ORIGINAL ARTICLE

Melanoma prone families with CDK4 germline mutation: phenotypic profile and associations with MC1R variants

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ABSTRACT

Background CDKN2A and CDK4 are high risk susceptibility genes for cutaneous malignant melanoma. Melanoma families with CDKN2A germline mutations have been extensively characterised, whereas CDK4 families are rare and lack a systematic investigation of their phenotype.

Methods All known families with CDK4 germline mutations (n=17) were recruited for the study by contacting the authors of published papers or by requests via the Melanoma Genetics Consortium (GenoMEL). Phenotypic data related to primary melanoma and pigmentation characteristics were collected. The CDK4 exon 2 and the complete coding region of the MC1R gene were sequenced.

Results Eleven families carried the CDK4 R24H mutation whereas six families had the R24C mutation. The total number of subjects with verified melanoma was 103, with a median age at first melanoma diagnosis of 39 years. Forty-three (41.7%) subjects had developed multiple primary melanomas (MPM). A CDK4 mutation was found in 89 (including 62 melanoma cases) of 209 tested subjects. CDK4 positive family members (both melanoma cases and unaffected subjects) were more likely to have clinically atypical nevi than CDK4 negative family members (p<0.001). MPM subjects had a higher frequency of MC1R red hair colour variants compared with subjects with one tumour (p=0.010).

Conclusion Our study shows that families with CDK4 germline mutations cannot be distinguished phenotypically from CDKN2A melanoma families, which are characterised by early onset of disease, increased occurrence of clinically atypical nevi, and development of MPM. In a clinical setting, the CDK4 gene should therefore always be examined when a melanoma family tests negative for CDKN2A mutation.

INTRODUCTION

Cutaneous malignant melanoma is characterised by a complex aetiology, involving both genetic and environmental risk factors. Approximately 5–10% of the melanoma cases occur in a familial setting, and two genes have so far been identified as high risk susceptibility genes for the disease: cyclin dependent kinase inhibitor 2A (CDKN2A)2 3 and cyclin dependent kinase 4 (CDK4).4 5

CDKN2A (MIM 600160) encodes two distinct proteins, p16INK4A and p14ARF; both are tumour suppressors involved in cell cycle inhibition through different pathways.6–8 In studies of melanoma families, the frequency of CDKN2A germline mutations is 20–40%, depending on the inclusion criteria.9

Common features of the CDKN2A melanoma families are early onset of disease and an increased risk of developing clinically atypical nevi, multiple primary melanomas (MPMs), and pancreatic cancer.10 11

CDK4 (MIM 123829) encodes the catalytic subunit of a heterodimeric Ser/Thr protein kinase, which together with its regulatory subunit (one of the D-type cyclins) is involved in controlling progression through the G1 phase of the cell cycle. Only 12 melanoma prone families with CDK4 germline mutations have been reported.4 5 12–18 All mutations are located in codon 24 in exon 2, resulting in either an Arg24His (R24H) or Arg24Cys (R24C) substitution. This changes the p16INK4A binding domain, leading to reduced p16INK4A inhibition of CDK4 kinase activity and, subsequently, to cell cycle progression.19 20

Fair skin, red/blonde hair colour, freckling, and sun sensitivity are established pigmentation related risk factors for melanoma development. Pigmentation phenotype is partly regulated by the melanocortin-1 receptor (MC1R) gene (MIM 155555), a low risk melanoma susceptibility gene that may act both dependently and independently of ultraviolet radiation to influence melanoma risk.21 22 MC1R encodes a seven-pass transmembrane, G-protein coupled receptor, which is involved in regulation of pheomelanin (yellow/red pigment) and eumelanin (black/brown pigment) production.23 The MC1R locus is highly polymorphic in the Caucasian population,24 and
certain variants have been associated with the red hair colour (RHC) phenotype; that is, red hair, fair skin, freckling, and the inability to tan.\textsuperscript{25, 26} It is mainly the RHC variants that have been associated with increased melanoma risk,\textsuperscript{27–29} although a link between non-RHC (NRHC) variants and melanoma has also been observed.\textsuperscript{30} Studies within CDKN2A melanoma families have established that common \textit{MC1R} variants act as modifier genes, in that carrying multiple variants is associated with increased melanoma risk. Several of these studies also reported an association between \textit{MC1R} variants and decreased age at melanoma diagnosis, particularly in MPM patients.\textsuperscript{31–35}

