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ORIGINAL ARTICLE

Melanoma in children and adolescents: analysis of susceptibility genes in 123 Italian patients

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Abstract

Background A polygenic inheritance involving high, medium and low penetrance genes has been suggested for melanoma susceptibility in adults, but genetic information is scarce for paediatric patients.

Objective We aim to analyse the major high and intermediate melanoma risk genes, *CDKN2A, CDK4, POT1, MITF* and *MC1R*, in a large multicentre cohort of Italian children and adolescents in order to explore the genetic context of paediatric melanoma and to reveal potential differences in heritability between children and adolescents.

Methods One-hundred-twenty-three patients (<21 years) from nine Italian centres were analysed for the *CDKN2A, CDK4, POT1*, *MITF,* and *MC1R* melanoma predisposing genes. The rate of gene variants was compared between sporadic, familial and multiple melanoma patients and between children and adolescents, and their association with clinicopathological characteristics was evaluated.

Results Most patients carried *MC1R* variants (67%), while *CDKN2A* pathogenic variants were found in 9% of the cases, the *MITF* E318K in 2% of patients and none carried *CDK4* or the *POT1* S270N pathogenic variant. Sporadic melanoma patients significantly differed from familial and multiple cases for the young age at diagnosis, infrequent red hair colour, low number of nevi, low frequency of *CDKN2A* pathogenic variants and of the *MC1R* R160W variant. Melanoma in children (≤12 years) had more frequently spitzoid histotype, were located on the head/neck and upper limbs and had higher Breslow thickness. The *MC1R* V92M variant was more common in children than in adolescents. *CDKN2A* common polymorphisms and *MC1R* variants were associated with a high number of nevi.

Conclusion Our results confirm the scarce involvement of the major high-risk susceptibility genes in paediatric melanoma and suggest the implication of *MC1R* gene variants especially in the children population.

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Conflict of interest

All authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this study

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Introduction

Melanoma is a rare disease in childhood and adolescence, with approximately 1%–4% of cutaneous melanomas (CM) occurring in patients younger than 21 years of age[1] and an incidence of 5–6 cases per million in the paediatric population.[2]

There are significant clinical and histopathological differences between childhood and adolescent melanoma supporting the hypothesis of biologically distinct diseases.[3] Children develop thicker lesions, mainly of the spitzoid and nodular subtype, frequently associated with a pre-existing congenital melanocytic nevus (CMN); they have higher rates of lymph node metastasis and more advanced stage at diagnosis than the adolescence counterpart.[4] Contrariwise, adolescents most commonly show superficial spreading melanoma (SSM) and mainly occurs *de novo* in patients with fair-coloured skin and sun exposure habits.[5]

Main risk factors for paediatric melanoma are conditions unique to the childhood population and include giant CMN and hereditary conditions such as xeroderma pigmentosum, immunodeficiency and albinism.[6] A history of sunburns and intermittent UV exposure has been associated with adolescence melanoma.[4,6] Other known risk factors are the presence of numerous acquired melanocytic nevi, the dysplastic nevus syndrome and a sun-sensitive phenotype.[3–6] Finally, although only a minority of paediatric cases have been reported in a familial context, the importance of a family history has been confirmed in several studies.[7,8]

A polygenic mechanism of inheritance involving high- and medium-/low-risk genes is known for adults with familial melanoma (FM) and multiple primary melanomas (MPM) and has been associated with an earlier age of development.[9] *CDKN2A* is the major melanoma high-risk gene and the frequency of germline pathogenic variants (PVs) varies between 8% and 40% of familial cases.[10] Additional genes, including *CDK4, POT1, BAP1, TERT* promoter, *ACD* and *TERF2IP,* have been reported to confer a high risk of melanoma susceptibility, although their pathogenic variants are extremely rare and all together contribute to <10% of adult heritability.[11] The hotspot S270N substitution of *POT1* gene has been reported as founder PV in Italy with a frequency comparable to that of *CDKN2A PVs* in Italian familial melanoma.[12] Inheritance of multiple medium-to-low risk variants, including *MC1R* variants and the *MITF* E318K substitution, may explain part of the missing heritability.[13–15] *MC1R* is a highly polymorphic gene and specific *MC1R* variants are classified as 'R' or 'r' alleles according to the strength of association with the red hair colour phenotype.[14,15] Overall, the presence of any *MC1R* variant is a risk factor for melanoma, with higher risk values observed for R alleles and occurrence of multiple variants.[16–18]

