MC1R variants in childhood and adolescent melanoma: a retrospective pooled analysis of a multicentre cohort

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Summary

Background **Germline variants in the melanocortin 1 receptor gene *(MC1R)* might increase the risk of childhood and adolescent melanoma, but a clear conclusion is challenging because of the low number of studies and cases. We assessed the association of *MC1R* variants with childhood and adolescent melanoma in a large study comparing the prevalence of *MC1R* variants in child or adolescent patients with melanoma to that in adult patients with melanoma and in healthy adult controls.**

Methods **In this retrospective pooled analysis, we used the M-SKIP Project, the Italian Melanoma Intergroup, and other European groups (with participants from Australia, Canada, France, Greece, Italy, the Netherlands, Serbia, Spain, Sweden, Turkey, and the USA) to assemble an international multicentre cohort. We gathered phenotypic and genetic data from children or adolescents diagnosed with sporadic single-primary cutaneous melanoma at age 20 years or younger, adult patients with sporadic single-primary cutaneous melanoma diagnosed at age 35 years or older, and healthy adult individuals as controls. We calculated odds ratios (ORs) for childhood and adolescent melanoma associated with *MC1R* variants by multivariable logistic regression. Subgroup analysis was done for children aged 18 or younger and 14 years or younger.**

Findings **We analysed data from 233 young patients, 932 adult patients, and 932 healthy adult controls. Children and adolescents had higher odds of carrying *MC1R* r variants than did adult patients (OR 1·54, 95% CI 1·02–2·33), including when analysis was restricted to patients aged 18 years or younger (1·80, 1·06–3·07). All investigated variants, except Arg160Trp, tended, to varying degrees, to have higher frequencies in young patients than in adult patients, with significantly higher frequencies found for Val60Leu (OR 1·60, 95% CI 1·05–2·44; p=0·04) and Asp294His (2·15, 1·05–4·40; p=0·04). Compared with those of healthy controls, young patients with melanoma had significantly higher frequencies of any *MC1R* variants.**

Interpretation **Our pooled analysis of *MC1R* genetic data of young patients with melanoma showed that *MC1R* r variants were more prevalent in childhood and adolescent melanoma than in adult melanoma, especially in patients aged 18 years or younger. Our findings support the role of *MC1R* in childhood and adolescent melanoma susceptibility, with a potential clinical relevance for developing early melanoma detection and preventive strategies.**

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Introduction

Cutaneous melanoma mainly occurs in adult patients and is rare in the paediatric population, with only 2% of all cutaneous melanoma cases diagnosed in patients younger than 20 years.[1–4] In the child and adolescent population, most cases of cutaneous melanoma are diagnosed among adolescents, with only 8% occurring in infancy and childhood.[5,6]

Differences exist between childhood or adolescent and adult cutaneous melanoma regarding clinical aspects, histopathological features, and disease staging.[2,7,8] Cutaneous melanoma in childhood is often amelanotic, shows broad histopathological variability, and can present with histological uncertainty and ambiguous atypical characteristics that do not allow a definite malignant or benign classification.[4,9] Children with cutaneous melanoma present at a more advanced stage of disease, with thicker lesions and higher rates of lymph node metastasis than do their adult counterparts, leading to a worse prognosis.[4,9] However, published studies have reported discordant data on survival rates.[5,10]

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Research in context

Evidence before this study

The development of melanoma in children and adolescents has been hypothesised to have a stronger genetic component than that of melanoma in adults. We searched PubMed for studies published up to July 31, 2018, on melanoma susceptibility in paediatric patients, without language or date restrictions. We used the search terms "pediatric melanoma" OR "childhood melanoma" OR "adolescent melanoma" AND "susceptibility" OR "predisposition" OR "genetics". We found that genetic predisposition for melanoma has been poorly investigated in childhood and adolescence because of the rarity of the disease. Most published research included few cases, mainly from single-institution cohorts, investigating the main susceptibility genes for melanoma *CDKN2A* (cyclin-dependent kinase inhibitor 2A), *CDK4* (cyclin-dependent kinase 4), and *MC1R* (melanocortin 1 receptor).

The overall findings reported a marginal role in paediatric patients of the two major melanoma susceptibility genes, *CDKN2A* and *CDK4*. By contrast, a high frequency of germline variants has been identified in the intermediate-penetrance *MC1R* gene, but the very low number of paediatric cases of melanoma made any significant conclusions impossible. Therefore, we hypothesised that a large-scale association study could explore the importance of the *MC1R* gene in paediatric melanoma predisposition.

Added value of this study

Our study assessed the effect of *MC1R* gene variants on paediatric melanoma susceptibility in a large case-case study, by comparing the prevalence of *MC1R* variants in child or adolescent patients with those in adult patients and in healthy controls. To our knowledge, our series of patients is the largest international multicentre cohort of paediatric patients with melanoma with available genetic data. Our pooled analysis showed that paediatric patients had a higher probability of carrying any *MC1R* variant than that of adult patients, suggesting a major role of *MC1R* variants, mainly r variants, in paediatric melanoma predisposition. Furthermore, r variants seemed to be most strongly associated with melanoma in patients aged 18 years or younger.

Implications of all the available evidence

We provided evidence of genetic determinants potentially involved in paediatric melanoma susceptibility. Our study represents a first step to comprehend the genetic background of paediatric melanoma and to elucidate the diversity of paediatric and adult melanoma, with potential clinical implications.

Whether adult melanoma and childhood and adolescent melanoma share a similar pathogenesis has long been a subject of debate. Major risk factors for paediatric cutaneous melanoma include giant congenital melanocytic naevi and hereditary conditions such as xeroderma pigmentosum, immunodeficiency, and albinism.[11] Other known risk factors common to paediatric and adult melanoma are family history of melanoma, dysplastic naevus syndrome, elevated number of acquired melanocytic naevi, red hair, sun-sensitive phenotype, and ultraviolet radiation (UV) exposure.[12,13]

It is uncertain whether childhood and adolescent cutaneous melanoma differs from the adult melanoma regarding genetic predisposition. The paediatric form of the disease is mostly sporadic, whereas adolescent cutaneous melanoma is sometimes observed in melanoma-prone families. In general, there is a higher proportion of germline mutation carriers among young patients with cancer than among older patients,[14] but whether this tendency holds true for cutaneous melanoma is unclear because of the rarity of this disease occurring in children or adolescents. On the basis of the few available studies,[12,15–21] child and adolescent patients have only rarely been found to carry germline mutations in the two high-penetrance melanoma genes, *CDKN2A* and *CDK4*, which are known to be significantly associated with melanoma in a familial context alone.