A systematic characterisation of melanoma prone families with \textit{CDK4} germline mutation has never been performed. Previous studies of such families have included a limited number of melanoma cases and have mainly served to confirm \textit{CDK4} as a high risk melanoma gene. Here, we report a joint investigation of all 12 published \textit{CDK4} melanoma families along with five unpublished pedigrees. The purpose was to examine the clinical phenotype of these families, including possible modifying effects of \textit{MC1R} variants, with the intent to inform genetic counselling internationally.

**METHODS**

**Recruitment of \textit{CDK4} mutated melanoma families and data collection**

Melanoma families with germline \textit{CDK4} mutations were recruited for this study either by contacting the authors of published papers\textsuperscript{4, 5, 12–18} or by requests for unpublished families via GenoMEL, the Melanoma Genetics Consortium (www.genomel.org). Seventeen families, including five unpublished, were enrolled (table 1).

Clinical examinations were performed by dermatologists or specifically trained research nurses, and phenotypic data were collected via a standardised form. Examiners were generally unaware of the genotype of individuals before recording clinical findings. Data collected included skin type using the ‘ Fitzpatrick classification’,\textsuperscript{36} eye and hair colour, and total number of nevi (>2 mm in diameter). Also the presence of clinically atypical nevi, defined as nevi >5 mm with irregular pigmentation and an irregular or diffuse border,\textsuperscript{37} was recorded. Diagnoses of cutaneous melanoma were confirmed by histology reports and/or medical records. For patients with MPMS, the total number of melanomas was recorded; however, age at melanoma diagnosis, anatomic location, and histological type were recorded only for the first three primaries. Anatomic location of the melanomas was categorised into head/neck, limbs, and trunk. Histologic type was recorded as superficial spreading melanoma (SSM), nodular melanoma (NM), lentigo malignant melanoma (LMM), in situ melanoma, and melanoma unclassified/classification unknown. No acral or mucosal melanomas were observed in our material. Information on non-melanoma cancers was collected for the melanoma cases and for those unaffected subjects who either had tested positive for a \textit{CDK4} mutation or were considered as obligate mutation carriers.

Written informed consent was obtained from the family members before enrolment. The study was performed according to the Helsinki declaration.

**DNA analysis of \textit{CDK4} and \textit{MC1R}**

DNA samples were available from 209 subjects. Altogether, DNA from 62 melanoma cases, 106 unaffected family members (melanoma status not given for three of these; they were considered as unaffected in this study), and 41 spouses were examined for mutations in \textit{CDK4} exon 2 and for variants in the coding region of \textit{MC1R}. The initial DNA analysis was performed by each individual research group, either by direct Sanger sequencing or by single strand conformation polymorphism screening; hence there was slight variation in protocols and primers.\textsuperscript{4, 5, 12–18}

**Statistical analysis**

Before the statistical analyses, hair and skin colour was grouped because of small sample sizes for these categorical variables: RHC versus all other hair colours, very fair/fair skin colour versus all other skin colours. For comparisons between subject groups (melanoma affected and unaffected \textit{CDK4} positive family members versus \textit{CDK4} negative family members and spouses) and different categorical variables (occurrence of clinically atypical nevi, melanoma status, hair and skin colour, \textit{MC1R} variant distribution), the Pearson $\chi^2$ test or the Fisher exact test were used depending on sample sizes. The non-parametric Mann–Whitney or Kruskal–Wallis tests were used to compare the continuous variable (age at first diagnosis) with the categorical variables (melanoma status, tumour location, histologic type, occurrence of clinically atypical nevi, \textit{MC1R} variant distribution).