It has long been debated if paediatric occurrence is linked to melanoma susceptibility genes already established in adult patients. However, genetic predisposition has been poorly investigated in childhood and adolescence due to the rarity of the disease. The few published genetic studies on paediatric melanoma susceptibility investigated only the variations in *CDKN2A, CDK4* and *MC1R* genes, with the majority of them including a small number of cases, mainly from a single institution.[7,16–22] Overall, they report that germline *CDKN2A* PVs are rare in paediatric melanoma,[7,16,17,23–25] while a high frequency of germline variants has been identified in *MC1R* gene.[19-20,22]

To the best of our knowledge, the differences in melanoma susceptibility between sporadic and familial cases, and between children and adolescents, have never been investigated.

Herein, we analysed the major high and intermediate melanoma risk genes, *CDKN2A, CDK4, POT1, MITF* and *MC1R*, in a large multicentre cohort of Italian children and adolescents, in order to explore the genetic context of paediatric melanoma and to reveal potential differences in heritability between children and adolescents.

Materials and methods

This multicentre cross-sectional study involved nine centres participating in the Italian Melanoma Intergroup (IMI). Investigators retrospectively reviewed their databases and identified 123 histologically confirmed CM diagnosed in patients <21 years of age. Sporadic, FM and MPM cases were included. Exclusion criteria were the followings: patients with atypical Spitzoid neoplasms/MELTUMP, ocular or mucosal melanomas. The exclusion of MELTUMP was motivated by the fact that it is not a histologically confirmed melanoma, therefore we thought that melanoma genes might not have a role in the susceptibility of these controversial lesions. Genomic DNA was already available

for 82 subjects included in research protocols previously approved by local Ethics Committees. For 41 patients, blood samples were prospectively collected during follow-up visits, as part of a multicentre research protocol (R58/14-IEO69).

Epidemiological and clinical data were collected by each investigator at the time of melanoma diagnosis. For a small number of sporadic cases (15/91, 16%), histopathological slide revision was performed as part of our previous publication.[22] All the data were centrally entered in a main database, data quality controls and data standardization were performed according to rules of the Melanocortin-1 receptor gene, Skin cancer and Phenotypic characteristics (M-SKIP) project.[26]

Genetic analysis

Genetic analyses were performed at five different centres, following a common experimental protocol. Briefly, genomic DNA was extracted from blood samples and analysed for mutations in *CDKN2A, CDK4, MC1R* and *MITF* by Sanger sequencing.[19,27–31] For the CDKN2A gene (reference sequence LRG_11), the common protocol included the sequencing of the 5′ UTR (from −157 bp), exons 1 alpha, 1 beta, 2 and 3, and the 3′ UTR (to +111 bp); the data of the −191 position were already collected by four centres and were retained in the analysis. For the CDK4 gene (reference sequence LRG_490), we sequenced the exon 2; for POT1, we analysed the position S270; for the MC1R (reference sequence NM_002386.4), the entire coding region and for the MITF (reference sequence LRG_776), the exon 9.

Statistical analysis

For genetic analysis, we considered as *CDKN2A* PVs variants all the coding substitutions and indels with a previously described pathogenic significance, while the known A148T (rs3731249) change and the non-coding polymorphisms were defined as single nucleotide polymorphisms (SNP). Regarding *MC1R* gene, we considered 'R' (D84E, R142H, R151C, R160W, D294H and I155T) and 'r' (V60L, V92M and R163Q) variants as previously reported.[21,32–34] Single polymorphisms analysis for *MC1R* compared carriers vs. non-carriers of each specific variant was performed. Sensitivity analyses comparing each *MC1R* variant with wild-type subjects were also conducted.

