SOLAR RADIATION AND MELANOMA EPIDEMIOLOGY IN NORWAY

Epidemiological data argue against a negative impact of sun exposure for certain types of melanoma

by

Emanuela Micu

Institute for Cancer Research - The Norwegian Radium Hospital
Department of Radiation Biology, Oslo University Hospital

Series of dissertations submitted to the
Faculty of Medicine, University of Oslo
No. 1675

ISBN 978-82-8264-093-0

Cover: Inger Sandved Anfinsen.
Printed in Norway: AIT Oslo AS.

Produced in co-operation with Akademika Publishing.
The thesis is produced by Akademika Publishing merely in connection with the thesis defence. Kindly direct all inquiries regarding the thesis to the copyright holder or the unit which grants the doctorate.

ACKNOWLEDGMENTS

The successful completion of this thesis would not have been possible without the professional and personal support from my colleagues and family.

The work of this thesis was carried out at the Department of Radiation Biology, The Norwegian Radium Hospital, Oslo University Hospital, Oslo, Norway, from 2008 to 2012 (PhD contract signed in May 2010). I am deeply grateful for the financial support provided by the Institute for Cancer Research and The Norwegian Radium Hospital Research Foundation.

I would like to express my deepest gratitude to my supervisor, Professor Johan Emilian Moan, who encouraged and mentored me throughout this endeavor. His efforts to create an international, stimulating and collaborative environment have been greatly appreciated.

My appreciation also goes to my co-supervisors, Professor Dr. Øyvind Sverre Bruland and Dr. Asta Juzeniene, for inspiring collaboration, constant guiding and support. Thank you, Asta, for our daily interesting discussions and for your constructive critical comments in writing manuscripts and thesis.

I am deeply grateful to my medical training supervisor, Professor Dr. Călin Giurcăneanu, whose broad knowledge and neverending enthusiasm for dermatology have been helpful and motivating to me.

Furthermore, I thank my collegues at the Departament of Radiation Biology; Vladimir Iani, Li-Wei Ma, Mantas Grigalavicius, Zivile Baturaite, Alina Porojnicu, Zoya Lagunova, and Patrycja Mikolajewska, for providing the friendliest atmosphere anyone can imagine.

My appreciation also goes to colleagues from the Cancer Registry of Norway; Trude Eid Robsahm, Siri Larønningen and Lidziya Vanahel, for interesting collaborative work.

I am also infinitely lucky to have the support of my loyal and loving family. I deeply appreciate the strong encouragement and support offered by my parents, Maricel and Eugenia,

my sister, Lidia, and my beloved husband, Sebastian. And last, but not least, thank you to my adorable two children, Toma and Ana, who have been growing up, discovering the world as I have during these past years as a PhD student. May their endless curiosity and tenacity be a genuine model of inspiration to all scientists, as they have been to me!

I. ABSTRACT

Norway has one of the highest melanoma incidences in the World, despite the low fluences of ultraviolet radiation. This has been partially explained by the phenotypic characteristics of the population. However, the correlation between melanoma and sun exposure is not yet fully understood.

In the present work, we performed a complex analysis of melanoma epidemiology, by analyzing time trends in gender, age, anatomic site and morphology specific incidence rates between 1965 and 2009, in south and north regions of Norway.

The main finding is that solar radiation may not have a negative role on certain types of melanoma, like for those on anatomic sites not directly exposed to the sun; moreover, the impact of sun exposure is different for various morphological types (nodular melanoma *versus* superficial-spreading melanoma), underlining the heterogeneity of melanoma.

Our updated time trends show different course by age, gender and anatomic site. More importantly, following a period with rapid increase in melanoma incidence, we observed stable incidence rates after 1990s, and even decreasing rates for young population. However, there are subtle differences between north and south regions, in particular for the female population. For melanoma on shielded sites (like perianal skin and anorectal mucosa) trends continue to be stable during a long period of time.

We found differences in the latitudinal gradient for melanoma incidence on different body sites with various sun exposure patterns. The latitudinal gradient was highest for trunk compared with head and neck, while no such latitudinal gradient was observed for melanomas on sites rarely (foot) or not exposed to the sun (anorectal and uveal melanomas).

Our data stress that solar radiation is a risk factor for all anatomic sun-exposed sites, emphasizing the role of intermittent exposures, in particular for young individuals.

ABBREVIATIONS

6-4 PP	Pyrimidine (6-4) pyrimidone photoproducts
8-oxo-dG	8-oxo-7,8-dihydro-2'-deoxyguanosine
A	Adenine
ACTH	Adrenocorticotropic hormone
AKT	A family of human protein kinases
ALM	Acral lentiginous melanoma
ARF	Alternative reading frame (ARF) of the CDKN2A locus
AS	Action spectrum
ASP	Agouti signalling protein
ASR	Age-standardized rate
Bcl-2	B-cell lymphoma oncogene
BRAF	Gene coding for the protein kinase B-raf (v-Raf murine sarcoma viral oncogene homolog B1)
BRCA1	Gene coding for breast cancer type 1 susceptibility protein
C	Cytosine
CDK	Cycline-dependent kinase gene
CDKN2A	Cycline-dependent kinase inhibitor 2A gene
CIE	Commission Internationale de l'Eclairage
CM	Cutaneous melanoma
CPD	Cyclobutane pyrimidine dimers
DBP	Vitamin D-binding protein
DNA	Deoxyribonucleic acid
EM	Extracutaneous melanoma
G	Guanine
ICD	International Classification of Diseases
INK4A	Alternative name for CDKN2A gene
KIT	Receptor tyrosine kinase
LMM	Lentigo maligna melanoma
MAPK	Mitogen-activated protein kinase
MC1R	Melanocortin 1 receptor
MITF	Microphtalmia-associated transcription factor
MoTNaC	Manual of Tumor Nomenclature and Coding
MSH	Melanocyte-stimulating hormone
NER	Nucleotide excision repair
NM	Nodular melanoma
NMSC	Non-melanoma skin cancer
NRAS	Neuroblastoma RAS viral oncogene homolog
OGG1	Oxoguanine glycosylase 1
PI3K	Phosphatidylinositide 3-kinase

PTEN	Gene coding for Phosphatase and tensin homolog protein
RAS	Gene family coding for Ras proteins (Rat Sarcoma proteins)
RB	Retinoblastoma
RGP	Radial growth phase
ROS	Reactive oxygen species
SSM	Superficial-spreading melanoma
T	Tymine
TNFα	Tumor necrosis factor α
TP53	Tumor protein 53 gene
USA	United States of America
UV	Ultraviolet
VDR	Vitamin D receptor
VGP	Vertical growth phase
W	World
WHO	World Health Organization

LIST OF PUBLICATIONS

This thesis is based on the following papers, which will be referred to by their Roman numerals in the text:

I. **Cicarma E**, Juzeniene A, Porojnicu AC, Bruland OS, Moan J. Latitude gradient for melanoma incidence by anatomic site and gender in Norway 1966-2007. J Photochem Photobiol B. 2010 Nov 3;101(2):174-8.

II. Moan J, **Cicarma E**, Setlow R Porojnicu AC, Grant WB, Juzeniene A. Time trends and latitude dependence of uveal and cutaneous malignant melanoma induced by solar radiation. Dermatoendocrinol. 2010 Jan;2(1):3-8

III. **Micu E**, Juzeniene A, Moan J. Comparison of the time and latitude trends of melanoma incidence in anorectal region and perianal skin with those of cutaneous malignant melanoma in Norway. J Eur Acad Dermatol Venereol. 2011 Dec;25(12):1444-9

IV. Juzeniene A, **Micu E**, Porojnicu AC, Moan J. Malignant melanomas on head/neck and foot: differences in time and latitudinal trends in Norway. J Eur Acad Dermatol Venereol. 2012 Jul;26(7):821-7

V. **Micu E**, Baturaite Z, Juzeniene A, Bruland ØS, Moan J. Superficial-spreading and nodular melanomas in Norway: a comparison by body site distribution and latitude gradients. Melanoma Res. 2012 Dec;22(6):460-5

TABLE OF CONTENT

1. INTRODUCTION

This thesis is devoted mainly to melanoma incidence in Norway, among the top countries in the world with high incidence rates (Fig. 1.1) of this most fatal type of skin cancer.

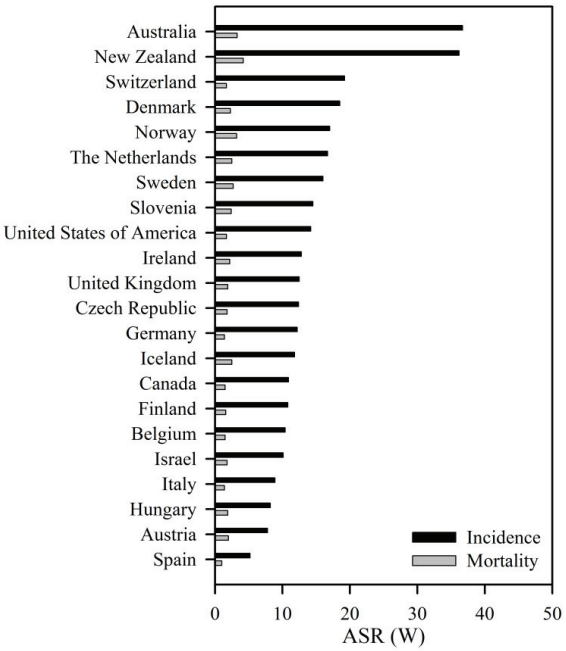

Figure 1.1. World incidence and mortality estimated rates of melanoma for both genders, GLOBOCAN 2008.[1] *ASR (W) age standardized rates per 100 000 to the World population.*

The peer-reviewed scientific papers that describe the work are found at the end of the thesis. This first chapter gives an overview of the general context and the aim of the thesis.

1.1. Background

Cancer is a leading cause of death, along with cardiovascular diseases, respiratory diseases and diabetes.[2] Incidence rates of the major types of cancer worldwide are shown in Fig. 1.2.

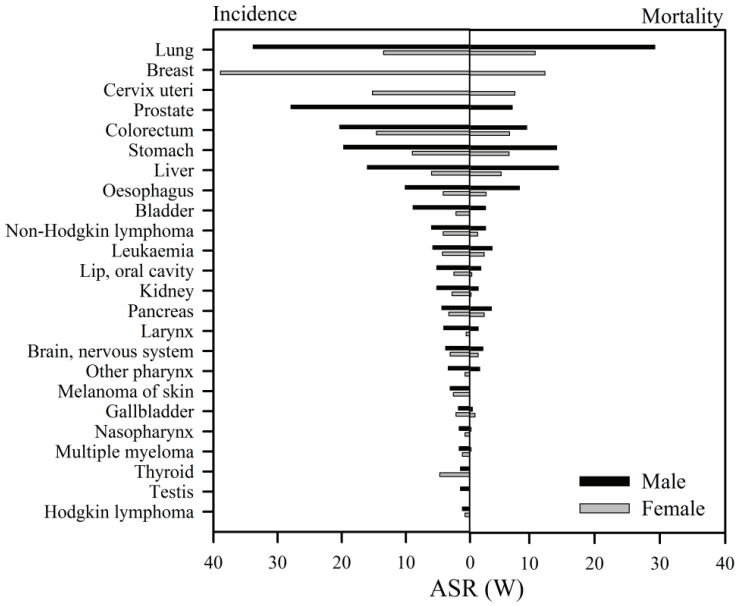

Figure 1.2. World incidence and mortality estimated rates of cancer, GLOBOCAN 2008.[1] *ASR (W) age standardized rates per 100 000 to the World population.*

The incidence rates of cancer in Norway (Fig. 1.3) have been increasing in the last decades, especially for the common cancers, like breast cancer in women, prostate cancer in men, colorectal and lung cancer for both sexes. However, for some cancers there is a declining or stabilizing trend observed in recent years, such as for breast or colon cancer.[3] For melanoma the authors observed an increasing trend in incidence for both sexes, melanoma being the second most rapidly increasing type of cancer in Norway, for the recent period, after the prostate cancer

for men and lung cancer for women. In this report, melanoma cases comprised 4.2% for women and 5% for men of all cancers for 2004-2008.

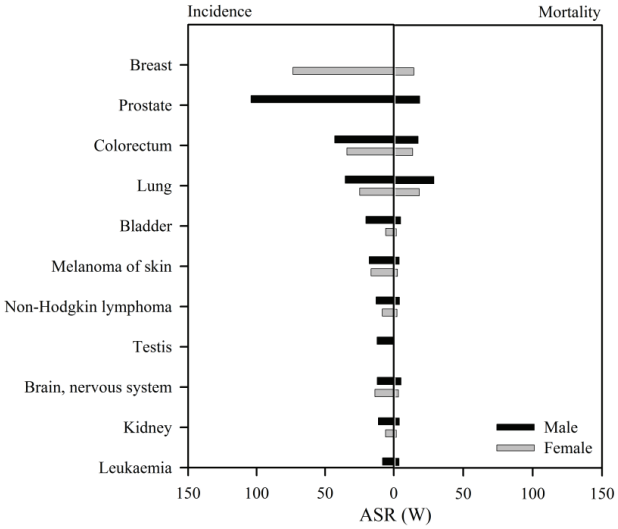

Figure 1.3. Incidence and mortality estimated rates of the major types of cancer in Norway, GLOBOCAN 2008.[1] *ASR (W) age standardized rates per 100 000 to the World population.*

An important environmental risk factor for melanoma is solar radiation, with ultraviolet (UV) radiation being a significant cause of the gene mutations.[4] However, the role of exposure to UV radiation in melanoma is complex, as melanoma may arise in anatomic sites that are not exposed to the sun, a theme that will be further detailed in this thesis.

It is essential to underline the dual role of solar radiation, as it has both beneficial and deleterious effects for human health (Fig. 2.1). Since ancient times, solar radiation has been associated with happiness and joy of living. Furthermore, solar radiation has been and it is used to treat various forms of illnesses, and it is the main source of vitamin D in humans. But not until the 20th century, its role of inducing skin cancers was recognized, the sun being amongst the first

agents acknowledged being carcinogenic to humans. Nevertheless, the common opinion in the general population is to associate a tanned person with health and beauty, and the use of sunbeds and sunlamps has been increasing for the last decades. All these factors, along with an improved surveillance of pigmented lesions, may have contributed to the so-called "epidemic" of melanoma.[5]

1.2. Aim and outline of the thesis

Our current knowledge about the relationship between solar radiation and human health is still at an early stage of development and the relationship with melanoma is far more complex than was previously recognized.

The general aim of the thesis is to improve our understanding about solar exposure and melanoma, by:

(i) analyzing trends of melanoma on different body sites and at different latitudes, in a country with a population at high risk for melanoma, and

(ii) shedding light upon some rare types of melanoma, such as melanoma on non sun-exposed anatomic sites, using descriptive epidemiology.

Chapter 2 is related to solar radiation and human health, focusing on positive (mainly vitamin D production) and negative aspects (mainly DNA damage).

Chapter 3 gives an overview of melanoma ethiopathogenesis and its epidemiology.

Chapter 4 describes the methods, data source and data analysis.

Chapter 5 gives an outline of the publications.

Chapter 6 discusses the main results of the work.

Chapter 7 contains the concluding remarks.

Chapter 8 presents future perspectives, related to the aim of the thesis.

2. SOLAR RADIATION AND HUMAN HEALTH

2.1. Historical background - changing perception about solar radiation

All forms of life on earth have evolved under the influence of solar radiation. Humans spend about half of their lives exposed to light from the sun. Positive effects of UV radiation have been demonstrated from ancient times, but in a more obvious manner at the end of the 19th century, when Downes and Bluntin discovered the bactericidal and fungicidal activity of UV *in vitro*. In 1890s, Finsen treated *lupus vulgaris* (a form of tuberculosis) with phototherapy. Many other skin diseases are successfully treated with phototherapy, even nowadays. Companies have thus started to produce artificial UV radiation sources. At those times, dermatologists reported associations between sun exposure and skin cancers, as some types of skin cancers usually occurred more commonly in outdoor workers. However, this association between sun exposure and skin cancer was not popular at the beginning of the century[6] and the public was not properly informed. Eventually, UV radiation was recognized as a carcinogen for skin and consequently, sunscreens development increased, the first commercial sunscreen being introduced in 1929.[6] Through the next decades and up to our present time, public health campaigns were initiated, to promote a safer sun exposure pattern. Nevertheless, the use of sunbeds and sunlamps is increasing. Many individuals that use sunbeds may not be aware of its dangers[7] and think that the use of sunbeds is safer than sun exposure, because the UV dosage is better controlled.

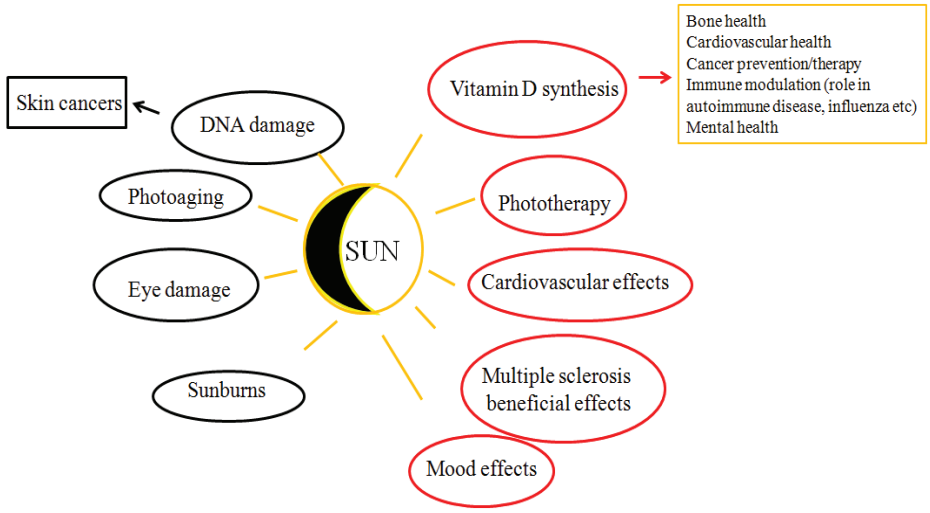

Figure 2.1. Graphical representation of the negative (black circles) and positive (red circles) effects of sun exposure.

Nowadays, we are facing a major dilemma: a strict "no sun policy" may lead to vitamin D deficiency, with several related health negative effects,[8] along with probably vitamin D independent effects (mood effects via β-endorphins release,[9;10] cardiovascular effects via UVA-induced nitric oxide and nitrite[11]) or unknown mechanism (like for multiple sclerosis[9]), while the increasing use of indoor tanning devices or sunbathing leads to skin cancers.[12;13] Fig. 2.1 shows a schematic representation of the positive and negative effects of sun exposure, some of them further detailed in the next chapters.

In order to inform the public properly about the benefits and dangers of the sun, we need to understand the complete context of sun exposure in the social and cultural meanings of our times.

2.2. Solar spectrum

The solar spectrum is composed of different wavelengths of radiation, having different photon energies. The three main regions with implications for all photobiological processes in plants and animals are: the UV region (short wavelengths that are not visible to humans, 100-400 nm), visible radiation (longer wavelengths than UV radiation, 400-760 nm), and infrared radiation (wavelengths > 760 nm, and also not visible to humans). Very little radiation below 300 nm reaches the surface of the earth, because it is absorbed by the ozone layer. Furthermore, emission from the sun is low above 1000 nm and atmospheric water absorbs strongly above 1000 nm. Thus, visible light and infrared radiation constitutes the major fraction of solar radiation reaching the earth surface.[14]

Even though UV radiation comprises only about 6-8% of the solar radiation reaching the earth surface, the UV region is of particular importance for human health. It is generally divided into three regions: the UVC region, which is defined as being in the wavelength region 100-280 nm (not reaching the earth surface), the UVB region (280-315 nm), and the UVA region (315-400 nm); the UVB-UVA cutoff at 320 nm is used in photodermatology.[15] UVA is 10 to 100 times more abundant than UVB, as UVB varies more dramatically than UVA due to differences in scattering and absorption (shorter wavelengths (blue) are scattered more than longer (red) wavelengths).

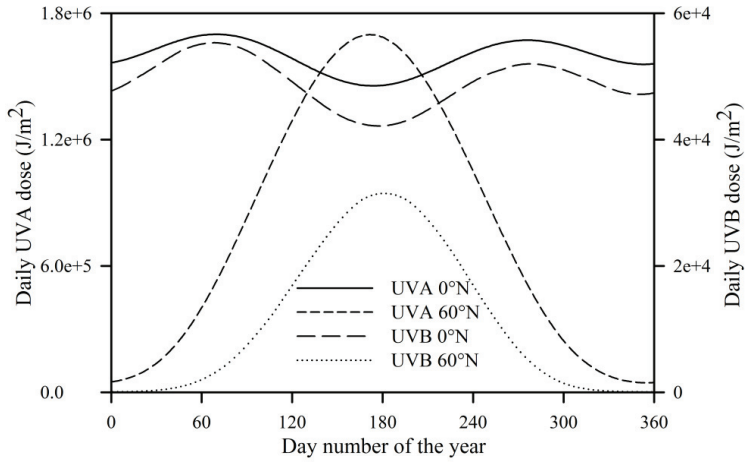

Figure 2.2. Daily integrated UVA and UVB doses on horizontal plane surface at sea level, on clear sky. [Data are kindly provided by Prof. Arne Dahlback, Department of Physics, University of Oslo, Norway. Daily ozone is taken from TOMS on NIMBUS 7 satellite and daily zonal total ozone columns (1979-1992 averages) are used].

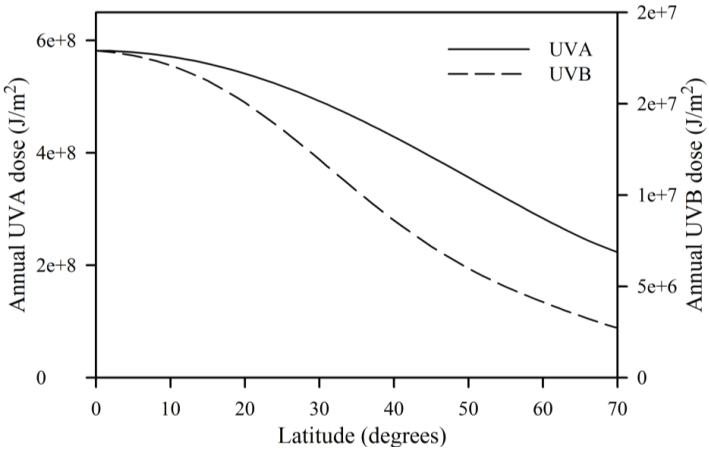

Figure 2.3. Annual integrated UVA and UVB doses on horizontal plane surface at sea level, on clear sky. [Data source the same as for Fig. 2.2.]

Several aspects have to be taken into account when analyzing the beneficial and harmful effects of sun exposure, in particular at higher latitudes, like in Norway.

The UV radiation reaching the earth surface depends on several factors: latitude, season, time of day, solar zenith angle, clouds, ozone layer, altitude, air pollution, surface reflections (snow etc).[16;17] The highest fluence rate of UV is around 11 am and 1 pm (when the solar zenith angle is smaller) and is very small near sunrise and sunset. Because UVB is more scattered and absorbed than UVA when the path length is longer, *i.e.* early morning and evening, UVB irradiance distribution during day is narrower compared with UVA and is more confined around noon, thus the ratio UVB/UVA has a maximum at noon. Concerning the season, UVB irradiance is more confined to the summer as compared with UVA, thus the ratio UVB/UVA is very small in the winter. At northern (higher) latitudes, due to increase in day length and path length of the solar beam and due to thicker ozone layer, we have less UVB, thus a smaller UVB/UVA ratio. Snow doubles the UVB exposure and glass absorbs UVB, not UVA. The clouds and air pollution reduce UVB more than UVA radiation. Figs. 2.2 and 2.3 show annual and daily calculated doses of UVB and UVA on horizontal plane surface at sea level.

2.3. Action spectra – different relevance of UVB and UVA in skin processes

Different biological effects of UV radiation have their own action spectrum (AS), and in some cases (like for melanoma), this spectrum is not known. An AS is a graphical representation of a photoresponse as a function of wavelength of light and it should mimic the absorption spectrum of the molecule that absorbs the light (the chromophore) and whose photochemical alteration causes the effect.

Most of the effects of sun exposure on the skin are due to wavelengths in the range 300-400 nm,[18] although for some rare disorders visible light is also responsible, like in the case of solar urticaria. As previously mentioned, UVB is more influenced by scattering and absorption than UVA is and is more absorbed in the epidermis, while UVA penetrates deeper.[19] Despite the

fact that UVA is more abundant in the atmosphere, UVB, having shorter wavelength, is more energetic and is more effective in inducing various biological effects.

Next, it will be briefly discussed the AS for biological processes with relevance for this thesis: vitamin D production and induction of skin cancer.

AS of vitamin D photosynthesis

The "official" AS for previtamin D_3 formation used nowadays is the Commission Internationale de l'Eclairage (CIE) AS from 2006[20] that has as a starting point the MacLaughlin *et al* AS from 1982.[21] The MacLaughlin' AS has maximum peak for previtamin D_3 production at 297 nm, with no production below 260 or above 315 nm. Thus, the CIE AS concludes that previtamin D_3 formation occurs almost entirely in the UVB region (280-315 nm), with only approximately 3-4% of the total production in the UVA region. The Lehamnn *et al* AS that uses a model of human skin[22] has a peak around 302 nm. Fig. 2.4 shows the MacLaughlin *et al* AS and the efficiency spectrum of vitamin D formation with the sun at midday in summer in Oslo, Norway, compared with the sun at equator.

AS for skin carcinogenesis

Since AS for human skin carcinogenesis is unknown as per the impossibility of obtaining such information from humans studies, substitutes are used, like erythema AS and immunosupression AS, or more directly from animal experiments.

The erythema AS, taken from the work of Anders *el al*[23] has a maximum in the UVB region (around 300 nm), similar to the standard erythema action spectra from the CIE but has also a distinct maximum in the UVA region (around 360 nm). The chromophore for erythema is considered to be DNA (the cyclobutane pyrimidine dimers (CPDs) induction).[24] A recent study showed that the AS for induction of CPDs matched the AS for tumor necrosis factor (TNF) α at 300 nm in the basal layer of the skin, showing that UVB-induced DNA photodamage is a major trigger for TNFα production. TNFα is one of the mediators of the photoimmunosupression.[25]

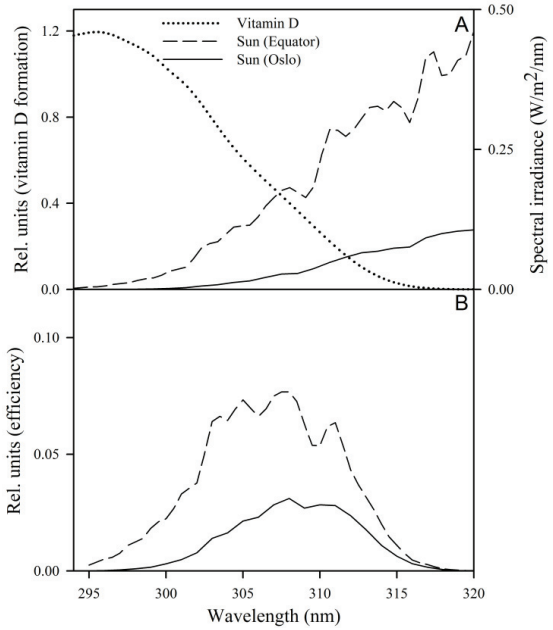

Figure 2.4. Spectral characteristics of the sun at noon midsummer in Oslo and at Equator [Data are kindly provided by Prof. Arne Dahlback, Department of Physics, University of Oslo, Norway] and action spectrum for vitamin D formation in human skin [taken from[21]] (A). Efficiency spectra for vitamin D formation with the sun in the two regions (B).

The immunosuppression AS, taken from the work of Halliday' group[26] shows a peak around 310 nm and a smaller peak at 370 nm and highlights the dominant role of longest UVA wavebands versus UVB, since UVA is more abundant at ground levels. In this AS, the UVA peak disappears at higher doses of UVA. Thus, the authors concluded that the wavebands important for immunosupression are: UVB 310 nm and UVA 360-380 nm, underlining the interactive effects of both, making the contribution of UVB and UVA dependent of sun exposure length and of the ratio UVA/UVB. The UVA peak from this immunosupression AS matches the

UVA peak in the erythema AS, and the UVA peak of the non-melanoma skin cancer (NMSC) AS in mice,[27] underlining the role of UVA in skin carcinogenesis.

2.4. Photoadaptation and skin sensitivity to solar radiation

Sensitivity to solar radiation is highly polymorphous for different persons and different body sites of the same person.[28;29] The individual sensitivity to sunburn and tanning defines the skin type. Sun-sensitive skin types I/II are at greater risk of skin cancer than sun-tolerant skin types III/IV.[30] Several factors influence skin response to sun exposure (erythemal sensitivity): skin color (the major determinant of response to UV radiation),[31] vascular responsiveness, epidermal thickness and hair follicle density. Overall, there is an anatomical site variation of the response to solar radiation: the face, neck and trunk are two-four times more sensitive than the limbs.[32]

The skin is capable of adapting to sun exposure and to amplify its ability to tolerate UV radiation by increasing the amount of melanin and by thickening of the skin (especially of the stratum corneum). However, this does not protect against DNA damage.[33] This is particularly important in the case of single exposures (*i.e.* daily), as the skin does not have enough time for repairing the injuries.[34] Unfortunately, there is no direct answer whether less frequent exposures that allow cells to recover are more useful for inducing UV adaptation without the additional risk of DNA damage, as, for example, before going to holidays in sunny countries.

2.5. Positive effects of ultraviolet radiation

Among the positive effects of solar radiation are: synthesis of vitamin D,[35] treatment of skin[36] and other diseases,[37;38] cardiovascular health,[11;39] and reducing the occurrence of several internal cancers.[35;40]

I will focus mainly on vitamin D photosynthesis and its implications in cancer, since this has been a main research topic in our group at Institute for Cancer Research, and since international research over the past decades has demonstrated its role in prevention and/or

treatment of various cancers (including ongoing research for melanoma), along with many other beneficial roles for human health.[41] Among our recent projects related to vitamin D is studying its role as an antiproliferative and prodifferentiation hormone *in vitro* to increase the effect of photodynamic therapy in cells of a specific form of skin cancer that usually responds poorly to this type of therapy.[42]

Another important application of UV radiation is phototherapy of skin diseases. In this field, our group investigated the magnitude of the increase in vitamin D levels after low doses of narrowband UVB phototherapy given to patients with chronic inflammatory skin disorders (*i.e.* psoriasis or atopic dermatitis). The main outcome was that even low doses of UVB provide a significant increase of the vitamin D status in people with low initial levels of calcidiol.[43]

2.5.1. Vitamin D metabolism

Vitamin D exists in two forms: vitamin D_3 (cholecalciferol), present and produced in animals, notably in fat fish, and vitamin D_2 (ergocalciferol), present in plants, notably in ergots, yeasts exposed to UV. In the skin, UVB radiation converts the precursor 7-dehydrochelosterol in the upper layers of the skin to previtamin D_3; vitamin D_3 will be formed over a few hours. These photochemical reactions are followed by sequential hydroxylations, first in the liver (where the prohormone calcidiol (25-hydroxyvitamin D_3) is formed, a metabolite that is used to assess "vitamin D status") and in the kidneys, forming calcitriol (1α,25-dihydroxyvitamin D_3), the most active vitamin D metabolite, that is responsible for the anticancer actions of vitamin D at the cellular level. Calcitriol is transported by vitamin D-binding protein (DBP) to target tissues that express the vitamin D receptor (VDR). In these target tissues, some of the calcitriol is converted to less-active metabolites, *i.e.* calcitroic acid.

Many extrarenal tissues (including malignant cells) are able to produce calcitriol from the circulating calcidiol.[44] Several cell types, among them keratinocytes, are able to directly convert vitamin D to calcitriol.[45]

2.5.2. Vitamin D and cancer

Since 1980s, there is growing consensus coming from ecological studies,[46] preclinical data (*in vitro* and *in vivo* experiments) and recently from more direct evidence that vitamin D influence cancer, with a direct inhibitory action (for recent reviews, reader is referred to[47;48]). The main anticancer mechanisms involve: i) antiproliferative actions: inhibition of cell growth (by targeting genes related to cell cycle control or by interfering with the actions of several growth factors) and induction of apoptosis and ii) prodifferentiation actions. More newly discovered actions with implications for cancer prevention and progression include: the anti-inflammatory actions,[49] suppression of angiogenesis,[50;51] regulation of the expression of a variety of genes involved in DNA repair,[52;53] and regulation of cells involved in innate and adaptative immune system.[54]

This entire evidence stands at the basis of development of clinical trials that use vitamin D, calcitriol or vitamin D analogs in several types of cancer, but at the present time there are few completed clinical trials to enable us to draw any firm conclusion about the efficacy of vitamin D in relation to cancer.

2.6. Negative effects of ultraviolet radiation

The negative effects of sun exposure include: DNA damage and mutations (that lead to skin cancer), sunburn, photoaging, eye damage, immunosuppression. Skin is exposed almost continuously to this environmental stress, and thus is prone of accumulating oncogenic damage that finally may lead to skin cancer. However, it is yet unclear how solar UV radiation is responsible for the variety of mutations found in relevant genes for skin cancer, especially in the case of melanoma.

2.6.1. Immunosuppression

An important aspect of UV radiation is immunomodulation, both potentiation and suppression. UV radiation causes both local and systemic immunosuppression (reviewed in[55]) that is suspected to play a role in skin carcinogenesis.[56] Susceptibility to immunosuppression

depends on skin type, although erythemal response is not a useful indicator of immunosuppression, especially in skin types I/II, in which immunosuppression is seen even at suberythemal exposure.[57]

2.6.2. DNA damage, repair and mutations

Both UVB and UVA can damage DNA, directly (UVB) or indirectly (UVA), but the relevance of consecutively induced mutations for skin carcinogenesis is still a matter of debate, especially in the case of UVA.

Cyclobutane pyrimidines dimers and 6-4 photoproducts

The main damage induced by UV radiation is the dimers formation between DNA bases pyrimidines (transition mutations) that leads to CPDs and pyrimidine (6–4) pyrimidone photoproducts (6-4 PPs). CPDs are formed between two adiacent pyrimidine bases: tymine, cytosine or 5-methylcytosine. Methylation of cytosine has been shown to enhance the formation of dimers when cells are exposed to UVB.[58] CPDs are induced both by UVB and UVA[59] (although at lower levels in the case of UVA than UVB and by different mechanism) and are considered "solar signature mutations". 6-4PPs are formed by complicated rearrangements, to a much lesser extent than CPDs; 6-4PPs that absorb around 320 nm can be photoisomerized to Dewar valence isomers.

UVB is acting mostly on DNA (direct excitation of the nucleobases). CPDs induced by UVB show a strong sequence dependence,[60] with three main types: TT > 6-4PP and TC sequence > TC. CPDs and 6-4PPs at CT and CC sites are formed in lower amounts and are poorly photoreactive, although CCs photoproducts are highly mutagenic.

DNA is a weak absorber of UVA[61] and UVA induces CPD in smaller yields than those induced by UVB.[62] For UVA other endogenous chromophores are important (acting in a photosensitization manner). The main CPDs induced by UVA are TTs,[63] that are not so mutagenic as CCs or CTs.[60;64] 6-4PPs were not detected in several UVA experimental

studies.[63;65] UVA induces CPDs (C to T transitions) not directly like in the case of UVB, but involving a triplet energy transfer mechanism.[65]

However, UVA induced CPDs may potentially be more dangerous than UVB induced CPDs, since they are more persistent,[66] and the UVA antimutagenic responses are not as effective as those induced by UVB,[59;67] when UVA and UVB are analyzed separately.

Oxidative damage

Oxidative damage involves mainly oxidation of purines (guanine), with formation of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxo-dG). 8-oxo-dG is the main oxidatively induced lesion by UVA, but also induced by UVB,[68] that causes G to T transversions[69] or A to C transversions.[70] UVA photooxidation also induces G to A transitions, probably via CPDs. Recently, it has been shown that CPDs induced by UVA are produced in larger amounts than oxidative lesions are.[65;71]

Beside these lesions, several other photoproducts occur to a smaller extent: photohydratation of cytosine (cytosine photohydrate), formation of adducts between adiacent bases, and single-strand breaks.[68]

If these photolesions are not repaired, mutations (*i.e.* transitions or transversions as mentioned above) will appear.[72] The main repair mechanism involves the nucleotide excision repair pathway. It is to be noted that 6-4PPs are repaired much faster than CPDs are.[73;74]

3. SKIN CANCER – MELANOMA

The three main types of skin cancers are basal cell carcinoma (BCC), the most frequent type, squamous cell carcinoma (SCC) and cutaneous melanoma (CM). BCC and SCC are the most common types of NMSC group. Our study is focused on melanoma, the most fatal of all skin cancers, whose incidence rate in most age groups has increased dramatically over the past decades, especially in Northern Europe.[1]

3.1. The melanocyte

The common origin of all melanomas is the melanocyte, a pigmented, highly dendritic cell, derived from the neural crest, which is located in various anatomic sites and has various functions (Table 3.1). In the skin, melanocytes are located in hair follicles and at the dermal-epidermal interface. Fig. 3.1 shows an exemplification of the skin structure, microscopically.

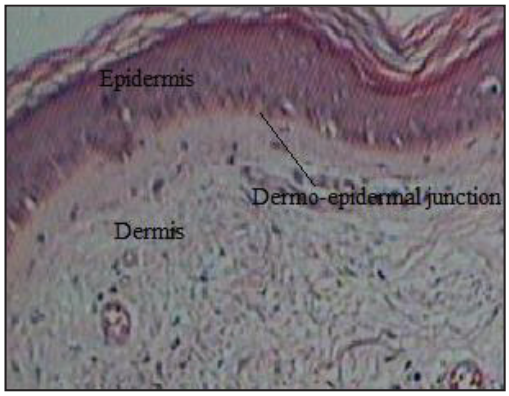

Figure 3.1. Histological structure of the skin. Image is kindly supplied by Irina Tudose, M.D., Elias Hospital, Bucharest, Romania (hemtoxylin-eosin, original magnification x40).

The main function of skin melanocytes is melanin synthesis and secretion. The melanin synthesis takes place in specialized organelles (melanosomes), of various morphologies and numbers. The melanosomes are transferred to keratinocytes,[75] where melanin protects the nucleus (forming supranuclear "caps") from the effects of UV radiation.[76]

Melanin is synthesized in two main forms: the brown-black eumelanin and the red-yellow pheomelanin. The melanin production is regulated by the melanocortin 1 receptor (MC1R). MC1R is controlled by the melanocyte-stimulating hormone (MSH) and adrenocorticotropic hormone (ACTH),[77] as well as by the MC1R antagonist, agouti signalling protein (ASP).[78] MC1R is highly polymorphic, and variations of this receptor are responsible for red hair and fair skin phenotype[79] and can confer a higher risk for melanoma development.

