



AUGUSTSSON, Agneta. MELANOCYTIC NAEVI, MELANOMA AND SUN EXPOSURE. 34 pp. Department of Dermatology, University of Göteborg, Göteborg, Sweden. Thesis defended 1991-10-30.

#### ABSTRACT

The naevus profile was examined in 379 randomly selected Swedes (30–50 years) and in 121 melanoma patients in the same age-range selected from the Regional Cancer Register. The total body count of common naevi (CN)  $\geq 2$  mm in the population was high (mean 67, range 1–300). Even so, the melanoma patients had almost twice as many CN as the controls (mean 113, range 13–347). The prevalence of clinical dysplastic naevi (DN) was 18% in the population and 56% in the patients. The corresponding figures for histologically diagnosed DN were 8% and 40% respectively. Subjects with dysplastic naevi had a significantly larger number of CN than those without DN. Subjects with a sun-sensitive skin,  $\geq 150$  naevi and presence of DN had a 50 times higher melanoma risk than those without these characteristics. For identifying subjects at risk of developing cutaneous malignant melanoma (CMM), clinically diagnosed DN was as good a discriminator as histologically diagnosed DN.

The numbers of naevi in different skin areas were tested for their power in predicting the total body naevus count. The strongest correlations were found between total counts and counts on the anterior surface of the thighs and the lateral aspect of the arms. Counts from either of these areas will provide a practical and satisfactory estimate of the total number of naevi.

To study the possible link between sun exposure, naevus formation and melanoma development, the distribution of CN, DN and CMM over the body surface was studied in the 121 melanoma patients and in 310 consecutive controls. The number of naevi was four times as high in a sun-exposed area on the lower back compared with in an adjacent sun-protected area on the buttocks, indicating that sunlight plays a role in naevus development.

Both CN and CMM were found to have a general distribution pattern over the body surface consistent with the idea that sun exposure is important for their formation.

The number of CN and CMM per unit body surface area was significantly higher in intermittently sun-exposed than in rarely or chronically exposed skin. The distribution pattern of DN was quite different from that of CN, with few DN on the sun-exposed upper chest and face and many DN in protected areas such as the buttocks and lower abdomen, indicating that DN may develop independently of sun exposure.

The probability of having a CMM in a specific area increased with increasing number of CN as well as of DN in that area. This site-specific melanoma risk might be the result of local insults of UV (ultraviolet)-irradiation to the melanocyte system and/or be explained by naevi being precursor lesions to CMM.

**Key words:** melanocytic naevus, dysplastic naevus, melanoma, UV-irradiation.

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

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- II Augustsson A, Stiernner U, Rosdahl I, Suurküla M. Common and dysplastic naevi as risk factors for cutaneous malignant melanoma in a Swedish population. *Acta Derm Venereol (Stockh)* 1991, in press.
- III Augustsson A, Stiernner U, Rosdahl I, Suurküla M. Melanocytic naevi in sun-exposed and protected skin in melanoma patients and controls. *Acta Derm Venereol (Stockh)* 1991, in press.
- IV Augustsson A, Stiernner U, Rosdahl I, Suurküla M. Regional distribution of melanocytic naevi in relation to sun exposure and site-specific counts predicting total number of naevi. Accepted for publication in *Acta Derm Venereol (Stockh)* 1991.
- V Stiernner U, Augustsson A, Rosdahl I, Suurküla M. Regional distribution of common and dysplastic naevi in relation to melanoma site and sun exposure. A case-control study. Submitted for publication in *Melanoma Research*.

# Contents

ABBREVIATIONS .....	6
INTRODUCTION .....	7
Risk factors for cutaneous malignant melanoma (CMM) .....	7
Exposure to ultraviolet irradiation .....	7
Other external factors .....	8
Constitutional factors .....	8
Common naevi (CN) .....	8
Dysplastic naevi (DN) .....	10
Melanoma prevention .....	10
AIMS OF THE STUDY .....	11
SUBJECTS AND METHODS .....	11
Population sample .....	11
Cases .....	12
Time for the study .....	12
Questionnaire .....	12
Skin examination .....	12
Statistical methods .....	14
RESULTS .....	14
Total body naevus count (I,II) .....	14
Clinically diagnosed dysplastic naevi (I,II) .....	15
Histologically diagnosed dysplastic naevi (I,II) .....	15
Relative risk for cutaneous malignant melanoma (II) .....	16
Naevus counts in a defined sun-exposed and sun-protected area (III) .....	17
Regional naevus distribution in men and women (IV) .....	18
Regional naevus distribution in relation to UV exposure patterns (IV) ..	18
Naevus counts in relation to anamnestic habits of UV exposure (IV) .....	19
Site-specific naevus counts predicting total body count (IV) .....	19
Regional distribution of common naevi in melanoma patients and controls (V) .....	20
Regional distribution of common naevi in relation to skin type and UV exposure in melanoma patients and controls (V) .....	20
Regional distribution of dysplastic naevi (IV,V) .....	20
Regional distribution of common naevi in subjects with and without DN (V) .....	21
Regional naevus distribution in relation to UV exposure in control subjects with and without clinical DN (IV) .....	21
Melanoma location in men and women in relation to UV exposure (V) ..	21
Melanoma location in relation to distribution of common and dysplastic naevi (V) .....	21

DISCUSSION..... 22  
    Methodological aspects ..... 22  
    General discussion ..... 23

SUMMARY AND CONCLUSIONS ..... 28

ACKNOWLEDGEMENTS ..... 29

REFERENCES ..... 30

## Abbreviations

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CN	Common naevus
CMM	Cutaneous malignant melanoma
DN	Dysplastic naevus
CI	Confidence interval
RR	Relative risk
Ex-Pr	Exposed – Protected
UV	Ultraviolet
UVA	Ultraviolet irradiation, wavelengths 315–400 nm

## Introduction

Cutaneous malignant melanoma (CMM) is the severest form of skin cancer and the incidence and mortality rate of CMM are increasing rapidly in Caucasian populations all over the world (1, 2, 3). UV (ultraviolet)-irradiation has been considered the most important etiological factor for CMM but its exact role in the pathogenesis of melanoma is not known. In recent years, it has been demonstrated that a large number of common naevi (CN) and the presence of dysplastic naevi (DN) are two major risk factors for developing CMM (4–15). Until 1986, when this investigation started, very little was known about the etiology of naevi and next to nothing was known about the naevus profile in the Swedish population.

### Risk factors for cutaneous malignant melanoma (CMM)

#### Exposure to ultraviolet irradiation

Squamous cell carcinoma has a well-established relationship with sun exposure. It occurs mainly in chronically UV-exposed skin areas such as the head, neck and hands, is far more common in outdoor than in indoor workers and increases exponentially in incidence with age (16). Furthermore, squamous cell carcinoma can easily be induced by UV-irradiation in laboratory animals (17).

Even though UV-irradiation is a well-known initiator and promoter of epidermal carcinogenesis, its potency as a complete carcinogen for melanocytic neoplasia has so far been unproven (18). Unfortunately, most laboratory animals have an epidermal melanocyte system quite different to that of humans and until recently it has not been possible to induce metastasizing melanoma in animals using repeated UV-irradiation.

However, new observations in the opossum now suggest that UV-irradiation alone may initiate melanoma of the skin capable of metastasizing (19). Even though direct evidence of a causal association between sun exposure and CMM in laboratory animals is scanty, epidemiological and clinical data in humans speak in favour of such a relationship. For example, subjects with a deficiency in enzyme repair of UV-induced DNA damage have a highly increased risk of CMM (20). CMM is more common in fair-skinned persons more susceptible to the harmful effects of UV-irradiation than in those with a darker complexion (21, 22). In blacks, CMM is extremely rare, but when it occurs it is mainly located in less pigmented skin areas (23). The incidence of CMM in Caucasians of the same skin type becomes higher the closer one lives to the equator (24). CMM has also been reported to be more common among migrants who have moved to countries with a sunnier climate than among those who have stayed in their place of birth (25, 26). In addition, CMM is rare in UV-protected skin areas, and the sex differences in melanoma site are consistent with differences in sun exposure due to clothing habits (27, 28, 25). Altogether, these findings speak in favour of an association between sun exposure and the development of CMM.

With the exception of lentigo maligna melanoma in the elderly, there are, however, no indications of a direct dose-response relationship between UV-irradiation and melanoma as reported for non-melanoma skin cancers. For example, the highest frequency of CMM is not in the body areas with the highest accumulated UV-dose. Instead, melanoma occurs most commonly on the back in men and on the legs in women (27, 28). Melanoma occurs at younger ages than does squamous cell carcinoma and is less common in outdoor than in indoor workers (25). Thus, it is evident that melanoma has a more complex relationship with sun exposure than has squamous cell carcinoma.

The function of the epidermal melanocytes is mainly to produce the pigment melanin which protects the skin and underlying structures

against UV-irradiation. Following sun exposure the melanocytes increase their melanin production. Thus, the regularly sun-exposed skin is protected by its tan while the intermittently exposed skin is less pigmented and unprepared for the harmful effects of UV-irradiation. It has therefore been proposed that intermittent periods of intense UV exposure are more harmful than moderate regular exposure (29). This idea is supported by the finding of an increased risk of CMM following intense sun exposure during recreational activities (30, 31). Reports of an elevated melanoma risk following severe sunburns (32, 33, 34, 31), lend further support to the idea that the pattern of UV exposure is more important for the development of CMM than the total accumulated UV dose. If this holds true, it could explain why CMM is less common in persons with outdoor work than in indoor workers only exposed to the sun during the weekends. It may also explain the sex difference in the distribution pattern of CMM over the body surface. Occasionally, CMM occurs in areas most often covered by clothes. If UV-irradiation is important for the formation of CMM, this seems to be a paradox. Recently, however, we found that UV-irradiation induces melanocyte proliferation not only in exposed but, via a systemic factor, also in covered skin areas (35). Thus, UV-irradiation may, due to its mitogenic effect on the melanocytes, play a role as a tumour promoter also in covered skin areas.