All observed \textit{MC1R} variants were recorded, but because many variants were rare, they were grouped before the statistical comparisons with phenotypic data. These comparisons were performed as follows: (1) The distribution of individuals with \textit{MC1R} consensus sequence, one and two \textit{MC1R} variants was compared between the different subject groups (analysis denoted ‘Number of \textit{MC1R} variants’). (2) The distribution of individuals with \textit{MC1R} consensus sequence, RHC, NRHC, and RHC+NRHC variants was compared between the different subject groups (analysis denoted ‘Type of \textit{MC1R} variants’). We observed no individuals with more than two \textit{MC1R} variants. The RHC variants were defined as D84E, R142H, R151C, R160W, and D294H, all associated with red hair phenotype.\textsuperscript{25, 26} Other non-synonymous \textit{MC1R} variants were labelled as NRHC. Synonymous \textit{MC1R} variants were excluded from all analyses. When analysing \textit{MC1R} variant distributions, the \textit{CDK4} negative family members and spouses were combined into a single control group.

Unconditional logistic regression analysis was used to assess whether atypical nevi status varied by melanoma affection and \textit{CDK4} carrier status when adjusted for age (age at last examination for unaffected subjects and age at diagnosis for melanoma patients). Statistical analyses were performed using the IBM Statistical Package for the Social Sciences, version 19 (SPSS Inc, Chicago, Illinois, USA) and SAS software (version 9.1.3, SAS Institute Inc, Cary, North Carolina, USA). $p$ values <0.05 were considered to represent significant associations. Also $p$ values between 0.05 and 0.10 are shown in the tables.

**RESULTS**

Seventeen familial melanoma pedigrees with \textit{CDK4} germline mutations (11 with the R24H mutation and six with R24C) were available for this study; 12 previously published and five unpublished (table 1). In these pedigrees, a total of 103 members with cutaneous malignant melanoma (=affected subjects) were recorded. DNA was available for 209 subjects of whom 89 were mutation carriers (62 affected, 27 unaffected), 79 were mutation negative unaffected family members, and the remaining were spouses (table 1). As expected, all spouses had a normal \textit{CDK4} exon 2 sequence. Among the 41 affected subjects for whom DNA was not available, seven were classified as obligate mutation carriers. Of unaffected subjects without available DNA, five were obligate mutation carriers.
Phenotypic characteristics of melanoma patients in CDK4 families

Phenotypic characteristics of the 103 malignant melanoma cases are presented in table 2. Age at first malignant melanoma diagnosis was available for 95 cases and ranged from 18–86 years, with a median age of 39 years. Most cases occurred in the fourth decade of life (31.6%), whereas age of onset above age 60 years was rare (7.4%). There was no statistically significant difference in distribution of age at first diagnosis between males and females, or between cases with and without available DNA.

Forty-three melanoma patients (41.7%) developed more than one primary tumour. The number of primaries ranged from 2–13. Altogether, 217 melanomas were reported for 102 affected subjects (data on the number of melanomas were missing for one subject with MPM). Patients with MPMs showed a significantly lower median age at first diagnosis than patients with single primary melanoma (SPM): 35 and 43 years, respectively (p=0.002). There was no difference in distribution of SPM and MPM by gender.

The melanomas occurred most frequently on the limbs (table 2), and subjects with their first melanoma on this location had a significantly lower age at first diagnosis (33.5 years) than subjects with melanomas located in the head and neck region (45.5 years) (p=0.018). The predominant histologic type was SSM (table 2). Subjects with SSM had a significantly lower median age at first diagnosis than individuals with NM and LMM (p=0.039). The median ages were 36.5, 54, and 64 years, respectively. Ten of the first melanomas were recorded as in situ cases with a median diagnosis age of 33 years.

We further evaluated the occurrence of clinically atypical nevi (table 3). Both affected and unaffected CDK4 positive subjects showed a significantly higher frequency of atypical nevi (70% and 75%) than the CDK4 negative subjects (26.5%) (p<0.001). The associations remained significant after age adjustment (affected CDK4 positive subjects: OR 6.08, 95% CI 2.51 to 14.76, p<0.001; unaffected CDK4 positive subjects: OR 7.37, 95% CI 1.99 to 27.39, p=0.003). The median age at first melanoma diagnosis for the atypical nevi positive patients was significantly lower (32.5 years) than for atypical nevi negative patients (40 years) (p=0.004).