Table 3.1. Locations and functions of melanocytes

Location	Function (s)	References
Skin – epidermis	Melanin synthesis	80;81
Skin – hair follicles and bulge region	Hair pigmentation, stem cell reservoir	81
Eye choroid and retinal pigment epithelium	Eye pigmentation/ vision, photoprotective effects, metabolism of the rod outer segments of the retina and retinoids, antioxidant	82;83
Ear – stria vascularis of the cohlea Inner ear	Hearing Balance	84 85
Brain (leptomeninges, substantia nigra, locus coeruleus)	Neuroendocrine and detoxification	86-88
Heart	Anti-inflammation	89;90
Adipose tissue	Antioxidant and anti-inflammatory properties	91
Lung	Unknown	92

Each melanocyte is surrounded by five keratinocytes and makes connections via its dendrites with 35-40 kearatinocytes, forming the epidermal melanin unit.[93] Within this unit, melanocyte growth and behaviour is controlled by keratinocytes, through growth factors, cell adhesion molecules or other factors.[94] Recently, this concept of epidermal melanin unit was extended to include also Langerhans cells (the main skin antigen-presenting cell type) forming "KLM"unit (K keratinocyte, L Langerhans cell, M melanocyte).[95] Furthermore, melanocytes interact with fibroblasts,[96] cutaneous axon terminals[97] and endothelial cells,[98] forming a skin-melanin unit.

3.2. Melanomagenesis

Currently, there are two theories for melanomagenesis: melanoma arising from pre-existing nevi and melanomas arising *de novo* (from previously normal skin). Fig. 3.2 shows a schematic representation of the current melanoma development models.

"Clark model"

Many authors regard nevi to be direct precursors of melanoma, with a stepwise tumor progression ("Clark model") from neavus → dysplastic naevus → radial growth phase (RGP) melanoma (within or very near to the epidermis, superficial-spreading melanoma (SSM)) → vertical growth phase (VGP) melanoma (invasion into the dermis, nodular melanoma (NM)) → metastatic melanoma. Yet, the molecular mechanism of malignant transformation form nevus to melanoma is not fully elucidated. A nevus is a clonal proliferation of melanocytes that has stopped growing (probably due to cellular senescence, which is considered a tumor suppressive mechanism[99;100]), a key difference from melanoma. Next, additional mutations that abrogate this oncogene-induced senescence are probably necessary in order to initiate progression to melanoma.[99]

De novo melanoma model

However, the Clark' model cannot explain the origin of all melanomas: the dysplastic nevus is not always seen prior to melanoma (most of melanomas do not arise from preexisting nevi[101;102]) and, in some cases, the VGP evolves without any radial component nor any significant epidermal involvement.[103] For explaining *de novo* melanomas, an alternative hypothesis is that melanomas arise from dermal melanocyte stem cells.[104] Other authors[105] have also hypothesized that different melanomas arise from distinct types of cutaneous stem cells: SSM arise from the epidermal basal layer stem cells, lentigo maligna melanoma from stem cells of hair follicles and NM from dermal stem cells.

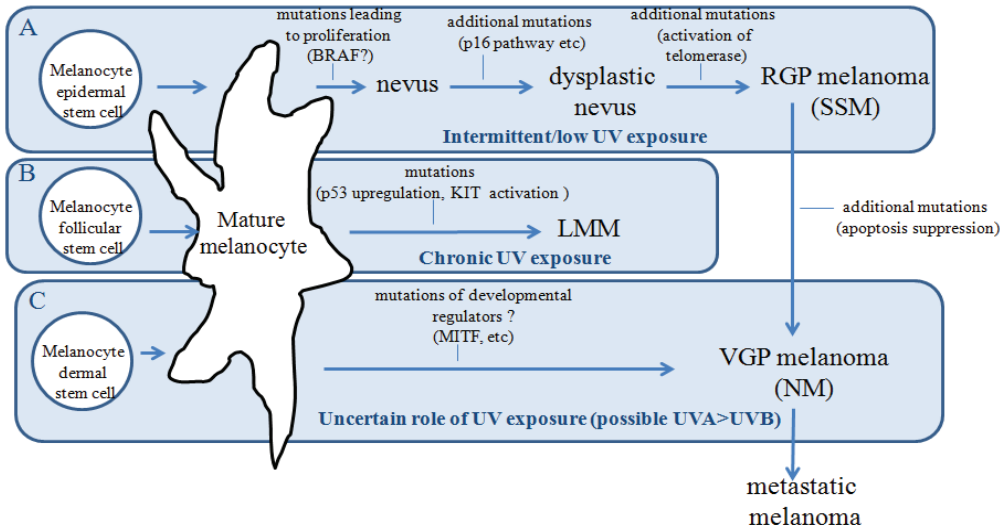

Figure 3.2. Hypothetical pathways for melanoma development (adapted from[100,105]).
"Clark model" pathway (intermittent UV radiation pathway, UV exposure necessary only for initiation in *nevus*-prone individuals) (A);[100] chronic UV radiation pathway (repeated UV exposures) (B);[140] "*de novo*" melanoma pathway (C).[104] *UV ultraviolet radiation, RGP radial growth phase, VGP vertical growth phase, SSM superficial-spreading melanoma, NM nodular melanoma, LMM lentigo maligna melanoma.*

With relevance for the purpose of this thesis is that different risk factors are implicated for melanoma arising from nevi and for melanoma arising *de novo*: Carli *et al*[101] found that history of sunburn was more important for melanomas arising from nevi, while light hair color was more significant found for *de novo* melanomas, thus the authors hypothesized that *de novo* melanoma could be caused by genetic factors in a process not requiring solar radiation.

3.2.1. Genetic changes in melanoma

Cell proliferation and terminal differentiation are regulated through signal transduction pathways and networks. Cancer appears to results from disturbances of these growth-controlling pathways, by mutations of oncogenes or tumor suppressor genes that are critically positioned within these cancer circuits.

I will briefly discuss the three major cancer circuits involved in melanoma: the ras signaling network, the CDKN2A/CDK4 network and the Bcl-2/p53 network; the main developmental regulators of melanocytes with implication for melanoma are mutations in KIT and MITF genes and activation of Wnt/β-Catenin pathway.

Oncogenes

The Ras signaling network has two distinct cascades: the Ras/MAPK cascade (with two key genes: NRAS and BRAF) and the Ras/PI3K/AKT cascade (PTEN being one of the main component of this pathway).

RAS is considered the first oncogene in melanoma[106] and mutations in NRAS, a isoform of the RAS family, are relatively common in sporadic melanoma.[107] Although NRAS mutations have been reported to be more common on chronically sun-exposed sites, Hocker *et al* [107] found that the most common NRAS mutations are not classic UVB-signature mutations, suggesting that sun exposure is not necessary for their induction. Also, melanoma found on intermittent sun-exposed sites seems not to carry these mutations.[108;109]

BRAF is mutated among different cancers and is the single most commonly mutated gene in sporadic melanoma.[110] The most common BRAF mutation is the T-A transversion at 1799 position (val600glu),[111] which is not a classic UV-signature change. Moreover, this mutation is found most frequently in tumors on intermittent sun-exposed sites, not on chronically sun-exposed sites.[112]

Tumor suppressor genes

Melanocytes that acquire BRAF mutation enter a state of cellular growth arrest (senescence), *i.e*. benign nevi, and do not progress to melanoma without cooperation of other pathways that can override this oncogene-induced senescence. Of the most well-described senescence barriers are $p16^{INK4A}$, $p14^{ARF}$, RB, and p53. $p16^{INK4A}$ and $p14^{ARF}$ are encoded from the CDKN2A locus. CDKN2A is a major tumor suppressor gene, as its alterations are found in 25-50% of familial melanomas[113;114] and in up to 30-70% of sporadic melanomas.[115;116]

One of the major tumor suppressor genes in human cancers is TP53 which initiates DNA repair and/or apoptosis when exposed to cellular stress (including UV irradiation). Although TP53 mutations are found in high percentage in human cancers,[117] it is less common in melanoma (closer to 13%[107] and between 20-40% in other studies). TP53 is also a target in UV carcinogenesis, as shown by mouse experiments[118;119] with special relevance for NMSC.[120]

The Bcl-2 network is considered one of the most crucial regulatory systems of melanoma cell apoptosis[121] and includes anti- and pro-apoptotic proteins.

Not all melanomas are genetically similar:[107;112] there is a partiality for NRAS and BRAF mutations for SSM and NM, while there is a lower rate of these mutations for acral lentiginous, lentigo maligna and non-cutaneous melanomas. Moreover, there are differences between anatomic sites: NRAS mutations are found on chronic sun-exposed sites, BRAF mutations on intermittent sun-exposed sites,[112;122] TP53 mutations are found more on head and neck regions,[123] but also on the legs[124] thus on sun-exposed anatomical locations. Mucosal melanomas have a higher rate of TP53 solar signature mutations compared with CM[125] and harbor KIT

mutations. Uveal melanoma harbor mutations for BRCA1-associated protein-1 and for the G(q) alpha subunits.[126]

In conclusion, there is a wide and complex variety of gene mutations in melanoma (for a comprehensive review, the reader is referred to the work of[138]) and a big challenge is to distinguish between the so-called "driver" mutations (that take place in cancer genes, which may be targets for therapy) and the "passenger" mutations (which have little clinical value, but may offer insights into the ethiopathogenesis of the cancer). It is not clear how and to what extent UV radiation is responsible for these genetic changes. In the following section I will address the relationship between UVB, UVA and melanoma.

Table 3.2. Risk factors for melanoma

UV exposure • Anatomic distribution by sex: intermittent sites dominate in both sexes[127] • Migration studies[128] • Differences by latitude of residence[129] • Racial differences[130;131]
Phenotype[132-134] • Pigmentary characteristics Blue eyes Blond, fair or red hair Light complexion • Response to sun exposure Freckling tendency Inability to tan Tendency to sunburn
Pre-existing melanocytic nevi[135]
Upper socioeconomic status[136]
Family history of melanoma History of prior melanoma[134]
Immunosuppression[137]
Pregnancy, estrogen use - controversial

3.2.2. Ultraviolet radiation and melanoma

The etiology of melanoma is multifactorial (Table 3.2), with UV being considered the most important environmental risk factor for the majority of melanomas. However, there is much debate about type, dose, duration and timing of UV exposure necessary for initiation and/or progression of melanoma. The occurrence of melanoma on sites that are rarely exposed to sunlight (like mucosal or uveal melanomas), suggests that DNA damage other than photoinduced may

be responsible for melanomagenesis, for example oxidative DNA damage and low inherent repair capacity of melanocytes from mucosal regions.[139]

The divergent pathway theory

Melanomas occurring on different anatomic sites are biologically different,[112;140-142] as briefly mentioned above, with sun exposure playing different roles in their etiology. Therefore, the current model is that for melanomas on chronic sun-exposed sites repeated sun exposure is the main risk factor, while for melanomas on less sun-exposed sites, genetic factors play essential etiological roles (these melanomas harbor BRAF mutations). The hypothesis is that in nevus-prone individuals, solar exposure may be necessary only for the first steps of the melanomagenesis, after which host factors become more important, while for melanoma not associated with nevi, chronic sun exposure function as a tumor-promoter in individuals with certain mutations, like TP53.[124] In the same direction, other authors consider that for melanomas not associated with nevi host susceptibility may be even more important than exposure to solar radiation.[101] Results from animal studies suggest that chronic sun exposure is not a factor for the initiation of melanoma.[143]

Both UVA and UVB play important roles in melanomagenesis

Several large human studies indicate a role of UV radiation in melanomagenesis, with pyrimidine dimers playing the major role, and, to a lesser extent, oxidative DNA damage.[4;107;144] Since both UVB and UVA induce pyrimidine dimers and ROS (as discussed in the previous chapter), a firm conclusion regarding the contribution of each wavelength to melanomagensis is not possible to be drawn yet.

Table 3.3 shows current evidence for the role of UVA and UVB in melanomagenesis. Hocker *et al*[107] found that UVB signature mutations (G:C>A:T transitions at dipyrimidine sites and GG:CC>AA:TT tandem alterations) were found at higher proportion at tumor suppressor loci (CDKN2A, TP53 and PTEN genes) compared with onocogene loci (NRAS and BRAF). The

authors did not find a high rate of UVA signature mutations (A:T>C:G transversions) in any of these sites.

UVB dominates the etiology in animal models, with major role for the initiation of melanoma.[145;146] A recent study[147] shows that the initiation of melanoma by UVB is pigment-independent (related to a direct DNA damage), in contrast to the UVA melanin-dependent mechanism. Moreover, there are other relevant biological differences between UVB and UVA: the basal layer of the skin, which contains the rapidly dividing cells likely to transform into skin cancer, is particularly sensitive to UVA.[148] This may be due to the higher proportion of UVA than UVB targeting basal layer or to reduction in repairing UVA-induced 8-oxodG by the enzyme glycosylase 1 OGG1 in the basal layer compared with upper layers of the epidermis, as OGG1 is expressed more abundantly in superficial layers than basal layer.[149]

Table 3.3. UVB, UVA and melanoma

Key points:

- Both UVA and UVB induce DNA damage by CPDs and ROS (see text for details), albeit:
  - DNA photon energy absorption is extremely low in the UVA range[150]
  - The UVA damage is smaller compared with UVB damage[62;71]
  - The number of UVA photons reaching the melanocytes at noon is 60 to 80 times greater than the number of UVB photons or 98-99% of photons reaching the basal layer[151]
  - UVB triggers adaptive responses in the skin (at the cellular level - involving p53[152] and $p16^{INK153}$ or at a tissue level - thickening of the skin) and the UVA antimutagenic response are not as effective as those induced by UVB[67] → UVA CPDs may be more dangerous than UVB CPDs
  - Only UVB induces vitamin D formation in the skin
- Melanoma on different anatomic sites harbor different gene mutations and sun exposure may have different roles for melanoma on chronic sun-exposed sites *versus* intermittent sun-exposed sites (see text)
- The solar spectrum contains both UVA and UVB and the limit between them is arbitrary, although in modern times there are several situations with "pure" UVA exposure: UVA phototherapy, sunbeds, sunscreens, exposure through window glass (in cars, offices etc)

UVA lines of evidence (against and pro):

- An ecological study by Garland *et al*[151] associated UVA with increased mortality rates
- The use of sunbeds (which contains mostly high doses of UVA) increases the risk of melanoma[154-156]
- Melanoma has been detected in unusual locations on the skin of sunbeds users[157]
- Use of sunscreens, which in the past did not filter UVA, may increase the melanoma risk[158]
- Animal models:
- In pigmented mice UVA induces melanoma[147]
- In Xiphophorus hybrid fish UVA induced melanoma,[159] although later on this was not confirmed[160]
- In opossum UVA induced melanoma precursors, but without progression to invasive melanoma[161]

UVB lines of evidence (against and pro):

- Animal models:
- Only UVB and not UVA induces melanoma in a transgenic mouse model[145]
- UVB induces SCC in mice,[162] but there is a smaller peak in the UVA region as well
- Mutations in tumor suppression genes like TP53 and CDKN2A contain UVB signature mutations and not UVA signature mutations
- Solar elastosis around melanoma lesions: the prevalence of solar elastosis varied by anatomic site of melanoma, with trunk melanoma having little elastosis compared with head and neck melanoma.[163] And since solar elastosis is linked to accumulated UVB exposure,[164] this indicates a low UVB exposure in melanoma

It is essential to remember that the limit between UVA and UVB is arbitrary (usually set at a wavelength above which DNA does not absorb) and that both wavelengths are part of the solar spectrum, thus making their relative contribution to melanomagenesis complex and perhaps synergistic.

3.3. Classification of melanomas

The classification of melanomas is still a matter of debate and the current WHO classification is based on Clark' four main growth patterns from the 1970s:[165] superficial spreading melanoma (SSM), lentigo maligna melanoma (LMM), acral lentiginous melanoma (ALM) and nodular melanoma (NM). These types are distinguished on the basis of the features of the intra-epidermal component of the tumor adjacent to any dermal invasive component or, in other words, by the absence (like in NM) or presence (like in SSM) of a radial growth phase.

But the impact of the Clark classification on clinical management has been limited, as there is no difference in overall survival among these types if stratified by tumor thickness.[166]

More recently, Bastian' group proposed a new classification system, based on the anatomical sites and the degrees of sun-damages to the skin: melanomas on skin without histopathologic signs of chronic sun-induced damage (that correspond to SSM), melanomas on skin with chronic sun-induced damage (solar elastosis) (correspond to LMM), melanomas on acral skin (correspond to ALM), which is less exposed to the sun and melanoma on mucosal membranes.[112] These melanomas exhibit distinct sets of genetic alterations. In this classification, there is no NM, as NM may arise in any anatomical site and the researchers did not find any unique feature that justify regarding NM as a unique type. However, recent data[167] indicate that NM is a distinct type of melanoma, as suggested by our own observations (Paper V).

A more recent concept[168] is to classify melanomas according to growth rate, clinical and epidemiologic findings: type I are fast growing melanomas, with stable incidence and bad prognosis (usually the thick type[169]), type II are slow increasing, located on intermittently sun-exposed sites and type III are slow increasing, located on continuously sun-exposed sites.

Moreover, melanomas may arise on extracutaneous sites: ocular melanoma, mucosal melanoma, leptomeningeal melanoma and rare cases of melanoma originating in some internal organs.

Melanoma is indeed a heterogeneous disease that may arise through different causal pathways, depending on the genetic background of the host, pattern of sun exposure and anatomical site of the target melanocyte. Thus, a unifying concept for a classification is difficult to develop.

3.4. Epidemiology of melanoma

3.4.1. Trends of melanoma incidence and mortality

Incidence

The incidence rates of melanoma have been increasing in most age groups for the last decades in many fair-skinned populations, with a doubling of rates every 10 to 20 years,[170] with the highest incidence rate observed in New Zeeland and Australia (GLOBOCAN estimates).[1] The highest incidence rate in Europe is seen in Scandinavian countries,[171;172] and the lowest in Mediterranean countries.[173;174] Beside this north-south gradient, there is a west-east gradient.[1;175] These incidence gradients are considered to be due to darken skin type in the South,[176] and may be relevant to consider the more sun seeking behavior in the North and in the West, due to higher income. However, in many central and east European countries, there are no centralized cancer registries of high quality to collect data properly.[175] Other factors contributing to the observed latitude gradient are differences in genetic susceptibility, local health system and prevention campaigns.

Potential factors contributing to this increase in incidence rates are:

1. An increase in sun exposure and changes of sun exposure pattern. Unlike NMSC that are caused by cumulative sun exposure, melanomas are often associated with intermittent (intentional) sun exposure.[177] Main arguments for the role of intermittent exposure are: i) the distribution favors anatomic sites that are intermittently exposed to the sun (like the trunk and limbs),[127] and ii) it is more common in people with indoor occupations (the ”white collar workers”), whose sun exposure is limited to

week-ends or vacations.[178] To be noted: LMM is not usually included in this analysis, since it is generally accepted to be associated with chronic sun exposure.

Factors related to increasing intentional sun exposures are:

- The increase in incidence was more rapid on skin areas exposed intermittently to the sun.[179-181]
- In the last decades, there has been an increase of outdoor sun-seeking behavior and of holidays spent in sunny countries during winter time, thus making intermittent exposure more frequent.[182]
- Changes in clothing habits after 1940s.
- The sunbed/indoor tanning use that has been associated with melanoma[155] has increased for the last decades.[183;184] Furthermore, a recent study from England points out that UV emissions are increasing due to development of high-power sunlamps.[185]
- Sunscreens use: sunscreen use may extend sun exposure duration[186] and thus leads to high risk behaviors: *i.e.* an increased number of sunburns among sunscreens users.

2. Improved surveillance and screening campaigns.[184;187;188] Related to this aspect, a recent study argued that the burden of melanoma increased incidence was independent of screening access, as the authors found doubling rates in all socioeconomic status groups.[5]

3. Overdiagnosis/underdiagnosis. Before the 1970s, early melanomas were almost always diagnosed as nevi or precancerous melanocytic lesions, and many pigmented lesions were not biopsied. Nowadays, there is a tremendous pressure and anxiety not to miss one single melanoma, as pointed out in a recent article by Weyers.[189] Thus, as the author mentions in his article, the increasing number of melanoma diagnosis

after 1970-80s is not caused only by overdiagnosis in present, but by underdiagnosis previously. Overdiagnosis may be the most difficult problem that a dermatopathologist faces today and may give a major contribution to the so called melanoma ”epidemic”.[190]

4. Changes and improvements in diagnostic criteria.

5. Increased average life-time; age is one of the strongest risk factors in all cancers, due to accumulating DNA damage.[191]

6. A possible existence of other yet unknown etiological factors.

Mortality

Despite this dramatic increase in incidence rates, mortality rates have more stable trends, with leveling off in many countries.[192]

Potential factors contributing to this discrepancy between a rapid increase in incidence and a less rapid increase in mortality rates are:

1. Diagnosis of thinner lesions and of *in situ* melanomas, which have more favorable prognosis.[193;194] Nevertheless, another study showed a persistent increase even among thicker lesions.[5]

2. Overdiagnosis and especially overdiagnosis of benign and/or borderline lesions.[190]

3. The very nature of melanoma: most melanomas have a slow growth over decades and sometimes regress spontaneously.[195;196]

4. Increased longevity.

5. Due to people not included in screening programs.

3.4.2. Gender, age, anatomic site and morphology

Several epidemiological studies have shown a variation of melanoma risk by anatomic site, gender and age, as pointed out also by other types of investigations discussed in the previous chapter.

Gender and age

In countries with a high melanoma incidence, such as Australia, a tendency towards melanoma in men is observed.[179;197] In Europe, GLOBOCAN 2008 estimates indicate a tendency towards women for most of the Western and Northern countries. Mortality rates are higher in men than in women in all Europe. A recent study showed a survival advantage for women, independent of gender differences in detection or diagnostic delay.[198] Women tend to be diagnosed at an earlier age than men,[199] and men exhibit higher increase in incidence over time than women.[179;197]

Anatomic site

Gender differences in melanoma incidence are more evident when anatomic sites are taken into account. The anatomic site distribution varies, not only by gender, but also by age,[179;200-202] with the most remarkable gender difference residing in the age-related change in trunk incidence.[203] In young male population, melanoma is observed more often on the trunk, while in young female population melanoma is seen on lower limbs, which are intermittent sun exposed sites (trunk being considered the least sun exposed, while limbs having intermediate sun exposure between trunk and head and neck[204]). Higher incidence rates of melanoma in older populations are observed on head and neck regions for both genders. When adjusted for the size of the anatomic area analyzed, CM most frequently arises on face and neck regions.[205]

The pattern of anatomic site distribution is currently explained by the divergent pathway theory. Trunk melanomas are considered to be more strongly related with host factors, like number of nevi, or with history of sunburn, sun exposure playing a role only in the initiation of

melanomagenesis.[124;146] Head and neck melanomas seem to be related with chronic exposures, as these melanomas are more likely to be associated with solar keratoses and appear in "low nevus count" people.[206;207] Limb melanomas are intermediate between trunk and head/neck melanomas, as both chronic sun exposure and exposures early in life may play significant roles.[208]

Anatomic site and latitude

There are differences of the above site distribution pattern (in particular for intermittent sun exposed sites) among different populations living at different latitudes, probably due to ambient sun exposure and clothing habits. A study that compared site distribution of CM in two different regions of the world (same ancestry, but different clime), Scotland and Queensland,[201] found that in the Scottish population higher incidence rates were observed on lower limbs with lower rates on upper limbs than in Queensland population; this difference was more evident among young females.

While recreational sun exposure is a strong predictor for anatomic sites that are intermittent sun-exposed (like trunk and limbs) at all latitudes, in regions with high environmental solar radiation, like in Australia, total sun exposure seems to increase the risk for head and neck melanoma.[135]

Morphology

SSM is the most frequent histopathologic subtype, followed by NM, LMM and ALM,[170] with different age and body site distribution. SSMs are more frequently diagnosed on intermittently exposed sites:[206;209;210] on the trunk in men and on the lower limbs in women; LLM is the most frequent type on head and neck sites. NM can be found on any part of the body, with no particular predilection. SSM is diagnosed at younger ages than NM or LMM: mean age for SSM is around 55 years, for NM is around 65 years and for LMM around 70.[170;211;212]

In general, SSM and LMM incidence rates have increased over time, more evidently so for SSM at older ages.[213;214] In recent years, a decreasing or stabilizing trend for SSM is

observed for the intermittent sites in Australia.[215] NM has a more stable incidence rate,[216;217] with a recently declining trend reported in Australia.[215]

3.4.3. Epidemiological controversies of ultraviolet radiation and melanoma

Several epidemiological observations are against the negative effect of solar radiation in CM:

1. The relation with latitude is inconsistent (*i.e.* Europe *versus* North America[218]) and smaller than for NMSC: the incidence rate of CM in Australia is only about two times higher than in the high-latitude country Norway, while the incidence rates of NMSC are 20 to 40 times higher.[219]
2. The prognosis is best for summer-autumn diagnosis.[220]
3. The prognosis seems to be best for tumors arising on skin areas with morphological signs of high UV exposure (elastosis).[163]
4. The mortality rates did not increase significantly in a period with increasing rates of incidence.[170]
5. Occupational sun exposure seems to be protective,[178] and also regular recreational sun exposure was found to be protective for melanoma on intermittent exposed sites.[221]

Summary points

1. Anatomic distribution varies by age, gender and pattern of sun exposure: intermittent sun-exposed sites in young populations and chronic sun-exposed sites in old populations.
2. Incidence is continuously increasing since several decades, but with stable mortality rates.
3. SSM and NM are the most frequent morphological types of melanoma, and have different epidemiological characteristics.
4. Melanoma may arise on non-cutaneous sites, as melanocytes are found in various anatomic places, but their incidence is very low (similar rates throughout world populations) and the effect of sun exposure is considered to be very small or absent.

3.5. Melanoma and vitamin D

Laboratory data

Currently, *in vitro* evidence of the involvement of vitamin D in melanoma (although the data are limited compared with other cancers) is increasing (for reviews, the reader is referred to[222;223]): VDR expression in malignant melanocytes, inhibition of growth (melanoma being the first cancer in which the antiproliferative and prodifferentiation actions of calcitriol were demonstrated[224;225] with more recent data[226;227]) and inhibition of metastasis.[228;229] However, not all melanoma cell lines respond to vitamin D,[230;231] probably due to a defect in VDR-mediated transcription.[231]

While some data suggest that vitamin D reduces UV-induced DNA damage in the skin, and, as a result, reduces UV-induced immunosuppression,[232] other data show that vitamin D has an immunosuppressive effect when analogues were applied topically to

irradiated skin.[233] Other studies show that vitamin D is photoprotective.[234] Taken together, these data suggest that vitamin D may have a role in melanoma prevention.

A number of studies explored the inheritance of polymorphisms in the gene coding for VDR as determinants of melanoma risk,[235;236] and the authors of a meta-analysis conclude that there is some genetic evidence supporting the view that vitamin D may have an effect on susceptibility to melanoma.[237]

Clinical data

The possible role of dietary vitamin D in melanoma risk was reported by a recent meta-analysis.[238] A large study[239] did not find any protective effect of combined dietary and supplemental intake of vitamin D, although there was a suggestion of a decreased risk for high supplemental use.

People with fair skin (that are at risk for melanoma) tend to have lower levels of vitamin D compared with people with darker skin.[240;241] This has been linked to sun avoidance.[241] Lower levels of vitamin D were associated with thicker melanoma and poorer survival.[242]

Overall, the current recommendation[223] is that people with risk factors for melanoma should avoid sun burns (the extreme avoidance of sun exposure in many melanoma patients is likely to lead to sub-optimal levels of vitamin D) and to supplement their diet with vitamin D (but not aiming for extreme levels, as the negative effects are unknown yet).

4. GENERAL METHODOLOGICAL CONSIDERATIONS

4.1. Definitions

Epidemiological research is based on quantifications of the frequency of a disease/event in one homogeneous, or more populations. The principal measures of occurrence of disease are prevalence and incidence. Prevalence is the proportion of existing cases (old and new) in a population at a single point in time. Incidence is more informative, as it quantifies the number of new cases of disease that develop in a population at risk, during a specified time interval, thus being unaffected by variations in life expectancy.

They are several types of incidence rates. The crude rates are calculated for the whole population and are widely used, because they are summary measures and easily interpreted. The crude rate is calculated by dividing the number of new cases observed for a given period of time by the corresponding number of persons-years in the population at risk.

For understanding certain epidemiological aspects of diseases, more detailed rates are used, for specific subgroups (strata) of the population (for example, age specific rates, which are crude rates for an age range). The adjusted rates are standardized rates to a reference population, a "standard population", with the general goal of comparability. The age standardized rate is calculated as the sum of the crude age specific rates multiplied by the respective proportions represented in the standard population.

4.2. Data sources and analysis

Population

The cases in our study were individuals in the Norwegian population who have had the disease (*i.e.* melanoma), identified through the Cancer Registry of Norway and the NORDCAN database (version 5.1, 03.2012). The Cancer Registry has systematically collected notifications on cancer incidence since 1952. The Cancer Registry Regulation require all hospitals, laboratories, general practitioners, individual physicians in Norway to report all new cases of cancer to the Registry within two months of diagnosis. More detailed information about the processes of cancer registration at the Registry is found in the recent publication "Cancer in Norway 2009".[3]

The melanoma cases are coded by topography according to the 7th revision of the International Classification of Diseases (ICD-7) with local modifications (the code 190.x for skin melanoma) and the morphologies for malignant melanomas according to MoTNaC (Manual of Tumor Nomenclature and Coding) and ICD-O-2. Different types of melanoma analyzed in the present work are summarized in Table 4.1.

Period of time

The period over which the data was collected varies between different subtypes of melanoma analyzed (Table 4.1), overall between 1965 and 2009.

Measures of occurrence

As a measure of melanoma occurrence, we used crude incidence rates (the number of new cases divided by the Norwegian population in the same period and sex), specific rates for a given age category (0-49 years and older than 50 years) and adjusted incidence rates (to the World standard population), for 3, 5, 6 or 20 years intervals per 100 000 persons or 1 000 000 persons (in the case of rare subtypes of melanoma), during a given time period (Table 4.1).

Anatomic sites and geographical regions

We evaluated the impact of certain variables that could have contributed to the observed association between the risk factor (presumably solar radiation) and outcome (melanoma diagnosis): gender, anatomic site, residential region.

Norway is divided in 20 counties (Fig. 4.1), stratified in this work in three regions: the south region of Norway is defined as referring to the counties 1, 2, 4, 6-11 – with mean latitude 59.5° and a relatively high annual ambient ultraviolet exposure, the mid/west region for counties 5, 12 and 14-17 – mean latitude 64°, with middle annual ambient UV exposure and the north region for the counties 18, 19 and 20 – with mean latitude 69.5° and a correspondingly low annual ambient UV exposure. This grouping also correlates with that used for SCC incidence rates, a skin cancer that is strongly associated with sun exposure.[243] The Southern and the Mid/Western regions in NORDCAN database are slightly different from the ones used in our study: southeastern region counties 1-10, western region counties 11, 12, 14 and central region counties 15-17. In our study, we excluded Oslo region (county 3) and we compared mainly North and South regions, for better clarity of the outcomes.

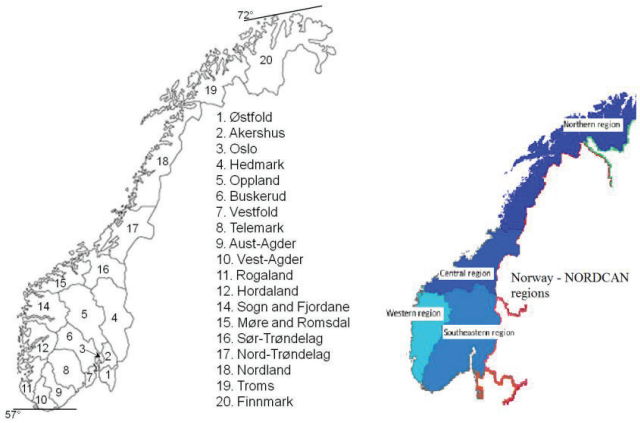

Figure 4.1. Norway counties.

Table 4.1. Types of melanoma analyzed

Cancer type	Period of inclusion	Number of cases*	Incidence measure	Analysis
Cutaneous melanoma (190.x)	1965-2009	31771	Age standardized rates for 5 years periods , per 100 000 persons	• Norway • Regions (by gender and two age groups)
• By anatomic site:	1966-2007			
- Head and neck (190.0)		4377	Specific rates, for 6 years periods, per 100 000 persons	• Regions (by gender)
- Trunk (190.1)		11627		
- Upper limbs (190.2)		3678		
- Lower limbs (190.4)		6306		
- Foot (190.3)		1119		
- Perianal skin (190.5)		65		
- Unspecified (190.9)		1586		
• By histological type**:	1978-2007			
• SSM (8743/3)		12934	Specific rates for 6 years periods, per 100 000 persons	• Regions • Anatomic site (only for NM and SSM, by gender and two age groups)
• NM (8721/3)		4686		
• LMM (8742/3)		892		
• ALM (8744/3)		201		
• Unspecified		6241		
Mucosal melanoma				
• Anorectal melanoma (154.0, 154.1)	1966-2007	75	Specific rates for 20 years periods, per 1 million persons	• Norway (by gender) • Regions
Ocular melanoma				
• Uveal melanoma	1993-2004	463	Specific rates for 3 years periods, per 100 000 persons	• Regions

*The total number of cases is taken from NORDCAN, the specific numbers were provided by the Cancer Registry of Norway. **SSM superficial-spreading melanoma, NM nodular melanoma, LMM lentigo maligna melanoma, ALM acral lentiginous melanoma.

The anatomic sites used for analysis are listed in Table 4.1 and were chosen according to presumed sun exposure patterns: chronic sun exposure (head and neck), intermittent (intentional) sun exposure (trunk and limbs) and no sun exposure (anorectal mucosa, perianal skin, and uvea).

Statistics

Statistical comparison was done using the computer programs SigmaPlot 10.0 software (Systat Software, Inc., Richmond, California, USA) and in Paper V SPSS (IBM SPSS Grad Pack 20.0, Chicago, Illinois, USA).

4.4. Strengths and limitations

Our study has some important strengths: the size of the cancer population (population based study group that allows to examine national trends and to estimate the cancer burden), the long follow up, the good quality of the cancer registration. Although ecological studies are important for generating hypothesis, the correlations found do not imply causality; however, the use of stronger clinical studies, like randomized clinical trials, are not ethical suitable in the case of sun exposure and skin cancer.

Another important limitation is that we used surrogate markers of solar exposure, such as residential region (latitude) and anatomic site (sun exposed *versus* non-sun-exposed sites), instead of direct information provided by the patients. However, these surrogate variables were chosen based on current available evidence and earlier experience.

The association between the risk factor and the outcome might be influenced by other confounding factors not analyzed, not available or unknown.

We were unable to verify the accuracy of the diagnosis and coding of different histological types of melanoma reported to the Norwegian Cancer Registry. Nevertheless, due to large numbers of cases, any variance in the diagnosis between regions or individual pathologists is likely to be minimal. Also, there was a large number of melanoma of

unspecified sites or of unspecified histology that may have affected the results, especially since a recent study observed that in Northern Europe the proportion of cases with clinical and unknown verification has increased with time.[244]

Epidemiology tends to homogenize and simplify reality. Still, analysis of classical epidemiological variable may provide interesting information about the relationship between sun exposure and melanoma and the impact of prevention campaigns.

5. SYNOPSIS OF PUBLICATIONS

Paper I

Aims To compare time trends and different anatomic localization of CM in north *versus* south regions of Norway.

Methods Latitude gradients and time trends for CM were analyzed using incident cases from the Norwegian Cancer Registry for the period 1966-2007. Body sites included in the analysis were head and neck, trunk, upper limbs and lower limbs, for both genders, in the south and north regions of Norway.

Results There is a latitude gradient for CM on all body sites included in the present study, with 2-2.5 times higher incidence rates in the south than in the north. The latitude gradients seem to be largest for the trunk. Melanomas on sites intermittently exposed to the sun (like the trunk) dominate both in the north and in the south, and this distribution has not changed over the years. A leveling off of the incidence rates are observed for both sexes and for all sites studied, after 1990s, except for the head and neck where the incidence rates have continued to increase slowly in the north as well as in the south.

Conclusion The latitude gradient support the role of solar radiation in melanomagenesis, for all body sites, but more convincing for intermittently sun-exposed sites.

Paper II

Aims To evaluate the role of solar radiation in uveal melanoma etiology, by comparing the time and latitude dependency of the incidence rates of this melanoma with CM in several Caucasian populations.

Methods Incidence rates for CM and uveal melanoma in Norway were provided by the Cancer Registry of Norway. Data for Sweden were taken from the work of Bergman *et al*[245] and for other populations included in the study from the work of Virgili *et al*.[246]

Results There is a marked north-south gradient of the incidence rates of CM in Norway, with three times higher rates in the south than in the north. No such gradient is found for uveal melanoma. In most populations the ratios of uveal melanoma incidence rates to those of CM tend to decrease with increasing CM rates. This is also true for Europe, in spite of the fact that in this region there is an inverse latitude gradient of CM, with higher rates in the north than in the south. In Norway the incidence rates of CM have increased until about 1990 but have been constant or even decreased (for young people) after that time. The uveal melanoma rates have been increasing after 1990. In most other populations the incidence rates of CM have been increasing until recently while those of uveal melanoma have been decreasing.

Conclusion The different time and latitudinal trends between uveal and cutaneous melanoma are strong arguments for different role played by solar radiation in their pathogenesis *i.e.* initiation *versus* protection.

Paper III

Aims To compare time and latitude trends of melanoma incidence in the anorectal region and perianal skin (non-sun-exposed sites) with those of CM (sun-exposed skin).

Methods We analyzed epidemiological data from the Cancer Registry of Norway for melanomas of the anorectal mucosa, perianal skin and overall CM, using crude incidence rates for two time periods: 1966-1986 and 1987-2007.

Results We found that melanoma incidence on these shielded sites tends to decrease or remain constant over a period during which the CM rates increase. Similar trend are found in the north and south regions of Norway. Comparison of latitudinal trends of the incidence rates of

CM and melanoma on these shielded sites shows that there is no latitude gradient for melanoma of the anorectal mucosa and perianal skin, whereas there is a strong one for CM.

Conclusion Solar radiation does not play the same role in perianal skin and anorectal mucosa as it has for CM, possible being even protective for these shielded sites.

Paper IV

Aims To compare the time and latitudinal trends of CM incidence on skin areas which are chronically (head and neck) and rarely (foot) exposed to UV radiation, in order to underline the role of pattern of sun exposure in melanomagenesis.

Methods We have analyzed epidemiological data from the Cancer Registry of Norway, for foot and head and neck melanoma for two time periods: 1966-1986 and 1987-2007.

Results CM incidence rate on head and neck has increased slowly with time, while incidence rates of foot CM have remained almost constant. There is a large north–south gradient in incidence rates of CM on head and neck in Norway, while there is almost no north–south gradient for CM incidence on foot.

Conclusion Chronic sun exposure is important for melanoma on head and neck region, but does probably play any role for foot melanoma.