#### **Other external factors**

Certainly, UV-irradiation cannot be the only environmental factor stimulating melanoma development. Other external factors such as diet, alcohol intake and tobacco smoking, as well as the use of hair dyes, have been investigated as potential risk factors for CMM (cf. 36). The results are inconsistent, however, and the occasional influence found for some of these variables on melanoma risk is weak and considered to be of limited importance.

#### **Constitutional factors**

The clustering of melanomas in certain families is well known. In recent years, the focus has been on familial melanomas associated with dysplastic naevi (see below).

Melanoma is far more common in whites than in those with dark skin (21). Within white populations, CMM is most common among subjects with fair skin, freckles, red or blond hair and blue or green eyes (37, 14). A tendency to burn in the sun as well as inability to tan are more often reported among subjects with than without CMM (7, 33). This is in line with the observations that melanoma patients have a reduced minimal erythema dose and a tendency to develop prolonged erythema after UV-irradiation (38, 39).

Hormones may play a role in the development of CMM. Some studies have reported a weak association between an increased melanoma risk and the use of oral contraceptives. Other investigators have not been able to confirm these findings, however. In a recent Danish case-control study, no correlations were found between risk of melanoma and age at menarche or menopause, duration of menstrual life, age at first pregnancy or number of pregnancies. Nor was there any association between melanoma and use of oral contraceptives or menopausal replacement therapy (40).

#### **Common Naevi (CN)**

The presence of a large number of CN is one of the strongest known risk factors for CMM (8, 10, 13, 14). The mean number of CN varies between different populations (41–44) but there seems to be a trend towards higher naevus counts as melanoma has become more common (45).

In addition to a risk marker, common naevi may also be precursor lesions to malignant melanoma. Up to 85% of all melanoma patients report a pre-existing naevus-like lesion at the melanoma site (46). Such anamnestic data must, however, be interpreted with caution. The strongest evidence of naevi being precursors to

melanoma comes from the finding of histological naevus remnants in up to 72% of all melanomas (46).

#### *Clinical presentation of common naevi*

There is a wide variation in the clinical presentation of CN. Starting as a hyperplasia of melanocytes along the basal lamina, a naevus appears in the skin as a minute dark brown macule. Gradually, naevus cell nests develop along the dermal-epidermal junction. This still macular "junctional naevus" enlarges to a diameter usually not exceeding half a centimeter. From the dermal-epidermal junction, some naevus cells descend into the underlying dermis. This "compound naevus" becomes somewhat lighter and rises slightly above the surface of the skin. With time all naevus cells become situated in the dermis, and the "dermal naevus" appears as a slightly pigmented to flesh-coloured papule. During the entire evolutionary sequence CN are round to ellipsoid with a regular border and are sharply demarcated from the surrounding skin. CN are most often uniformly pigmented, typically tan or brown. Individual naevi may stop at any point in their development or they may, after the dermal stage, finally undergo involution (47).

A congenital melanocytic naevus, already present at birth, has been reported in 1 – 5% of all newborn infants (48). In contrast, CN appear first in early childhood, increase in number up to adult life and thereafter decrease in number so that in old age few naevi if any are observed. This model of the life cycle of CN is based on point observations of individuals of different age-groups.

#### *Etiology of common naevi*

There is a general impression that a large naevus number is more common in individuals of certain families but no detailed investigations are available on the genetics of CN. As for melanoma, it is possible that CN occurs in subjects with genetically susceptible melanocytes which

receive some additional stimuli (49). UV-irradiation is the most obvious environmental stimulus inducing proliferation of melanocytes in the skin (50). The results from some previous studies indicate that UV-irradiation plays a role in the formation of melanocytic naevi. Nicholls reported that sun-exposed skin reaches peak values of naevi earlier in life than do protected skin areas (42). An association has been reported between an increasing number of naevi and an increasing number of painful sunburns in childhood (51, 31). Kopf et al. reported three times as many elevated naevi on the lateral, sun-exposed, aspect of the arms compared with on the medial protected side (52). A higher prevalence of naevi in white than in darker races is in line with the idea that UV-irradiation plays a role in naevus development (22, 23). Available data are, however, not ideal to compare and within Caucasian populations there is no obvious association between skin type and proneness to form naevi (44).

Apart from UV-irradiation, very few environmental factors have been considered to stimulate naevus formation. In one investigation, Armstrong et al studied the influence of dietary variables on naevus formation (51). They observed that consumption of alcohol as well as intake of retinol had a weak protective effect on naevus development. The biological significance of these findings is hard to evaluate.

The assumption that melanocytic naevi are hormone responsive is based on the observation that naevi darken and enlarge in some women during pregnancy and the finding of an increasing naevus number during puberty (42, 44). It is, however, possible that the increase in number during puberty is solely an expression of the normal evolution of naevi independent of the hormonal status. Neither MacKie et al. (44) nor English et al. (53) found any correlation between total naevus counts and hormonal factors when studying parity and the use of oral contraceptives.

There are occasional reports on eruptive naevi after exacerbation of severe bullous dermatoses (54, 55), intense UVA exposure (56) and cytostatic or immunosuppressive therapy (57, 58). In

a recent study, it was found that children who had completed a course of chemotherapy had higher naevus counts than controls and higher counts than those currently undergoing treatment (59). This is interesting and implies that immunological mechanisms might be of importance for naevus formation.

### **Dysplastic Naevi (DN)**

Dysplastic naevi are general markers of an increased melanoma risk (9, 10, 13, 60). In addition, DN constitute precursor lesions to CMM (46, 60, 61). In order to assess the melanoma risk of an individual with DN, Kraemer et al (62) proposed the current classification of kindreds with dysplastic naevi: type A with sporadic occurrence of DN; type B with familial occurrence of DN; type C with sporadic DN and melanoma; type D1 with familial DN and one family member with melanoma, and type D2 with familial DN and at least two family members with melanoma.

#### *Clinical presentation of dysplastic naevi*

Dysplastic naevi were first recognised by Clark et al (63) and by Lynch et al (64) in 1978 in certain melanoma-prone families. Elder et al reported DN in association with non-familial melanoma and later the occurrence of sporadic DN was described (65, 66, 67). The life cycle of DN is not completely known but there seem to be no differences in the clinical presentation of the hereditary and the sporadic form of DN. A DN starts as a brown macular lesion, which initially cannot be differentiated from a common junctional naevus. Most DN, however, continue to grow beyond the size of CN to reach a diameter larger than half a centimeter. The border become irregular and ill-defined and the colour become speckled with variations from dark brown to tan and pink. The DN commonly consists of both a macular and a papular component, causing a pebbled surface or a lesion of the so called

“fried-egg type”. Similar to CN, individual DN may stop at any point in their development (47).

DN may appear anywhere on the body surface but are most often located on the trunk (63, 61). Unlike CN, hereditary DN also frequently occur in areas that do not receive much sun exposure such as the buttock area. The regional distribution of sporadic DN has so far not been described. DN already appear during childhood but their typical clinical characteristics can often not be recognized before early puberty. In contrast to CN, new DN continue to appear throughout life. Subjects with the hereditary form of DN may have more than a hundred lesions, while subjects with the sporadic form usually have only a few DN. The prevalence of DN reported from different populations varies from 2 to 17% (66, 67, 68, 13).

The histological criteria of a dysplastic naevus were first described by Clark et co-workers (47, 69), stressing the occurrence of random melanocytic atypia as an obligatory feature for the histological diagnosis. This is at variance with other pathologists, cf. Sagebiel (70). The National Institutes of Health consensus conference in 1984 did not emphasize cellular atypicality but instead suggested that the histological diagnosis DN could be based only on architectural alterations superimposed on a junctional or compound naevus (71).

#### *Etiology of dysplastic naevi*

Apart from the heredity, very little is known concerning the etiology of DN. As for CN, UV-irradiation has been suggested to stimulate the formation of DN (72, 18).

### **Melanoma prevention**

Public prevention programmes have been initiated in many countries in attempt to reduce the incidence of melanoma. General educational campaigns on early tumour signs and advice to

reduce risk behaviour in the sun have been given through the media. In addition, various target groups, e.g. schoolchildren, sun-catchers at the beach and people practising various outdoor activities have been approached in an attempt to influence the lifestyle towards less excessive and intense sun exposure. Increasing knowledge on the etiology of malignant melanoma will in the future improve our chances of more optimal primary prevention.

So far, we still lack a curative treatment for advanced melanoma. The prognosis is correlated to the thickness of the tumour. Since excision of early lesions remains the only effective cure, screening examinations of the skin to find pre-invasive melanomas will be one important step to reduce the mortality rate. The characterization of melanoma-prone risk groups is therefore of great importance. It is known that kindreds with DN belonging to a D2 family have a cumulative lifetime incidence of melanoma that approaches 100% (62, 61). Detailed surveillance programmes already exist for this group of patients. However, this high-risk group constitutes only a minor part of all melanoma patients. To address a larger risk group with screening programmes it will be necessary to have data on the occurrence of various risk factors, for example the number of CN and the prevalence of DN in the general population.

