Malignant melanoma-Risk factors and the CDKN2A mutation in relation to phenotypes and other cancers.

Nielsen, Kari

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Malignant melanoma

-Risk factors and the *CDKN2A* mutation in relation to phenotypes and other cancers.

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CONTENTS

ORIGINAL PAPERS 5
ABBREVIATIONS 7
ABSTRACT 11
INTRODUCTION 13
1. The human skin 13
2. Nevus/naevus/mole 21
3. Atypical mole syndrome (AMS) 25
4. Oncogenesis in general 29
5. Malignant melanoma 31
6. Risk factors for melanoma 39
7. Cancers associated with melanoma 49
AIMS OF THE THESIS 53
MATERIAL AND METHODS 55
1. Background data 55
2. Study design, Study populations and Methods 57
3. Statistical methods 61
RESULTS 63
1. Study I 63
2. Study II 65
3. Study III 69
4. Study IV 73
DISCUSSION 79
1. Methodological considerations 79
2. General discussion 83
CONCLUSIONS 95
FUTURE PROSPECTS 97
SAMMANFATTNING PÅ SVENSKA 99
ACKNOWLEDGEMENTS 103
REFERENCES 105
GLOSSARY/EXPLANATIONS 115

ORIGINAL PAPERS

The current thesis is based on results reported in the following papers, which will be referred to in the text by their Roman numerals.

I. Nielsen K, Ingvar C, Måsbäck A, Westerdahl J, Borg A, Sandberg T, Jonsson N, Nagel J, Olsson H. (2004)

Melanoma and nonmelanoma skin cancer in patients with multiple tumours--evidence for new syndromes in a population-based study.

Br J Dermatol. Mar;150(3):531-6.

II.* Nielsen K, Måsbäck A, Bladström A, Lundgren L, Jonsson N, Borg Å, Ingvar C, Olsson H. (2008) Epub 2007

Confirmed cancer trends in families of patients with multiple cancers including cutaneous melanoma.

Br J Dermatol. Feb;158(2):429-31.

III. Nielsen K, Harbst K, Måsbäck A, Jönsson G, Borg Å, Olsson H, Ingvar C.

Swedish *CDKN2A* mutation carriers do not present the Atypical Mole Syndrome phenotype. *Accepted for publication in Melanoma Research*

IV. Nielsen K, Måsbäck A, Olsson H, Ingvar C.

A prospective study of 40 000 women regarding host factors, UV exposure and sunbed use in relation to risk and anatomic site of malignant melanoma.

Submitted

* Supplement.

ABBREVIATIONS

ACTH	Adrenocorticotropic hormone/Corticotropin
AK	Actinic keratosis
AKT=PKB	Oncogene *AKT1*, which encodes AKT=PKB (protein kinase B)
α-MSH	α-Melanocyte Stimulating Hormone/Melanotropin
AMN	Acquired melanocytic nevus=“common” nevus, (i.e. not congenital nevus)
AMS	Atypical mole syndrome
ASIP=ASP	Agouti signalling protein
BCC	Basal cell carcinoma= basalioma
BCL-2	B-cell leukaemia/lymphoma 2 protein
BRAF	V-raf murine sarcoma viral oncogene homolog B1
CAN	Clinically atypical nevus/nevi
CDKN2A	Cyclin dependent kinase inhibitor 2A
CI	Confidence Interval
CMM	Cutaneous malignant melanoma
CREB	cAMP response-element binding protein
CSC/TSC	Cancer stem cell /Tumour stem cell
DNS	Dysplastic nevus syndrome
ERK	Extra cellular signal-regulated kinase
ET-1	Endothelin-1
ETR	Endothelin receptor
FAMMM	Familial atypical multiple mole and melanoma syndrome
G 0/1/2	Gap phase 0/1/2
GWAS	Genome-wide association study

HDM2/MDM2	Human homolog of MDM2 (murine double minute oncogene).
HPV	Human papilloma virus
HR	Hazard Ratio
HRAS/H-RAS	see RAS
IGF	Insulinlike growth factor
IGFBP7	IGF binding protein 7
KC	Keratinocyte
KRAS/K-RAS	see RAS
MAPK	Mitogen-activated protein (MAP) kinase
MATP/SLC45A2	Membrane-associated transporter protein. Gene *MATP*, alternative title *SLC45A2*
MC	Melanocyte
MC1R	Melanocortin-1 Receptor
MEK	MAP/ERK kinase. See Glossary: MAPK
MISS	Melanoma Inquiry in Southern Sweden
MITF	Microophtalmia- associated transcription factor
MM	Malignant melanoma
MN	Melanocytic nevus
MSH, α-MSH	Melanocyte-Stimulating Hormone/melanotrophin
MTAP/MSAP	Methylthioadenosine phosphorylase
NC	Nevus cell/ nevocyte
NMSC	Non-melanoma skin cancer
NO	Nitric oxide
NRAS/N-RAS	see RAS
OCA 1	Oculocutaneous albinism type 1 (tyrosinase-negative), mutated gene: *TYR*
OCA 2	Oculocutaneous albinism type 2 (tyrosinase-positive), mutated gene: *OCA2=P gene*

OMIM	Online Mendelian Inheritance in Man (http://www.ncbi.nlm.nih.gov.ludwig.lub.lu.se/omim)
OR	Odds Ratio
p53=TP53	Tumour Protein p53
PI3K	Phosphoinositide-3-kinase/phosphatidylinositol-3-kinase
PKB	Protein kinase B, see AKT
POMC	ProOpioMelanoCortin
PTEN	Phosphatase and tensin homolog
RAS/Ras	Rat Sarcoma viral oncogene homolog (see Glossary HRAS/NRAS/KRAS)
RB=Rb	Retinoblastoma
RHC	Red hair colour
RR	Relative risk
SCC	Squamous cell carcinoma
SIR	Standardized Incidence Ratios
SLC24A4	Solute carrier family 24 A4, see Glossary
SLC24A5	Solute carrier family 24 A5, see Glossary
SMR	Standardized Mortality/Morbidity Ratio
SNP	Single Nucleotide Polymorphism
TSC/CSC	Tumour stem cell /Cancer stem cell
TP53=p53	Tumour Protein p53
TYR	Tyrosinase
TYRP1=TRP	Tyrosinase-related protein 1
UVA	Ultraviolet A radiation (320-400nm)
UVB	Ultraviolet B radiation (290-320nm)
UVR	Ultraviolet radiation (200-400nm)

ABSTRACT

Background: Cutaneous malignant melanoma (CMM) is an increasingly common cancer in fair-skinned people. The purpose of this thesis was to study high-risk patients with multiple tumours including a CMM, high-risk families with the unique Swedish germline mutation in *CDKN2A(113insArg)*, as well as study risk factors for CMM in women.

Methods: Tumours associated with CMM, in individuals/probands with four or more primary tumours including at least one CMM were genotyped. The probands were further sub-grouped according to subsequent cancers (Paper I). Possible similarities in tumour patterns were studied in their close relatives (Paper II*). Further, melanoma-prone families in southern Sweden with the *113insArg/CDKN2A* mutation were phenotyped and genotyped (Paper III). Finally, a population-based cohort of originally 40.000 women was prospectively followed for 18 years regarding CMM after answering a questionnaire about CMM risk factors (Paper IV).

Results: Papers I-II: The mutation was overrepresented in probands with multiple CMM. Non-mutation probands presented e.g. Neural System Tumours (NSTs), adenocarcinomas and non-melanoma skin cancer (NMSC), which were also seen in their relatives. For the relatives an overall increased risk for cancer was seen. Paper III: Positive mutation status was associated with clinically atypical nevi (CAN), and CMM diagnosis with red hair colour and CAN. No CMM were diagnosed in non-mutation carriers. The overall total nevus count (median 12, IQR: 5-25) and rate of individuals affected by CAN (14%), were lower in these families than shown in previous, population-based, Swedish studies. No atypical mole syndrome (AMS) phenotype was seen. Paper IV: Family history and ≥1 nevus on the left arm were risk factors for CMM, irrespective of age of the participants. Younger women with a history of frequent sunbed use had an additionally increased risk for CMM. CMM on the trunk were associated with a family history of CMM, a high nevus number and the youngest age at diagnosis.

Conclusions: The *113insArg/CDKN2A* mutation in these melanom-prone families is difficult to diagnose dermatologically, but the presence of CMM seems to be completely associated with the mutation. Hence, mutation carriers must be followed-up by dermatologists irrespective of phenotype. The population-based risks for CMM in southern Swedish women seem to be associated with a family history of CMM, a higher nevus number and, for younger women, the use of sunbeds. *Supplement.

INTRODUCTION

1. The human skin

The skin is the largest human organ with a surface area of 1.5-2m^2 and consists of the three layers epidermis, dermis and subcutis. In the skin hair follicles, sweat and sebaceous glands are embedded and the three basic layers are complemented and joined by three networks that link the skin to the rest of the body: blood vessels, lymphatic system and cutaneous nerves. These networks provide e.g. nutrition and oxygen supply, and are vital for fluid balance, skin immune responses and skin sensation. The skin constitutes an important barrier against outer dangers as mechanical trauma, infections, chemical irritants, Ultraviolet radiation (UVR) and free radicals (which can harm DNA and cell membranes), toxins and heat.

The most superficial part of the skin is the epidermis **(Figure 1.)**, an avascular layer, mainly composed of keratinocytes (KC) as well as smaller populations of pigment producing melanocytes (MC) and mechanosensory Merkel cells. The immune system is also present in the epidermis, as the migratory Langerhans cells and intraepidermal T-cells.

Beneath the epidermis is the dermoepidermal junction with the basal layer, and underlying this is the dermis, which mainly consists of connective tissue with dermal fibroblasts and a complex network of vessels, nerves, eccrine glands and hair follicles.

The third layer of the skin is the subcutis (or subcutaneous fat), providing thermal insulation and acting as a shock absorber and a hormone factory.

1.1 Melanocytes

Melanocytes are the neural crest-derived dendritic cells, which are found in the epidermis, in the inner ear, in the uveal tract, in the leptomeninges and in the hair follicles. They can also be found in the mucosal membranes and in the gastrointestinal tract and common for all anatomical sites is that increased numbers of MCs indicate lentigo, melanocytic nevi or malignant melanoma.

In the epidermis they are evenly dispersed along the basal layer at the dermoepidermal junction, where approximately every 10^{th} cell is a MC **(Figure 1.)**.

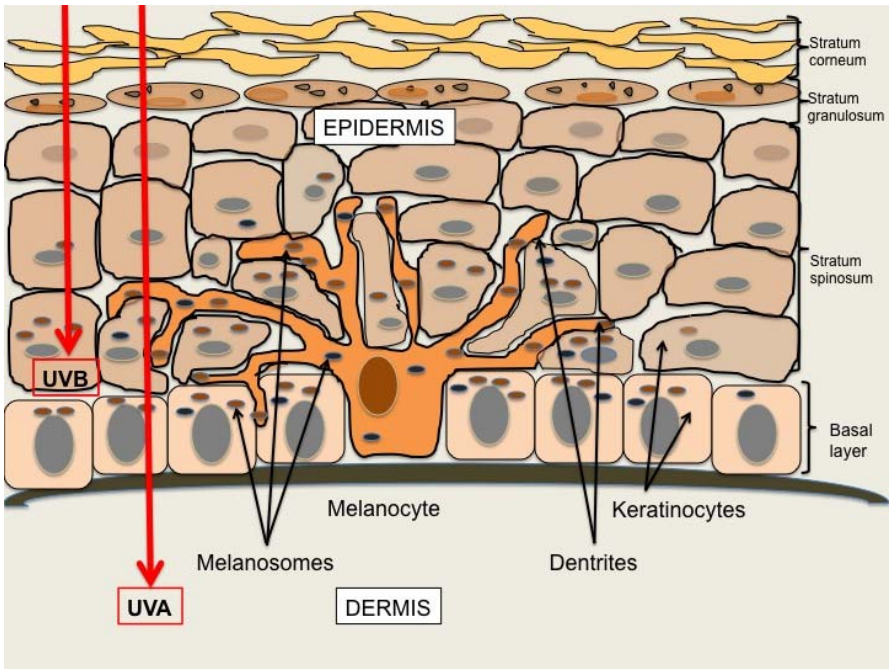

Figure 1. The epidermal skin and the UVR penetrance.

The figure shows the dermis, the epidermal melanin-unit, the four layers of the epidermis and how deep the different UV radiation types (UVA and UVB) penetrate the skin layers.

They produce the pigment melanin (for melanogenesis see below) within melanosomes, organelles that are transferred to surrounding keratinocytes and hair follicle cells. This pigment production determines human hair and skin colour and protects the receiving cells nuclei/DNA from damage by UV light. The way that melanin protects the DNA is by absorption and scattering of the UVR (Park et al. 2009). One melanocyte takes care of and provides melanin to up to 36 KCs in the surrounding area, named the epidermal melanin unit (Jimbow et al. 1976). The synthesis and transfer of melanin are regulated by several paracrine (mainly from the KCs) and autocrine factors, in response to both endogenous and exogenous stimuli, where UVR is one important factor (Tsatmali et al. 2002; Park et al. 2009). The MCs are also susceptible to UVR and oxidative stress, factors that may create genetic mutations in the MCs and thus capable of inducing malignant transformations.

1.2 Melanin synthesis

Melanin is produced in three versions, the reddish-yellow pheomelanin and the brown-black eumelanins (DHICA-melanin [brown] and DHI-melanin [black] (Ito

and Wakamatsu 2003) **(Figure 2.)**). They have different functions where eumelanin is photoprotective and acts as a scavenger to Reactive Oxygen Species (ROS), unlike pheomelanin that is probably harmful after UVR exposure, via the generation of free radicals/ROS (Ito and Wakamatsu 2003; Park et al. 2009).

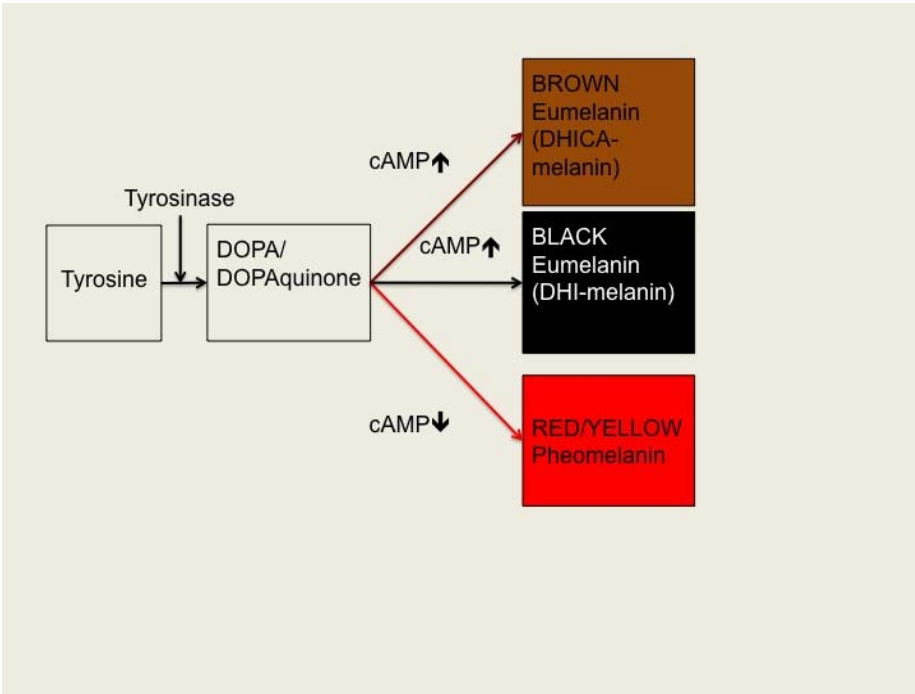

Figure 2. Melanin synthesis

The amino acid tyrosine is catalyzed by tyrosinase to DOPA/DOPAquinone and further to eumelanin or pheomelanin, dependent on the activity of tyrosinase, the level of cyclic AMP (cAMP) and the presence of cysteine. DHICA-eumelanin is brown, DHI-eumelanin is black.

The key factor in the production of melanin is the amino acid tyrosine, but tyrosinase is the rate-limiting enzyme for melanogenesis. Tyrosinase catalyzes the conversion of L-tyrosine to DOPA and further to DOPAquinone, which is required for the synthesis of both eumelanin and pheomelanin. The formation of pheomelanin requires the presence of cysteine as well as less tyrosinase activity and less cyclic AMP (cAMP) than does the formation of eumelanin. The activity of tyrosinase is enhanced by DOPA and is stabilized by tyrosinase-related-protein 1 (TRP1), a transmembrane protein of the melanosome. If the tyrosinase gene (*TYR*) is mutated, no melanin can be produced (oculocutaneous albinism type IA). The type and ratio of melanin produced (eu/pheo) depends on several different

melanogenic enzymes, the melanosome proteins (e.g. P protein and TRP1) and the availability of cysteine.

Several factors, including e.g. α-MSH (α-melanocyte stimulating hormone, see below), Agouti signalling protein (ASP/ASIP), endothelin-1 (ET-1), basic fibroblast growth factor (bFGF) and UVR, influence the activity of the most important proteins involved in the melanogenesis (Park et al. 2009).

1.3 Basal Skin Pigmentation (skin colours)

The human skin colours/photo types **(Table 1.)** are mainly dependent on the content and type of melanin distributed to the KCs, and on factors produced by KCs to regulate pigmentation (Yamaguchi and Hearing 2009).

Skin type	Typical features	Tendency to burn	Ability to tan
I	Pale white skin	Always burns	Never tans
II	Fair skin	Burns easily	Tans poorly
III	Darker white skin	Burns moderately	Tans, sometimes after a slight burn
IV	Light brown skin	Burns minimally	Tans easily
V	Brown skin	Rarely burns	Tans darkly easily
VI	Dark brown or black skin	Never burns	Always tans darkly

Table 1. Skin phototypes

The table is based on the research conducted by TB Fitzpatrick et al (Fitzpatrick 1988), where the skin phototypes are classified according to UVR reaction.

Dark skin contains the same number and density of MCs as fair skin, and the basal pigmentation ("constitutive pigmentation") thus depends on the level of the melanogenic activity of the MCs and on the transfer of melanosomes to the surrounding KCs. This activity is mirrored in the number of produced melanosomes and in the efficiency of transporting the melanosomes to the KCs. The size of the melanosomes (smaller in fair skin), the type of melanin produced (eumelanin/pheomelanin ratio) and the rate of melanosome-degradation (occurring in mid-epidermis) of the KCs (smaller degrades faster) are also important elements in the basal pigmentation. Eumelanin is present in large amounts in individuals with dark skin and hair **(Figure 2.)** whereas pheomelanin predominates in individuals with lower skin types (I-II) and red hair (Park et al. 2009). The

genetics of human pigmentation is not wholly understood, but many genes, and polymorphisms within these gene loci, are involved in different ways such as *TYR, OCA2, TYRP1, POMC, ASIP, MC1R* and *SLC45A2* (see Abbreviations and Glossary), where *MC1R* is one of the best studied (see below) (Sturm 2009; Yamaguchi and Hearing 2009). The genetics of pigmentation is also tightly connected to the risk for melanoma (Duffy et al. 2009).

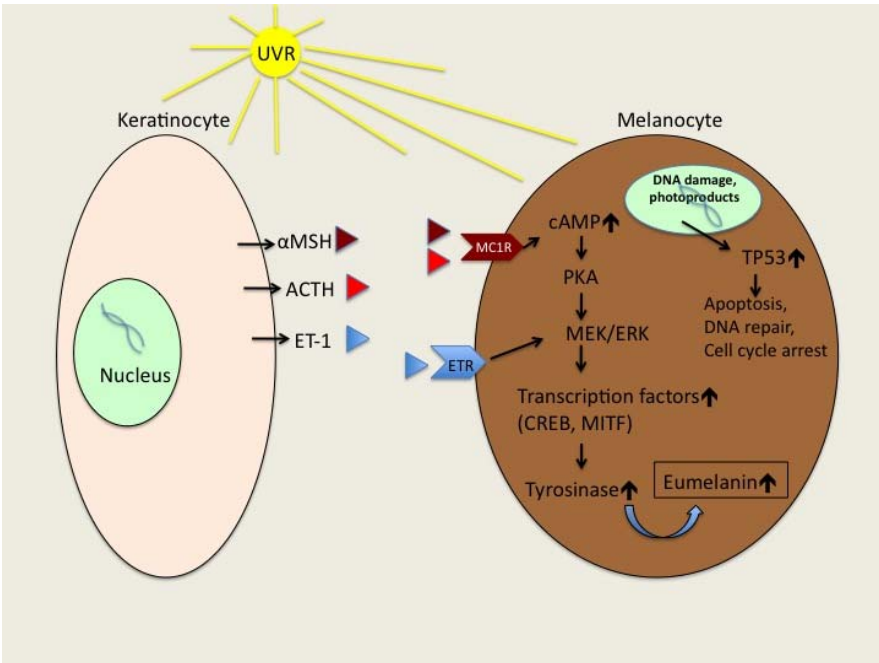

Figure 3. Some of the UVR effects on MCs and KCs.

UVR exposure results in α-Melanocyte stimulating hormone (α-MSH), Adrenocorticotropic hormone (ACTH) and Endothelin (ET-1) release from the keratinocytes (KCs). α-MSH and ACTH bind to the melanocortin-1-receptor (MC1R), increase cyclic AMP (cAMP) and activate Protein kinase A (PKA), finally leading to increased levels of tyrosinase and hence increased eumelanin synthesis. UVR also damages the DNA of the cells, thereby activating the tumour protein p53 (TP53)-pathway to enhance DNA repair, apoptosis and allow for cell cycle arrest. ETR=Endothelin receptor, MEK/ERK Mitogen-activated protein kinase 2/Extracellular signal-regulated kinase.

