Melanoma is hereditary in approximately 10% of cases. Familial melanoma is also known as familial atypical multiple mole melanoma (FAMMM) syndrome. The clinical diagnostic criteria are defined as the occurrence of invasive cutaneous melanoma in two or more first-degree relatives or three or more family members (irrespective of the degree of relationship) on the same side of the family.

Many members of melanoma families exhibit atypical nevi; however, the occurrence of clinically atypical nevi is not required for the diagnosis. The presence of multiple atypical nevi in family members implies that they are three times more likely to be carriers of a mutation in a melanoma-predisposing gene than their relatives without atypical moles.

Germline mutations in CDKN2A are found in about 40% of melanoma families. CDKN2A encodes two distinct proteins, p16INK4 and p14ARF, which function as tumor suppressors. In the Netherlands, the p16-Leiden (19-base pair deletion) is the most prevalent CDKN2A germline mutation.

Besides hereditary factors, multiple studies have explored other risk factors for melanoma. The development of nevi begins in childhood. The number of acquired melanocytic nevi is an independent risk factor for cutaneous melanoma.

Atypical nevi strongly correlate with melanoma risk. Some studies have investigated the site-specific relationship between nevi and melanoma. Melanomas occurred more fre-
quently in individuals with a high number of melanocytic nevi at the same site where the melanoma originated.

One study compared the site-specific risk of sporadic melanoma with the site-specific distribution of nevi in childhood. This study has found associations between the nevus distribution in children and the melanoma distribution in adults. However, this study compared a cohort of children with a different cohort of adults, and sporadic cases of melanoma, not the familial type we report, were involved. To our knowledge, no studies have been published so far that have compared the nevus distribution and nevus count in childhood with the melanoma distribution in adulthood in the same cohort.

Methods

From 1985 to 1990, a total of 133 children from 34 families who fulfilled the diagnostic requirements of familial melanoma were photographed at the Department of Dermatology at the Leiden University Medical Center, Leiden, the Netherlands. At that time, the \textit{CDKN2A} gene had not yet been discovered. At the moment of photographing, the children were between 2 and 18 years old. The children were included by chance (they accompanied their parents) and were photographed with the oral consent of their parents. All people who were included in the study received a letter of invitation for a second visit so far that have compared the nevus distribution and nevus count in childhood with the melanoma distribution in adulthood in the same cohort.

![Figure. Multiple Mole Melanoma Mutation Carrier](image)

A 10-year-old child from a familial atypical multiple mole melanoma family who was a mutation carrier. A, Anterior trunk; B, posterior trunk and buttocks; C, anterior legs; and D, posterior legs.

Nevi were defined as pigmented macules or papules with a minimal diameter of 2 mm, excluding freckles, café au lait macules, and warts. The number of atypical nevi was noted, defined as predominantly flat or macular, with 3 or more of the following 5 ABCDE features: asymmetry, indistinct borders, variation in color, diameter of 5 mm or larger, and erythema. The total number of melanocytic nevi 5 mm or larger in diameter, including typical and atypical nevi, was also documented. Nevi were counted per body part. Some parts of the bodies, such as the flanks, were not photographed separately and could not be counted. These body parts were equal for all children (Figure).

Persons included in this study were all members of familial melanoma families under study at our department and were seen on several research occasions; however, they were not necessarily under regular surveillance. Review of patient medical records and pedigrees was used to confirm the diagnoses and to gather information about the chance of having a gene mutation. From 2004 onward, these families were invited to have their DNA tested. Information was collected about a proven p16-Leiden mutation, p14ARF mutation, or an unknown gene mutation (this means that a melanoma patient in the family was tested but no \textit{CDKN2A} mutation was found). If no genetic testing was performed in that family, this was also noted. Therefore, 3 types of families were included: \textit{CDKN2A}-positive families, \textit{CDKN2A}-negative families, and families without DNA testing. Persons were categorized into 4 different groups. The first group consisted of people with a proven \textit{CDKN2A} mutation. The second group included people at 50% risk for a \textit{CDKN2A} mutation and people from non-\textit{CDKN2A} families who had a first-degree relative with melanoma. The third group contained persons with a 25% risk of a \textit{CDKN2A} mutation and people who had a second-degree relative with melanoma. The last group included people who were proven to have no gene mutation or whose parents or grandparents were proven to have no gene mutation. In \textit{CDKN2A}-positive families, members with a proven absence of that gene mutation are strictly speaking no longer patients; however, their melanoma risk is reported to be still slightly higher than the popu-
analyses, and software, version 17 (SPSS Inc). Survival analyses, correlation and feet.