## Aims of the study

- to investigate the number of common naevi (CN) and the prevalence of dysplastic naevi (DN) in an adult Swedish population (I)
- to study the importance of melanocytic naevi (CN and DN) as risk markers for CMM (II)
- to identify individuals at risk for developing CMM (II)
- to study the possible role of UV-irradiation in the development of melanocytic naevi (III, IV, V)
- to evaluate CN and DN as precursor lesions to CMM by determining the regional distribution of naevi in relation to melanoma site (V)

## Subjects and methods

### Population sample

From the census file in Göteborg, a random sample of 500 individuals aged 30–50 years was drawn. A letter was sent to each person offering a free skin examination by a dermatologist. Two reminder letters were sent to those not responding. At this time, 31 subjects (6%) had either moved from the area, lacked a permanent address, were severely ill or were deceased. Of the remaining 469, 383 (82%) were examined. Four of them were excluded as they were non-Caucasians. Of the drop-outs, 27 (6%) were interviewed by telephone but declined further participation (most often due to lack of time) and 59 (12%) could not be reached. The 379 subjects analysed (183 men and 196 women), equaled 76% of the original 500 subjects and had a mean age of 42.5 (range 34–52) years at examination. Data from these individuals are presented in *paper I*. In *paper II*, one individual has been excluded from analysis because a malignant melanoma was diagnosed during the course of the study.

*Papers III–V*: these investigations started when 69 subjects were already examined. The 310 consecutive subjects (152 men, 158 women, mean age 41.4 years) were included.

## Cases

All patients 30–50 years of age with a history of CMM and living in Göteborg were selected from the Regional Cancer Register,  $n=197$ . Of these, 154 (78%) were still alive. In all but two cases permission to contact the patient was obtained from his/her physician. Like the control subjects, the patients were offered a free skin examination. Ten patients declined participation and an additional 5 could not be reached. The remaining 137/152 (90%) were examined. Of these, 16 patients were excluded from analysis, 6 because tumour material could not be found for histological reevaluation and 10 because the melanoma diagnosis could not be verified on re-examination. Two patients were retained in the study although material from their primary melanoma could not be traced. In these cases the diagnosis was verified by histological examination of metastases.

Of the 119 melanomas re-evaluated, 55 were classified as superficially spreading melanoma, 16 as nodular melanoma, 9 as lentigo maligna melanoma, and 8 as spindle cell melanoma. As many as 31 of the melanomas could not be classified at re-evaluation partly due to the minimal amount of melanoma tissue remaining in the paraffin blocks. Due to the young population studied patients with lentigo maligna melanoma were not excluded. The histological re-examination was performed by one pathologist (M.S.). Altogether 121 melanoma patients (52 men, 69 women, mean age 43.5 years) were included for analysis and are presented in *papers II, III and V*.

## Time for the study

The study was performed during the winter seasons 1986–88.

## Questionnaire

At the time of the examination the patient and the doctor together filled in the questionnaire.

Information was collected regarding pigmentary phenotypic characteristics such as skin type according to Melski (73), hair and eye colour as well as on ethnic origin, heredity for CMM and use of oral contraceptives (papers I and II). The estimate of life-time amount of UV exposure included occupation and spare time exposure, number of weeks in a sunny climate, number of burns, age at first burn and use of UVA sunbeds (paper IV).

## Skin examination

All subjects had a total body skin examination and all macular or raised lesions  $\geq 2$  mm considered to be melanocytic naevi were counted. It was sometimes difficult to differentiate between naevi, freckles and lentigines. If in doubt, the lesion was not counted. All patients and controls were examined by one and the same dermatologist (A.A.). In 20 randomly selected control subjects, independent naevus counts were performed by another dermatologist (I.R.). The oncologist (U.S.) participated in the examination of the patients.

The major clinical criterion for a dysplastic naevus was a diameter  $\geq 5$  mm. In addition, at least two of the following criteria were required: an ill-defined or irregular border, speckled pigmentation, erythema or a pebbled surface (74). All DN were photographed and re-evaluated by the other two clinicians.

A pigmented naevus was considered to be a congenital melanocytic naevus if  $\geq 10$  mm and anamnesticly apparent during the first year of life.

All subjects with clinical DN, except a few melanoma patients with advanced disease, were offered excision of the lesions for histological examination. One pathologist (M.S.) examined all biopsies without knowing if the lesion was excised from a control subject or a melanoma patient. To further avoid bias in the histological diagnosis, some flat common naevi were excised and the slides were added to those of clinical

DN. The histological criteria used for DN are described in detail in papers I and II. Lesions fulfilling only some of the criteria for the diagnosis of DN were registered separately as naevi with dysplastic features.

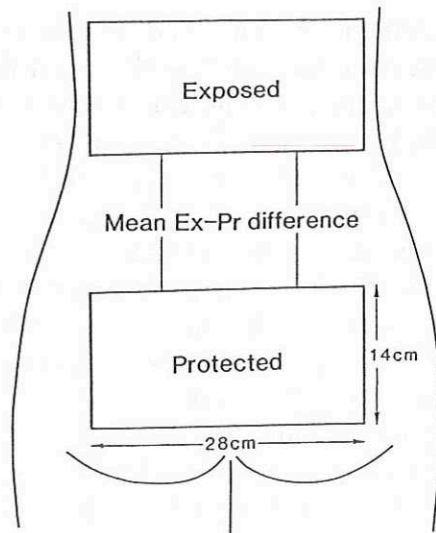


Fig. 1 Schematic figure illustrating the size and location of the sun-exposed and the sun-protected area.

The number of melanocytic naevi  $\geq 2$  mm was counted separately in two 14 x 28 cm large areas, one sun-protected on the buttocks and one adjacent sun-exposed on the lower back (Fig. 1). The area was defined on a transparent sheet and the median line was placed in the natal cleft. The sheet was then moved upwards so that the two studied areas were 14 cm apart. These areas were selected to study the most sun-protected skin area in both sexes and an adjacent area usually exposed to the sun during the summer and when sun-bathing. These anatomical areas were further selected to avoid skin exposed to repeated mechanical trauma and to minimise counting errors due to freckling. The difference in naevus count between the exposed and the protected area in each individual was calculated and will be referred to as the Ex-Pr difference (Exposed-Protected) (paper III).

The exact location of each CN, DN and CMM was plotted on three separate schematic figures. The melanoma location was registered in the

centre of the scar. To compare naevus counts between different body sites, the figures were later divided into 16 separate areas (A-P)(Fig. 2). The areas were defined taking clothing habits and general behaviour in the sun into account. The areas H and J are most often covered by clothes even when sun-bathing. These two areas and area C, the medial aspect of the arms, were considered rarely UV exposed. The areas A and F were considered chronically UV exposed as they are regularly exposed all year round when a person is out of doors. With the exception of areas B, E and P (scalp, palms and soles), the remaining areas were considered intermittently exposed to UV-irradiation. During the long winter season in Sweden these areas are generally covered and become less pigmented but during vacations in sunnier climates and during our short summer they usually receive a high and intense UV dose. The number of naevi and melanoma per unit surface area was calculated using the estimates of body surface area by Lund and Browder (75) with minor adjustments indicated on the schematic figure (papers IV and V).

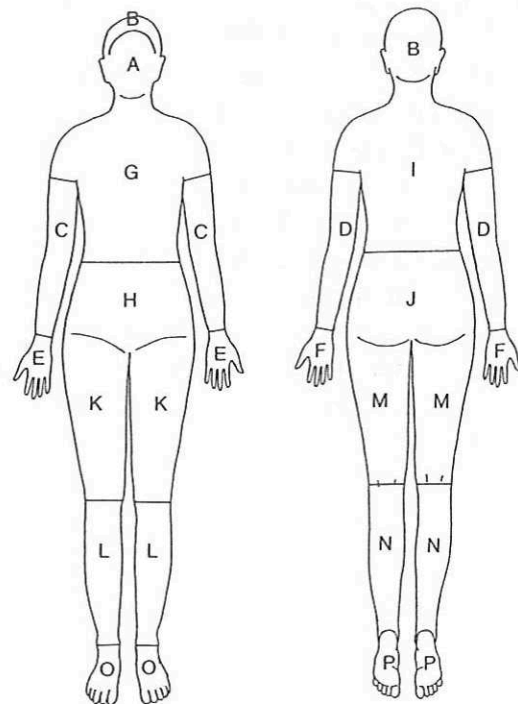


Fig. 2 Schematic figure illustrating the 16 areas (A-P) studied.

## Statistical methods

Spearman's rank test was used for the correlation analyses. For comparisons between groups, we used Wilcoxon's two-sample test. For comparisons of proportions between groups, Fisher's exact test was used. Trends in contingency tables were analysed using the Mantel-Haenszel chi square test (76). A stepwise logistic regression analysis was applied in order to assess the risk factors that contribute independently of each other to the risk of melanoma. The relative risk with confidence limits adjusted for important confounding variables was calculated.

Wilcoxon's test for paired observations was used when naevus counts between the sun-exposed and the protected area were compared and when the number of CN and CMM per unit surface area were compared between areas of different UV-exposure categories. To study the correlation between the number of naevi in each defined area (A-P) and the total body naevus count, Pearson's correlation test and a standard regression analysis were used.