We compared clinico-pathological and genetic features among sporadic, FM and MPM cases, and between children (<12 years) and adolescents by using the non-parametric Wilcoxon two-independent sample test for continuous variables, and the Chi-Square test or Fisher Exact test, as appropriate, for categorical variables. The same tests were used to investigate the association between the most frequent *CDKN2A* and *MC1R* alterations with clinical and epidemiological characteristics. Missing data were excluded by the analysis. The statistical analysis was carried out using SAS software, version 9.2 (SAS Institute, Cary, NC, USA).

Results

Overall, 123 histologically confirmed CM cases diagnosed in patients younger than 21 years of age were included in the study. The majority of patients (59%) was females, had brown hair (74%), dark eyes (72%) and developed melanoma on the trunk (41%; Table 1). Most melanomas (71%) were of the SSM subtype, and the mean Breslow thickness of the first melanoma was 1.81 ± 2.0 mm. Melanoma was associated with a CMN in 12 of 102 (12%) patients.

Overall, 91 of 123 (74%) were sporadic single primary cases, 18 (15%) FM and 14 (11%) MPM. MPM patients included 8 sporadic and 6 familial melanoma patients. Sporadic single primary patients were younger ($P = 0.03$), less frequently red haired ($P = 0.03$) and carried a lower number of common melanocytic nevi than FM or MPM patients ($P = 0.05$; Table 1).

According to age at first diagnosis, 105 were adolescents (85%) and 18 children (15%). Primary melanomas of children differed from those of the adolescents for histotype, being more frequently spitzoid and less frequently SSM ($P < 0.0001$), for higher Breslow thickness (2.88 ± 2.27 vs. 1.63 ± 1.92; $P = 0.03$) and for the more frequent occurrence in the head/neck region and upper limbs than trunk and lower limbs ($P = 0.01$; Table 2).

Genetic susceptibility

Analysis of high-penetrance genes identified germline *CDKN2A* PVs in 11 of 123 (9%) patients, while PVs in *CDK4* exon 2 and the POT1 S270N variant were not detected in our sample. Among *CDKN2A* PVs, the G101W was detected in five (5/11, 45%) cases (1 sporadic and 4 FM patients) and the R24P in two (18%), while L63M, P48T, R58X and the delCDKN2A in one case each (9%). In addition to PVs, we observed a high frequency of CDKN2A polymorphisms (57/123, 46%), mainly the c.-191G>A (33/83 with available information, 40%) and 3′UTR polymorphisms (55/123, 45%). Regarding intermediate penetrance genes, 3 (3/123, 2.4%) patients harboured the *MITF* p.E318K substitution, whereas the majority of cases (83/122 with available information, 67%) carried at least one *MC1R* variant (Table 3).

CDKN2A PVs were detected at higher frequency in MPM (50%) and in FM patients (29%) than in sporadic cases (2%; $P < 0.0001$). Same results were observed for the *MC1R* R160W variant (21%, 13% and 2% respectively, $P = 0.01$; Table 3).

Regarding age at diagnosis, no association was found with *CDKN2A,* while we observed that the *MC1R* V92M variant was significantly more frequent in children than adolescents (Table 4) and was associated with a lower age at diagnosis ($P = 0.03$, Table S1).