Paper V

Aims To compare SSM and NM incidence in Norway, by latitude gradient and body site distribution. SSM and NM are two major histological types of melanoma. Intermittent sun exposure seems to play a major role in SSM, which, overall, has an increasing incidence rate during the last decades. However, the relationship with sun exposure is more complex in the case of NM, as the latter may arise on any body part and has a more stable incidence rate.

Methods The study was based on official reports from the Cancer Registry of Norway, using melanoma incidence rates for a period of 30 years (1978-2007), by age, gender, anatomic site (trunk and head and neck) and region of Norway (South *versus* North).

Results Our results show that in Norway, SSM is more strongly related to intermittent sun exposure than NM, as it arises mostly on the trunk as compared with the head and neck. Moreover, SSM has a significantly higher incidence in the Southern regions of Norway, whereas for NM, the north–south gradient is not statistically significant.

Conclusion Our work underlines that SSM and NM are distinct forms of melanoma as based on different epidemiological characteristics. Based on differences in the body site distribution and latitudinal gradient, NM may be less UV related than SSM.

6 DISCUSSION OF THE RESULTS

6.1. Overall melanoma incidence rates in Norway

The melanoma incidence rates in Norway are among the highest in the World (and in Europe) (Fig. 1.1), with increasing rates up until 1985-1995. A levelling off is observed after this time point, more evident for the young population for which there is a decreasing trend[247] (Fig. 6.1). This time trend is similar in the north and south regions of Norway for male population, but with subtle differences for young female population (Fig. 6.2).

The levelling off is probably associated with campaigns against large sun exposures and, consequently, increasing awareness in the population after 1980s.[248;249] Stabilizing incidence rates are observed also in other parts of the world, like Australia, New Zeeland, US, Canada or Israel, in particular for young persons, but with increasing trends in Southern and Eastern Europe.[215;248;250] However, following the period with stable rates, we observed a slight but not significant increasing trend for female melanoma in the

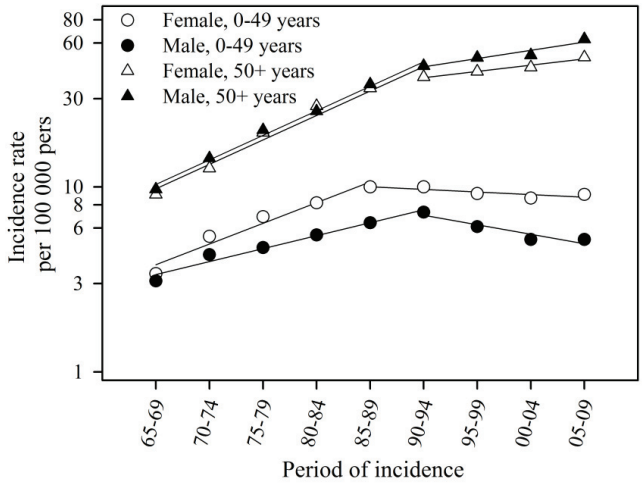

Figure 6.1. Time trends of melanoma incidence in two age groups. Incidence rates are ASR (world population) from NORDCAN database.

north region of Norway (Fig. 6.2 B). Increasing trend for the recent period in women is also observed in Denmark[251] and in the USA.[252]

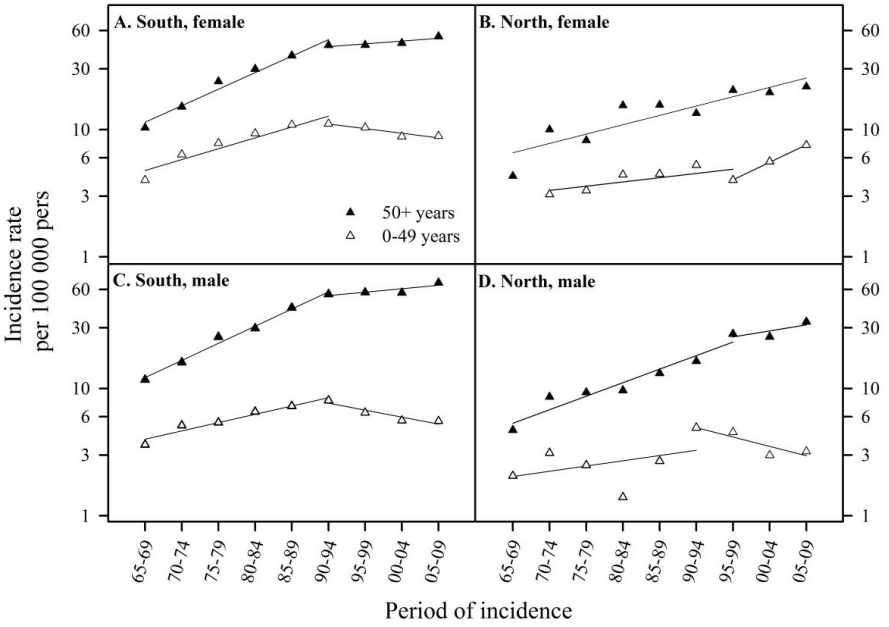

Figure 6.2. Time trends of melanoma incidence in south and north regions of Norway. Incidence rates are ASR (world population) from NORDCAN database.

Even though the annual doses of UV radiation are low in Norway, the CM incidence is high, possibly due to factors like: pigmentary characteristics (fair skin type, blond hair and blue eyes), positive attitudes towards outdoor activities and holidays to sunny countries (acute and intense intermittent sun exposures) and sunbed use,[253] (which was found to be associated with melanoma risk (reviewed in[254])). In Norway, the temperatures are cool and the days are longer during the summer, thus encouraging people to remain in the sun for longer periods when

outdoors, in contrast with Mediterranean countries where the hot temperatures do not allow people to stay outdoor during middle day.

The larger variation of the UVB than UVA intensity at Northern latitudes makes UVA more important, recent evidence showing that UVA may play a significant role in melanomagenesis, previously underestimated (as discussed in chapter 2).

The Norwegian population has a good vitamin D status, estimated at levels above 75 nmol/L for a significant fraction of the population during summer and autumn and 50-65 nmol/L during winter,[255] current studies arguing for a protective role of vitamin D in melanoma (as discussed in chapter 3.5) and thus a possible explanation of the observed levelling off of the incidence rates.

6.2. Trends by age and gender

Melanoma incidence varies by gender between different age groups. In younger persons (0-49 years), the incidence is significantly higher in women than in men, while in older persons (>50 years) the incidence is higher in men than in women, but the difference does not reach statistical significance (Fig. 6.1). The predominance of men in the older group became more evident after 1985s, possibly due to behavioural changes in men. The gender difference has been partially explained by different sun exposure behaviour or clothing habits,[256] supported by the different anatomic site distribution in men and women (see below).

Several studies have reported a diagnosis delay in males that might explain the higher incidence in older men than in women: men are less likely to self-detect their melanomas,[169] have a lower awareness of skin cancer risk,[257] and are less likely engaged in preventive behaviors.[258] Concerning hormone influence, estrogens seem not to affect melanoma.[259]

6.3. Trends by anatomic site

We have analyzed melanoma anatomic distribution in men and women by comparing sites with supposedly different sun exposure patterns: chronic exposed sites (head and neck),

intermittent exposed sites (trunk and limbs, with a particular focus on the foot, which has little sun exposure) and non-sun-exposed sites (anorectal mucosa, perianal skin and uveal melanomas). Furthermore, we analyzed the data separately for north and south regions of Norway.

Melanoma on intermittent sun-exposed sites dominates, both in the north and in the south regions of Norway: trunk for men and both trunk and lower limbs for women (Paper I) (Fig. 6.3). In the north, following a period with stable rates we observed an increase in female melanoma incidence for all intermittent exposed sites analyzed (in particular upper limbs) (Fig. 6.3 B).

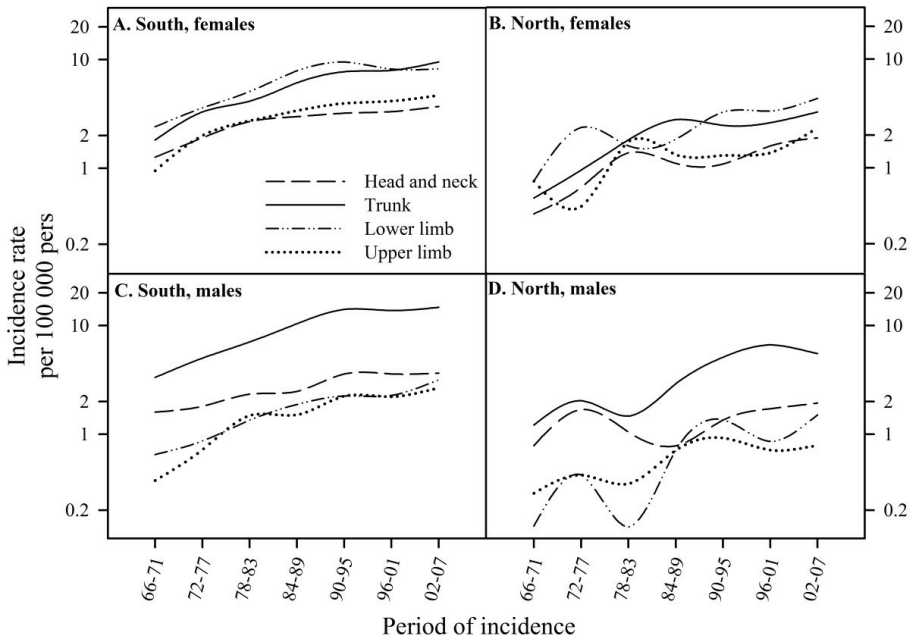

Figure 6.3. Time trends of melanoma incidence by anatomic site, region and gender. Incidence rates are crude rates from the Cancer Registry of Norway database.

Melanoma incidence rates for head and neck are significantly higher in the older group than in the younger group (Fig. 6.4), possible because the low level of annual radiation in Norway induce head and neck melanoma only after long duration of cumulative exposure, as Whiteman *et al* observed for Scotland as well,[201] in agreement with the "divergent pathway hypothesis". In our study, in older group, the intermittent sites (trunk) significantly dominate only for males, and not for females. Trunk melanoma in older men has to be addressed vigorously in health messages, as it has one of the highest rates of increase in incidence in the last decade. An indication of success of public campaigns is the recent stabilization of the trunk melanoma in young population in Australia.[215]

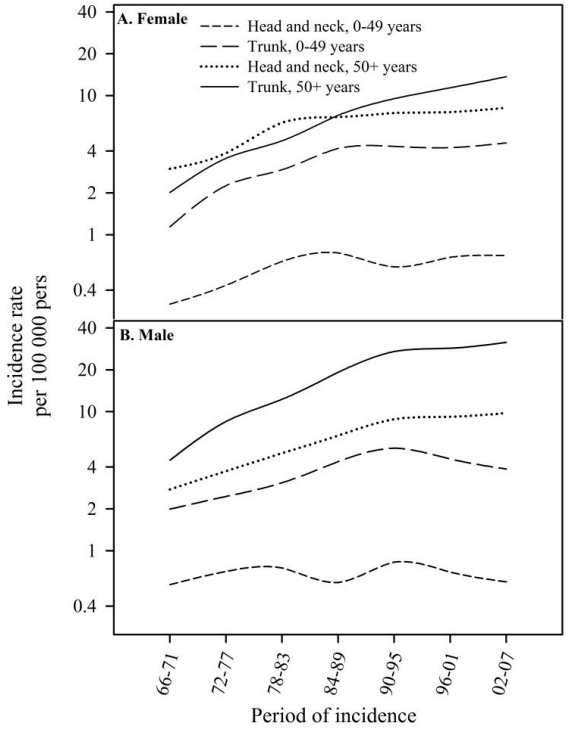

Figure 6.4. Time trends of melanoma incidence by anatomic site and gender in two age groups.
Incidence rates are crude rates from the Cancer Registry of Norway database.

Opposite time trends for different anatomic localizations

Our work revealed different time trends between various types of melanoma with different sun exposures patterns:

- Trunk melanomas rates tend to increase at higher rate than those for head and neck melanomas, while foot melanomas tend to remain nearly constant, in the same period of time (Papers I and IV) (Fig. 6.5).

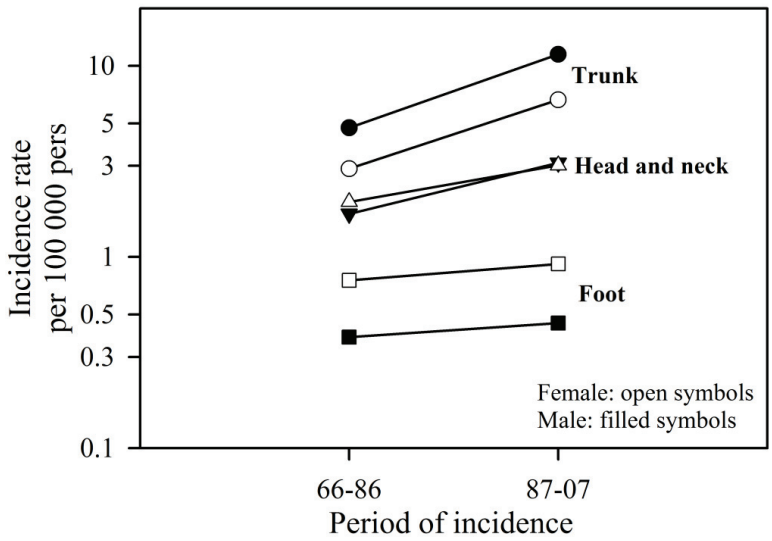

Figure 6.5. Different time trends of melanoma incidence in three anatomic sites with different sun exposure patterns.
Incidence rates are crude rates from the Cancer Registry of Norway database.

- Perianal and anorectal melanoma incidence rates tend to remain constant over a period during which the CM rates have increased (Paper III) (Figs. 6.6 and 6.7).

- After 1990s, CM incidence rates tend to level off, a time trend not seen for uveal melanomas in Norway (Paper II) (Fig. 6.8).

These different time trends indicate that there is difference between the pathogenic mechanism of melanomas on various anatomic sites, which harbour different mutations (discussed in the chapter 3.2) which may require tissue-specific environments for progression in their malignant transformation (as pointed out in a recent study of mucosal melanomas[260]). Different body sites differ with respect to density of melanocytes,[261] thickness of the skin,[262;263] or hair follicle density,[264] all of these factors influencing skin sensitivity to solar radiation.

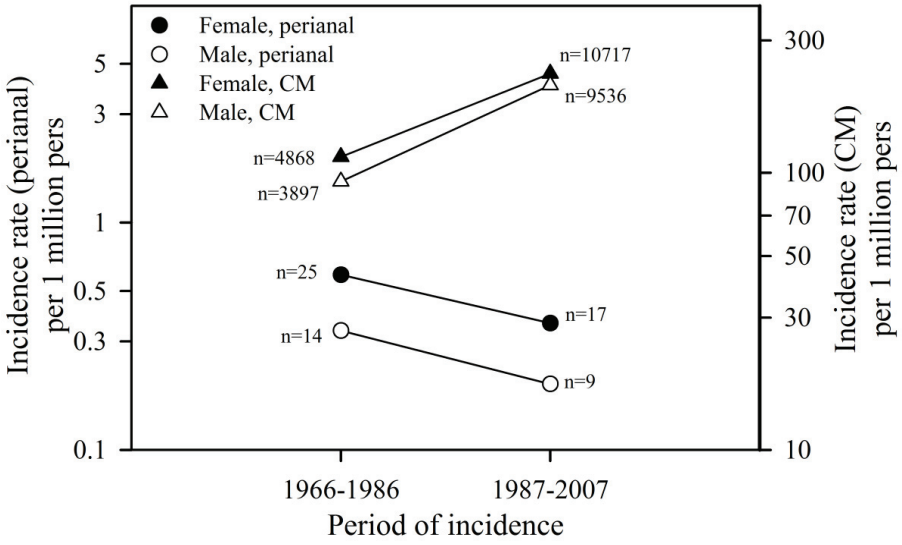

Figure 6.6. Comparison of perianal melanoma and cutaneous melanoma time trends in Norway.

Incidence rates are crude rates from the Cancer Registry of Norway database. *CM cutaneous melanoma.*

Gender-related differences in anatomic distribution of melanomas are not easily explained. The most plausible explanation may be related to different patterns of sun exposure,

with behavioural factors influencing the site distribution.[256] Different fashions of clothing were thought to explain the excess of female melanoma on the legs.[265] However, for the last decades, clothing habits did not differ much between men and women: men expose both their trunk and their legs during recreational activities, and women wear clothes that often leave part of the trunk and legs exposed to the sun during summer. Despite this similarity in clothing pattern, melanoma on the legs of men exhibits much lower rates than melanoma on the trunk, possibly because legs are less sensitive to the sun than the trunk.[29;32] The higher rate of female melanoma on the legs has been explained by some authors by the low sun protection afforded by women stockings[266] or by hair removal practices with consecutive hair alterations.[267] A better explanation may be an inherited sex-dependent tendency to develop nevi (and thus melanomas) on a given anatomic site,[268] in particular in the case of trunk melanomas. The genetic susceptibility of melanoma on different anatomic sites may have different impact in men and women, possibly being modulated by endocrine sex hormones, as expression of MC1R is influenced by specific endocrine sex hormones.[269;270]

6.4. Latitude gradients

There is a strong north-south gradient of the CM incidence rates (Fig. 6.2) that persists even when different body sites are analyzed (Paper I) (Fig. 6.3). However, we found no latitude gradient for the foot (Paper IV), the anorectal region (Paper III) and uveal tract (Paper II). The latitude gradient suggests that UV is a strong risk factor for the vast majority of melanomas (*i.e.* those on sun exposed skin), as the latitude gradient of the calculated solar UV fluence[271] agrees well with the observed melanoma latitude gradient, but not a risk factor for melanomas for which we did not find a latitude gradient. This hypothesis is supported also by the different time trends of these types of melanomas when compared with CM, as discussed above. However, the number of anorectal melanomas was too low to allow a firm conclusion.

As the observed latitude gradient was largest for the trunk, compared with head and neck, intermittent sun exposure pattern seems more important in the south region. In the south regions there are more factors contributing to an increased intermittent exposure than in the north: the

warmer and more agreeable climate promotes more outdoor activities; due to urbanization people are more likely to work indoor than outdoor, and due to higher income people have possibly spent more holidays in sunny countries.

Another factor that varies with latitude is the vitamin D level: the highest level is observed in south region (around 70 mmol/L)[272;273] and it is 21% lower in the north.[274] Thus, for anorectal or uveal melanoma, for which solar radiation seems to have a protective effect, vitamin D may be the mediator.

6.5. Extracutaneous melanoma

6.5.1. Epidemiology

Extracutaneous melanomas (EM) are rare tumours, with poor prognosis[275] and very few studies are population-based.[244;275] The most common EM are ocular melanomas (eyelid, conjunctival and uveal melanomas), followed by mucosal melanomas.[275;276] Despite the common cellular origin with CM, they show different epidemiological characteristics.

Age distribution

Mucosal melanomas have an older age distribution curve compared with CM, with a median age of ~70 years in some studies;[275;277] in our study, only 10% of anorectal melanoma cases in males (three cases of a total of 30) were diagnosed under 50 years of age and non in women, for a period of 40 years analyzed (1966-2007).

Racial differences

While CM are 10-20 higher in white populations than in dark skin populations, mucosal melanomas are only two times higher in white than in dark skin populations[276;278] or show similar rates in these two racial groups.[279]

The risk for uveal melanoma is much lower for dark skin individuals than for white skin individuals in the US.[280;281]

Thus, constitutive melanin pigmentation seems to act in a protective manner (*i.e.* as antioxidant) even for sites that are not reached by UV radiation.

Time trends

Several studies have shown differences in time trend between melanoma on shielded and non-shielded sites, in different parts of the world. A recent study from the Netherlands showed that EM had no definite time trend, whereas for CM there was an increase in incidence.[275]

In Sweden, anorectal melanoma incidence has remained constant over a period where CM incidence has increased strongly;[282] in Queensland, one study found no change in the incidence rates of anorectal melanoma despite a very high incidence rate of CM;[283] in Norway, anorectal melanoma tend to remain constant, whereas CM incidence increased in the last decades (Fig. 6.7). Thus, there is agreement between our work and those of others in other countries.

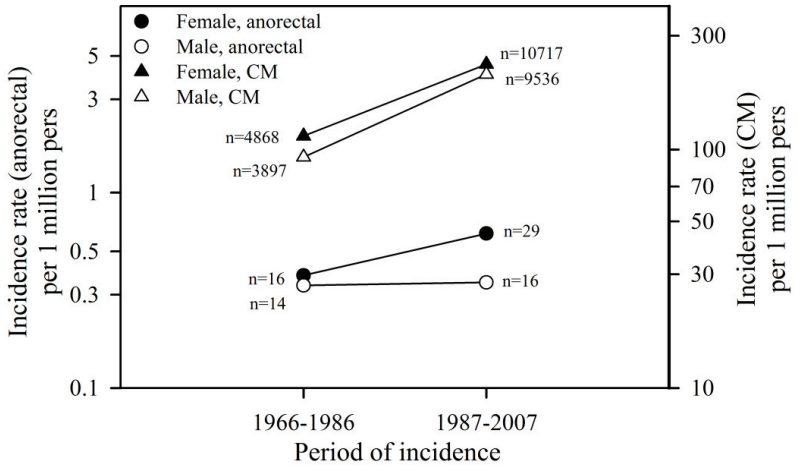

Figure 6.7. Comparison of anorectal melanoma and cutaneous melanoma time trends in Norway.

Incidence rates are crude rates from the Cancer Registry of Norway database. *CM cutaneous melanoma.*

Ocular melanoma epidemiology is more similar with CM, showing increasing trends;[279] however, when stratified by subtype, ocular melanomas show different time trend: conjunctival (external) melanomas are more similar with CM, while uveal (internal) melanoma (iris, choroidal and ciliary body melanomas) rates remained stable[279;284;285] in countries with increasing trends for CM (Paper II). Uveal melanoma show similar incidence rates all over the world: in North America, Europe or Australia, despite the different incidence for CM.[281] Surprisingly, in Norway, we observed an increasing trend for uveal melanoms for a period for which CM incidence has a stable or a decreasing trend (Fig. 6.9).

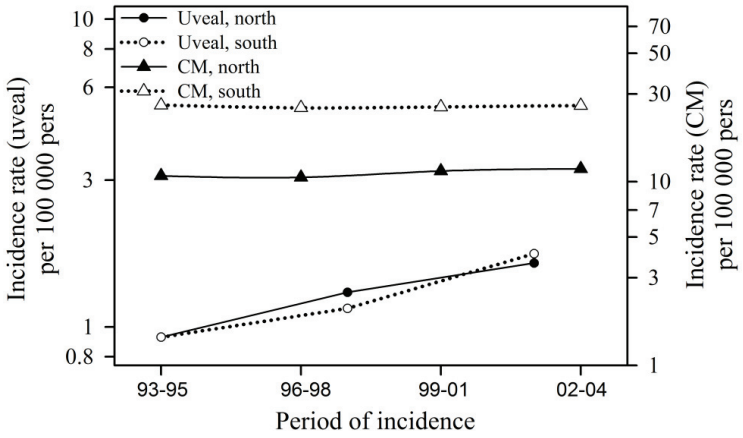

Figure 6.8. Comparison of uveal melanoma and cutaneous melanoma in south and north regions of Norway, both genders combined.
Incidence rates are crude rates from the Cancer Registry of Norway database.*CM cutaneous melanoma.*

Latitude gradients

In Europe, uveal melanomas tend to decrease from north to south.[244;246] As skin and eye pigmentation generally increases from north to south in Europe,[176] it has been proposed

that melanin has a protective effect against uveal melanoma,[83] with light iris color being a risk factor for ocular melanoma,[286] the same as fair skin is.[287] In Norway, for the period 1993-2004, we found no latitude gradient for uveal melanomas, while there is a strong one for CM (Fig. 6.8). The Norwegian population is homogeneous concerning pigmentary characteristics. This may explain why we did not find any latitudinal gradient. But when considering countries with high incidence rates of CM by latitude, ratio of the incidence rates of uveal melanomas to those of CM appear to increase with increasing rates of CM incidence, thus with decreasing annual UV exposure (Paper II).

Nor for anorectal melanomas did we find any latitudinal gradient (Fig. 6.9), but the number of cases was too low to allow us to draw any firm conclusion. One study from the USA found higher rates of mucosal melanomas (vulvar and anorectal) in the North,[288] while CMs show an opposite latitudinal trend in the white population.[289]

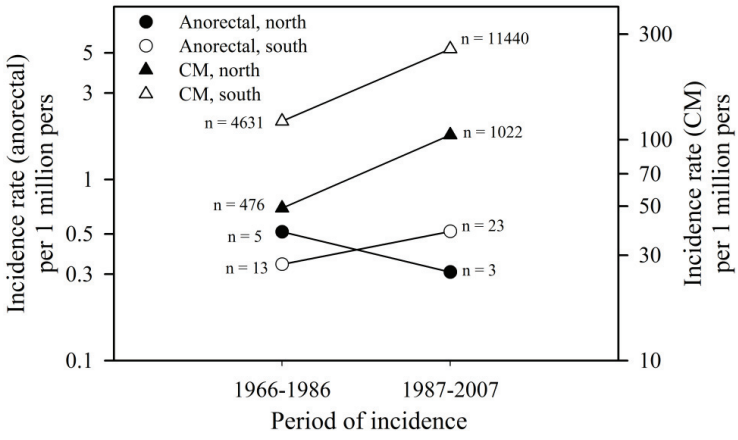

Figure 6.9. Comparison of anorectal melanoma and cutaneous melanoma in south and north regions of Norway, both genders combined.
Incidence rates are crude rates from the Cancer Registry of Norway database. *CM cutaneous melanoma.*

6.5.2. Extracutaneous melanomas and solar radiation

EM cannot be reached directly by sun exposure. Thus, the negative role of UV radiation for these cancers is controversial. In light of the epidemiological evidence described above, our hypothesis is that sun exposure may have a protective systemic effect against melanomas on shielded sites via vitamin D synthesis, in the same way as vitamin D acts for some internal cancers. The pathogenesis of these subtypes of melanoma is thus multifactorial, as supported also by genetic studies. A study[125] found solar signature mutations for TP53 in mucosal melanomas and chronically sun exposed melanomas, identical to those observed for internal cancers. Also, KIT is the most frequent altered oncogene in mucosal melanomas,[290;291] but its mutations are also found on the skin with chronic sun damage.[292;293] Thus, the induction of these particular mutations is not solely due to UV radiation.

As for uveal melanoma, much controversy still exists. UVB cannot reach the uveal tract and only small fluences of UVA may reach this target.[294] Furthermore, a recent meta-analysis did not find any strong evidence for a role of sun exposure in uveal melanoma;[295] however, outdoor work was found to be a risk factor for uveal melanoma in some studies.[287] The recent discoveries of the genetic mutations in uveal melanomas (for BRCA1-associated protein-1 and for the G(q) alpha subunits[126]) do not imply any major role of sun exposure, but rather point towards host susceptibility.

6.6. Trends by morphology of cutaneous melanoma

Melanoma is a highly heterogeneous cancer with various clinical and histological types, some of them being difficult to diagnose even by experts. SSM and NM are the most common types (Table 6.1.), and therefore we focused our analysis on them (Paper V).

Table 6.1. Number of cases of the main histological types of melanoma, by gender, Norway, 1978-2007.

Gender	Number of melanoma cases*					
	Total cases	**SSM**	**NM**	**LMM**	**ALM**	**Unspecified**
Female	13606	7249	2237	546	124	3245
Male	11737	5685	2449	346	77	2996

*Number of cases is provided by the Cancer Registry of Norway. *SSM superficial-spreading melanoma, NM nodular melanoma, LMM lentigo maligna melanoma, ALM acral lentiginous melanoma.*

SSM is usually associated with a pre-existing nevus, and it is found on intermittent sun-exposed body sites. It is characterized by a population of atypical melanocytes with a monomorphous appearance, with a "pagetoid" distribution throughout the epidermis (radial growth).[296]

NM has a rapid evolution and usually begins *de novo* in an involved skin, both on intermittently and on chronic sun-exposed sites.[296;297] It arises at dermo-epidermal junction and extends vertically into the dermis (vertical growth), with little intraepidermal growth (conjointly with the underlying dermis).

6.6.1. Time trends

In Norway, both NM and SSM show an increase in incidence up until 1990s with a stabilizing trend after that, both in the north and in the south regions of Norway (Fig. 6.10).

However, we observed a recent slight increase in incidence in the North region (for NM in both genders and for SSM in women). We found a decreasing trend in the case of SSM incidence in young male population in the recent period (Fig. 6.11).

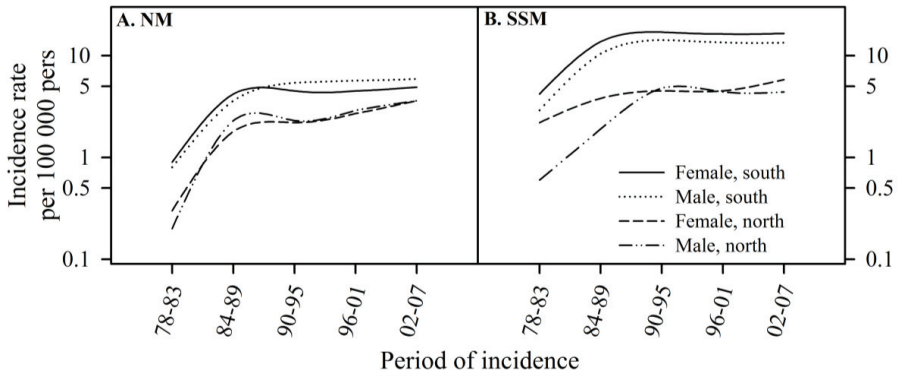

Figure 6.10. Time trends of nodular melanoma and superficial-spreading melanoma in south and north regions of Norway.
Incidence rates are crude rates from the Cancer Registry of Norway database. *NM nodular melanoma, SSM superficial-spreading melanoma.*

Increasing trends have been reported for the last decades for SSM, in Europe (Germany,[213] Italy[214;298]) and in the USA, in particular for women,[199] with a shifting toward earlier stages of SSM.[299] Stabilizing trends of SSM for recent years are observed in Australia, for intermittent sun-exposed body sites.[215] NM has been reported to have a more stable incidence[216;299] and does not show any significant difference in thickness over time.[299;300] A recent decline of NM incidence on intermittent body sites was recently reported for Australia.[215]

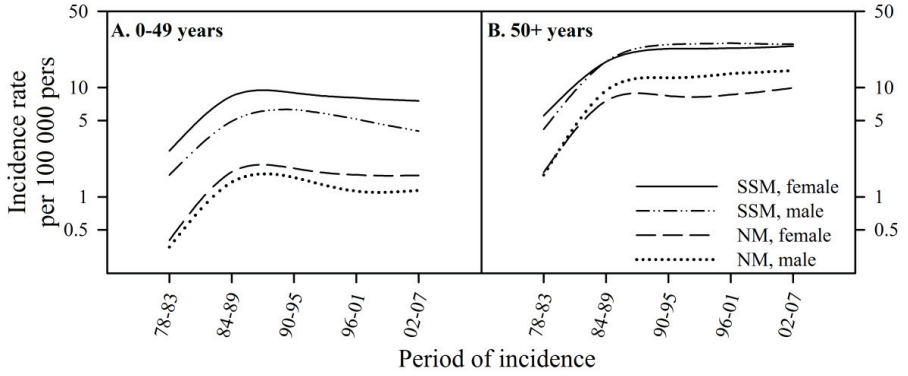

Figure 6.11. Time trends of nodular melanoma and superficial-spreading melanoma in two age groups.

Incidence rates are crude rates from the Cancer Registry of Norway database. *NM nodular melanoma, SSM superficial-spreading melanoma.*

6.6.2. Age, gender and anatomic site

We did not find any significant difference between gender distribution of SSM and NM, as did other studies in Northern Europe,[301] although some studies argue for a male tendency in the case of NM.[297;302]

In younger age groups, the NM incidence was insignificant compared with the incidence of SSM (Fig. 6.11), NM being a cancer of older age.[297;303] Even though SSM had also a higher incidence in older group than in younger group, this predilection was significant only for women.

For men, we observed higher rates of both SSM and NM on the trunk as compared with head and neck and limbs (Fig. 6.12). For women, the predilection for intermittent sun-exposed sites (trunk and lower limbs) was significant in the case of SSM and not for NM. On the head and neck, we found similar ratios between incidence rates of NM and SSM in men, but higher rates of SSM in women. Thus, for men the anatomical site distribution was more or less similar for SSM and NM (favouring the trunk). For women only SSM showed a partiality for

intermittently exposed body sites, while NM occurred with similar rates on chronic and intermittent sun-exposed sites. Overall, intermittent exposure patterns seem more important for SSM than for NM, in particular for women.

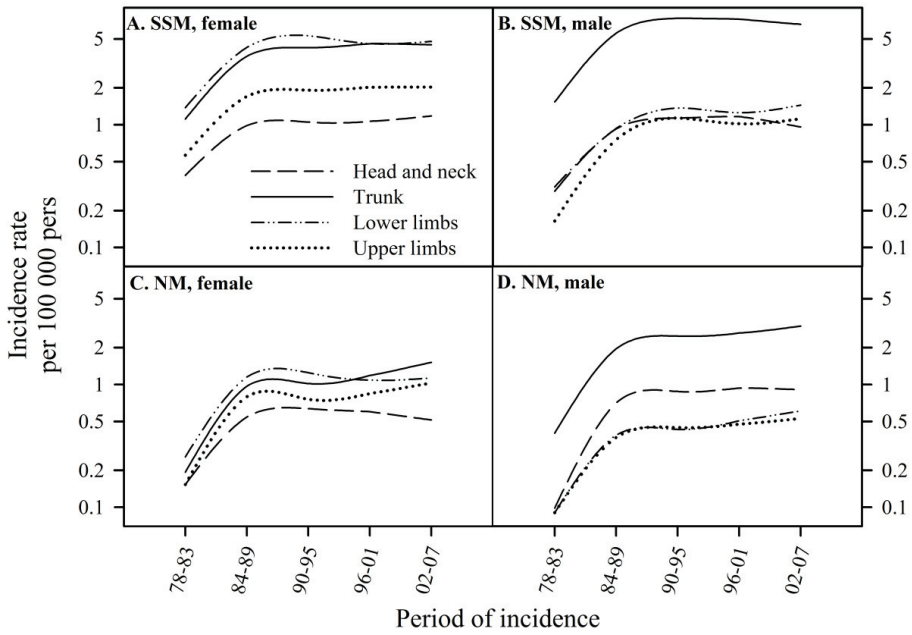

Figure 6.12. Time trends of nodular melanoma and superficial-spreading melanoma by anatomic site and gender.

Incidence rates are crude rates from the Cancer Registry of Norway database. *NM nodular melanoma, SSM superficial-spreading melanoma.*

6.6.3. Latitude gradient

We observed a significant latitude gradient for both SSM and NM when the whole time period is considered (Fig. 6.10). The gradient was larger for SSM than for NM. In the south, SSM was more frequent than NM. In the north, this SSM propensity was largest for women. At the date of publication of this thesis, there were no other population-based studies concerning any latitudinal gradient for different morphological types of melanoma to be found in the literature.

7. CONCLUSIONS

This work was aimed at evaluating the association between solar radiation and melanoma risk in Norway by analyzing several variables: age, gender, anatomic site, morphology and residential region, in a population-based retrospective study.

- CM incidence in Norway is among the highest in the World, with marked increase in all age groups until 1990s. After that time stable rates and even decreasing trends were found for young persons. However, we observed an increasing trend in the last years for females in the north region, seemingly caused by an increase of melanoma on the intermittent sites;
    - Similar time trends were observed for both of the main morphological types, SSM and NM.
    - For some rare types of melanoma, *i.e.* those on shielded sites, the rates have been constant over a long period of time.
    - The gender distribution of CM differs by age: a female tendency in younger age groups and male tendency in older age groups.
- The anatomical distribution in both genders favors intermittently sun-exposed sites, similar patterns were found in the north and south regions. Notably, there is a decreasing trend for trunk melanoma in younger men, but increasing trends for trunk melanoma in older groups, for both genders. Head and neck melanoma has more stable rates. Thus, an intermittent sun exposure seems to be the most important factor for melanoma incidence, in both younger and older age groups, regardless of latitude.

  - When analyzing the anatomical distribution separately for SSM and NM, we did not find a tendency for intermittently exposed sites in the case of female NM.

- CM incidence displays a north-south gradient, with significant higher rates in the south. This supports the assumption that solar radiation is an important factor for melanoma risk.

  - For some rare types of melanoma, those on anatomic sites that are not exposed to the sun, we did not find any latitudinal gradient.

  - When analyzing latitudinal gradients by morphological types, we did not observe any significant gradient in the case of NM, while there is significant one for SSM. In conclusion, solar radiation is correlated more strongly with SSM than with NM.

Overall, our data stress that solar radiation is a risk factor for all anatomic sun-exposed sites, emphasizing the role of intermittent exposures, in particular for young individuals. This is true even in northern regions with low annual fluences of UV radiation. We did find some subtle differences between genders and regions that warrant further investigations. The novelty of our work is that for melanomas on shielded sites, there is a different time course than for melanoma on non-shielded sites and a lack of latitudinal gradient. These are important arguments for a different causal pathway between them, even though they share a common cellular origin.

8. FUTURE PERSPECTIVES

Melanoma is cancer, and, like all other forms of cancers, lowering its impact is best approached by prevention. Melanoma is characterized by high visibility and accessibility; however, as Neville Davis, MD, surgeon form Queensland wrote, melanoma "writes its message in the skin with its own ink and it is there for all of us to see. Some see, but do not comprehend."

It is essential to recognize that sun exposure is one factor than can be modified, which is not always possible for other forms of cancer. Individuals with genetic/phenotypic risk factors should avoid intense sun exposure. However, many of these individuals, especially teenagers, may not pay enough attention to it and may not adopt healthy protective behaviors. This may have significant clinical implications. Health-related behavior is partially influenced by individuals' perception of a variety of factors: personal susceptibility, nature and severity of the disease, available treatments, and causes of the disease. The development of effective healthcare services, particularly for those at increased risk, as well for the entire human population, requires insights into how the body is perceived. This perception should not only refer to the physical aspect, but also to the body as a cultural phenomenon, where values, meanings and social, political and economical conditions are embodied and communicated. We need to better understand how individuals perceive melanoma as a fatal disease, to increase the compliance of the patient, and to improve communication between patients and healthcare professionals.

The increase in incidence of melanoma of the trunk in older male individuals may reflect lifestyles changes that took place around 1940s. Comparison of site-specific sun-exposure histories between them, and those of female population of the same cohort or those of male population from earlier generations, may add valuable information about the relevance of behavioral pattern. Also, analysis of lifestyles in northern regions, compared with southern regions (including dietary differences), in particular for women, may explain some of the

discrepancy in melanoma incidence between these regions. We will continue our work by analyzing and comparing mortality and survival rates in northern *versus* southern regions, by gender, anatomic sites and morphology types, along with more standardized measurements of vitamin D status, in order to assess the impact vitamin D has.