The *MC1R* (melanocortin 1 receptor) gene is a key determinant of human pigmentation.[22] *MC1R* is highly polymorphic in the general population, and specific variants were defined as R (Asp84Glu, Arg142His, Arg151Cys, Ile155Thr, Arg160Trp, Asp294His) or r (Val60Leu, Val92Met, Arg163Gln) alleles, according to their strength of association with the red hair colour phenotype.[23] Extensive in vitro and in vivo evidence showed that both R and r alleles produce hypomorphic proteins with compromised activity compared with native MC1R function.[22] The R alleles have been found to have a major effect on pigmentation and UV sensitivity.[22,23] By contrast, r alleles confer normal or slightly impaired MC1R activity, resulting in a low-strength association with the fair skin phenotype.[23]

Natural *MC1R* variation is an established risk factor for cutaneous melanoma across multiple populations worldwide.[24] The risk of cutaneous melanoma is higher for carriers of an *MC1R* variant than for wild-type individuals, with the strongest association among carriers of R alleles and multiple variants.[24] *MC1R* variants confer a significant increased risk of cutaneous melanoma in darkly pigmented individuals, highlighting the effect of *MC1R* through non-pigmentary pathways.[25,26] Moreover, *MC1R* variant genotypes are associated with phenotypic characteristics of melanoma[27] and melanocytic naevi[28] and seem to influence the somatic mutational load in adult cutaneous melanoma.[29] Young patients (aged 20 years or younger) with cutaneous melanoma have an elevated prevalence of *MC1R* variants, but the low number of available studies, coupled with the small

number of cases per study, makes drawing clear conclusions a challenge.[18–20]

To help elucidate the role of *MC1R* in childhood and adolescent cutaneous melanoma and to better understand the genetic and clinical diversity of the disease across age, with potential clinical effects in terms of early melanoma detection and preventive strategies, we assessed these tumours in a large multicentre cohort pooled from the M-SKIP (melanocortin 1 receptor skin cancer and phenotypic characteristics) Project, the Italian Melanoma Intergroup (IMI), and other European groups. The aims of our study were to compare the prevalence of *MC1R* variants between young patients and healthy controls, with a case-control study design, and between young patients and adult patients, with a case-case study design.

Methods

Study design and participants

We analysed a large, multicentre cohort pooled from the M-SKIP Project, the IMI, and other European groups (appendix), including participants from 11 countries (Australia, Canada, France, Greece, Italy, the Netherlands, Serbia, Spain, Sweden, Turkey, and the USA; figure 1). Our analysis included children and adolescents diagnosed with sporadic single-primary cutaneous melanoma at age 20 years or younger, adult patients with sporadic single-primary cutaneous melanoma diagnosed at age 35 years or older, and healthy adult individuals as controls. Because age is a continuous variable, and an exact age cutoff between adolescents and adults would not be expected, we excluded melanoma cases diagnosed in the age range of 21–34 years to avoid a possible overlap between categories, and thus enable comparison between groups with distinct clinical and genetic characteristics. Because of the known challenges in diagnosing paediatric melanoma[30–32] and to decrease misdiagnosis, participating investigators were asked to provide the original histopathological reports and representative glass slides for central review. Only patients for whom the original histopathological report was available were eligible. Additionally, we restricted the study to cases with complete *MC1R* genotyping. We excluded familial melanoma cases, cases with a history of cancer at any site other than non-melanoma skin cancer, atypical spitzoid neoplasms or melanocytic tumors of uncertain malignant potential, and ocular and mucosal melanomas.

Detailed information on recruitment is reported in the appendix. Ethics committee approval was obtained at each institution in which new blood samples were drawn. For each young patient, four adult patients and four healthy controls were randomly selected from the same parent study that provided the young patient. When this was not possible, adult patients and controls were selected from a study that was done in the nearest geographical proximity to the parent study of the young patient (appendix; figure 1). Written consent was obtained from adult and older adolescent patients and the parents of young patients.

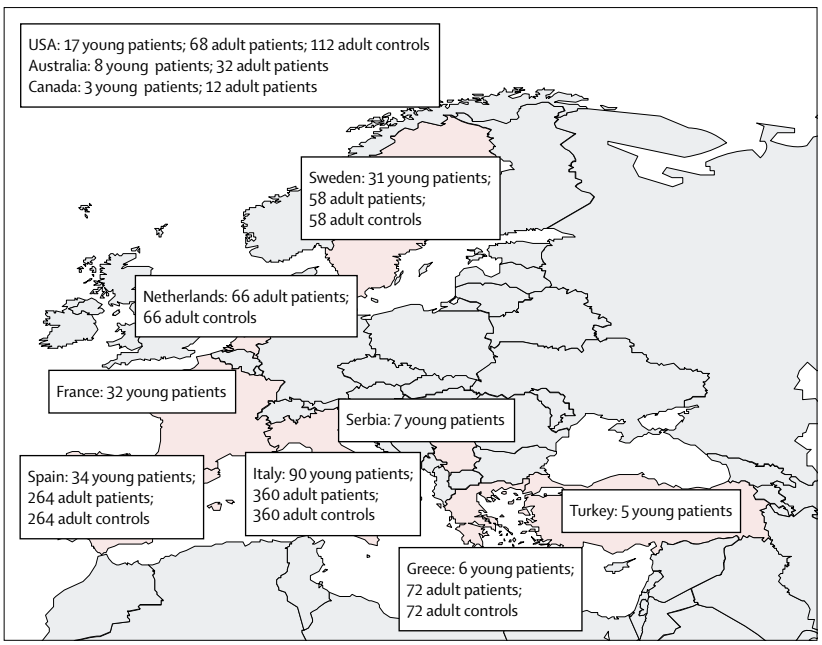

Figure 1: **Geographical areas of participant recruitment**

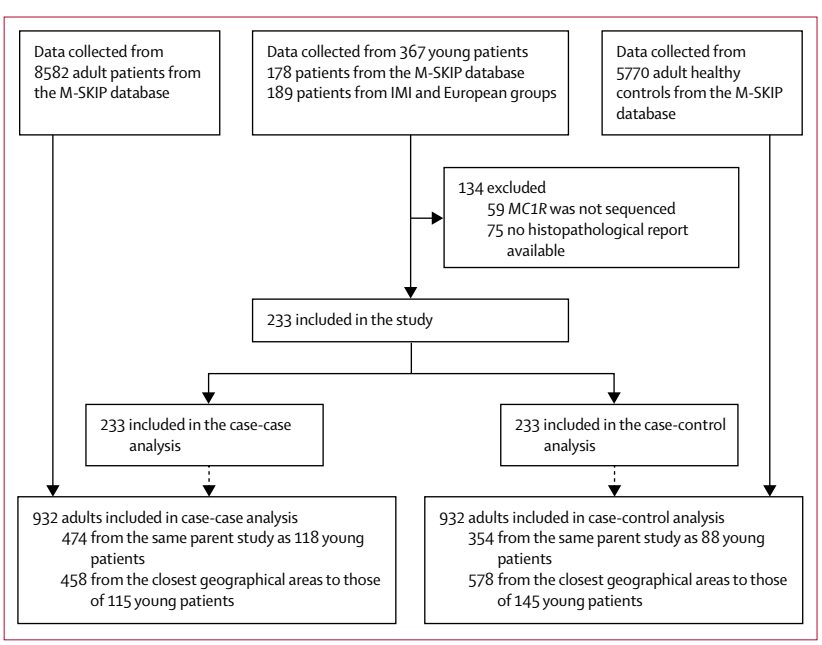

Figure 2: **Flow chart of participants included in the analysis**
Young patients were aged 20 years or younger, whereas adult patients and healthy controls were aged 35 years or older. M-SKIP=melanocortin 1 receptor skin cancer and phenotypic characteristics Project. IMI=Italian Melanoma Intergroup. *MC1R*=melanocortin 1 receptor.