There was no difference in distribution of hair and skin colour between the affected and unaffected CDK4 positive family members and the CDK4 negative family members (see online supplementary table 1). We also tested for phenotypic differences between subjects carrying the R24H and R24C mutations. No statistically significant differences were seen with regard to age at first melanoma diagnosis or the occurrence of MPM and clinically atypical nevi (see online supplementary table 2).

Concerning non-melanoma cancers, 33 cases were found in 25 of the 105 subjects where information on other cancers had been specified (see online supplementary table 3). Non-melanoma skin cancers and female related cancers were most frequently observed. Two cases of pancreatic cancer were seen. Ages of onset of the non-melanoma cancers were in a range expected in normal populations.

MC1R variants

Altogether, 15 different MC1R variants were observed in our material. Eleven variants predicted non-synonymous amino acid changes (V60L, V60R, D84E, V92M, R142H, R142S, R151C, I155T, R160W, R163Q, D294H), three variants corresponded to synonymous amino acid changes (V60L, V60R, D84E, V92M, R142H, R142S, R151C, I155T, R160W, R163Q, D294H), and one was an insertion at the nucleotide level (86insA). V60R and R142S have, to our knowledge, not been reported before.

We did not see any differences in distribution of MC1R variants in our cohort. None of the affected CDK4 positive carriers had MC1R variants.
Table 2  Phenotypic and genotypic characteristics of melanoma cases in families with CDK4 germline mutations

<table>
<thead>
<tr>
<th>Variable*</th>
<th>Number</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (N=103)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>44</td>
<td>42.7</td>
</tr>
<tr>
<td>Female</td>
<td>59</td>
<td>57.3</td>
</tr>
<tr>
<td>Number of primary melanomas in affected subjects (N=103)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>One</td>
<td>60</td>
<td>58.3</td>
</tr>
<tr>
<td>Multiple</td>
<td>43</td>
<td>41.7</td>
</tr>
<tr>
<td>Mean</td>
<td>2.1</td>
<td>–</td>
</tr>
<tr>
<td>CDK4 mutation status of affected subjects (N=103)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subjects (N=77) in R24H families</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CDK4 mutation positive</td>
<td>41</td>
<td>39.8</td>
</tr>
<tr>
<td>CDK4 mutation negative</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Obligate CDK4 mutation carrier†</td>
<td>7</td>
<td>6.8</td>
</tr>
<tr>
<td>Unknown mutation status†</td>
<td>29</td>
<td>28.2</td>
</tr>
<tr>
<td>Subjects (N=26) in R24C families</td>
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<td></td>
</tr>
<tr>
<td>CDK4 mutation positive</td>
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<td>20.4</td>
</tr>
<tr>
<td>CDK4 mutation negative</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Obligate CDK4 mutation carrier†</td>
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<td>0.0</td>
</tr>
<tr>
<td>Unknown mutation status†</td>
<td>5</td>
<td>4.9</td>
</tr>
<tr>
<td>Age at first melanoma diagnosis (N=95)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;30 years</td>
<td>20</td>
<td>21.1</td>
</tr>
<tr>
<td>30–39 years</td>
<td>30</td>
<td>31.6</td>
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<tr>
<td>40–49 years</td>
<td>24</td>
<td>25.3</td>
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<tr>
<td>50–59 years</td>
<td>14</td>
<td>14.7</td>
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<tr>
<td>≥60 years</td>
<td>7</td>
<td>7.4</td>
</tr>
<tr>
<td>Missing data‡</td>
<td>8</td>
<td>–</td>
</tr>
<tr>
<td>Mean (years)</td>
<td>40.4</td>
<td>–</td>
</tr>
<tr>
<td>Median (years)</td>
<td>39.0</td>
<td>–</td>
</tr>
<tr>
<td>Anatomic location (N=140)§</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Head/neck</td>
<td>31</td>
<td>22.1</td>
</tr>
<tr>
<td>Limbs</td>
<td>66</td>
<td>47.1</td>
</tr>
<tr>
<td>Trunk</td>
<td>43</td>
<td>30.7</td>
</tr>
<tr>
<td>Missing data‡</td>
<td>28</td>
<td>–</td>
</tr>
<tr>
<td>Anatomic location, first primary melanoma only (N=81)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Head/neck</td>
<td>17</td>
<td>21.0</td>
</tr>
<tr>
<td>Limbs</td>
<td>34</td>
<td>42.0</td>
</tr>
<tr>
<td>Trunk</td>
<td>30</td>
<td>37.0</td>
</tr>
<tr>
<td>Missing data‡</td>
<td>21</td>
<td>–</td>
</tr>
<tr>
<td>Histologic type (N=95)§</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SSM</td>
<td>71</td>
<td>74.7</td>
</tr>
<tr>
<td>NM</td>
<td>3</td>
<td>3.2</td>
</tr>
<tr>
<td>LMM</td>
<td>1</td>
<td>1.1</td>
</tr>
<tr>
<td>In situ melanoma</td>
<td>20</td>
<td>21.1</td>
</tr>
<tr>
<td>Melanoma unclassified or classification unknown†</td>
<td>73</td>
<td>–</td>
</tr>
<tr>
<td>Histologic type, first primary melanoma only (N=48)</td>
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<td></td>
</tr>
<tr>
<td>SSM</td>
<td>34</td>
<td>70.8</td>
</tr>
<tr>
<td>NM</td>
<td>3</td>
<td>6.3</td>
</tr>
<tr>
<td>LMM</td>
<td>1</td>
<td>2.1</td>
</tr>
<tr>
<td>In situ melanoma</td>
<td>10</td>
<td>20.8</td>
</tr>
<tr>
<td>Melanoma unclassified or classification unknown†</td>
<td>54</td>
<td>–</td>
</tr>
</tbody>
</table>