GLOBOCAN project offers valuable estimates about melanoma epidemiology in the world, with NORDCAN database offering information for Nordic European countries. Nevertheless, many countries lack quality cancer registry. It would be of major importance to improve melanoma registration in the future, especially from output and private settings.

Although the link between melanoma and ultraviolet radiation is unequivocal, current evidence shows some ultraviolet-independent carcinogenetic mechanisms as well. These later mechanisms warrant further investigation in order to properly inform the public and help elaborating optimal prevention strategies for susceptible individuals. Future time will prove whether sun is the most important risk factor for certain group risk individuals or certain melanoma subtypes.

REFERENCES

1. Ferlay, J., Shin, H. R, Bray, F., Forman, D., Mathers, C., and Parkin, D. M. GLOBOCAN 2008 v2.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 10 [Internet]. 2010. Lyon, France, International Agency for Research on Cancer. 31-1-2013.
   Ref Type: Serial (Book,Monograph)

2. World Health Report. The Global Burden of Disease, 2004 update. 2008. Geneva, Switzerland, World Health Organisation.
   Ref Type: Report

3. Larsen IK, Grimsrud TK, Haldorsen T *et al*. *Cancer in Norway 2009 - Cancer incidence, mortality, survival and prevalence in Norway*. Oslo: Cancer Registry of Norway, 2011.

4. Pleasance ED, Cheetham RK, Stephens PJ *et al*. A comprehensive catalogue of somatic mutations from a human cancer genome. *Nature.* 2010; **463**: 191-6.

5. Linos E, Swetter SM, Cockburn MG *et al*. Increasing burden of melanoma in the United States. *J.Invest Dermatol.* 2009; **129**: 1666-74.

6. Bolanca Z, Bolanca I, Buljan M *et al*. Trends, habits and attitudes towards suntanning. *Coll.Antropol.* 2008; **32 Suppl 2:143-6.**: 143-6.

7. Mawn VB, Fleischer AB, Jr. A survey of attitudes, beliefs, and behavior regarding tanning bed use, sunbathing, and sunscreen use. *J.Am.Acad.Dermatol.* 1993; **29**: 959-62.

8. Thacher TD, Clarke BL. Vitamin D insufficiency. *Mayo Clin.Proc.* 2011; **86**: 50-60.

9. Baarnhielm M, Hedstrom AK, Kockum I *et al*. Sunlight is associated with decreased multiple sclerosis risk: no interaction with human leukocyte antigen-DRB1*15. *Eur.J.Neurol.* 2012; **19**: 955-62.

10. Skobowiat C, Dowdy JC, Sayre RM *et al*. Cutaneous hypothalamic-pituitary-adrenal axis homolog: regulation by ultraviolet radiation. *Am.J.Physiol Endocrinol.Metab.* 2011; **301**: E484-E493.

11. Feelisch M, Kolb-Bachofen V, Liu D *et al*. Is sunlight good for our heart? *Eur.Heart J.* 2010; **31**: 1041-5.

12. Dore JF, Chignol MC. Tanning salons and skin cancer. *Photochem.Photobiol.Sci.* 2012; **11**: 30-7.

13. Gandini S, Autier P, Boniol M. Reviews on sun exposure and artificial light and melanoma. *Prog.Biophys.Mol.Biol.* 2011; **107**: 362-6.

14. Frederick JE, Lubin D. Possible long-term changes in biologically active ultraviolet radiation reaching the ground. *Photochem.Photobiol.* 1988; **47**: 571-8.

15. Lucas, R, McMichael, T, Smith, W, and Armstrong, B. Solar ultraviolet radiation: Global burden of disease from solar ultraviolet radiation. Prüss-Üstün, A, Zeeb, H, Mathers, C, and Repacholi, M. 2006. **World Health Organization 2006**. Environmental burden of disease.
Ref Type: Report

16. Godar DE. UV doses worldwide. *Photochem.Photobiol.* 2005; **81**: 736-49.

17. Seckmeyer G, Pissulla D, Glandorf M *et al*. Variability of UV irradiance in Europe. *Photochem.Photobiol.* 2008; **84**: 172-9.

18. Diffey BL. Human exposure to ultraviolet radiation. In: *Photodermatology* (Hawk,JLM, ed). London: Arnold, 1999: 5-24.

19. Bruls WA, Slaper H, van der Leun JC *et al*. Transmission of human epidermis and stratum corneum as a function of thickness in the ultraviolet and visible wavelengths. *Photochem.Photobiol.* 1984; **40**: 485-94.

20. CIE. Action spectrum for the production of previtamin D3 in human skin. Technical Report 174. 2006. International Commission on Illumination.
Ref Type: Report

21. MacLaughlin JA, Anderson RR, Holick MF. Spectral character of sunlight modulates photosynthesis of previtamin D3 and its photoisomers in human skin. *Science.* 1982; **216**: 1001-3.

22. Lehmann B, Genehr T, Knuschke P *et al*. UVB-induced conversion of 7-dehydrocholesterol to 1alpha,25-dihydroxyvitamin D3 in an in vitro human skin equivalent model. *J.Invest Dermatol.* 2001; **117**: 1179-85.

23. Anders A, Altheide HJ, Knalmann M *et al*. Action spectrum for erythema in humans investigated with dye lasers. *Photochem.Photobiol.* 1995; **61**: 200-5.

24. Young AR, Chadwick CA, Harrison GI *et al*. The similarity of action spectra for thymine dimers in human epidermis and erythema suggests that DNA is the chromophore for erythema. *J.Invest Dermatol.* 1998; **111**: 982-8.

25. Rivas JM, Ullrich SE. The role of IL-4, IL-10, and TNF-alpha in the immune suppression induced by ultraviolet radiation. *J.Leukoc.Biol.* 1994; **56**: 769-75.

26. Damian DL, Matthews YJ, Phan TA *et al*. An action spectrum for ultraviolet radiation-induced immunosuppression in humans. *Br.J.Dermatol.* 2011; **164**: 657-9.

27. de Gruijl FR, Sterenborg HJ, Forbes PD *et al*. Wavelength dependence of skin cancer induction by ultraviolet irradiation of albino hairless mice. *Cancer Res.* 1993; **53**: 53-60.

28. Ha T, Javedan H, Waterston K *et al*. The relationship between constitutive pigmentation and sensitivity to ultraviolet radiation induced erythema is dose-dependent. *Pigment Cell Res.* 2003; **16**: 477-9.

29. Waterston K, Naysmith L, Rees JL. Physiological variation in the erythemal response to ultraviolet radiation and photoadaptation. *J.Invest Dermatol.* 2004; **123**: 958-64.

30. Armstrong BK, Kricker A. Skin cancer. *Dermatol.Clin.* 1995; **13**: 583-94.

31. Wagner JK, Parra EJ, Norton L *et al*. Skin responses to ultraviolet radiation: effects of constitutive pigmentation, sex, and ancestry. *Pigment Cell Res.* 2002; **15**: 385-90.

32. Olson RL, Sayre RM, Everett MA. Effect of anatomic location and time on ultraviolet erythema. *Arch.Dermatol.* 1966; **93**: 211-5.

33. Bataille V, Bykov VJ, Sasieni P *et al*. Photoadaptation to ultraviolet (UV) radiation in vivo: photoproducts in epidermal cells following UVB therapy for psoriasis. *Br.J.Dermatol.* 2000; **143**: 477-83.

34. de WS, Vink AA, Roza L *et al*. Solar-simulated skin adaptation and its effect on subsequent UV-induced epidermal DNA damage. *J.Invest Dermatol.* 2001; **117**: 678-82.

35. Holick MF. Vitamin D, Sunlight and Cancer Connection. *Anticancer Agents Med.Chem.* 2012.

36. Walker D, Jacobe H. Phototherapy in the age of biologics. *Semin.Cutan.Med.Surg.* 2011; **30**: 190-8.

37. Taylor SL, Kaur M, LoSicco K *et al*. Pilot study of the effect of ultraviolet light on pain and mood in fibromyalgia syndrome. *J.Altern.Complement Med.* 2009; **15**: 15-23.

38. Schwartz HP, Haberman BE, Ruddy RM. Hyperbilirubinemia: current guidelines and emerging therapies. *Pediatr.Emerg.Care.* 2011; **27**: 884-9.

39. Oplander C, Volkmar CM, Paunel-Gorgulu A *et al*. Whole body UVA irradiation lowers systemic blood pressure by release of nitric oxide from intracutaneous photolabile nitric oxide derivates. *Circ.Res.* 2009; **105**: 1031-40.

40. Grant WB. Update on Evidence that Support a Role of Solar Ultraviolet-B Irradiance in Reducing Cancer Risk. *Anticancer Agents Med.Chem.* 2012.

41. Holick MF. Vitamin D: A millenium perspective. *J.Cell Biochem.* 2003; **88**: 296-307.

42. Cicarma E, Tuorkey M, Juzeniene A *et al*. Calcitriol treatment improves methyl aminolaevulinate-based photodynamic therapy in human squamous cell carcinoma A431 cells. *Br.J.Dermatol.* 2009; **161**: 413-8.

43. Cicarma E, Mork C, Porojnicu AC *et al*. Influence of narrowband UVB phototherapy on vitamin D and folate status. *Exp.Dermatol.* 2010; **19**: e67-e72.

44. Zehnder D, Bland R, Williams MC *et al*. Extrarenal expression of 25-hydroxyvitamin d(3)-1 alpha-hydroxylase. *J.Clin.Endocrinol.Metab.* 2001; **86**: 888-94.

45. Lehmann B, Meurer M. Extrarenal sites of calcitriol synthesis: the particular role of the skin. *Recent Results Cancer Res.* 2003; **164:135-45.**: 135-45.

46. Grant WB, Mohr SB. Ecological studies of ultraviolet B, vitamin D and cancer since 2000. *Ann.Epidemiol.* 2009; **19**: 446-54.

47. Krishnan AV, Feldman D. Mechanisms of the anti-cancer and anti-inflammatory actions of vitamin D. *Annu.Rev.Pharmacol.Toxicol.* 2011; **51:311-36. doi: 10.1146/annurev-pharmtox-010510-100611.**: 311-36.

48. Fleet JC, DeSmet M, Johnson R *et al*. Vitamin D and cancer: a review of molecular mechanisms. *Biochem.J.* 2012; **441**: 61-76.

49. Krishnan AV, Feldman D. Molecular pathways mediating the anti-inflammatory effects of calcitriol: implications for prostate cancer chemoprevention and treatment. *Endocr.Relat Cancer.* 2010; **17**: R19-R38.

50. Ben-Shoshan M, Amir S, Dang DT *et al*. 1alpha,25-dihydroxyvitamin D3 (Calcitriol) inhibits hypoxia-inducible factor-1/vascular endothelial growth factor pathway in human cancer cells. *Mol.Cancer Ther.* 2007; **6**: 1433-9.

51. Bao BY, Yao J, Lee YF. 1alpha, 25-dihydroxyvitamin D3 suppresses interleukin-8-mediated prostate cancer cell angiogenesis. *Carcinogenesis.* 2006; **27**: 1883-93.

52. Akutsu N, Lin R, Bastien Y *et al*. Regulation of gene Expression by 1alpha,25-dihydroxyvitamin D3 and Its analog EB1089 under growth-inhibitory conditions in squamous carcinoma Cells. *Mol.Endocrinol.* 2001; **15**: 1127-39.

53. Swami S, Raghavachari N, Muller UR *et al*. Vitamin D growth inhibition of breast cancer cells: gene expression patterns assessed by cDNA microarray. *Breast Cancer Res.Treat.* 2003; **80**: 49-62.

54. Cantorna MT, Zhu Y, Froicu M *et al*. Vitamin D status, 1,25-dihydroxyvitamin D3, and the immune system. *Am.J.Clin.Nutr.* 2004; **80**: 1717S-20S.

55. Ullrich SE. Mechanisms underlying UV-induced immune suppression. *Mutat.Res.* 2005; **571**: 185-205.

56. Kripke ML. Skin cancer, photoimmunology, and urocanic acid. *Photodermatol.* 1984; **1**: 161-3.

57. Kelly DA, Young AR, McGregor JM *et al*. Sensitivity to sunburn is associated with susceptibility to ultraviolet radiation-induced suppression of cutaneous cell-mediated immunity. *J.Exp.Med.* 2000; **191**: 561-6.

58. You YH, Pfeifer GP. Similarities in sunlight-induced mutational spectra of CpG-methylated transgenes and the p53 gene in skin cancer point to an important role of 5-methylcytosine residues in solar UV mutagenesis. *J.Mol.Biol.* 2001; **%19;305**: 389-99.

59. Kappes UP, Luo D, Potter M *et al*. Short- and long-wave UV light (UVB and UVA) induce similar mutations in human skin cells. *J.Invest Dermatol.* 2006; **126**: 667-75.

60. Douki T, Cadet J. Individual determination of the yield of the main UV-induced dimeric pyrimidine photoproducts in DNA suggests a high mutagenicity of CC photolesions. *Biochemistry.* 2001; **40**: 2495-501.

61. Sutherland JC, Griffin KP. Absorption spectrum of DNA for wavelengths greater than 300 nm. *Radiat.Res.* 1981; **86**: 399-409.

62. Runger TM, Kappes UP. Mechanisms of mutation formation with long-wave ultraviolet light (UVA). *Photodermatol.Photoimmunol.Photomed.* 2008; **24**: 2-10.

63. Tewari A, Sarkany RP, Young AR. UVA1 induces cyclobutane pyrimidine dimers but not 6-4 photoproducts in human skin in vivo. *J.Invest Dermatol.* 2012; **132**: 394-400.

64. Pfeifer GP, You YH, Besaratinia A. Mutations induced by ultraviolet light. *Mutat.Res.* 2005; **571**: 19-31.

65. Douki T, Reynaud-Angelin A, Cadet J *et al*. Bipyrimidine photoproducts rather than oxidative lesions are the main type of DNA damage involved in the genotoxic effect of solar UVA radiation. *Biochemistry.* 2003; **42**: 9221-6.

66. Xu G, Snellman E, Jansen CT *et al*. Levels and repair of cyclobutane pyrimidine dimers and 6-4 photoproducts in skin of sporadic basal cell carcinoma patients. *J.Invest Dermatol.* 2000; **115**: 95-9.

67. Runger TM, Farahvash B, Hatvani Z *et al*. Comparison of DNA damage responses following equimutagenic doses of UVA and UVB: a less effective cell cycle arrest with UVA may render UVA-induced pyrimidine dimers more mutagenic than UVB-induced ones. *Photochem.Photobiol.Sci.* 2012; **11**: 207-15.

68. Ravanat JL, Douki T, Cadet J. Direct and indirect effects of UV radiation on DNA and its components. *J.Photochem.Photobiol.B.* 2001; **63**: 88-102.

69. Shibutani S, Takeshita M, Grollman AP. Insertion of specific bases during DNA synthesis past the oxidation-damaged base 8-oxodG. *Nature.* 1991; **349**: 431-4.

70. Cheng KC, Cahill DS, Kasai H *et al*. 8-Hydroxyguanine, an abundant form of oxidative DNA damage, causes G----T and A----C substitutions. *J.Biol.Chem.* 1992; **267**: 166-72.

71. Mouret S, Baudouin C, Charveron M *et al*. Cyclobutane pyrimidine dimers are predominant DNA lesions in whole human skin exposed to UVA radiation. *Proc.Natl.Acad.Sci.U.S.A.* 2006; **103**: 13765-70.

72. ya-Grosjean L, Dumaz N, Sarasin A. The specificity of p53 mutation spectra in sunlight induced human cancers. *J.Photochem.Photobiol.B.* 1995; **28**: 115-24.

73. Mitchell DL, Nairn RS. The biology of the (6-4) photoproduct. *Photochem.Photobiol.* 1989; **49**: 805-19.

74. Besaratinia A, Kim SI, Pfeifer GP. Rapid repair of UVA-induced oxidized purines and persistence of UVB-induced dipyrimidine lesions determine the mutagenicity of sunlight in mouse cells. *FASEB J.* 2008; **22**: 2379-92.

75. Tolleson WH. Human melanocyte biology, toxicology, and pathology. *J.Environ.Sci.Health C.Environ.Carcinog.Ecotoxicol.Rev.* 2005; **23**: 105-61.

76. Montagna W, Carlisle K. The architecture of black and white facial skin. *J.Am.Acad.Dermatol.* 1991; **24**: 929-37.

77. Millington GW. Genomic imprinting and dermatological disease. *Clin.Exp.Dermatol.* 2006; **31**: 681-8.

78. Suzuki I, Tada A, Ollmann MM *et al*. Agouti signaling protein inhibits melanogenesis and the response of human melanocytes to alpha-melanotropin. *J.Invest Dermatol.* 1997; **108**: 838-42.

79. Valverde P, Healy E, Jackson I *et al*. Variants of the melanocyte-stimulating hormone receptor gene are associated with red hair and fair skin in humans. *Nat.Genet.* 1995; **11**: 328-30.

80. Yamaguchi Y, Brenner M, Hearing VJ. The regulation of skin pigmentation. *J.Biol.Chem.* 2007; **282**: 27557-61.

81. Tolleson WH. Human melanocyte biology, toxicology, and pathology. *J.Environ.Sci.Health C.Environ.Carcinog.Ecotoxicol.Rev.* 2005; **23**: 105-61.

82. Bok D. The retinal pigment epithelium: a versatile partner in vision. *J.Cell Sci.Suppl.* 1993; **17:189-95.**: 189-95.

83. Hu DN, Simon JD, Sarna T. Role of ocular melanin in ophthalmic physiology and pathology. *Photochem.Photobiol.* 2008; **84**: 639-44.

84. Tachibana M. Sound needs sound melanocytes to be heard. *Pigment Cell Res.* 1999; **12**: 344-54.

85. Takeda K, Takahashi NH, Shibahara S. Neuroendocrine functions of melanocytes: beyond the skin-deep melanin maker. *Tohoku J.Exp.Med.* 2007; **211**: 201-21.

86. Goldgeier MH, Klein LE, Klein-Angerer S *et al*. The distribution of melanocytes in the leptomeninges of the human brain. *J.Invest Dermatol.* 1984; **82**: 235-8.

87. Zecca L, Bellei C, Costi P *et al*. New melanic pigments in the human brain that accumulate in aging and block environmental toxic metals. *Proc.Natl.Acad.Sci.U.S.A.* 2008; **105**: 17567-72.

88. Sulzer D, Bogulavsky J, Larsen KE *et al*. Neuromelanin biosynthesis is driven by excess cytosolic catecholamines not accumulated by synaptic vesicles. *Proc.Natl.Acad.Sci.U.S.A.* 2000; **97**: 11869-74.

89. Brito FC, Kos L. Timeline and distribution of melanocyte precursors in the mouse heart. *Pigment Cell Melanoma Res.* 2008; **21**: 464-70.

90. Yajima I, Larue L. The location of heart melanocytes is specified and the level of pigmentation in the heart may correlate with coat color. *Pigment Cell Melanoma Res.* 2008; **21**: 471-6.

91. Randhawa M, Huff T, Valencia JC *et al*. Evidence for the ectopic synthesis of melanin in human adipose tissue. *FASEB J.* 2009; **23**: 835-43.

92. Ferrans VJ, Yu ZX, Nelson WK *et al*. Lymphangioleiomyomatosis (LAM): a review of clinical and morphological features. *J.Nippon Med.Sch.* 2000; **67**: 311-29.

93. Chu D, Haake A, Holbrook K *et al*. The Structure and Development of the Skin. In: *Fitzpatrick's Dermatology in General Medicine* (Freedberg,IM, Eisen,AZ, Wolff,K et al, eds), 6 edn. New-York: McGraw-Hill, 2003: 12.

94. Haass NK, Smalley KS, Li L *et al*. Adhesion, migration and communication in melanocytes and melanoma. *Pigment Cell Res.* 2005; **18**: 150-9.

95. Nordlund JJ. The melanocyte and the epidermal melanin unit: an expanded concept. *Dermatol.Clin.* 2007; **25**: 271-81, vii.

96. Buffey JA, Messenger AG, Taylor M *et al*. Extracellular matrix derived from hair and skin fibroblasts stimulates human skin melanocyte tyrosinase activity. *Br.J.Dermatol.* 1994; **131**: 836-42.

97. Hara M, Toyoda M, Yaar M *et al*. Innervation of melanocytes in human skin. *J.Exp.Med.* 1996; **184**: 1385-95.

98. Kim EJ, Park HY, Yaar M *et al*. Modulation of vascular endothelial growth factor receptors in melanocytes. *Exp.Dermatol.* 2005; **14**: 625-33.

99. Vredeveld LC, Possik PA, Smit MA *et al*. Abrogation of BRAFV600E-induced senescence by PI3K pathway activation contributes to melanomagenesis. *Genes Dev.* 2012; **26**: 1055-69.

100. Bennett DC. Human melanocyte senescence and melanoma susceptibility genes. *Oncogene.* 2003; **%19;22**: 3063-9.

101. Carli P, Massi D, Santucci M *et al*. Cutaneous melanoma histologically associated with a nevus and melanoma de novo have a different profile of risk: results from a case-control study. *J.Am.Acad.Dermatol.* 1999; **40**: 549-57.

102. Bevona C, Goggins W, Quinn T *et al*. Cutaneous melanomas associated with nevi. *Arch.Dermatol.* 2003; **139**: 1620-4.

103. Segura S, Pellacani G, Puig S *et al*. In vivo microscopic features of nodular melanomas: dermoscopy, confocal microscopy, and histopathologic correlates. *Arch.Dermatol.* 2008; **144**: 1311-20.

104. Hoerter JD, Bradley P, Casillas A *et al*. Extrafollicular dermal melanocyte stem cells and melanoma. *Stem Cells Int.* 2012; **2012:407079. doi: 10.1155/2012/407079. Epub;%2012 May 10.**: 407079.

105. Zalaudek I, Marghoob AA, Scope A *et al*. Three roots of melanoma. *Arch.Dermatol.* 2008; **144**: 1375-9.

106. Albino AP, Le SR, Oliff AI *et al*. Transforming ras genes from human melanoma: a manifestation of tumour heterogeneity? *Nature.* 1984; **308**: 69-72.

107. Hocker T, Tsao H. Ultraviolet radiation and melanoma: a systematic review and analysis of reported sequence variants. *Hum.Mutat.* 2007; **28**: 578-88.

108. van EA, Zerp SF, van der FS *et al*. Relevance of ultraviolet-induced N-ras oncogene point mutations in development of primary human cutaneous melanoma. *Am.J.Pathol.* 1996; **149**: 883-93.

109. Jiveskog S, Ragnarsson-Olding B, Platz A *et al*. N-ras mutations are common in melanomas from sun-exposed skin of humans but rare in mucosal membranes or unexposed skin. *J.Invest Dermatol.* 1998; **111**: 757-61.

110. Davies H, Bignell GR, Cox C *et al*. Mutations of the BRAF gene in human cancer. *Nature.* 2002; **417**: 949-54.

111. Platz A, Egyhazi S, Ringborg U *et al*. Human cutaneous melanoma; a review of NRAS and BRAF mutation frequencies in relation to histogenetic subclass and body site. *Mol.Oncol.* 2008; **1**: 395-405.

112. Curtin JA, Fridlyand J, Kageshita T *et al*. Distinct sets of genetic alterations in melanoma. *N.Engl.J.Med.* 2005; **353**: 2135-47.

113. Soufir N, Avril MF, Chompret A *et al*. Prevalence of p16 and CDK4 germline mutations in 48 melanoma-prone families in France. The French Familial Melanoma Study Group. *Hum.Mol.Genet.* 1998; **7**: 209-16.

114. Goldstein AM, Chan M, Harland M *et al*. High-risk melanoma susceptibility genes and pancreatic cancer, neural system tumors, and uveal melanoma across GenoMEL. *Cancer Res.* 2006; **66**: 9818-28.

115. Bartkova J, Lukas J, Guldberg P *et al*. The p16-cyclin D/Cdk4-pRb pathway as a functional unit frequently altered in melanoma pathogenesis. *Cancer Res.* 1996; **56**: 5475-83.

116. Walker GJ, Flores JF, Glendening JM *et al*. Virtually 100% of melanoma cell lines harbor alterations at the DNA level within CDKN2A, CDKN2B, or one of their downstream targets. *Genes Chromosomes.Cancer.* 1998; **22**: 157-63.

117. Greenblatt MS, Bennett WP, Hollstein M *et al*. Mutations in the p53 tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. *Cancer Res.* 1994; **54**: 4855-78.

118. Kress S, Sutter C, Strickland PT *et al*. Carcinogen-specific mutational pattern in the p53 gene in ultraviolet B radiation-induced squamous cell carcinomas of mouse skin. *Cancer Res.* 1992; **52**: 6400-3.

119. Dumaz N, van Kranen HJ, de VA *et al*. The role of UV-B light in skin carcinogenesis through the analysis of p53 mutations in squamous cell carcinomas of hairless mice. *Carcinogenesis.* 1997; **18**: 897-904.

120. Ziegler A, Leffell DJ, Kunala S *et al*. Mutation hotspots due to sunlight in the p53 gene of nonmelanoma skin cancers. *Proc.Natl.Acad.Sci.U.S.A.* 1993; **90**: 4216-20.

121. Soengas MS, Lowe SW. Apoptosis and melanoma chemoresistance. *Oncogene.* 2003; **%19;22**: 3138-51.

122. Maldonado JL, Fridlyand J, Patel H *et al*. Determinants of BRAF mutations in primary melanomas. *J.Natl.Cancer Inst.* 2003; **95**: 1878-90.

123. Straume O, Akslen LA. Alterations and prognostic significance of p16 and p53 protein expression in subgroups of cutaneous melanoma. *Int.J.Cancer.* 1997; **74**: 535-9.

124. Whiteman DC, Parsons PG, Green AC. p53 expression and risk factors for cutaneous melanoma: a case-control study. *Int.J.Cancer.* 1998; **77**: 843-8.

125. Ragnarsson-Olding BK, Karsberg S, Platz A *et al*. Mutations in the TP53 gene in human malignant melanomas derived from sun-exposed skin and unexposed mucosal membranes. *Melanoma Res.* 2002; **12**: 453-63.

126. Harbour JW. Update in uveal melanoma. *Clin.Adv.Hematol.Oncol.* 2012; **10**: 459-61.

127. Elwood JM. Melanoma and sun exposure. *Semin.Oncol.* 1996; **23**: 650-66.

128. Langley RG, Sober AJ. A clinical review of the evidence for the role of ultraviolet radiation in the etiology of cutaneous melanoma. *Cancer Invest.* 1997; **15**: 561-7.

129. Mack TM, Floderus B. Malignant melanoma risk by nativity, place of residence at diagnosis, and age at migration. *Cancer Causes Control.* 1991; **2**: 401-11.

130. Cormier JN, Xing Y, Ding M *et al*. Ethnic differences among patients with cutaneous melanoma. *Arch.Intern.Med.* 2006; **166**: 1907-14.

131. Cress RD, Holly EA. Incidence of cutaneous melanoma among non-Hispanic whites, Hispanics, Asians, and blacks: an analysis of california cancer registry data, 1988-93. *Cancer Causes Control.* 1997; **8**: 246-52.

132. Holman CD, Armstrong BK. Pigmentary traits, ethnic origin, benign nevi, and family history as risk factors for cutaneous malignant melanoma. *J.Natl.Cancer Inst.* 1984; **72**: 257-66.

133. Elwood JM, Gallagher RP, Hill GB *et al*. Pigmentation and skin reaction to sun as risk factors for cutaneous melanoma: Western Canada Melanoma Study. *Br.Med.J.(Clin.Res.Ed).* 1984; **288**: 99-102.

134. Gandini S, Sera F, Cattaruzza MS *et al*. Meta-analysis of risk factors for cutaneous melanoma: III. Family history, actinic damage and phenotypic factors. *Eur.J.Cancer.* 2005; **41**: 2040-59.

135. Chang YM, Newton-Bishop JA, Bishop DT *et al*. A pooled analysis of melanocytic nevus phenotype and the risk of cutaneous melanoma at different latitudes. *Int.J.Cancer.* 2009; **124**: 420-8.

136. Rimpela AH, Pukkala EI. Cancers of affluence: positive social class gradient and rising incidence trend in some cancer forms. *Soc.Sci.Med.* 1987; **24**: 601-6.

137. Kubica AW, Brewer JD. Melanoma in immunosuppressed patients. *Mayo Clin.Proc.* 2012; **87**: 991-1003.

138. Hocker TL, Singh MK, Tsao H. Melanoma genetics and therapeutic approaches in the 21st century: moving from the benchside to the bedside. *J.Invest Dermatol.* 2008; **128**: 2575-95.

139. Wang HT, Choi B, Tang MS. Melanocytes are deficient in repair of oxidative DNA damage and UV-induced photoproducts. *Proc.Natl.Acad.Sci.U.S.A.* 2010; **107**: 12180-5.

140. Walker G. Cutaneous melanoma: how does ultraviolet light contribute to melanocyte transformation? *Future.Oncol.* 2008; **4**: 841-56.

141. Green A. A theory of site distribution of melanomas: Queensland, Australia. *Cancer Causes Control.* 1992; **3**: 513-6.

142. Hacker E, Muller K, Whiteman DC *et al*. Reduced expression of IL-18 is a marker of ultraviolet radiation-induced melanomas. *Int.J.Cancer.* 2008; **123**: 227-31.

143. Fernandez AA, Paniker L, Garcia R *et al*. Recent advances in sunlight-induced carcinogenesis using the Xiphophorus melanoma model. *Comp Biochem.Physiol C.Toxicol.Pharmacol.* 2012; **155**: 64-70.

144. Berger MF, Hodis E, Heffernan TP *et al*. Melanoma genome sequencing reveals frequent PREX2 mutations. *Nature.* 2012; **485**: 502-6.

145. De Fabo EC, Noonan FP, Fears T *et al*. Ultraviolet B but not ultraviolet A radiation initiates melanoma. *Cancer Res.* 2004; **64**: 6372-6.

146. Mitchell D, Fernandez A. The photobiology of melanocytes modulates the impact of UVA on sunlight-induced melanoma. *Photochem.Photobiol.Sci.* 2012; **11**: 69-73.

147. Noonan FP, Zaidi MR, Wolnicka-Glubisz A *et al*. Melanoma induction by ultraviolet A but not ultraviolet B radiation requires melanin pigment. *Nat.Commun.* 2012; **3:884. doi: 10.1038/ncomms1893.**: 884.

148. Agar NS, Halliday GM, Barnetson RS *et al*. The basal layer in human squamous tumors harbors more UVA than UVB fingerprint mutations: a role for UVA in human skin carcinogenesis. *Proc.Natl.Acad.Sci.U.S.A.* 2004; **101**: 4954-9.

149. Javeri A, Huang XX, Bernerd F *et al*. Human 8-oxoguanine-DNA glycosylase 1 protein and gene are expressed more abundantly in the superficial than basal layer of human epidermis. *DNA Repair (Amst).* 2008; **7**: 1542-50.

150. de Gruijl FR. Photocarcinogenesis: UVA vs UVB. *Methods Enzymol.* 2000; **319:359-66.**: 359-66.

151. Garland CF, Garland FC, Gorham ED. Epidemiologic evidence for different roles of ultraviolet A and B radiation in melanoma mortality rates. *Ann.Epidemiol.* 2003; **13**: 395-404.

152. Decraene D, Smaers K, Maes D *et al*. A low UVB dose, with the potential to trigger a protective p53-dependent gene program, increases the resilience of keratinocytes against future UVB insults. *J.Invest Dermatol.* 2005; **125**: 1026-31.

153. Chazal M, Marionnet C, Michel L *et al*. P16(INK4A) is implicated in both the immediate and adaptative response of human keratinocytes to UVB irradiation. *Oncogene.* 2002; **21**: 2652-61.

154. Gallagher RP, Spinelli JJ, Lee TK. Tanning beds, sunlamps, and risk of cutaneous malignant melanoma. *Cancer Epidemiol.Biomarkers Prev.* 2005; **14**: 562-6.

155. Veierod MB, Weiderpass E, Thorn M *et al*. A prospective study of pigmentation, sun exposure, and risk of cutaneous malignant melanoma in women. *J.Natl.Cancer Inst.* 2003; **95**: 1530-8.

156. Cicarma E, Porojnicu AC, Lagunova Z *et al*. Sun and sun beds: inducers of vitamin D and skin cancer. *Anticancer Res.* 2009; **29**: 3495-500.

157. Higgins EM, Du Vivier AW. Possible induction of malignant melanoma by sunbed use. *Clin.Exp.Dermatol.* 1992; **17**: 357-9.

158. Autier P, Dore JF, Schifflers E *et al*. Melanoma and use of sunscreens: an Eortc case-control study in Germany, Belgium and France. The EORTC Melanoma Cooperative Group. *Int.J.Cancer.* 1995; **61**: 749-55.

159. Setlow RB, Grist E, Thompson K *et al*. Wavelengths effective in induction of malignant melanoma. *Proc.Natl.Acad.Sci.U.S.A.* 1993; **90**: 6666-70.

160. Mitchell DL, Fernandez AA, Nairn RS *et al*. Ultraviolet A does not induce melanomas in a Xiphophorus hybrid fish model. *Proc.Natl.Acad.Sci.U.S.A.* 2010; **107**: 9329-34.

161. Ley RD. Ultraviolet radiation A-induced precursors of cutaneous melanoma in Monodelphis domestica. *Cancer Res.* 1997; **57**: 3682-4.

162. de Gruijl FR, Sterenborg HJ, Forbes PD *et al*. Wavelength dependence of skin cancer induction by ultraviolet irradiation of albino hairless mice. *Cancer Res.* 1993; **53**: 53-60.

163. Berwick M, Armstrong BK, Ben-Porat L *et al*. Sun exposure and mortality from melanoma. *J.Natl.Cancer Inst.* 2005; **97**: 195-9.

164. Kligman LH, Sayre RM. An action spectrum for ultraviolet induced elastosis in hairless mice: quantification of elastosis by image analysis. *Photochem.Photobiol.* 1991; **53**: 237-42.

165. Clark WH, Jr., From L, Bernardino EA *et al*. The histogenesis and biologic behavior of primary human malignant melanomas of the skin. *Cancer Res.* 1969; **29**: 705-27.

166. Koh HK, Michalik E, Sober AJ *et al*. Lentigo maligna melanoma has no better prognosis than other types of melanoma. *J.Clin.Oncol.* 1984; **2**: 994-1001.

167. Greenwald HS, Friedman EB, Osman I. Superficial spreading and nodular melanoma are distinct biological entities: a challenge to the linear progression model. *Melanoma Res.* 2012; **22**: 1-8.

168. Lipsker D. Growth rate, early detection, and prevention of melanoma: melanoma epidemiology revisited and future challenges. *Arch.Dermatol.* 2006; **142**: 1638-40.

169. Liu W, Dowling JP, Murray WK *et al*. Rate of growth in melanomas: characteristics and associations of rapidly growing melanomas. *Arch.Dermatol.* 2006; **142**: 1551-8.

170. Garbe C, Leiter U. Melanoma epidemiology and trends. *Clin.Dermatol.* 2009; **27**: 3-9.

171. Mansson-Brahme E, Johansson H, Larsson O *et al*. Trends in incidence of cutaneous malignant melanoma in a Swedish population 1976-1994. *Acta Oncol.* 2002; **41**: 138-46.

172. Osterlind A. Malignant melanoma in Denmark. Occurrence and risk factors. *Acta Oncol.* 1990; **29**: 833-54.

173. Balzi D, Carli P, Giannotti B *et al*. Cutaneous melanoma in the Florentine area, Italy: incidence, survival and mortality between 1985 and 1994. *Eur.J.Cancer Prev.* 2003; **12**: 43-8.

174. Ocana-Riola R, Martinez-Garcia C, Serrano S *et al*. Population-based study of cutaneous malignant melanoma in the Granada province (Spain), 1985-1992. *Eur.J.Epidemiol.* 2001; **17**: 169-74.

175. Forsea AM, Del M, V, de VE *et al*. Melanoma incidence and mortality in Europe: new estimates, persistent disparities. *Br.J.Dermatol.* 2012; **167**: 1124-30.

176. Jablonski NG, Chaplin G. The evolution of human skin coloration. *J.Hum.Evol.* 2000; **39**: 57-106.

177. International Agency for Research on Cancer. *IARC monographs on the evaluation of carcinogenic risks to humans*. (55). 1992. Lyon, *IARC Press*. *Solar and ultraviolet radiation*. Ref Type: Serial (Book,Monograph)

178. Gandini S, Sera F, Cattaruzza MS *et al*. Meta-analysis of risk factors for cutaneous melanoma: II. Sun exposure. *Eur.J.Cancer.* 2005; **41**: 45-60.

179. Bulliard JL, Cox B, Semenciw R. Trends by anatomic site in the incidence of cutaneous malignant melanoma in Canada, 1969-93. *Cancer Causes Control.* 1999; **10**: 407-16.

180. Chen YT, Zheng T, Holford TR *et al*. Malignant melanoma incidence in Connecticut (United States): time trends and age-period-cohort modeling by anatomic site. *Cancer Causes Control.* 1994; **5**: 341-50.

181. Dal H, Boldemann C, Lindelof B. Does relative melanoma distribution by body site 1960-2004 reflect changes in intermittent exposure and intentional tanning in the Swedish population? *Eur.J.Dermatol.* 2007; **17**: 428-34.

182. Westerdahl J, Olsson H, Ingvar C *et al*. Southern travelling habits with special reference to tumour site in Swedish melanoma patients. *Anticancer Res.* 1992; **12**: 1539-42.

183. Lazovich D, Forster J. Indoor tanning by adolescents: prevalence, practices and policies. *Eur.J.Cancer.* 2005; **41**: 20-7.

184. Robinson JK, Rigel DS, Amonette RA. Trends in sun exposure knowledge, attitudes, and behaviors: 1986 to 1996. *J.Am.Acad.Dermatol.* 1997; **37**: 179-86.

185. Tierney P, Ferguson J, Ibbotson S *et al*. Nine out of 10 sunbeds in England emit ultraviolet radiation levels that exceed current safety limits. *Br.J.Dermatol.* 2013; 10.

186. Autier P, Boniol M, Dore JF. Sunscreen use and increased duration of intentional sun exposure: still a burning issue. *Int.J.Cancer.* 2007; **121**: 1-5.

187. Swerlick RA, Chen S. The melanoma epidemic. Is increased surveillance the solution or the problem? *Arch.Dermatol.* 1996; **132**: 881-4.

188. Welch HG, Woloshin S, Schwartz LM. Skin biopsy rates and incidence of melanoma: population based ecological study. *BMJ.* 2005; **331**: 481.

189. Weyers W. The 'epidemic' of melanoma between under- and overdiagnosis. *J.Cutan.Pathol.* 2012; **39**: 9-16.

190. Glusac EJ. The melanoma 'epidemic', a dermatopathologist's perspective. *J.Cutan.Pathol.* 2011; **38**: 264-7.

191. Moriwaki S, Ray S, Tarone RE *et al*. The effect of donor age on the processing of UV-damaged DNA by cultured human cells: reduced DNA repair capacity and increased DNA mutability. *Mutat.Res.* 1996; **364**: 117-23.