Procedures

For 135 young patients from the M-SKIP Project, and 48 from the IMI and European groups, *MC1R* sequencing had already been done in study-specific laboratories

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	Young patients (n=233)	Adult patients (n=932)	p value*	Adult controls (n=932)	p value*
Sex	..	..	0·025	..	0·00030
Male	95 (41%)	456 (49%; n=931)	..	500 (54%; n=926)	..
Female	138 (59%)	475 (51%; n=931)	..	426 (46%; n=926)	..
Median Breslow thickness, mm (IQR)	0·93 (0·50–2·10)	1·00(0·50–2·40)	0·16	NA	NA
Median common melanocytic naevi, count (IQR)	30 (15–64)	25 (10–45)	0·00070	21 (5–30)	<0·0001
Any atypical melanocytic naevi	49 (43%; n=113)	165 (30%; n=556)	0·010	46 (9%; n=501)	<0·0001
Melanoma body site	..	..	0·037	..	NA
Head or neck	27 (12%; n=222)	127 (16%; n=808)	..	NA	..
Trunk	91 (41%; n=222)	313 (39%; n=808)	..	NA	..
Upper limbs	11 (5%; n=222)	90 (11%; n=808)	..	NA	..
Lower limbs	75 (34%; n=222)	236 (29%; n=808)	..	NA	..
NOC†	18 (8%; n=222)	42 (5%; n=808)	..	NA	..
Histolopathogical subtype	..	..	<0·0001	..	NA
LMM	0 (0%)	50 (7%; n=719)	..	NA	..
NM	33 (17%; n=198)	127 (18%; n=719)	..	NA	..
SSM	124 (63%; n=198)	493 (69%; n=719)	..	NA	..
ALM	7 (3%; n=198)	39 (5%; n=719)	..	NA	..
Spitzoid	13 (7%; n=198)	2 (<1%; n=719)	..	NA	..
Others‡	21 (11%; n=198)	8 (1%; n=719)	..	NA	..
Hair colour	..	..	0·73	..	0·00030
Red	14 (7%; n=213)	55 (6%; n=895)	..	24 (3%; n=709)	..
Blonde	60 (28%; n=213)	216 (24%; n=895)	..	129 (18%; n=709)	..
Brown	139 (65%; n=213)	609 (68%; n=895)	..	535 (76%; n=709)	..
NOC†	0 (0%)	15 (2%; n=895)	..	21 (3%; n=709)	..
Eye colour	..	..	0·011	..	<0·0001
Blue	65 (36%; n=181)	420 (50%; n=845)	..	330 (47%; n=709)	..
Brown	77 (42%; n=181)	314 (37%; n=845)	..	364 (51%; n=709)	..
Black	2 (1%; n=181)	2 (<1%; n=845)	..	5 (1%; n=709)	..
Green, grey, hazel	5 (3%; n=181)	0 (0%)	..	0 (0%)	..
NOC†	32 (18%; n=181)	109 (13%; n=845)	..	10 (1%; n=709)	..
Skin type	..	..	0·67	..	0·015
I	16 (8%; n=210)	59 (7%; n=838)	..	26 (4%; n=682)	..
II	68 (33%; n=210)	320 (36%; n=838)	..	191 (28%; n=682)	..
III	94 (44%; n=210)	400 (45%; n=838)	..	378 (55%; n=682)	..
IV	32 (15%; n=210)	59 (13%; n=838)	..	87 (13%; n=682)	..
Any solar lentigines	15 (15%; n=100)	321 (75%; n=428)	<0·0001	203 (68%; n=299)	<0·0001

Data are n (%), unless otherwise specified. NA=not applicable. NOC=not otherwise classifiable. LMM=lentigo maligna melanoma. NM=nodular melanoma. SSM=superficial spreading melanoma. ALM=acral lentiginous melanoma. *Logistic regression model, adjusted by matching stratum variable. †This group includes patients with doubtful or mixed information, thus not classifiable. ‡Other subtypes among children or adolescents include nevoid (n=4), epithelioid (n=3), desmoplastic (n=1), and others not specified (n=13); subtypes among adults include epithelioid (n=5), nevoid (n=1), desmoplastic (n=1), and others not specified (n=1).

Table 1: **Characteristics of the study population**

(appendix). For the remaining 50 young patients from IMI and European groups who provided new blood or saliva samples, *MC1R* genotyping was done centrally at the University of L'Aquila (L'Aquila, Italy) and done as described elsewhere.[33]

Statistical analysis

A complete description of the statistical analysis is available in the appendix. Briefly, we analysed the associations between risk factors and young melanoma by logistic regression in comparison with two reference groups, adult patients and healthy controls, with adjustment for study or geographical location.

We compared the frequency of any *MC1R* variants among children or adolescents with that of adult patients and controls by logistic regression, with adjustment for study or geographical location. These comparisons were repeated for any *MC1R* R variant, for any r variant, for a score calculated by summing across the *MC1R* alleles, which gives a value of 1 to r and 2 to R variants (as proposed elsewhere),[34] and for each of the nine most prevalent *MC1R* variants and any rare *MC1R* variants (presence or absence).

	All studied patients					Patients with centralised confirmed melanoma diagnosis				
	Young patients (n=233)	Adult patients (n=932)	p value*	Adult controls (n=932)	p value*	Young patients (n=64)	Adult patients (n=256)	p value*	Adult controls (n=256)	p value*
Any *MC1R* variants	173 (74%)	662 (71%)	0·33	550 (59%)	<0·0001	46 (72%)	193 (75%)	0·56	145 (57%)	0·028
Any R variants	86 (37%)	350 (38%)	0·86	238 (26%)	0·00060	24 (38%)	102 (40%)	0·73	58 (23%)	0·016
Any r variants	115 (49%)	420 (45%)	0·24	370 (40%)	0·0077	29 (45%)	115 (45%)	0·95	102 (40%)	0·43
Score	..	..	0·85	..	<0·0001	..	..	0·38	..	0·0029
0	60 (26%)	270 (29%)	..	382 (41%)	..	18 (28%)	63 (25%)	..	111 (43%)	..
1	71 (30%)	260 (28%)	..	261 (28%)	..	16 (25%)	70 (27%)	..	77 (30%)	..
2	64 (27%)	227 (24%)	..	201 (22%)	..	20 (31%)	72 (28%)	..	47 (18%)	..
3	28 (12%)	106 (11%)	..	57 (6%)	..	7 (11%)	24 (9%)	..	15 (6%)	..
≥4	10 (4%)	69 (7%)	..	31 (3%)	..	3 (5%)	27 (11%)	..	6 (2%)	..
Any Val60Leu variants	77 (33%)	270 (29%)	0·22	251 (27%)	0·060	24 (38%)	82 (32%)	0·40	70 (27%)	0·11
Any Asp84Glu variants	3 (1%)	14 (2%)	0·81	7 (1%)	0·43	1 (2%)	3 (1%)	0·80	1 (0%)	0·32
Any Val92Met variants	30 (13%)	115 (12%)	0·82	115 (12%)	0·83	9 (14%)	25 (10%)	0·32	29 (11%)	0·55
Any Arg142His variants	7 (3%)	34 (4%)	0·63	22 (2%)	0·57	0 (0%)	12 (5%)	0·98	11 (4%)	0·98
Any Arg151Cys variants	30 (13%)	142 (15%)	0·36	91 (10%)	0·17	11 (17%)	45 (18%)	0·94	23 (9%)	0·060
Any Ile155Thr variants	4 (2%)	18 (2%)	0·83	15 (2%)	0·91	1 (2%)	5 (2%)	0·84	2 (1%)	0·57
Any Arg160Trp variants	21 (9%)	93 (10%)	0·66	63 (7%)	0·23	7 (11%)	29 (11%)	0·92	15 (6%)	0·16
Any Arg163Gln variants	13 (6%)	59 (6%)	0·67	34 (4%)	0·18	0 (0%)	17 (7%)	0·97	7 (3%)	0·98
Any Asp294His variants	19 (8%)	54 (6%)	0·18	37 (4%)	0·0089	4 (6%)	17 (7%)	0·91	8 (3%)	0·25

