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.

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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,[1] 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, $p16^{INK4A}$ and $p14^{ARF}$; 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 $p16^{INK4A}$ binding domain, leading to reduced $p16^{INK4A}$ 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.[25 26] It is mainly the RHC variants that have been associated with increased melanoma risk,[27–29] although a link between non-RHC (NRHC) variants and melanoma has also been observed.[30] Studies within *CDKN2A* melanoma families have established that common *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 *MC1R* variants and decreased age at melanoma diagnosis, particularly in MPM patients.[31–35]

A systematic characterisation of melanoma prone families with *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 *CDK4* as a high risk melanoma gene. Here, we report a joint investigation of all 12 published *CDK4* melanoma families along with five unpublished pedigrees. The purpose was to examine the clinical phenotype of these families, including possible modifying effects of *MC1R* variants, with the intent to inform genetic counselling internationally.

METHODS

Recruitment of *CDK4* mutated melanoma families and data collection

Melanoma families with germline *CDK4* mutations were recruited for this study either by contacting the authors of published papers[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 features. Data collected included skin type using the 'Fitzpatrick classification',[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,[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 *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 *CDK4* and *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 *CDK4* exon 2 and for variants in the coding region of *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.[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 *CDK4* positive family members versus *CDK4* negative family members and spouses) and different categorical variables (occurrence of clinically atypical nevi, melanoma status, hair and skin colour, *MC1R* variant distribution), the Pearson χ^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, *MC1R* variant distribution).

All observed *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 *MC1R* consensus sequence, one and two *MC1R* variants was compared between the different subject groups (analysis denoted 'Number of *MC1R* variants'). (2) The distribution of individuals with *MC1R* consensus sequence, RHC, NRHC, and RHC+NRHC variants was compared between the different subject groups (analysis denoted 'Type of *MC1R* variants'). We observed no individuals with more than two *MC1R* variants. The RHC variants were defined as D84E, R142H, R151C, R160W, and D294H, all associated with red hair phenotype.[25 26] Other non-synonymous *MC1R* variants were labelled as NRHC. Synonymous *MC1R* variants were excluded from all analyses. When analysing *MC1R* variant distributions, the *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 *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 *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 *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.

Table 1 Overview of the studied melanoma families with *CDK4* germline mutations

Family designation	Laboratory number of family	Mutation	Subjects with verified cutaneous melanoma in pedigree	Total number of subjects with analysed DNA	Mutation positive family members	Mutation negative family members	Spouses	Reference
Norway-1	–	Arg24His	28	108	33	48	27	5
USA-1	8302	Arg24Cys	9	29	12	10	7	4
USA-2	8290	Arg24Cys	6	12	7	5	0	4
UK-1	301	Arg24His	4	5	2	2	1	5
UK-2	1119	Arg24Cys	5	7	4	3	0	Unpublished
Latvia-1	247	Arg24His	5	5	2	3	0	13
Latvia-2	268	Arg24His	5	6	3	3	0	16
Latvia-3	M679	Arg24His	5	3	2	0	1	Unpublished
Australia-1	60007	Arg24His	10	3	2	0	1	5
Italy-1	FM029	Arg24His	4	6	4	1	1	15
Italy-2	501153	Arg24Cys	1	2	1	1	0	18
France-1	759	Arg24His	6	14	9	3	2	12
France-2	–	Arg24His	2	4	3	0	1	14
France-3	–	Arg24Cys	2	1	1	0	0	Unpublished
France-4	14648	Arg24Cys	3	1	1	0	0	Unpublished
France-5	–	Arg24His	2	1	1	0	0	Unpublished
Greece-1	–	Arg24His	6	2	2	0	0	17
Total			103	209	89*	79	41	

*Sixty-two of the mutation positive family members had melanoma and 27 were unaffected.

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 patients: 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 (A166A, Q233Q, T314T), and one was an insertion at the nucleotide level (86insA). V60R and R142S have, to our knowledge, not been reported before. R160W, R151C, and V60L were the most frequently observed variants. Five RHC variants were recorded: D84E, R142H, R151C, R160W, D294H. We classified all remaining non-synonymous *MC1R* variants and 86insA to the NRHC group.

There were no significant differences in *MC1R* variant distribution between the *CDK4* negative family members and spouses. A control group was therefore established consisting of all *CDK4* negative subjects. Comparison of the affected *CDK4* mutation carriers with the *CDK4* negative control group

Table 2 Phenotypic and genotypic characteristics of melanoma cases in families with *CDK4* germline mutations

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

*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

	CDK4 negative family members	*CDK4* positive family members			
		Affected		Unaffected	
Clinically atypical nevi*	N=49 (%)	N=50 (%)	p Value†	N=20 (%)	p Value†
Present	13 (26.5)	35 (70.0)	<0.001	15 (75.0)	<0.001
Not present	36 (73.5)	15 (30.0)		5 (25.0)	

*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.

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.