*One melanoma case was recorded as MPM, but with no information on the actual number of melanomas. It was therefore excluded when calculating the mean number of melanomas and when summarising anatomic location and histologic type.
†DNA was not available for these cases.
‡Missing data are not included in the parentheses (N=) and not included when calculating percentages.
§For persons with MPM, information about the first three registered tumours was recorded.
LMM, lentigo malignant melanoma; MPM, multiple primary melanomas; NM, nodular melanoma; SSM, superficial spreading melanoma.

Table 3  Occurrence of clinically atypical nevi in families with CDK4 germline mutations

<table>
<thead>
<tr>
<th>Clinically atypical nevi*</th>
<th>CDK4 negative family members</th>
<th>CDK4 positive family members</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Affected (%)</td>
<td>p Value‡</td>
</tr>
<tr>
<td>Present</td>
<td>13 (26.5)</td>
<td>35 (70.0)</td>
</tr>
<tr>
<td>Not present</td>
<td>36 (73.5)</td>
<td>15 (30.0)</td>
</tr>
</tbody>
</table>

*Data on CDK4 mutation status and clinically atypical nevi were available for 119 subjects.
‡CDK4 negative family members were compared with affected and with unaffected CDK4 positive family members, respectively.

DISCUSSION

This study presents the largest dataset on melanoma families with CDK4 germline mutations to date, and is the first systematic evaluation of their phenotype and the influence of MC1R variants. We examined 17 families from eight countries that included 103 subjects with a verified melanoma diagnosis. The families carried either an R24H or R24C mutation, and we were not able to reveal any clinical differences between carriers of the two CDK4 mutations.

Early onset of disease is a characteristic feature of hereditary cancers. In this study, median age at first melanoma diagnosis was 39 years, about 15 years earlier than in the general Caucasian population. Thus, 21.1% of the melanoma patients in the CDK4 families had been diagnosed before the age of 30 years, whereas only 7.4% were diagnosed at age 60 years or older. Based on all individuals for which clinical information was available at age 50 years (or later), the mutation penetrance at this age was 74.2%. This confirms CDK4 as a highly penetrant melanoma risk gene. However, since most of the younger mutation carriers are now enrolled in screening programmes where severely dysplastic or borderline lesions are removed, the true lifetime melanoma risk of carrying a CDK4 germline mutation might be difficult to assess, assuming that such lesions are precursors.