Data are n (%). For each group of children and adolescents, the adult patients or healthy controls matched for study and geographical frequency were used as comparison groups. The score was calculated by summing across the *MC1R* alleles, which gives a value of 1 to r and 2 to R variants.[34] R variants include Asp84Glu, Arg142His, Arg151Cys, Ile155Thr, Arg160Trp, Asp294His, and other rare variants classified as R according to the algorithm proposed by Davies et al.[34] The r variants include Val60Leu, Val92Met, Arg163Gln, and other rare variants classified as r according to the algorithm proposed by Davies et al.[34] MC1R=melanocortin 1 receptor. *Logistic regression model, adjusted by matching stratum variable.

Table 2: **Association between *MC1R* variants and childhood or adolescent melanoma in all study patients and in the subgroup of patients with a confirmed melanoma diagnosis after centralised slide review**

We then used multivariable unconditional logistic regression models to calculate the odds ratio (OR) for *MC1R* variants after adjusting for study or geographical location and other covariables (as available) including sex, melanoma body site, histopathological subtype, hair colour, and skin type. We also did a sensitivity analysis with multivariable conditional logistic regression models. Because of the retrospective and multicentre nature of the study, information on covariables was not available for all the patients. Covariables with more than 30% of missing data were not included in the models, whereas multiple imputation models were done for variables with less than 30% of missing data (appendix).

The primary analysis compared the entire sample of young patients with adult controls and adult patients. Considering the possible misdiagnosis in young patients, we repeated the primary analysis including only the subgroup of young patients with cutaneous melanoma diagnosis confirmed after central slide review. We then calculated a modified OR, applying the method proposed by Manfred Green[35] that incorporates adjustment based on the predictive value of a positive test. We also did sensitivity analyses on the subgroup of young and adult patients coming from the same parental study and on the overall sample after the exclusion of patients without confirmed diagnosis. Subgroup analyses were done according to age at diagnosis of young patients.

Generally, p values lower than 0·05 were considered statistically significant. However, we also calculated p values corrected for false discovery rate (FDR) to take into account multiple comparisons. We used SAS software (version 9.4) and STATA (version 15) for our analyses.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

We retrospectively collected data up to Dec 31, 2016, of 367 young patients, 8582 adult patients, and 5770 adult controls (figure 2). For 59 young patients, information on *MC1R* was not available either because of patients' death (two patients) or refusal to participate in the study (n=57). Among the remaining 308 patients, 75 had no original histopathological report available, leaving 233 young patients for inclusion in the statistical analysis. For the selected 932 adult patients, 474 were from the same parent study as the young patients and 458 came from a geographically close study population. For the selected 932 adult controls, 354 were from the same parent study

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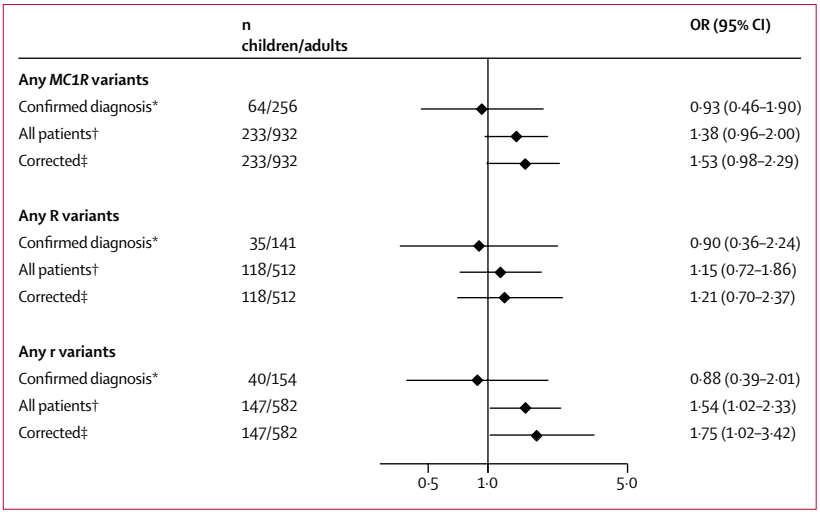

Figure 3: **Covariable-adjusted odds ratio (OR) for the association between any *MC1R* variants, R variants, and r variants and childhood and adolescent melanoma compared with adult melanoma**

All ORs were adjusted by sex, matching stratum variable, melanoma body site and histopathological subtype, hair colour, and skin type. For each OR, the comparison groups included child and adolescent patients matched (4:1) with adult patients by study or geographical area. The reference category for OR was *MC1R* wild-type (WT) individuals. Numbers of children or adolescents and adults reported here are the total numbers of patients included in each analysis, independently by *MC1R* status. For the analysis on any R variant versus WT, patients carrying only r variants were excluded, and for the analysis on any r variant versus WT, patients carrying only R variants were excluded. R variants include Asp84Glu, Arg142His, Arg151Cys, Ile155Thr, Arg160Trp, Asp294His, and other rare variants classified as R according to the algorithm proposed by Davies and colleagues.[34] The r variants include Val60Leu, Val92Met, Arg163Gln, and other rare variants classified as r according to the algorithm proposed by Davies and colleagues.[34] *MC1R*=melanocortin 1 receptor. *Calculated on the subgroup of patients with confirmed diagnosis of melanoma after centralised pathological review of glass slides. †Calculated on the whole sample of 233 child and adolescent patients. ‡Corrected for probability of misdiagnosis, by combining information from OR of confirmed diagnoses and OR of all patients, as suggested elsewhere.[35]

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See Online for appendix

as the young patients and 578 came from a geographically close study population. Young patients had a median age of 18 years (IQR 15–19), adult patients had a median age of 55 years (45–67), and adult healthy controls had a median age of 50 years (43–59). The total count of common melanocytic naevi was higher in young patients than in either adult patients or controls (table 1). Young patients had a higher proportion of atypical melanocytic naevi than those of adult patients and adult controls. We found differences between young patients and adult patients regarding the histopathological subtype of melanomas and the body site where they occurred. Children and adolescents had a lower prevalence of blue eyes than that of adult patients or controls, and they were less likely to have solar lentigines than adult patients or controls (table 1).