The CDKN2A -191G>A polymorphism was associated with a high number of common nevi ($P = 0.007$; Fig. 1a and Table S2), the presence of atypical nevi (76% vs. 40% of non-carriers, $P = 0.05$, Table S2) and blonde hair colour (0/3 red,

Table 1 Descriptive characteristics of the study population according to sporadic, familial and multiple melanoma. Reported tumour characteristics for multiple melanoma refer to the first primary melanoma

	All cases (*N* = 123) *N* (%)	**Sporadic single primary cases (*N* = 91) *N* (%)**	**Familial cases (*N* = 24†) *N* (%)**	**Multiple melanoma cases (*N* = 14†) *N* (%)**	***P*-value**
Age‡	16 ± 4	16 ± 4	18 ± 2	16 ± 3	**0.03**
Female sex	73 (59%)	52 (57%)	14 (58%)	11 (79%)	0.40
Dark eye colour§	63 (72%)	44 (70%)	13 (68%)	8 (80%)	0.56
Hair colour					**0.03**
Red	7 (8%)	2 (3%)	3 (16%)	4 (40%)	
Blonde	12 (14%)	10 (16%)	2 (11%)	0 (0%)	
Brown	65 (74%)	49 (78%)	13 (68%)	5 (50%)	
Black	4 (5%)	2 (3%)	1 (5%)	1 (10%)	
Skin type					0.49
I	7 (8%)	4 (6%)	1 (5%)	3 (30%)	
II	39 (45%)	26 (42%)	10 (53%)	6 (60%)	
III	33 (38%)	25 (40%)	7 (37%)	1 (10%)	
IV	8 (9%)	7 (11%)	1 (5%)	0 (0%)	
Presence of freckles	15 (18%)	9 (16%)	5 (25%)	3 (30%)	0.40
Common nevi number‡	42 ± 27	39 ± 27	52 ± 26	52 ± 27	**0.05**
Presence of atypical nevi	34 (48%)	23 (47%)	8 (44%)	7 (88%)	0.81
Any sunburn	46 (53%)	30 (49%)	13 (65%)	6 (60%)	0.29
Histotype of the primary melanoma					0.06
NM	19 (20%)	18 (25%)	1 (5%)	0 (0%)	
SSM	67 (71%)	45 (63%)	18 (95%)	9 (90%)	
ALM	2 (2%)	2 (3%)	0 (0%)	0 (0%)	
Spitzoid	4 (4%)	4 (6%)	0 (0%)	0 (0%)	
Others¶	3 (3%)	2 (3%)	0 (0%)	1 (10%)	
Body site of the primary melanoma					0.08
Arms/upper limbs	14 (12%)	11 (13%)	2 (8%)	3 (23%)	
Head and neck	17 (14%)	15 (17%)	1 (4%)	1 (8%)	
Trunk	48 (41%)	32 (37%)	13 (54%)	6 (46%)	
Lower limbs	37 (31%)	29 (33%)	6 (25%)	3 (23%)	
Others	2 (2%)	0 (0%)	2 (8%)	0 (0%)	
Breslow thickness of the primary melanoma‡	1.81 ± 2.01	2.06 ± 2.20	1.14 ± 0.99	1.15 ± 1.37	0.16
Melanoma arising in congenital nevi	12 (12%)	7 (10%)	3 (13%)	3 (27%)	0.28

N = 52 missing data for atypical nevi; *N* = 46 for common nevi; *N* = 39 for freckles; *N* = 36 for skin type and sunburn; *N* = 35 for eye colour and hair colour; N = 28 for histotype; *N* = 21 for congenital nevi; *N* = 11 for Breslow thickness and *N* = 5 for body site. Significant *P*-values (<0.05) are in bold.
ALM, acral lentigo melanoma; NM, nodular melanoma; SSM, superficial spreading melanoma.
**P*-value for the difference between the following two groups: sporadic single primary (*N* = 91 subjects) vs. familial and/or multiple melanoma cases (*N* = 32 patients). Non-parametric Wilcoxon two-independent sample test for continuous variables and Chi-Square test or Fisher Exact test for categorical variables, as appropriate.
†Included six familial cases with multiple primary melanomas.
‡Mean ± SD.
§Dark eye colour: brown and black; fair eye colour: blue, green, grey and hazel.
¶Epithelioid (*N* = 1), Equine type (*N* = 1) and Desmoplastic (*N* = 1).