1.4 Facultative Skin Pigmentation (Tanning)

Tanning is when pigmentation increases above baseline as an effect of UVR exposure **(Figure 3.)**.

Tanning ability is highly influenced by the genes responsible for skin pigmentation and thus the response to UVR, where mutated genes e.g. lead to defect melanin production or defect response to receptor stimulation. UVR affects MCs both direct and indirect (via effect on KCs). In response to UVR the KCs secrete paracrine factors as α-MSH (melanocyte stimulating hormone), ACTH (Adrenocorticotropic hormone), ET-1 (endothelin-1), NO (nitric oxide) and several growth factors, which all in different ways increase the melanogenesis (Park et al. 2009). The direct UVR effects on MCs include e.g.: an increase in MC size and dendricity, an increase in the transcription and activity of tyrosinase, damage of DNA (giving harmful photoproducts), increased activity of tumour protein p53/TP53/p53, production of NO, increased numbers and activities of the melanocortin-1 receptors (MC1R) on the cell surface (see below) and effects on proliferation and survival. These events finally elevate the responsiveness of the MCs to α-MSH, increase eumelanin synthesis and enhance melanosome transfer (Rouzaud et al. 2005; Sturm 2009). Studies have shown a role for DNA photoproducts in the transformation of a MC to a melanoma (Abdel-Malek et al. 2008; Ibrahim and Brown 2008), and UVR exposure to existing pheomelanin leads to the production of ROS/free radicals (Park et al. 2009), which probably contributes to the increased incidence of CMM and NMSC observed in red hair colour (RHC) phenotypes (Raimondi et al. 2008).

More specifically, Ultraviolet A radiation (UVA, 320-400 nm) is thought to mediate one of its tanning effects through oxidation of pre-existing melanin, which results in immediate pigment darkening (which fades after 8 hours). Delayed tanning is visible 48 to 72 hours after UVA and Ultraviolet B radiation (UVB, 290-320 nm)) exposure, which is dependent on new melanin formation via increased transcription of essential factors and eventually increased tyrosinase activity.

The inability of redheads to tan after UVR exposure can partly be explained by a dysfunction of melanin synthesis related to MC1R **(Figure 3.)** (Abdel-Malek et al. 2008; Raimondi et al. 2008).

1.5 α-Melanocyte stimulating hormone/ α-MSH

α-MSH is one of the melanocortin peptides (α-MSH, ACTH, and beta-endorphin), which plays an important role in the melanogenesis by inducing MC differentiation and melanin production. All melanocortin peptides are produced by the proteolytic cleavage of the precursor protein POMC (ProOpioMelanoCortin) and exert their effects through melanocortin receptors (MCR, see below). α-MSH is produced in the pituitary gland, but also in the human skin by KCs, MCs and Langerhans cells. Increased production of α-MSH by KCs is seen after UVR exposure **(Figure 3.)**. α-MSH together with ET-1 have been shown to: activate the AKT-pathway (a prosurvival signalling pathway), enhance DNA repair (nucleotide excision repair [NER]), inhibit production of ROS and DNA photoproducts after UVR exposure and finally to promote eumelanin-synthesis. In summary, all these functions lead to increased survival of MCs after UV irradiation (Abdel-Malek et al. 2008).

1.6. Melanocortin-1 receptor/MC1R

There are five major human melanocortin receptors and the most common seen on the surface of melanocytes is the melanocortin-1 receptor (MC1R). Epidemiologic studies have revealed more than 80 allelic variants (mutations/polymorphisms) of the *MC1R* gene (Raimondi et al. 2008). These polymorphisms are important for basal skin and hair colours in different ethnic groups (Park et al. 2009; Sturm 2009).

The wild type allele of the *MC1R* that is predominant in the African continent is associated with dark skin and hair, as a result of promoted eumelanin production.

When α-MSH (or ACTH) binds to a functional MC1R, the levels of cAMP increase and the levels of tyrosinase and several melanogenic enzymes are increased by transcription. With plenty of tyrosinase around, the synthesis of eumelanin is favoured instead of pheomelanin **(Figure 3.)** (Raimondi et al. 2008).

In melanocytes with functional/wild type *MC1R*, the UVR-response with increased α-MSH allows MCs and KCs to survive with genomic stability (see α-MSH). With non-functional *MC1R* (mutations/polymorphisms) the effect is thought to be the reverse, with compromised NER capacity, due to the inability to respond to melanocortins and the shift to pheomelanin production/ROS and increased risk of skin cancer. Mutations/polymorphisms in *MC1R* are thus associated with "red hair colour phenotype" (RHC) and the skin phototype I-II (Raimondi et al. 2008). If the antagonist to α-MSH, ASP/ASIP binds to MC1R by way of competitive inhibition, this favours the production of pheomelanin in a way similar to when MC1R is non-functional (Gudbjartsson et al. 2008).

The phenotypic advantages, with MC1R-variants, lighter skin and bright/red hair colours in northern countries with scarce UV radiation, are thought to be related to the vitamin D synthesis and the bone metabolism (Tsatmali et al. 2002; Abdel-Malek et al. 2008).

2. Nevus/naevus/mole

A melanocytic nevus (MN) is by definition a benign clonal proliferation of non-dendritic melanocytes “nevus cells”/nevocytes (NCs) arranged in nests, and is the most common human tumour. Nevi can be either congenital or acquired (AMN)/”common nevi” **(Figure 4.)**. Even though acquired nevi are common, their pathogenesis is poorly understood (Krengel 2005; Zalaudek et al. 2007; Zhu et al. 2007). Trigger factors are e.g. early sun exposure (in childhood) and hormones (puberty) but recent research has shown that about 60% of nevi are genetically determined (Wachsmuth et al. 2001; Falchi et al. 2009). The first common nevi appear during childhood and the peak of number of nevi occurs around 35 years of age, thereafter most nevi slowly undergo senescence after age 50/60. Eventually, they become more and more dermal and disappear, probably due to regression and involution (Zalaudek et al. 2007).

Acquired nevi are often divided into three types, depending on where the nests of NCs are found in the skin; junctional nevi show nests of NCs at the dermo-epidermal junction, compound nevi show nests both at the dermoepidermal junction and in the dermis, and dermal nevi show nests in the dermis only. Increased numbers of AMN/common nevi are independent markers of an increased risk for melanoma (Bauer and Garbe 2003; Gandini et al. 2005).

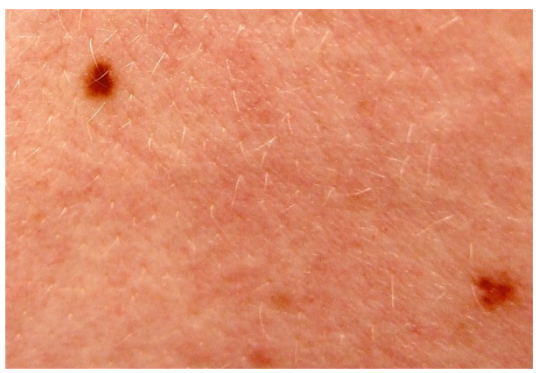

Figure 4. Common/AMN nevi.

2.1 Clinically Atypical nevi (CAN)

Clinically atypical nevi/Atypical moles/Clark nevi/Dysplastic nevi are all names for the same nevus entity, but there is still no consensus on the nomenclature of this group. A problem is that the clinical features all too often do not correspond to the histopathological features, as a clinically atypical nevus can lack histological

signs of dysplasia and vice versa. The histopathological criteria are thus controversial without any general agreement (Zalaudek et al. 2007).

Clinically, CAN are pigmented macules, papules or plaques, most common on the scalp and trunk and with clinical features resembling melanoma such as asymmetry, red-brown-black colour, faded, indistinct or notched borders, fried egg appearance with a papule within a macula, concentric circles resembling a target (cockade nevus) and diameter > 5mm (Bauer and Garbe 2003).

An estimated two to eight percent of the population have CAN in the US, while one study from northern Sweden showed a population-based prevalence of 11% (Karlsson et al. 2000). Another study from the Swedish west coast reported population-based figures of as high as 18% (Augustsson et al. 1991). The onset of CAN is usually in older children and young adults and CAN can be sporadic (nonfamilial), as well as familial **(Figure 5.)** or part of a syndrome (see below).

The presence of atypical nevi independently indicates a higher risk of melanoma, as they can be both markers of and possibly even potential precursors for melanoma (Friedman et al. 2009). In case-control studies (which have shown an excess prevalence of CAN in melanoma cases relative to controls) and in cohort studies (which have shown an excess in melanoma incidence both in familial melanoma kindreds with CAN and in patients with sporadic CAN relative to the general population) this correlation, with a higher melanoma risk, has been observed (Bauer and Garbe 2003; Gandini et al. 2005; Chang et al. 2009). Moreover, there seem to be a dose-response relation between melanoma risk and the total number of CAN (Gandini et al. 2005).

The causal genes that create the phenotype with excess CAN have not yet been fully elucidated, but several candidates exist. Data suggests possible linkage to loci on chromosomes 5, 7 and 9. However, the *Cyclin-dependent kinase inhibitor 2A* (*CDKN2A*) locus on chromosome 9 shows linkage only to a small subgroup of patients (Newton Bishop and Gruis 2007; de Snoo et al. 2008).

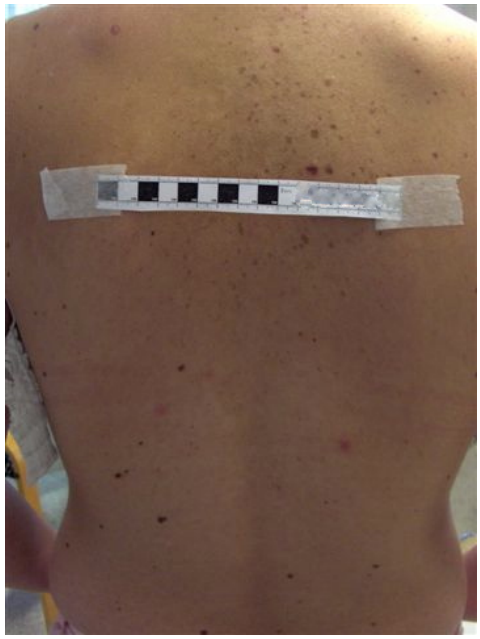

Figure 5. Atypical nevi on the back of a patient.

The patient presented multiple primary melanomas and reported a weak family history of melanoma (one additional melanoma case).

3. Atypical mole syndrome (AMS)

The observation that individuals with melanoma, from melanoma-prone families, often had an abnormal nevus phenotype with multiple common and atypical nevi/moles was first described in 1978 by Clark as the "B-K-mole syndrome" (Clark et al. 1978), named after the initials of affected family members. This phenotype has later been variously known as the familial atypical multiple mole and melanoma syndrome (FAMMM) (Lynch et al. 1978)), the dysplastic nevus syndrome (DNS) or the atypical mole syndrome (AMS). Different definitions of the same syndrome have been proposed, and thus literature is sometimes difficult to compare (Slade et al. 1995), but in 1983 Kraemer et al described a further subdivision of the DNS, dependent on different melanoma risk categories, and on how many individuals per kindred that were affected by melanoma and atypical nevi, respectively (Kraemer et al. 1983). **(Table 2.)** The initial study about the B-K-mole syndrome referred e.g. to the last category "D-2", but often different categories are intended when studies in general are referring to AMS/DNS, and the non-specific nomenclature can be confusing as AMS/DNS can be demonstrated both as a sporadic form and in different familial settings, and simultaneously with or without melanoma(s) (Slade et al. 1995).

	Description	Proband status DN/AN	Proband status MM	Family status DN/AN	Family status MM
A	**Sporadic DNS (Non-familial)**	Proband has DN/AN	-	-	-
B	**Familial DNS**	Proband has DN/AN	-	≥1 member has DN/AN	-
C	**Sporadic DNS/ Sporadic MM**	Proband has DN/AN	Proband has ≥1 MM	-	-
D-1	**Familial DNS and MM**	Proband has DN/AN	Proband has ≥1 MM	≥1 member has DN/AN	1 member has MM
D-2	**Familial DNS and MM**	Proband has DN/AN	Proband has ≥1 MM	≥2 members have DN/AN	≥2 members have MM

Table 2. Subgroups of the dysplastic nevus syndrome.

Description of the sporadic and the subgroups of the familial Atypical Mole Syndromes/ Dysplastic Nevus Syndromes, according to Kraemer et al (Kraemer et al. 1983). The subgroup "D-2" is the group that is most commonly referred to as the "AMS/DNS", when this is mentioned in familial studies. DN= dysplastic nevus, DNS= Dysplastic nevus syndrome, AN=Atypical Nevus, MM= malignant melanoma

Just as the description of the syndrome is somewhat arbitrary, different authors have differently described the characteristics of the AMS phenotype (de Snoo et al. 2008). However, most authors define the phenotype as one that presents "large numbers" of common nevi over 2 mm in diameter and ≥2 CAN.

Nevertheless, a more specific scoring system has been developed by Bishop et al., (Newton Bishop et al. 1994) to easier define this phenotype and to avoid inter-observer disagreements (Bishop et al. 2000). Individuals are considered to have the AMS phenotype if they score 3 or more using this system **(Table 3.)**.

In conclusion, the presence of the AMS phenotype is associated with an increased risk of melanoma, both in the general population (as sporadic AMS/DNS) and in families, and therefore this phenotype is important to recognize (Bishop et al. 2000; Gandini et al. 2005; Chang et al. 2009) especially as the AMS phenotype has also been found in some families with susceptibility for pancreas cancer, in addition to melanoma (Bergman et al. 1990; Lynch et al. 2008).

AMS Phenotypic characteristics	Point
≥100 nevi, >2mm (≥50 if under 20, or more than 50 years)	1 point
≥2 CAN	1 point
≥1 nevus in the anterior scalp	1 point
1 nevus on the buttocks or ≥2 nevi on the dorsum of the feet	1 point
≥1 nevus in iris*	1 point

Table 3. The original AMS scoring system according to Newton Bishop et al.

Patients are considered to have the "AMS phenotype" if the total score is 3 or more. *An iris naevus was defined as a pigmented maculae in the iris of at least 2 mm in diameter viewed with the naked eye (Newton Bishop et al. 1994).

4. Oncogenesis in general

Cancer is a complex collection of diseases in different organs. The mechanisms that cause cancer development are far from elucidated but comprise both environmental and genetic factors, but genetic changes are "the cornerstone of cancer" (WHO/IARC 2008). Cancer cells have defects in regulatory systems that control the normal lifespan of a cell. The *normal* balance between proliferation and apoptosis (programmed cell death) is controlled by mechanisms that tightly regulate e.g. the processes of the cell cycle and cell division. Under normal cellular conditions, DNA is constantly slightly damaged (DNA breaks) by exogenous (environmental) and endogenous genotoxic agents such as UVR exposure, but these breaks are normally repaired or, if too much damage is present, the cell is forced to commit "suicide" (apoptosis) as a mechanism of cancer avoidance. If this balance is disrupted, it results in uncontrolled cell division and rapid proliferation of harmed cells, eventually forming a tumour of deregulated cells (Hanahan and Weinberg 2000; WHO/IARC 2008). Most cancer cells have probably recruited one or several of the following characteristics: self-sufficiency in growth signals, insensitivity to anti-growth signals, possibility to escape from apoptosis, metastatic potential, an infinite replicative possibility and promoted angiogenesis. In order for cells to transform to cancer, genes that regulate these systems must be changed (Hanahan and Weinberg 2000) and most probably a series of several mutations in these genes is required.

Two classes of genes are especially important in the causation of cancer: tumour suppressor genes and proto-oncogenes. When mutations result in loss-of-function of tumour suppressor genes no further inhibition help to regulate the cell functions that e.g. normally control cell numbers. Mutations in proto-oncogenes have a dominant effect (gain-of-function) and the mutant genes (oncogenes) lead e.g. to increased activity in signalling cascades, further stimulating cell growth, cell cycle progression, cell proliferation and metastasis. Thus, tumour progression can often be correlated with mutations that activate specific oncogenes and inactivate specific tumour suppressor genes.

5. Malignant melanoma

5.1 Definition

A malignant melanoma (MM) is a melanocyte-derived cancer, which most often is found in the skin (*cutaneous* malignant melanoma, CMM) but can be found in all organs that harbour melanocytes, e.g. the ears, the eyes, the mucosal membranes (nose, oral cavity, anorectal mucosa and the genitourinary mucosa), the central nervous system (leptomeningeal melanoma) and in the gastrointestinal tract. It is the most malignant skin cancer type and causes the majority of skin cancer related deaths. CMM is among the most common types of cancer in young adults.

Melanomas can arise de novo in the skin (about 70%) or have a common nevus or a clinically atypical nevus as a precursor lesion (in about 30%) (Bevona et al. 2003). As long as the CMM grows in the epidermis the tumour is characterized as *in situ*, but when it grows down in the dermis it is invasive with a potential to metastasize. The histopathological examination of a melanoma always includes the measure of depth in millimetre, according to Breslow, and the measure of invasion to different skin layers (I to V), according to Clark **(Table 4.)**.

CLARK LEVEL	INVASION LEVEL
I	Intraepidermal growth/in situ
II	Cells reaching the papillary dermis
III	Cell occupation and expansion of the papillary dermis
IV	Cells invading the reticular dermis
V	Cells invading the subcutaneous fat

Table 4. Measure of invasion depth according to Clark.

CMM can further be divided clinico-histopathologically into four main types according to growth patterns: superficial spreading melanoma (SSM), nodular melanoma (NM), acral lentiginous melanoma (on hands and feet) and lentigo malignant melanoma (LMM).

5.2 Epidemiology

In Sweden cutaneous melanoma was the sixth and eighth most common cancer in women and men respectively in 2007, and the latest reported incidence of invasive melanoma was 2333 cases (1182 women, 1151 men). The intraepidermal melanomas (in situ) added another 1146 cases (606 women, 540 men) (Socialstyrelsen 2008). The last reported average annual increase of all cancers in Sweden was 1.1% for women and 1.7% for men, but for melanoma this increase was 3.8% for women and 3.6% for men and thereby the most rapidly increasing malignant tumour in Sweden **(Figure 6.)** (Socialstyrelsen 2008). This rapid increase is evident also in other fair-skinned populations all over the world (Lens and Dawes 2004). In Australia, which has the highest CMM incidence rates worldwide, the age-adjusted incidence rates were 34.6 (women) and 46.1 (men) per 100 000 individuals in year 2005 (AIHW/AACR 2008). The corresponding numbers in Sweden 2007 approached 24 (women) and 26.5 (men) per 100 000 individuals, reflecting that Sweden has some of the highest rates after Australia and New Zealand (WHO/IARC 2008).

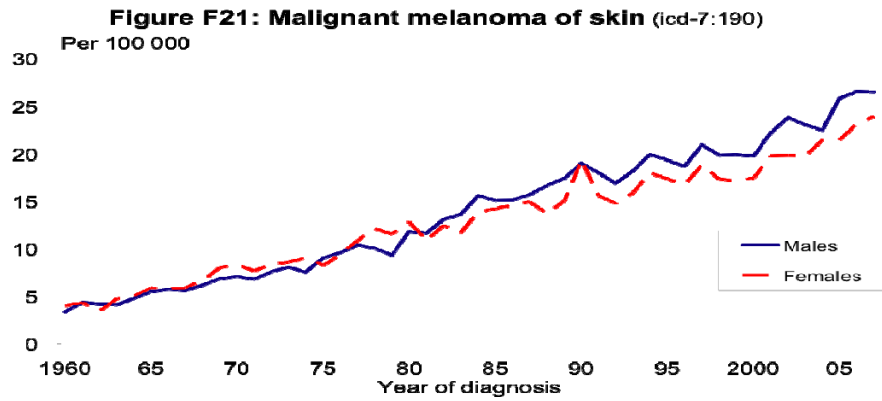

Figure 6. Trends in melanoma incidence in Sweden.

The rates are age standardized according to the Swedish population January 1, 2000. Adopted from Cancer Incidence in Sweden 2007 (Socialstyrelsen 2008).

5.3 Melanoma pathogenesis-cell signalling networks and tumour stem cells.

The pathogenesis of the MC transformation into a melanoma cell is complex and the exact mechanisms are still not wholly understood. Both inherited and somatic

genetic events probably contribute, as well as environmental factors and increased production of ROS in the MC (Meyskens et al. 2004; Miller and Mihm 2006; Gray-Schopfer et al. 2007; Sekulic et al. 2008; Nelson and Tsao 2009).

At least three changes are required to transform a MC into an invasive melanoma; (i) something that initiates clonal expansion of the MC (e.g. mutations in proto oncogenes), (ii) events that make the MC overcome cell senescence (e.g. influencing cell-cycle control), and (iii) something that reduces or suppresses the apoptotic ability of the MCs (Bennett 2008).

If mutagenic DNA in the MC escapes repair before cell division and if the cell cycle regulating mechanisms simultaneously cease to function, the melanocytes with DNA damage begin to divide in an uncontrolled manner, thus leading to the formation of a melanocytic tumour, a melanoma. Hence, dysregulations of the MC cell cycle control, of cell signalling mechanisms and aberrations in transcriptional control are all mechanisms underlying the oncogenesis of melanoma, together with other factors as availability of nutrients, activation of cell surface receptors and level of cellular stress (Hocker et al. 2008; Sekulic et al. 2008).

Recent research has also added theories about melanoma stem cells, which might probably change the view upon melanoma pathogenesis in the future. The mechanisms involved (so far known) will be briefly reviewed below.