Table 2 shows the frequencies of any *MC1R* variants, any R variants, any r variants, *MC1R* score, and any of the nine most prevalent *MC1R* variants in the young patients, adult patients, and adult controls in our study. With a univariable analysis, we found no significant differences in frequency of *MC1R* variants between young and adult patients. However, young patients had significantly higher frequencies of any variants, R variants, r variants, and *MC1R* score than those of healthy controls, supporting the role of *MC1R* in melanoma susceptibility. We found eight rare *MC1R* variants in young patients: 86insA (two patients), Val51Ala, Thr95Met, Val122Met, Arg151His, Ala218Thr, Phe258Leu, Lys278Glu, (one patient each). No association was found between childhood and adolescent melanoma and any *MC1R* rare variant (data not shown).

Among the 233 young patients in our cohort, representative histopathological slides of the tumour were available for 85 patients and were centrally reviewed for quality control by a dermatopathologist (DM). These 85 patients had clinicopathological characteristics similar to those of 148 patients for whom glass slides were not reviewed (appendix). The original diagnosis of melanoma was confirmed in 64 (75%) of 85 patients. The samples of the other 21 (25%) patients were deemed not representative, were difficult to interpret for technical reasons, or were reclassified as atypical melanocytic naevi, atypical junctional melanocytic proliferations, pagetoid melanocytosis overlying congenital naevi, or ambiguous atypical melanocytic proliferations with spitzoid features. In the reclassified cases, serial unstained slides or paraffin blocks were not available, and thus additional immunohistochemical or molecular analyses, which would have clarified interpretation, were precluded. Such doubtful cases were independently reviewed by a second dermatopathologist (FF), but the conflicting discrepancy with the original diagnosis remained unresolved. The median Breslow thickness was 1·00 mm (IQR 0·50–1·90) in the 64 patients with a confirmed diagnosis and 0·45 mm (0·10–0·75) in the 21 patients for whom the original diagnosis was not confirmed (p=0·0005; appendix). No other clinicopathological features differed between the two groups (appendix).

The frequencies of *MC1R* variants in this subgroup of 64 children or adolescents with a confirmed diagnosis after histopathological review and in the matched 256 adult patients, and 256 controls were similar to those reported for the primary analysis (table 2).

We found that children or adolescents with cutaneous melanoma had significantly higher odds of carrying any r variants than those of adult patients (OR 1·54, 95% CI 1·02–2·33; FDR-corrected p=0·17; figure 3). Concerning specific *MC1R* variants, we found a positive association for all *MC1R* variants with childhood and adolescent melanoma, except for the Arg160Trp variant (figure 4). We found a statistically significant association for Val60Leu (p=0·04, FDR-corrected p=0·17) and Asp294His (p=0·04, FDR-corrected p=0·17) variants in both the primary analysis and after correction for possible misdiagnosis (figure 4). Similar results were obtained in the sensitivity analysis with conditional logistic regression models (appendix) and by excluding the 21 children and adolescents without centrally confirmed diagnosis (appendix). Finally, when we repeated the primary analysis on the subgroups of young patients and adult patients from the same parental study, we obtained stronger associations with childhood and adolescent

melanoma than those of the primary analysis for carriers of any *MC1R* variant (OR 2·04, 95% CI 1·19–3·50), r variants (2·61, 1·43–4·73), and Val60Leu (2·67, 1·44–4·95) and Asp294His variants (3·12, 1·08–9·03; appendix).

We did a subgroup analysis by age at diagnosis (patients who were 18 years or younger and patients who were 14 years or younger at diagnosis), and we observed a significantly higher frequency of r variants in patients aged 18 years or younger than in adult patients (OR 1·80, 95% CI 1·06–3·07; FDR-corrected p=0·61; table 3). The corresponding OR for patients aged 14 years or younger was even higher, but was not significant because of the small number of patients.

We also did a case-control analysis comparing young patients with melanoma with healthy adult controls (appendix). We found a significantly higher risk of childhood and adolescent melanoma in carriers of any *MC1R*, R, and r variants, as well as for the most common *MC1R* Val60Leu, Val92Met, Arg151Cys, Arg163Gln, and Asp294His variants compared with that in healthy controls. Results remained significant after correction for multiple comparison, except for the Val92Met variant (FDR-corrected p=0·07).

Discussion

Our pooled-analysis showed that young patients had significantly higher frequencies of any *MC1R* variants, R variants, and r variants than those of healthy controls, supporting the role of *MC1R* variants as genetic risk factors for childhood and adolescent cutaneous melanoma. We also found that the frequency of r variants was elevated in young patients compared with that of adult patients. The effect of r variants was supported by analyses limited to individuals aged 18 years or younger and was even stronger, but not significantly, for children aged 14 years or younger, suggesting a higher prevalence of *MC1R* variants in childhood melanoma. The *MC1R* Val60Leu and Asp294His variants showed the most robust association with melanoma in childhood and adolescence, even after correction for possible misdiagnosis.

Childhood and adolescent melanoma has been reported to occur most commonly in white people and in girls.[2,10,13] In line with two previous studies,[12,13] we found that young patients with melanoma are characterised by a fairer phenotype than that of healthy controls, including traits such as red hair and skin type. By contrast, young patients presented with more darkly pigmented characteristics, such as brown eyes, skin type III or IV, and a lower prevalence of freckles compared with those of location-matched adult patients. Consistent with most published studies,[2,11,36] our young patients showed a high number of melanocytic naevi, both common and atypical, and developed melanomas mainly on the lower limbs and the trunk. Childhood and adolescent melanoma was more commonly diagnosed as nodular melanoma compared with its adult counterpart. Spitzoid melanomas