In order to assess the correlation between clustering of naevi and presence of CMM within a small skin area, we applied a procedure which is not affected by the correlation between the total body count of naevi and CMM. For each area in each individual, a score was calculated measuring the relative clustering of naevi within the area,  $(P-E)/S$ .  $P$  is the quotient between the number of naevi in the area and the total body count.  $E$  is the mean proportion of naevi in the same area for all individuals and  $S$  is the corresponding standard deviation. By logistic regression analysis, the probability of melanoma in the area was estimated as a function of the score. Two-sided tests were used.

## Results

### Total body naevus count (I, II)

The melanoma patients had almost twice as many melanocytic naevi as the controls. The mean total body naevus count (common and dysplastic naevi) was 115 (median 89) versus 67 (median 54) in the controls,  $p < 0.001$ . There was a wide range in interindividual naevus number both in the melanoma group (13-355) and in the controls (1-305). It is interesting to note that as many as 26% of the melanoma patients had 150 naevi or more. The corresponding figure for the controls was 7%. Only 2% of the patients but 18% of the controls had less than 25 naevi. More details of the naevus distribution in the population are given in table 1. The number of naevi was not influenced by age, sex or skin type in either group. In this study on Caucasians, there was no correlation between the number of naevi and ethnic origin, heredity for CMM, hair colour, eye colour or use of oral contraceptives.

Eighty patients (66%) had had 1-23 naevi excised before or after the diagnosis of melanoma. Altogether as many as 310 histologically verified naevi had been removed and were included in our counts. Of these 310 naevi, 286 were histologically re-evaluated to find naevi fulfilling the criteria for DN excised before this entity was described. Those not re-examined had been excised from the face or neck region and were histologically diagnosed as intradermal naevi. Some of the controls had a history of an occasional skin lesion previously excised. With a few exceptions, these lesions had not been sent for histological examination and therefore were not included in our counts. Even if all these lesions were included, it would not have influenced the mean naevus count significantly.

A melanocytic naevus  $\geq 10$  mm and present at birth was considered to be congenital. Such naevi were found in 9.9% ( $n = 12$ ) of the patients and in 5.8% ( $n = 22$ ) of the controls.

*Table 1* The numerical distribution of common naevi (CN) in relation to dysplastic naevi (DN).

		Number of CN/individual															
		0-24	25-49	50-74	75-99	100-124	125-149	150-174	175-199	200-224	225-249	250-274	275-299	300-324	325-349		
Controls (n=378)		66	98	63	36	26	6	3	6	2	1	3	0	0	0	0	0
		1	10	7	10	8	8	2	3	1	0	1	0	0	0	0	0
		0	0	3	2	4	4	0	2	1	0	0	0	1	0	0	0
																0	Number of DN/ indi- vidual
																1-2	
																≥3	

		Number of CN/individual															
		0-24	25-49	50-74	75-99	100-124	125-149	150-174	175-199	200-224	225-249	250-274	275-299	300-324	325-349		
Mela- noma patients (n=121)		1	16	17	7	3	4	3	0	1	0	0	1	0	0	0	0
		1	2	8	10	1	1	2	2	1	1	1	2	1	0	0	0
		0	0	3	7	4	6	2	5	2	1	0	2	1	2	0	0
																0	Number of DN/ indi- vidual
																1-2	
																≥3	

### Clinically diagnosed dysplastic naevi (I, II)

One or more clinical DN were found in 56% (CI 47–65%) of the melanoma patients and in 18% (CI 14–22%) of the controls,  $p < 0.001$ . When the diagnosis DN was re-evaluated from photographs there was good agreement between the three clinicians. The minor difference did not change the prevalence figures. The group of melanoma patients with clinical DN includes 10 subjects without clinical DN at the time of the examination but with an earlier diagnosis of histologically verified DN. Of the 286 previously excised lesions 44 were histological DN at re-evaluation.

Subjects with DN had a markedly larger mean total number of naevi than subjects without DN. In the melanoma group, those with DN had a mean total naevus count of 144 (median 117), compared with 78 (median 59) for those without DN,  $p < 0.001$ . The corresponding figure in the

control group for subjects with DN was 107 naevi (median 102) and for those without 58 (median 47),  $p < 0.001$ .

As expected, melanoma patients had a more sun-sensitive skin than controls,  $p < 0.001$ . Control subjects with clinical DN had a more sun-sensitive skin type than those without DN,  $p < 0.001$ . No such difference was found between melanoma patients with and without DN, probably due to the larger proportion of subjects with a sun-sensitive skin in this group as a whole. Neither in the patients nor in the controls did sex or age have any influence on the presence of clinical DN.

### Histologically diagnosed dysplastic naevi (I, II)

Forty-eight of the 58 melanoma patients with clinical DN at the examination had 1–8 naevi ex-

cised. In the controls, 65 of 69 subjects with DN had 1–6 naevi removed. Forty per cent (CI 31–49%) of all melanoma patients had at least one histologically verified DN. The prevalence of histologically verified DN in the control group was 8% (CI 5–11%),  $p < 0.001$ .

In the control group, a total of 125 clinically diagnosed DN were excised. Of these, 45 were histologically DN (30 subjects) and 24 demonstrated some dysplastic features (16 subjects). The remaining 56 biopsies were histologically diagnosed as CN. Most DN diagnosed as CN histologically were less cellular than the rest of the lesions. In 25 of 65 control subjects with DN, one relatively flat naevus clinically diagnosed as a CN was excised as a histological control. Five of these naevi fulfilled the histological criteria for the diagnosis of DN. All these naevi were small, however, and the difficulty in differentiating between CN and DN microscopically in-

creases with smaller size and less cellularity.

The 30 control subjects with histologically verified DN had a significantly larger mean number of CN (119) than the rest of the controls (63),  $p < 0.001$ . It is interesting to note that the 16 individuals with clinical DN that demonstrated only dysplastic features, histologically, had a higher mean count of CN (111) than controls without clinical DN (58),  $p < 0.001$ . Furthermore, even the 19 subjects with clinical DN diagnosed as CN histologically had a significantly larger mean number of CN (81) than controls without clinical DN (58),  $p < 0.01$ .

## Relative risk for cutaneous malignant melanoma (II)

The crude relative risk (RR) for CMM was calculated for all variables studied. A significantly

Table 2 Risk Factors for CMM

Variable	Category	Cases n=121	Controls n=378	Relative risk crude (95% CI)	Relative risk adjusted (95% CI)*
Skin type	III and IV	84	348	1	3.6 (1.9–6.9)
	I and II	37	30	5.1 (3.0–8.7)	
Hair colour	dark brown	10	59	1	1.9 (0.8–4.8)
	light brown	59	235	1.5 (0.7–3.1)	
	red/blond	28/24	17/67	3.6 (1.7–7.7)	
Eye colour	brown/mixed	13	77	1	1.4 (0.7–3.0)
	blue/grey	76	244	1.8 (1.0–3.5)	
	green	32	57	3.3 (1.6–6.9)	
Total body naevus count	1–74	48	247	1	1.2 (0.7–2.2)
	75–149	41	105	2.0 (1.2–3.2)	
	≥150	32	26	6.3 (3.5–11.6)	
Number of clinical DN	0	53	310	1	2.5 (1.3–4.5)
	1–2	33	51	3.8 (2.2–6.4)	
	≥3	35	17	12.0 (6.3–23.0)	
Presence of histological DN	No	72	348	1	4.6 (2.5–8.4)**
	Yes	49	30	7.9 (4.7–13.3)	

\* The stepwise logistic regression model included skin type, hair colour, eye colour, total body naevus count, number of clinical DN, age and sex.

\*\* Tested in a separate analysis excluding the variable "Number of clinical DN".

increased relative risk was found for skin type, hair colour, eye colour, total number of naevi, number of clinical DN and the presence of histologically verified DN (Table 2). In a stepwise logistic regression procedure, the adjusted RR was calculated for all risk factors together with sex and age. The number of clinical DN and the presence of histologically verified DN were tested separately. The risk of having a melanoma was most strongly correlated to the presence of 3 or more clinical DN, RR 12.0. It remains the strongest risk factor (RR 5.6) even after adjustment for the contribution of all other variables to the relative risk. The presence of histologically verified DN was also a strong risk factor both before (RR 7.9) and after adjustment (RR 4.6). In addition, an increasing melanoma risk with an increasing total number of naevi was found. After adjustment, this relative risk decreased and was statistically significant only at the level  $\geq 150$  naevi. A sun-sensitive skin, red or blond hair and green eye colour constitute three other important risk factors for melanoma. After correction for the other associated phenotypic variables, red or blond hair colour was no longer an independent risk factor. However, when analysing the trend from dark to red or blond hair a lighter hair colour remained a significant melanoma risk factor,  $p < 0.001$ .

The relative risk for subjects regarding three of the most important risk factors is shown in table 3. Subjects with clinical DN,  $\geq 150$  naevi and a sun-sensitive skin have a 50 times higher melanoma risk than subjects without clinical DN, with few naevi and with good tanning ability.

The probability (in per cent) of correctly classifying a person as a melanoma case or a control was compared based on the presence of clinically versus histologically verified DN using a logistic regression model. Clinical DN turned out to be an as good or even better prognostic tool than histologically verified DN (64% versus 61%).