192. Bosetti C, La VC, Naldi L *et al*. Mortality from cutaneous malignant melanoma in Europe. Has the epidemic levelled off? *Melanoma Res.* 2004; **14**: 301-9.

193. Crocetti E, Carli P. Changes from mid-1980s to late 1990s among clinical and demographic correlates of melanoma thickness. *Eur.J.Dermatol.* 2003; **13**: 72-5.

194. Garbe C, McLeod GR, Buettner PG. Time trends of cutaneous melanoma in Queensland, Australia and Central Europe. *Cancer.* 2000; **89**: 1269-78.

195. Grafton WD. Regressing malignant melanoma. *J.La State Med.Soc.* 1994; **146**: 535-9.

196. Saleh D, Peach AH. Ultra-late recurrence of malignant melanoma after 40 years of quiescent disease. *J.Surg.Oncol.* 2011.

197. Marrett LD, Nguyen HL, Armstrong BK. Trends in the incidence of cutaneous malignant melanoma in New South Wales, 1983-1996. *Int.J.Cancer.* 2001; **92**: 457-62.

198. Joosse A, Collette S, Suciu S *et al*. Superior outcome of women with stage I/II cutaneous melanoma: pooled analysis of four European Organisation for Research and Treatment of Cancer phase III trials. *J.Clin.Oncol.* 2012; **%20;30**: 2240-7.

199. Johnson TM, Dolan OM, Hamilton TA *et al*. Clinical and histologic trends of melanoma. *J.Am.Acad.Dermatol.* 1998; **38**: 681-6.

200. Elwood JM, Gallagher RP. Body site distribution of cutaneous malignant melanoma in relationship to patterns of sun exposure. *Int.J.Cancer.* 1998; **78**: 276-80.

201. Whiteman DC, Bray CA, Siskind V *et al*. A comparison of the anatomic distribution of cutaneous melanoma in two populations with different levels of sunlight: the west of Scotland and Queensland, Australia 1982-2001. *Cancer Causes Control.* 2007; **18**: 485-91.

202. Bulliard JL, Cox B. Cutaneous malignant melanoma in New Zealand: trends by anatomical site, 1969-1993. *Int.J.Epidemiol.* 2000; **29**: 416-23.

203. Perez-Gomez B, Aragones N, Gustavsson P *et al*. Do sex and site matter? Different age distribution in melanoma of the trunk among Swedish men and women. *Br.J.Dermatol.* 2008; **158**: 766-72.

204. Bulliard JL, De WD, Fisch T *et al*. Detailed site distribution of melanoma and sunlight exposure: aetiological patterns from a Swiss series. *Ann.Oncol.* 2007; **18**: 789-94.

205. Green A, MacLennan R, Youl P *et al*. Site distribution of cutaneous melanoma in Queensland. *Int.J.Cancer.* 1993; **53**: 232-6.

206. Whiteman DC, Watt P, Purdie DM *et al*. Melanocytic nevi, solar keratoses, and divergent pathways to cutaneous melanoma. *J.Natl.Cancer Inst.* 2003; **95**: 806-12.

207. Siskind V, Whiteman DC, Aitken JF *et al*. An analysis of risk factors for cutaneous melanoma by anatomical site (Australia). *Cancer Causes Control.* 2005; **16**: 193-9.

208. Green AC, Siskind V. Risk factors for limb melanomas compared with trunk melanomas in Queensland. *Melanoma Res.* 2012; **22**: 86-91.

209. Anderson WF, Pfeiffer RM, Tucker MA *et al*. Divergent cancer pathways for early-onset and late-onset cutaneous malignant melanoma. *Cancer.* 2009; **115**: 4176-85.

210. Newell GR, Sider JG, Bergfelt L *et al*. Incidence of cutaneous melanoma in the United States by histology with special reference to the face. *Cancer Res.* 1988; **48**: 5036-41.

211. Cox NH, Aitchison TC, Sirel JM *et al*. Comparison between lentigo maligna melanoma and other histogenetic types of malignant melanoma of the head and neck. Scottish Melanoma Group. *Br.J.Cancer.* 1996; **73**: 940-4.

212. Garbe C, Orfanos CE. Epidemiology of malignant melanoma in central Europe: risk factors and prognostic predictors. Results of the Central Malignant Melanoma Registry of the German Dermatological Society. *Pigment Cell Res.* 1992; **Suppl 2:285-94.**: 285-94.

213. Lasithiotakis KG, Leiter U, Gorkievicz R *et al*. The incidence and mortality of cutaneous melanoma in Southern Germany: trends by anatomic site and pathologic characteristics, 1976 to 2003. *Cancer.* 2006; **107**: 1331-9.

214. Levi F, Te VC, Randimbison L *et al*. Trends in incidence of various morphologies of malignant melanoma in Vaud and Neuchatel, Switzerland. *Melanoma Res.* 2005; **15**: 73-5.

215. Youl PH, Youlden DR, Baade PD. Changes in the site distribution of common melanoma subtypes in Queensland, Australia over time: implications for public health campaigns. *Br.J.Dermatol.* 2012; 10-2133.

216. Lipsker DM, Hedelin G, Heid E *et al*. Striking increase of thin melanomas contrasts with stable incidence of thick melanomas. *Arch.Dermatol.* 1999; **135**: 1451-6.

217. Shaikh WR, Xiong M, Weinstock MA. The contribution of nodular subtype to melanoma mortality in the United States, 1978 to 2007. *Arch.Dermatol.* 2012; **148**: 30-6.

218. Crombie IK. Variation of melanoma incidence with latitude in North America and Europe. *Br.J.Cancer.* 1979; **40**: 774-81.

219. Moan J, Porojnicu AC, Dahlback A. Ultraviolet radiation and malignant melanoma. *Adv.Exp.Med.Biol.* 2008; **624:104-16. doi: 10.1007/978-0-387-77574-6_9.**: 104-16.

220. Boniol M, Armstrong BK, Dore JF. Variation in incidence and fatality of melanoma by season of diagnosis in new South Wales, Australia. *Cancer Epidemiol.Biomarkers Prev.* 2006; **15**: 524-6.

221. Newton-Bishop JA, Chang YM, Elliott F *et al*. Relationship between sun exposure and melanoma risk for tumours in different body sites in a large case-control study in a temperate climate. *Eur.J.Cancer.* 2011; **47**: 732-41.

222. Osborne JE, Hutchinson PE. Vitamin D and systemic cancer: is this relevant to malignant melanoma? *Br.J.Dermatol.* 2002; **147**: 197-213.

223. Field S, Newton-Bishop JA. Melanoma and vitamin D. *Mol.Oncol.* 2011; **5**: 197-214.

224. Colston K, Colston MJ, Feldman D. 1,25-dihydroxyvitamin D3 and malignant melanoma: the presence of receptors and inhibition of cell growth in culture. *Endocrinology.* 1981; **108**: 1083-6.

225. Frampton RJ, Omond SA, Eisman JA. Inhibition of human cancer cell growth by 1,25-dihydroxyvitamin D3 metabolites. *Cancer Res.* 1983; **43**: 4443-7.

226. Evans SR, Houghton AM, Schumaker L *et al*. Vitamin D receptor and growth inhibition by 1,25-dihydroxyvitamin D3 in human malignant melanoma cell lines. *J.Surg.Res.* 1996; **61**: 127-33.

227. Reichrath J. Vitamin D and the skin: an ancient friend, revisited. *Exp.Dermatol.* 2007; **16**: 618-25.

228. Yudoh K, Matsuno H, Kimura T. 1alpha,25-dihydroxyvitamin D3 inhibits in vitro invasiveness through the extracellular matrix and in vivo pulmonary metastasis of B16 mouse melanoma. *J.Lab Clin.Med.* 1999; **133**: 120-8.

229. Hansen CM, Madsen MW, Arensbak B *et al*. Down-regulation of laminin-binding integrins by 1 alpha,25-dihydroxyvitamin D3 in human melanoma cells in vitro. *Cell Adhes.Commun.* 1998; **5**: 109-20.

230. Danielsson C, Fehsel K, Polly P *et al*. Differential apoptotic response of human melanoma cells to 1 alpha,25-dihydroxyvitamin D3 and its analogues. *Cell Death.Differ.* 1998; **5**: 946-52.

231. Reichrath J, Rech M, Moeini M *et al*. In vitro comparison of the vitamin D endocrine system in 1,25(OH)2D3-responsive and -resistant melanoma cells. *Cancer Biol.Ther.* 2007; **6**: 48-55.

232. Mason RS, Sequeira VB, Dixon KM *et al*. Photoprotection by 1alpha,25-dihydroxyvitamin D and analogs: further studies on mechanisms and implications for UV-damage. *J.Steroid Biochem.Mol.Biol.* 2010; **121**: 164-8.

233. Damian DL, Kim YJ, Dixon KM *et al*. Topical calcitriol protects from UV-induced genetic damage but suppresses cutaneous immunity in humans. *Exp.Dermatol.* 2010; **19**: e23-e30.

234. Gupta R, Dixon KM, Deo SS *et al*. Photoprotection by 1,25 dihydroxyvitamin D3 is associated with an increase in p53 and a decrease in nitric oxide products. *J.Invest Dermatol.* 2007; **127**: 707-15.

235. Halsall JA, Osborne JE, Potter L *et al*. A novel polymorphism in the 1A promoter region of the vitamin D receptor is associated with altered susceptibilty and prognosis in malignant melanoma. *Br.J.Cancer.* 2004; **91**: 765-70.

236. Li C, Liu Z, Wang LE *et al*. Haplotype and genotypes of the VDR gene and cutaneous melanoma risk in non-Hispanic whites in Texas: a case-control study. *Int.J.Cancer.* 2008; **122**: 2077-84.

237. Randerson-Moor JA, Taylor JC, Elliott F *et al*. Vitamin D receptor gene polymorphisms, serum 25-hydroxyvitamin D levels, and melanoma: UK case-control comparisons and a meta-analysis of published VDR data. *Eur.J.Cancer.* 2009; **45**: 3271-81.

238. Gandini S, Raimondi S, Gnagnarella P *et al*. Vitamin D and skin cancer: a meta-analysis. *Eur.J.Cancer.* 2009; **45**: 634-41.

239. Asgari MM, Maruti SS, Kushi LH *et al*. A cohort study of vitamin D intake and melanoma risk. *J.Invest Dermatol.* 2009; **129**: 1675-80.

240. Glass D, Lens M, Swaminathan R *et al*. Pigmentation and vitamin D metabolism in Caucasians: low vitamin D serum levels in fair skin types in the UK. *PLoS.One.* 2009; **4**: e6477.

241. Malvy DJ, Guinot C, Preziosi P *et al*. Relationship between vitamin D status and skin phototype in general adult population. *Photochem.Photobiol.* 2000; **71**: 466-9.

242. Newton-Bishop JA, Beswick S, Randerson-Moor J *et al*. Serum 25-hydroxyvitamin D3 levels are associated with breslow thickness at presentation and survival from melanoma. *J.Clin.Oncol.* 2009; **27**: 5439-44.

243. Armstrong BK, Kricker A, English DR. Sun exposure and skin cancer. *Australas.J.Dermatol.* 1997; **38 Suppl 1:S1-6.**: S1-S6.

244. Mallone S, De VE, Guzzo M *et al*. Descriptive epidemiology of malignant mucosal and uveal melanomas and adnexal skin carcinomas in Europe. *Eur.J.Cancer.* 2012; **48**: 1167-75.

245. Bergman L, Seregard S, Nilsson B *et al*. Uveal melanoma survival in Sweden from 1960 to 1998. *Invest Ophthalmol.Vis.Sci.* 2003; **44**: 3282-7.

246. Virgili G, Gatta G, Ciccolallo L *et al*. Incidence of uveal melanoma in Europe. *Ophthalmology.* 2007; **114**: 2309-15.

247. Moan J, Dahlback A, Lagunova Z *et al*. Solar radiation, vitamin D and cancer incidence and mortality in Norway. *Anticancer Res.* 2009; **29**: 3501-9.

248. de VE, Bray FI, Coebergh JW *et al*. Changing epidemiology of malignant cutaneous melanoma in Europe 1953-1997: rising trends in incidence and mortality but recent stabilizations in western Europe and decreases in Scandinavia. *Int.J.Cancer.* 2003; **%20;107**: 119-26.

249. Koh HK, Geller AC. Melanoma control in the United States: current status. *Recent Results Cancer Res.* 1995; **139:215-24.**: 215-24.

250. Erdmann F, Lortet-Tieulent J, Schuz J *et al*. International trends in the incidence of malignant melanoma 1953-2008-are recent generations at higher or lower risk? *Int.J.Cancer.* 2013; **132**: 385-400.

251. Fuglede NB, Brinck-Claussen UO, Deltour I *et al*. Incidence of cutaneous malignant melanoma in Denmark, 1978-2007. *Br.J.Dermatol.* 2011; **165**: 349-53.

252. Purdue MP, Freeman LE, Anderson WF *et al*. Recent trends in incidence of cutaneous melanoma among US Caucasian young adults. *J.Invest Dermatol.* 2008; **128**: 2905-8.

253. Moan, J., Baturaite, Z., Grigalavicius, M., and Juzeniene, A. The UV dilemma: Melanomagenesis versus vitamin D photosynthesis. 2013.
Ref Type: Unpublished Work

254. Cicarma E, Porojnicu AC, Lagunova Z *et al*. Sun and sun beds: inducers of vitamin D and skin cancer. *Anticancer Res.* 2009; **29**: 3495-500.

255. Porojnicu, A. C. Seasonal variations of cancer prognosis and of vitamin D status - A relationship? 46. 2008. Oslo, Norway, University of Oslo.
Ref Type: Thesis/Dissertation

256. Bulliard JL, Cox B. Site distribution of melanomas of the upper and lower limbs. *Melanoma Res.* 1997; **7**: 436-7.

257. Devos SA, Baeyens K, Van HL. Sunscreen use and skin protection behavior on the Belgian beach. *Int.J.Dermatol.* 2003; **42**: 352-6.

258. Courtenay WH, Keeling RP. Men, gender, and health: toward an interdisciplinary approach. *J.Am.Coll.Health.* 2000; **48**: 243-6.

259. Gupta A, Driscoll MS. Do hormones influence melanoma? Facts and controversies. *Clin.Dermatol.* 2010; **28**: 287-92.

260. Monsel G, Ortonne N, Bagot M *et al*. c-Kit mutants require hypoxia-inducible factor 1alpha to transform melanocytes. *Oncogene.* 2010; **29**: 227-36.

261. Rees JL. Genetics of hair and skin color. *Annu.Rev.Genet.* 2003; **37:67-90.**: 67-90.

262. Sandby-Moller J, Poulsen T, Wulf HC. Epidermal thickness at different body sites: relationship to age, gender, pigmentation, blood content, skin type and smoking habits. *Acta Derm.Venereol.* 2003; **83**: 410-3.

263. Lock-Andersen J, Therkildsen P, de Fine OF *et al*. Epidermal thickness, skin pigmentation and constitutive photosensitivity. *Photodermatol.Photoimmunol.Photomed.* 1997; **13**: 153-8.

264. Otberg N, Richter H, Schaefer H *et al*. Variations of hair follicle size and distribution in different body sites. *J.Invest Dermatol.* 2004; **122**: 14-9.

265. Lee JA. Melanoma and exposure to sunlight. *Epidemiol.Rev.* 1982; **4:110-36.**: 110-36.

266. Sinclair SA, Diffey BL. Sun protection provided by ladies stockings. *Br.J.Dermatol.* 1997; **136**: 239-41.

267. Garcia AM, McLaren CE, Meyskens FL, Jr. Melanoma: is hair the root of the problem? *Pigment Cell Melanoma Res.* 2011; **24**: 110-8.

268. Autier P, Boniol M, Severi G *et al*. Sex differences in numbers of nevi on body sites of young European children: implications for the etiology of cutaneous melanoma. *Cancer Epidemiol.Biomarkers Prev.* 2004; **13**: 2003-5.

269. Scott MC, Suzuki I, bdel-Malek ZA. Regulation of the human melanocortin 1 receptor expression in epidermal melanocytes by paracrine and endocrine factors and by ultraviolet radiation. *Pigment Cell Res.* 2002; **15**: 433-9.

270. Mogil JS, Wilson SG, Chesler EJ *et al*. The melanocortin-1 receptor gene mediates female-specific mechanisms of analgesia in mice and humans. *Proc.Natl.Acad.Sci.U.S.A.* 2003; **100**: 4867-72.

271. Porojnicu AC, Robsahm TE, Dahlback A *et al*. Seasonal and geographical variations in lung cancer prognosis in Norway. Does Vitamin D from the sun play a role? *Lung Cancer.* 2007; **55**: 263-70.

272. Holvik K, Meyer HE, Sogaard AJ *et al*. Pakistanis living in Oslo have lower serum 1,25-dihydroxyvitamin D levels but higher serum ionized calcium levels compared with ethnic Norwegians. The Oslo Health Study. *BMC.Endocr.Disord.* 2007; **7:9.**: 9.

273. Lagunova Z, Porojnicu AC, Lindberg F *et al*. The dependency of vitamin D status on body mass index, gender, age and season. *Anticancer Res.* 2009; **29**: 3713-20.

274. Brustad M, Alsaker E, Engelsen O *et al*. Vitamin D status of middle-aged women at 65-71 degrees N in relation to dietary intake and exposure to ultraviolet radiation. *Public Health Nutr.* 2004; **7**: 327-35.

275. Koomen ER, de VE, van Kempen LC *et al*. Epidemiology of extracutaneous melanoma in the Netherlands. *Cancer Epidemiol.Biomarkers Prev.* 2010; **19**: 1453-9.

276. McLaughlin CC, Wu XC, Jemal A *et al*. Incidence of noncutaneous melanomas in the U.S. *Cancer.* 2005; **103**: 1000-7.

277. Chang AE, Karnell LH, Menck HR. The National Cancer Data Base report on cutaneous and noncutaneous melanoma: a summary of 84,836 cases from the past decade. The American College of Surgeons Commission on Cancer and the American Cancer Society. *Cancer.* 1998; **83**: 1664-78.

278. Hu DN, Yu GP, McCormick SA. Population-based incidence of vulvar and vaginal melanoma in various races and ethnic groups with comparisons to other site-specific melanomas. *Melanoma Res.* 2010; **20**: 153-8.

279. Tsai T, Vu C, Henson DE. Cutaneous, ocular and visceral melanoma in African Americans and Caucasians. *Melanoma Res.* 2005; **15**: 213-7.

280. Hu DN. Photobiology of ocular melanocytes and melanoma. *Photochem.Photobiol.* 2005; **81**: 506-9.

281. Singh AD, Rennie IG, Seregard S *et al*. Sunlight exposure and pathogenesis of uveal melanoma. *Surv.Ophthalmol.* 2004; **49**: 419-28.

282. Ragnarsson-Olding BK, Nilsson PJ, Olding LB *et al*. Primary ano-rectal malignant melanomas within a population-based national patient series in Sweden during 40 years. *Acta Oncol.* 2009; **48**: 125-31.

283. Miller BJ, Rutherford LF, McLeod GR *et al*. Where the sun never shines: anorectal melanoma. *Aust.N.Z.J.Surg.* 1997; **67**: 846-8.

284. Singh AD, Turell ME, Topham AK. Uveal melanoma: trends in incidence, treatment, and survival. *Ophthalmology.* 2011; **118**: 1881-5.

285. Bergman L, Seregard S, Nilsson B *et al*. Incidence of uveal melanoma in Sweden from 1960 to 1998. *Invest Ophthalmol.Vis.Sci.* 2002; **43**: 2579-83.

286. Weis E, Shah CP, Lajous M *et al*. The association between host susceptibility factors and uveal melanoma: a meta-analysis. *Arch.Ophthalmol.* 2006; **124**: 54-60.

287. Schmidt-Pokrzywniak A, Jockel KH, Bornfeld N *et al*. Positive interaction between light iris color and ultraviolet radiation in relation to the risk of uveal melanoma: a case-control study. *Ophthalmology.* 2009; **116**: 340-8.

288. Weinstock MA. Epidemiology and prognosis of anorectal melanoma. *Gastroenterology.* 1993; **104**: 174-8.

289. Eide MJ, Weinstock MA. Association of UV index, latitude, and melanoma incidence in nonwhite populations--US Surveillance, Epidemiology, and End Results (SEER) Program, 1992 to 2001. *Arch.Dermatol.* 2005; **141**: 477-81.

290. Omholt K, Grafstrom E, Kanter-Lewensohn L *et al*. KIT pathway alterations in mucosal melanomas of the vulva and other sites. *Clin.Cancer Res.* 2011; **17**: 3933-42.

291. Beadling C, Jacobson-Dunlop E, Hodi FS *et al*. KIT gene mutations and copy number in melanoma subtypes. *Clin.Cancer Res.* 2008; **14**: 6821-8.

292. Handolias D, Salemi R, Murray W *et al*. Mutations in KIT occur at low frequency in melanomas arising from anatomical sites associated with chronic and intermittent sun exposure. *Pigment Cell Melanoma Res.* 2010; **23**: 210-5.

293. Curtin JA, Busam K, Pinkel D *et al*. Somatic activation of KIT in distinct subtypes of melanoma. *J.Clin.Oncol.* 2006; **24**: 4340-6.

294. Boettner E, Wolter JR. Transmission of the Ocular Media. *Investigative Ophthalmology and Vision Science* 1962; **6**: 776-83.

295. Shah CP, Weis E, Lajous M *et al*. Intermittent and chronic ultraviolet light exposure and uveal melanoma: a meta-analysis. *Ophthalmology.* 2005; **112**: 1599-607.

296. Langley RG, Barnhill R, Mihm M *et al*. Neoplasms: Cutaneous Melanoma. In: *Fitzpatrick's Dermatology in General Medicine* (Freedberg,IM, Eisen,AZ, Wolff,K et al, eds). New-York: McGraw-Hill Professional, 2003.

297. Chamberlain AJ, Fritschi L, Giles GG *et al*. Nodular type and older age as the most significant associations of thick melanoma in Victoria, Australia. *Arch.Dermatol.* 2002; **138**: 609-14.

298. Crocetti E, Carli P. Only superficial spreading melanoma is causing the melanoma epidemics? *Eur.J.Epidemiol.* 2004; **19**: 91-2.

299. Warycha MA, Christos PJ, Mazumdar M *et al*. Changes in the presentation of nodular and superficial spreading melanomas over 35 years. *Cancer.* 2008; **113**: 3341-8.

300. Crocetti E, Caldarella A, Chiarugi A *et al*. The thickness of melanomas has decreased in central Italy, but only for thin melanomas, while thick melanomas are as thick as in the past. *Melanoma Res.* 2010; **20**: 422-6.

301. Osterlind A, Hou-Jensen K, Moller JO. Incidence of cutaneous malignant melanoma in Denmark 1978-1982. Anatomic site distribution, histologic types, and comparison with non-melanoma skin cancer. *Br.J.Cancer.* 1988; **58**: 385-91.

302. Hersey P, Sillar RW, Howe CG *et al*. Factors related to the presentation of patients with thick primary melanomas. *Med.J.Aust.* 1991; **154**: 583-7.

303. Murray CS, Stockton DL, Doherty VR. Thick melanoma: the challenge persists. *Br.J.Dermatol.* 2005; **152**: 104-9.

I

Journal of Photochemistry and Photobiology B: Biology 101 (2010) 174–178

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

Latitude gradient for melanoma incidence by anatomic site and gender in Norway 1966–2007

Emanuela Cicarma [a,*,1], Asta Juzeniene [a], Alina C. Porojnicu [a], Øyvind S. Bruland [b], Johan Moan [a,c]

[a] Department of Radiation Biology, The Norwegian Radium Hospital, Oslo University Hospital, Montebello, 0310 Oslo, Norway
[b] Department of Medical Oncology & Radiotherapy, The Norwegian Radium Hospital, 0310 Oslo, Norway
[c] Department of Physics, University of Oslo, 0316 Oslo, Norway

ARTICLE INFO

Article history:
Received 4 November 2009
Received in revised form 11 February 2010
Accepted 1 April 2010
Available online 6 April 2010

Keywords:
Melanoma
Epidemiology
Latitude
Anatomic site

ABSTRACT

Latitude gradients and time trends for cutaneous malignant melanoma (CMM) were analyzed using incident cases from the Norwegian Cancer Registry for the period 1966–2007. Sex and various anatomic regions of the body were taken into account, for better understanding of the role of ultraviolet radiation in CMM etiology.

There is a latitude gradient for CMM on all body sites included in the present study, with 2–2.5 times higher incidence rates in the south. The latitude gradients seem to be largest for the trunk. Melanomas on sites intermittently exposed to the sun (like the trunk) dominate both in the north and in the south and this distribution has not changed over the years. A leveling off of the incidence rates are observed for both sexes and for all sites studied, after 1985–1995, slightly more in the south than in the north, except for the head and neck where the incidence rates have continued to increase slowly in the north as well as in the south. The leveling off of melanoma trend is probably associated with melanoma prevention campaigns and with increasing awareness, although vitamin D could play a role.

1. Introduction

Cutaneous malignant melanoma (CMM) has until about 1990 been one of the fastest growing cancers in most white populations [1]. The increase has been rather dramatic in Northern Europe, especially in Norway [2,3]. Among the environmental etiological factors, exposure to solar ultraviolet radiation (UV) is the most important one [4], although there are other important risk factors like individual phenotype (among which the number of melanocytic nevi is considered to be the strongest one [5]) and family history of melanoma [6].

In the case of CMM the role of exposure to UV radiation is complex and to some extent controversial [7–9]. This is due to several epidemiological observations: the anatomic distribution favors body localizations that are not regularly exposed to the sun [1], while chronic sun exposure may have a certain protective effect [10,11]. Moreover, the progression of CMM seems to be slower for those lesions localized on skin that receives large UV exposures, and regular sun exposure has been associated with increased survival from melanoma [12]. This apparent protective effect of the sun may be due to vitamin D formation, as vitamin D in the skin may induce photoprotection [13], along with other biological positive effects, like regulation of cell growth and differentiation, and modulation of the immune system [14].

Melanoma incidence displays a north–south gradient in homogeneous populations [15,16], which is a strong argument for the role of UV exposure as a CMM carcinogen. However, this gradient is smaller for CMM than for non-melanoma skin cancers (NMSC) [17]. This is an argument for the role of UVA in CMM generation and of UVB in NMSC generation as we have proposed [17]. Furthermore, the pattern of UV exposure (chronic versus intermittent) has different impact in populations living at different latitudes: recreational sun exposure and sunburn are strong predictors for CMM at all latitudes, while occupational and total sun exposures appear to be correlated with CMM at low latitudes [18].

We have in the present work analyzed the latitude gradients for CMM by body site and gender, in a country situated at high latitude and stretching over a long north–south distance, in order to gain better understanding of the role of UV radiation in CMM etiology.

2. Materials and methods

2.1. Data

The analysis was based on newly diagnosed cases of CMM in Norway from 1966 to 2007. Data on incidence cases according to

* Corresponding author. Tel.: +47 22934260; fax: +47 22934270.
E-mail address: emanuela.cicarma@rr-research.no (E. Cicarma).
[1] Present address: Dermatology Department, Elias Emergency University Hospital, 17, Marasti, Bucharest 011461, Romania. Tel.: +40 21 3161600; fax: +40 21 3173052.

1011-1344/$ - see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jphotobiol.2010.04.002

sex, anatomical site, region, and calendar period were provided by the Cancer Registry of Norway. Melanoma cases have been registered in this registry since 1953, and reporting to the registry is compulsory, by law. The registry receives reports both from the laboratories and from the clinicians. The melanoma cases are coded according to the seventh revision of the International Classification of Diseases (ICD-7 code 190) with some modifications. In the site specific analysis we included only CMM of the head and neck (ICD 190.0), of the trunk (ICD 190.1), upper extremity (ICD 190.2), and lower extremity (ICD 190.4).

Norway is located at high latitudes (ranging from approximately 57–72°N) and is, in this work, divided in three residential regions, according to the calculated levels of UV exposure [19]: southeast region (high annual ambient UV exposure, mean latitude 59.5°), midwest region (mean latitude 62.5°) and north region (low annual ambient UV exposure, mean latitude 69.5°). The cloud cover is taken into account. For better clarity, we used for further analysis only the southeast (south) and the north regions. The main city, Oslo, was excluded from the analysis, as Oslo is the most urbanized region of Norway.

2.2. Analysis

To compare rates through calendar periods (time trends) we used crude incidence rates (incidence number divided by the Norwegian population in the same period and sex). The values plotted represent incidence rates for 6 year periods per 100,000 persons, from 1966 until 2007, using a logarithmic scale, since the relationship is not linear. Time trends were estimated by single exponential fitting for the data from 1966–1989 and from 1989–2007 or by best-fitting a regression line for all data points in other cases, using SigmaPlot 10.0 software from Systat Software, Inc. (Richmond, CA, USA). Significant p was considered <0.05. Using the same software we calculated doubling time of the incidence rates for two time periods: 1966–1989 and 1984–2007.

3. Results

3.1. Overall melanoma incidence

There is a clear latitude gradient for both sexes, with higher melanoma incidence in the south than in the north. With the present regional division, the incidence rates are about 2–2.5 times larger in the south than in the north, and the rates are slightly larger for women than for men, notably in the south. The incidence rates increased sharply until 1985–1995, but were almost constant after that time point (Fig. 1). It may seem that after the 1980s, the incidence rates were increasing slightly in the north but not in the south (see the doubling times as given in Table 1). However, the differences are small and not significant.

3.2. Sex and anatomic site

The distribution on anatomic sites differ between men and women except for head and neck, were the CMM incidence rates were almost similar for the two sexes (data not shown). Thus we grouped men and women together for this particular localization (Fig. 2).

For head and neck melanoma, a slow increase of the rates were seen for the whole period, and in contrast with the other localizations, no decrease took place after 1985 (Fig. 2). The incidence rates were almost twice as large in the south as in the north (Fig. 2). This is a similar difference as found for the total rates (Fig. 1) as well as for all the rates on the other localizations studied (see below).

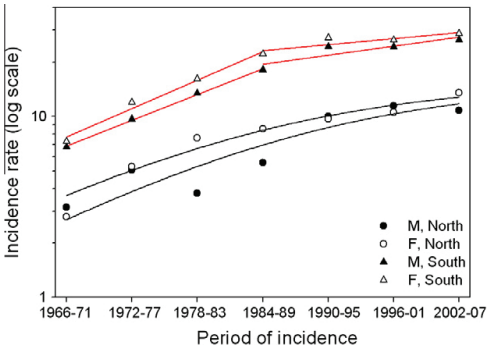

Fig. 1. Trends in melanoma incidence, males and females, in the north and in the south regions of Norway, 1966–2007. Incidence rates are cases per 100,000 persons, Norwegian population, for 6 year periods. F stands for female, M stands for male.

For the trunk, the incidence rates were higher for men than for women in both regions (Fig. 3). The change in the increase of the rates was notably evident for women in the northern region. In this case the rates were almost constant after 1984–1989 (Fig. 3, Table 1).

For the upper extremity, there were too few cases to any reliable analysis of trend changes. In this case the rates were larger for women than for men and about twice as large in the south as in the north (Fig. 4).

For the lower extremity, the incidence is higher for women in the south and was almost constant after 1984–1990, while in the other cases there were too few cases to allow any reliable trend analysis to be made (Fig. 5 and Table 1).

There was a similar CMM distribution by body site in the north as in the south, but different for men and women. For men the highest incidence rates were found for the trunk, followed by the head and the neck, the lower limbs and the upper limbs, while for women the highest incidence rates were found for lower limbs, followed by the trunk, the upper limbs and the head and neck (Fig. 6). This relative distribution has not changed over the two calendar periods analyzed: 1966–1986 and 1987–2007.

4. Discussion

This article presents an update of the clinical epidemiology of melanoma in Norway, one of the countries with the highest melanoma incidence rate in Europe. The melanoma incidence rate in Norway is high despite the relatively low intensity of ultraviolet radiation. This has been documented and discussed for a long time. Among the reasons for this are host factors like pigmentary characteristics (skin type, hair and eye color) and positive attitudes to recreational activities, due to increase socioeconomic status. Fair skin type, blue eyes and blond hair are associated with increased risk for CMM [6,20] and are all characteristics of the Scandinavian populations. Fair skin individuals have low amounts of the protective eumelanin in their epidermis, and are therefore more susceptible to UV radiation [21]. Holidays in sunny countries are considered an estimate for the intermittent sun exposure, a well known risk factor for melanoma [22,23] and many Norwegians go regularly to vacations in Mediterranean countries [24]. It has been speculated that there may be a relationship between melanoma incidence and vacations abroad, among Norwegians [25,26], but this needs further research.

Another important aspect is the increase of solar UV irradiance, due to suggested depletion of the stratospheric ozone that could

Table 1
Estimated doubling time for melanoma incidence, by body site, gender and region, for two time periods: 1966–1989 and 1984–2007, except for the head and neck melanoma, for which the data shown are for the whole period 1966–2007.

Body site	Gender*	South (doubling time in years ± SE)		North (doubling time in years ± SE)	
		1966–1989	1984–2007	1966–1989	1984–2007
Trunk	M	11.5 ± 0.3	40.8 ± 19.01	18.6 ± 10.5	19.13 ± 10.4
	F	11.8 ± 1.18	30.8 ± 6.07	7.4 ± 0.4	Stationary (77.01 ± 78.6)
Upper extremity	M	9.6 ± 1.6	24.4 ± 7.9	16.3 ± 7.4	Stationary (80.2 ± 103)
	F	12.3 ± 2.4	41 ± 5.6	13.7 ± 11.5	23.7 ± 12.1
Lower extremity	M	12.8 ± 0.95	26.4 ± 7.05	8.3 ± 3.6	23.18 ± 19.3
	F	11.8 ± 0.76	Negative	15.58 ± 7.3	15.85 ± 4.5
Head and neck	M + F	17.28 ± 5.6		17.9 ± 7.8	
All melanomas	M	13.07 ± 0.28	36.25 ± 13.6	17.25 ± 5.92	19.5 ± 9.44
	F	12.78 ± 1.13	54.42 ± 22.7	14.01 ± 3.04	28.33 ± 4.8

* F stands for female, M stands for male.

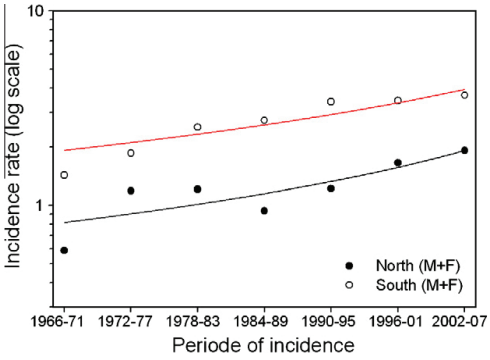

Fig. 2. Trends of the head and neck melanoma incidence, in the north and in the south regions of Norway, 1966–2007. Incidence rates are cases per 100,000 persons, Norwegian population, for 6 year periods. F stands for female, M stands for male.

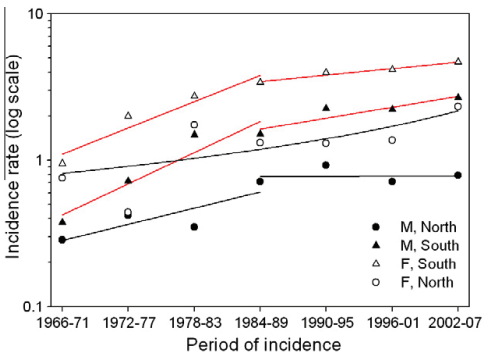

Fig. 4. Trends of the upper extremity melanoma incidence, in the north and in the south regions of Norway, 1966–2007. Incidence rates are cases per 100,000 persons, Norwegian population, for 6 year periods. F stands for female, M stands for male.

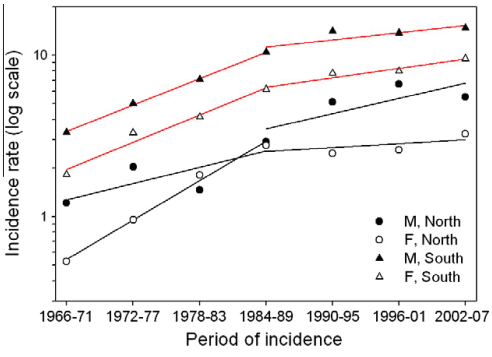

Fig. 3. Trends of the trunk melanoma incidence, in the north and in the south regions of Norway, 1966–2007. Incidence rates are cases per 100,000 persons, Norwegian population, for 6 year periods. F stands for female, M stands for male.

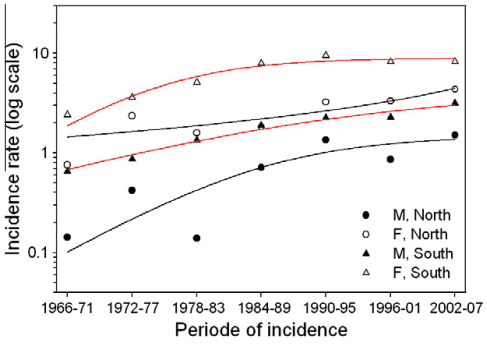

Fig. 5. Trends of the lower extremity melanoma incidence, in the north and in the south regions of Norway, 1966–2007. Incidence rates are cases per 100,000 persons, Norwegian population, for 6 year periods. F stands for female, M stands for male.

have significant impact on melanoma incidence in the future [26,27]. However, the ozone depletion has stopped and is even reversing in the north so that this will supposedly not play any large role in the future, as earlier suggested.

Overall, the rates increased until 1985–1995 but after that time point the rates were, with few exceptions, almost constant, in agreement with our earlier findings [28,29]. The changes in the trends taking place after 1985–1995 are probably associated with prevention campaigns and with increasing awareness, that leads to changes in UV pattern exposures and to an early detection of suspected (premalignant) lesions [3,30]. Another explanation for the leveling off of the incidence rates could be related to vitamin D, as Norway has relatively high vitamin D levels [31,32].

A leveling off of the incidence rates are, as mentioned, observed for both sexes and for all sites studied, after 1985–1995, except for the head and neck where the incidence rates have continued to increase slowly in the north as well as in the south (perhaps due to aging of the population). Overall, time trends seem to level off

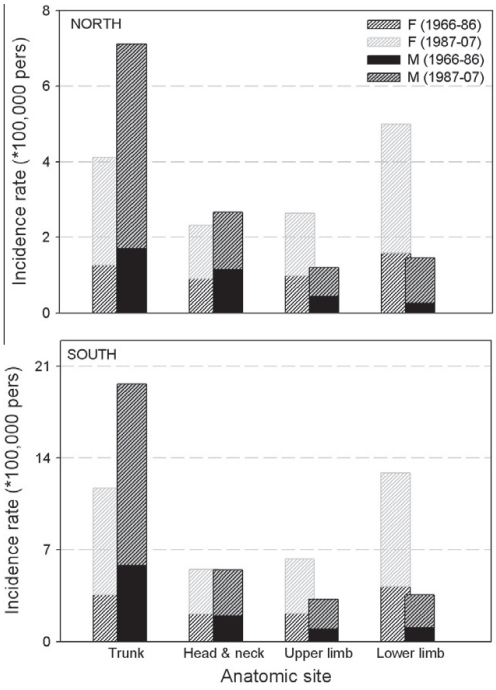

Fig. 6. Anatomic distribution of melanomas by region and gender, for two time periods: 1966–1986 and 1987–2007.

slightly more in the south than in the north after the 1985–1995, both for men and women, more obvious for CMM on the intermittent sun-exposed sites. After this time point CMM on lower extremity of women in the south seems to have the most favorable trend, but stable incidence rates were also observed for CMM on the trunk of women in the north.