5.3.1 Three cell signalling networks; CDKN2A/RAS/Apoptosis

Three major, interacting, signalling networks have been shown to be important: (i) the tumour-constraining CDKN2A network, (ii) the growth-promoting RAS signalling network and (iii) the key downstream regulator of apoptosis, the BCL-2/p53 network (Hocker et al. 2008).

- **The tumour-limiting *CDKN2A* network;**

One of the major genes involved in melanoma pathogenesis is found on chromosome 9 (9p21), and the gene is called *CDKN2A* (formerly *MTS1/p16/p16 [INK4A].* The gene (OMIM#600160) encodes two gene products through alternative splicing: p16/INK4A and p14ARF (alternative reading frame). **(Figure 7.)** Both are acting as tumour suppressors.

The tumour-constraining *CDKN2A* network regulates two critical cell cycle regulatory pathways, the Retinoblastoma (Rb/RB) pathway and the TP53/p53 pathway. Wild type *CDKN2A* prevents cancer formation by mediating a senescence-like state upon oncogenic stress, by the proteins acting as a brake on the cell cycle (p16) and by stabilizing p53 (p14ARF) respectively. If *CDKN2A*

function is lost the opposite will occur with uninhibited cellular division, growth and proliferation (Udayakumar and Tsao 2009).

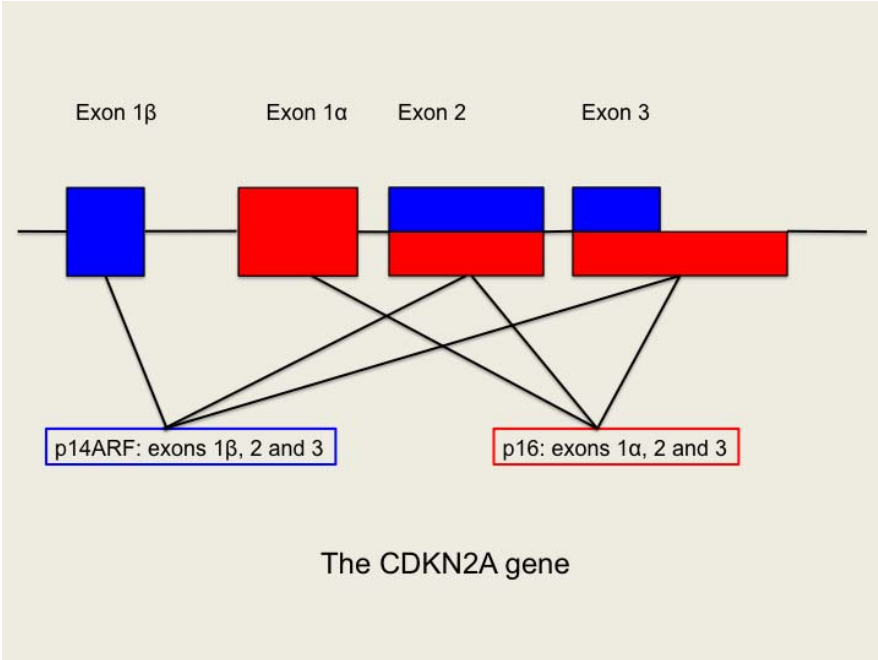

Figure 7. The CDKN2A gene.

- **The growth-promoting Ras signalling network with two cascades; (RAS/MAPK and RAS/PI3K/AKT).**

The *NRAS* proto-oncogene encodes the N-Ras/NRAS protein and is frequently (20%) found in its mutated oncogenic form in melanoma (Hocker et al. 2008; Platz et al. 2008). Mutated forms of the RAS protein are constantly active, and in this state they activate e.g. the plasma membrane bound protein B-Raf/BRAF (a kinase) and the PI3K (phossphatidylinositol-3-kinase).

The RAS signalling network regulates cell growth, survival and invasion through two cascades; (i) the RAS/BRAF/MAPK (mitogen-activated protein kinase) pathway, a major stimulus of melanocytic proliferation and (ii) the RAS/PI3K/AKT signalling stream, a promoter of melanoma progression and anti-apoptosis/survival. Mutated RAS constantly activates both the MAPK and PI3K pathways, resulting in effects on both transcriptional cell cycle regulation and apoptotic signalling (**Figure 8.**).

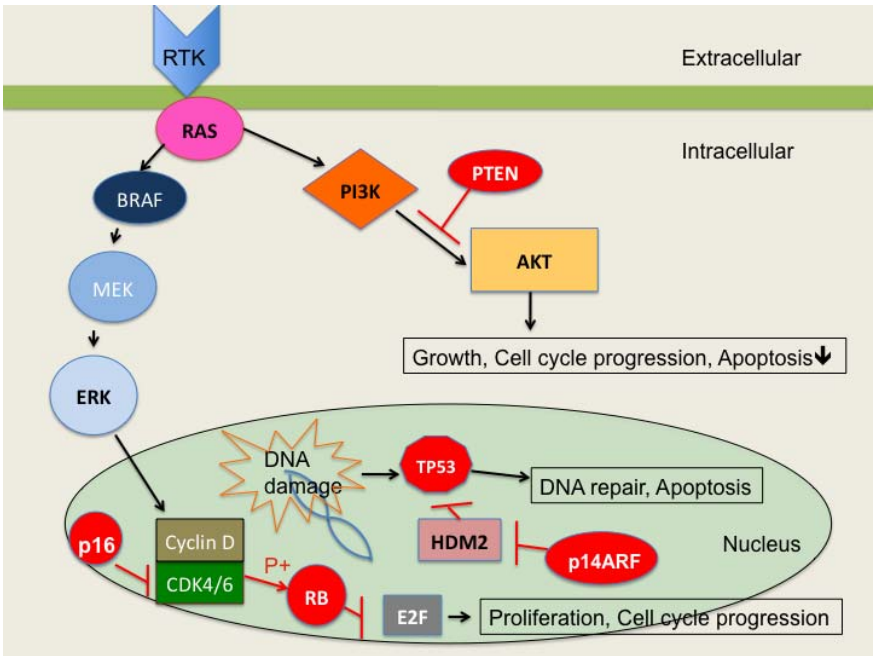

Figure 8. Cell signalling networks.

The RAS network with two cascades and the important steps with p16, p14ARF, Rb and TP53, regulating proliferation, DNA repair and apoptosis as well as growth and prosurvival signalling. RTK= receptor tyrosine kinase, RB= Rb= Retinoblastoma protein, which in a native (hypophosphorylated state) binds and inhibits the E2F transcription factor, thus preventing G1-to-S transition of the cell cycle, P+= phosphorylation of RB, thereby inhibiting RB. E2F=a transcription factor. P16 inhibits the Cyclin D1/CDK4-mediated phosphorylation of RB. P14ARF inhibits human homolog of double minute 2 (HDM2), which otherwise inhibits the action of/accelerates the degradation of p53/TP53.

Downstream, activated ERK/MAPK transfers signals (via e.g. influence on transcription factors) that regulate several cell processes as proliferation, differentiation, angiogenesis and survival (Sekulic et al. 2008). Isolated activation of the MAPK pathway, as constitutively activated (mutated) *BRAF*, results in secretion of Insulin-like growth factor binding protein 7 (IGFBP7), which in turn induces senescence through suppression of MAPK, as seen in common nevi. Melanomas however, block the IGFBP7 expression (in a so far unknown way) and escape this negative feed back loop, which leads to uncontrolled proliferation (Sekulic et al. 2008).

The PI3K/AKT pathway is important in regulation of apoptosis, cell cycle progression, cell growth (cell mass increase), cell proliferation and survival gene transcription.

Alterations in the PI3K pathway have been reported in 50-60% of melanomas. A further key component of the PI3K pathway is *PTEN* (phosphatase and tensin homolog), another tumour suppressor gene, which is commonly altered (12-50%) in melanoma, and which is the second most frequently mutated gene in human cancers after *TP53* (Yin and Shen 2008). Wild type PTEN acts as a tumour suppressor by removing phosphate groups and deactivates/dephosphorylates PI3K, and thereby suppresses cell survival and cell proliferation.

Loss of *PTEN* is often found in combination with *BRAF* mutations in melanomas on sites without chronic sun-exposure (Curtin et al. 2005). *PTEN* has further been shown to be mutated in gliomas, endometrial, breast, prostate and kidney cancers as well as in melanomas, and germline mutations of *PTEN* have been found in cancer susceptibility syndromes (e.g. Cowden syndrome) (Steck et al. 1997; Hocker et al. 2008; Yin and Shen 2008).

The effector of the PI3K pathway is Akt/AKT=protein kinase B/PKB, and the subsequent proliferation, survival and invasion are promoted through AKT. AKT is inhibiting apoptotic processes, thereby promoting cell survival (see below).

- **The regulator of melanoma cell apoptosis; the BCL-2/p53 network.**

Two apoptotic pathways exist and converge: the intrinsic and the extrinsic. Both pathways lead to the activation of effector caspases that finally mediate cell death and they are important in understanding the survival as well as the chemotherapeutic resistance of melanomas (Soengas and Lowe 2003).

The intrinsic (mitochondrial) pathway is activated through several factors (including hypoxia, loss of growth factors and DNA damage) and is regulated by the big B-cell lymphoma (BCL)-family of proteins. These proteins can be either carcinogenic/antiapoptotic/pro-survival (e.g. BCL-2, BCL-XL and MCL-1) or proapoptotic (BID, BAD, Bim, PUMA, NOXA, BAX and BAK). The proteins function to regulate and execute the intrinsic pathway of apoptosis, where BAX and BAK are finally responsible for mitochondrial permeabilization, which in turn leads to activation of caspases and apoptosis. The MAPK and PI3K/AKT pathways (see above) interact with the intrinsic pathway (Wang et al. 2007) and p53 upregulates the transcription of several pro-apoptotic genes e.g. *BAX, PUMA* and *NOXA* (Soengas and Lowe 2003; Smalley 2009).

The extrinsic (death receptor) pathway involves activation of "death receptors" in the plasma membrane and downstream activation of caspases. This activation of caspases finally leads to apoptosis, as well as an activation of BID that interferes with the intrinsic pathway.

The balance between apoptosis and survival is delicate, both in normal MCs, nevi and melanomas, and high levels of antiapoptotic proteins have been found both in MCs, common nevi and in melanoma, which could partly explain the resistance to apoptosis (Soengas and Lowe 2003; Smalley 2009).

5.3.2 The tumour stem cell theory.

A new model of cancer development has been suggested involving research about tumour /cancer stem cells (TSC/CSC). The model suggests that tumours, like melanoma, contain a subset of cells that is capable of both self-renewal and of giving rise to differentiated progeny (Grichnik 2008; La Porta 2009).

If the growth potential of melanomas is based on a rare subset of melanoma stem cells, it is important to find out how to eradicate these cells. The TSC theory might explain how thin melanomas can metastasize and why metastatic melanomas are so difficult to treat. Nevertheless, the cell of origin to the TSC is still not known.

6. Risk factors for melanoma

Risk factors can be divided into environmental and genetic. Gene-environment interactions are also likely to play a role. Genetic factors are represented by the genotypes (and accordingly expressed as phenotypes), while environmental factors are mainly represented by UVR and medical therapy e.g. immunosuppression **(Table 5.)** (Ford et al. 1995; Bataille et al. 1996; Westerdahl et al. 2000; Gandini et al. 2005; Gandini et al. 2005; Gandini et al. 2005; Bataille and de Vries 2008; WHO/IARC 2008; Caini et al. 2009; Chang et al. 2009; Chang et al. 2009).

6.1 Ultraviolet radiation - sun and sunbeds

The major environmental risk factor for melanoma is UVR exposure, a fact that is supported by several epidemiological studies and migration studies (Augustsson et al. 1992; Westerdahl et al. 1994; Elwood and Jopson 1997; Westerdahl et al. 2000; Bataille et al. 2004; Bataille et al. 2005; Whiteman et al. 2006; Hocker and Tsao 2007; Ibrahim and Brown 2008; Sekulic et al. 2008; WHO/IARC 2008). The main source for UVA and UVB is the sun but other sources are sunbeds and sun lamps.

Wavelengths in the shorter UVB range (280-320 nm) are absorbed by the epidermis while UVA waves (320-400 nm) penetrate more deeply and reach the basal layer of the epidermis and the dermal connective tissue **(Figure 1.)**.

UVR causes genetic changes in the skin, impairs cutaneous immune function, increases the local production of growth factors, and induces the formation of DNA-damaging reactive oxygen species (ROS) that affect both KCs and MCs (Miller and Mihm 2006). It is well known that UVA acts through ROS and that UVB does direct harm to DNA in the melanocytes (see below) but the exact molecular basis for the induction of a CMM is still unclear (Hocker and Tsao 2007; Bennett 2008).

The genotoxic effects of UVB radiation are mediated by the direct absorption of photons by DNA, causing photoproducts. Incorrect repair of these lesions leads to mutations in the cells in the epidermis. UVA acts by indirect effects mediated by ROS, which are generated through photoactivation of endogenous photosensitizers (e.g. porphyrins, quinones) and induce various DNA damages, including DNA breaks and oxidative modifications of nucleic bases (Marrot and Meunier 2008).

Genetically related risk factors	**Environmentally related risk factors**	**Relative risk for melanoma***
Risk genes (mutations in e.g. CDKN2A, MC1R, pigmentary genes)		**
	UVR: sunshine, sunbeds	2-3***
High number of common nevi (>100)	High number of common nevi (>100)	1-11 (Bishop et al. 2000; Gandini et al. 2005; Thompson et al. 2005)
Clinically atypical nevi (>2)	Clinically atypical nevi (>2)	2-11 (Bishop et al. 2000; Gandini et al. 2005; Thompson et al. 2005)
AMS phenotype, no family history		≈7 (Newton 1993)
AMS phenotype + family history of both CAN and MM (DNS-D2)		≈500 (Newton 1993; Slade et al. 1995)
Skin type I, II (see pigmentary genes)		≈2
DNA repair defects (e.g. Xeroderma Pigmentosum)		"High" (Cleaver 2005)
Immunosuppression (congenital)	Immunosuppression (acquired)	1-5
Family history of melanoma ****		35-70
Personal history of melanoma		8
History of sunburns (due to skin type)	History of sunburns (due to UV exposure)	2-5
	Patterns of UVexposure; chronic, intermittent	2-5 (Slade et al. 1995)

Table 5. Risk factors for melanoma, and relative risks when appropriate.

*The risk estimates are collected from (Thompson et al. 2005) or from the cited reference(s). **depends on single or multiple mutation(s) in gene(s), ***depends on frequency of exposure and age at exposure, **** strong family history (≥3 first degree relatives affected).

A meta-analysis of melanoma-case control studies showed that intermittent sun exposure and history of severe sunburn were most strongly associated with melanoma while occupational (cumulative) exposure seemed to be protective in some studies (Gandini et al. 2005). One biologic explanation to the effect of intermittent exposure is that it causes DNA damage in MCs but not enough to cause apoptosis. An accumulation of genetic mutations caused by incomplete DNA repair from every intermittent exposure could lead to malignant transformation over time.

The risk associated with use of sunbeds is debated, as most epidemiological studies have found an increased risk, especially in young users, but some have not (Westerdahl et al. 1994; Westerdahl et al. 2000; Veierod et al. 2003; Bataille et al. 2004; Bataille et al. 2005; IARC 2006). One hypothesis is that host response to UVR is more important than the dose and type of UVR exposure (Bataille et al. 2004; Bataille and de Vries 2008).

In addition, it is problematic to estimate the individual's exposure to UVR in retrospective studies, as study participants estimate the used amounts of sunscreen, the amount of UVR exposure and when the UVR exposure occurred subjectively and sometimes after a long latency (Veierod et al. 2003; Bataille et al. 2005). Moreover, multiple sunburns during childhood and adolescence and the increased risk of melanoma associated with that, might be influenced of recall bias and modified by the phenotype (Pho et al. 2006). These potential risks of bias have been of great concern when case control studies have been discussed.

6.2 Nevi, CAN and AMS

The presence of giant congenital nevi, many AMN/common nevi, and the presence of CAN, with or without a family history of melanoma, are all independent risk factors for melanoma (Slade et al. 1995; Gandini et al. 2005; Tannous et al. 2005; Sekulic et al. 2008). A meta-analysis of observational studies found that the phenotype with "≥100 common nevi" was associated with a seven times higher risk of melanoma compared to a phenotype with 0-15 nevi. Individuals with five CAN had a six times higher risk for melanoma than people with no CAN at all (Gandini et al. 2005).

Numerous nevi might indicate previous exposure(s) to UVR (UVR thereby independently causing both a larger number of nevi and an increased melanoma risk) but it could also indicate a genotype associated with a tendency to form nevi. Studies have shown that up to 60% of nevi are genetically determined. This genotype might also indicate a greater tendency to form melanoma, but so far no major nevi+melanoma gene(s) has been identified except for *CDKN2A* in some melanoma-prone families. However, a genome-wide association study (GWAS)

recently identified a number of single-nucleotide polymorphisms (SNPs) associated with both risk of melanoma and a high number of nevi. Among the SNPs reported, was one located adjacent to the gene locus of *CDKN2A* (chromosome 9p) in the *MTAP* gene and one in the gene *PLA2G6* in chromosome 22 (Falchi et al. 2009).

The AMS phenotype is also independently a risk factor for melanoma (see Introduction, chapter 3.).

6.3 Skin, eye and hair phenotypes

Red/blond hair, freckles, inability to tan and blue/grey eye colour are all factors associated with an increased risk for melanoma. The skin types I and II **(Table 1.)** present fair skin, poor tanning ability, a tendency to burn easily and often additionally light/red hair, light eye colours and the presence of freckles. These phenotypic characteristics are well known to have an association with the genes that regulate skin colour and pigmentation. One of these key genes is *MC1R* (see above). Some variants of *MC1R* are more strongly associated with melanoma as well as with red hair/fair skin, while others are only associated with red hair or red hair/fair skin but not with melanoma (Raimondi et al. 2008).

Other phenotypic risk factors that have been studied are e.g. indicators of actinic skin damage and a history of a previous premalignant or invasive nonmelanoma skin cancer (NMSC) (i.e. Actinic keratoses [AK], Squamous Cell Carcinoma [SCC]). These risk factors are also indicators of cumulative sun exposure. In addition, the freckling phenotype is also closely connected to the amount of UVR that the individuals have been exposed to (Gandini et al. 2005). This stresses the delicate balance between hereditary and environmentally causes of melanoma.

6.4 Immunosuppression

Individuals with an immune deficiency (e.g. organ transplant recipients with immunosuppressive therapy and individuals with HIV/AIDS) have an increased risk to develop cancer, and the risk of melanoma has been shown to be 1.24 in HIV/AIDS patients and 2.34 in transplant patients. The comparable risk of NMSC was 4.11 for HIV/AIDS patients and 28.62 for transplant patients (Grulich et al. 2007).

6.5 Inherited susceptibility to melanoma/Genetics

About 90 % of all melanomas are sporadic and only 10% of all cases of melanoma occur in patients who have a hereditary predisposition for melanoma **(Figure 9.)** (Thompson et al. 2005).

In the high-risk melanoma-prone families several cases of melanoma are present in multiple generations. The affected family members often develop multiple primary melanomas and are diagnosed at a younger age than non-familial sporadic cases. The observed increased melanoma risk in *relatives* of melanoma cases is probably caused by both genetic factors and shared environmental exposure (Ford et al. 1995; Begg et al. 2004).

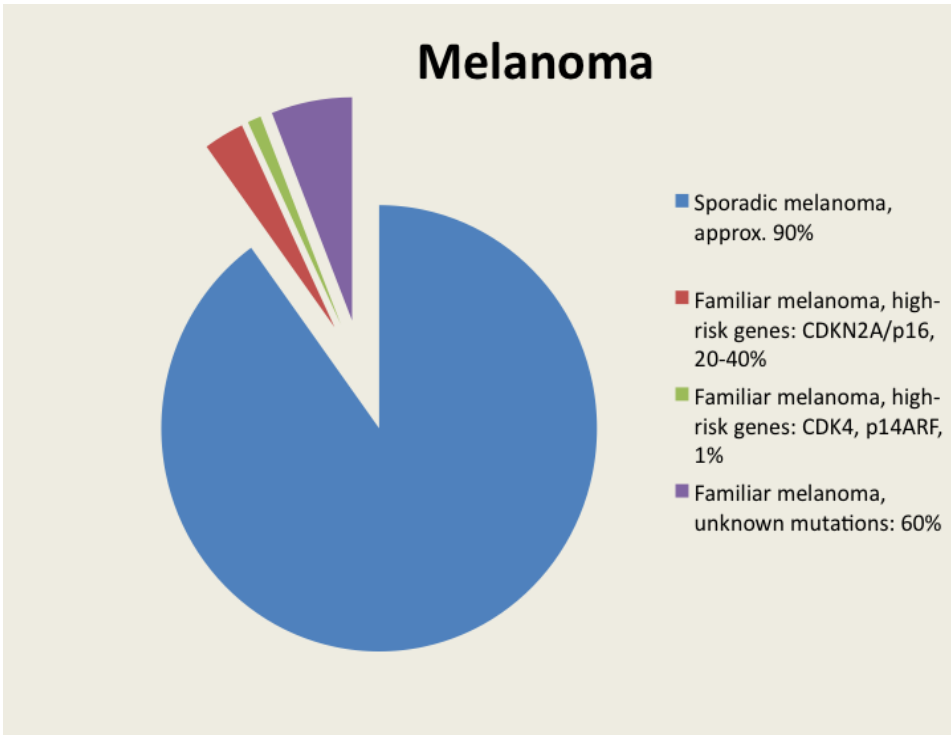

Figure 9. The ratio of sporadic and familiar melanoma.