Table 3 Relative Risk for CMM\*

Skin type	I + II		III + IV	
	No	Yes	No	Yes
Presence of clinical DN				
Number of naevi				
1-74	4	14	1	3
75-149	8	26	2	6
$\geq 150$	16	50	4	11

\* The table gives the relative risk for different groups defined by combining different variables compared with the group with the lowest risk (=1).

### Naevus counts in a defined sun-exposed and sun-protected area (III)

Both patients and controls had more naevi in the sun-exposed area just above the waist than in the protected area on the buttocks,  $p < 0.001$  (Fig. 1). The mean naevus count in the exposed area was in patients 6.5 (range 0-29) and in controls 3.7 (range 0-20). The corresponding figures for the protected area were in patients 1.4 (range 0-8) and in controls 0.9 (range 0-12) (Table 4).

The mean Ex-Pr difference was larger in patients, 5.1, than in controls, 2.8,  $p < 0.001$  (Table 4), but corresponded to a more than four-fold increase in mean naevus count in exposed skin in both groups.

Melanoma patients had more naevi than controls both in the protected,  $p < 0.01$ , and in the exposed skin area,  $p < 0.001$ . The difference between the two groups was more pronounced in the exposed area (Table 4). For both groups, the interindividual variation in naevus count was greater in the exposed than in the protected area.

For further analysis, patients and controls were divided into subgroups with and without clinically diagnosed DN (Table 4). Melanoma patients with DN had more naevi in protected skin than those without DN,  $p < 0.05$ . The same tendency was seen in controls with and without DN. The difference in naevus counts between

**Table 4** Mean (median) number of naevi in the defined areas on the buttocks and the lower back.

		Protected area	Exposed area	Ex-Pr difference
Controls, all	(n=310)	0.9 (1)	3.7 (3)	2.8
Controls with clinical DN	(n=59)	1.3 (1)	6.2 (5)	4.9
Controls without clinical DN	(n=251)	0.8 (0)	3.1 (2)	2.3
Melanoma patients, all	(n=121)	1.4 (1)	6.5 (5)	5.1
Melanoma patients with clinical DN	(n=68)	1.7 (1)	8.8 (7)	7.1
Melanoma patients without clinical DN	(n=53)	1.1 (1)	3.7 (3)	2.6

the subgroups with and without DN was even more pronounced in the exposed area,  $p < 0.001$  in both patients and controls. However, melanoma patients with DN had more naevi than controls with DN in both areas studied,  $p < 0.05$ . No significant difference in naevus counts was found between patients without DN and controls without DN. For all subgroups, the exposed area had more naevi than the protected area,  $p < 0.001$ .

Both patients and controls with DN had a significantly larger mean Ex-Pr difference than patients and controls without DN,  $p < 0.001$ . Melanoma patients with DN had a mean Ex-Pr difference of 7.1, and the corresponding figure for controls with DN was 4.9. Melanoma patients without DN had a mean Ex-Pr difference of 2.6, and the corresponding figure for controls without DN was 2.3. The mean Ex-Pr difference did not differ significantly between patients and controls with DN, or between patients and controls without DN (Table 4).

#### Regional naevus distribution in men and women (IV)

In both sexes, there was a wide variation in naevus concentration between the different defined areas A–P. After adjustment for the individual total naevus count, the mean number of naevi in each area was compared between the two sexes. Men had significantly more naevi than women on the chest, the back and the buttocks, while women had more naevi than men on the face, the anterior surface of the thighs and the lower limbs.

#### Regional naevus distribution in relation to UV exposure patterns (IV)

The skin areas were classified as rarely (C, H, J), intermittently (D, G, I, K, L, M, N, O) or chronically (A, F) exposed. The mean number

of naevi per unit surface area was significantly larger in the UV-exposed skin (0.78) than in skin areas rarely exposed (0.53),  $p < 0.001$ , supporting the idea that UV-irradiation promotes naevus development. To study the "naevogenic" effect of different patterns of UV exposure, intermittently and chronically exposed areas were studied separately. Intermittently exposed skin had a high mean count of naevi per unit surface area (0.81), while chronically exposed areas had a mean count comparable with that of rarely exposed areas (0.41).

### Naevus counts in relation to anamnestic habits of UV exposure (IV)

A significant inverse correlation was found between the total body naevus count and the amount of occupational sun exposure. That is, subjects with predominantly indoor work had higher mean naevus counts than outdoor workers (70 versus 50),  $p < 0.05$ . No significant correlation was found between the total number of naevi and other parameters grading the life-time amount of UV exposure. Surprisingly, there was no correlation between the total naevus count and the number of weeks in southern countries. Subjects reporting more than three burns had, however, a higher mean count (83) than the rest of the sample (65),  $p < 0.05$ . No correlation was found between age at first burn and the total body naevus count.

### Site-specific naevus counts predicting total body count (IV)

The number of naevi in each area (A–P) was tested for its power in predicting the total body naevus count. In both sexes, there were strong, highly significant correlations between total counts and counts on the anterior surface of the thighs, the lateral aspect of the arms and the back,  $p < 0.001$  (corr.coeff. 0.82–0.88). The excellent correspondence between counts on the

anterior surface of the thighs and the total body count ( $r^2 = 0.72$ ) is shown by a regression plot (Fig. 3a). An even better correspondence ( $r^2 = 0.87$ ) was obtained if counts from the anterior surface of the thighs and the lateral aspect of the arms were summed (Fig. 3b).

Fig. 3a

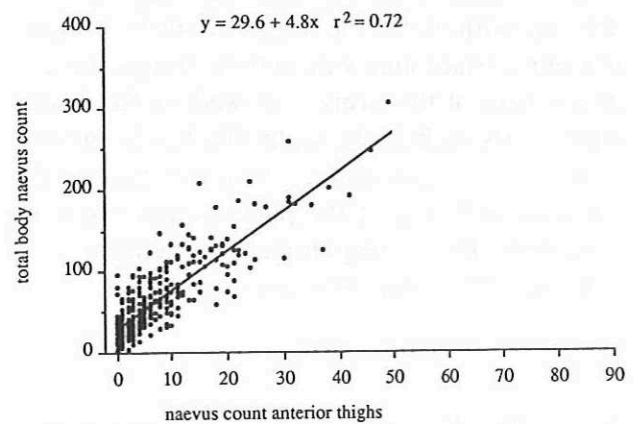


Fig. 3b

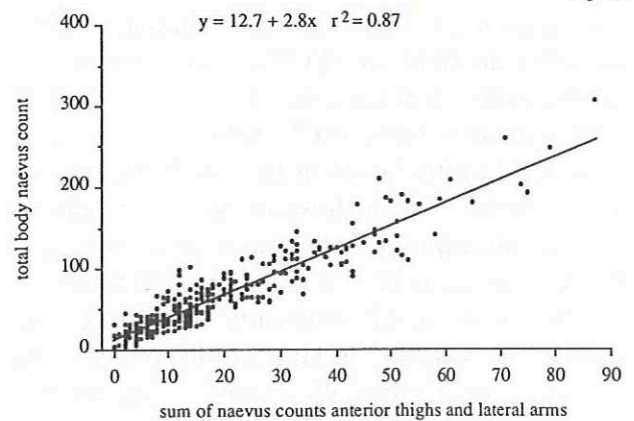


Fig. 3 Correlation between individual total body naevus count and

- counts on the anterior surface of the thighs.
- sum of counts on the anterior surface of the thighs and the lateral aspect of the arms

### Regional distribution of common naevi in melanoma patients and controls (V)

With the exception of the dorsum of the hands and the soles, the melanoma patients had significantly more CN than the controls in all areas studied (A–P). After adjustment for the total number of CN in each individual, this difference remained only for the lower legs and the dorsum of the feet. There was a wide variation in the concentration of naevi over the body surface. The site with by far the largest number of naevi per unit surface area was in both groups the lateral aspect of the arms, followed by the back, while areas such as the scalp, the hands and the soles had very low counts. Five per cent of the controls and 14% of the patients had naevi on the palms. The corresponding prevalence for the soles was 15% and 18% respectively.

### Regional distribution of common naevi in relation to skin type and UV exposure in melanoma patients and controls (V)

Skin type did not have any major impact on the regional distribution of CN either in the melanoma group or in the controls.

The mean number of CN per unit surface area was significantly larger in the intermittently exposed areas than in those considered rarely or chronically exposed. This was true for both patients and controls. The mean difference in counts between intermittently and rarely exposed skin was twice as large in the patients as in the controls. Chronically exposed skin had the lowest naevus counts in both groups.