0%; 5/6 blonde, 83%; 18/35 brown, 51%; 0/4 black, 0%, $P = 0.02$, Table S2). Similarly, *CDKN2A* polymorphisms in 3′ UTR were associated with the presence of atypical nevi (59% vs. 32% of non-carriers, $P = 0.03$, Table S2).

Overall, patients with *MC1R* variants showed lower Breslow thickness ($P = 0.02$) and presented a higher number of common nevi than non-carriers ($P = 0.006$; Table S1 and Fig. 1b). This last association was stronger for carriers of *MC1R* R variants ($P = 0.0008$; Fig. 1c). As expected, R variants were associated with fair skin and red hair colour, while r variants with darker hair colour (Table S1). Looking at specific *MC1R* variants, R151C was more frequent in patients with CM localized on head and neck (35% vs. 0% upper limbs, 11% trunk, 8% lower limbs and 0% others, $P = 0.04$, Table S1), while four of the six

Table 2 Descriptive characteristics of the study population according to age at melanoma diagnosis

	All cases (*N* = 123) *N* (%)	Cases ≤12 years (*N* = 18) *N* (%)	Cases >12 years (*N* = 105) *N* (%)	*P*-value*
Female sex	73 (59%)	13 (72%)	60 (57%)	0.23
Familial or multiple	32 (26%)	4 (22%)	28 (27%)	0.69
Dark eye colour†	63 (72%)	7 (78%)	56 (71%)	1.00
Hair colour				0.58
Red	7 (8%)	0 (0%)	7 (9%)	
Blonde	12 (14%)	1 (11%)	11 (14%)	
Brown	65 (74%)	7 (78%)	58 (73%)	
Black	4 (5%)	1 (11%)	3 (4%)	
Skin type				0.85
I	7 (8%)	0 (0%)	7 (9%)	
II	39 (45%)	5 (56%)	34 (44%)	
III	33 (38%)	4 (44%)	29 (37%)	
IV	8 (9%)	0 (0%)	8 (10%)	
Presence of freckles	15 (18%)	1 (11%)	14 (19%)	1.00
Common nevi number§	42 ± 27	39 ± 31	42 ± 27	0.64
Presence of atypical nevi	34 (48%)	2 (33%)	32 (49%)	0.68
Any sunburn	46 (53%)	3 (33%)	43 (55%)	0.30
Histotype of the primary melanoma				**<0.0001**
NM	19 (20%)	4 (27%)	15 (19%)	
SSM	67 (71%)	4 (27%)	63 (79%)	
ALM	2 (2%)	1 (7%)	1 (1%)	
Spitzoid	4 (4%)	4 (27%)	0 (0%)	
Others‡	3 (3%)	2 (13%)	1 (1%)	
Body site of the primary melanoma				**0.01**
Arms/upper limbs	14 (12%)	5 (31%)	9 (9%)	
Head and neck	17 (14%)	5 (31%)	12 (12%)	
Trunk	48 (41%)	4 (25%)	44 (43%)	
Lower limbs	37 (31%)	2 (13%)	35 (34%)	
Other	2 (2%)	0 (0%)	2 (2%)	
Breslow thickness of the primary melanoma§	1.81 ± 2.01	2.88 ± 2.27	1.63 ± 1.92	**0.03**
Melanoma arising in congenital nevi	12 (12%)	2 (14%)	10 (11%)	0.67

Significant *P*-values (<0.05) are in bold.
ALM, acral lentigo melanoma; NM, nodular melanoma; SSM, superficial spreading melanoma.
**P*-value for the difference between patients older and younger than 12 years old. Non-parametric Wilcoxon two-independent sample test for continuous variables and Chi-Square test or Fisher Exact test for categorical variables, as appropriate.
†Fair eye colour: blue, green, grey and hazel; dark eye colour: brown and black.
‡Epithelioid (*N* = 1), Equine type (*N* = 1) and Desmoplastic (*N* = 1).
§Mean ± SD.

patients with D294H variant had CM on upper limbs ($P = 0.01$). Children and adolescents with melanoma raised on congenital nevus were more frequently carriers of R151C and R150W variants ($P = 0.05$ and 0.03, respectively, Table S1).