The high-risk melanoma-prone families are under intense research and several underlying genes have been revealed. The melanoma genetics consortium, GenoMEL, (www.genomel.org) is an organisation with melanoma research groups from 14 countries around the world pooling data, in order to elucidate the genetic field of melanoma and with a special interest for familiar melanoma (Newton Bishop and Bishop 2005; Goldstein et al. 2006; Goldstein et al. 2007; Harland et al. 2008; Borges et al. 2009; Leachman et al. 2009).

The heritable genes for melanoma susceptibility can vary from rare high-risk, high-penetrance genes to low-risk, low-penetrance genes that are rather common in populations (Hocker et al. 2008; Meyle and Guldberg 2009; Nelson and Tsao

2009; Udayakumar and Tsao 2009) **(Table 6.)**. It is thought that the high-risk, high penetrance genes are responsible for the rare familial melanomas but not for the sporadic cases, which are instead probably caused by more common allelic variants ("polymorphisms") in the moderate risk or low-risk genes (Bennett 2008; Nelson and Tsao 2009). Some of the genes will be highlighted below.

High-risk genes	Moderate risk gene	Low-risk genes
CDKN2A-p16	*MC1R*	*CCND1 (cyclin D1)*
CDKN2A-p14ARF		*PTEN*
CDK4		*MITF*
Rb/RB		*BRAF*
		NRAS
		TP53
		Pigmentary genes

Table 6. Genes associated with the development of melanoma.

- ***CDKN2A-the major high-risk gene***

The major gene involved in melanoma development is the tumour suppressor gene *CDKN2A*, mentioned above (see cell signalling networks, **Figures 7, 8.**). Studies have shown that this high-risk gene is mutated in 20-40% of melanoma-prone families (with ≥3 members affected by melanoma), as compared to a mutation frequency of about 1-2 % in population-based melanoma patients (Thompson et al. 2005). Mutations in the gene have also been linked to an increased risk of other tumours as e.g. pancreatic cancer (PC) and breast cancer (Borg et al. 2000; de Snoo et al. 2008; Landi 2009). The encoded two gene products (p16, which is a negative regulator of cell cycle progression, and p14ARF which stabilizes p53) are both tumour suppressors. The exons 1α, 2 and 3 form p16 and the exons 1β, 2 and 3 form the p14ARF protein, and hence mutations in the *CDKN2A* gene can alter one or both proteins and their functions, depending on where the mutations are found.

Most mutations occur in exons 1α or 2, affecting the p16 and/or the p14ARF proteins, but some families show mutations in exon 1β only, which merely therefore affects the p14ARF protein.

More than 60 mutations are described affecting the p16 protein, but uniquely for Sweden there is a specific germline mutation (*CDKN2A/113insArg*), which

explains the majority of *CDKN2A* mutations found (Borg et al. 1996; Platz et al. 1997; Borg et al. 2000; Hashemi et al. 2001; Hashemi et al. 2002). Analysis has shown that the age of this founder mutation must be approximately 2000 years (Hashemi et al. 2001) and thus the majority of the Swedish melanoma-prone families (with this germline mutation) are anciently related.

The functional (wild type) protein p16 acts by inhibiting the cyclin-dependent kinase 4/6 (CDK4/6) and cyclin D1 (CCND1)-mediated phosphorylation of the retinoblastoma protein (RB/Rb) **(Figure 8.)**. As hypophosphorylated (native/active status), the RB protein binds and represses the E2F transcription factor (which in turn normally induces the transcription of S-phase genes) and hence induces cell cycle arrest by preventing G1-to-S transition **(Figure 10.)**.

On the contrary, when the *CDKN2A* gene is mutated the p16 protein cannot inhibit CDK4, resulting in inactive (phosphorylated) RB/Rb, and thus the cells are allowed to pass the G1/S checkpoint and can undergo unregulated cell division (Nelson and Tsao 2009).

The second gene product p14ARF binds to and prevents human double minute-2 (HDM2) from accelerating the degradation of p53, thus possessing tumour-suppressive effects.

p53 normally senses genetic damage and allows pause for DNA repair or activates apoptosis if there is too much DNA damage. Decreased p53 leads to genetic instability when mutations and other genetic damage are left.

A mutated p14ARF does not inhibit HMD2, which in turn accelerates the destruction of p53 and thus enhances growth and survival of altered/damaged cells instead of cell cycle arrest and apoptosis.

Families with mutations in the exon 1β resulting in a mutated p14ARF protein have e.g. shown an association between melanoma and neural system tumours (NST) (Randerson-Moor et al. 2001).

- ***CDK4 and RB/Rb--other high-risk genes***

As mentioned, the normal p16 protein binds to (and inhibits) the action of CDK4. Mutations in the *CDK4* gene make the CDK4 resistant to p16 inhibition and thus give the same phenotypic results as for a non-functional p16. Not inhibited CDK4 interacts with CCND1 and phosphorylates the RB/Rb protein, hence making RB/Rb inactive and allowing cells to unregulated pass the G1/S checkpoint **(Figure 10.)** (Nelson and Tsao 2009).

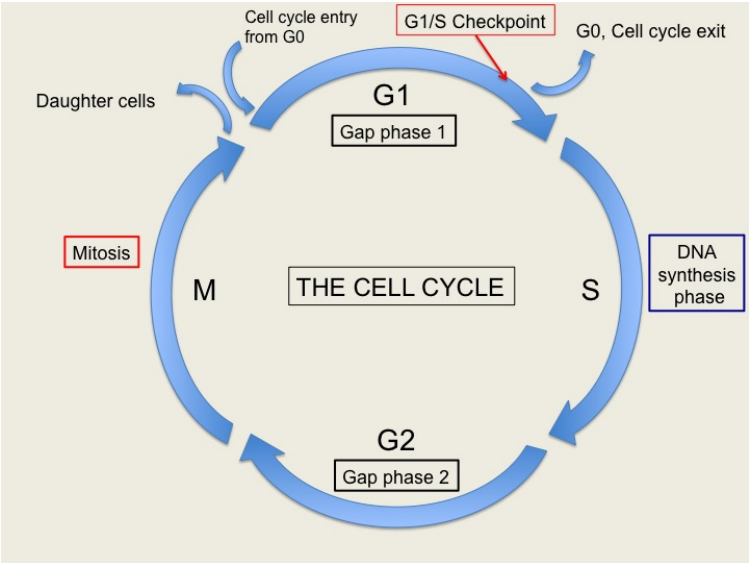

Figure 10. The cell cycle of eukaryotic cells.

G0= Resting/Senescent or quiescent phase, G1= Gap phase 1, G2= Gap phase 2, S= DNA synthesis phase, M= Mitotic phase, the cell division phase.

CDK4 mutations have been identified in about ten melanoma-prone families in the world (Zuo et al. 1996; Soufir et al. 1998; Molven et al. 2005; Meyle and Guldberg 2009; Pjanova et al. 2009). Moreover, *CDK4* amplification has been observed in sporadic melanomas, especially in acral and mucosal melanomas (Curtin et al. 2005).

Families with germline mutations in the retinoblastoma gene (*RB1*), especially inherit a risk for retinoblastomas, but individuals who survive the retinoblastoma tumour also have a high risk for developing melanomas, as RB/Rb no longer can bind E2F and prevent unregulated cell division (Kleinerman et al. 2005). A *RB1* mutation is found in approximately 6 % of sporadic melanomas (Bennett 2008).

- ***MC1R, a moderate-risk gene***

Mutations or variants (SNPs, polymorphisms) in the *MC1R* gene cancel the signalling cascade that leads to elevation of intracellular cAMP in the MCs, which in turn favours pheomelanogenesis instead of eumelanogenesis **(Figures 2, 3)**. That is why many carriers of *MC1R* variants present the phenotype with fair skin (skin type I/II), red or blond hair and sun sensitiveness **(Table 1.)**. An increased melanoma risk has been associated with the variants Asp84Glu/D84E, Arg142His/R142H, Arg151Cys/R151C, Ile155Thr/I155T, Arg160Trp/R160W, Arg163Gln/R163Q and Asp294His/D294H, with different Odds Ratios (ORs)

ranging from 1.42 to 2.45 (Raimondi et al. 2008). A single *MC1R* variant might confer a 2.7-fold risk for melanoma, while the presence of two variants might lead to a 3.6-fold risk (Udayakumar and Tsao 2009).

However, all variants are not associated with an increased melanoma risk or the phenotype with red hair and fair skin, which make the associations complex. Nevertheless, the variants D84E, R151C, R160W, D294H as well as R142H have all been associated with the red hair colour (RHC) phenotype. On the contrary, V60L and V92M do not seem to be correlated to either risk of melanoma or red hair colour, while I155T and R163Q are associated with risk of melanoma, but not with red hair colour (Raimondi et al. 2008). Other studies have suggested that an interaction between *MC1R* variants and *CDKN2A* mutations may independently play a role in melanoma risk, possibly by way of modifying the gene penetrance of *CDKN2A* mutations (Box et al. 2001; Goldstein et al. 2005).

- ***Pigmentary genes and other low risk genes***

The association between phenotypic characteristics regarding human pigmentation, as e.g. skin/eye/hair colour, and the risk for melanoma has been known for a long time (Gandini et al. 2005). Worldwide, variations in human pigmentation are very common, and thus several gene variants/polymorphisms in pigmentary genes are commonly found. They confer much lower risk levels for melanoma, but according to the common presence the risks are still evident at a population level. Several phenotypic characteristics are inter-related and probably interact in determining melanoma risks. Today more than 120 genes are known to contribute in determining the basal and acquired skin pigmentation (Udayakumar and Tsao 2009).

Recently, GWAS have revealed several SNPs associated with pigment synthesis and host factor pigmentation traits (e.g. skin colour, hair colour, freckles and eye colours)(Sulem et al. 2007; Gudbjartsson et al. 2008; Sulem et al. 2008). The genes involved in different ways in the pigmentation process are e.g. *TYR, TYRP1, POMC, ASIP, OCA2, SLC45A2/MATP*, *SLC24A4* and *SLC24A5* (Bishop et al. 2009; Nan et al. 2009; Udayakumar and Tsao 2009). Different SNPs close to or encompassing these genes have been examined in relation to melanoma risk (Gudbjartsson et al. 2008; Bishop et al. 2009; Nan et al. 2009). One recent study by GenoMEL, showed e.g. increased risk for melanoma associated with SNPs in loci correlated to the *MC1R* gene on chromosome 16, the *TYR* gene on chromosome 11 and a in a locus adjacent to the *MTAP* gene (flanking the *CDKN2A* gene) on chromosome 9 (Bishop et al. 2009).

7. Cancers associated with melanoma

For a member of a very melanoma-prone family (≥3 first-degree relatives affected), the relative risk for an individual with this strong family history for developing a cutaneous melanoma can be as high as 30-70, compared to the risk for developing a melanoma in the general population (Kefford et al. 1999). The high risk in these families is most probably a result of shared genes and shared environment, and just as melanoma is a result of both environmental and genetic risk factors, including several acquired and inherited genetic mutations, the risk of developing additional, *non-melanoma cancers* might be increased, both for patients affected by melanoma and for their close relatives. Investigating the prevalence and risks for non-melanoma cancers among melanoma cases and their relatives might give clues to a better understanding of cancer predisposition and the genes involved (Larson et al. 2007).

In Sweden it has e.g. been shown that when parents had a single melanoma diagnosis, irrespective of a familial setting and depending on invasive or *in situ* diagnosis in the parents, the familial risk (Standardized Incidence Ratio, SIR) of invasive melanoma in offspring varied between 2.4 and 4.0 respectively, with the highest risk associated with *in situ* melanoma in the parents (Hemminki et al. 2003). Another study has shown that first-degree relatives of melanoma patients have a 2- to 3-fold increased risk for melanoma development (Larson et al. 2007).

In melanoma-prone families, with the specific high-risk *CDKN2A* mutation, additional associations apart from melanoma, to breast cancer (Ghiorzo et al. 1999; Borg et al. 2000; de Snoo et al. 2008), pancreatic cancer (Ghiorzo et al. 1999; Borg et al. 2000; Goldstein 2004; Goldstein et al. 2006; de Snoo et al. 2008), childhood cancer (Magnusson et al. 2008) and lung cancer (Borg et al. 2000; de Snoo et al. 2008) have been found, probably dependent on the non-functional gene products of the mutated tumour suppressor gene.

Other associations between melanoma and non-melanoma cancers (not always found in connection with a *CDKN2A* mutation) have been reported, and e.g. the risk for developing a second primary cancer in patients affected by melanoma has been studied.

For example, the reported overall risk (SIR) of a second primary (non-melanoma) cancer in a melanoma-patient has been shown to be 1.23 in Sweden (Tuohimaa et al. 2007) and more than doubled (2.06) in Northern Ireland (Cantwell et al. 2009).

Although NMSCs (mainly SCC and to some extent Basal Cell Carcinoma [BCC]), are often the most reported second primaries (Wassberg et al. 1996; Wassberg et al. 1999), associations with a diagnosis of melanoma and e.g. Neural System

Tumours (NST) (Azizi et al. 1995; Bahuau et al. 1997; Hemminki et al. 2003; Nielsen et al. 2004; Marian et al. 2005; Pasmant et al. 2007; Nielsen et al. 2008), Non-Hodgkin's lymphoma (NHL) (Lens and Newton-Bishop 2005), prostate cancer (Hemminki and Chen 2005), lung cancer (Yarbrough et al. 1996; Debniak et al. 2003), kidney cancer (Schmid-Wendtner et al. 2001) and liver cancer (Debniak et al. 2003) have also been shown.

There are different explanations of why specific tumour types are associated to melanomas. Shared environmental risk factors (e.g. UVR) and genes associated with sensitivity to UVR exposure could to some extent explain the increased risks for other skin cancers, and the shared embryonic origin (neural crest) of the nervous system and the melanocytes might possibly explain some of the associations found for NST. Most explanations probably exist within the field of molecular genetics, where intense research is ongoing to find the responsible gene(s), DNA copy number variations (CNVs) and/or SNP(s). Many observed changes involve tumour suppressor genes, genes involved in DNA repair, apoptosis regulation, or cell-cycle regulatory factors, which could have important influences not only on predisposition for melanoma, but also on other cancer types.

Regarding the co-morbidity of NST and melanoma, a syndrome merely including astrocytomas and melanoma (OMIM # 155755), was first described in one family from the Mayo Clinic, Minnesota in 1993 by Kaufman et al. (Kaufman et al. 1993), where later studies revealed deletions of the whole *CDKN2A* gene, though excluding the *CDKN2B/p15* gene, adjacent to *CDKN2A* **(Figure 11.)** (Bahuau et al. 1998).

In 1995, several Jewish families with clustering of NST and melanoma were reported by Azizi et al. (Azizi et al. 1995), but no associations with the *CDKN2A* gene were found when the families were genetically tested (Marian et al. 2005). However, a French family was reported by Bahuau et al. in 1997 (Bahuau et al. 1997), where both melanoma and NST (not merely astrocytomas) were found, and the genetic tests later revealed a large deletion of the chromosome 9p, including both *CDKN2A/p16/p14ARF* and the adjacent *CDKN2B/p15* (Bahuau et al. 1998).

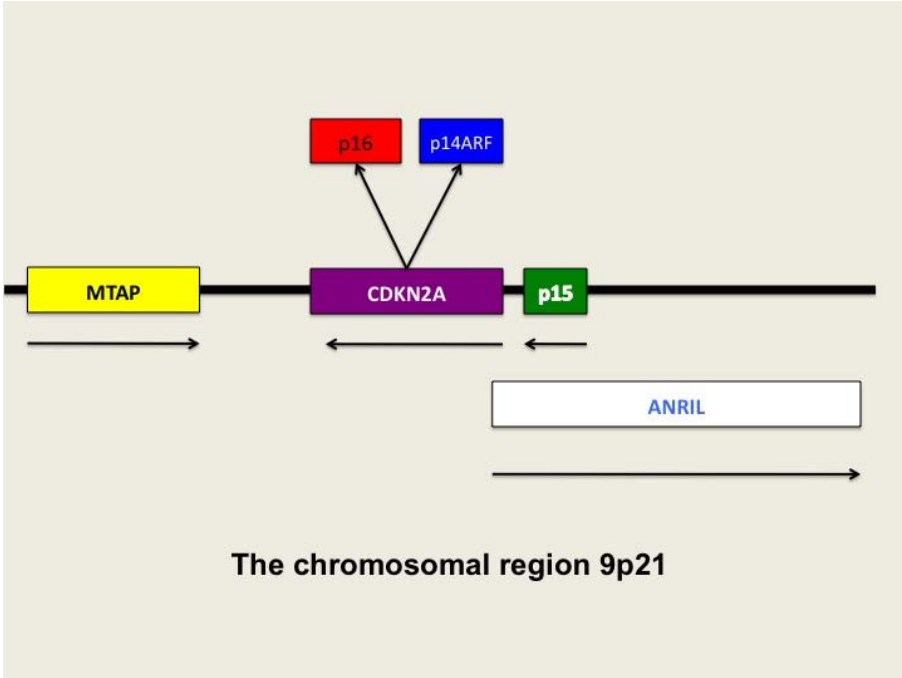

Figure 11. The chromosomal region 9p21.

The figure shows some of the adjacent genes on chromosome 9, important in understanding where the different mutations/SNPs in studies are located. MTAP=metylthioadenosine phosphorylase, p15= CDKN2B, ANRIL =CDKN2B antisense RNA = CDKN2BAS.

In 2001, Randerson-Moor et al. described yet another U.K. family with clustering of melanoma and NST. The family showed a germline deletion of the *CDKN2A* exon 1β, the *p14ARF*-specific exon of the *CDKN2A* gene (Randerson-Moor et al. 2001). This family did not show the melanoma-astrocytoma syndrome but a single individual presented pituitary macro-adenoma, uterine adenocarcinoma, thyroid adenoma, a neurilemmoma and multiple melanomas simultaneously, in addition to melanoma cases in three first-degree relatives.

Further, in a very large study of melanoma-prone families in different parts of the world, only weak associations to NST and mutations in *CDKN2A(ARF)* were found (Goldstein et al. 2006).

Nevertheless, in 2007 Pasmant et al. re-examined the family described by Bahuau (Bahuau et al. 1998), and suggested the possibility of a new gene within this large deleted locus, possibly involved in both melanoma-NST syndrome families, in somatic tumours and in melanoma-prone families with no identified *CDKN2A/p16* mutations. They posed that the newly identified gene *ANRIL* (*CDKN2B* antisense RNA [Noncoding]) as well as the metylthioadenosine phosphorylase (*MTAP*)

gene, both deleted within the gene cluster previously described, might be of importance for cancer predisposition (Pasmant et al. 2007).

On the contrary, Solomon et al. were not able to verify that a suspected gene on 9p23 (*PTPRD*) was mutated, either in melanoma-NST kindreds or in familial melanoma (Solomon et al. 2009). Thus, the pathogenesis of this co-morbidity is not fully understood and more research is needed.

Other syndromes that might involve melanoma diagnoses are e.g. the Li-Fraumeni syndrome, where a mutation in the major tumour suppressor gene *TP53* is responsible for the many cancers, including melanoma, which can affect a single individual (Upton et al. 2009), or Bloom syndrome, where growth inhibition, light sensibility and high occurrence of earl onset cancers characterise the phenotype (Broberg et al. 2007; Broberg et al. 2009).

Further, individuals affected by e.g. Xeroderma Pigmentosum type A (XPA), an autosomal recessive disorder caused by mutations in the *XPA* gene (OMIM # 611153) on chromosome 9 (9q22.3), are at increased risk for skin cancers, including melanoma, as the *XPA* gene codes for a nucleotide excision repair (NER) enzyme.

The NER enzymes are responsible for DNA repair and removal of UVR-induced photoproducts, and mutations in the corresponding genes result in defect DNA repair capability and therefore several DNA mutations are gathered. This predisposes already at a very early age for all kinds of skin cancers in e.g. *XPA* mutation carriers (Cleaver 2005). (Other XP types exist (XP B-G) but will not be reviewed here.)

AIMS OF THE THESIS

The overall aims of the thesis were to examine risk factors for malignant melanoma and to verify the clinical presentation of high-risk patients.

The specific issues in our studies were:

Study I

-Is the *CDKN2A* mutation overrepresented in patients with four or more cancers, including at least one melanoma, and do specific patterns of associated cancers disclose?

Study II

-Are the same trends of cancers detectable among the closest relatives of probands in Paper I?

Study III

-Do high-risk patients, harbouring the *CDKN2A* mutation, present a specific phenotype?

-Is this phenotype identical with the AMS phenotype?

Study IV

-Will prospective collection of data give other results than those from retrospective case-control studies?

-Which population-based risk factors predispose for melanoma in women?

-Phenotypic characteristics of female melanoma patients?

-Risk factors in relation to anatomic site of melanoma?

MATERIAL AND METHODS

1. Background data

1.1 The Swedish population and medical regions

In 2007 the population in Sweden was in total 9148092 individuals, 4604370 women and 4543722 men. The medical regions divide Sweden in six parts reflecting also the six regional cancer registries. The Southern Swedish Health Care Region comprises the counties of Skåne, Blekinge, Kronoberg and the southern part of the county Halland. This region is located around the 56th latitude north, with totally approximately 1.6 millions (18 %) of Swedish residents, mostly of Caucasian origin.