### Regional distribution of dysplastic naevi (IV, V)

The exact location of each DN was plotted on separate schematic figures (Fig. 4a+b). In the 310 controls, one or more clinical DN were diag-

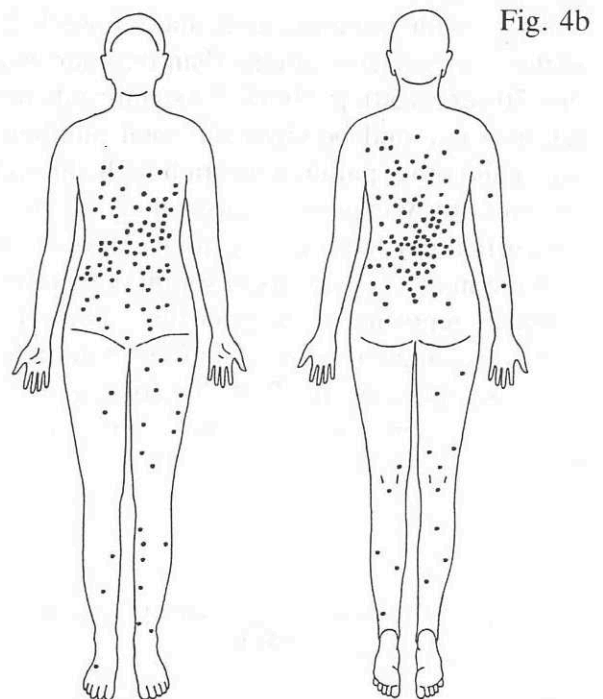
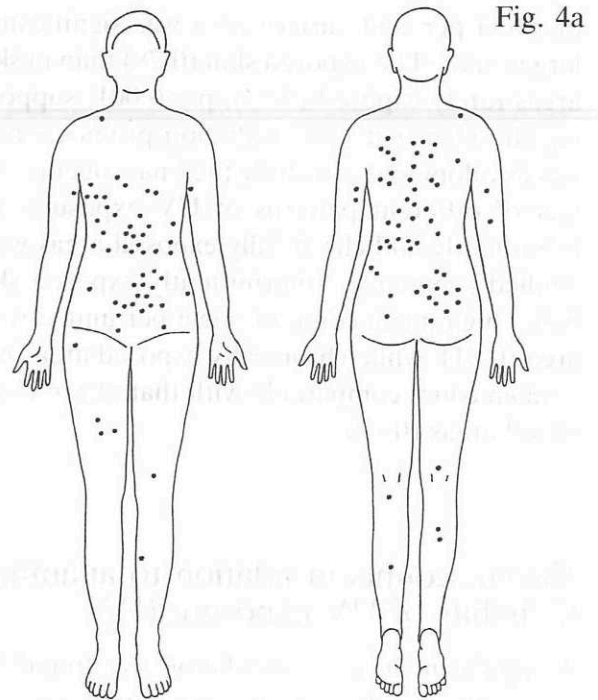


Fig. 4 Regional distribution of dysplastic naevi in

- a. 59 control subjects (n=117).
- b. 68 melanoma patients (n=215).

Each dot represents one naevus.

nosed in 59 individuals and altogether 117 DN were found. In 68 of the 121 melanoma patients a total of 215 DN were registered. This includes 44 DN diagnosed on histological re-examination of naevi excised before this study started. The regional distribution of DN in the melanoma patients was similar to that of the controls.

### Regional distribution of common naevi in subjects with and without DN (V)

Subjects with DN, cases as well as controls, had more CN in all areas except on the scalp, the hands and the soles than subjects without DN. The relative naevus distribution (% of total number of naevi per individual) was, however, very similar in the two groups.

### Regional naevus distribution in relation to UV exposure in control subjects with and without clinical DN (IV)

The mean naevus count per unit surface area was higher in intermittently UV-exposed than in rarely exposed areas in both subjects with (1.25 versus 0.89) and without DN (0.70 versus 0.45),  $p < 0.001$ . The difference in counts between intermittently and rarely exposed skin was, however, larger in subjects with than in those without DN.

### Melanoma location in men and women in relation to UV exposure (V)

The site distribution of 127 CMM in the 121 patients is shown in figure 5. The number of CMM per unit surface area was compared between the areas A to P grouped into the three exposure categories used. The concentration of CMM in intermittently exposed skin was significantly higher than in both rarely and chronically exposed skin areas,  $p < 0.001$ .

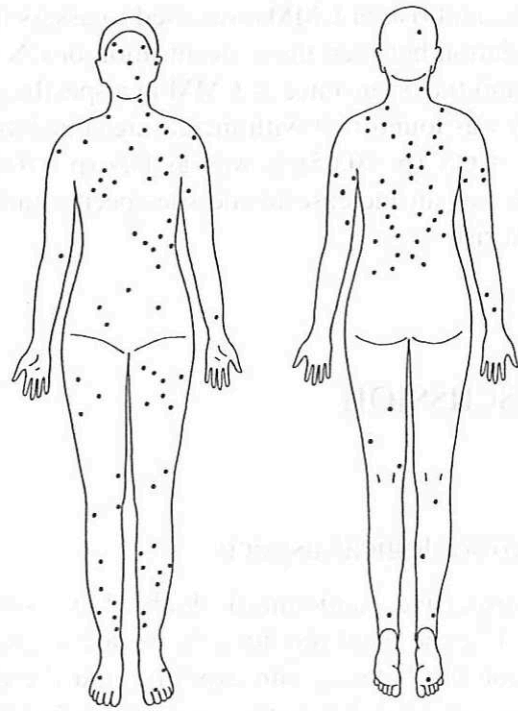


Fig. 5 Site distribution of 127 CMM in 121 patients.

There was a male predominance for CMM on the trunk ( $p < 0.01$ ), while CMM on the upper and lower limbs was more common in females,  $p < 0.05$ . Skin type had no influence on the site distribution of CMM.

### Melanoma location in relation to distribution of common and dysplastic naevi (V)

The distribution pattern of CN over the body surface showed a marked resemblance to that of melanoma. There was, however, no conformity between the distribution of DN and CMM on the upper chest and in the head and neck region, where no DN were found but a significant number of melanomas. In the buttock area, where few melanomas were located, we found several DN (Fig. 4a+b, Fig. 5). On the other hand, when a statistical method (not affected by the correlation between the total body naevus

counts and risk of CMM) was used to assess the correlation between the concentration of CN or DN and the occurrence of CMM in a specific area, it was found that with increasing concentration of CN ( $p < 0.05$ ) as well as DN ( $p < 0.01$ ) there was an increase in the site-specific melanoma risk.

## Discussion

### Methodological aspects

A prospective, randomized, double-blind study would be the ideal way to demonstrate a causal relationship between sun exposure and the development of melanocytic naevi and melanoma in humans. Such controlled experimental studies are for obvious reasons impossible to perform. We are therefore left with epidemiological studies, with all their pitfalls and limitations, to elucidate such a relationship.

We selected subjects between 30 and 50 years of age since most CN as well as DN have appeared by this time. Also, it is in this age-group that the incidence of melanoma begins to rise and preventive measures become important. The control population was randomly selected from a census file. The participation rate was high. The sample is therefore considered representative of the Swedish population in this age-group.

All patients with the diagnosis of CMM between 1964 and 1986 were selected from the Regional Cancer Register. The participation rate was 90%. Seventy-five per cent of the cases had their melanomas diagnosed less than 10 years and 95% less than 15 years before the investigation started. The 10 and 15-year relative survival for patients in the actual sampling frame is 80% and 75% respectively. As we studied a selection of survivors, it was important to analyse if a more favourable prognosis might be consistent with certain phenotypic characteris-

tics. The melanoma patients whose tumours were diagnosed within the last 5 years did not, however, differ from those with an earlier diagnosis in any of the variables studied. This led to the conclusion that the patients in this study do not differ significantly from melanoma cases being diagnosed today.

All patients included had their melanoma diagnosis confirmed by re-evaluation of their tumour sections. In this study, 7.6% of the original melanoma cases were excluded because the diagnosis could not be verified on re-examination. In a previous study, randomly re-evaluating the diagnosis of malignant melanoma in the National Cancer Register, an incorrect diagnosis was found in 3.7% of the cases (77). Higher figures of misdiagnosis have, however, been reported for melanoma survivors (78).

The high naevus counts in this study might to some extent be explained by the age-range studied and the fact that virtually the whole skin surface was examined. Furthermore, our counts include most previously excised and histologically verified naevi. The possible recall bias among melanoma patients regarding the number of excised skin lesions was eliminated by registering all scars. Precautions were taken not to misdiagnose other pigmented lesions as naevi. To reach maximal consistency in the counts, all subjects were examined by one and the same dermatologist (A.A.). In 20 randomly selected control subjects, independent naevus counts were performed by another dermatologist (I.R.). There were no systematic differences in the counts between the two observers and the overall deviation was less than 10%. To minimize the influence of sun-tan and freckles, the study was performed during the winter. If there were still difficulties in the clinical judgement of a pigmented lesion, it was not counted. Nevertheless, all the lesions diagnosed clinically as naevi and excised by us were confirmed to be naevi histologically. With all these precautions, we are convinced that our high naevus counts are not overestimated.

The lack of animal models makes it difficult to get direct and detailed evidence of the relation-

ship between UV-irradiation and naevus evolution. In this study, we have compared the number of naevi in a defined sun-exposed area on the lower back with the number in an adjacent protected area on the buttocks. Obviously, in humans there are no skin areas totally unexposed to UV-irradiation during life available for this kind of investigation. We chose to study the buttock area, which we considered one of the most UV-protected areas. Still, one has to be aware of the fact that this area is occasionally UV-exposed, especially during childhood. Sporadic exposure of the buttock area would, however, imply that we have underestimated the role of UV-irradiation in naevus development. In addition, the areas were selected to avoid the possible influence of repeated mechanical stimuli on naevus formation and to minimize the counting errors due to freckling.

Other factors such as hormones or medications may stimulate naevus development but it seems unlikely that they would influence the two areas differently. We therefore believe that the simplest and most obvious explanation for the observed difference in naevus number between the two areas is the difference in UV dose.