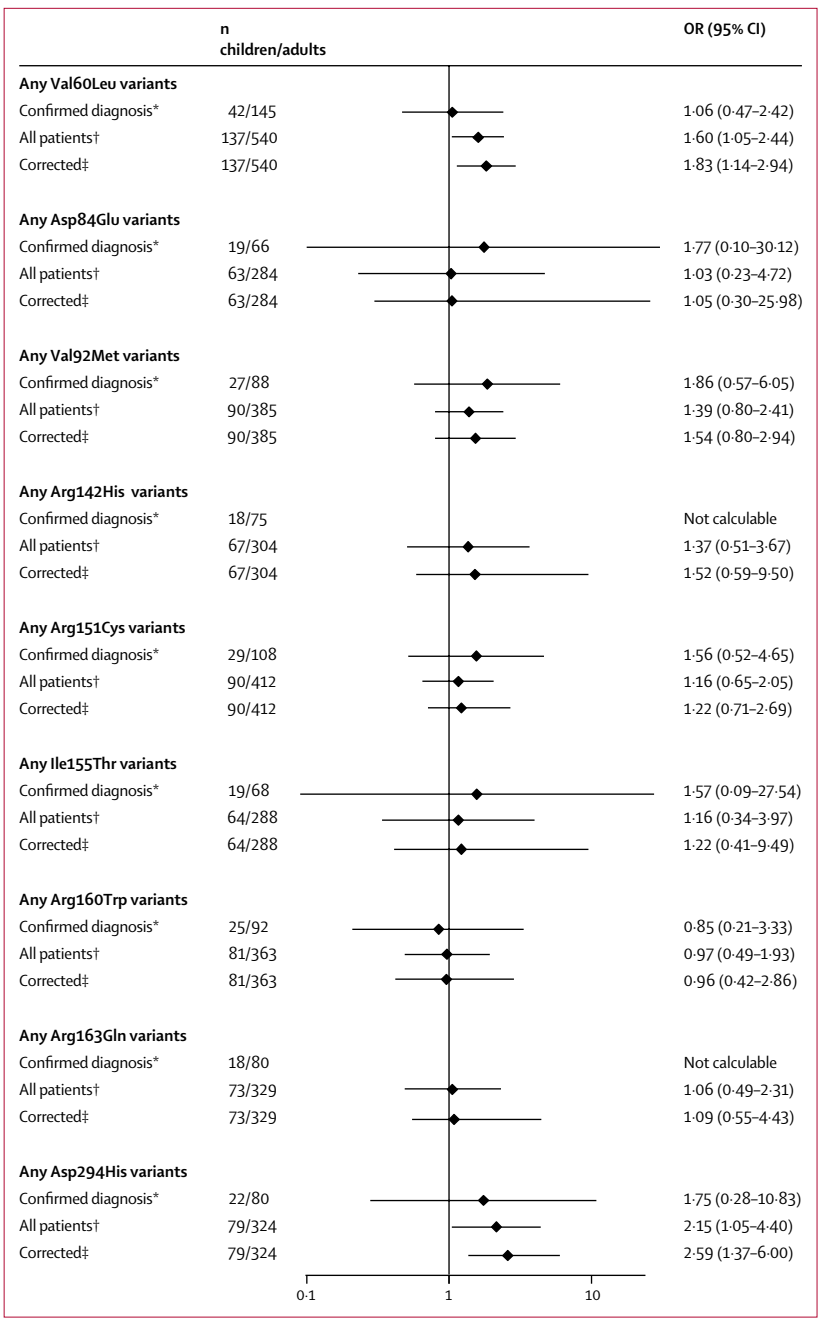

Figure 4: **Covariable-adjusted odds ratio (OR) for the association between the nine most prevalent *MC1R* variants and childhood and adolescent melanoma compared with adult melanoma**

All ORs were adjusted by sex, matching stratum variable, melanoma body site and histopathological type, hair colour, and skin type. For each OR, the comparison groups included young patients matched (4:1) with adult patients by study or geographical area. The reference category for OR was *MC1R* wild-type (WT) individuals. Numbers of children or adolescents and adults reported here are the total numbers of patients included in each analysis, independently by *MC1R* status. For the analysis on each variant versus WT, patients carrying only other *MC1R* variants were excluded. R variants include Asp84Glu, Arg142His, Arg151Cys, Ile155Thr, Arg160Trp, Asp294His, and other rare variants classified as R according to the algorithm proposed by Davies and colleagues.[34] The r variants include Val60Leu, Val92Met, Arg163Gln, and other rare variants classified as r according to the algorithm proposed by Davies and colleagues.[34] *MC1R*=melanocortin 1 receptor. *Calculated on the subgroup of patients with confirmed diagnosis of melanoma after centralised pathological review of glass slides. †Calculated on the whole sample of 233 young patients. ‡Corrected for probability of misdiagnosis, by combining information from OR of confirmed diagnoses and OR of all patients, as suggested elsewhere.[35]

	Patients aged ≤18 years (n=148)		Patients aged ≤14 years (n=52)	
	Young patients/adult patients	OR (95% CI)	Young patients/adult patients	OR (95% CI)
Any variants	148/592	1·45 (0·89–2·34)	52/208	1·86 (0·69–5·03)
Any R variants	73/330	0·99 (0·52–1·89)	27/115	1·63 (0·42–6·36)
Any r variants	98/374	1·80 (1·06–3·07)	35/142	2·27 (0·76–6·83)
Any Val60Leu variants	88/357	1·59 (0·91–2·76)	31/136	2·27 (0·76–6·80)
Any Asp84Glu variants	43/192	0·97 (0·13–6·99)	15/73	Not calculated
Any Val92Met variants	61/253	1·62 (0·79–3·33)	18/93	0·95 (0·11–7·97)
Any Arg142His variants	45/201	1·32 (0·34–5·13)	13/80	Not calculated
Any Arg151Cys variants	56/277	0·82 (0·38–1·80)	18/97	0·61 (0·10–3·88)
Any Ile155Thr variants	43/192	1·13 (0·17–7·64)	15/71	Not calculated
Any Arg160Trp variants	55/234	1·08 (0·45–2·58)	21/87	3·57 (0·62–20·52)
Any Arg163Gln variants	51/219	1·61 (0·61–4·22)	16/84	0·68 (0·03–14·81)
Any Asp294His variants	52/272	1·47 (0·58–3·70)	14/86	Not calculated

Odds ratios (ORs) were adjusted by sex, matching stratum variable, melanoma body site and histological subtype, and skin type. Hair colour was not included because this category had more than 30% of missing data for these groups of patients. For each OR, the comparison group included 4:1 frequency-matched adult patients by study or geographical area. The reference category for ORs were *MC1R* wild-type individuals. The number of children and adults reported here are the total number of patients included in each analysis, independently by *MC1R* status. For the analysis on each variant versus wild type, patients carrying only other *MC1R* variants were excluded. R variants include Asp84Glu, Arg142His, Arg151Cys, Ile155Thr, Arg160Trp, Asp294His, and other rare variants classified as R according to the algorithm proposed by Davies et al.[34] The r variants include Val60Leu, Val92Met, Arg163Gln, and other rare variants classified as r according to the algorithm proposed by Davies et al.[34] *MC1R*=melanocortin 1 receptor.

Table 3: **Subgroup analysis by age at diagnosis**

were more frequently identified in young patients, whereas lentigo maligna melanomas were only seen in adulthood.

The effect of *MC1R* variants in childhood and adolescent melanoma was investigated in small series of patients.[18–20] One study[19] published in 2009, identified *MC1R* variants in 12 (57%) of 21 patients, with a higher frequency of *r* than that of *R* variants. More recently, two case series reported *MC1R* variants in ten (43%) of 23 patients[18] and in four (67%) of six.[20] In our pooled-analysis, *MC1R* variants were detected in 74% of young patients.