1.2 The Swedish National Population Registry

Since 1991 the Swedish Tax Agency (Skatteverket) deals with the population registration in Sweden. From the beginning of the 17th century, the church and the local parishes were responsible for this census administration. All persons registered in Sweden are given a personal identity number as identification and this number is kept for their whole life. The identity number gives information about date of birth (six digits), a birth number (three digits) and one control digit. The Registry contains information about who lives in the country and where they live as well as information about name, identity, family relationships, any immigration to and emigration from Sweden, death and place of burial (Skatteverket 2007).

1.3 Cancer Registration in Sweden

The National Swedish Cancer Registry was established in 1958 and according to regulations by the National Board of Health and Welfare a double reporting system is connected to all the Regional Cancer Registries, where both the responsible physician and the responsible pathologist independently must report all new cases of malignant tumours. Six regional cancer registries cover Sweden and send information annually of newly registered cases to the National Cancer Registry. All definitely malignant diseases as well as all carcinoid tumours, all tumours (including benign tumours) of the central nervous system, endocrine glands (excluding benign tumours of the thyroid) and some precancerous lesions must be reported. If a person has multiple primary tumours, each tumour is

registered separately. Information in the registries includes the personal registration number, the malignant disease, sex, diagnosing hospital, site of tumour, histological type, stage according to appropriate staging systems and date of diagnosis. Data from the Cause of Death Registry is also available in the Cancer Registry with information about date of death and cause of death according to the International Classification of Diseases, tenth revision, ICD-10, (or earlier ICD-versions 1958-1997). The unique personal identification number assigned to every citizen in Sweden makes matches to the cancer registries easy and reliable (Socialstyrelsen 2008).

2. Study design, Study populations and Methods

2.1 Study I

- Design

The hypothesis of an enrichment of the *CDKN2A* mutation among patients with several primary tumours including one or more melanomas was tested together with hypotheses of possibly different etiologies of the CMM and hence different tumour aggregations according to age at melanoma diagnosis or to one or multiple melanoma diagnoses.

- Patients

The overall study population in study I consisted of all patients in the Southern Swedish Regional Cancer Registry diagnosed between 1958 and 1999, with four or more primary tumours (n=394). To be included in the final analysis a further selection of these patients was made, in favour of patients with four or more primary tumours of the same or different origin and of which at least one tumour should be a cutaneous melanoma.

- Methods

Available (two patients were impossible to test) paraffin-embedded tissue blocks or peripheral blood samples were tested for the unique Swedish germline mutation *113insArg* in the *CDKN2A* gene (Borg et al. 1996).

2.2 Study II

- Design

The study was based on the hypothesis that some of the cancers found among the index patients in Study I might be found also in first- and second-degree relatives, as a result of shared genes, shared behavioural patterns or shared environment.

- Patients

The first- and second-degree relatives (n=521) of the index patients (probands) in Study I were included in the study.

- Methods

The relatives were traced through the National Swedish Population Registry and through parish records. A match of the personal identity numbers of the relatives was made with the National Cancer Registry and the Population Registry to identify cancer diagnoses and deaths. All tumours, *in situ* as well as invasive, were included. Tumour distribution, among relatives as individuals and according to previously described subgroups of the corresponding probands, was analysed. All NMSC were SCC and not BCC.

2.3 Study III

- Design

The ambition of the study was to phenotype and genotype mutation carriers and non-carriers in the families living in the Southern Swedish Health Care Region with a hereditary melanoma-predisposing *CDKN2A(113insArg)* mutation. The aim was to verify, if possible, the high-risk phenotype of mutation carriers.

- Patients

The definition of a family with hereditary melanomas was "a family with two or more cutaneous melanoma cases in first or second-degree relatives". From a previous study of possible melanoma inheritance (Borg et al. 2000), seven families (originally nine of 52 tested) were identified with a verified *113insArg* mutation. The additional two families included in this study reported a family history of melanoma in an unpublished population-based case-control study about risk factors for melanoma, obtained 1995-1996. In that study 47 individuals reported a positive family history, and of those two individuals/probands fulfilled the family criteria as well as tested positive for a *113insArg* mutation.

- Methods

Via the index patients all nine melanoma-prone families were invited to an educational research lecture about melanoma research. In connection with the lecture, a total skin examination, including a total nevus count, was offered to all participants. On the same occasion all participants older than 18 years were offered a blood sample test (voluntarily) for genotyping of *CDKN2A(113insArg)* and *MC1R.* Family pedigrees were constructed. Melanoma cases were verified in the National Cancer Registry and histopathologically re-examined. Risk factors for melanoma, in relation to phenotypic and genotypic characteristics, were evaluated.

2.4 Study IV

- Design

The study design was to prospectively investigate risk factors for melanoma, in a cohort of originally 40.000 Swedish women with no prior cancer diagnoses.

- Patients

One thousand women from each birth cohort in the period between 1926 and 1965 were randomly selected from the Regional Population Registry/Census Registry. The women were of Swedish/Caucasian origin and lived in the Southern Swedish Health Care Region.

- Methods

A questionnaire about risk factors for melanoma, Melanoma Inquiry of Southern Sweden (MISS I), was sent to the cohort in 1990-92, at the inception of the study. The cohort was then followed with regard to any cutaneous melanoma diagnoses until any event of death, a cutaneous melanoma diagnosis or until December 31, 2008. A match with the National Cancer Registry and the Cause of Death Registry was made in January 2009 to verify cutaneous melanoma cases. Available histopathological slides of the melanoma until 2006 were re-examined by one pathologist.

Reported risk factors for melanoma were examined in relation to age group, site of melanoma and histological type of melanoma. The age groups were chosen, with the breakpoint of the year 1950 (i.e. younger or older than 40 years at baseline), because sunbed use has been shown to be more common in younger individuals (Westerdahl et al. 1994, Veierod et al. 2003).

3. Statistical methods

P-values ≤0.05 were considered significant in all four studies when appropriate.

3.1 Study I

The Wilcoxon Mann-Witney test was used when the difference in age distribution and mutation prevalence between the different groups were analysed and exact OR and Confidence Intervals (CI) were calculated. Age- and sex-specific expected values were calculated for NST and for NMSC according to ICD -7.

3.2 Study II

The cancer distribution was analysed and standardized morbidity ratios (SMRs) were calculated by means of the indirect standardization method. The Swedish population was used as a reference.

3.3 Study III

Median and interquartile ranges (IQR) were used when showing nevus counts, age at diagnosis and for the presentation of Breslow thickness. To be able to compare the results about total nevus counts with the current literature, both median and mean counts (±Standard Deviation [SD]) were presented, although the counts were not normally distributed.

Fisher's exact test was used for comparison of proportions (frequencies) of the most common MC1R variants between groups (e.g. mutation carriers vs. non-carriers, melanoma cases vs. no melanoma diagnosis). It was also used to compare the presence of CAN in mutation carriers vs. non-carriers. Wilcoxon Mann-Whitney test was used when the distribution of nevi (continuous variable) was compared in mutation carriers vs. non-carriers. To examine the independent risk factors for a melanoma diagnosis and for the presence of a *CDKN2A(113insArg)* mutation respectively, a backward stepwise logistic regression test was applied. The assigned family identification numbers were used in the multivariate analyses to avoid difficulties in interpreting the results with respect to familial dependence. Linear regression test was used when comparing mutation carriers and non-carriers regarding their total mean number of nevi.

3.4 Study IV

Frequencies were analysed for comparison of proportions between melanoma cases and controls, using Chi-squared test or two-sided Fisher's exact test. These univariate analyses were presented with OR and 95% CI.

Multivariate analyses were performed (Cox proportional hazard regression) to evaluate the risk factors that contributed independently of each other to the risk of melanoma in relation to (i) the whole cohort, (ii) the cohort sub-divided in two age groups (women younger or older than 40 years respectively), (iii) the histological sub-groups and (iiii) the anatomical sites of the melanoma. In the multivariate analyses, adjustments for year of birth, and for all the other tested risk factors in the tables were made simultaneously.

RESULTS

1. Study I

Searching the Cancer Register for the defined specific inclusion criteria resulted in 44 individuals who were included in the final analyses.

One blood sample and 41 paraffin-embedded tissue blocks were tested for the mutation *113insArg* in the *CDKN2A* gene (Borg et al. 1996) and four mutation carriers (9%) were found; three with multiple melanomas and one with a single melanoma.

The cohort was subdivided into three groups according to hypotheses about possibly different etiological genesis of the melanomas; A) Multiple melanoma cases in addition to other tumour types, B) Single melanoma cases with mostly adenocarcinomas in addition, and C) Late-onset, single melanoma cases in addition to NMSC and/or other tumour types.

The three groups, with the additional tumour types, are presented in **Figure 12.**. Number of patients (not number of diagnoses), sex ratios, number of *CDKN2A* mutation carriers, additional tumour types and median age at (first) melanoma diagnosis are shown.

In Group A several cases of NST and NMSC were observed, and only a few other tumour types were diagnosed, where prostate cancer was the third most common diagnosis. Significantly more NST and NMSC respectively, were seen in Group A: five observed cases vs. 0.2 expected ($p < 0.0001$) and six observed cases vs. 0.2 expected $p < 0.0001$). None of the NST affected individuals was a mutation carrier. Median age at (first) melanoma diagnosis was 60 years, which was the youngest age at melanoma diagnosis observed in the three groups, and median age of death was 81 years, which was significantly younger than in Group C ($p<0.005$) but not compared to Group B ($p=0.21$).

In Group B two cases of NST were observed and according to the subdivision of groups no NMSC was observed. Instead, the predominant pattern of cancer types were of adenocarcinomal origin, such as prostate cancer, breast cancer, kidney cancer (renal cell carcinoma) and colon cancer. The NSTs and pancreatic carcinomas observed were not associated with the mutation carrier. Median age for the melanoma diagnosis was 67 years and median age of death was 77 years, i.e. the youngest age of death in the studied groups.

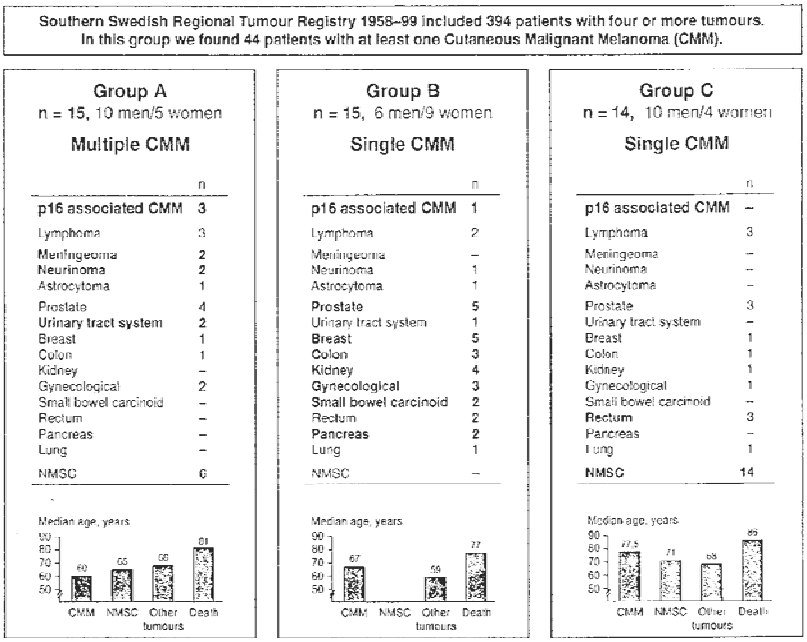

Figure 12. Subgroups in study I.

Numbers of individuals, sex ratios, number of *CDKN2A* mutation carriers, associated tumours and median ages for different tumour types for Groups A, B and C. Total numbers of diagnoses (if multiple primaries) are not demonstrated. CMM= cutaneous malignant melanoma, NMSC= nonmelanoma skin cancer (only including squamous cell carcinomas), *p16=CDKN2A* gene. The median age of CMM diagnosis in Group C should be 78 years.

The expected rate for NST in a normal population comparable with group B was shown to be 0.2, vs. the observed rate of 2 NST diagnoses (p=0.04).

In Group C significantly more NMSC were seen (14 observed cases vs. 0.5 expected, $p < 0.0001$), and several individuals (n=10) were affected by multiple NMSC primaries. Seven of the participants were affected by a NMSC prior to their melanoma diagnosis. No NST case was diagnosed in this group. Both median age of melanoma diagnosis (78 years) and median age of death (86 years) were the highest observed in the study. A statistically significant difference in median age at melanoma diagnosis was seen both when comparing Group B (67 years) with Group C (78 years) ($p<0.04$) and when comparing Group A and C (see above).

2. Study II

624 first-and second-degree relatives of the 44 probands from the previous study were identified, but 103 were excluded due to emigration, incomplete personal identification number or death before the founding of the Cancer Registry (1958). Thus, the studied cohort included 521 individuals, of which 84 were related to *CDKN2A* mutation carrier probands and 437 were related to non-carrier probands.

In total, an overall increased risk for cancer was observed (SMR 1.4, 95% CI; 1.1-1.7) among the relatives (n=521). The increased risk was highest for melanoma (SMR 8.7, 95% CI; 5.2-13.5). A significantly increased risk was also noted for colon cancer (SMR 2.0, 95% CI; 1.0-3.5). It was noteworthy that also the risk for NST was increased (SMR 2.2, 95% CI; 0.7-5.2), although not statistically significant. **(Table 7.)**

Tumours	**SMR (95% CI)**	
All tumours	1.4	(1.1-1.7)
CMM	8.7	(5.2-13.5)
NST	2.2	(0.7-5.2)
NMSC	1.4	(0.4-3.2)
Corpus uteri	2.4	(0.7-6.1)
Colon cancer	2.0	(1.0-3.5)
Breast cancer	0.9	(0.4-1.8)
Pancreatic cancer	1.4	(0.3-4.0)

Table 7. The risk for cancer (all relatives).

The table shows the cancer risk as standardized morbidity ratio (SMR) with 95% Confidence Interval (CI) for all included relatives, irrespective of possible inheritance of a *CDKN2A* mutation. CMM= cutaneous malignant melanoma, NMSC= nonmelanoma skin cancer, NST= neural system tumours

The relatives were subsequently divided into two groups (related to mutation carriers or not) and the relatives of mutation carriers were hence excluded, due to the expected influence of the mutation on the results. The SMRs of relatives of non-mutation carriers are presented in **Table 8**, showing that the risk of getting a

melanoma was decreased but still high (SMR 3.7, 95% CI; 1.5-7.6) and the SMR for NST was 2.1 (95% CI; 0.6-5.4).

Tumours	SMR (95% CI)
All tumours	1.1 (0.9-1.5)
CMM	3.7 (1.5-7.6)
NST	2.1 (0.6-5.4)
NMSC	1.3 (0.4-3.3)
Colon cancer	2.0 (0.9-3.7)
Breast cancer	0.9 (0.3-1.9)
Pancreatic cancer	0.6 (0.01-3.2)

Table 8. The risk for cancer in relatives of non-mutation carries.

A tendency to a slightly increased total risk of malignant tumours and an increased risk for melanoma (CMM) were observed in relatives of non-mutation carriers. Neural System Tumours (NST), colon cancer, Non Melanoma Skin Cancer (NMSC) and breast cancer were the other most common malignancies diagnosed. SMR= standardized morbidity ratio

The cohort was further divided into the three groups previously constructed according to the probands. The groups (small sample-sizes) are presented including and excluding the relatives of the mutation carriers respectively (**Table 9.**).

Neural System Tumours were diagnosed in the group consisting of mainly adenocarcinomal tumours besides a melanoma (Group B), and the SMR was far higher than expected (SMR 7.6, 95% CI; 2.1-19.4).

In the corresponding “proband Group B” in study I, the number of NST was likewise increased, but in that study the highest incidence of NST was seen in non-mutation carriers in “proband Group A”, which was not confirmed in this study.

Tumour	Group A*, n=207 SMR (95% CI)	Group A, n= 141 SMR (95% CI)	Group B*, n=157 SMR (95%CI)	Group B, n= 139 SMR (95% CI)	Group C, n=157 SMR (95% CI)
All tumours	1.2 (0.9-1.6)	0.6 (0.4-1.0)	1.7 (1.2-2.4)	1.8 (1.2-2.5)	1.3 (0.9-1.9)
CMM	12.3 (6.4-21.5)	2.7 (0.3-9.7)	5.3 (1.1-15.4)	2.0 (0.1-10.9)	6.2 (1.7-15.9)
NST	1.0 (0.03-5.7)	-	6.6 (1.8-16.9)	7.6 (2.1-19.4)	-
NMSC	0.6 (0.01-3.0)	-	1.0 (0.03-5.6)	-	3.5 (0.7-10.2)
Colon cancer	1.9 (0.6-4.4)	1.6 (0.3-4.7)	3.3 (1.1-7.8)	4.0 (1.3-9.4)	-
Breast cancer	0.3 (0.01-1.6)	-	2.9 (1.0-6.2)	3.3 (1.2-7.1)	-
Pancreatic cancer	1.0 (0.03-5.5)	-	3.5 (0.4-12.6)	-	-

Table 9. Analysis of relatives by subgroup.

Interestingly, although excluding relatives of mutation carriers, Group A showed a tendency for an increased risk applied to cutaneous malignant melanoma (CMM), amplified by the presence of relatives of mutation carriers. Group B showed an increased risk of CMM, tumours of adenocarcinomal origin and of neural system tumours (NST). Nonmelanoma skin cancer (NMSC) and CMM were most common in Group C. Pancreatic carcinoma was only present among relatives to mutation carriers. *Relatives to mutation-carriers included.

Other similarities between the two studies were that colon cancer and breast cancer were overrepresented in Group B and that NMSC only were overrepresented in Group C.

3. Study III

Eight of nine possible families in the Southern Health Care Region were included in the study. One family presented two individuals with melanoma, all the remaining seven families presented three or more individuals with melanoma. The members of the ninth family were unable to be present during the offered phenotyping events and the family was thus excluded.

In total 97 individuals participated, of whom 93 were older than 18 years and thus were both genotyped and phenotyped. No participant over 18 years declined to be part of the genotyping. The median age of all participants was 39 years (IQR 30-51).

Of the 93 genotyped participants, 38 were mutation carriers and 55 were non-carriers of the *CDKN2A(113insArg)* mutation.

A pedigree of one of the included families is shown in **Figure 13.**

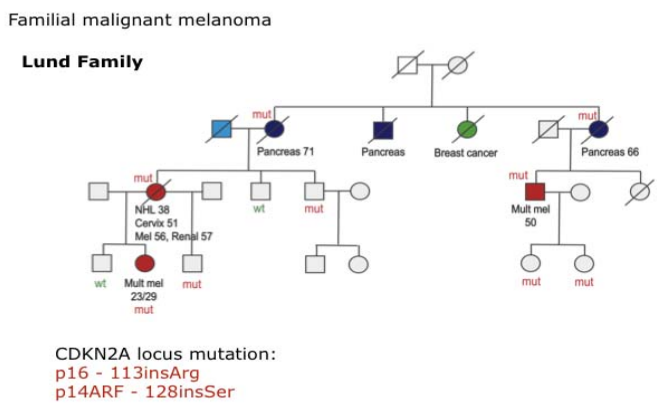

Figure 13. Family pedigree.

A pedigree of one of the included families showing mutation status, age at melanoma or age at other cancer diagnosis, cancer types and gender. Circles account for women, rectangles for men. Mut= mutation carrier, wt= wild type= no mutation, NHL=Non-Hodgkin's lymphoma, Mel= melanoma, Mult mel= multiple melanoma.

Total median nevus count and the corresponding mean counts are presented for all participants and for the different subgroups analysed in the study **(Table 10.)**.

CAN were seen in 14 (14%) of all participants. In the constructed pedigrees for each family, all melanoma diagnoses were verified in the Cancer Register. Totally 41 individuals (of whom 21 were deceased) in the eight pedigrees were affected by ≥1 melanoma. Of the 20 affected individuals who were alive, 16 (80%) participated in the study. The characteristics of the participating families are presented in **(Table 11.)**.

Total nevus counts	All participants	Mutation-negatives	Mutation-carriers	Melanoma patients	Individuals affected by CAN
Median (IQR)	12 (5-25)	8 (4-13)	24 (12-47)	29 (21-59)	30 (17-75)
Mean (±SD)	22 (28)	16 (29)	31 (26)	40 (28)	45 (34)

Table 10. Total nevus counts.

Total nevus counts (median and mean) are presented for the whole cohort and for different subgroups, respectively. IQR=interquartile range, SD= standard deviation, CAN= clinically atypical nevi.

Family	No. of participants per family	Total no. of individuals in each pedigree affected by CMM *	Number of individuals affected by CMM**	Number of invasive CMM**	Number of in situ CMM**	Number of mutation carriers**	Number of individuals affected by CAN**
1	32	3 (5)	4	6	2	10	4
2	8	2 (2)	1	2	1	4	4
3	6	1 (2)	1	1	-	2	-
4	7	2 (2)	2	3	-	5	1
5	18	3 (6)	1	5	1	4	1
6	20	4 (3)	4	6	1	8	3
7	4	3 (1)	1	1	-	3	-
8	2	2 (0)	2	5	1	2	1
All	97	20 (21)	16	29	6	38	14

Table 11. Included families.

The table shows number of phenotyped participants per family, number of individuals in the kindreds affected by melanoma, number of study participants affected by melanoma, melanoma cases, number of mutation carriers and number of study participants affected by CAN. *Number of all individuals, alive, affected by CMM and number of additionally affected individuals who are deceased in brackets. **Participating in the present study. CMM= cutaneous malignant melanoma, no.= number, CAN= clinically atypical nevi

3.1 Melanoma patients

Median age at first diagnosis of melanoma was 36 years (IQR 35-39) and all patients with a melanoma diagnosis were *113insArgCDKN2A* mutation carriers. Nine (56%) of the 16 examined patients affected by melanoma had a history of multiple primaries.