It may be argued that the difference in naevus number is not primarily due to the variation in UV dose but instead to a difference in the melanocyte population density between the two areas. There are, however, no indications of such regional variations. Furthermore, in the skin of the adult, Szabó has demonstrated that the distribution of naevi is not directly related to the basal melanocyte population density (79).

The two defined areas studied in paper III are rather small and to exclude the possibility that our results reflect only local phenomena we extended the investigation by studying the naevus distribution over the entire body surface. The classification of the areas (A–P) (Fig. 2) into different exposure categories was based on general clothing habits in our climate and behaviour in the sun. While it might be possible to prospectively study the exact UV dose in various body regions over a short time period (80), it is impossible to accurately determine the life-time

dose. Obviously, individual anamnestic data on the amount of UV-exposure in different body regions is of little value. Although our classification of the body areas in different exposure categories is simplified and includes approximations, it probably gives an overall estimate of the pattern of UV exposure in a Scandinavian population.

The areas classified as chronically exposed, i.e. the face (A) and the dorsum of the hands (F), constitute a small part of the body surface and findings from these areas should therefore be interpreted with some caution.

Since some of the analyses were performed on naevus counts per unit surface area, the estimates of area sizes are crucial. The areas H and J on the lower trunk are the most difficult ones to estimate. We therefore tested the validity of our conclusions based on data from these two areas. Most important for our conclusions was that the areas H and J were not overestimated. For that reason, we tested our counts for the possibility that the areas instead of 14% equalled 10% of the body surface. It was reassuring to find that this intentional underestimation did not change our conclusions.

## General discussion

It has been suggested that melanocytic naevi may be formed as a result of multiple somatic mutations (81). According to this theory, the early appearance of naevi in sun-exposed compared with in protected areas is attributed to the mutagenic effects of UV-irradiation. That naevi appear in relatively protected areas later in life might be due to the more gradual accumulation of mutational effects in clones of melanocytes in these areas.

In most previous observations indicating an association between UV-irradiation and naevus development, the UV dose is based on anamnes-

tic data. Kopf et al. introduced a more objective method of studying the UV-effects on naevus formation. In a study of volunteers, they compared the number of raised naevi in two separate areas on the arms, one sun-exposed and one protected area (52). We used a similar approach, but to avoid interference by freckles on the arms we compared naevus counts in a sun-exposed area on the lower back with counts in a protected area on the buttocks. Using a non-freckled area, we were able to count naevi in all stages of development. In agreement with the findings by Kopf et al., we found significantly more naevi in the sun-exposed compared with in the protected skin (52). This was true for both melanoma patients and controls. In addition, we studied the naevus distribution over the entire body surface. In conformity with the finding from the two restricted areas on the back and buttocks in paper III, there was a significantly higher concentration of naevi also in the other sun-exposed areas compared with in the more protected ones. It seems unlikely that this naevus distribution pattern is solely genetically determined and independent of UV exposure. If this was the case, we find it hard to explain why the naevus distribution is in concordance with the distribution of UV-irradiation over the body surface. We believe that our finding of more naevi in sun-exposed skin areas than in protected areas is attributed to the effects of UV-irradiation. This is based on the assumption that melanocytes are randomly susceptible to UV-induced mutations leading to naevus induction.

In analogy to what is the case for melanoma, there are reports suggesting that the pattern of sun exposure is more important than the total accumulated UV-dose in the development of naevi also. For example, areas exposed to short periods of intense UV-irradiation like the lateral aspect of the arms and the back have more naevi than areas more regularly exposed (42, 44). In addition, Gallagher et al. have found that naevi are more common in children on intermittently exposed body sites than on constantly or minimally sun-exposed sites (82). In this study, we found a similar naevus distribution in adults,

that is, a higher mean naevus count per unit surface area in intermittently UV-exposed skin areas than in chronically exposed skin. This might be due to the fact that the regularly exposed skin is protected against the immediate UV-insults by its tan, while intermittently exposed skin is unprepared. In agreement with this reasoning, we found that subjects who reported more than three burns had a higher mean total naevus count than the rest of the sample and outdoor workers more regularly exposed to the sun had lower mean total naevus counts than indoor workers. Altogether, our results support the idea that intermittent UV-exposure has a more potent "naevogenic" effect than chronic exposure. More information is needed on the biology and the life cycle of melanocytic naevi. With our present knowledge, it cannot be excluded that chronic UV exposure in some way also stimulates the maturation and/or the disappearance of naevi (52, 51).

Except for the association between high naevus counts and indoor occupation and several burns, no correlation was found between the number of naevi and other anamnestic parameters grading the life-time amount of UV exposure. It is obvious that data acquired by trying to memorize the amount of UV-exposure since childhood are unreliable and that comparisons between individual estimates are impossible to evaluate. Information on occupation and several burns are probably the most reliable data.

In consistency with previous studies on Caucasians, we found differences in naevus patterns between the two sexes (42, 43, 44). Naevus counts on the trunk were higher in men than in women, while women had more naevi on the face and the lower limbs. If sun exposure is important in naevus development, this finding might reflect different clothing habits and/or different behaviour in the sun between the sexes.

In subjects with the dysplastic naevus syndrome, Kopf et al. reported a larger number of CN and clinical DN in a sun-exposed area on the back than in a less exposed area on the buttocks (72). They suggested that sunlight promotes the

development of DN as well as CN. We also found more DN on the lower back than on the buttocks when these areas were studied separately in paper III. On the other hand, when the distribution of DN over the entire body surface was investigated, we found large numbers of DN in protected areas such as the lower abdomen and the buttocks as a whole (area H and J) and only a few DN in UV-exposed areas as the lateral aspect of the arms, the upper chest and the head and neck region (Fig. 4 a+b). The distribution pattern of DN thus did not support the hypothesis that UV-irradiation is a main etiological factor for DN.

The overall distribution of CMM in this study is in general agreement with what has been reported by others (27, 28). The concentration of CMM was higher in intermittently than in chronically exposed skin areas, while the lowest concentration of CMM in this age-group was found in rarely exposed skin. Intermittent, intense UV exposure has both mutagenic and mitogenic effects on the melanocytes (50, 83). Chronically exposed skin is more protected against the immediate mutagenic UV-insults by a slight tan. Gilchrest et al. have reported that the melanocyte population density in chronically exposed skin is constantly high (84) and we have, in a previous report (35), demonstrated that a high melanocyte population density minimizes the proliferative response to UV-irradiation. A high mitotic activity might be the first step in tumour promotion (85, 18). Therefore, the constantly high melanocyte population density in chronically UV-exposed skin might be protective. This does not exclude a high melanoma rate in chronically exposed skin. In intermittently exposed skin, the carcinogenic effect of UV-irradiation might be due to a synergistic or additive effect of mutation and proliferation, while in chronically exposed skin it might mainly be due to accumulated mutagenic effects. Such possibilities are interesting to consider in view of the long induction time for lentigo maligna melanoma in chronically exposed skin.

Both CN and CMM have distribution patterns

over the body surface consistent with the idea that UV-irradiation is important in their formation. Furthermore, there was good agreement between the overall distribution of CN and CMM in this study. This co-variation is in line with the concept that UV-irradiation might be a common etiological factor for both tumours.

It is reasonable to assume that immunological mechanisms are in some way involved in the development of melanoma and naevi. In mice, Kripke et al. have shown that UV-irradiation induces a failure of immune rejection of tumour tissue (86). In human skin influenced by sun exposure local defects in cell-mediated immunity have also been reported (87). In accordance with these observations, the role of UV-irradiation in the development of melanoma and naevi might partly be indirect by altering the immunological response to tumour growth. The dramatic increase in naevus number noted in children after treatment with chemotherapy is of great interest in this context (59, 88). The immune response of sun-exposed skin and its influence on the development of naevi and melanoma is an interesting field for future research.

Swedes have more than twice as many melanocytic naevi as previous populations studied (41-44). Even so, melanoma patients have almost twice as many naevi as the controls. The finding of more naevi in melanoma patients than in controls is in agreement with the results from other case-control studies but the counts in this study are again extremely high (10, 13). Intermittent UV exposure was shown to have the most potent "naevogenic" effect and we propose that the genetic proneness to form naevi might be due to a genetic difference in the UV susceptibility of the melanocytes. Based on our findings, it is reasonable to assume that the high naevus counts in Swedes reflect the Scandinavian sunbathing habits of intense but short UV exposure periods with long intervals. As many of our melanoma patients had reduced their UV exposure after being diagnosed, it is possible that the difference in naevus counts between patients and controls might be even larger.

If UV-irradiation is a main etiological factor

for naevus development, one might expect that subjects with a more sun-sensitive skin would have higher naevus counts than subjects with good tanning ability. The mean total body naevus count was, however, not influenced by skin type in this study. A similar observation has been reported by MacKie et al. (44). The relationship of skin type to naevus number is complex since skin type is also a determinant of a person's behaviour in the sun.

It is now well established that the number of naevi in an individual is a strong indicator of his/her risk of developing melanoma. In previous reports, the number of naevi has been assessed by various counting procedures (8, 14). So far, there are few population studies on naevus counts from the whole body (41–44, 89). This is probably due to the fact that total body counts are time-consuming and impracticable to perform. For large population studies, it would therefore be of great value to find smaller, well-defined areas predicting an individual's total body naevus count. Such a simplified counting procedure to identify individuals at risk would also be useful in melanoma screening programmes. English et al. demonstrated strong correlations between total number of naevi and counts on each of the upper limbs, the lower limbs and the trunk (89). In this study, we found strong correlations between total counts and counts from even smaller areas such as the anterior surface of the thighs and the lateral aspect of the arms. Unfortunately, it is difficult for non-professionals to differentiate between naevi and freckles on the lateral aspect of the arms. Therefore, for larger screening programmes based on self-examination, the anterior surface of the thighs seems to be the most suitable area to count. For investigations conducted by medically trained observers, naevus counts from the anterior surface of the thighs together with the lateral aspect of the arms would give even better predictive values of the total body count.