We found that 41.7% of the melanoma subjects developed more than one primary melanoma, a frequency comparable to that observed in families with CDKN2A mutations.11 38 39

Regarding clinically atypical nevi, a significantly higher occurrence revealed no significant differences in the MC1R variant distribution (table 4). However, when comparing the unaffected CDK4 mutation carriers with the CDK4 negative control group, the former group showed a lower number of RHC variants (p=0.012). The unaffected CDK4 mutation carriers also had a significantly lower number of RHC variants compared with the affected CDK4 carriers (p=0.042) (table 4).

Finally, we investigated the MC1R variant distribution in MPM and SPM subjects (table 4). We found no statistically significant difference in the number of MC1R variants, but the MPM subjects were more likely to carry RHC variants (p=0.010). There were no significant associations between age at first melanoma diagnosis and MC1R variant distribution (tested in SPM and MPM subjects, both separately and combined). Similarly, there were no significant differences in the MC1R variant distribution in subjects with and without clinically atypical nevi.
was observed in the CDK4 mutation carriers compared with the CDK4 negative family members (table 3). Again, this is similar to findings in CDKN2A families.\textsuperscript{40,41} The presence of clinically atypical nevi has been suggested to be a modifier of melanoma risk in CDKN2A mutation carriers,\textsuperscript{40,41} and we observed that among affected subjects, the median age at first melanoma diagnosis was 7.5 years lower in atypical nevi positive than in negative family members. On the other hand, the frequency of these nevi was similar in affected and unaffected CDK4 positive subjects (table 3).

Unfortunately, a high number of the melanoma cases were unclassified with regard to histology, or classification could not be obtained from the patients’ records. This mainly concerned the oldest cases, as histology data generally became more complete for more recent cases. Nevertheless, the most frequent histologic type was SSM (74.7%), as in CDKN2A families.\textsuperscript{39} The relatively high frequency of in situ melanomas (21.1%) may be influenced by increased surveillance of melanoma prone families.

We tried to assess non-melanoma cancers in our material, but encountered some obstacles. Firstly, most participating laboratories had collected anamnestic cancer data only from melanoma cases and CDK4 positive family members, and not from CDK4 negative members or spouses. Secondly, the CDK4 families stemmed from many countries and populations, with varying background incidences and different national registration systems for cancer. Thus, we were prevented from performing formal analyses to test whether the observed incidences of non-melanoma cancers (see online supplementary table 3) were higher than expected. Still, the frequencies of breast cancer and non-melanoma skin cancer might suggest that CDK4 mutation carriers could be at an increased risk. Sun exposure is an environmental determinant of risk for all skin cancers and an over-representation of non-melanoma skin cancer would therefore not be surprising. However, for all observed non-melanoma cancers, the median age of onset was similar to that of sporadic cases, so our data should be interpreted with caution.

When investigating the MC1R variant distribution, we observed that unaffected, CDK4 positive family members had a disproportionately low frequency of RHC variants, suggesting a biological influence. This difference may, however, be related to the smaller number of subjects in the unaffected, CDK4 positive group. Additionally, these subjects were generally younger (median age 28 years at last examination) than their affected relatives. It is therefore likely that some of the unaffected, CDK4 positive subjects eventually develop melanoma.

Looking at melanoma cases only, we found that the MPM subjects had a higher frequency of RHC type variants than the SPM subjects (table 4). Moreover, although not reaching statistical significance, MPM subjects also had the highest frequency of any MC1R variant (86.7% compared with 63.3% in SPM). These findings are consistent with previous observations in CDKN2A melanoma families.\textsuperscript{11,38,39} We did not find any modifying effects of MC1R variants upon age at disease onset in the CDK4 families, in contrast to what has been reported for CDKN2A.\textsuperscript{31–35}

The current study has some limitations. Collection of data and biological material was performed by various groups in several different countries, and the data diverged in completeness. Small sample size due to lack of complete data contributes to low power in some statistical analyses and prohibited us from evaluating each MC1R variant separately. Despite these limitations, our study provides results informative for the clinical evaluation of CDK4 pedigrees.