Phenotypic risk factors associated with a diagnosis of melanoma were red hair colour (OR: 9.1, 95% CI 1.5-54.2) and the presence of ≥1 CAN (OR: 5.7, 95% CI 1.5-22.3).

The most common *MC1R* variants found in melanoma patients were, apart from the wild type (WT) *MC1R* (25%); R151C (13%) and V60L (13%). Percentages of

number of affected variants were; one variant 38% and two variants 38%. No one showed three or more *MC1R* variants.

Six of the 16 individuals with melanoma presented CAN; five showed 1 CAN each and one showed 3 CAN. No one fulfilled the criteria for the AMS phenotype according to the AMS scoring system developed by Bishop et al (Newton Bishop et al. 1994).

3.2 Mutation carriers

Sixteen (42%) of the 38 mutation carriers presented one or multiple melanomas and CAN were presented in 11 (29%) of all mutation carriers. The only significant phenotypic risk factor associated with a positive mutation status, was the presence of CAN (OR: 6.9, 95% CI 1.7-27.9). Compared with non-mutation carriers, the mutation positive individuals had significantly more nevi on all examined anatomical sites.

The most common *MC1R* variants found, apart from WT (26%) were R151C (11%) and D84E (11%). 8% of the mutation carriers had three *MC1R* variants, while 21% had two variants and 45% had one variant.

3.3 Individuals affected by CAN

Of the 14 individuals who presented CAN, six (43%) were affected by melanoma and eleven (79%) had a positive mutation status. However, total number of CAN per individual was low and 12 (86%) showed merely a single CAN. The additional two individuals showed two and three CAN, respectively, but none fulfilled the criteria for the AMS phenotype. The median (and mean) nevus count was highest for participants affected by CAN **(Table 10.)**.

4. Study IV

4.1 The initial cohort and melanoma diagnoses

The population-based cohort of 29475 participating women was studied regarding differences (frequencies) of reported risk factors among participants with a melanoma diagnosis compared with those not affected by melanoma. Differences, with a higher frequency reported in melanoma patients, were noted for family history, freckles, high number of nevi, red hair colour, high number of sunbathing vacations, high number of sunburns in childhood and adolescence and high use of sun lamps.

During totally eighteen years of follow-up, 215 melanoma diagnoses (155 invasive [72%], 60 *in situ* [28%]) were reported in 206 women. Eight women (4%) had multiple primaries. The median Breslow thickness was 0.75 mm (IQR: 0.51-1.43), based on the available 140 primary, invasive melanomas. The other histopathological features of the melanomas are presented in **Table 12. (a, b, c)**.

Histological Type	**Number (%)**
-SSM	88 (57)
-SSM in situ	26 (43)
-NM	19 (12)
-NM in situ	N/A N/A
-LMM	21 (14)
-LM	19 (32)
-ALM	6 (4)
-ALM in situ	0 (0)
-Unclassified	21 (14)
-Unclassified in situ	15 (25)
Ulceration*	22 (13)
Regression*	70 (41)
Nevus remnants*	73 (43)

Primary Tumour site	Number (%)
-Head/Neck	17 (11)
-Head/Neck in situ	17 (28)
-Trunk	47 (30)
-Trunk in situ	20 (33)
-Upper limbs	28 (18)
-Upper limbs in situ	12 (20)
-Lower limbs	59 (38)
-Lower limbs in situ	10 (17)
-Unclassified site	4 (3)
-Unclassified site in situ	1 (2)

Level of invasion (Clark)	Number (%)
-I (in situ)	59 (40)
-II	51 (35)
-III	45 (31)
-IV	21 (14)
-V	3 (2)
-Unclassified	30 (20)

Table 12 a) b) c). Melanoma characteristics.

The table shows characteristics of melanomas as histological type, presence of ulceration/regression/nevus remnants (a), primary anatomical tumour site (b) and Clark level distribution (c). SSM= superficial spreading melanoma, NM= nodular melanoma, LMM= lentigo maligna melanoma, LM= lentigo maligna N/A= not applicable *Based on the reviewed melanoma cases until December 2006, n=170, 8 melanomas not possible to assess.

In the multivariate analysis of the whole cohort, the risk factors for melanoma were: a reported heredity/family history of melanoma (HR, 3.7; 95% CI, 2.0-6.8) and low numbers of nevi (HR, 1.8; 95% CI, 1.1-3.2) as well as high numbers of nevi (HR, 2.9; 95% CI, 1.7-5.0) (compared to no nevi) **(Tables 13 and 14)**.

4.2 Risk factors for melanoma with respect to age groups

The risk factors for melanoma, with respect to the two subgroups consisting of women younger or older than 40 years at the inception of the study, did slightly differ, and for younger women the following significant risk factors were found: reported heredity (HR, 3.1; 95% CI, 1.1-9.0), high numbers of nevi (HR, 2.5; 95% CI, 1.2-5.3) and highest degree of sunbed use (HR, 2.5; 95% CI, 1.0-6.2). For older women increased risks were shown for reported heredity (HR, 4.2; 95% CI, 2.0-8.8) and for both low (HR, 2.6; 95% CI, 1.3-5.0) and high numbers of nevi (HR, 2.9; 95% CI, 1.3-6.4). A tendency for an association (HR, 1.4; 95% CI, 1.0-2.0) between ever going on sunbathing vacations (versus never) and risk of melanoma was also demonstrated **(Tables 13 and 14)**.

Risk factors	**All women**	**P-value**	**Women born 1925-50 HR (95% CI)**	**P-value**	**Women born 1951-65 HR (95% CI)**	**P-value**
Family history/Heredity vs. no heredity	3.7 (2.0-6.8)	<0.001	4.2 (2.0-8.8)	<0.001	3.1 (1.1-9.0)	0.03
Low no. of nevi vs. 0 nevi	1.8 (1.1-3.2)	0.03	2.6 (1.3-5.0)	0.006	1.0 (0.4-2.5)	1.0
High no. of nevi vs. 0 nevi	2.9 (1.7-5.0)	<0.001	2.9 (1.3-6.4)	0.01	2.5 (1.2-5.3)	0.02
Hair colour red vs. brown/black	1.6 (0.5-5.6)	0.5	3.1 (0.7-15.4)	0.2	0.8 (0.1-6-0)	0.8
Haircolour light blond vs. brown/black	1.6 (0.7-3.6)	0.3	1.9 (0.6-6.2)	0.3	1.3 (0.4-3.6)	0.7
Hair colour blond vs. brown/black	1.0 (0.6-1.8)	0.9	1.4 (0.6-3.1)	0.4	0.8 (0.4-1.7)	0.5
Hair colour other vs. brown/black	0.5 (0.2-1.2)	0.1	0.6 (0.2-1.7)	0.3	N/A*	

Continued table

Risk factors	All women	P-value	Women born 1925-50 HR (95% CI)	P-value	Women born 1951-65 HR (95% CI)	P-value
Freckles ever vs. no freckles ever	1.2 (0.7-1.8)	0.5	1.2 (0.7-2.1)	0.6	1.1 (0.5-2.1)	0.9
Sunburn childhood ever vs. never	1.3 (1.0-1.7)	0.1	1.2 (0.8-1.7)	0.4	1.4 (0.9-2.1)	0.2
Sunburn adolescence ever vs. never	0.9 (0.7-1.4)	0.8	1.2 (0.7-1.9)	0.6	0.7 (0.4-1.3)	0.3
Sunburn adult ever vs. never	1.1 (0.8-1.5)	0.8	1.0 (0.6-1.4)	0.7	1.5 (0.9-2.6)	0.1
Blisters ever vs. never	0.9 (0.6-1.5)	0.7	0.6 (0.3-1.2)	0.2	1.4 (0.7-3.0)	0.3
Ulcers ever vs. never	0.7 (0.3-1.5)	0.4	0.6 (0.2-1.9)	0.3	0.9 (0.3-2.3)	0.8

Table 13. Melanoma risk (host factors) in relation to all women and to the two age groups.

Multivariate (Cox regression) analysis of risk of melanoma correlated to host factors and stratified for all women and for the chosen age groups. Data are adjusted for all the variables in tables 13 and 14 simultaneously, and presented with Hazard Ratios (HRs) and 95% Confidence intervals (CI). * N/A= not applicable, due to low numbers in this age group. Hair colour medium blond= combined variable of dark blond and medium blond. Hair colour "other"= greyish, grey and white.

Risk factors	All women	P-value	Women born 1925-50 HR (95% CI)	P-value	Women born 1951-65 HR (95% CI)	P-value
Sun vacations winter ever vs. never	1.0 (0.8-1.5)	0.7	1.3 (0.9-1.9)	0.2	0.9 (0.5-1.4)	0.5
Sunbathing vacations ever vs. never	1.3 (1.0-1.8)	0.06	1.4 (1.0-2.0)	0.07	1.2 (0.8-2.0)	0.4
Sun lamp use*						
1-10 times/year	0.8 (0.2-3.2)	0.7	1.0 (0.2-4.1)	1.0	N/A**	
>10 times/year	1.6 (0.2-11.9)	0.6	2.5 (0.3-19.5)	0.4	N/A**	
Sunbed use*						
1-10 times/year	1.0 (0.6-1.6)	0.9	0.7 (0.3-1.5)	0.4	1.6 (0.7-3.5)	0.2
>10 times/year	1.5 (0.8-2.8)	0.2	1.2 (0.5-3.0)	0.7	2.5 (1.0-6.2)	0.05
Sunscreen use ever vs. never	1.0 (0.8-1.3)	0.9	0.9 (0.7-1.2)	0.5	1.2 (0.8-1.8)	0.3

Table 14. Melanoma risk (UVR exposure and sunscreen use) in relation to all women and to the two age groups.

Multivariate (Cox regression) analysis of risk of melanoma correlated to UVR exposure and sunscreen use and stratified for all women and for the chosen age groups. Data are adjusted for all the variables in tables 13 and 14 simultaneously, and presented with Hazard Ratios (HRs) and 95% Confidence intervals (CI). *Reference: no/never use of sun lamp/sunbed ** N/A=not applicable (too few users).

4.3 Risk factors in relation to anatomical site and histological type of melanoma

Different ages at diagnosis were observed for the three examined anatomical sites of melanoma; the median age for trunk melanoma was 52.0 years, for limbs 60.0 years and for head and neck melanomas 66.5 years. A common risk factor for all sites was heredity for melanoma, with the highest risk observed (HR, 8.2; 95% CI, 3.0-22.6) for limbs. Further, regarding melanomas on the limbs tendencies for

associations with high numbers of nevi (HR, 2.3; 95% CI, 1.0-5.4) and freckles (HR, 1.9; 95% CI, 0.9-3.9) were observed. Trunk melanomas were associated with a high nevus count (HR, 3.0; 95% CI, 1.2-7.3) and head/neck melanomas were associated with sunbathing vacations (HR, 2.5; 95% CI, 1.2-5.3).

The histopathological subtypes were examined regarding associations to certain risk factors but no statistically significant findings were observed in our model.

DISCUSSION

1. Methodological considerations

1.1 Study I

As a descriptive study it was only hypothesis-generating, and little attempt was made to infer causation. Further, the observed whole cohort and the hypothetical subgroups were small, which is a limitation to a study with respect to e.g. chance findings. Nonetheless, the cohort consisted of all available cases during 40 years found in the Cancer Register and for a hypothesis-generating study the material was substantial.

1.2 Study II

The hypothesis that some of the cancers found among the index patients in Study I might be found also in first- and second-degree relatives was tested. The fact that no information about exposure data or mutation status of the relatives was available was however an inevitable limitation. The results of shared genes, shared behavioural patterns or shared environment, were not possible to verify, only to hypothesize. The numbers of the different subgroups were small, although all available first-and second-degree relatives were included.

1.3 Study III

The ideal study situation would be to phenotype all known *CDKN2A(113insArg)* families in Sweden, and all patients with a melanoma diagnosis within those families, but this is difficult to accomplish due to limited time and monetary resources and hence the families in southern Sweden were chosen for the study. Efforts were taken to reduce selection bias of the families, and all known families in the selected region were invited on several occasions. Further, the statistical analyses were adjusted for familial dependence, and therefore this bias ought to be reduced. The studied cohort and the subgroups within the cohort were nevertheless small, even though eight of nine families and 80% of all living melanoma patients participated.

The stepwise backward logistic regression test applied, allowed us to evaluate the most important risk factors with regard to different aspects of the study participants. Still, some correlations between different constitutional factors, e.g.

freckles and red hair colour, blond hair and blue eye colour, CAN and common nevi were difficult to exclude.

The study participants were examined by different physicians, which might generate different results regarding e.g. nevus counts. In an ideal study situation, one experienced dermatologist would examine all subjects, but this was impossible due to the study design, with the invitation of more than one family to each research event. We did not examine any systematic differences in the counts between the observers, but all physicians were experienced in the area of nevus counts, and miss-classification of nevus with regard to freckles, lentigo or seborrhoeic keratoses should be diminutive.

All skin examinations were performed during winter, to minimize the influence of natural solar tanning, although influence of sunbed use and sunbathing vacations were not possible to exclude.

The original AMS score constructed by Bishop et al 1994 (Newton Bishop et al. 1994), was used to evaluate if an AMS phenotype was present. The reason for using this score instead of arbitrary definitions of the AMS phenotype depended on the possibility to easier compare our results with other studies using this system and to avoid the risk of possible inter-observer bias (Bishop et al. 2000).

1.4 Study IV

As a closed prospective cohort study, the information about risk factors/exposure in study IV was obtained prior to the outcome (melanoma diagnosis), and as such the risk of selection bias was eliminated and the risk of misclassification/information bias, as recall bias, was reduced (Veierod et al. 2003; Bataille et al. 2005). However, the applied questionnaire (MISS I) was constructed in the late eighties, when the knowledge about the correlation between the risk factors sunbed use and e.g. leisure time spent outdoors, both reflecting intermittent UVR exposure, was merely emerging. A limitation mirroring this absence of knowledge was that we could not analyse the significance of every kind of intermittent UVR exposures other than those requested in the questionnaire.

Confounding factors, always present to some extent influencing study results, were important to try to adjust for, both in the study design and in the analyses. We tried to take this into account e.g. when adjusting for several risk factors simultaneously, but we were not able to adjust for all possible confounding factors simply due to the way the questions in the applied questionnaire were constructed (prior to today's greater knowledge of all possible confounders).

Furthermore, the study design restricted to participants with no prior cancer diagnosis before enrolment, might admit some selection bias and all data about the

non-responders (approx. 25% of the initial cohort) were unknown, which might be another limitation of the study.

2. General discussion

2.1 Study I and II

In the cohort of rare individuals in study I (with ≥4 tumours of which ≥ 1 was a melanoma), the preformed hypotheses (according to possible different etiological genesis of cutaneous melanoma such as (i) patients with multiple melanoma, (ii) patients with a single melanoma and an overrepresentation of adenocarcinomas and (iii) patients with a late-onset, single melanoma in addition to NMSC) were partly supported. Most *CDKN2A(113insArg)* mutation carriers were found in Group A presenting multiple melanomas. In addition, the age at first melanoma diagnosis was lower in Group A than in the Groups B and C. Similar results, depending on a germline mutation in *CDKN2A,* have previously been reported in Sweden, with regard to patients with multiple primary melanomas (Hashemi et al. 2000) and in the U.K., for individuals with familial melanoma and in individuals with multiple primaries (MacKie et al. 1998). However, one mutation carrier was found in the group with mostly adenocarcinomas (NHL, Kidney cancer, Cervical cancer), in addition to the single melanoma, but this patient showed to be a member of a known melanoma-predisposed family with a strong family history of melanoma and PC.

We also assumed that late-onset melanomas could be associated with DNA repair defects (Broberg et al. 2007; Broberg et al. 2009), and hence probably a vulnerability to other DNA repair defect-related tumours, such as NMSC. Unfortunately we could not examine this genetically, i.e. in any NER- associated genes, or in some of the homologous recombination associated genes (*BLM/RMI1/TOP3A*) (Broberg et al. 2007; Broberg et al. 2009) but the hypothesis was somewhat further supported, as the individuals in this Group (C), in 4 of 14 cases developed no other cancer types than NMSC and melanoma. In addition, the predisposition for developing NMSC seemed to be transferred to the first-and second-degree relatives (SMR 3.5 [0.7-10.2]).

In study I the relationship between melanoma and NSTs was most obvious for non-mutation carriers with multiple melanoma (Group A), but NSTs were present also among non-mutation carriers in Group B. Among the corresponding relatives in study II, the possible transfer of susceptibility to NST was only present in Group B among non-mutation carriers (SMR 7.6 [2.1-19.4]). In the corresponding Group A, one NST case was found, and this time among one relative of a mutation carrier, disparate of the former findings. The possibility of this being a chance finding cannot be excluded.

The well-studied U.S. family, which was the first reported with the astrocytoma-melanoma syndrome (Kaufman et al. 1993), was followed by a population-based study in Israel (Azizi et al. 1995) that showed an increased risk for NST in melanoma patients and in their relatives. More families with melanoma and NST (not merely astrocytomas) were reported in 1997-98 from France by Bahuau (Bahuau et al. 1997; Bahuau et al. 1998), and in 2001 from the U.K. (Randerson-Moor et al. 2001). The genetical predisposition for both tumours has been thoroughly studied in e.g. families with this proneness, but different deletions and mutations have been reported. Nevertheless, most results involve the chromosome 9, and more precisely the *CDKN2A/p16/p14ARF/CDKN2B* loci, where the locus for 1β exon of *p14ARF* is one of the most suspected regions to be relevant (Randerson-Moor et al. 2001; Mistry et al. 2005; Pasmant et al. 2007). However, in a large Israeli study, no mutations were found in these loci (Marian et al. 2005), and in an analysis of melanoma-prone families in different continents no or weak associations to *CDKN2A/p16* and *CDKN2A/p14ARF* were seen (Goldstein et al. 2006). In addition, Solomon et al reported that no mutations in the *PTPRD* gene, adjacent to *CDKN2A* on chromosome 9, were found in familial melanoma or families with the melanoma-astrocytoma syndrome (Solomon et al. 2009).

Studies in families prone to brain tumours (gliomas) have been conducted, and recently one study reported about risks among individuals with a family history of brain tumours (Scheurer et al. 2007). They presented a study of first-degree relatives to glioma patients showing an increased risk for brain tumours (SIR 2.14), melanoma (SIR 2.02) and for sarcoma (SIR 3.83). For comparison, previously reported risks for cancer in this population have been 1.0-1.8 for any cancer, and 1.0-9.0 for brain tumours. They also observed an excess of pancreatic cancers, which they could not explain (Scheurer et al. 2007).

A nation-wide study in Sweden about patients with primary brain tumours, showed slightly increased risk for melanoma in first-degree relatives but not in spouses, also supporting a genetic rather than an environmental predisposition for both tumour types (Malmer et al. 2003). Further, in two recent reports, SNPs associated with a susceptibility to gliomas were studied (GWAS) and associations to SNPs on 9p21, near *CDKN2B/p15*, and on 9p21.3 (*CDKN2A-2B*), were seen **(Figure 9.)** (Shete et al. 2009; Wrensch et al. 2009).

However, these studies did not include information about melanoma diagnoses, if any, but the authors speculated about the role of *CDKN2B/p15* as an important "back-up" under special circumstances, when *CDKN2A* does not function. Wrensch et al further discussed the role of Tumour Growth Factor-β (TGF-β) on inducing *CDKN2B/p15* and the SNPs involved in this signalling-pathway (Wrensch et al. 2009).

The disparate associations seen for pancreatic cancer (PC) among the probands and the relatives in our cohorts in studies I and II, are somewhat difficult to explain, and chance findings cannot be excluded as sample sizes were small. The PC cases in study I were not mutation carriers, in contrast to in study II, where all the PC cases were relatives of mutation carriers.

PC in the familial melanoma context, have been especially associated to subgroups of mutations in the *CDKN2A* gene, e.g. the Swedish germline mutation *113insArg* and the founder mutation in the Netherlands, "p16-Leiden" (Borg et al. 2000; de Snoo et al. 2008). In the Netherlands >60% of these families reported occurrence of PC in the family (de Snoo et al. 2008). However, in a GenoMEL report with results from different parts of the world only 28% of all the melanoma-prone families (with *CDKN2A* mutations) showed associations to PC. On the other hand, out of these melanoma-prone families with PC, 74% showed a *CDKN2A* mutation. No associations between PC and mutations in *p14ARF* or *CDK4* were reported (Goldstein et al. 2006).

Overall PC incidence shows the highest rates in Northern and Eastern Europe (Stolzenberg-Solomon 2009) and risk factors associated with PC are smoking, diabetes, obesity and chronic pancreatitis, apart from genetical factors/familial PC (Landi 2009). Interestingly, a high sun exposure has been associated with lower PC death rates in some studies and the role of Vitamin D has been debated (Stolzenberg-Solomon 2009).

For sporadic PC, many genes have been shown to be mutated at a low frequency, and several mutations in several signalling pathways are probably important for the development of PC (Landi 2009), and thus our findings might reflect either chance findings or the complex nature of both sporadic and familial PC.

Squamous cell carcinoma (in our report defined as NMSC) were occurring at a higher than expected degree in Group "C". In addition, the age at diagnosis of the melanoma in the proband group was the oldest observed, which is in line with current research.