In this study, a prevalence of clinical DN of 18% was found in the population sample and the prevalence among melanoma patients was three times as high, 56%. These figures are high com-

pared with what has been reported from Australia and Germany but similar to those reported from California (9, 90, 13). The prevalence of histological DN in this study was 8% in the population, compared with 40% in the melanoma patients. In a study on Californian patients seen in a dermatology practice, histologically confirmed DN was found in 4.9% (67) and in a French investigation of non-familial melanoma cases, 18% had histologically verified DN (91). We are not aware of any study comparing the histological findings in cases and controls.

Studies on the prevalence of histologically diagnosed DN are difficult to compare, primarily due to differences in the criteria required for the histological diagnosis. Kelly et al. excised 118 clinically diagnosed DN with an overall clinicopathological correlation of 75% (74). In our study, the clinical diagnosis of DN could only be verified histologically to a limited extent. This might partly be due to the fact that almost all clinically diagnosed DN were excised and not only the most typical ones. Histological criteria of dysplasia have been reported in 14%–29% of naevi clinically considered to be non-dysplastic (74, 92). This is in agreement with our finding that 20% of the clinically diagnosed CN excised as histological controls also showed dysplasia microscopically. All these lesions were small and relatively flat and all were excised in subjects with additional clinical DN. In this context, one must bear in mind that dysplastic naevi may appear throughout life and as they appear they cannot clinically be differentiated from a common naevus. This finding needs further penetration and it will be important to perform a comparative study between the histological appearance of CN from subjects with and without clinical DN.

Swerdlow et al have reported that subjects with "clinically atypical" naevi without dysplastic histology are also at increased risk for melanoma (10). This is in line with our finding that the clinically diagnosed DN is as good a discriminator as the histologically diagnosed for identifying individuals at risk of developing CMM. This is an important result since a simple skin ex-

amination is a fast and inexpensive method of identifying subjects at risk.

It is interesting that subjects with clinical DN had nearly twice as many CN as those without DN. This was true for both melanoma patients and controls. The risk of having at least one DN increased with the number of CN. There was, however, no correlation between the individual number of DN and CN (Table 1). Furthermore, the distribution pattern of DN over the body surface was clearly different from that of CN. These two observations speak against a systematic error in the clinical judgement. In addition, subjects with DN had a larger Ex-Pr difference and a larger difference in naevus concentration between intermittently and rarely exposed skin than subjects without DN. This was true for both melanoma patients and controls and might reflect different habits of sun exposure and/or an increased UV sensitivity in subjects with DN. Altogether, controls with DN had a naevus pattern very similar to that of melanoma patients with DN. The naevus profile in melanoma patients without DN was in conformity with that of controls without DN (Table 4). The finding in this study that controls with DN had a more sun-sensitive skin type than those without suggests that DN is a marker for an increased UV sensitivity. On the other hand, the distribution pattern of DN over the body surface indicates that DN as such may develop independently of UV-irradiation.

The finding of a more sun-sensitive skin in melanoma patients than in controls is in accordance with a number of previous reports (37, 38). Red or blond hair is another well-established risk factor, although it becomes less important when adjusted for other associated pigmentary phenotypic characteristics. In other populations, blue eye colour is a well-documented risk factor. There was no association between blue/grey eye colour and melanoma in this study, probably because blue eyes are such a common phenotypic feature in Swedes. Instead, green eye colour was found to be a significant risk factor even after adjustment for other variables.

In this study, both a large number of CN and

the presence of DN constitute independent risk factors for CMM even though their strong covariation partly weakens their individual power. Besides being a marker of an increased melanoma risk, naevi are also potential precursors of CMM. Previous investigators have not been able to document any association between a large number of naevi in a specific body area and a local melanoma risk (10, 93). Using a statistical method which is not affected by the correlation between the total body naevus count and risk of CMM, we have demonstrated an increasing site-specific melanoma risk with increasing concentrations of CN as well as DN. This site-specific melanoma risk might be the result of local insults of UV-irradiation to the melanocytic system and/or be due to the fact that naevi are precursor lesions to CMM.

Malignant melanoma is a tumour with a rapidly increasing incidence and with a considerable mortality. In its early phase, most melanomas are curable by simple surgical excision. Furthermore, trained observers are able to recognize most early melanomas by performing a total body skin examination. Few other malignant diseases can be diagnosed by such a simple, painless and inexpensive procedure. For these reasons, melanoma is an ideal tumour for screening and surveillance programmes. This study focused on identifying subjects at high risk of melanoma. A sun-sensitive skin, a large number of naevi and the presence of DN contribute to a high melanoma risk. When combining these factors, a subgroup with a 50 times higher melanoma risk than those without these three characteristics can be identified (Table 3). Extrapolating these results to the entire Swedish population, this subgroup would constitute approximately 3% of the inhabitants of the age range studied but only about 5% of the melanoma cases are recruited from the actual risk group. Therefore, the value of surveillance of these subjects can be questioned.

Many crucial questions concerning the model for and the value of melanoma screening are still unanswered. For example, how accurate is screening and who should do it? Who should be

screened? How do we find subjects at high risk and how do we influence them to attend screening programmes? How cost-effective is screening?

Since 1985, a regular voluntary screening programme for melanoma/skin cancer has been available in the USA. A recent study from Massachusetts demonstrated that most individuals attending such a programme were at risk for skin cancer (94). Almost 80% had at least two risk factors for melanoma/skin cancer, indicating that these self-selected subjects constituted an appropriate population for screening. In Scotland, it was found that a relatively short-term public and professional education campaign rapidly reduced the number and proportion of thick melanomas with a poor prognosis (95).

An intensified public education programme concerning early melanoma signs and balanced recommendations regarding sun awareness seem to be the most reasonable preventive steps in Sweden today. Such campaigns must include immediate access to medical attention. For such purposes, Pigmented Lesion Clinics already exist at most dermatology clinics. High-risk groups, such as the one defined in this study, are important targets to reach with information. Nevertheless, one must bear in mind that despite intensive information and screening programmes there are always subjects ignoring early symptoms of disease. Bringing forward the positive message of cure with early melanoma detection will hopefully reduce fear and attract even subjects less prone to seek medical care.

## Summary and conclusions

In this thesis, the naevus profile was studied in 379 subjects (30–50 years) from a Swedish census file and in 121 melanoma patients, in the same age-range, from the Regional Cancer Reg-

ister. Special interest was focused on the possible link between UV-irradiation, naevus formation and melanoma development.

1. Four times as many naevi were found in a sun-exposed area on the back compared with in an adjacent protected area on the buttocks. When the entire body surface was studied, the concentration of naevi was significantly higher in UV-exposed skin than in protected areas, indicating that UV-irradiation plays a role in the development of melanocytic naevi. Furthermore, intermittent UV exposure seems to have the most "naevogenic" effect while chronic UV exposure might even be protective.
2. There was good agreement between the distribution of CN and CMM over the body surface. This co-variation lends further support to the idea that CN is a precursor to CMM. The similarity in distribution pattern may also be explained by both tumours sharing (a) common etiological factor(s).
3. The distribution pattern of DN was different from that of CN, with many DN in some rarely UV-exposed skin areas and virtually no DN in some of the exposed areas. This suggests that DN may develop independently of UV exposure.
4. Swedes have more than twice as many melanocytic naevi as what has been reported from populations in other countries. This might reflect the Scandinavian sunbathing habits of intense but short UV exposure periods with long intervals.
5. Melanoma patients had twice as many naevi as the controls, indicating different habits of sun exposure and/or an increased proneness to form naevi following UV exposure.
6. We found strong correlations between the total body naevus count and the number of naevi on the anterior surface of the thighs as

well as on the lateral aspect of the arms. Counts from any of these areas will provide a satisfactory estimate of the total number of naevi in large population studies as well as for screening purposes.

7. Close to one out of five in this Swedish population had at least one naevus fulfilling the clinical criteria of a dysplastic naevus. The prevalence of DN in the melanoma patients was three times as high.
8. The most important phenotypic risk factor for CMM was the presence of three or more clinical DN. A large number of melanocytic naevi, a sun-sensitive skin and green eye colour were other important independent risk factors.
9. Clinically diagnosed DN was as good a discriminator as histologically diagnosed DN for identifying individuals at risk of developing CMM.
10. Combining the three risk factors a sun-sensitive skin, a large number of naevi and the presence of DN, a subgroup with a 50 times higher melanoma risk than those without these characteristics can be identified.
11. Subjects with DN, patients as well as controls, had nearly twice as many CN as subjects without DN. Furthermore, they had a larger difference in naevus counts between the UV-exposed and the protected skin areas. This might reflect different habits of sun exposure and/or an increased UV sensitivity.
12. The probability of having a CMM in a specific area increased with increasing number of CN and/or DN in that area. This site-specific risk might be due to the fact that CN and DN are precursor lesions to CMM or due to (a) common etiological factor(s) for naevi and melanoma.

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