutations [25]. This may be the cause of an increased formation and proliferation of nevi (considered melanoma precursors), which has been correlated with increased risk of melanoma [26,27].

Since telomere length and dysfunction and the presence of variants in telomere-related genes have been associated with cancer, the aim of our study was to evaluate the association between genetic variants in telomerase gene, *TERT*, *plus two SNPs in two TERT regulatory genes*, *previously significant SNPs* (*one SNP in TRF1*, and another one in the telomere-related region *TERT-CLPTM1L*) *with* telomere length and melanoma susceptibility. Therefore, we have undertaken a comprehensive case–control study in blood of 970 Spanish cases and 733 Spanish controls. This is the first time that telomere length has been evaluated in Spanish melanoma blood samples.

2. Materials and methods

2.1. Study subjects and data collection

The genotyping case–control study was carried out in 648 Spanish patients with melanoma and 381 volunteer cancer-free control samples in a first phase, plus 322 more Spanish cases of melanoma and 352 control samples in a second validation phase. All of them were recruited from several hospitals from Madrid (Hospital La Paz, Hospital Ramón y Cajal, Hospital Gregorio Marañón), Castellon (Hospital General de Castellon), and Las Palmas de Gran Canaria (Hospital Doctor Negrín).

All participants provided written informed consent, and the study was approved by the Ethics Committee of INCLIVA Health Research Institute of Valencia, Spain. All participants were non-related Caucasians of Spanish origin, with the same ethnic background [28]. A comprehensive standardised questionnaire was used to collect information on pigmentation characteristics such as eye, hair and skin colour, number of nevi, presence of lentigines, sun exposure habits and personal plus family history of melanoma, cancer or any other skin disease. Each individual questionnaire has been guided by an expert clinician or a trained nurse. For cases only, tumour characteristics were added and medical data were obtained via medical exploration. Patients with acral or multiple melanoma were excluded from the study, as well as control individuals with suspected personal or family history of melanoma (Table 1).

2.2. DNA extraction and quantification

Genomic DNA from individuals was isolated from peripheral blood lymphocytes (PBLs). This was done using the traditional saline method or the DNAzol procedure (Invitrogen, Eugene, OR, United States of America (USA)). DNA concentration was quantified