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

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

MC1R variant distribution	*CDK4* negative family members and spouses* N=115 (%)	*CDK4* positive family members† Unaffected N=23 (%)	p Value§	Affected N=60 (%)	p Value§	p Value¶	Number of primary melanomas‡ SPM N=30 (%)	MPM N=30 (%)	p Value**
Number of *MC1R* variants									
0 (consensus sequence)	23 (20.0)	10 (43.5)	0.071	15 (25.0)	NS	NS	11 (36.7)	4 (13.3)	0.070
1 variant	71 (61.7)	10 (43.5)		32 (53.3)			15 (50.0)	17 (56.7)	
2 variants	21 (18.3)	3 (13.0)		13 (21.7)			4 (13.3)	9 (30.0)	
Type of *MC1R* variants									
0 (consensus sequence)	23 (20.0)	10 (43.5)	0.012	15 (25.0)	NS	0.042	11 (36.7)	4 (13.3)	0.010
RHC only	48 (41.7)	3 (13.0)		23 (38.3)			7 (23.3)	16 (53.3)	
NRHC only	31 (27.0)	9 (39.1)		14 (23.3)			10 (33.3)	4 (13.3)	
Both RHC and NRHC	13 (11.3)	1 (4.4)		8 (13.3)			2 (6.7)	6 (20.0)	

**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.
†Melanoma status and *MC1R* data were available for 83 of 89 *CDK4* positive family members.
‡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.
MPM, multiple primary melanomas; NRHC, non-red hair colour; RHC, red hair colour; SPM, single primary melanoma.

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.[40 41] The presence of clinically atypical nevi has been suggested to be a modifier of melanoma risk in *CDKN2A* mutation carriers,[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.[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 overrepresentation 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 disproportionally 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.[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*.[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.[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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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. SJS, HHV, and IMB carried out genotyping, performed genetic counselling or dermatological examinations, and provided and interpreted clinical data for the Norwegian family. MAT, MH, JAN-B, AO, DP, RV, NKH, JMP, PGr, CC, PGh, LP, MFA, BBdeP, MB, H-HH, NS, TJ, ABD-M, JT, SD, TM-D, LT, AJS, and HT carried out genotyping, performed genetic counselling and dermatological examinations, and provided and interpreted clinical data for all other families included in the study. AM managed and designed the study in collaboration with AMG and LAA. The writing group consisted of HEP, XRY, AMG, LAA, and AM. All other authors have read and commented on the manuscript, and approved the final version.

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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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Supplementary Table 1. *Distribution of pigmentation phenotypes in CDK4-positive and CDK4-negative family members*

	Skin color N (%)						Hair color N (%)					
Family members	N	Very fair	Fair	Olive	Brown	*P*-value*	N	Red	Blond	Brown	Black	*P*-value*
Affected and *CDK4*-positive	46	8 (17.4)	27 (58.7)	7 (15.2)	4 (8.7)	NS	49	8 (16.3)	20 (40.8)	17 (34.7)	4 (8.2)	NS
Unaffected and *CDK4*-positive	19	0 (0.0)	14 (73.7)	3 (15.8)	2 (10.5)	NS	19	0 (0.0)	8 (42.1)	10 (52.6)	1 (5.3)	NS
CDK4-negative	53	4 (7.6)	33 (62.3)	10 (18.9)	6 (11.3)	-	62	6 (9.7)	26 (41.9)	30 (48.4)	0 (0.0)	-

* *CDK4*-negative family members were compared with affected and with unaffected *CDK4*-positive family members, respectively.

NS = non-significant *P*-value

Supplementary Table 2. *Phenotypic characteristics of family members testing positive for the CDK4 mutations R24C or R24H*

Variable	R24C	R24H	*P*-value
Age at first diagnosis (N=62)			
N	21	41	
Median (years)	33.0	39.0	NS
Mean (years)	36.2	40.0	
	N (%)	**N (%)**	
Number of primary melanomas (N=62)			
SPM	10 (47.6)	21 (51.2)	NS
MPM	11 (52.4)	20 (48.8)	
Atypical nevi (N=70)			
Present	19 (82.6)	31 (66.0)	NS
Not present	4 (17.4)	16 (34.0)	

NS = non-significant *P*-value; SPM = single primary melanoma; MPM = multiple primary melanoma

Supplementary Table 3. *Presence of non-melanoma cancers in melanoma cases and CDK4 mutation carriers*

Cancer type*	N		Age at diagnosis†
Non-melanoma skin cancer	**12**		
	8	Squamous cell carcinomas	73.5
	4	Basal cell carcinomas	68
Female-related cancer	**12**		
	9	Breast cancers	50
	1	Phyllodes tumor	45
	1	Borderline ovarial tumor	41
	1	Uterine cervix cancer	77
Gastro-intestinal cancer	**5**		
	2	Pancreatic cancers	60
	1	Stomach cancer	-
	1	Colon cancer	70
	1	Rectal cancer	43
Other cancers	**4**		
	2	Lung cancers	69
	1	Prostate cancer	70
	1	Malignant lymphoma	73

* The table is based on the 105 subjects with melanoma and/or verified *CDK4* mutation (including obligate carriers) where information on other cancers had been specified on the data collection forms. Altogether, 33 non-melanoma cancers were recorded in 25 subjects.

† Median age when more than one case.