The increasing trend in CMM incidence in the north might have several explanations. In the north, due to the chilly climate, the limbs usually are covered up even in summer time, thus they are exposed preferentially during holidays, making the intermittent exposure more acute and intense. The low temperature itself might contribute, as suggested by Christophers, in 1998 [9]. The reduction in exposed skin surface by clothing, beside already lower fluencies of the UVB rate in the north than in the south, further diminishes the natural production of vitamin D, which is considered to play an important role for protection against UV radiation and melanoma [12,13]. UVB latitude gradient is more prominent than UVA gradient, as UVB varies relatively more at high latitudes than at low latitudes (due to absorption of UVB by ozone layer), thus making the UVA/UVB ratio larger at higher latitudes [17]. The role of UVA in DNA damage at higher latitudes, in fair skin populations, is suggested also by Gorham et al. [33]. As people probably will continue to have intermittent/acute sun exposures and use inadequate sunscreens, the melanoma incidence may continue to rise, especially in susceptible individuals, like Scandinavian populations.

There is a latitude gradient for CMM on all anatomic localizations included in the present study, with 2–2.5 times higher incidence rates in the south. The latitude gradients seem to be largest for the trunk.

The latitude gradient of the calculated solar UV fluence [19] agrees well with the latitude gradient for CMM incidence rates, suggesting that UV exposure is a strong risk factor. We have earlier concluded that in Norway the north–south gradients are similar for CMM and squamous cell carcinoma [29], but when larger populations are taken into account the latitude gradients for CMM are smaller than those for NMSC [15,17]. We have attributed this to the fact that the latitude gradient for UVB is larger than that for UVA [17], as discussed above. UVB (but not UVA) is considered to be a strong carcinogen for NMSC, while UVA almost certainly plays a role for CMM [23,34]. However, the north–south distance is not large enough in Norway to allow any detailed analysis.

In Norway, the main urbanized regions are located in the south and CMM is more prevalent in urban than in rural regions [15]. The warmer climate in the south may stimulate vacation-related intermittent sun exposures. Also, because of the urbanization and higher income, people are more likely to work indoor than outdoor and to spent their holidays abroad, in sunny countries, all of which are risk factors for melanoma [25,26,35] and could also play some role for the observed latitude gradient.

The head and neck are considered to be sites with chronic sun exposure, while the trunk and limbs are presumably sites with more intermittent sun exposure. CMM on intermittent-exposed sites seems to dominate, both in the north and in the south: the trunk for men and lower limbs for women. But even for women, the lower limbs are followed very closely by the trunk in recent years, a trend evident notably in the south. Thus, the trunk is becoming the major site of CMM in both sexes, as observed also by Lipsker et al. [36]. This distribution of CMM supports the hypothesis that the pattern of sun exposure is important for skin carcinogenesis, intermittent exposure patterns being most dangerous with respect to CMM [1,37], in agreement with our earlier work [29].

For CMM a "divergent pathway" model has been proposed, including a site dependent susceptibility of melanocytes to carcinogenesis [38,39]. Recent studies seem to support the existence of at least two different pathways to CMM [40,41], that appear to be variable and depend on the host: (i) the melanocytes of people with a low tendency to develop nevi require chronic sun exposure in order to initiate the development of CMM, and these CMMs tend to occur most frequently on the head and neck, and (ii) in nevus-prone individuals melanocytes require little sun exposure in order to become neoplastic, and in this case CMMs tend to occur more frequently on the trunk. A third pathologic pathway is proposed for fast-growing CMMs, with bad prognosis, and these CMMs are seemingly not sun-induced [36]. Genetic studies have provided support for these "divergent pathways" [42].

Understanding these concepts may be very important for future strategies of CMM prevention. Further epidemiological research may help in better understanding CMM etiology and in analyzing differences among different subtypes of CMMs, in populations with different skin types and living at different latitudes.

Acknowledgements

We acknowledge the Cancer Registry of Norway (Institute of Population-based Cancer Research), for providing us the epidemiological data, with special thanks to Siri Larønningen and Lidziya Vanahel.

References

[1] C. Garbe, U. Leiter, Melanoma epidemiology and trends, Clin. Dermatol. 27 (2009) 3–9.
[2] Cancer Incidence in Five Continents, vol. IX, IARC Scientific Publications, Lyon, 2007.
[3] E. de Vries, F.I. Bray, J.W. Coebergh, D.M. Parkin, Changing epidemiology of malignant cutaneous melanoma in Europe 1953–1997: rising trends in incidence and mortality but recent stabilizations in western Europe and decreases in Scandinavia, Int. J. Cancer 107 (2003) 119–126.

[4] B.K. Armstrong, A. Kricker, How much melanoma is caused by sun exposure?, Melanoma Res 3 (1993) 395–401.
[5] A.J. Swerdlow, A. Green, Melanocytic naevi and melanoma: an epidemiological perspective, Br. J. Dermatol. 117 (1987) 137–146.
[6] S. Gandini, F. Sera, M.S. Cattaruzza, P. Pasquini, R. Zanetti, C. Masini, P. Boyle, C.F. Melchi, Meta-analysis of risk factors for cutaneous melanoma: III. Family history, actinic damage and phenotypic factors, Eur. J. Cancer 41 (2005) 2040–2059.
[7] N. Cascinelli, R. Marchesini, Increasing incidence of cutaneous melanoma, ultraviolet radiation and the clinician, Photochem. Photobiol. 50 (1989) 497–505.
[8] A. Baker-Blocker, Ultraviolet radiation and melanoma mortality in the United States, Environ. Res. 23 (1980) 24–28.
[9] A.J. Christophers, Melanoma is not caused by sunlight, Mutat. Res. 422 (1998) 113–117.
[10] P.J. Nelemans, F.H. Rampen, D.J. Ruiter, A.L. Verbeek, An addition to the controversy on sunlight exposure and melanoma risk: a meta-analytical approach, J. Clin. Epidemiol. 48 (1995) 1331–1342.
[11] J.M. Elwood, J. Jopson, Melanoma and sun exposure: an overview of published studies, Int. J. Cancer 73 (1997) 198–203.
[12] M. Berwick, B.K. Armstrong, L. Ben-Porat, J. Fine, A. Kricker, C. Eberle, R. Barnhill, Sun exposure and mortality from melanoma, J. Natl. Cancer Inst. 97 (2005) 195–199.
[13] R. Gupta, K.M. Dixon, S.S. Deo, C.J. Holliday, M. Slater, G.M. Halliday, V.E. Reeve, R.S. Mason, Photoprotection by 1, 25 dihydroxyvitamin D3 is associated with an increase in p53 and a decrease in nitric oxide products, J. Invest. Dermatol. 127 (2007) 707–715.
[14] B. Lehmann, Role of the vitamin D3 pathway in healthy and diseased skin – facts, contradictions and hypotheses, Exp. Dermatol. 18 (2009) 97–108.
[15] J. Moan, A. Dahlback, Ultraviolet radiation and skin cancer: epidemiological data from Scandinavia, in: L. Bjørn, J. Moan, W. Nultsch, A. Young (Eds.), Environmental UV Photobiology, Plenum Press, New York, 1993, pp. 255–293.
[16] J.M. Elwood, J.A. Lee, S.D. Walter, T. Mo, A.E. Green, Relationship of melanoma and other skin cancer mortality to latitude and ultraviolet radiation in the United States and Canada, Int. J. Epidemiol. 3 (1974) 325–332.
[17] J. Moan, A. Dahlback, R.B. Setlow, Epidemiological support for an hypothesis for melanoma induction indicating a role for UVA radiation, Photochem. Photobiol. 70 (1999) 243–247.
[18] Y.M. Chang, J.H. Barrett, D.T. Bishop, B.K. Armstrong, V. Bataille, W. Bergman, M. Berwick, P.M. Bracci, J.M. Elwood, M.S. Ernstoff, R.P. Gallagher, A.C. Green, N.A. Gruis, E.A. Holly, C. Ingvar, P.A. Kanetsky, M.R. Karagas, T.K. Lee, M.L. Le, R.M. Mackie, H. Olsson, A. Osterlind, T.R. Rebbeck, P. Sasieni, V. Siskind, A.J. Swerdlow, L. Titus-Ernstoff, M.S. Zens, J.A. Newton-Bishop, Sun exposure and melanoma risk at different latitudes: a pooled analysis of 5700 cases and 7216 controls, Int. J. Epidemiol. 38 (2009) 814–830.
[19] A.C. Porojnicu, T.E. Robsahm, A. Dahlback, J.P. Berg, D. Christiani, O.S. Bruland, J. Moan, Seasonal and geographical variations in lung cancer prognosis in Norway. Does Vitamin D from the sun play a role?, Lung Cancer 55 (2007) 263–270
[20] M.B. Veierod, H.O. Adami, E. Lund, B.K. Armstrong, E. Weiderpass, Sun and solarium exposure and melanoma risk: effects of age, pigmentary characteristics, and nevi, Biomarkers Prev. 19 (2010) 111–120.
[21] T. Tadokoro, Y. Yamaguchi, J. Batzer, S.G. Coelho, B.Z. Zmudzka, S.A. Miller, R. Wolber, J.Z. Beer, V.J. Hearing, Mechanisms of skin tanning in different racial/ethnic groups in response to ultraviolet radiation, J. Invest Dermatol. 124 (2005) 1326–1332.
[22] S. Gandini, F. Sera, M.S. Cattaruzza, P. Pasquini, O. Picconi, P. Boyle, C.F. Melchi, Meta-analysis of risk factors for cutaneous melanoma: II. Sun exposure, Eur. J. Cancer 41 (2005) 45–60.
[23] J. Moan, A.C. Porojnicu, A. Dahlback, Ultraviolet radiation and malignant melanoma, Adv. Exp. Med. Biol. 624 (2008) 104–116.
[24] Statistics Norway, 2010, <http://www.ssb.no/ferie_en>.
[25] Y.Z. Agredano, J.L. Chan, R.C. Kimball, A.B. Kimball, Accessibility to air travel correlates strongly with increasing melanoma incidence, Melanoma Res. 16 (2006) 77–81.
[26] G. Bentham, A. Aase, Incidence of malignant melanoma of the skin in Norway, 1955–1989: associations with solar ultraviolet radiation, income and holidays abroad, Int. J. Epidemiol. 25 (1996) 1132–1138.
[27] J.M. Elwood, Epidemiology of malignant melanoma: its relationship to ultraviolet radiation and ozone depletion, in: R. Russel-Jones, T. Wigley (Eds.), Ozone depletion: health and environmental consequences, John Wiley, Chichester, 1989, pp. 169–189.
[28] J. Moan, A.C. Porojnicu, A. Dahlback, R.B. Setlow, Addressing the health benefits and risks, involving vitamin D or skin cancer, of increased sun exposure, Proc. Natl. Acad. Sci. USA 105 (2008) 668–673.
[29] J. Moan, A. Dahlback, Z. Lagunova, E. Cicarma, A.C. Porojnicu, Solar radiation, vitamin D and cancer incidence and mortality in Norway, Anticancer Res. 29 (2009) 3501–3509.
[30] H.K. Koh, A.C. Geller, D.R. Miller, R.A. Lew, The early detection of and screening for melanoma, Cancer 75 (1995) 674–683.
[31] M. Brustad, E. Alsaker, O. Engelsen, L. Aksnes, E. Lund, Vitamin D status of middle-aged women at 65–71 degrees N in relation to dietary intake and exposure to ultraviolet radiation, Public Health Nutr. 7 (2004) 327–335.
[32] Z. Lagunova, A.C. Porojnicu, F. Lindberg, S. Hexeberg, J. Moan, The dependency of vitamin D status on body mass index, gender, age and season, Anticancer Res. 29 (2009) 3713–3720.
[33] E.D. Gorham, S.B. Mohr, C.F. Garland, G. Chaplin, F.C. Garland, Do sunscreens increase risk of melanoma in populations residing at higher latitudes?, Ann Epidemiol. 17 (2007) 956–963.
[34] S.Q. Wang, R. Setlow, M. Berwick, D. Polsky, A.A. Marghoob, A.W. Kopf, R.S. Bart, Ultraviolet A and melanoma: a review, J. Am. Acad. Dermatol. 44 (2001) 837–846.
[35] I.S. Silva, C.D. Higgins, T. Abramsky, M.A. Swanwick, J. Frazer, L.M. Whitaker, M.E. Blanshard, J. Bradshaw, J.M. Apps, D.T. Bishop, J.A. Newton-Bishop, A.J. Swerdlow, Overseas sun exposure, nevus counts, and premature skin aging in young English women: a population-based survey, J. Invest. Dermatol. 129 (2009) 50–59.
[36] D. Lipsker, F. Engel, B. Cribier, M. Velten, G. Hedelin, Trends in melanoma epidemiology suggest three different types of melanoma, Br. J. Dermatol. 157 (2007) 338–343.
[37] M. Berwick, A. Lachiewicz, C. Pestak, N. Thomas, Solar UV exposure and mortality from skin tumors, Adv. Exp. Med. Biol. 624 (2008) 117–124.
[38] A. Green, A theory of site distribution of melanomas: Queensland, Australia, Cancer Causes Control 3 (1992) 513–516.
[39] D.C. Whiteman, P.G. Parsons, A.C. Green, p53 expression and risk factors for cutaneous melanoma: a case-control study, Int. J. Cancer 77 (1998) 843–848.
[40] D.C. Whiteman, P. Watt, D.M. Purdie, M.C. Hughes, N.K. Hayward, A.C. Green, *Melanocytic nevi*, solar keratoses, and divergent pathways to cutaneous melanoma, J. Natl. Cancer Inst. 95 (2003) 806–812.
[41] C.M. Olsen, M.S. Zens, T.A. Stukel, C. Sacerdote, Y.M. Chang, B.K. Armstrong, V. Bataille, M. Berwick, J.M. Elwood, E.A. Holly, C. Kirkpatrick, T. Mack, J.N. Bishop, A. Osterlind, A.J. Swerdlow, R. Zanetti, A.C. Green, M.R. Karagas, D.C. Whiteman, Nevus density and melanoma risk in women: a pooled analysis to test the divergent pathway hypothesis, Int. J. Cancer 124 (2009) 937–944.
[42] J.A. Curtin, J. Fridlyand, T. Kageshita, H.N. Patel, K.J. Busam, H. Kutzner, K.H. Cho, S. Aiba, E.B. Brocker, P.E. LeBoit, D. Pinkel, B.C. Bastian, Distinct sets of genetic alterations in melanoma, New Engl. J. Med. 353 (2005) 2135–2147.

II

Dermato-Endocrinology 2:1, 3-8; January/February/March 2010; © 2010 Landes Bioscience

Time trends and latitude dependence of uveal and cutaneous malignant melanoma induced by solar radiation

Johan Moan,[1,2] Emanuela Cicarma,[1] Richard Setlow,[3] Alina C. Porojnicu,[1] William B. Grant[4] and Asta Juzeniene[1,*]

[1]Department of Radiation Biology; Institute for Cancer Research; The Norwegian Radium Hospital; Oslo University Hospital; Montebello, Oslo Norway; [2]Department of Physics; University of Oslo; Oslo, Norway; [3]Biology Department; Brookhaven National Laboratory; Upton, NY USA; [4]Sunlight, Nutrition and Health Research Center (SUNARC); San Francisco, CA USA

Key words: uveal melanoma, cutaneous malignant melanoma, ultraviolet radiation, vitamin D, latitude

Abbreviations: CMM, cutaneous malignant melanoma (CMM); UV, ultraviolet; UVA, ultraviolet A; UVB, ultraviolet B

In order to evaluate the role of solar radiation in uveal melanoma etiology, the time and latitude dependency of the incidence rates of this melanoma type were studied in comparison with those of cutaneous malignant melanoma (CMM). Norway and several other countries with Caucasian populations were included. There is a marked north-south gradient of the incidence rates of CMM in Norway, with three times higher rates in the south than in the north. No such gradient is found for uveal melanoma. Similar findings have been published for CMM in other Caucasian populations, with the exception of Europe as a whole. In most populations the ratios of uveal melanoma incidence rates to those of CMM tend to decrease with increasing CMM rates. This is also true for Europe, in spite of the fact that in this region there is an inverse latitude gradient of CMM, with higher rates in the north than in the south.

In Norway the incidence rates of CMM have increased until about 1990 but have been constant or even decreased (for young people) after that time, indicating constant or decreasing sun exposure. The uveal melanoma rates have been increasing after 1990. In most other populations the incidence rates of CMM have been increasing until recently while those of uveal melanoma have been decreasing. These data generally support the assumption that uveal melanomas are not generated by ultraviolet (UV) radiation and that solar UV, via its role in vitamin D photosynthesis, may have a protective effect.

Introduction

Ultraviolet B (UVB) radiation from the sun is carcinogenic and certainly a main cause of skin cancers, most likely including melanoma.[1-7] However, UVB can practically not reach melanocytes in the uveal tract, where melanomas sometimes arise.[8-10] Ultraviolet A (UVA) radiation which may be inducing cutaneous malignant melanoma (CMM),[11-13] may reach some of the melanocytes in the eye, although at much lower fluence rates than those reaching melanocytes in the skin.[9] Thus, while it is almost universally accepted that solar radiation is a cause of CMM,[1-6,14-16] this is not likely to be true for uveal melanomas. In fact, epidemiological investigations indicate an inverse latitude gradient for uveal melanomas in the US,[10] and choroidal melanomas are not distributed as might be expected if UV played a major role.[17,18] However, some studies indicate a positive correlation between sun exposure and ocular melanoma,[17,19-25] so the question is not settled yet. For choroidal and ciliary body melanoma (melanoma of the uveal tract) solar radiation may play a role since these localizations can be reached by UV according to some authors.[25] However, this is not certain since ciliary body and choroidal melanocytes are covered internally by densely pigmented retinal or ciliary pigment epithelium and externally by nontransparent sclera. Furthermore, UV must pass the cornea, the lens and the vitreous region before reaching the pigment epithelia. It has been stated that only 0.1% UVA can pass an adult lens.[26] Thus, only small fluences of even UVA can probably reach the melanocytes in the ciliary body and the choroid.[18] Also other authors argue in agreement with this,[27,28] so the last word has probably not been said on this matter.

In the present work we followed two possible ways to elucidate this problem further: (1) Comparisons of north-south gradients for cutaneous and uveal melanomas, and (2) Comparisons of time trends of the two melanoma types. Increasing time trends of CMM incidence rates indicate increasing sun exposure. In many populations, including the Norwegian, CMM rates increased with doubling times of about 15–20 years up to 1985–1990.[3,16,29] After that time the increase is less marked in many countries,[30,31] and in Norway even a decrease has been observed for young people.[16,29]

*Correspondence to: Asta Juzeniene; Email: asta.juzeniene@rr-research.no
Submitted: 02/22/10; Accepted: 03/01/10
Previously published online: www.landesbioscience.com/journals/dermatoendocrinology/article/11745

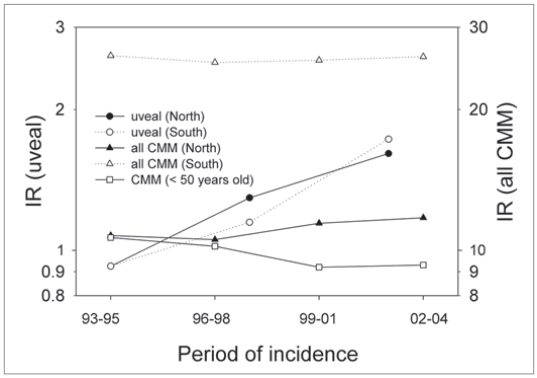

Figure 1. Time trends of the incidence rates (IR) of CMM and uveal melanoma in Norway for the period 1993–2004. For CMM over all data (men and women, age adjusted. Rates for persons below 50 years (all country) are shown separately).

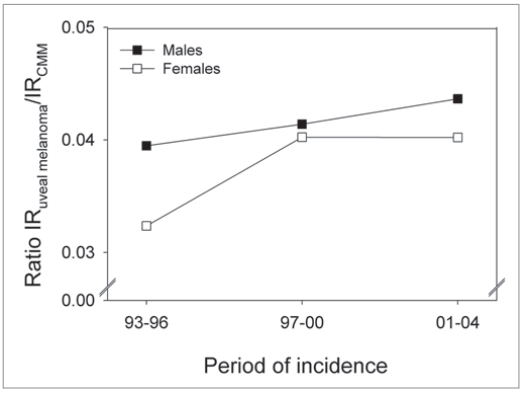

Figure 2. The ratio of the number of uveal melanomas to that of CMM in Norway for three periods. Data shown separately for women and men.

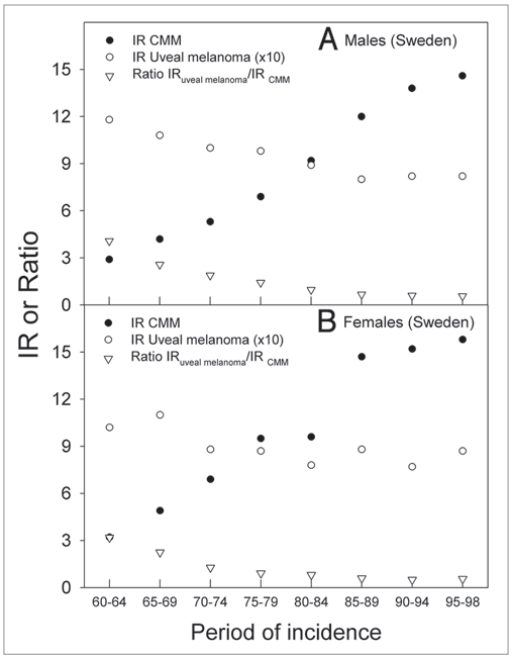

Figure 3. The age adjusted incidence rates (IR) of CMM (per 10,000) and uveal melanoma (per 10,00,000) in Sweden, (A) for men, (B) for women. The ratio of the incidence rates of uveal melanoma to CMM is shown at the bottom of the panels in open triangles.

Here we show that after 1990 there has been an increase in uveal melanoma rates in Norway, opposite to what is found for CMM. There is no north-south gradient for uveal melanoma in the time period investigated (1993–2004), while there is a prominent north-south gradient for CMM. These data will be compared with data for other populations of Caucasians, and, together with these data, discussed in view of sun exposure, melanoma generation and vitamin D photosynthesis. A protective role of vitamin D may apply for melanomas[15,29] as well as for internal cancers.[32-37]

Results

Figure 1 shows a comparison of epidemiological data of CMM and uveal melanoma in Norway. Unfortunately, we have data for uveal melanoma only from 1993. The incidence rates of CMM for both sexes together, all ages included, have been constant. However, the rates for persons younger than 50 years have decreased in the same period. The rates for uveal melanomas have increased significantly. For CMM there is a strong north-south gradient, while for uveal melanoma there is no such gradient.

The ratio of uveal melanoma cases to CMM cases has increased somewhat for both men and women in the same period (**Fig. 2**).

For Sweden data for uveal melanoma are available for a longer time, and the time trends from 1960 to 1998 are shown in **Figure 3** (A for males and B for females). Until about 1990 the CMM rates increased sharply, as in Norway,[29] while the uveal melanoma rates decreased in the same period. Thus, the ratio of uveal melanoma rates to CMM rates decreased strongly (**Fig. 3**). After 1990, however, the rates of CMM and those of uveal melanoma have been almost constant for both sexes.

The incidence rates of CMM are about three times higher in Australia and New Zealand than in Scandinavia and in The British Isles, while those of uveal melanomas are not much different (**Fig. 4**). For these countries the ratio of incidence rates of uveal melanomas to CMM are strongly decreasing with

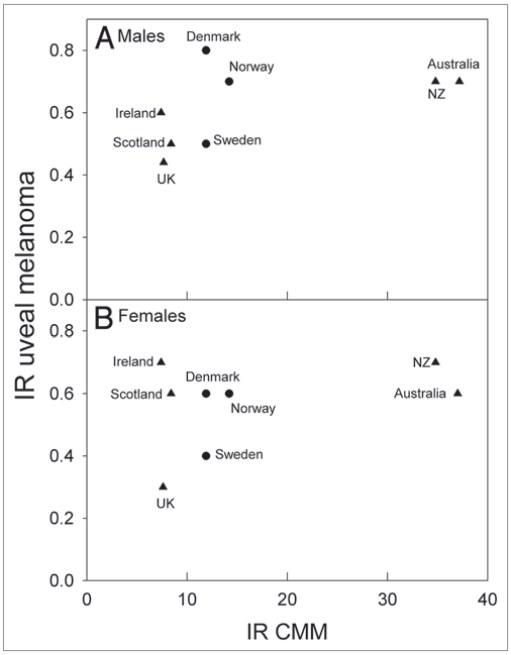

Figure 4. Age adjusted incidence rates (IR) of uveal melanomas as a function of the incidence rates of CMM (A men, B women) for some countries selected on the basis of our earlier CMM work.[12]

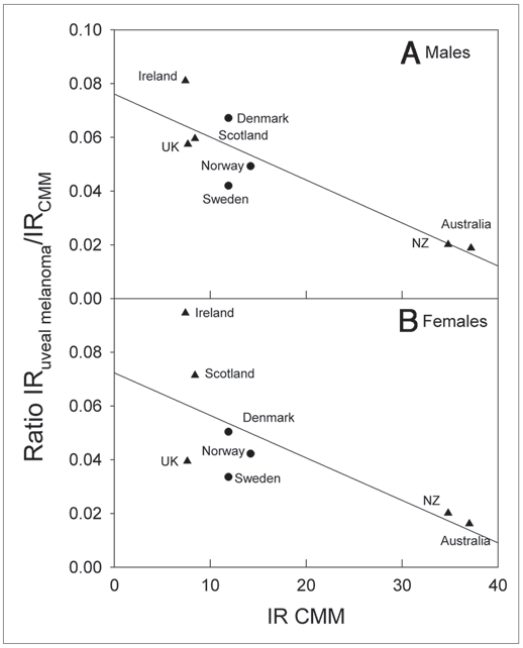

Figure 5. The ratio of the incidence rates (IR) of uveal melanoma to CMM as a function of the incidence rates of CMM for the same countries as those included in **Figure 4**.

increasing CMM rates (**Fig. 5**). This is true both when UK, Ireland, Scotland and Scandinavia are considered alone, and when also Australia and New Zealand are brought into the picture (**Fig. 5**). When those European countries, for which relevant data are available, are included, inverse latitudinal gradients are found, both for CMM and for uveal melanoma (**Fig. 6A and B**). However, the ratio of the rates of uveal melanoma to CMM increases with increasing CMM rates (**Fig. 6C**).

Discussion

Time trends. This is, to our knowledge, the first time uveal melanoma trends are analyzed for a population and a period in which CMM rates tend to decrease with time. Surprisingly, an increase in uveal melanoma rates is found. The opposite is observed for populations and periods of increasing CMM incidence trends. This is exemplified by the data for Sweden, where, until 1990, CMM incidence rates increased rapidly, with a doubling time of about 15–20 years,[15] as in practically all countries where such investigations have been carried out.[1,15] Such increasing trends of CMM rates have been observed for a long time (**Fig. 7**), and is generally explained by increasing sun exposure, notably in vacations and holydays (intermittent exposures). The rates of conjunctival melanomas in the US have increased in the period 1973–1999, as have those of CMM,[40] while the rates of uveal melanomas have remained stable.[41] The conjunctiva can be reached even by UVB radiation. For about the same time period even decreasing trends of uveal melanoma rates have been reported for Sweden and some other countries (**Fig. 7**, reviewed in refs. 1, 38, 39 and 42). As will be discussed below, in connection with latitude trends, a possibility exists that the decreasing time trends of uveal melanomas for periods of increasing time trends of CMM can be explained by a protective role of solar radiation on uveal melanomas. This might take place via vitamin D photosynthesis:[43] In time periods when CMM rates have been increasing, such as in Sweden before 1990 (**Fig. 2**) and in many other countries with Caucasian populations,[1-3,14] the sun exposure of the people have supposedly been increasing, leading to increasing vitamin D synthesis in skin, the uveal rates have generally decreased.[1] Exactly opposite trends are found for Norway after 1990 (**Fig. 1**). These data support the assumption of a protective role of solar radiation in relation for uveal melanomas. According to the CMM rates the sun exposure has increased in Sweden from 1960 to 1998 (**Fig. 3**). This agrees with the fact that also the incidence of cataract, which is caused by sun exposure also increased in Sweden from 1992 to 2000.[44]

Latitude trends. The Norwegian data show a strong north-south gradient of CMM rates (**Fig. 1**, reviewed in refs. 2 and 12). No such gradient is found for uveal melanoma (**Fig. 1**). In the US, as in Norway, the CMM rates increase with decreasing latitude.[2,3]

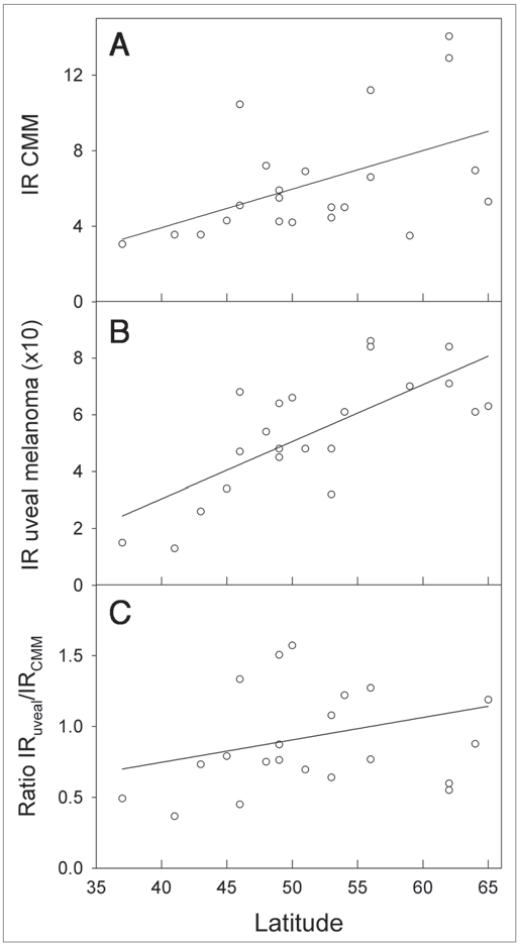

Figure 6. Incidence rates (IR) of CMM per 1,00,000 (A), uveal melanoma per 10,00,000 (B) and the ratio (C) as functions of latitude. Melanoma data were extracted from the work of Virgili et al.[38] Data refer to the period 1983–1994 and are from Ragusa (37°C), Tarragona (41°C), Navarra (43°C), Parma (45°C), Geneva (46°C), Slovenia (46°C), Bas Rhin (48°C), Saarland (49°C), CalvadosGen (49°C), Slovakia (49°C), Cracow (50°C), Eindhoven (51°C), West Midlands (53°C), Mersey (53°C), Yorkshire (54°C), Scotland (56°C), Denmark (56°C), Estonia (59°C), Sweden (62°C), Norway (62°C), Finland (64°C) and Iceland (65°C).

The uveal melanoma rates in the US show a significant opposite latitudinal trend,[10] even if Hawaii is omitted from the picture. This is interpreted as an indication of a protective role of solar radiation.[10] As for Norway, there is no significant latitudinal gradient for uveal melanomas when The British Isles, Scandinavia, Australia and New Zealand are considered (**Fig. 4**). Clearly, the higher the CMM rates are, the lower are the ratios of the rates of uveal melanoma to CMM (**Fig. 5**). The same is true for Norway (**Fig. 1**), and it seems that in these populations solar radiation is generating CMM, but not uveal melanomas. However, in these countries no protective role, as in the US, is indicated. The discrepancy of these data may be related to a possible genetical north south gradient of susceptibility to uveal melanoma, either in the US or in the countries included in **Figures 4 and 5**. Such gradients should certainly be taken into account: In Europe CMM rates are higher in the north (Scandinavia) than in the south (**Fig. 6A**). This is likely to be caused by differences in pigmentation. However, even when considering these countries with increasing CMM rates from south to north, the ratio of the incidence rates of uveal melanomas to those of CMM appears to increase with increasing rates of CMM incidence, i.e., with increasing latitude and decreasing annual UV exposure (**Fig. 6B and C**). This may be related to the observation that blue eyes, which are more prevalent in the north, are risk factors for ocular melanoma.[18,45-47] The fact that the relative risk of uveal melanomas can be as much as 19 times smaller for black persons than for white persons in the US,[18] probably indicates that melanin itself acts as a protectant.[38]

The role of solar radiation and vitamin D in uveal melanoma etiology. UV is likely to be the main carcinogen for CMM, as has been discussed by many of the authors cited above, although some controversies still exist.[48] Both UVB and UVA may play roles. The uveal tract, however, cannot be reached by UVB,[9] and, since most uveal melanomas arise in the choroid layer,[49] to which very little UVA can penetrate,[9] solar radiation is unlikely to be significantly involved in the etiology of uveal melanomas. This conclusion is strengthened by the observation that melanomas are evenly distributed on the choroid, while this is not true for solar radiation.[17] The time trends discussed above, and partly also the latitudinal trends, suggest that solar radiation is a main carcinogen for CMM, (possibly also for the rare, conjunctival melanomas[18] whose changing incidence patterns coincide with those of CMM in the US[40]), but may act as a protectant against uveal melanomas, possibly through its generation of vitamin D:[10] The time trends for uveal melanomas and for CMM are generally opposite. How can we then explain why there in some countries are no latitudinal gradients of uveal melanomas? Two factors should be considered in future investigations of this: Intermittent versus constant UV exposure, and latitudinal gradients of constitutive melanin (genetically determined). Firstly, CMM is more closely related to intermittent UV exposure than to total exposure,[1,40,43,48-50] while the vitamin D status is likely to be dependent on total exposure. The genetic impact on CMM rates among Caucasians is demonstrated by **Figure 6A**, which shows that the incidence rates of CMM decrease with decreasing latitude in Europe. The melanin pigmentation generally increases from north to south in Europe,[51] leading to decreasing rates of CMM and of vitamin D photosynthesis.[52] Unfortunately, no reliable comparisons of the vitamin D status in north and south Europe are available. The rates of uveal melanomas tend to decrease from north to south in Europe (in contrast to what is found in the US), and so does the ratio of the incidence rates for uveal melanomas to those of CMM (**Fig. 6**). It has been proposed that melanin by itself has a protective effect against uveal melanomas,[53] which may contribute to explain

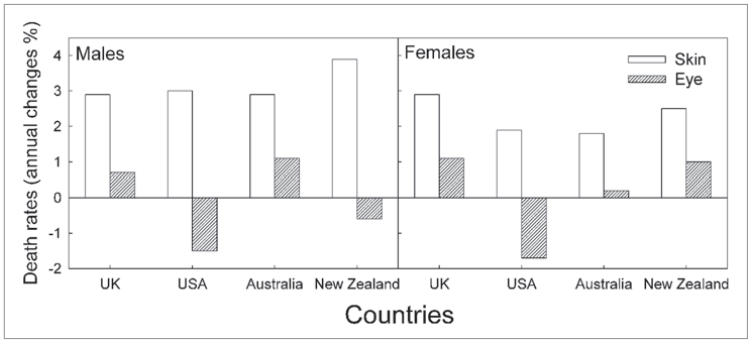

Figure 7. The annual percent changes of CMM and uveal melanomas death rates as taken from references.[1,39] Average values for the time period from 1951 to 1978.

the European data shown in the **Figure 6**. A protective role of solar radiation on CMM, in addition to an inductive role as discussed above, is indicated by the following observations: (1) The prognosis of CMM is best for summer-autumn diagnosis.[15] (2) The prognosis of CMM appears to be best for tumours arising on skin areas with signs of high UV exposure.[54] (3) The CMM incidence rates in the US have increased rapidly in the period 1992–2004, with a doubling time as short as 10 years, whereas the mortality rates have not increased significantly,[55] and it was concluded that screening associated diagnosis of thinner melanomas could not explain the increasing incidence rates. A decline of the latitudinal effect of CMM mortality rates and a stabilization of the rates in the US was predicted already in 1997.[56] Even earlier it was shown that outdoor work does little to increase the CMM risk.[45,57,58] A meta-analysis of the relation between intermittent and chronic UV exposure and uveal melanoma indicates a slightly protective role of outdoor and leisure life.[59]

Limitations of the work. First of all uveal melanomas are rare, so in many cases it is not possible to give good 95% confidence intervals. Secondly, the induction time may be different for different types of melanoma. Lentigo maligna melanoma, for instance, occurs late in life and is probably related to accumulated UV dose, in contrast to CMM, which, as stated above, seems to be related more to intermittent exposures. Thirdly, as also stated above, melanin, notably eumelanin, may act as a protectant by itself, i.e., through chemical protection and not only through UV absorption. The north south gradient of CMM in Europe may be related to such protection.

Conclusions. Comparisons of time trends and latitudinal trends of the incidence rates of CMM and uveal melanoma among Caucasians indicate that solar radiation is likely to act ac a carcinogen for CMM but not for uveal melanoma. For the latter melanoma type, solar radiation may even seem to act in a protective manner: Time trends, as well as latitudinal trends, of CMM and uveal melanoma are generally opposite in agreement with this hypothesis.

The situation is complicated in the central and northern European countries where there is an increase in CMM rates from south to north and a slightly decreasing trend of uveal melanoma. This may be related to an increase in pigmentation, possibly also to an increace in the eumelanin/pheomelanin ratio, from north to south in Europe.

Materials and Methods

In our study we used data from various sources. The incidence data for CMM and uveal melanoma in Norway are obtained from The National Cancer Registry, a population-based registry that since 1953 collects data on cancer incidence and survival.[60] In the case of uveal melanoma data are available only after 1993 when this localization was coded separately. The data for uveal melanoma in Sweden have been extracted from the work of Bergman et al.[61] while for the European plot the data are from the publication by Virgili et al.[38] The annual percent changes of CMM and uveal melanomas death rates are taken from references.[1,39] The other epidemiological data used in this work are retrieved from the online data base of the International Agency for Research on Cancer (IARC).[62]

We performed two main types of analyses: time trends for the two cancer types studied and the correlation between the occurrence of uveal melanoma and the incidence of CMM. Where it was possible we have stratified our data by age (two groups, younger and older than 50 years) and by gender since both of these factors affect the incidence rates of melanomas.

Acknowledgements

The present work was supported by the Research Council of Norway (Norges forskningsråd) and the Norwegian Cancer Society (Kreftforeningen).