ventralthorax, dorsalthorax, buttocks, upperlegs, lowerlegs, but the same as for the first visit. The melanocytic nevi of the second count were arranged into several localizations (left or right where appropriate): face, scalp, hands, lower arms, upper arms, ventral thorax, dorsal thorax, buttocks, upper legs, lower legs, and feet.

Analyses of the data were performed with SPSS statistical software, version 17 (SPSS Inc). Survival analyses, correlation analy- ses, and t tests were used. Logistic regression analyses were used to examine associations with gene mutations and removed lesions. The occurrence of melanoma was evaluated with survival analyses using the Kaplan-Meier method, log-rank tests, and the Cox proportional hazards regression tests. Proportions were compared using 2-sided t tests. Cox proportional hazards regression was used to estimate the risk of melanoma. We also evaluated random-effects models (frailty models in the case of the Cox proportional hazards model) to account for family effects. Only 34 families were present in the data set; therefore, only models with few covariates could be fitted reliably. Because the random effect in the considered models was never statistically significant, we only describe models without random effects. Tests were considered statistically significant at $P \leq .05$.

Results

Of the 133 participants included in this study, 52 (39.1%) were female and 81 (60.9%) were male. The mean age was 13 years at childhood and 24 years at adulthood. The mean count of nevi in childhood was 36. Forty people were seen for a second nevus count; their mean count was 39 in childhood and 121 in adulthood (Table 1).

Fifteen people (11.3%) had at least one melanoma or melanoma in situ (4 males and 11 females); 7 people had more than one melanoma. The mean age at which the participants developed their first melanoma was 26 years. One person died of melanoma at the age of 38 years.

Eighty-eight of the 133 study participants (66.2%) were members of families with a proven p16-Leiden mutation; 8 people (6.0%) were family members with a proven mutation in the CDKN2A gene that affected purely p14ARF.

Of all study participants, 12 (9.0%) were proven to have a gene mutation. On the basis of their position in the pedigree, 52 people (39.1%) had a gene mutation risk of 50%, 41 (30.8%) had a risk of gene mutation of 25% or less, and 28 (21.1%) were proven to have no gene mutation themselves or no gene mutation in their parents or grandparents.

Two groups of gene mutation risk were formed: 50% or greater and 25% or less. The group with a 50% or greater risk of gene mutation consisted of 64 people (48.1%); those with a 25% or less risk consisted of 69 people (51.9%).

Of the 15 people with at least one melanoma, 10 were carriers of the p16-Leiden mutation; 4 people were not formally tested. In the families of 2 of these 4 people, the p16-Leiden gene mutation was determined in other members, and in the families of the remaining 2 people, no genetic testing was performed. One person with a melanoma was proven to have no gene mutation, whereas in his family the p16-Leiden mutation was verified in a different family member (phenocopy phenomenon). In all 247 removed lesions, 22 melanomas and 5 melanomas in situ were found.

The total number of nevi in childhood was a significant (hazard ratio [HR], 1.02; 95% CI, 1.00-1.03; $P = .04$) predictor of the development of melanoma later in life. The total nevus count on the legs in childhood was on its own significantly (HR, 1.04; 95% CI, 1.02-1.06; $P < .001$) associated with melanoma. Analyses were also performed in the 2 gene mutation risk groups ($\geq 50\%$ and $\leq 25\%$). The total number of nevi in childhood was a significant (HR, 1.02; 95% CI, 1.00-1.03; $P = .03$) predictor of the development of melanoma in the group with a 50% or greater risk of a gene mutation but not in the group with a risk of 25% or less (HR, 0.98; 95% CI, 0.87-1.11; $P = .75$). When the different body parts were examined, the same results were observed. The nevus count on the legs in childhood was a significant predictor (HR, 1.04; 95% CI, 1.03-1.05; $P < .001$) of melanoma in the 50% or greater risk group. The total count of childhood nevi with a diameter of 5 mm or larger was not a significant predictor of melanoma; however, childhood nevi 5 mm or larger on the buttocks gave a high risk of melanoma (HR, 1.47; 95% CI, 1.98-44.38; $P = .005$), specifically in the high-risk group (HR, 6.47; 95% CI, 1.36-30.75; $P = .02$). The total count of atypical nevi in childhood yielded a higher risk of melanoma (HR, 1.21; 95% CI, 1.02-1.44; $P = .03$). Atypical nevi on the ventral body side and atypical nevi on the buttocks in childhood accounted for a higher risk of melanoma (HR, 1.48; 95% CI, 1.02-2.14; $P = .04$; and HR, 14.00; 95% CI, 2.94-66.55; $P = .001$; respectively) (Table 2).