Our findings showed a stronger role of *MC1R* r variants in childhood and adolescent melanoma than in adult melanoma, suggesting an involvement of biological pathways other than pigmentation and UV-sensitivity, such as antioxidant defences, DNA repair, and cell proliferation.[22,24,37] Indeed, MC1R signalling was found to be crucial for melanocyte key processes,[38] showing that *MC1R* variants, combined with HERC2/*OCA2* alleles, can determine the number of naevi bigger than 2 mm in sunburned children.[39]

In our study, the *MC1R* variants Val60Leu and Asp294His showed significantly higher prevalence in childhood and adolescent melanoma than in the adult form of the disease. The role of Val60Leu in adult melanoma is controversial, and the magnitude of risk varies across populations.[40] A positive association of Val60Leu with melanoma has been reported in the Mediterranean area, where Val60Leu is the most frequent of all variants.[40] The Asp294His variant is common in individuals with the red hair colour phenotype. The association of Asp294His with melanoma risk shows the heterogeneity between northern and southern European populations, where individuals who are more darkly pigmented are at higher risk of melanoma associated with Asp294His than are northern, less pigmented populations.[41]

To the best of our knowledge, our series of childhood and adolescent melanoma patients is the largest international multicentre cohort published so far with available *MC1R* genetic data. The large number of young patients with melanoma, and comparable adult patients, provide powerful estimates of the association between *MC1R* variants and childhood and adolescent melanoma within different populations. Another strength of our study was the centralised data quality control and statistical analysis that provided consistency across the numerous parent studies in defining and adjusting for important covariates. Histopathological centralised review of a third of the patients allowed us to calculate association estimates in a subset of children or adolescents with a histologically confirmed diagnosis and was helpful for calculating corrected risk estimates considering the issue of misdiagnosis.

Young patients with melanoma represent a heterogeneous group, including neonates, children, and adolescents, with various distinct presentations.[9] Childhood melanoma might indeed differ from adolescent melanoma, and both might differ from adult melanoma.[4] To further address heterogeneity between melanomas developed at different ages, we did a stratified analysis for patients aged 14 years or younger and 18 years or younger. Our non-significant findings from the younger subgroup might have resulted from decreased power related to the small sample size (59 patients) of this subgroup, whereas a separate multivariable analysis limited to children aged 10 years or younger was not possible because of the low number of patients (23 patients). In our child and adolescent sample, we had more darkly pigmented patients from southern European countries than from northern European origin, which might have resulted in high frequencies of r variants, more common in southern Europe than in northern Europe.[42] However, because young patients were compared with adult patients and controls from the same geographical areas, we do not believe this affected our results. Indeed, a sensitivity analysis done in the subgroup of young patients with adult patients sampled from the same parent study provided similar results. A centralised review of all melanomas would be desirable but, unfortunately, it was not feasible because of the retrospective nature of the study. To reduce disease misclassification, we excluded from the analysis patients whose histopathological reports were not available. We also provided risk estimates corrected for our observed

misclassification prevalence among patients with histopathological centralised review, a group that was representative of the entire cohort of young patients. Nevertheless, we should note that this correction could not provide an exact estimate of the associations, as in a sample with only centrally confirmed diagnosed cases, and some imprecision of estimates could therefore not be ruled out. Because our cohort did not include patients with familial melanoma and the major susceptibility genes are rarely mutated in young patients,[12,15,17,20] we did not analyse *CDKN2A* and *CDK4* genes in our patients. It is possible that other major melanoma predisposition genes might influence the risk of disease in children and adolescents, but the absence of genetic data on these genes, such as *BAP1*, prevented the analysis of possible gene–gene interactions. Finally, although we did a high number of statistical tests, we allowed unadjusted p values to guide the interpretation of our results. Because of the exploratory, rather than confirmatory, nature of this study, we believe that our approach of describing the tests of significance we did, as advised by Thomas V Perneger,[43] is appropriate. However, to directly address the issue of multiple testing, we also presented FDR-corrected p values.

In conclusion, our pooled analysis showed that natural variations in *MC1R* are a genetic risk factor for childhood and adolescent cutaneous melanoma, as well as for adult cutaneous melanoma. *MC1R* variants, mainly r alleles, were suggested to have a greater role in childhood and adolescent melanoma than in adult melanoma, possibly through a pigmentation-independent pathway. Additionally, we observed a stronger effect of r variants when the analysis was restricted to patients with melanoma aged 18 years or younger. Our study contributes to the comprehension of the genetic background of paediatric melanoma and elucidates the genetic diversity of paediatric and adult melanoma, with potential clinical relevance for developing early melanoma detection and preventive strategies.

Contributors

CP and SR did the literature search. SR, SG, PM, PAK, JCG-B, HN, MTL, JL, JN-B, FS, and MCF contributed to study design. M-FA, FD, BB-dP, VH, AEC, HA-C, SBG, RPG, LM, RZ, TD, NET, CBB, MB, SP, MP, EN, PG, CMe, AMM, MR, SB, EP, LKS, FBa, GG, AS, FO, FA, RF-d-M, PQ, GR, AR, EM, IS, PAK, MAP, and MCF contributed to data collection. DM and FF did the histophatological review. CP and CMa did the molecular analysis of *MC1R* for new samples. FBo and SR analysed the data. CP, DM, SG, PAK, MTL, JL, MCF, and SR contributed to data interpretation. CP, FBo, and CMa wrote the manuscript. FB did the figures.

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Declaration of interests

We declare no competing interests.