The etiology of SCC is multifactorial, but the main risk factor for SCC is cumulative UVB exposure, causing harm to DNA. Other causes are human papilloma viruses (HPV), radiation therapy, arsenic exposure, hereditary syndromes (e.g. Xeroderma Pigmentosum and Oculocutaneous albinism) and other genetic factors as well as immunosuppression (WHO/IARC 2008). **(Figure 14.)**

In our study no information about e.g. sun exposure or immunosuppressive therapy was available, and thus we could merely speculate about the underlying causes in our cohorts. As the trend for NMSC was observed also in study II, it is tempting to speculate about possible genetic factors as mutated *TP53* or decreased

function of NER-enzymes or other factors involved in DNA repair (Marrot and Meunier 2008; Broberg et al. 2009).

The co-morbidity with melanoma could partly be explained by the same risk factors (Wassberg et al. 1999; Hemminki and Dong 2000; Lindstrom et al. 2007) and no *CDKN2A* germline mutation was found in this group, which otherwise could explain a melanoma susceptibility. The highest age at melanoma diagnosis observed in this group is in line with that not inherited, but acquired mutations could be responsible for the development of both cancer types (Leiter and Garbe 2008; WHO/IARC 2008).

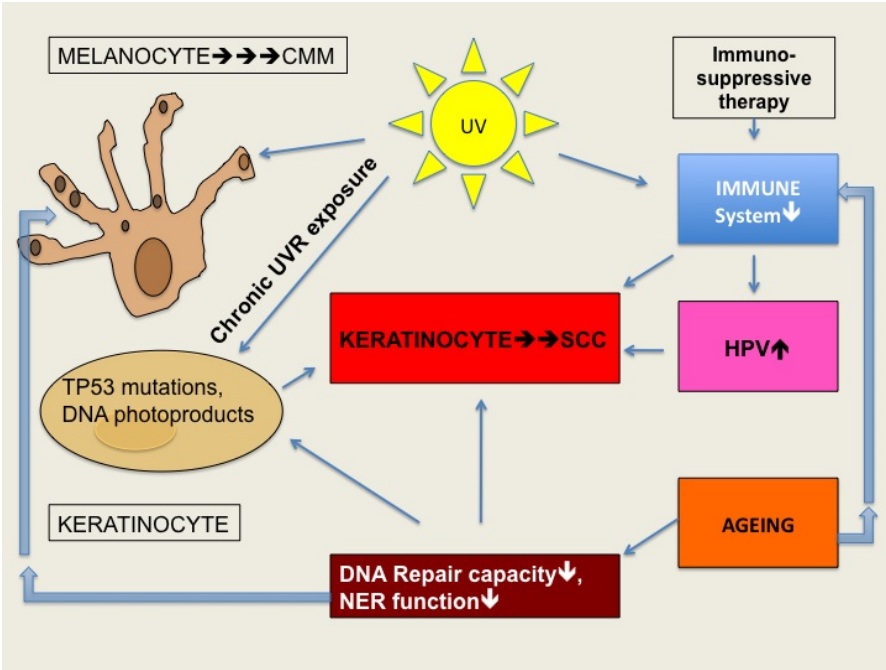

Figure 14. Pathogenesis of Squamous cell carcinoma.

The partly shared pathogenesis of melanoma and squamous cell carcinoma (SCC) is illustrated by UVR exposure affecting the immune system, the melanocytes and the keratinocytes. UVR leads to *TP53* mutations and DNA photoproducts in the cells. Impaired DNA repair capacity due to e.g. aging, leads to cumulative mutations and hence constitute the basis for clonal expansions of cells with harmed DNA. Ageing, immunosuppressive drugs as well as UVR affect the immune system, leading to increased susceptibility to infections as HPV, and probably also to impaired resistance to malignant transformation. NER=nucleotide excision repair, HPV= human papilloma virus, CMM= cutaneous malignant melanoma

2.2 Study III

A positive mutation status was correlated to ≥1 CAN, but not to any other examined phenotypic characteristics as heavy freckling, hair colour or eye colour. No melanoma cases were found among non-mutation carriers. If these findings could be verified in larger sample sizes, this might implicate that genotyped individuals without a *113insArgCDKN2A* mutation and with no other risk factors, do not have to be part of a surveillance program in pigment lesion clinics, as the risk for melanoma is probably comparable with that of the general population. This is important both in an economical aspect for the health care systems and for the affected individuals. The potential risk for other cancers in a non-mutation carrier belonging to a *113insArgCDKN2A* mutation-positive family is however not so easy to speculate about as limited data is available. On the contrary, confirmed mutation carriers with a possible risk of developing not merely melanomas but other cancers, as PC and breast cancer, should be assessed both by a dermatologist as well as by a clinical geneticists (Leachman et al. 2009).

Study III further presented evidence that the risk of melanoma in melanoma-prone families was strongly correlated to: a positive mutation status, the presence of ≥1 CAN and red hair colour. The possibility that a mutation in the *MC1R* gene might add a sensibility to develop melanoma, through impact on *CDKN2A* penetrance, might be the clue to this association with the red hair colour as reported by others (Box et al. 2001; Goldstein et al. 2005).

A high number of common nevi as well as the presence of atypical nevi are well-known risk factors for melanoma (Briollais et al. 1996; Briollais et al. 2000; Gandini et al. 2005; Thompson et al. 2005; Miller and Mihm 2006; Sekulic et al. 2008; Chang et al. 2009). The nevus phenotype is thus a marker for an increased risk of melanoma and that is why our study focused on nevus counts.

A higher number of nevi (defined in our study as n>50) were seen only in few participants, and this could not be shown to be correlated either to mutation status or a melanoma diagnosis. Anyhow, mutation carriers showed a higher total median count than non-mutation carriers (24 versus 8 nevi), melanoma patients showed a median count of 29 nevi and patients with ≥1 CAN showed the highest median count of 30 nevi. The whole cohort showed a median count of 12 nevi.

The comparison to other Swedish studies about nevus counts is inevitable, and the reported population-based median count in northern Sweden (Storuman) was 15 nevi (Karlsson et al. 2000), in western Sweden (Göteborg) it was 53 nevi (Augustsson et al. 1991) and finally in eastern Sweden (Linköping) the count was 23 nevi (Bishop et al. 2000) respectively. The median count for individuals with ≥1 CAN was 68 nevi in northern Sweden and 102 nevi in Göteborg, as compared with 30 nevi in our study.

Our overall lower counts, despite of the setting with high-risk families and not a population-based cohort, should not be dependent of a higher or lower median age of the cohort. The median age of the participants was 39 years (IQR: 30-51) and thus other explanations as genetical or environmental differences are more probable.

The overall expression of CAN in all relatives was intermediate (14%), compared to earlier studies in Sweden which showed population-based rates of 11% (Karlsson et al. 2000), 18% (Bishop et al. 2000), and 18% (Augustsson et al. 1991), respectively.

We could not verify any AMS phenotype, as defined by Bishop et al (Newton Bishop et al. 1994), in our study. Our findings with low numbers of nevi and few CAN in the cohort are in contrast to the population-based high numbers of common nevi and CAN previously reported in western Sweden. Although our cohort was based on melanoma-prone families and associations were observed between the presence of CAN and a melanoma diagnosis as well as between the presence of CAN and a positive mutation status, the AMS phenotype was not diagnosed either among mutation carriers or among non-carriers.

The etiology of common, as well as atypical/dysplastic, nevi are still not fully elucidated and both environmental and genetic factors are suggested to be important. The risk factors for nevi tend to parallel those for melanoma (UVR exposure, fair skin type, sun burn tendency) making research more complex. One confounding factor may be that many nevi might indicate a high exposure to UVR, possibly independently causing both a higher number of nevi and an increased risk of melanoma. In addition, up to 90% of melanomas and benign melanocytic nevi carry activating mutations in one of the two key MAPK pathway genes; oncogene *BRAF* or the oncogene *NRAS,* (Pollock et al. 2003; Hocker et al. 2008) but *NRAS* and *BRAF* activating mutations are in themselves insufficient to lead to malignant transformation.

As nevi have a "life span" of their own, with a start in infanthood, a peak in the middle age (about 35-40 years) and a slow disappearance in old age, the biology behind indicates a tight genetic regulation of cellular melanocytic/nevocytic proliferation and senescence, which may be vulnerable to both genetical and environmental factors (Wachsmuth et al. 2001). It is thought that each nevus represents a clonal expansion of a single MC (Hui et al. 2001), which harbours a/several somatic mutation(s) in a senescence or cell cycle regulatory gene (Zhu et al. 2007). The two common mutations found in nevi in the *BRAF* and *NRAS* genes (Eskandarpour et al. 2003; Poynter et al. 2006), are quite commonly found also in melanomas, though in the case of melanomas partly dependent on anatomical site (Curtin et al. 2005).

However, we did not investigate any other genes than *CDKN2A* and *MC1R*, and hence we could not evaluate the presence of *BRAF/RAS* mutations, if any.

The most studied environmental factor influencing nevus counts is UVR exposure, and several studies have reported higher nevus counts in sun-exposed areas on the body compared to protected areas (Augustsson et al. 1992; Stierner et al. 1992; Wachsmuth et al. 2005), which might support that UVR is nevogenic.

In a recent meta-analysis of risk factors for melanoma Gandini et al reported that the presence of large numbers of nevi (101-120 nevi) compared with <15 nevi (RR=6.89) as well as 5 CAN vs. 0, (RR=6.86) were associated with a risk for melanoma (Gandini et al. 2005). This was partly supported in our study, by the observed association of the presence of CAN and an increased risk for melanoma.

The CAN expression in melanoma patients in our study was higher (38%), but still low, compared with earlier studies on melanoma patients in Sweden where Augustsson presented a population-based rate of 56% (Augustsson et al. 1991) and Erlandson showed a rate of 88% in a cohort of familial melanoma patients (Erlandson et al. 2007). In familial melanoma cases internationally the rates have been shown to be 57% in the UK and 54% in the Netherlands, respectively (Bergman et al. 1986; Newton Bishop et al. 1994).

The findings might reflect differences in both UVR exposure and in the underlying genetical causes for CAN development, and strengthen the assumption that the *CDKN2A(113insArg)* mutation probably not is the only explanation for the etiology of CAN.

The Leiden-group has e.g. recently performed a GWAS, with the aim to find possible atypical nevus susceptibility genes, and they reported about strong associations to the locus 7q21.3 on chromosome 7, a region containing the *CDK6* gene (de Snoo et al. 2008). As the Swedish and the Dutch melanoma-prone families with a *CDKN2A* mutation share several features, such as an increased incidence of PC and breast cancer in addition to the disparate findings of an atypical nevus phenotype (present in about half of the families in the Netherlands), it would therefore probably be interesting to study this chromosomal region also in the Swedish families in the future.

2.3 Study IV

In a large population-based cohort of women, followed prospectively, we evaluated the possible risk factors for melanoma and found evidence that a reported family history of melanoma and a high nevus count were the most important factors overall.

The heredity/family history was reported in 9.5% of the individuals affected by CMM, which is in line with previous studies (Ford et al. 1995; Gandini et al. 2005), and the attributed risk (Hazard Ratio) was 3.7 (95% CI, 2.0-6.8), which is higher than reported in previous studies from Sweden (OR: 1.8, 95% CI, 1.0-3.3) (Westerdahl et al. 1994) and the pooled results in a recent meta-analysis by Gandini et al (RR=1.74, 95% CI, 1.41-2.14) (Gandini et al. 2005). This is probably not due to different definitions in the questionnaires of the included studies, as most studies regarded first-degree relatives, but instead it might reflect the selection of included cohorts in the meta-analysis, or describe a true risk in our female population. Higher risks, more similar to our finding, have been reported in separate studies (Gandini et al. 2005). In our study we had no possibility to genotype the participants, and we can only speculate about the possible factors responsible for the reported family history.

The use of self-reported nevus count on the left arm as a proxy for total nevus count might be a limitation of our study, but previous studies on nevus counts of specific sites as a predictor of total counts have refuted this assumption (English et al. 1988; Augustsson et al. 1992), thus supporting our method and results regarding nevus counts.

However, the participants were unskilled in recognising nevi and another limitation might be possible miss-classifications of nevi, with the subsequent possible risk for over- or underestimations of the number of nevi. With regard to the prospective study design, there is however no reason to believe that the melanoma cases would miss-classificate and overestimate their numbers of nevi more than the women without a melanoma diagnosis.

A high total nevus count is a well-known risk factor for melanoma. Our finding of the high nevus count on the left arm being associated with an increased risk for melanoma (HR: 2.9, 95% CI, 1.7-5.0), proved to be in line with previous Swedish studies of Westerdahl et al (OR: 3-3.2) (Westerdahl et al. 1994; Westerdahl et al. 2000) and of Augustsson et al (RR: 1.2-2.6) (Augustsson et al. 1992).

High numbers of nevi also showed an association with melanomas on the trunk, in line with the findings of Chang et al (Chang et al. 2009) and of Caini et al (Caini et al. 2009) and further supporting the hypothesis of divergent pathways for melanoma development.

This hypothesis was described by Green and Whiteman et al (Green et al. 1993; Whiteman et al. 1998; Whiteman et al. 2003; Whiteman et al. 2006), as well as independently by Masback et al in a different version (Masback et al. 1999), but in short it suggests that melanomas at different body sites may arise through different causal pathways.

Whiteman et al have expounded the theory of site-dependent sensibility of melanocytic proliferations and propose that: (i) lesions at chronically UVR exposed sites (e.g. head/neck), show associations with low-risk genes, exogenous proliferative factors and a phenotype with "nevus-resistance" and melanocytic stability as opposed to (ii) melanomas on intermittently exposed sites (e.g. trunk), which show associations with "nevus-proneness", endogenous proliferative factors, high-risk genes, freckles and melanocytic instability (Green et al. 1993; Whiteman et al. 1998; Whiteman et al. 2003; Whiteman et al. 2006; Olsen et al. 2009).

A related theory is based on the recent research about the different molecular genetic profiles of melanomas (and nevi) at different body sites, where *NRAS* mutations are more frequent in melanomas on chronically UVR-exposed sites (Jiveskog et al. 1998; Curtin et al. 2005), as opposed to melanomas from intermittently exposed skin, which mainly harbours an activated form of BRAF (i.e. *BRAF* mutations). In addition, melanomas from chronically sun-exposed skin, acral melanoma and mucosal melanoma do not harbour *BRAF* mutations (Curtin et al. 2005).

Additional support for the divergent pathway theory was seen in our study regarding freckles associated with limb melanomas and regarding different median age at diagnosis, where melanomas on the trunk were associated with the youngest age, and melanomas on the head/neck were associated with the oldest age at diagnosis.

However, our finding that sunbathing vacations, as a substitute for intermittent UVR exposure, were associated with head/neck melanoma was in contrast to the theory, but nevertheless supported by the findings of Caini et al in a recent meta-analysis (Caini et al. 2009). The possibly increased risk for head/neck melanoma due to chronic exposure, if any, was not possible to evaluate with regard to the design of the questionnaire.

We could not confirm a relationship between intermittent UVR exposure and trunk melanoma, like most other studies have done (Elwood and Jopson 1997; Elwood and Gallagher 1998; Cho et al. 2005; Chang et al. 2009). One explanation could be that this is due to the small sample sizes of each anatomical site. A longer follow-up time, with larger sample sizes, will probably lend better statistical power to future results.

Sunbed and sun lamp use were studied, and an increased risk for melanoma was observed for younger women in the setting of frequent sunbed use. The univariate analysis indicated an overall high ever use of artificial UVR device both among melanoma cases and controls, in line with previous Swedish studies (Boldeman et al. 1997; Bataille et al. 2005).

The number of sun lamp users was limited, and hence we were unable to show any differences with respect to main type of UVR exposure, i.e. UVA (sunbeds) vs. UVB (sun lamps). The finding that the increased risk for melanomas was only significant in younger women is also similar to previous reports from Scandinavia, where Westerdahl et al showed that individuals younger than 30 years had the highest OR: 7.7, (95% CI, 1.0-63.6) for exposure >10 times/year vs. never (Westerdahl et al. 1994) and Veieröd et al showed strong correlations between young women, sunbed use and melanoma (Veierod et al. 2003).

The underlying explanation for this relationship might possibly be that it takes less time (from UVA exposure to melanoma development) and/or less amount of UVA to create a melanoma, or to prepare the way for other melanoma initiating processes, compared to the amount of sun exposure needed and the latency observed between merely sunlight exposure and melanoma development. (Godar et al. 2009; Kirkwood and Eggermont 2009).

In addition, as almost 100% of the skin area is exposed in sunbeds this gives very high doses of UVA in a short time (Wester et al. 1999) (compared to the skin area exposed outdoors) and this might possibly, to a certain extent, explain the shorter delay between sunbed use and melanoma diagnosis compared to earlier observed time delay between mainly solar exposure and melanoma development. However, a limitation of our study was that we could not clearly discriminate the impact of sunbed use from overall intermittent or e.g. leisure time sun exposure outdoors, due to the construction of questions in the applied questionnaire. It is quite possible that more thorough analyses of type and length of sun exposure in our cohort could have had impact on our results. More adjustments to variates about sun exposure might possibly have attenuated the results observed for sunbed use, as these two risk factors have been shown to be correlated. In conclusion, the potential risk for melanoma development after sunbed use is still not fully elucidated (Bataille et al. 2004; Bataille and de Vries 2008; IARC 2006).

No phenotypic host factors, apart from high numbers of nevi, showed associations with increased risk for melanoma, which was somewhat unexpected but might nevertheless reflect this population-based cohort truly. In a recent meta-analysis about risk factors, and especially constitutional factors, higher risks were shown for red hair colour (RR 3.64), high density of freckles (RR 2.1) and blue eye colour vs. dark colour (RR 1.47) (Gandini et al. 2005). Our results might be due to several causes; the low sample sizes in each category, the self-reported phenotypic traits, which might have been under- or overestimated, or the fact that we adjusted the model for all analysed host factors simultaneously.

A strength with our study was that a validation of the MISS I-questionnaire was already performed, showing fair to good test-retest reliability (Westerdahl et al. 1996), and that the questionnaire was administered as early as in 1990, when

media coverage was less intense in this field, and the study participants were probably not that influenced and aware of possible relationships between different types of host factors and UVR exposure.

Still, some recall bias or chance findings could not be excluded. In addition, the number of melanoma patients was quite small and therefore the statistical power could be somewhat limited. However, the major strength was the prospective cohort study design.

CONCLUSIONS

- The specific Swedish *CDKN2A(113insArg)* mutation was only present in 9% of the individuals with ≥4 tumours including ≥1 melanoma, thus probably influencing only a minority of the different cancer types that the investigated cohort developed.
- Verified *CDKN2A(113insArg)* mutation carriers more often developed multiple melanomas, and presented their first melanoma at a younger age than an average melanoma patient.
- Neural System Tumours have been found in two different settings of non-mutation carriers in our studies: among patients with multiple melanomas, and among first- and second-degree relatives to patients with melanoma and several adenocarcinomas.
- An overall increased risk for cancer was detected among first- and second-degree relatives to patients with ≥4 tumours including ≥1 melanoma, independent of proband mutation status, and mainly dependent on an increased risk for melanoma.
- *CDKN2A(113insArg)* mutation carriers did not present a specific phenotype, but the presence of ≥1 CAN was associated with having the mutation.
- Total nevus count in the melanoma-prone families was lower than in previously population-based published studies from the Swedish west coast, but higher than in a study from Northern Sweden.
- CAN were present only in 14% of the whole cohort, and in 56% of patients with ≥1 melanoma, which again was lower than in the population-based findings from the Swedish west coast (18% and 88%, respectively).
- The criteria for the AMS phenotype were not fulfilled by any of the participating individuals from the melanoma-prone families.
- The main risk factors for melanoma in Swedish women were high numbers of common nevi and a family history of melanoma, for younger women an additionally increased risk was seen for frequent use of sunbeds.
- Nevi on the trunk were associated with a younger age at diagnosis and with high numbers of nevi. Head/neck melanomas were associated with the oldest age at diagnosis and with sunbathing vacations.

FUTURE PROSPECTS

The results of the presented studies left ample room for further questions, and below are some of the aspects on possible future research presented.

- To examine the genotype and phenotype (e.g. prevalence of pigmented nevi and clinically atypical nevi) in a randomized population-based adult (30-50 years) cohort (men and women) from southern Sweden. The aim would be to repeat, as exact as possible, the previously published population-based studies from northern and western Sweden (Karlsson et al, Augustsson et al) about prevalence of common AMN and atypical/dysplastic nevi, to find out if/how the populations differ, when it comes to nevus counts and other phenotypic factors. A complementary genotyping based on the currently known genes/SNPs/CNVs associated with melanoma risk and nevogenesis would probably be further elucidating.

-To evaluate if answering the questionnaire (MISS I) and participation in the MISS study could be melanoma-preventive. The findings in study IV indicated that the melanomas diagnosed were thinner than previously reported in the population, and that a high degree (28%) of the melanomas were not invasive but *in situ* tumours. The study participants might have become more careful with exposing themselves to UVR after enrolment in the study, or the participating women might differ from a similar population-based matched cohort of women. A study design with 2 controls (matched for age, diagnosis, residence, and ethnic origin) for each melanoma case from study IV, might give clues to whether the study population in study IV is representative of the population or not.

- A register-based study about the non-responders in study IV, to search for possible confounding factors that contributed to their choice not to participate. It would further be interesting to investigate if an over-representation of melanoma is present in this sub-cohort.

-The MISS cohort is further followed and a new questionnaire was sent to the participants in the year 2000 (MISS II) and a third questionnaire will be sent to the remaining cohort in 2010 (MISS III). The MISS II questionnaire covers the issues in MISS I in addition to more thoroughly asked questions about physical activity, weight/BMI, prescribed drugs, skin type and reaction to UVR exposure. The possibility to evaluate all these risk factors, after a longer follow-up time, and probably some added cases of melanoma, will be interesting.