The total count of nevi in adulthood did not indicate a significant risk of melanoma ($n = 40$). When the distribution of the nevi was taken into account, the nevus count on the lower half of the body (legs and buttocks) indicated a higher risk of melanoma. Analysis of the upper half of the body revealed a converse association with melanoma (HR, 0.97; 95% CI, 0.94-1.00; $P = .04$) (Table 3).

In the 50% or greater risk group, the total nevus count in adulthood was a risk indicator for melanoma (HR, 1.01;
95% CI, 1.00-1.05; \( P = .03 \)) as was the nevus count on the lower half of the body (HR, 1.02; 95% CI, 1.01-1.03; \( P = .01 \)). No association between the risk of melanoma and nevus size of 5 mm or larger in adulthood or the atypical nevus count was found.

The number of melanocytic lesions that were removed produced a higher risk of melanoma in the whole group (HR, 1.27; 95% CI, 1.23-1.31; \( P < .001 \)) and especially in the 50% or greater risk group.

An association was found between the numbers of nevi on a certain site (distribution) in childhood and the site of melanoma in adulthood (\( n = 133 \); correlation, 0.89; 95% CI, 0.67-0.96; \( P < .001 \)) and especially in the 50% or greater risk group.

Gene mutation status (ie, \( CDKN2A \) mutation carriers) obviously was a significant (HR, 118.10; 95% CI, 23.65-910.51) predictor of melanoma. No association was found between the count and distribution of nevi, numbers of nevi 5 mm or larger, numbers of atypical nevi, and the number of removed lesions as predictors for gene mutation status.

### Discussion

This study examined the numbers and distribution of acquired melanocytic nevi in a cohort of children from familial melanoma families as a risk indicator for melanoma later in life. A group of 133 children with varying degrees of risk of being a mutation carrier on the basis of genetic testing in adulthood or their position in the pedigree were included, and 20 years later 15 people had developed 27 melanomas. A subgroup of adults who were seen for a second nevus count consisted of 40 people who were part of the cohort of 133 children. This small group might be biased at inclusion because it can be expected that these persons are under regular surveillance because of development of melanoma or their type and number of nevi. Nevertheless, this group had significant results that are important for clinical practice.

Concerning the role of the total number of nevi as a risk indicator for melanoma later in life, we found that the total nevus count in childhood was a significant risk factor for melanoma. A childhood nevus count of 5 mm or larger on the buttocks also resulted in a higher risk of melanoma. Nevi 5 mm
Table 2. Childhood Risk Factors for Melanoma