Discussion

We analysed known melanoma susceptibility genes in paediatric patients, showing differences among sporadic, familial and multiple melanoma cases and between children and adolescents. In the overall patient population, we identified a low frequency of PVs in *CDKN2A* and *MITF* genes and a high rate of *MC1R* variants. Patients with sporadic, FM and MPM significantly differed for age at diagnosis, hair colour, number of nevi and for frequency of *CDKN2A* mutations and *MC1R* R160W variant. Regarding age groups, melanoma in patients aged <12 years showed a high Breslow thickness, head/neck and upper limbs localization and higher frequency of spitzoid subtype in comparison with adolescents. The MC1R V92M variant was more frequent in children than in adolescents and was suggested to be associated with earlier age at diagnosis.

Table 3 Genetic characteristics of the study population according to sporadic, familial and multiple melanoma. Reported tumour characteristics for multiple melanoma refer to the first primary melanoma

	All cases (*N* = 123) *N* (%)	**Sporadic single primary cases (*N* = 91) *N* (%)**	**Familial cases (*N* = 24†) *N* (%)**	**Multiple melanoma cases (*N* = 14†) *N* (%)**	***P*-value‡**
CDKN2A mutations§	11 (9%)	2 (2%)	7 (29%)	7 (50%)	**<0.0001**
CDKN2A SNPs (any)	57 (46%)	45 (49%)	10 (42%)	6 (43%)	0.24
CDKN2A SNP -191G>A¶	33 (40%)	22 (39%)	10 (48%)	3 (30%)	0.75
CDKN2A SNP 3′UTR††	55 (45%)	43 (47%)	10 (42%)	6 (43%)	0.33
CDKN2A (any)‡‡	83 (68%)	64 (70%)	15 (65%)	8 (57%)	0.35
R variants	36 (30%)	24 (26%)	9 (39%)	6 (43%)	0.19
r variants	55 (45%)	45 (49%)	9 (39%)	2 (14%)	0.10
V60L	45 (37%)	37 (41%)	7 (30%)	2 (14%)	0.14
R151C	15 (12%)	12 (13%)	1 (4%)	2 (14%)	0.76
V92M	10 (8%)	7 (8%)	2 (9%)	1 (7%)	0.71
R160W	7 (6%)	2 (2%)	3 (13%)	3 (21%)	**0.01**
R142H	6 (5%)	4 (4%)	1 (4%)	1 (7%)	0.64
D294H	6 (5%)	4 (4%)	1 (4%)	2 (14%)	0.64
R163Q	3 (2%)	2 (2%)	1 (4%)	0 (0%)	1.00
D84E	1 (1%)	1 (1%)	0 (0%)	0 (0%)	1.00
MITF	3 (3%)	2 (2%)	1 (5%)	0 (0%)	1.00

Significant *P*-values (<0.05) are in bold.
R, red hair colour variant; r, not red hair colour variant; SNP, single nucleotide polymorphism; UTR, untranslated region.
†Included six familial cases with multiple primary melanomas.
‡*P*-value for the difference between sporadic single primary and familial and multiple melanoma cases. Chi-Square test or Fisher Exact test, as appropriate.
§G101W was detected in five cases (a sporadic and four familial cases, two of which presented multiple melanoma), R24P in two cases, while L63M, P48T, R58X and *del*CDKN2A in one case respectively.
¶Evaluated by four centres (*N* = 83 patients: 57 sporadic, 16 familial, 5 multiple and 5 familial and multiple).
††SNPs detected in the 3′UTR are c.*29G/C (rs11515) and c.*69C/T occurring in 31% and 16% of the studied population respectively. The SNPs 3′UTR are in linkage disequilibrium with the SNP -191G>A, with an estimated week degree of correlation among the alleles (https://ldlink.nci.nih.gov/).
‡‡One patient (familial) with missing information on MC1R.