Melanoma families with CDK4 germline mutations are very uncommon. However, codon 24 of this gene is likely to be a mutational hotspot and CDK4 families have been found in various countries, with several independent origins suggested by haplotype analysis.\textsuperscript{5,13,15} Our study suggests that CDK4 melanoma families are phenotypically similar to the CDKN2A families with regard to age of melanoma diagnosis, tumour localisation, histological type, and increased incidence of MPM and clinically atypical nevi. A general influence of MC1R variants on melanoma risk is seen in both types of melanoma families, although there may be some differences. We therefore conclude that it is not possible to distinguish CDK4 melanoma families from those with CDKN2A mutation based on the phenotype. The clinical implication is that, although CDK4 mutation carriers are rarely seen, exon 2 of this gene should be examined in melanoma families seeking gene testing whenever tests are negative for CDKN2A.

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Table 4 Frequencies of MC1R variants in families with CDK4 germline mutations

<table>
<thead>
<tr>
<th>MC1R variant distribution</th>
<th>CDK4 negative family members and spouses*</th>
<th>CDK4 positive family members†</th>
<th>Number of primary melanomas‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=115 (%)</td>
<td>N=23 (%) p Value§</td>
<td>N=60 (%) p Value$</td>
</tr>
<tr>
<td>Number of MC1R variants</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 (consensus sequence)</td>
<td>23 (20.0)</td>
<td>10 (43.5) 0.071</td>
<td>15 (25.0) NS</td>
</tr>
<tr>
<td>1 variant</td>
<td>71 (61.7)</td>
<td>10 (43.5) 32 (53.3)</td>
<td>13 (21.7)</td>
</tr>
<tr>
<td>2 variants</td>
<td>21 (18.3)</td>
<td>3 (13.0)</td>
<td>4 (13.3) 9 (30.0)</td>
</tr>
<tr>
<td>Type of MC1R variants</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 (consensus sequence)</td>
<td>23 (20.0)</td>
<td>10 (43.5) 0.012</td>
<td>15 (25.0) NS</td>
</tr>
<tr>
<td>RHC only</td>
<td>48 (41.7)</td>
<td>3 (13.0)</td>
<td>7 (23.3) 16 (53.3)</td>
</tr>
<tr>
<td>NRHC only</td>
<td>31 (27.0)</td>
<td>9 (39.1) 14 (23.3)</td>
<td>10 (33.3) 4 (13.3)</td>
</tr>
<tr>
<td>Both RHC and NRHC</td>
<td>13 (11.3)</td>
<td>1 (4.4) 8 (13.3)</td>
<td>2 (6.7) 6 (20.0)</td>
</tr>
</tbody>
</table>

*MC1R data were available for 76 of 79 CDK4 negative family members and for 39 of 41 spouses. In these groups, the distributions of number and type of MC1R variants were very similar, and the two groups were combined into a single control group for the statistical analyses.
†The number of primary melanomas and MC1R data were available for 60 of the 103 melanoma subjects.
‡The control group was compared with unaffected and affected CDK4 positive family members, respectively.
§Unaffected mutation carriers were compared with affected mutation carriers.
¶Subjects with SPM and MPM were compared with each other with regard to MC1R variant distribution.
NS=non-significant p value.

SPM, multiple primary melanomas; NRHC, non-red hair colour; RHC, red hair colour; SPM, single primary melanoma.
Cancer genetics

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Contributors
HEP performed genotyping, collected all data from the participating research groups, tabulated the results, carried out the statistical analyses, and wrote the first manuscript draft. XRY and AMG provided data on the American families, and participated in the interpretation of data and statistical analyses. SJ, HHV, and IMB carried out genotyping, performed genetic counselling or dermatological examinations, and provided and interpreted clinical data for the Norwegian family. AM managed and designed the study in collaboration with AMG and LAA. All other authors have read and commented on the manuscript, and approved the final version.

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Competing interests
None.

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Obtained.

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Correction

Puntervoll HE, Yang XR, Vetti HH et al. Melanoma prone families with CDK4 germline mutation: phenotypic profile and associations with MC1R variants. *J Med Genet* 2013;50:264-270. In this paper, one of the MC1R mutations (V60R) was incorrectly reported. The DNA sample has turned out to contain the previously described V60L mutation. Under the section head *MC1R* variants (on the third page of the article) the second sentence beginning ‘Eleven variants…’ should say ‘Ten variants predicted non-synonymous amino acid changes …’. Neither the statistical analyses nor the conclusions drawn in the paper are affected by this error.

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