References

1. Lee JA. Melanoma and exposure to sunlight. Epidemiol Rev 1982; 4:110-36.
2. Moan J, Dahlback A. Ultraviolet Radiation and Skin Cancer: Epidemiological Data from Scandinavia. In: Young AR, Young A, Bjorn LO, Moan J, Nultsch W. Environmental UV Photobiology. New York: Plenum Press 1993; 255-93.
3. Weinstock MA. Ultraviolet Radiation and Skin Cancer: Epidemiological Data from the United States and Canada. In: Young AR, Young A, Bjorn LO, Moan J, Nultsch W. Environmental UV Photobiology. New York: Plenum Press 1993; 295-344.
4. Elwood JM, Jopson J. Melanoma and sun exposure: an overview of published studies. Int J Cancer 1997; 73:198-203.
5. Gandini S, Sera F, Cattaruzza MS, Pasquini P, Picconi O, Boyle P, Melchi CF. Meta-analysis of risk factors for cutaneous melanoma: II. Sun exposure. Eur J Cancer 2005; 41:45-60.
6. Pleasance ED, Cheetham RK, Stephens PJ, McBride DJ, Humphray SJ, Greenman CD, et al. A comprehensive catalogue of somatic mutations from a human cancer genome. Nature 2010; 463:191-6.
7. Situm M, Buljan M, Bulic SO, Simic D. The mechanisms of UV radiation in the development of malignant melanoma. Coll Antropol 2007; 31:13-6.
8. Egan KM, Seddon JM, Glynn RJ, Gragoudas ES, Albert DM. Epidemiologic aspects of uveal melanoma. Surv Ophthalmol 1988; 32:239-51.
9. World Health Organization. The effects of solar UV radiation on the eye. Geneva: World Health Organization 1994; 1-56.
10. Yu GP, Hu DN, McCormick SA. Latitude and incidence of ocular melanoma. Photochem Photobiol 2006; 82:1621-6.
11. Setlow RB, Grist E, Thompson K, Woodhead AD. Wavelengths effective in induction of malignant melanoma. Proc Natl Acad Sci USA 1993; 90:6666-70.
12. Moan J, Dahlback A, Setlow RB. Epidemiological support for an hypothesis for melanoma induction indicating a role for UVA radiation. Photochem Photobiol 1999; 70:243-7.
13. Wang SQ, Setlow R, Berwick M, Polsky D, Marghoob AA, Kopf AW, Bart RS. Ultraviolet A and melanoma: a review. J Am Acad Dermatol 2001; 44:837-46.
14. Muir CS. Malignant melanoma of skin. In: Magnus K. Trends in Cancer Incidence. Washington New York London: Hemisphere Publishing Corporation 1982; 363-85.
15. Moan J, Porojnicu AC, Dahlback A. Epidemiology of cutaneous malignant melanoma. In: Ringborg U, Brandberg Y, Breitbart EW, Greinert R. Skin cancer prevention. New York: Informa Healthcare 2007:179-201.
16. Moan J, Porojnicu AC, Dahlback A. Ultraviolet radiation and malignant melanoma. Adv Exp Med Biol 2008; 624:104-16.
17. Schwartz LH, Ferrand R, Boelle PY, Maylin C, D'Hermies F, Virmont J. Lack of correlation between the location of choroidal melanoma and ultraviolet-radiation dose distribution. Radiat Res 1997; 147:451-6.
18. Hu DN. Photobiology of ocular melanocytes and melanoma. Photochem Photobiol 2005; 81:506-9.
19. Tucker MA, Shields JA, Hartge P, Augsburger J, Hoover RN, Fraumeni JF Jr. Sunlight exposure as risk factor for intraocular malignant melanoma. N Engl J Med 1985; 313:789-92.
20. Swerdlow AJ, Storm HH, Sasieni PD. Risks of second primary malignancy in patients with cutaneous and ocular melanoma in Denmark 1943–1989. Int J Cancer 1995; 61:773-9.
21. Graham S, Marshall J, Haughey B, Stoll H, Zielezny M, Brasure J, West D. An inquiry into the epidemiology of melanoma. Am J Epidemiol 1985; 122:606-19.
22. Dolin PJ, Johnson GJ. Solar ultraviolet radiation and ocular disease: a review of the epidemiological and experimental evidence. Ophthalmic Epidemiol 1994; 1:155-64.
23. Dolin PJ, Foss AJ, Hungerford JL. Uveal melanoma: is solar ultraviolet radiation a risk factor? Ophthalmic Epidemiol 1994; 1:27-30.
24. Seddon JM, Gragoudas ES, Glynn RJ, Egan KM, Albert DM, Blitzer PH. Host factors, UV radiation and risk of uveal melanoma. A case-control study. Arch Ophthalmol 1990; 108:1274-80.
25. Vajdic CM, Kricker A, Giblin M, McKenzie J, Aitken J, Giles GG, Armstrong BK. Sun exposure predicts risk of ocular melanoma in Australia. Int J Cancer 2002; 101:175-82.
26. Boettner EA, Walter JR. Transmission of the ocular media. Invest Ophthalmol Vis Sci 1962; 1:776-83.
27. Li W, Judge H, Gragoudas ES, Seddon JM, Egan KM. Patterns of tumor initiation in choroidal melanoma. Cancer Res 2000; 60:3757-60.
28. Horn EP, Hartge P, Shields JA, Tucker MA. Sunlight and risk of uveal melanoma. J Natl Cancer Inst 1994; 86:1476-8.
29. Moan J, Porojnicu AC, Dahlback A, Setlow RB. Addressing the health benefits and risks, involving vitamin D or skin cancer, of increased sun exposure. Proc Natl Acad Sci USA 2008; 105:668-73.
30. Gaudette LA, Gao RN. Changing trends in melanoma incidence and mortality. Health Rep 1998; 10:29-41.
31. Lens MB, Dawes M. Global perspectives of contemporary epidemiological trends of cutaneous malignant melanoma. Br J Dermatol 2004; 150:179-85.
32. Robsahm TE, Tretli S, Dahlback A, Moan J. Vitamin D3 from sunlight may improve the prognosis of breast-, colon- and prostate cancer (Norway). Cancer Causes Control 2004; 15:149-58.
33. Moan J, Porojnicu A, Lagunova Z, Berg JP, Dahlback A. Colon cancer: prognosis for different latitudes, age groups and seasons in Norway. J Photochem Photobiol B 2007; 89:148-55.
34. Porojnicu AC, Lagunova Z, Robsahm TE, Berg JP, Dahlback A, Moan J. Changes in risk of death from breast cancer with season and latitude: sun exposure and breast cancer survival in Norway. Breast Cancer Res Treat 2007; 102:323-8.
35. Porojnicu AC, Dahlback A, Moan J. Sun exposure and cancer survival in Norway: changes in the risk of death with season of diagnosis and latitude. Adv Exp Med Biol 2008; 624:43-54.
36. Deeb KK, Trump DL, Johnson CS. Vitamin D signalling pathways in cancer: potential for anticancer therapeutics. Nat Rev Cancer 2007; 7:684-700.
37. Giovannucci E. Vitamin D status and cancer incidence and mortality. Adv Exp Med Biol 2008; 624:31-42.
38. Virgili G, Gatta G, Ciccolallo L, Capocaccia R, Biggeri A, Crocetti E, et al. Incidence of uveal melanoma in Europe. Ophthalmology 2007; 114:2309-15.
39. Strickland D, Lee JA. Melanomas of eye: stability of rates. Am J Epidemiol 1981; 113:700-2.
40. Yu GP, Hu DN, McCormick S, Finger PT. Conjunctival melanoma: is it increasing in the United States? Am J Ophthalmol 2003; 135:800-6.
41. Singh AD, Topham A. Incidence of uveal melanoma in the United States: 1973-1997. Ophthalmology 2003; 110:956-61.
42. Stang A, Parkin DM, Ferlay J, Jockel KH. International uveal melanoma incidence trends in view of a decreasing proportion of morphological verification. Int J Cancer 2005; 114:114-23.
43. Osborne JE, Hutchinson PE. Vitamin D and systemic cancer: is this relevant to malignant melanoma? Br J Dermatol 2002; 147:197-213.
44. Lundstrom M, Stenevi U, Thorburn W. The Swedish National Cataract Register: A 9-year review. Acta Ophthalmol Scand 2002; 80:248-57.
45. Gallagher RP, Elwood JM, Rootman J, Spinelli JJ, Hill GB, Threlfall WJ, Birdsell JM. Risk factors for ocular melanoma: Western Canada Melanoma Study. J Natl Cancer Inst 1985; 74:775-8.
46. Holly EA, Aston DA, Char DH, Kristiansen JJ, Ahn DK. Uveal melanoma in relation to ultraviolet light exposure and host factors. Cancer Res 1990; 50:5773-7.
47. Hu DN, Yu GP, McCormick SA, Schneider S, Finger PT. Population-based incidence of uveal melanoma in various races and ethnic groups. Am J Ophthalmol 2005; 140:612-7.
48. Shuster S. Is sun exposure a major cause of melanoma? No. BMJ 2008; 337:764.
49. Grin JM, Grant-Kels JM, Grin CM, Berke A, Kels BD. Ocular melanomas and melanocytic lesions of the eye. J Am Acad Dermatol 1998; 38:716-30.
50. Oliveria SA, Saraiya M, Geller AC, Heneghan MK, Jorgensen C. Sun exposure and risk of melanoma. Arch Dis Child 2006; 91:131-8.
51. Jablonski NG, Chaplin G. The evolution of human skin coloration. J Hum Evol 2000; 39:57-106.
52. Zittermann A, Schleithoff SS, Koerfer R. Putting cardiovascular disease and vitamin D insufficiency into perspective. Br J Nutr 2005; 94:483-92.
53. Hu DN, Simon JD, Sarna T. Role of ocular melanin in ophthalmic physiology and pathology. Photochem Photobiol 2008; 84:639-44.
54. Berwick M, Lachiewicz A, Pestak C, Thomas N. Solar UV exposure and mortality from skin tumors. Adv Exp Med Biol 2008; 624:117-24.
55. Linos E, Swetter SM, Cockburn MG, Colditz GA, Clarke CA. Increasing Burden of Melanoma in the United States. J Invest Dermatol 2009.
56. Lee JA. Declining effect of latitude on melanoma mortality rates in the United States. A preliminary study. Am J Epidemiol 1997; 146:413-7.
57. Goodman KJ, Bible ML, London S, Mack TM. Proportional melanoma incidence and occupation among white males in Los Angeles County (California, United States). Cancer Causes Control 1995; 6:451-9.
58. Armstrong BK. Epidemiology of malignant melanoma: intermittent or total accumulated exposure to the sun? J Dermatol Surg Oncol 1988; 14:835-49.
59. Shah CP, Weis E, Lajous M, Shields JA, Shields CL. Intermittent and chronic ultraviolet light exposure and uveal melanoma: a meta-analysis. Ophthalmology 2005; 112:1599-607.
60. Moller B, Aagnes B. Cancer in Norway 2005. Cancer incidence, mortality, survival and prevalence in Norway. In: Bray F, ed. Oslo, Norway: Cancer Registry of Norway 2006.
61. Bergman L, Seregard S, Nilsson B, Ringborg U, Lundell G, Ragnarsson-Olding B. Incidence of uveal melanoma in Sweden from 1960 to 1998. Invest Ophthalmol Vis Sci 2002;43:2579-83.
62. The International Agency for Research on Cancer (IARC), Cancer Incidence in Five Continents Volumes I to IX, 2010, http://ci5.iarc.fr/CI5i-ix/ci5i-ix.htm (accessed January 2010).

III

DOI: 10.1111/j.1468-3083.2011.04023.x JEADV

ORIGINAL ARTICLE

Comparison of the time and latitude trends of melanoma incidence in anorectal region and perianal skin with those of cutaneous malignant melanoma in Norway

E. Micu,[†,*] A. Juzeniene,[†] J. Moan[†,‡]

[†]Department of Radiation Biology, The Norwegian Radium Hospital, Oslo University Hospital, Oslo, Norway
[‡]Institute of Physics, University of Oslo, Blindern, Oslo, Norway
*Correspondence: E. Micu. E-mail: emanuela.cicarma@rr-research.no

Abstract

Background Melanoma incidence is increasing in many parts of the world. The main environmental risk factor is exposure to solar radiation. However, melanomas may arise also on non-sun-exposed areas (uveal and mucosal melanomas) and little is known about a possible relationship between sun exposure and melanoma on such locations.

Objectives We have compared the time and latitude trends of melanoma incidence in the anorectal region and perianal skin (non-sun-exposed sites) with those of cutaneous malignant melanoma (CMM) (sun-exposed skin) to gain more information about the relationship between sun exposure and melanoma on such sites.

Methods We analysed epidemiological data from the Cancer Registry of Norway for melanomas of the anorectal mucosa, perianal skin and overall CMM for the time period 1966–2007.

Results We found that melanoma incidence on these shielded sites tends to decrease or remain constant over a period during which the CMM rates increase. This is true both in the North and in the South regions of Norway. Comparison of latitudinal trends of the incidence rates of CMM and melanoma on these shielded sites shows that there is no latitude gradient for melanoma of the anorectal mucosa and perianal skin, whereas there is a strong one for CMM.

Conclusions The time and latitudinal trends are likely to support the assumption that melanomas on these shielded sites are not generated by ultraviolet radiation. Possible causes and significances of these trends are discussed.

Received: 30 August 2010; Accepted: 31 January 2011

Conflict of interest

None declared.

Funding sources

This study was supported by the Research Foundation of the Norwegian Radium Hospital and the Norwegian Cancer Society (Kreftforeningen).

Introduction

Malignant melanoma is a heterogeneous disease with many atypical variants. Many genetic and environmental factors may play roles as risk factors. For cutaneous malignant melanoma (CMM), the main environmental risk factor is exposure to solar radiation. Supposedly due to increasing exposure, its incidence has been increasing in most parts of the world until recently.[1] However, despite their common origin with CMM (the melanocytes), the aetiological factors for mucosal melanomas remain obscure. These sites are not subjected to direct sun exposure, and their incidence rates have been relatively constant over time, at least in comparison with CMM.[2,3]

The US National Cancer Data Base has reported that mucosal melanoma represents the third most common site of primary melanomas after the skin and eye, with anorectal malignant melanoma (AMM) being the second most frequent mucosal site after the head and neck.[4] Still, mucosal melanomas remain rare tumours and are difficult to diagnose. AMM is of particular relevance for the study of risk factors other than sun exposure and for *de novo* melanoma development.

In this study, we have studied time and latitude trends of the incidence rates of melanoma in the anorectal region and perianal skin (non-sun-exposed sites), in comparison with those of CMM

on more or less sun-exposed skin. We have used epidemiological data for a period of 40 years in Norway.

Materials and methods

We analysed epidemiological data from the Cancer Registry of Norway for the anorectal region, perianal skin and overall skin melanomas for the time period 1966–2007. The melanoma cases are coded by topography according to the 7th revision of the International Classification of Diseases (ICD-7) with local modifications (the code 190.x for skin melanoma). The Cancer Registry of Norway has not begun to use ICD-O-2 until 1993 and the data before 1993 have not been recoded in ICD-O-2; since 1993, there is a semi-automatic conversion of ICD-O-2 codes into ICD-7 codes. For all sites other than the skin, to ensure consistent data extraction, we combined the ICD-7 code for the specific location and the morphologies for malignant melanomas according to Manual of Tumor Nomenclature and Coding (MoTNaC) and ICD-O-2. Thus, we identified the perianal skin melanoma according to the ICD-7 specific code 190.5 (it does not include the melanoma of the scrotum, coded as 190.6) and the AMM cases according to the ICD-7 code 154.0 and 154.1 and ICD-O-2 codes C20.9, C21.0, C21.1, C21.2 and C21.8, combined with the morphology codes for malignant melanoma according to MoTNaC and ICD-O-2.

We used crude incidence rates (incidence number divided by the Norwegian population in the same period and region) to compare time trends between CMM and AMM and perianal melanomas for the entire Norway as well as separately for the North and South regions of this country. Norway is divided into 20 counties; the South region of Norway is defined in this study as referring to the counties 1, 2, 4 and 6–11 (mean latitude 59.5° with high annual ambient ultraviolet exposure[5]), and the North region to the counties 18, 19 and 20 (mean latitude 69.5° with low annual ambient ultraviolet exposure[5]). The values plotted represent incidence rates for 20-year periods per 1 000 000 persons (as the case numbers are low for melanoma on non-sun-exposed sites), for the following time frames: 1966–1986 and 1987–2007, using a logarithmic scale. [Correction added on 10 September 2011, after first online publication: The years "1966–1989 and 1989–2007" have been changed to "1966–1986 and 1987–2007".] Crude time trends have been estimated by fitting a regression line for the two data points (corresponding to the two periods analysed), using SigmaPlot 10.0 software from Systat Software, Inc. (Richmond, CA, USA). $P < 0.05$ was considered significant.

Results

During the study period (1966–2007), there were 75 cases of AMM (30 men and 45 women) and 65 cases of perianal melanomas (23 men and 42 women). The vast majority of patients was a population older than 50 years (data not shown), with a higher incidence rate in women than in men (statistical significant in the case of perianal melanoma). Concerning CMM, there were 29 018 cases registered during the 40 years we have analysed, specifically 15 584 female patients and 13 433 male patients.

In the present analysis, we found that the rates of melanoma incidence on shielded sites, like anorectal mucosa and perianal skin, tend to remain constant or decrease over a time period with increasing CMM rates, with a possible exception for AMM in women, which tends to increase slightly, although non-significantly (Figs 1 and 2). Table 1 shows a significant difference between the two periods included in the analysis (1966–1986 and 1987–2007) for the CMM incidence rates, but with no statistically significant difference with regard to the other types of melanomas.

Furthermore, we combined the AMM cases with the perianal skin melanoma cases, men and women, to have access to a

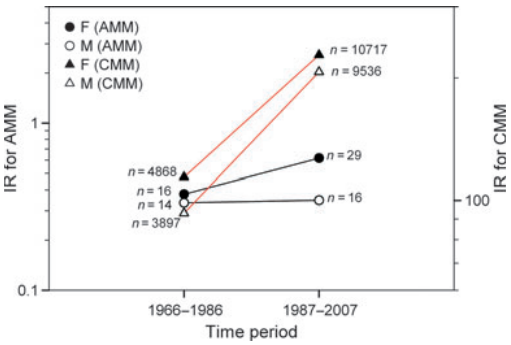

Figure 1 Time trends of anorectal melanoma (AMM) incidence in comparison with cutaneous malignant melanoma (CMM) incidence for two time periods, 1966–1986 and 1987–2007. Incidence rates (IR) are crude rates per one million persons, Norwegian population, log scale. *N = number of cases, M = male, F = female*.

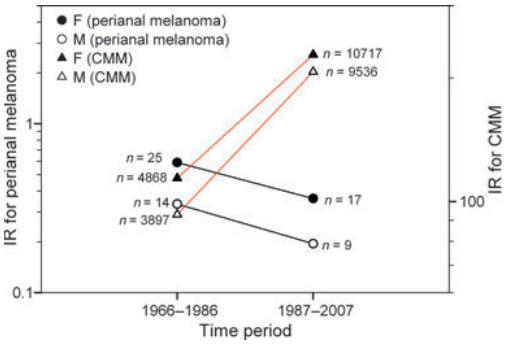

Figure 2 Time trends of perianal melanoma incidence in comparison with cutaneous malignant melanoma (CMM) incidence for two time periods, 1966–1986 and 1987–2007. Incidence rates (IR) are crude rates per one million persons, Norwegian population, log scale. *N = number of cases, M = male, F = female.*

Table 1 Comparison between two time periods: 1966–1986 and 1987–2007 of different anatomic localizations of melanoma in Norway

Type of melanoma (male and female combined)	1966–1986		1987–2007		*t*-test
	Number of cases	Incidence rate*	Number of cases	Incidence rate*	
All cutaneous melanoma	8765	103.12	20253	216.99	$P < 0.001$
Anorectal melanoma	30	0.35	45	0.48	$P > 0.05$
Perianal skin melanoma	39	0.46	26	0.27	$P > 0.05$
Anorectal + perianal skin melanomas	69	0.81	71	0.75	$P > 0.05$

*Incidence rates are crude incidence rates per one million persons, Norwegian population.

Table 2 Comparison between the North and the South regions of Norway during 1966–2007 of different anatomic localizations of melanoma

Type of melanoma (male and female combined)	North*		South*		*t*-test
	Number of cases	Incidence rate†	Number of cases	Incidence rate†	
All cutaneous melanoma	1498	76.88	16071	187.74	$P < 0.001$
Anorectal melanoma	8	0.41	36	0.42	$P > 0.05$
Perianal skin melanoma	7	0.35	31	0.37	$P > 0.05$
Anorectal + perianal skin melanomas	15	0.76	67	0.80	$P > 0.05$

*The North region of Norway has a mean latitude of 69.5° and the South region, 59.5°.
†Incidence rates are crude incidence rates per one million persons, Norwegian population.

considerable number of cases to ensure a better statistical analysis of time trends for the North and South regions of Norway. We did not find any significant latitude gradient for AMM and perianal melanoma, for the time frame 1966–2007, in contrast with CMM, for which there is a significant latitude gradient, between the North and the South regions (Table 2). When we analysed the time trend in these two regions, we found that the incidence rates of CMM have increased for both the North and the South regions of Norway, with a significant latitude gradient for both time periods studied (Fig. 3). For the other types of melanoma, there was a constant time trend for both regions, although we did observe a non-significant decrease in the incidence rate in the North region in the recent period (Fig. 3).

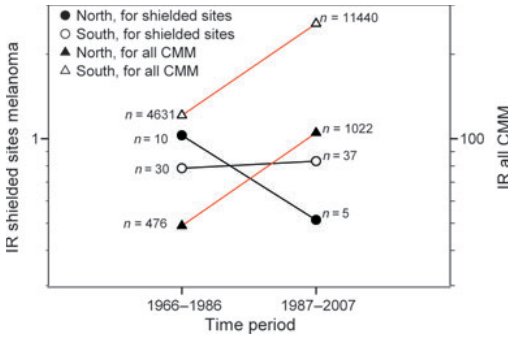

Figure 3 Time trends in the North and South regions of Norway for shielded sites (anorectal and perianal melanoma combined) and cutaneous malignant melanoma (CMM), 1966–2007. The north region has a mean latitude of 69.5° and the South region, 59.5°. Incidence rates (IR) are crude rates per one million persons, Norwegian population, male and female cases combined, log scale. *N = number of cases, M = male, F = female.*

Discussion

Intermittent sun exposure and sunburn are likely to be strong predictors for CMM.[6] However, melanomas may arise also on non-sun-exposed areas (like mucosal melanomas and uveal melanomas), and little is known about a possible relationship between sun exposure and melanoma on such areas other than the fact that direct sun exposure can be ruled out. Most of the information about the incidence of mucosal melanomas, in general, and of AMM, in particular, comes from case reports, but estimation of true incidence rates can be achieved from population-based data, such as national cancer registries. A few such studies are reported in the literature, mainly with regard to AMM.[4,7–10]

The higher rates among women (both on skin and perianal regions) are difficult to explain. There is no solid evidence linking female sex hormones to melanoma risk,[11–14] although oestrogens are known to increase the number of melanocytes and modify their melanin content.[15] The expression of the human melanocortin-1

receptor gene is also modulated by the paracrine and endocrine sex hormones.[16] Contrary to incidence rates of CMM in European countries, in Australia and North America, there is a higher incidence rate of CMM in males than in females;[1] as for AMM in Australia, the male preponderance is also visible.[8]

Comparisons of latitudinal gradients and/or time trends of the incidence rates of melanomas on shielded sites (like mucosal and uveal melanomas) with those on non-shielded sites often show differences,[2,10,17,18] as in our current study. In some cases, incidence rates of melanoma on shielded sites tend to increase from South to North, whereas for melanoma on sun-exposed skin areas, the incidence rates decrease from South to North. For example, in USA, the uveal melanoma rates show an opposite latitudinal trend as compared with CMM.[18] In Norway, we have found no North–South gradient for uveal melanoma, whereas there is a strong one for CMM.[19,20] In USA, Weinstock has also reported a higher incidence of vulvar melanoma and AMM in the North.[10] CMM incidence rates continue to increase both in the North and in the South regions of Norway, and show a North–South gradient for different cutaneous sites as recently described by our research group,[20] but not for the shielded sites analysed in the current study, for which the time trend is constant or decreasing both in the North and in the South regions and the latitude gradient is not evident (although we must allow for possible errors due to low number of cases).

As in our study, as well as in the one by Ragnarsson-Olding *et al.*, from Sweden,[9] AMM incidence rates tend to remain constant, whereas those for CMM in these countries have increased in the last decades when compared with earlier periods. For southern latitudes, like in Queensland, Miller *et al.*[8] have found no change in the incidence rates of AMM despite the very high incidence rate of CMM in this region. Other authors have found an increased incidence rate of AMM in some regions of the USA in recent years,[4,7] perhaps due to some other aetiological factors, like HIV infection.[4]

Time and latitudinal trends are likely to support the assumption that melanomas on shielded sites are not generated by ultraviolet (UV) radiation, a hypothesis reviewed in the work of Row and Weiser.[21] The possibility exists that solar UV radiation, probably via its role in vitamin D photosynthesis in exposed skin, may have a systemic, protective effect against melanomas on shielded sites (in the same way as for some internal cancers[22,23]) in addition to its melanomagenic effect on the exposed skin, as we have proposed earlier for vulvar melanomas.[24] Vitamin D is considered the precursor of a hormone (1,25-dihydroxyvitamin D, $1,25(OH)_2D$), which has many biological effects in the skin, such as regulation of cell growth and differentiation and modulation of the immune system.[25] The anti-cancer effect of $1,25(OH)_2D$ has been documented in different melanoma cell lines expressing vitamin D nuclear receptor, VDR,[26] but the relationship between vitamin D induced by sun exposure and melanoma is far more complex than for other systemic cancers.[27]

The protective role of vitamin D for mucosal melanomas is supported by the higher incidence of AMM in people with darker skin pigmentation,[10,21,28] as individuals with darker skin need higher doses of UVB to induce the same amount of vitamin D as compared with individuals with lighter skin,[29] and many African-Americans are vitamin D-deficient.[30] Other researchers have found a lower incidence of mucosal melanomas (in particular vulvar melanomas) in dark-pigmented individuals, possibly related to the antioxidant properties of melanin rather than its photoscreening effects.[31] Overall, when white skin/dark skin ratios are calculated, there is a lower racial difference for mucosal melanomas than for cutaneous melanomas[31,32]. Thus, factors other than solar radiation may also act for mutagenesis on these sites, like the factors present in the mucus membrane, as hypothesized by Hu *et al.*[31]

Increased availability of the immunohistochemistry methods, improvement of endoscopy and increased longevity may influence the incidence rates of AMM. More and more tumours in the anorectal region that were initially considered to be other types of cancer, i.e. leiomyosarcomas, small cell carcinomas or lymphoma, are being classified later on as melanomas due to immunohistochemical analysis.[33,34] Despite this, AMMs remain rare tumours with poor prognosis.[35]

For a long time, melanoma of the gastro-intestinal tract has not been considered as a primary lesion, but as a metastatic one.[36] It is still difficult to diagnose and the following criteria are suggested for considering a melanoma to be primary in the gastro-intestinal tract: the presence of atypical melanocytes along the basal epithelium, the absence of melanomas elsewhere and no history of metastatic melanoma.[37] It is associated with the melanocytes that have migrated from the neural crest or from the mucocutaneous junctions. The melanocytes exert complex functions other than production, transport and transfer of melanin to the keratinocytes.[38] Many other cells than keratinocytes interact actively with melanocytes and even elements of the vascular system and nerve connections influence their functions. Although melanin synthesis is the principal function of melanocytes,[39] they also reside in invisible anatomical areas such as stria vascularis of the cochlea,[40] leptomeninges[41] or retinal pigment,[42] where their role is mainly to counteract oxidative stress. They may also contribute to regional immune responses.[38,43] Thus, mucosal/anorectal melanocytes may also play an important role against oxidative stress, and malignant transformation may be related to oxidative stress in these regions or to the immunosuppression for various reasons, especially because these types of melanomas occur mostly in patients older than 50 years, suggesting the typical progressive acquisition of genetic mutations that ultimately lead to a malignant phenotype.

Several studies classify mucosal melanomas to be different from CMM at the molecular level, even though mucosal melanomas display histological and immunohistochemical features similar to those of CMM. The exon 15 BRAF mutation (V599E) is highly expressed in CMM, more frequently in sites intermittently exposed to UV,[44] whereas it is not so frequently observed in mucosal

melanomas.[45] Another genetic difference is the KIT a receptor tyrosine kinase mutation. KIT is thought to play an important role in melanocyte development and differentiation.[46] It is expressed in normal melanocytes, benign nevi and in *in situ* melanomas, but it tends to be reduced in invasive melanomas.[47,48] Recent studies have shown that KIT is an important oncogene for mucosal melanomas, for melanomas on the acral skin and on the skin with chronic sun damage,[49–51] but, as recently pointed out,[52] the melanocytes harbouring KIT mutations require a tissue-specific environment (such as hypoxia) to progress in their malignant transformation. In addition, a subset of mucosal melanomas with activating KIT mutations responds to KIT inhibitors,[49,53] showing that these KIT mutants are promising therapeutic targets.

We were unable to verify the accuracy of diagnosis and coding of these rare tumours reported to the Norwegian Cancer Registry. The errors may have affected the results, and the directions and roles of such effects are uncertain.

Each melanoma subtype may have unique aetiological initiating factors and may be influenced by its microenvironment with different progression pathways[54] for which the solar radiation may play different roles.

Acknowledgements

We acknowledge the Cancer Registry of Norway (Institute of Population-based Cancer Research) for providing us the epidemiological data, with special thanks to Siri Larønningen, and the financial support of the Norwegian Cancer Society (Kreftforeningen) and The Research Foundation of the Norwegian Radium Hospital.

References

1 MacKie R.M., Hauschild A., Eggermont AM. Epidemiology of invasive cutaneous melanoma. *Ann Oncol* 2009; **20**(Suppl. 6): vi1–vi7.
2 Chang AE, Karnell LH, Menck HR. The National Cancer Data Base report on cutaneous and non-cutaneous melanoma: a summary of 84,836 cases from the past decade. The American College of Surgeons Commission on Cancer and the American Cancer Society. *Cancer* 1998; **83**: 1664–1678.
3 Ross MI, Stern SJ. Mucosal melanomas. In Balch CM, Houghton AN, Milton GW, Sober AJ, Soong S-J, eds. *Cutaneous Melanoma*, 3rd edn. J.B. Lipincott, Philadelphia, 1998: 195–206.
4 Cagir B, Whiteford MH, Topham A, Rakinic J, Fry RD. Changing epidemiology of anorectal melanoma. *Dis Colon Rectum* 1999; **42**: 1203–1208.
5 Porojnicu AC, Robsahm TE, Dahlback A *et al.* Seasonal and geographical variations in lung cancer prognosis in Norway. Does Vitamin D from the sun play a role? *Lung Cancer* 2007; **55**: 263–270.
6 Elwood JM, Gallagher RP. Body site distribution of cutaneous malignant melanoma in relationship to patterns of sun exposure. *Int J Cancer* 1998; **78**: 276–280.
7 Cote TR, Sobin LH. Primary melanomas of the esophagus and anorectum: epidemiologic comparison with melanoma of the skin. *Melanoma Res* 2009; **19**: 58–60.
8 Miller BJ, Rutherford LF, McLeod GR, Cohen JR. Where the sun never shines: anorectal melanoma. *Aust N Z J Surg* 1997; **67**: 846–848.
9 Ragnarsson-Olding BK, Nilsson PJ, Olding LB, Nilsson BR. Primary ano-rectal malignant melanomas within a population-based national patient series in Sweden during 40 years. *Acta Oncol* 2009; **48**: 125–131.
10 Weinstock MA. Epidemiology and prognosis of anorectal melanoma. *Gastroenterology* 1993; **104**: 174–178.
11 Karagas MR, Zens MS, Stukel TA *et al.* Pregnancy history and incidence of melanoma in women: a pooled analysis. *Cancer Causes Control* 2006; **17**: 11–19.
12 Lea CS, Holly EA, Hartge P *et al.* Reproductive risk factors for cutaneous melanoma in women: a case-control study. *Am J Epidemiol* 2007; **165**: 505–513.
13 MacKie RM, Bray CA. Hormone replacement therapy after surgery for stage 1 or 2 cutaneous melanoma. *Br J Cancer* 2004; **90**: 770–772.
14 Smith MA, Fine JA, Barnhill RL, Berwick M. Hormonal and reproductive influences and risk of melanoma in women. *Int J Epidemiol* 1998; **27**: 751–757.
15 Jee SH, Lee SY, Chiu HC, Chang CC, Chen TJ. Effects of estrogen and estrogen receptor in normal human melanocytes. *Biochem Biophys Res Commun* 1994; **199**: 1407–1412.
16 Scott MC, Suzuki I, bdel-Malek ZA. Regulation of the human melanocortin 1 receptor expression in epidermal melanocytes by paracrine and endocrine factors and by ultraviolet radiation. *Pigment Cell Res* 2002; **15**: 433–439.
17 Ragnarsson-Olding B, Johansson H, Rutqvist LE, Ringborg U. Malignant melanoma of the vulva and vagina. Trends in incidence, age distribution, and long-term survival among 245 consecutive cases in Sweden 1960–1984. *Cancer* 1993; **71**: 1893–1897.
18 Yu GP, Hu DN, McCormick SA. Latitude and incidence of ocular melanoma. *Photochem Photobiol* 2006; **82**: 1621–1626.
19 Moan J, Cicarma E, Setlow R, Porojnicu AC, Grant WB, Juzeniene A. Time trends and latitude dependence of uveal and cutaneous malignant melanoma induced by solar radiation. *Dermato-Endocrinology* 2010; **2**: 1–6.
20 Cicarma E, Juzeniene A, Porojnicu AC, Bruland OS, Moan J. Latitude gradient for melanoma incidence by anatomic site and gender in Norway 1966–2007. *J Photochem Photobiol B* 2010; **101**: 174–178.
21 Row D, Weiser MR. Anorectal melanoma. *Clin Colon Rectal Surg* 2009; **22**: 120–126.
22 Moan J, Porojnicu AC, Robsahm TE *et al.* Solar radiation, vitamin D and survival rate of colon cancer in Norway. *J Photochem Photobiol B* 2005; **78**: 189–193.
23 Schwartz GG, Skinner HG. Vitamin D status and cancer: new insights. *Curr Opin Clin Nutr Metab Care* 2007; **10**: 6–11.
24 Moan J, Porojnicu AC, Dahlback A, Grant WB, Juzeniene A. Where the sun does not shine: Is sunshine protective against melanoma of the vulva? *J Photochem Photobiol B* 2010; **101**: 179–183.
25 Lehmann B. The vitamin D3 pathway in human skin and its role for regulation of biological processes. *Photochem Photobiol* 2005; **81**: 1246–1251.
26 Evans SR, Houghton AM, Schumaker L *et al.* Vitamin D receptor and growth inhibition by 1,25-dihydroxyvitamin D3 in human malignant melanoma cell lines. *J Surg Res* 1996; **61**: 127–133.
27 Osborne JE, Hutchinson PE. Vitamin D and systemic cancer: is this relevant to malignant melanoma? *Br J Dermatol* 2002; **147**: 197–213.
28 Ahmad M, Mamoon N, Khan AH. Anorectal melanoma in northern Pakistan. *J Pak Med Assoc* 1992; **42**: 155–157.
29 Armas LA, Dowell S, Akhter M *et al.* Ultraviolet-B radiation increases serum 25-hydroxyvitamin D levels: the effect of UVB dose and skin color. *J Am Acad Dermatol* 2007; **57**: 588–593.
30 Tseng M, Giri V, Bruner DW, Giovannucci E. Prevalence and correlates of vitamin D status in African American men. *BMC Public Health* 2009; **9**: 191.
31 Hu DN, Yu GP, McCormick SA. Population-based incidence of vulvar and vaginal melanoma in various races and ethnic groups with comparisons to other site-specific melanomas. *Melanoma Res* 2010; **20**: 153–158.

32 Neugut AI, Kizelnik-Freilich S, Ackerman C. Black-white differences in risk for cutaneous, ocular, and visceral melanomas. *Am J Public Health* 1994; **84**: 1828–1829.
33 van Schaik PM, Ernst MF, Meijer HA, Bosscha K. Melanoma of the rectum: a rare entity. *World J Gastroenterol* 2008; **14**: 1633–1635.
34 Maqbool A, Lintner R, Bokhari A, Habib T, Rahman I, Rao BK. Anorectal melanoma – 3 case reports and a review of the literature. *Cutis* 2004; **73**: 409–413.
35 Brady MS, Kavolius JP, Quan SH. Anorectal melanoma. A 64-year experience at Memorial Sloan-Kettering Cancer Center. *Dis Colon Rectum* 1995; **38**: 146–151.
36 Backman H, Davidsson L. Metastases of malignant melanoma in the stomach and small intestine. *Acta Med Scand* 1965; **178**: 329–335.
37 Manouras A, Genetzakis M, Lagoudianakis E *et al.* Malignant gastrointestinal melanomas of unknown origin: should it be considered primary? *World J Gastroenterol* 2007; **13**: 4027–4029.
38 Plonka PM, Passeron T, Brenner M *et al.* What are melanocytes really doing all day long...? *Exp Dermatol* 2009; **18**: 799–819.
39 Lin JY, Fisher DE. Melanocyte biology and skin pigmentation. *Nature* 2007; **445**: 843–850.
40 Tachibana M. Sound needs sound melanocytes to be heard. *Pigment Cell Res* 1999; **12**: 344–354.
41 Goldgeier MH, Klein LE, Klein-Angerer S, Moellmann G, Nordlund JJ. The distribution of melanocytes in the leptomeninges of the human brain. *J Invest Dermatol* 1984; **82**: 235–238.
42 Bharti K, Nguyen MT, Skuntz S, Bertuzzi S, Arnheiter H. The other pigment cell: specification and development of the pigmented epithelium of the vertebrate eye. *Pigment Cell Res* 2006; **19**: 380–394.
43 Zecca L, Zucca FA, Wilms H, Sulzer D. Neuromelanin of the substantia nigra: a neuronal black hole with protective and toxic characteristics. *Trends Neurosci* 2003; **26**: 578–580.
44 Deichmann M, Krahl D, Thome M, Wust K, Hassanzadeh J, Helmke B. The oncogenic B-raf V599E mutation occurs more frequently in melanomas at sun-protected body sites. *Int J Oncol* 2006; **29**: 139–145.
45 Helmke BM, Mollenhauer J, Herold-Mende C *et al.* BRAF mutations distinguish anorectal from cutaneous melanoma at the molecular level. *Gastroenterology* 2004; **127**: 1815–1820.
46 Wehrle-Haller B. The role of Kit-ligand in melanocyte development and epidermal homeostasis. *Pigment Cell Res* 2003; **16**: 287–296.
47 Montone KT, van BP, Elenitsas R, Elder DE. Proto-oncogene c-kit expression in malignant melanoma: protein loss with tumor progression. *Mod Pathol* 1997; **10**: 939–944.
48 Willmore-Payne C, Holden JA, Tripp S, Layfield LJ. Human malignant melanoma: detection of BRAF- and c-kit-activating mutations by high-resolution amplicon melting analysis. *Hum Pathol* 2005; **36**: 486–493.
49 Antonescu CR, Busam KJ, Francone TD *et al.* L576P KIT mutation in anal melanomas correlates with KIT protein expression and is sensitive to specific kinase inhibition. *Int J Cancer* 2007; **121**: 257–264.
50 Rivera RS, Nagatsuka H, Gunduz M *et al.* C-kit protein expression correlated with activating mutations in KIT gene in oral mucosal melanoma. *Virchows Arch* 2008; **452**: 27–32.
51 Ashida A, Takata M, Murata H, Kido K, Saida T. Pathological activation of KIT in metastatic tumors of acral and mucosal melanomas. *Int J Cancer* 2009; **124**: 862–868.
52 Monsel G, Ortonne N, Bagot M, Bensussan A, Dumaz N. c-kit mutants require hypoxia-inducible factor 1alpha to transform melanocytes. *Oncogene* 2010; **29**: 227–236.
53 Lutzky J, Bauer J, Bastian BC. Dose-dependent, complete response to imatinib of a metastatic mucosal melanoma with a K642E KIT mutation. *Pigment Cell Melanoma Res* 2008; **21**: 492–493.
54 Takata M, Murata H, Saida T. Molecular pathogenesis of malignant melanoma: a different perspective from the studies of melanocytic nevus and acral melanoma. *Pigment Cell Melanoma Res* 2010; **23**: 64–71.