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References

1 Nikolaou V, Stratigos AJ. Emerging trends in the epidemiology of melanoma. *Br J Dermatol* 2014; **170:** 11–19.
2 Strouse JJ, Fears TR, Tucker MA, Wayne AS. Pediatric melanoma: risk factor and survival analysis of the surveillance, epidemiology and end results database. *J Clin Oncol* 2005; **23:** 4735–41.
3 Austin MT, Xing Y, Hayes-Jordan AA, Lally KP, Cormier JN. Melanoma incidence rises for children and adolescents: an epidemiologic review of pediatric melanoma in the United States. *J Pediatr Surg* 2013; **48:** 2207–13.
4 LaChance A, Shahriari M, Kerr PE, Grant-Kels JM. Melanoma: kids are not just little people. *Clin Dermatol* 2016; **34:** 742–48.
5 Lorimer PD, White RL, Walsh K, et al. Pediatric and adolescent melanoma: a national cancer data base update. *Ann Surg Oncol* 2016; **23:** 4058–66.
6 Steliarova-Foucher E, Colombet M, Ries LAG, et al. International incidence of childhood cancer, 2001–10: a population-based registry study. *Lancet Oncol* 2017; **18:** 719–31.
7 Livestro DP, Kaine EM, Michaelson JS, et al. Melanoma in the young—differences and similarities with adult melanoma: a case-matched controlled analysis. *Cancer* 2007; **110:** 614–24.
8 Berg P, Lindelöf B. Differences in malignant melanoma between children and adolescents. A 35-year epidemiological study. *Arch Dermatol* 1997; **133:** 295–97.
9 Tracy ET, Aldrink JH. Pediatric melanoma. *Semin Pediatr Surg* 2016; **25:** 290–98.
10 Averbook BJ, Lee SJ, Delman KA, et al. Pediatric melanoma: analysis of an international registry. *Cancer* 2013 **119:** 4012–19.
11 Pappo AS. Melanoma in children and adolescents. *Eur J Cancer* 2003; **39:** 2651–61.
12 Youl P, Aitken J, Hayward N, et al. Melanoma in adolescents: a case-control study of risk factors in Queensland, Australia. *Int J Cancer* 2002; **98:** 92–98.
13 Whiteman DC, Valery P, McWhirter W, Green AC. Risk factors for childhood melanoma in Queensland, Australia. *Int J Cancer* 1997; **70:** 26–31.
14 Ripperger T, Bielack SS, Borkhardt A, et al. Childhood cancer predisposition syndromes—a concise review and recommendations by the Cancer Predisposition Working Group of the Society for Pediatric Oncology and Hematology. *Am J Med Genet A* 2017; **173:** 1017–37.
15 Berg P, Wennberg AM, Tuominen R, et al. Germline *CDKN2A* mutations are rare in child and adolescent cutaneous melanoma. *Melanoma Res* 2004; **14:** 251–55.
16 Whiteman DC, Milligan A, Welch J, Green AC, Hayward NK. Germline *CDKN2A* mutations in childhood melanoma. *J Natl Cancer Inst* 1997; **89:** 1460.
17 Hocevar M, Avbelj M, Perić B, Zgajnar J, Besić N, Battelino T. High prevalence of germline *CDKN2A* mutations in Slovenian cutaneous malignant melanoma families. *Croat Med J* 2006; **47:** 851–54.
18 Lu C, Zhang J, Nagahawatte P, et al. The genomic landscape of childhood and adolescent melanoma. *J Invest Dermatol* 2015; **135:** 816–23.
19 Daniotti M, Ferrari A, Frigerio S. Cutaneous melanoma in childhood and adolescence shows frequent loss of INK4A and gain of KIT. *J Invest Dermatol* 2009; **129:** 1759–68.
20 Rabbie R, Rashid M, Arance AM, et al. Genomic analysis and clinical management of adolescent cutaneous melanoma. *Pigment Cell Melanoma Res* 2017; **30:** 307–16.
21 Wilmott JS, Johansson PA, Newell F, et al. Whole genome sequencing of melanomas in adolescent and young adults reveals distinct mutation landscapes and the potential role of germline variants in disease susceptibility. *Int J Cancer* 2019; **144:** 1049–60.
22 Beaumont KA, Shekar SN, Newton RA, et al. Receptor function, dominant negative activity and phenotype correlations for *MC1R* variant alleles. *Hum Mol Genet* 2007; **16:** 2249–60.
23 Dessinioti C, Antoniou C, Katsambas A, Stratigos AJ. Melanocortin 1 receptor variants: functional role and pigmentary associations. *Photochem Photobiol* 2011; **87:** 978–87.
24 Kanetsky PA, Rebbeck TR, Hummer AJ, et al. Population-based study of natural variation in the melanocortin-1 receptor gene and melanoma. *Cancer Res* 2006; **66:** 9330–37.

25 Pasquali E, García-Borrón JC, Fargnoli MC, et al. *MC1R* variants increased the risk of sporadic cutaneous melanoma in darker-pigmented Caucasians: a pooled-analysis from the M-SKIP project. *Int J Cancer* 2015; **136:** 618–31.

26 Tagliabue E, Gandini S, Bellocco R, et al. *MC1R* variants as melanoma risk factor independent of at-risk phenotypic characteristics: a pooled-analysis from the M-SKIP project. *Cancer Manag Res* 2018; **10:** 1143–54.

27 Fargnoli MC, Sera F, Suppa M, et al. Dermoscopic features of cutaneous melanoma are associated with clinical characteristics of patients and tumours and with *MC1R* genotype. *J Eur Acad Dermatol Venereol* 2014; **28:** 1768–75.

28 Vallone MG, Tell-Marti G, Potrony M, et al. Melanocortin 1 receptor (MC1R) polymorphisms' influence on size and dermoscopic features of nevi. *Pigment Cell Melanoma Res* 2018; **31:** 39–50.

29 Robles-Espinoza CD, Roberts ND, Chen S, et al. Germline *MC1R* status influences somatic mutation burden in melanoma. *Nat Commun* 2016; **7:** 12064.

30 Massi D, Tomasini C, Senetta R, et al. Atypical Spitz tumors in patients younger than 18 years. *J Am Acad Dermatol* 2015; **72:** 37–46.

31 Elmore JG, Barnhill RL, Elder DE, et al. Pathologists' diagnosis of invasive melanoma and melanocytic proliferations: observer accuracy and reproducibility study. *BMJ* 2017; **357:** j2813.

32 Busam KJ, Murali R, Pulitzer M, et al. Atypical spitzoid melanocytic tumors with positive sentinel lymph nodes in children and teenagers, and comparison with histologically unambiguous and lethal melanomas. *Am J Surg Pathol* 2009; **33:** 1386–95.

33 Pellegrini C, Di Nardo L, Cipolloni G, et al. Heterogeneity of BRAF, NRAS, and TERT promoter mutational status in multiple melanomas and association with MC1R genotype: findings from molecular and immunohistochemical analysis. *J Mol Diagn* 2018; **20:** 110–22.

34 Davies JR, Randerson-Moor J, Kukalizch K, et al. Inherited variants in the *MC1R* gene and survival from cutaneous melanoma: a BioGenoMEL study. *Pigment Cell Melanoma Res* 2012; **25:** 384–94.

35 Green MS. Use of predictive value to adjust relative risk estimates biased by misclassification of outcome status. *Am J Epidemiol* 1983; **117:** 98–105.

36 Berk DR, LaBuz E, Dadras SS, et al. Melanoma and melanocytic tumors of uncertain malignant potential in children, adolescents and young adults—the Stanford experience 1995–2008. *Pediatr Dermatol* 2010; **27:** 244–54.

37 Sanchez PC, Noda AY, Franco DD, Lourenço SV, Sangueza M, Neto CF. Melanoma in children, adolescents, and young adults: a clinical pathological study in a Brazilian population. *Am J Dermatopathol* 2014; **36:** 620–28.

38 D'Mello SA, Finlay GJ, Baguley BC, Askarian-Amiri ME. Signaling pathways in melanogenesis. *Int J Mol Sci* 2016; **17:** e1144.

39 Barón AE, Asdigian NL, Gonzalez V, et al. Interactions between ultraviolet light and *MC1R* and *OCA2* variants are determinants of childhood nevus and freckle phenotypes. *Cancer Epidemiol Biomarkers Prev* 2014; **23:** 2829–39.

40 Mills O, Messina JL. Pediatric melanoma: a review. *Cancer Control* 2009; **16:** 225–33.

41 Gerstenblith MR, Goldstein AM, Fargnoli MC, Peris K, Landi MT. Comprehensive evaluation of allele frequency differences of MC1R variants across populations. *Hum Mutat* 2007; **28:** 495–505.

42 Guida S, Bartolomeo N, Zanna PT, et al. Sporadic melanoma in south-eastern Italy: the impact of melanocortin 1 receptor (MC1R) polymorphism analysis in low-risk people and report of three novel variants. *Arch Dermatol Res* 2015; **307:** 495–503.

43 Perneger TV. Adjusting for multiple testing in studies is less important than other concerns. *BMJ* 1999; **318:** 1288.