Melanoma is rare in paediatric patients and differs from the adult counterpart for distinct demographic, clinico-pathological and biological presentation.[3] The baseline demographic and clinical aspects of our cohort are in line with previous evidences, as we found a primarily adolescent distribution with most diagnoses after 12 years of age.[35,36] Moreover, our patients showed a high number of melanocytic nevi, both common and atypical, and a high frequency of non-spitzoid melanoma mainly located on the limbs and trunk.[4,6]

A family history of melanoma represents an important risk factor for the development of the disease in childhood and adolescence.[6,17,23,37] Youl et al. (2002) estimated an OR for paediatric occurrence of 3.8 (95% CI 1.6–9.4) in a cohort of adolescent Australian melanoma patients with a first-degree affected relative.[7] More recently, a 6- to 28-fold higher percentage of paediatric cases was reported in melanoma-prone families compared with the general US population, regardless of *CDKN2A* status.[8] Family history was described in 10%–32% of paediatric patients worldwide,[7,16,17,23–25] but in only 1.5% of cases in a previous Italian study.[38] In our cohort, a positive family history was observed in 20% of patients. Differences between the two Italian cohorts could be due to the design of the studies (retrospective vs. prospective) and baseline characteristics of patients.

In our study, FM and MPM patients were more frequently red haired and had a higher number of nevi than sporadic patients, suggesting a major contribution of genetic predisposing factors in high-risk patients. Unexpectedly, although anticipation of diagnosis is typically associated with a family history, we observed a significant earlier melanoma occurrence in sporadic than in familial patients. However, the lack of previous studies directly comparing familial vs. sporadic melanoma patients makes difficult to include this finding in a literature context.

Inactivating mutations in high-risk susceptibility genes explain the majority of hereditary melanoma. However, only few studies analysed melanoma predisposition in the paediatric population.[7,16–22] The reported frequency of *CDKN2A* PVs in previous studies[8,18–24,39] varied from 0% to 4.7%, while no PVs were identified in the *CDK4* gene.[7,17–20] When information about family history was available, these studies showed contrasting results. *CDKN2A* PVs were reported in 10%–20% of familial paediatric patients in two studies,[16,17] while Youl *et al.* (2002)[7] did not find PVs in this age group in a familial context. We observed an overall low *CDKN2A* mutation rate (9%), but, when

Table 4 Genetic characteristics of the study population according to age at melanoma diagnosis

	All cases (*N* = 123) *N* (%)	Cases ≤ 12 years (*N* = 18) *N* (%)	Cases > 12 years (*N* = 105) *N* (%)	*P*-value*
CDKN2A mutations	11 (9%)	1 (6%)	10 (10%)	1.00
CDKN2A SNPs (any)	57 (46%)	6 (33%)	51 (49%)	0.23
MC1R (any)†	83 (68%)	10 (56%)	73 (70%)	0.22
R variants	36 (30%)	4 (22%)	32 (31%)	0.58
r variants	55 (45%)	8 (44%)	47 (45%)	0.95
V60L	45 (37%)	6 (33%)	39 (38%)	0.74
R151C	15 (12%)	2 (11%)	13 (13%)	1.00
V92M	10 (8%)	4 (22%)	6 (6%)	**0.04**
R160W	7 (6%)	0 (0%)	7 (7%)	0.59
D294H	6 (5%)	0 (0%)	6 (6%)	0.59
R142H	6 (5%)	1 (6%)	5 (5%)	1.00
R163Q	3 (2%)	0 (0%)	3 (3%)	1.00
D84E	1 (1%)	1 (6%)	0 (0%)	0.15
MITF	3 (3%)	1 (6%)	2 (2%)	0.38

Significant *P*-values (<0.05) are in bold.
R, red hair colour variant; r, not red hair colour variant; SNP, single nucleotide polymorphism; UTR, untranslated region.
**P*-value for the difference between patients older and younger than 12 years old. Chi-Square test or Fisher Exact test, as appropriate.
†One patient (familial) with missing information on MC1R.