IV

DOI: 10.1111/j.1468-3083.2011.04162.x JEADV

ORIGINAL ARTICLE

Malignant melanomas on head/neck and foot: differences in time and latitudinal trends in Norway

A. Juzeniene,[†,*] E. Micu,[†] A.C. Porojnicu,[†] J. Moan[†,‡]

[†]Department of Radiation Biology, Institute for Cancer Research, the Norwegian Radium Hospital, Oslo University Hospital, Montebello 0310 Oslo, and [‡]Institute of Physics, University of Oslo, Blindern 0316 Oslo, Norway
*Correspondence: A. Juzeniene. E-mail: asta.juzeniene@rr-research.no

Abstract

Background Cutaneous malignant melanoma (CMM) incidence continues to increase in many parts of the world. Solar ultraviolet (UV) radiation is the main environmental risk factor for CMM. Different body locations are subjected to different doses and exposure patterns of solar UV. Time and latitudinal trends of CMMs on shielded and exposed skin give valuable information about the aetiology of these cancers. In this study, we have compared the time and latitudinal trends of CMM incidence on skin areas which are chronically (head and neck) and rarely (foot) exposed to UV radiation, to gain more information about the relationship between sun doses, exposure patterns and melanomagenesis.

Methods We have analysed epidemiological data from the Cancer Registry of Norway, for foot and head and neck CMM for two time periods: 1966–1986 and 1987–2007.

Results Cutaneous malignant melanoma incidence rate on head and neck has increased with time, while incidence rates of foot CMM have remained almost constant with time in Norway. There is a large north–south gradient in incidence rates of CMM on head and neck in Norway, while there is almost no north–south gradient for CMM incidence on foot.

Conclusions Comparisons of time trends and latitudinal trends of the incidence rates of CMM on head/neck and on foot indicate that solar radiation plays a role in the induction of the former CMM but probably not for the latter.

Received: 1 February 2011; Accepted: 6 June 2011

Conflict of interest

None declared.

Funding sources

None.

Introduction

Malignant melanoma (MM) arises in activated and genetically altered melanocytes. MM occurs mainly on the skin, but may also occur on mucosa in oral and genital regions (vulvar MM) and in the eye (ocular MM, consisting of uveal and conjunctiva MMs). For cutaneous MM (CMM), the main environmental risk factor is exposure to solar radiation.[1–3] The overall incidence rate of CMM is increasing in most parts of the world.[4] CMM may occur on different anatomical sites which get different doses of ultraviolet (UV): Head and neck are more often exposed to solar radiation than foot.

In this study, we have compared the time and latitude trends of the incidence rates of foot CMM with those of head and neck CMM. We have used epidemiological data for a period of 40 years, in Norway.

Materials and methods

We have analysed epidemiological data from the Cancer Registry of Norway, for head/neck and foot and overall CMMs for the time period 1966–2007. The melanoma cases are coded by topography, according to the 7th revision of the International Classification of Diseases (ICD-7) with local modifications (the code 190.x for skin melanoma). The Cancer Registry of Norway did not use ICD-O-2 until 1993 and the data before 1993 has not been recoded to ICD-O-2; since 1993, there is a semi-automatic conversion of ICD-O-2 to ICD-7 codes. Thus, we have identified head/neck skin melanomas according to the ICD-7 specific code 190.0, foot skin melanomas according to the ICD-7 specific code 190.3 and the total CMM cases according to the ICD-7 code 190.0, 190.1, 190.2, 190.3, 190.4, 190.5, 190.6 (only for males), 190.7, 190.8 and 190.9.

Crude incidence rates (incidence number divided by the Norwegian population in the same period and region) was used to compare time trends between foot CMM and head and neck CMM for the north, mid/west and south regions of this country. Age-adjusted rates are usually used for comparison between different populations, but to compare trends in the same population (Norwegian), crude rates were used in the present work as they are more suitable because of the low number of foot CMM cases in the different regions. In general, age-adjusted rates and crude rates are almost similar and possibly small differences will play no role for the present discussion.

Assignment of the Norwegian counties into three regions was based on ambient annual UV doses, calculated and measured as earlier described.[5,6] Norway is divided in 20 counties (Fig. 1). The south region of Norway is defined in this work as referring to the counties 1, 2, 4 and 6–11 (mean latitude 60°, with highest annual ambient UV exposure), the mid/west region for counties 5, 12 and 14–17 (mean latitude 64°, with middle annual ambient UV exposure) and the north region for the counties 18, 19 and 20 (mean latitude 70°, with lowest annual ambient UV exposure).[5,6]

The values plotted represent incidence rates for 20 year periods per 100 000 persons, for the following time periods: 1966 to 1986 and 1987 to 2007.

Relative melanoma density was calculated for CMM as previously described:[7] crude incidence rates of CMM on a given body localization (head/neck and foot) were divided by the fraction of the total body area occupied by the given localization. The values of the fraction of the skin areas of different sites are taken from Cross *et al.*,[8] and are 8.4% for head/neck and 7.0% for foot.

Figure 1 The counties of Norway.

A *t*-test was used for the comparison of the results using SigmaPlot 11.0 software from Systat Software, Inc. (Richmond, CA, USA). Significant *P*-value was considered below 0.05.

Results

The number of cases of foot, head/neck and total CMM for the period 1966–2007 are given in Table 1. The incidence numbers of new cases of CMM occurring on head/neck are similar in females and in males ($P = 0.740$), while the incidence number of foot CMM was two times higher in women than in men ($P < 0.001$).

Table 2 gives the numbers for two separate periods (1966–1986 and 1987–2007) and subdivided for three regions of Norway: north (an average annual UV dose of 26×10^4 J/m^2), mid/west (an average annual UV dose of 30.5×10^4 J/m^2) and the south (an average annual UV dose of 37×10^4 J/m^2).[5,6] The average, annual UV doses are CIE-weighted (i.e. mainly made up of UVB radiation, CIE – Commission internationale de l'éclairage or the International Commission on Illumination) and determined as earlier described together with the numbering of the Norwegian counties.[5,6] The calculated doses are relevant for real exposures of human skin as demonstrated by the age-adjusted incidence rates for squamous cell carcinoma (1965–1992), a skin cancer form expected to be related to the total, lifelong UV exposure, which are as expected: 2.7, 4.0 and 6.5 for the north, the mid/west and the south region, respectively.[6] A more detailed discussion of the data for squamous cell carcinoma can be found in reference 6. There is a large and statistically significant north–south gradient for CMM on head/neck, for both periods and for both genders (Table 2). For CMM on the foot, the gradient is much smaller and statistically insignificant almost in all cases (Table 2). For CMM on head/neck, the incidence rates have increased significantly ($P < 0.001$) from the first to the second period of observation, while for CMM on the foot, in most cases, there is only a small increase. The time trend is analysed more in detail in Fig. 2 where data for the whole country are included. The same time trends are revealed for both genders: a strong and almost continuous increase of CMM rates on head/neck, and no increase of foot CMM rates.

Table 1 The number of cases and distribution of foot CMM, head and neck CMM and total CMM in females and males in Norway for 1966–2007

Gender	Head/neck CMM	Foot CMM	Total CMM
Females	2241 (51.2%)* (14.4%)†	752 (67.2%)* (4.8%)†	15585 (53.7%)*
Males	2136 (48.8%)* (15.9%)†	367 (32.8%)* (2.7%)†	13433 (46.3%)*
Females and males	4377 (15.1%)†	1119 (3.9%)†	29018

*% by gender.
†% of total number of cases of CMM.
CMM, cutaneous malignant melanoma.

Table 2 Comparison between two time periods, 1966–1986 and 1987–2007, of foot melanoma, head and neck for north, mid/west and south regions of Norway

Location of CMM		1966–1986		1987–2007		Increase (%), ‡ P (periods)
		Number of cases	Incidence rate* (density)†	Number of cases	Incidence rate* (density)†	
Foot	Females					
	North	25	0.53 (7.5)	31	0.64 (9.2)	21 ($P = 0.39$)
	Mid/west	82	0.64 (9.2)	143	1.00 (14.4)	56 ($P = 0.01$)
	Southwest	135	0.70 (10.1)	206	0.92 (13.1)	31 ($P = 0.08$)
	Males					
	North	15	0.30 (4.3)	15	0.31 (4.4)	3 ($P = 0.96$)
	Mid/west	49	0.38 (5.5)	68	0.48 (6.9)	26 ($P = 0.21$)
	Southwest	74	0.39 (5.6)	101	0.46 (6.6)	18 ($P = 0.30$)
Head and neck	Females					
	North	43	0.90 (10.7)	68	1.41 (16.7)	57 ($P < 0.001$)
	Mid/west	259	2.02 (24.0)	427	3.00 (35.6)	49 ($P < 0.001$)
	Southwest	406	2.12 (25.1)	760	3.38 (40.1)	59 ($P < 0.001$)
	Males					
	North	57	1.15 (13.6)	74	1.51 (17.9)	31 ($P < 0.001$)
	Mid/west	194	1.51 (17.9)	419	2.96 (35.1)	96 ($P < 0.001$)
	Southwest	373	1.96 (23.2)	770	3.50 (41.5)	79 ($P < 0.001$)

*Incidence rates are crude incidence rates per 100 000 persons, Norwegian population.
†Relative melanoma density (Incidence rate/area).
‡Increase in incidence rates from the first to second period.
CMM, cutaneous malignant melanoma.

In Fig. 3, these data are shown for the three different regions of Norway. There was no statistically significant difference, neither for the two periods nor for the genders in the risk of foot CMM between people living in the north or the south (Fig. 3). However, large differences were observed for head/neck CMM (Fig. 3).

In Fig. 4, the relative CMM density, defined as relative incidence rate on a given body localization divided by the skin area of that localization. Essentially, this gives the relative number for melanoma incidence rate per unit skin area.

Discussion

Head/neck is the area of the human body the most exposed to the sun. It is commonly believed that UVB, and possibly UVA, from solar radiation are the main melanomagenic agents for humans.[7,9–11] Possible mechanisms for CMM carcinogenesis have been reviewed by several authors.[1,12,13] Increasing trends of CMM rates have been observed for a long time and are generally explained by increased sun exposure, notably in vacations and holidays (intermittent exposures).[14,15] However, in a recent article by Newton-Bishop *et al.*,[16] it was found that sun exposure during weekends or holidays may be protective for CMM in populations living at high latitudes. Earlier diagnosis of CMM related to the use of novel diagnostic techniques, improved screening and surveillance programmes and also some diagnostic drift towards classifying benign lesions as stage 1 CMM may also partly contribute to the reported increasing CMM rates.[17,18] However, the delay in diagnosis of CMM, both by the patient and by the professional, is relatively small in Norway compared with that in studies in other countries.[19,20]

In many populations, including the Scandinavian, the Australian and that in the USA, CMM rates have increased with doubling times of about 15–20 years and even as short as 5–10 years in some cases, up to 1985–1990.[21,22] After that time, the increase is less marked in many countries,[23,24] and, occasionally, a decrease has been observed for young people.[22]

In agreement with earlier investigations, we find that the overall CMM incidence rate on head/neck has increased with time (Fig. 2, Table 2). Such trends of skin cancer are commonly attributed to increasing sun exposure.[3,15,25] However, the incidence rates of almost all cancers have increased in Norway over the same period as studied here.[7] Thus, one cannot be sure that the entire increase in CMM incidence is due to increasing UV exposure. The incidence rate of foot CMM remains almost constant (Fig. 2). In any case, the general increase in head/neck CMM incidence is larger than that of foot CMM (Fig. 2).

In agreement with earlier work,[15,26] there is a large north–south gradient in incidence rates of CMM on head/neck in Norway (Figs 3,4; Table 2). Such a north–south trend is one of the major arguments for the melanomagenic action of solar radiation. Norway is a country particularly suited for such studies, as the population of the country is homogenous with respect to skin type, and as it stretches over a long north–south distance with

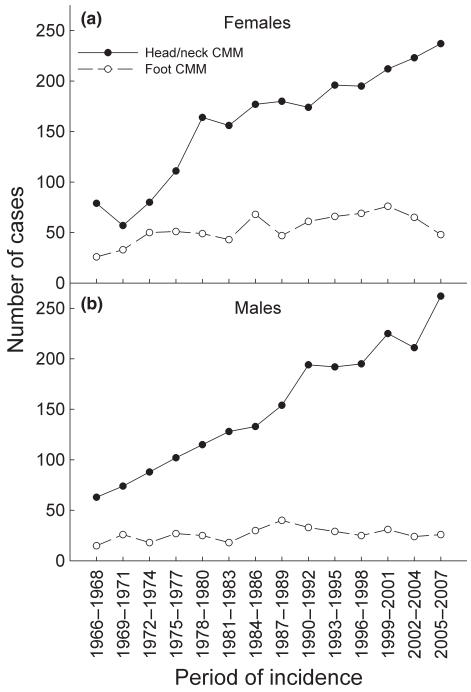

Figure 2 Number of new cases of head/neck and foot cutaneous malignant melanoma (CMM) in females (a) and males (b) in Norway since 1966.

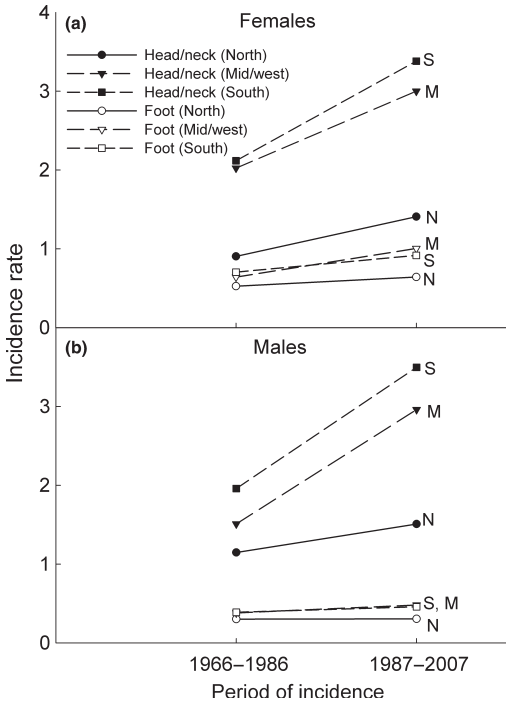

Figure 3 Crude incidence rates per 100 000 for females (a) and males (b) of head/neck and foot cutaneous malignant melanoma (CMM) for two time periods (1966–1986 and 1987–2007) in the north (N), the mid/west (M) and the south (S) regions of Norway.

about 30% larger annual UV dose and about three times higher incidence rates of squamous cell carcinomas of the skin in the south than in the north.[5,6,15,27] In contrast to CMM on head/neck, there is almost no north–south gradient for CMM incidence on foot (Figs 3,4; Table 2). Despite the fact that the annual UV dose is 21% larger in the south region than in the mid/west region, there is no significant difference in foot CMM between these two regions (Figs 3,4; Table 2). However, the incidence rates are lower in the north region than in the two other regions. This small north–south gradient may be related to a fraction of the foot melanomas occur on the lower part of the legs which are occasionally sun exposed. The fact that the rates of CMMs on this location are larger for women than for men supports this assumption.

Does solar radiation protect against foot melanomas? The possibility exists that solar UV radiation, via its role in vitamin D photosynthesis,[28] may have a systemically working protective effect against melanoma on body localizations exposed to minimal sun doses, as earlier proposed for the uveal tract and for the vulva.[29,30] The relationship between vitamin D and CMM has recently been reviewed.[31,32] Vitamin D binds to the vitamin D receptor (VDR) resulting in transcription of a number of genes playing roles in inhibition of MAPK signalling, induction of apoptosis and cell-cycle inhibition, and therefore, vitamin D has anti-proliferative and pro-apoptotic effects in different cells.[31] The vitamin D receptor has been identified in normal melanocytes and in melanoma cell lines.[32] *In vitro* results suggest that vitamin D has the same anti-proliferative effects on melanoma cells.[31] A few studies have demonstrated relationships of functional polymorphisms in the vitamin D receptor with melanoma risk or tumour aggressiveness.[31,32] Much remains unknown about vitamin D and melanoma. However, the avoidance of suboptimal vitamin D levels (<75 nmol/L) are likely to be beneficial for CMM patient.

The highest levels of serum 25-hydroxyvitamin D in Norway (around 70 nmol/L) are observed in healthy Norwegians living in the south part (Oslo region).[33,34] The concentrations of serum 25-hydroxyvitamin D seem to be 8% and 21% lower in the mid/west part (Bergen) and in the north (Tromsø region) in comparison with the south part the of Norway.[35,36] While annual UV radiation is 7% and 40% lower in the mid/west and the north parts than in the south part of Norway.[5] In the time

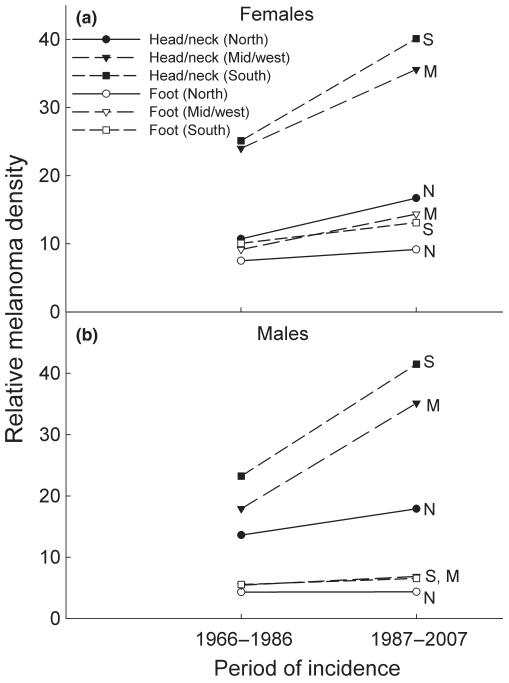

Figure 4 Relative melanoma densities (crude incidence rates divided by fractional body area of skin on foot or head/neck) for females (a) and males (b) of head/neck and foot cutaneous malignant melanoma (CMM) for two time periods (1966–1986 and 1987–2007) in the north (N), the mid/west (M) and the south (S) regions of Norway.

period when total CMM rates have been increasing, as in most Caucasian populations until about 1990,[21,22,26,37,38] the intermittent sun exposure of these populations have supposedly been increasing, perhaps partly because of increased duration of vacations and travelling frequency to sunny locations.[39] This leads, not only to higher incidence rates of CMM, but also to an increase in vitamin D synthesis in skin, and thus, to higher vitamin D levels. Time trends of vitamin D levels have not been reliably determined so far.

It might be argued that CMM may be related to UVA which produces no vitamin D. Thus, in the USA, mortality rates of non-malignant skin cancer have decreased since the 1960s, while mortality rates of CMM have increased.[40] These divergent trends may be related to the increased use of sunscreens that block UVA less well than UVB.[41]

Ultraviolet A and UVB vary differently with latitude because UVB is more scattered in the atmosphere than UVA and because some UVB, but not UVA, is absorbed by the ozone layer.[42]

Cutaneous malignant melanoma is more common among indoor workers than among outdoor workers, as farmers and fishermen.[43–45] It is possible that vitamin D synthesis may inhibit already initiated CMMs; thus reverting or slowing down melanomagenesis.[43] Unfortunately, no reliable and standardized comparisons of the vitamin D status, neither between different time periods nor between different geographical locations in Norway, are available.

A protective role of solar radiation against CMM, in addition to its inductive role discussed above, is indicated by the following observations: (i) the prognosis of CMM is best for summer–autumn diagnosis;[46] (ii) the prognosis of CMM appears to be the best for tumours arising on skin areas with morphological signs of high UV exposure[47] possibly related to UV-induced elastosis;[48] and (iii) CMM incidence rates in USA have increased rapidly in the period 1992–2004, with a doubling time as short as 10 years, whereas the mortality rates have not increased significantly.[49] Diagnosis of thinner melanomas related to screening projects could not explain the increasing incidence rates.[49] A decline of the latitudinal effect of CMM mortality rates and a stabilization of the rates in USA was predicted already in 1997.[50] Even earlier, it was shown that outdoor work contributes little to increase the CMM risk.[51]

There are limited data to support a role for vitamin D in CMM prevention, although some have hypothesized such a role.[14,16,52–54] A recently published cohort study gave no evidence for a protective effect of a greater vitamin D intake on the melanoma risk.[55,56] However, there are genetic data to suggest that inherited variation in the vitamin D receptor (VDR) gene is associated with melanoma risk.[54]

The 7-dehydrocholesterol (7-DHC) present in the keratinocytes of the epidermis is photolysed to cutaneous previtamin D_3 when irradiated with UVB radiation. From previtamin D_3 vitamin D_3 is generated in a thermal process, taking about one day at 37 °C. Vitamin D_3 is bound to D-binding protein (DBP) and transported by the circulation to the liver, where it is hydroxylated to calcidiol ($25(OH)D_3$). Then it is once more bound to DBP and transported to the kidneys and several other tissues for another hydroxylation, now forming calcitriol ($1,25(OH)_2D_3$), which is the active hormone, crucial for bone formation and maintenance. Calcidiol seems to play an important role in the defence against several diseases, such as influenza, cancer, diabetes, multiple sclerosis, mood disorders, *etc.* [57]

It has been demonstrated that reduced $25(OH)D_3$ levels in patients correlate with more aggressive melanoma[58] and high levels are associated with thinner tumours and better survival from CMM;[59] $1,25(OH)_2D_3$ has the ability to regulate cell proliferation, differentiation, migration and apoptosis.

Constitutive melanin pigmentation seems to act in a protective way by itself (as an antioxidant), even for mucosa and skin at sites not reached by solar UV radiation. Thus, there are generally lower or similar rates of vulvar, uveal and acral CMMs among Africans

as among Caucasians living in the same geographical area.[60–64] However, acral CMM is the prevalent form of CMM in darker populations.[64,65]

Sun exposure, severe sunburns, family or personal history of previous CMM seem not be associated with acral CMM.[66–68] Trauma in the area,[66,69] distinct genetic profile (KIT gene mutations [Kit is a receptor tyrosine kinase that binds stem cell factor])[70] or microenvironment[71] have been identified as risk factors for the development of acral CMM.

Conclusions

Comparisons of time trends and latitudinal trends of the incidence rates of CMM on head/neck and on foot indicate that solar radiation is a carcinogen for the former CMM but not for the latter. Taking the general increase in cancer incidence rates into account, one cannot exclude the possibility that solar radiation may act in a protective manner, likely through production of vitamin D. This should be further investigated.

Acknowledgements

The present work was supported by the Research Council of Norway (Norges forskningsråd), by the Norwegian Cancer Society (Kreftforeningen) and by Oslo University Hospital.

References

1 Garibyan L, Fisher DE. How sunlight causes melanoma. *Curr Oncol Rep* 2010; **12**: 319–326.
2 Seo SJ, Fisher DE. Melanocyte photobiology, ultraviolet radiation and melanoma. *G Ital Dermatol Venereol* 2010; **145**: 603–611.
3 Tang MS. Ultraviolet a light: potential underlying causes of melanoma. *Future Oncol* 2010; **6**: 1523–1526.
4 MacKie RM, Hauschild A, Eggermont AM. Epidemiology of invasive cutaneous melanoma. *Ann Oncol* 2009; **20**(Suppl 6): vi1–vi7.
5 Porojnicu AC, Lagunova Z, Robsahm TE *et al.* Changes in risk of death from breast cancer with season and latitude: sun exposure and breast cancer survival in Norway. *Breast Cancer Res Treat* 2007; **102**: 323–328.
6 Moan J, Porojnicu A, Lagunova Z *et al.* Colon cancer: prognosis for different latitudes, age groups and seasons in Norway. *J Photochem Photobiol B* 2007; **89**: 148–155.
7 Moan J, Porojnicu AC, Dahlback A. Ultraviolet radiation and malignant melanoma. *Adv Exp Med Biol* 2008; **624**: 104–116.
8 Cross A, Collard M, Nelson A. Body segment differences in surface area, skin temperature and 3D displacement and the estimation of heat balance during locomotion in hominins. *PLoS ONE* 2008; **3**: e2464.
9 Setlow RB. Spectral regions contributing to melanoma: a personal view. *J Investig Dermatol Symp Proc* 1999; **4**: 46–49.
10 Moan J, Dahlback A, Setlow RB. Epidemiological support for an hypothesis for melanoma induction indicating a role for UVA radiation. *Photochem Photobiol* 1999; **70**: 243–247.
11 De Fabo EC. Initial studies on an in vivo action spectrum for melanoma induction. *Prog Biophys Mol Biol* 2006; **92**: 97–104.
12 Walker G. Cutaneous melanoma: how does ultraviolet light contribute to melanocyte transformation? *Future Oncol* 2008; **4**: 841–856.
13 Di Lucca J, Guedj M, Descamps V *et al.* Interactions between ultraviolet light exposure and DNA repair gene polymorphisms may increase melanoma risk. *Br J Dermatol* 2010; **162**: 891–893.
14 Elwood JM, Gallagher RP, Hill GB *et al.* Cutaneous melanoma in relation to intermittent and constant sun exposure – the Western Canada Melanoma Study. *Int J Cancer* 1985; **35**: 427–433.
15 Moan J, Dahlback A. The relationship between skin cancers, solar radiation and ozone depletion. *Br J Cancer* 1992; **65**: 916–921.
16 Newton-Bishop JA, Chang YM, Elliott F *et al.* Relationship between sun exposure and melanoma risk for tumours in different body sites in a large case-control study in a temperate climate. *Eur J Cancer* 2011; **47**: 732–741.
17 Stang A, Lampert T, Uhlemann T, *et al.* Skin cancer mortality in Germany before and after the post-communist transition. *Int J Dermatol* 2009; **48**: 363–370.
18 Levell NJ, Beattie CC, Shuster S *et al.* Melanoma epidemic: a midsummer night's dream? *Br J Dermatol* 2009; **161**: 630–634.
19 Helsing P, Faye R, Langmark F. Cutaneous malignant melanoma. Correlation between tumor characteristics and diagnostic delay in Norwegian patients. *Eur J Dermatol* 1997; **7**: 359–361.
20 Faye RS, Helsing P, Langmark F. [Diagnostic delay in malignant melanoma]. *Tidsskr Nor Laegeforen* 2000; **120**: 1023–1025.
21 Weinstock MA. Ultraviolet radiation and skin cancer: epidemiological data from the United States and Canada. In Young AR, Young A, Bjorn LO *et al.*, eds. *Environmental UV Photobiology*. Plenum Press, New York, 1993: 295–344.
22 Moan J, Porojnicu AC, Dahlback A *et al.* Addressing the health benefits and risks, involving vitamin D or skin cancer, of increased sun exposure. *Proc Natl Acad Sci USA* 2008; **105**: 668–673.
23 Gaudette LA, Gao RN. Changing trends in melanoma incidence and mortality. *Health Rep* 1998; **10**: 29–41.
24 Lens MB, Dawes M. Global perspectives of contemporary epidemiological trends of cutaneous malignant melanoma. *Br J Dermatol* 2004; **150**: 179–185.
25 Benjamin CL, Melnikova VO, Ananthaswamy HN. Models and mechanisms in malignant melanoma. *Mol Carcinog* 2007; **46**: 671–678.
26 Cicarma E, Juzeniene A, Porojnicu AC *et al.* Latitude gradient for melanoma incidence by anatomic site and gender in Norway 1966-2007. *J Photochem Photobiol B* 2010; **101**: 174–178.
27 Porojnicu AC, Dahlback A, Moan J. Sun exposure and cancer survival in Norway: changes in the risk of death with season of diagnosis and latitude. *Adv Exp Med Biol* 2008; **624**: 43–54.
28 Holick MF. Evolution and function of vitamin D. *Recent Results Cancer Res* 2003; **164**: 3–28.
29 Moan J, Cicarma E, Setlow R *et al.* Time trends and latitude dependence of uveal and cutaneous malignant melanoma induced by solar radiation. *Dermatoendocrinol* 2010; **2**: 3–8.
30 Moan J, Porojnicu AC, Dahlback A *et al.* Where the sun does not shine: is sunshine protective against melanoma of the vulva? *J Photochem Photobiol B* 2010; **101**: 179–183.
31 Egan KM. Vitamin D and melanoma. *Ann Epidemiol* 2009; **19**: 455–461.
32 Field S, Newton-Bishop JA. Melanoma and vitamin D. *Mol Oncol* 2011; **25**: 197–214.
33 Holvik K, Meyer HE, Sogaard AJ *et al.* Pakistanis living in Oslo have lower serum 1,25-dihydroxyvitamin D levels but higher serum ionized calcium levels compared with ethnic Norwegians. The Oslo Health Study. *BMC Endocr Disord* 2007; **7**: 9.
34 Lagunova Z, Porojnicu AC, Lindberg F *et al.* The dependency of vitamin D status on body mass index, gender, age and season. *Anticancer Res* 2009; **29**: 3713–3720.
35 Christensen MH, Lien EA, Hustad S *et al.* Seasonal and age-related differences in serum 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D and parathyroid hormone in patients from Western Norway. *Scand J Clin Lab Invest* 2010; **70**: 281–286.
36 Brustad M, Alsaker E, Engelsen O *et al.* Vitamin D status of middle-aged women at 65-71 degrees N in relation to dietary intake and exposure to ultraviolet radiation. *Public Health Nutr* 2004; **7**: 327–335.
37 Lasithiotakis KG, Leiter U, Gorkievicz R *et al.* The incidence and mortality of cutaneous melanoma in Southern Germany: trends by

anatomic site and pathologic characteristics, 1976 to 2003. *Cancer* 2006; **107**: 1331–1339.
38 Moan J, Dahlback A. Ultraviolet radiation and skin cancer: epidemiological data from Scandinavia. In Young AR, Young A, Bjorn LO *et al.*, eds. *Environmental UV Photobiology*. Plenum Press, New York, 1993: 255–93.
39 Autier P, Dore JF, Gefeller O *et al.* Melanoma risk and residence in sunny areas. EORTC Melanoma Co-operative Group. European Organization for Research and Treatment of Cancer. *Br J Cancer* 1997; **76**: 1521–1524.
40 Grant WB. Solar ultraviolet irradiance and cancer incidence and mortality. *Adv Exp Med Biol* 2008; **624**: 16–30.
41 Gorham ED, Mohr SB, Garland CF *et al.* Do sunscreens increase risk of melanoma in populations residing at higher latitudes? *Ann Epidemiol* 2007; **17**: 956–963.
42 Moan J. *Visible Light and UV Radiation*. In Brune D, Hellborg R, Persson BRR *et al.*, eds. *Radiation at Home, Outdoors and in the Workplace*. Scandinavian Science Publisher, Oslo, 2001: 69–85.
43 Garland FC, White MR, Garland CF *et al.* Occupational sunlight exposure and melanoma in the U.S. Navy. *Arch Environ Health* 1990; **45**: 261–267.
44 Holman CD, Mulroney CD, Armstrong BK. Epidemiology of preinvasive and invasive malignant melanoma in Western Australia. *Int J Cancer* 1980; **25**: 317–323.
45 Chang YM, Barrett JH, Bishop DT *et al.* Sun exposure and melanoma risk at different latitudes: a pooled analysis of 5700 cases and 7216 controls. *Int J Epidemiol* 2009; **38**: 814–830.
46 Moan J, Porojnicu AC, Dahlback A. Epidemiology of cutaneous malignant melanoma. In Ringborg U, Brandberg Y, Breitbart EW *et al.*, eds. *Skin Cancer Prevention*. Informa Healthcare, New York, 2007: 179–201.
47 Berwick M, Armstrong BK, Ben-Porat L *et al.* Sun exposure and mortality from melanoma. *J Natl Cancer Inst* 2005; **97**: 195–199.
48 Lee EY, Williamson R, Watt P *et al.* Sun exposure and host phenotype as predictors of cutaneous melanoma associated with neval remnants or dermal elastosis. *Int J Cancer* 2006; **119**: 636–642.
49 Linos E, Swetter SM, Cockburn MG *et al.* Increasing burden of melanoma in the United States. *J Invest Dermatol* 2009; **129**: 1666–1674.
50 Lee JA. Declining effect of latitude on melanoma mortality rates in the United States. A preliminary study. *Am J Epidemiol* 1997; **146**: 413–417.
51 Goodman KJ, Bible ML, London S *et al.* Proportional melanoma incidence and occupation among white males in Los Angeles County (California, United States). *Cancer Causes Control* 1995; **6**: 451–459.
52 Vollmer RT. Solar elastosis in cutaneous melanoma. *Am J Clin Pathol* 2007; **128**: 260–264.
53 Rigel DS. Cutaneous ultraviolet exposure and its relationship to the development of skin cancer. *J Am Acad Dermatol* 2008; **58**: S129–S132.
54 Randerson-Moor JA, Taylor JC, Elliott F *et al.* Vitamin D receptor gene polymorphisms, serum 25-hydroxyvitamin D levels, and melanoma: UK case-control comparisons and a meta-analysis of published VDR data. *Eur J Cancer* 2009; **45**: 3271–3281.
55 Asgari MM, Maruti SS, Kushi LH *et al.* A cohort study of vitamin D intake and melanoma risk. *J Invest Dermatol* 2009; **129**: 1675–1680.
56 Rosso R, Kim N, Kirsner RS. Vitamin D intake and melanoma risk. *J Invest Dermatol* 2009; **129**: 1598.
57 Reichrath J, Nurnberg B. Cutaneous vitamin D synthesis versus skin cancer development: The Janus faces of solar UV-radiation. *Dermatoendocrinol* 2009; **1**: 253–261.
58 Nurnberg B, Graber S, Gartner B *et al.* Reduced serum 25-hydroxyvitamin D levels in stage IV melanoma patients. *Anticancer Res* 2009; **29**: 3669–3674.
59 Newton-Bishop JA, Beswick S, Randerson-Moor J *et al.* Serum 25-hydroxyvitamin D3 levels are associated with breslow thickness at presentation and survival from melanoma. *J Clin Oncol* 2009; **27**: 5439–5444.
60 Stevens NG, Liff JM, Weiss NS. Plantar melanoma: is the incidence of melanoma of the sole of the foot really higher in blacks than whites? *Int J Cancer* 1990; **45**: 691–693.
61 Tsai T, Vu C, Henson DE. Cutaneous, ocular and visceral melanoma in African Americans and Caucasians. *Melanoma Res* 2005; **15**: 213–217.
62 Hu DN, Yu GP, McCormick SA. Population-based incidence of vulvar and vaginal melanoma in various races and ethnic groups with comparisons to other site-specific melanomas. *Melanoma Res* 2010; **20**: 153–158.
63 Gloster HM Jr, Neal K. Skin cancer in skin of color. *J Am Acad Dermatol* 2006; **55**: 741–760.
64 Bradford PT, Goldstein AM, McMaster ML *et al.* Acral lentiginous melanoma: incidence and survival patterns in the United States, 1986-2005. *Arch Dermatol* 2009; **145**: 427–434.
65 Forman SB, Ferringer TC, Peckham SJ *et al.* Is superficial spreading melanoma still the most common form of malignant melanoma? *J Am Acad Dermatol* 2008; **58**: 1013–1020.
66 Phan A, Touzet S, Dalle S *et al.* Acral lentiginous melanoma: a clinicoprognostic study of 126 cases. *Br J Dermatol* 2006; **155**: 561–569.
67 Nagore E, Pereda C, Botella-Estrada R *et al.* Acral lentiginous melanoma presents distinct clinical profile with high cancer susceptibility. *Cancer Causes Control* 2009; **20**: 115–119.
68 Albreski D, Sloan SB. Melanoma of the feet: misdiagnosed and misunderstood. *Clin Dermatol* 2009; **27**: 556–563.
69 Rolon PA, Kramarova E, Rolon HI *et al.* Plantar melanoma: a case-control study in Paraguay. *Cancer Causes Control* 1997; **8**: 850–856.
70 Godshalk SE, Paranjape T, Nallur S *et al.* A variant in a MicroRNA complementary site in the 3′ UTR of the KIT oncogene increases risk of acral melanoma. *Oncogene* 2011; **30**: 1542–1550.
71 Monsel G, Ortonne N, Bagot M *et al.* c-Kit mutants require hypoxia-inducible factor 1alpha to transform melanocytes. *Oncogene* 2010; **29**: 227–236.

Errata

Page: 2, Figure 1.1 legend: a line was added: *World population*.

Page: 8, line 13: was “into three regions; the UVC region, which is defined as being in the wavelength region from 100-280”, corrected to “into three regions: the UVC region, which is defined as being in the wavelength region 100-280”.

Page: 17: line spacing was added before the paragraph “Beside these lesions”.

Page: 20, line: 15: was: “progression from neavus → dysplastic naevus”, corrected to: “progression (“Clark model”) from neavus → dysplastic naevus”.

Page: 22, line 23: was “melanoma caused by intermittent”, corrected to: “melanoma found on intermittent”.

Page: 24, new tab space before the second paragraph “In conclusion […]”; last line of the page was: “damage other than those photoinduced may” and corrected to: “damage other than photoinduced may”.

Page: 31, line: 2: was “underdiagnosis earlier”, corrected to “underdiagnosis previously”.

Page: 35, line: 18: a bracket was added: “of calcitriol were demonstrated with more recent data)”.

Page: 36, line: 13: was “people with white skin”, corrected to: “people with darker skin”.

Page: 38, line: 14: was “The different types of […] are summarized in the Table 4.1.”, corrected to “Different types of […] are summarized in Table 4.1.”.

Page: 46, line: 8: was “Our work underline”, corrected to “Our work underlines”.

Page: 49, line: 5: was “Nevertheless, the Norwegian population”, corrected to” The Norwegian population”, with new paragraph.

Page: 52, Figure 6.5 legend was: “female filled symbols, male open symbols”, corrected to “female open symbols, men filled symbols”.

Page: 62, line 10: was “was significant, but only in the”, corrected to “was significant in the”.

Page: 65, line 10: was “Similar […] for the both of”, corrected to “Similar […] for both of”.