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>HR (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total nevus count in childhood</td>
<td>1.02 (1.00-1.03)</td>
<td>.04</td>
</tr>
<tr>
<td>Nevus count on legs in childhood</td>
<td>1.04 (1.02-1.06)</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Total nevus count in childhood ≥50% risk</td>
<td>1.02 (1.00-1.03)</td>
<td>.03</td>
</tr>
<tr>
<td>Total nevus count in childhood ≥25% risk</td>
<td>0.98 (0.87-1.11)</td>
<td>.75</td>
</tr>
<tr>
<td>Total nevus count in childhood ≥5 mm</td>
<td>1.05 (0.97-1.14)</td>
<td>.21</td>
</tr>
<tr>
<td>Nevus count in childhood ≥5 mm on buttocks</td>
<td>9.36 (1.98-44.38)</td>
<td>.005</td>
</tr>
<tr>
<td>Total nevus count in childhood ≥5 mm and ≥50% risk</td>
<td>1.04 (0.97-1.12)</td>
<td>.28</td>
</tr>
<tr>
<td>Nevus count in childhood ≥5 mm on buttocks and ≥50% risk</td>
<td>6.47 (1.36-30.75)</td>
<td>.02</td>
</tr>
<tr>
<td>Total atypical nevus count in childhood</td>
<td>1.21 (1.02-1.44)</td>
<td>.03</td>
</tr>
<tr>
<td>Atypical nevus count in childhood on buttocks</td>
<td>14.00 (2.94-66.55)</td>
<td>.001</td>
</tr>
<tr>
<td>Atypical nevus count in childhood on ventral body site</td>
<td>1.47 (1.02-2.14)</td>
<td>.04</td>
</tr>
<tr>
<td>Total atypical nevus count in childhood ≥50% risk</td>
<td>1.19 (1.00-1.41)</td>
<td>.047</td>
</tr>
<tr>
<td>Total atypical nevus count in childhood ≥25% risk</td>
<td>0.08 (0.03-0.43)</td>
<td>.57</td>
</tr>
</tbody>
</table>

Abbreviation: HR, hazard ratio.

Table 3. Adulthood Risk Factors for Melanoma

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>HR (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total nevus count in adulthood</td>
<td>1.00 (0.99-1.01)</td>
<td>.23</td>
</tr>
<tr>
<td>Nevus count in adulthood for upper half of the body</td>
<td>0.97 (0.94-1.00)</td>
<td>.04</td>
</tr>
<tr>
<td>Nevus count in adulthood for lower half of the body</td>
<td>1.05 (1.01-1.09)</td>
<td>.01</td>
</tr>
<tr>
<td>Total nevus count in adulthood ≥50% risk</td>
<td>1.01 (1.00-1.05)</td>
<td>.03</td>
</tr>
<tr>
<td>Total nevus count in adulthood ≥25% risk</td>
<td>0.99 (0.93-1.04)</td>
<td>.61</td>
</tr>
</tbody>
</table>

Abbreviation: HR, hazard ratio.

or larger have previously been described as a risk factor of melanoma in multiple studies.13 Buttock moles are a component of the so-called atypical mole syndrome phenotype, a complex risk phenotype for melanoma.16 There is no clue as to how or why these nevi preferentially appear on the buttocks. The total count of atypical nevi in childhood also yielded an increased risk of melanoma. This correlation was reported in multiple previous studies.8

The ages of the included children were between 2 and 18 years; this is the age range in which nevus counts in individuals vary significantly.9 The association between the number of nevi and melanoma will certainly differ between very young children and older children, which is why we divided the children into 2 groups (ie, <13 years and ≥13 years old) and performed the analyses on these prepubertal and postpubertal subgroups. The analyses revealed that nevi larger than 5 mm on the buttocks and the total number of atypical nevi in the age group younger than 13 years were still indicative of an increased risk of melanoma (P = .007 and P = .004, respectively). In the 13 years or older group, these associations were lost, which might be a result of the small group size or reflect the variability in nevus counts in this age group. It could also be that buttock nevi are a much more specific indicator of risk when present before puberty.

Earlier population studies3,8,10,14 have found that high numbers of nevi in both childhood and adulthood increase the risk of melanoma. Surprisingly, adulthood nevus count was not significantly associated with melanoma in our data, which seems to be due to lack of power. Still, our finding is compatible with a positive association of nevus count with increased risk of death by 0.4% per nevus.

During the years we have followed up our study population, they received sun exposure counseling that was especially aimed at children. It seems plausible that young individuals from melanoma families under surveillance for many years might have lower nevus counts. Previous studies8,9 have found that numbers of nevi 5 mm or larger and atypical nevi in adulthood were strongly correlated with melanoma. Our findings could not confirm this report, which might be because of the size of the research group. Furthermore, some of the 40 people who had a second nevus count presented themselves without any nevi 5 mm or larger (n = 7) and/or atypical nevi (n = 22), which reflects the variable phenotype in familial melanoma.4

Analysis of melanoma and number of lesions removed were performed in all 133 cases from the patient records, the hospital oncology registry, and the research database. Some data may be missing because our findings could be checked by personal history in only 40 people. All the families who were included in this study have been included in family studies for many years and usually report all cancer cases. However, we may have missed melanomas. People who were seen for a second nevus count were between the ages of 21 and 41 years. If the cohort had been followed up for a longer period, more melanomas would have developed. It is therefore suspected that the analyses would then have resulted in more significant outcomes; therefore, we consider our findings conservative.