- For the subgroup of melanoma patients in paper IV who got their diagnosis in the time interval between the two questionnaires, MISS I and II, one could study the existence of any recall bias regarding UVR exposure and thus estimate its

magnitude in an ideal setting as the exposure information was gathered both before and after the diagnosis.

- To apply the findings (from study I and II) about cancers associated with melanoma to the population-based MISS cohort, and thus to investigate the MISS cohort regarding other simultaneous cancer diagnoses and specific patterns of associated cancers, completed with information about the medical history as well as UVR and drug exposure.

SAMMANFATTNING PÅ SVENSKA

Huden är kroppens största organ och i ett av dess skikt, längst ned i överhuden, finns pigmentproducerande celler (melanocyter) jämnt fördelade i tillväxtlagret (basalcellslagret) bland övriga hudceller (keratinocyter) **(Bild 1.)**. Oavsett hudfärg har alla människor ungefär lika många och lika tätt mellan sina melanocyter men de kan vara mer eller mindre aktiva (delvis genetiskt styrt) och programmerade att tillverka olika färgämnen/pigment, vilket resulterar i olika hudfärg. Melanocyterna producerar pigmenten, eumelanin (brunsvart) och pheomelanin (rödgult) **(Bild 2.)**, vars huvuduppgifter är att skydda hudcellernas/keratocyternas arvsmassa i cellkärnorna, DNA, mot den ultravioletta strålningen från solen. Melanocyterna har långa "tentakler" sträckta in mellan hudcellerna för att kunna nå och leverera pigment till keratocyterna.

Melanocyterna ingår även i "tentakellösa ansamlingar" i huden (oftast godartade) som kliniskt motsvaras av nevi ("leverfläckar/födelsemärken") **(Bild 4.)**. Ibland kan dessa nevi se atypiska ut (kliniskt atypiska nevi/CAN) utan att de behöver utvecklas till melanom, men ibland är dessa atypiska nevi korrelerade med en ökad risk för att man ska få melanom. Detta hänger ihop med hur många CAN man har själv och om det finns sådana nevi i släkten. Hur riskökningen hänger ihop med antalet vanliga nevi och vad som orsakar att vissa nevi blir atypiska vet man inte exakt idag.

Solljus och solarier är våra största UV-källor idag, med olika spektrum av UVB respektive UVA-ljus. I solljus finns ca 6% UVB och resten är UVA. I solarier är huvuddelen av UV-ljuset av UVA-typ och knappt 1% är UVB-ljus. När huden utsätts för UV-ljus ökar produktionen av nytt melanin, det redan befintliga melaninet mörknar och huden blir långsamt brunare och skyddas mot strålningen. UV-ljus kan dock också orsaka skada i huden genom att förstöra DNA och orsaka mutationer, direkt eller indirekt via fria radikaler, och utgör på så vis en cancerframkallande riskfaktor **(Bild 3.)**. Vi vet också idag att UV-ljus bidrar till för tidigt åldrande/rynkor av huden (ffa UVA som bryter ned bindväven/kollagenet i läderhuden) men även till viktig D-vitaminproduktion (UVB).

Malignt melanom är den tumörtyp som ökat explosionsartat de senaste årtiondena i världens ljushyade befolkning. Malignt melanom utgår från förändrade, muterade, melanocyter som därmed undgått det stränga maskineri av kontroll som normalt finns i kroppen, programmerat i cellernas arvsmassa (DNA). Istället delar de sig ohämmat, vägrar att dö och på så vis bildas tumörer (melanom). Melanom uppstår framför allt i huden de novo (ca 70%) men kan även uppstå i redan

befintliga nevi (knappt 30%) eller i andra organ där det finns pigmentbildande celler, t.ex. i ögat.

Malignt melanom i huden är den allvarligaste hudcancersjukdomen eftersom den till skillnad från annan hudcancer (basalcells- och skivepitelcancer) har en större benägenhet att sprida sig (metastasera) och har en högre dödlighet (mortalitet). Melanom drabbar även yngre åldersgrupper.

Hudmelanom är ungefär lika vanligt bland kvinnor som bland män men vanligare hos kvinnor i de lägre åldersgrupperna (15-55 år) och motsvarande för män i de övre åldersgrupperna (>55 år). De riktigt unga melanompatienterna (15-25 år) är i 70% av fallen kvinnor. I cirka 10% av alla fall finns en ärftlig faktor bakom benägenheten att utveckla melanom och då ser man flera fall av melanom bland de närmaste släktingarna och ibland flera melanom hos samma individ. Bland svenska personer med denna familjebakgrund är den mest kända orsaken en mutation i en gen på kromosom 9 (*CDKN2A113insArg*) men i de flesta fall med flera melanom i familjen har man ännu inte hittat den/de bakomliggande genetiska orsaken/orsakerna.

Diagnosen melanom får man genom att läkare, med vana av diagnostisering av hudtumörer, gör en noggrann inspektion av hudkostymen, helst med hjälp av handhållet mikroskop/lupp, s.k. dermoskop eller dermatoskop. Om melanom inte kan uteslutas opereras hudförändringen bort i sin helhet och skickas för mikroskopisk undersökning och diagnostik (PAD) till en histopatolog.

Prognosen vid melanom är varierande. Ju tunnare tumören är vid upptäckt desto bättre prognos. Många som kommer till diagnos och operation i ett tidigt stadium botas idag helt. Om tumören däremot hunnit växa djupt och/eller metastaserat är prognosen mycket sämre. Detta innebär att man måste eftersträva att hitta och operera alla melanom så tidigt som möjligt. Preventivt arbete med t.ex. information och råd till riskgrupper samt identifiering och uppföljning av högriskgrupper kan vara värdefullt ur både ett samhälls- och individperspektiv.

I studie I studerade vi individer som var och en fått fyra eller fler cancertyper, varav minst en cancer skulle vara av hudmelanomtyp. Vi tittade på vilka andra typer av cancer som personerna fått och såg att de som fått *flera* melanom ofta insjuknade i sitt första melanom vid en yngre ålder (ca 60 år) än de som bara fått ett melanom gjorde. De med *flera* (“multipla”) melanom hade också i större utsträckning än de andra deltagarna en speciell, nedärvd mutation i kromosom 9 (*CDKN2A/113insArg*) **(Bild 7.)**. Den specifika mutationen är känd för att bidra till en starkt ökad risk för melanom i enstaka specifika familjer i Sverige. Vi såg också att grupperna med patienter med *ett* melanom förutom de andra cancerdiagnoserna i stor utsträckning drabbades av cancertyperna: (i) adenocarcinom (t.ex. bröstca, tjocktarmsca., prostataca. och njurca.) (Grupp ”B”), eller (ii) annan hudcancer

(skivepitelcancer) (Grupp ”C”) **(Bild 12.)**. Personerna i Grupp C drabbades av sina melanom mycket senare än personerna i Grupp A och B (genomsnittsålder ca 78 år). Vi hittade också, totalt sett, fler tumörer med nervsystem-ursprung “Neural System Tumours” (i både Grupp A och i Grupp B) än vad som kunde förväntas, och detta kan möjligen ha en förklaring i melanocyternas och nervcellernas gemensamma embryonala ursprung, eller bero på riskfaktorer eller genetiska förändringar som vi inte känner till idag. Inga av dessa nervsystemstumörer fanns dock hos personerna med den undersökta mutationen.

I studie II studerade vi första- och andragradssläktingar (syskon, föräldrar, barn, syskonbarn, far/morföräldrar, föräldrars syskon) till personerna i den första studien. Släktingarna kontrollerades i det nationella cancerregistret avseende eventuella cancersjukdomar. En indelning av släktingarna gjordes, efter den gruppindelning som visades i studie I, och de mönster av cancertyper som hittades i studie I jämfördes mot de cancertyper som hittades hos motsvarande släktingar. Man kunde se att totalt sett hade släktingarna en överrisk att drabbas av cancer (oavsett diagnos) men att den risken framförallt berodde på en ökad risk för melanom **(Tabellerna 7 och 8)**. Cancermönstren gick också igen avseende adenocarcinom och skivepitelcancer och även här sågs en högre än förväntad andel av nervsystemrelaterade tumörer hos släktingarna, denna gång tydligast hos släktingarna i ”Grupp B”, men inte i Grupp A.

I studie III inbjöds alla familjemedlemmar, i de nio kända familjerna i södra sjukvårdsregionen med en ärftlig benägenhet för melanom, att delta i en föreläsning om ny melanomforskning samt till en helkroppsundersökning avseende nevi och eventuella misstänkta melanom. Blodprovtagning erbjöds personer över 18 år för kompletterande gentestning av den kända svenska melanommutationen (*CDKN2A 113insArg*) samt av pigmententerings-relaterade genen MC1R.

Studien visade att personer med en mutation i *CDKN2A* i större utsträckning hade kliniskt atypiska nevi jämfört med sina släktingar utan mutation och att alla personer som fått melanom var bärare av mutationen. De som fått melanom visade statistiska samband med rött hår och kliniskt atypiska nevi **(Tabellerna 10 och 11)**. Överlag hade dock alla släktingar ganska få nevi, färre än vad man sett i tidigare svenska studier från ex. Göteborg och vad detta beror på är svårt att veta. Ingen av de undersökta personerna kunde klassas att ha en s.k. AMS-fenotyp (med >100 nevi, >2 kliniskt atypiska nevi och nevi på ovanliga ställen som fötterna/skinkorna), vilket man sett på vissa andra håll i världen för personer i familjer med en motsvarande mutation.

I studie IV följdes en stor grupp friska kvinnor (40.000 st.) från 1990 och fram till 2008. I studiens början fick de svara på ett formulär om riskfaktorer för melanom (solvanor, solariebruk, resvanor/solsemester, solbrännor tidigare i livet och om det fanns melanom i släkten). Studiens upplägg eliminerade därmed risken för att personer som insjuknar i melanomsjukdomen ”minns” händelser bakåt i tiden (om t.ex. solvanor) på ett annat sätt än de som inte drabbats av melanom, ett faktum som flertalet studier inom området inte kunnat bortse från eftersom patienterna oftast tillfrågats efter att de fått sin diagnos. Under studiens 18 år hade 206 kvinnor utvecklat 215 melanom. Enkätsvaren från de kvinnor som fått melanom och de kvinnor som inte fått melanom jämfördes. En hög andel (28%) av melanomen var *in situ*-melanom (växande bara i epidermis). De flesta tumörerna var tunna (median 0.75 mm), diagnostiserades på benen och var av typen ”ytligt växande” (SSM). Riskfaktorerna för melanom visade sig vara delvis olika för yngre (<40 år) och äldre (>40 år) kvinnor, men alla hade ökad risk om de (i) hade melanom i släkten och (ii) om de hade några eller många nevi (jämfört med de med inga nevi alls). De *yngre* hade dessutom ökad risk för melanom om de solade solarium ofta och de *äldre* om de hade åkt på solsemester **(Tabellerna 13 och 14)**. Risken för melanom på bålen och på armar/ben var kopplad till många nevi medan risken för melanom på huvud/hals var kopplad till solsemestrar.

Sammanfattningsvis så har studierna bidragit med mer kunskap om (i) vilka tumörtyper som kan vara associerade med melanomsjukdom, (ii) hur högriskpersoners hudkostym ser ut eller *inte* ser ut och (iii) vilka riskfaktorer kvinnor har för att utveckla melanom.

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GLOSSARY/EXPLANATIONS

Some of the used molecular and genetically related terms are described, not always in alphabetical order but in order of context.

G1 phase Gap 1 phase of the eukaryotic cell-division cycle, between the end of M phase (mitosis/cell division) and the start of S phase (DNA synthesis).

Germline The lineage of germ cells (which contribute to the formation of a new generation of organisms), as distinct from somatic cells (which form the body and leave no descendants).

Mitogen A mitogen is a chemical substance, usually some form of a protein, which encourages a cell to commence cell division, triggering mitosis.

Mitogens trigger signal transduction pathways in which mitogen-activated protein kinase is involved, leading to mitosis.

Oncogene An altered gene whose product can act in a dominant fashion to help make a cell cancerous. Typically, an oncogene is a mutant form of a normal gene (proto-oncogene) involved in the control of cell growth or division.

Proto-oncogene Normal gene, usually concerned with the regulation of cell proliferation that can be converted into a cancer-promoting oncogene by mutation.

Protein kinase =Phosphotransferase. Enzyme that transfers the terminal phosphate group of ATP to a specific amino acid of a target protein, i.e. phosphorylates proteins. Examples of protein kinases are PKA, AKT/PKB, BRAF, MEK, MAPK,

Purine Constitutes of a pyrimidine ring fused to an imidazole ring. Examples of purines are Adenine and Guanine. Purines make up one of the two groups of nitrogenous bases, the building blocks of DNA and RNA, together with

pyrimidines. Purines are also important as components in e.g. ATP, GTP and cyclic AMP. Other purines are caffeine, theobromine and uric acid.

In DNA: Adenine bonds to Thymine (AT) and Guanine bonds to Cytosine (GC), respectively. (In RNA, Adenine bonds to Uracil instead).

Pyrimidine One of the two categories of nitrogen-containing ring compounds ("bases") found in DNA and RNA. Cytosine, thymine (in DNA) and uracil (in RNA) are pyrimidines, which form hydrogen bonds with their complementary purines Adenine and Guanine in DNA.

Senescence A name for the growth arrest in eukaryotic cells, which occurs after a specific number of cell divisions, i.e. the state when normal cells lose the ability to divide. The specific number of cell divisions is based on the characteristic for a species.

Stem cell Relatively undifferentiated cell that can continue dividing indefinitely, throwing off daughter cells that can undergo terminal differentiation into particular cell types.

Tumour suppressor gene Gene that appears to prevent formation of cancer. E.g. loss-of-function mutations in such genes enhance susceptibility to cancer.

AKT Akt/AKT1/Protein kinase B/PKB. Three AKT genes exist in humans (Akt1-3). The genes code for enzymes that are members of the serine/threonine-specific protein kinase family. AKT is inhibiting apoptotic processes through the PI3K/AKT pathway, thereby promoting cell survival.

BCL-2 B-cell leukaemia/lymphoma 2 protein. A total of 25 genes in the *Bcl-2 family* are known to date and can be either pro-apoptotic (e.g. BID, BAD, Bim, PUMA, NOXA, BAX and BAK) or anti-apoptotic=carcinogenic=pro-survival (e.g. BCL-2, BCL-XL and MCL-1). The proteins function to regulate and execute the intrinsic (mitochondrial) pathway of apoptosis, where BAX and BAK are finally responsible for mitochondrial permeabilization and apoptosis.

BRAF V-raf murine sarcoma viral oncogene homolog B1. *BRAF* is the gene that codes for the protein B-RAF/BRAF. BRAF/B-Raf (OMIM #164757) is a protein kinase, which functions in (phosphorylates and activates) the MAPK/ERK signal transduction pathway as part of a protein kinase cascade (as a MAP kinase kinase kinase (MAP3K)). BRAF becomes activated when it binds to RAS/Ras and thus phosphorylates MAP2K/MEK, which in turn phosphorylates a mitogen-activated protein kinase (MAPK)/ERK. (See MAPK/ERK pathway). When the gene *BRAF* is mutated (oncogene), the gene product BRAF is changed and can increase the growth and spread of cancer cells through constant activation of the downstream pathway.

CREB cAMP response-element binding protein. Functions as a transcription factor, regulating the expression of other genes. The activation of PKA by cAMP (when MSH has bound to MC1R) leads to increased activity of CREB and e.g. increased activity of tyrosinase

HDM2/MDM2 Human homolog of MDM2 (murine double minute oncogene). Marks proteins for degradation in proteasome through ubiquitination. Binds especially to p53, which leads to inactivation/degradation of p53. P14ARF inhibits the function of HDM2.

MAPK/ERK Mitogen-activated protein (MAP) kinase 1. Downstream kinase involved in a pathway that responds to extra cellular stimuli (mitogens) and downstream regulates cellular activities as cell differentiation and cell proliferation by phosphorylating nuclear targets. MAPK was formerly called "extra cellular signal-regulated kinases" (ERK).

MAPK/ERK pathway An important signalling pathway involved in melanoma pathogenesis, and primarily involved in the regulation of cell growth. Technically, (B)RAF/(MAP3K), MEK/(MAP2K) and MAPK1/(ERK) are all mitogen-activated kinases part of the MAPK/ERK pathway, which first upstream event is the activation of (B)RAF by RAS.

MATP/SLC45A2 Gene *MATP*, alternative title *SLC45A2,* located on chromosome 5 (5p13.2), which encodes MATP (Membrane-associated transporter protein), involved in inducing melanisation of melanosomes, thus influencing normal human pigmentation. Mutations in this gene are a cause of oculocutaneous albinism type IV and polymorphisms (SNPs) in the gene are associated with skin and hair colour variations as well as with melanoma.

MITF Microophtalmia-associated transcription factor. The *MITF* gene codes for a transcription factor (a family of at least nine isoforms (Park 09)) that regulates melanocyte development, function and survival. It acts both as an oncogene in some melanomas and modulates the state of MC differentiation. MIFF activity and stability is regulated by its phosphorylation state. It exerts effects on cell-cycle progression genes (*CDKN2A, p21, TBX2),* differentiation genes *(MART-1, TYR etc.),* motility genes *(C-MET),* melanin synthesis genes *(TYR, TRP-1, TRP-2, PKC-β),* melanosomal genes (with gene products Rab27a, Pmel17) and apoptotic genes *(BCL-2 and HIF1a).*

MTAP Gene on chromosome 9 (9p21), adjacent to genes *CDKN2A* and *CDKN2B.* SNPs in this locus have been associated (weakly) with melanoma risk and nevus counts. Often co-deleted with the *CDKN2A* gene in many cancers. Gene product: the enzyme Methylthioadenosine phosphorylase.

NRAS see RAS.

OCA2/P gene Gene on chromosome 15 (15q11.2-q12), which encodes a gene product (P-protein) that acts as a transmembrane transporter protein in melanosomes, involved in the regulation of pH of melanosomes. Mutations in the gene are associated with oculocutaneous albinism type II (MIM#203200). SNPs in this gene are associated with variations of eye/skin/hair pigmentation in humans and influences risk of melanoma.

PI3K Phosphoinositide-3-kinase, a kinase which becomes activated e.g. by RAS, and which further activates AKT by phosphorylation (which can be inhibited by PTEN). The PI3K/AKT pathway is important in melanoma development as it has antiapoptotic effects and thus promotes melanoma progression.

p53/TP53 Tumour protein p53. (Gene: *TP53*, (OMIM #191170), locus on chromosome 17 (17p13.1). p53 functions as a tumour suppressor via its activity as a transcription factor, it regulates target genes responsible for apoptosis, senescence, DNA repair and induction of cell cycle arrest. Is regulated by several factors, including cellular and oncogenic stress that activates p53. Senses genetic damage and allows pause for DNA repair or activates apoptosis if too much DNA damage. Decreased p53 lead to genetic instability when mutations are left

unrepaired. Mutated *TP53* is part of several cancer syndromes, e.g. the Li-Fraumeni syndrome.

PTEN Phosphatase and tensin homolog. *PTEN* acts as a tumour suppressor gene and is located on chromosome 10 (10q23.3). The gene product PTEN functions as an important tumour suppressor, by negatively regulating Akt/PKB signalling pathway (by catalysing the dephosphorylation of PIP3 and inhibition of the AKT signalling pathway). This pathway signals cells to stop dividing and causes cells to undergo apoptosis when necessary. *PTEN* mutations and deletions occur in many cancers, e.g. Cowdens syndrome, where the defective protein is unable to stop cell division or the signals to abnormal cells to force them to undergo apoptosis.

RAS/Ras RAS viral (v-ras) oncogene homolog, named for the proto-oncogene *Ras*, first identified in viruses that cause rat sarcomas, and the most famous member of a large family of G-proteins/GTP-binding proteins that helps to pass on signals from cell-surface receptors to the nucleus (i.e. a signal transduction protein). Three human *Ras* proto-oncogenes with three protein isoforms exist: *HRAS, KRAS* and *NRAS. NRAS* is most common of the isoforms involved in melanomas (OMIM #164790). RAS activates a number of pathways, but an especially important one seems to be the mitogen-activated protein kinase (MAPK) pathway, which transmits signals downstream to other protein kinases and gene regulatory proteins, and where activated RAS in the beginning activates the protein kinase activity of RAF kinase (BRAF). In the field of melanoma cell signalling pathways, RAS also activates the cell-signalling cascade of PI3K (see PI3K). Mutations in the *Ras/RAS* genes can permanently activate the RAS proteins, thus acting as oncogenes. Receptors: RTK (receptor tyrosine kinase, Kit).

SLC24A4 Gene on chromosome 14 (14q32.12), which encodes a sodium/potassium/calcium exchanger (Solute carrier family 24 A4) that has a role in human skin/hair/eye pigmentation

SLC24A5 Gene on chromosome 15 (15q21.1), which encodes an intracellular membrane protein (sodium/potassium/calcium exchanger) that has a role in human skin/hair/eye pigmentation.

TRP1/TYRP1 Tyrosinase-related protein 1, a transmembrane protein of the melanosomes, which stabilizes (and possibly activates) the enzyme tyrosinase by forming a complex with tyrosinase. Involved in human pigmentation. Gene *TRP1/TYRP1*

TYR Tyrosinase, a transmembrane protein in the melanosomal membrane. Acts as the key enzyme in melanin synthesis where it e.g. catalyzes the first step in melanin synthesis (tyrosine to DOPA), thus involved in human pigmentation. Gene *TYR*. Mutations in *TYR* are e.g. the cause of oculocutaneous albinism type I (OCA I).