Our analysis, not surprisingly, confirmed the findings of other publications1,2 that the presence of a CDKN2A mutation yields a higher risk of melanoma compared with families without such a mutation, implicating a lower melanoma risk in families with unresolved genetic mechanisms. Regarding the distribution of childhood nevi, the analyses revealed a high association with melanoma distribution in adulthood. This correlation could also be found in the distribution of adulthood nevi and melanoma distribution. Melanoma in adulthood tends to develop more frequently on the sites with the highest nevus count in adulthood according to the literature.9-11

Several correlations could not be found in the 25% or less group, which can be viewed as a matched control group, supporting the positive correlations we found in the 50% or greater group. The 25% or less group consisted of children from the same families who shared the same genetic background and environmental exposure. They were included completely at random, years before the identification of the CDKN2A gene. Both risk groups are expected to contain a few people who belong to the other group, so-called misclassifications. These misclassifications dilute findings but have nevertheless led to significant outcomes. Children and young adults most often do...
not want to be genetically tested for financial reasons, which prevented us from definitively classifying all individuals.

The association between the occurrence of nevi and the CDKN2A mutation is puzzling. The children included in our study have been selected solely on the basis of multiple melanoma patients in their family. Later, in 21 of the 34 families, a CDKN2A mutation was found. Linkage analysis performed in 1993 on the Leiden FAMMM families provided evidence of linkage of the atypical nevi phenotype to the melanoma locus on chromosome 9p21. Inclusion of family members with 10 or more atypical nevi and/or melanoma as affected individuals led to a slight decrease in the logarithm of the odds score compared with inclusion of melanoma patients alone. Broadening the inclusion criteria to persons with 5 or fewer atypical nevi as affected individuals resulted in a remarkably decreased logarithm of the odds score, suggesting that the presence of atypical nevi could not be ascribed entirely to a melanoma susceptibility gene in that locus. Several years later, a genome-wide scan that investigated the genetic component of atypical nevi in these large extended founder pedigrees with many family members with atypical nevi again did not reveal a nevus locus on chromosome 9p. The strongest evidence of an atypical nevus susceptibility gene was mapped to chromosome band 7q21.3, a region containing the candidate gene CDK6. Making use of advanced technological genome-wide association studies, Falchi et al reported a nevus-associated variant of MTAP adjacent to CDKN2A on chromosome 9p21. Other nevus-associated variants were identified on chromosome 22q13. In addition, protecting variants in the NID1 gene on chromosome 1q42 were recently identified. We do not know yet whether the currently identified nevus-related variants segregate with extensive moles in families.

On the basis of the results of the current study, physicians should take additional care of children from families at high risk of melanoma. Children with a high number of melanocytic nevi and children with atypical nevi should be included in a surveillance program with their parents. Buttocks should be checked for moles. In addition, sun avoidance information should be provided, with emphasis on the prevention of sunburns. Despite the difficulty in performing long-term follow-up studies in children from families with melanoma, results of these studies provide us with additional information about risk factors and may help to detect melanoma as early as possible.

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Dr S Vredenborg and Bergman had full access to all the data in the study and take responsibility for the integrity of all the data and the accuracy of the data analysis.

Study concept and design: Vredenborg, Kukutsch, Bergman.

Acquisition of data: Vredenborg, Boonk, Gruis, Out-Luijting.

Analysis and interpretation of data: Vredenborg, Böhringer, Kukutsch, Bergman.

Drafting of the manuscript: Vredenborg, Böhringer, Kukutsch, Bergman.

Critical revision of the manuscript for important intellectual content: All authors.

Statistical analysis: Vredenborg, Böhringer.

Administrative, technical, or material support: Vredenborg, Boonk, Gruis, Out-Luijting, Kukutsch, Bergman.

Study supervision: Vredenborg, Kukutsch, Bergman.

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