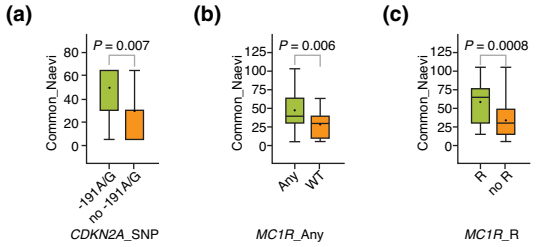

Figure 1 Box plot representing the association of common nevi number with the presence of (a) CDKN2A SNP c.-191G>A, (b) MC1R (any variant) and (c) MC1R R variants. Minimum and maximum are depicted by black whiskers, the box signifies the upper and lower quartiles, and the median and mean are represented by a black line and a small cross within the box respectively. For CDKN2A SNP c.-191G, the median is equal to the third quartile both for patients with and without the mutation.

we categorized patients, significant differences were detected among sporadic, FM and MPM, with a frequency of 2%, 29% and 50% respectively. The majority of our patients carried the G101W variant that is the most common PV in families from Mediterranean European countries.[10,19,40] Interestingly, in line with previous findings, we identified a higher number of patients with *CDKN2A* polymorphisms, mainly the -191G>A and the 3′UTR changes, in FM and MPM than in sporadic patients.

Regarding intermediate-risk genes, we found a high rate of *MC1R* variants (67%) and a low frequency of the *MITF* E318K substitution (2%). *MC1R* polymorphisms have been investigated in childhood and adolescent melanoma in few studies, with frequencies ranging from 57% to 74%.[18–20,22] In our published pooled analysis in a worldwide cohort, we demonstrated that paediatric patients had a higher probability of carrying any *MC1R* variant than adults, mainly r variants (OR; 1.75; 95%CI: 1.02–3.42). We now support the potential role of *MC1R* variants as genetic risk factors for childhood and adolescent melanoma in Italy. Notably, the R160W variant was significantly more frequent in FM and MPM cases than in sporadic melanoma. This amino acid substitution is common in individuals with the red hair colour phenotype and is less frequent in Italy than in other Caucasian samples.[41]

Age-based differences exist between children and adolescent melanoma, possibly indicating a distinct disease biology.[5] In patients ≤12 years, we confirmed specific and unique clinical characteristics of patients, as darker phenotypes and of melanoma, as anatomical location, histological subtype and thickness. As in large retrospective studies,[2,4–6] NM occurring on the head and neck or on the extremities was more common in children, while SSM melanoma mainly developed in the older group on the trunk. In addition, we found that children had significantly thicker tumours than adolescents, supporting the evidence that younger patients are more likely to have worse prognostic factors at the time of detection. It is strongly debated[24] if this is due to a delay in diagnosis due to challenging lesions of childhood and/or to differences in growth dynamics, i.e. for Spitzoid melanoma that is more frequent in children than in adolescents and has a vertical and fast growth.

In our recent pooled analysis on *MC1R* variants in paediatric melanoma patients, we demonstrated a role of r polymorphisms in melanoma predisposition of patients aged <18 and hypothesized their stronger involvement in those aged <14 years. Herein, we found that the V92M r polymorphism was associated with an earlier age at diagnosis and was observed at a higher frequency in children compared to adolescents. Interestingly, the V92M variant has been recently associated with the increasing size of the CMN, implying a growth-promoting effect of these alleles in childhood.[42] Interestingly, our results also confirmed the association of other two *MC1R* variants, R151C and R160W, with melanoma raised on CNM.

A genetic predisposition to increasing number of common nevi was observed in our study for CDKN2A -191G>A and for *MC1R* variants, with R160W as the mostly associated with a high number of common nevi. The same variant has been previously associated with the presence of atypical nevi in healthy controls, suggesting a possible impact on nevogenesis.[6] The higher number of common nevi for carriers of *MC1R* variants may also explain the lower Breslow thickness observed for these patients: it is indeed probable that children and adolescent accede earlier

and more frequently to clinical controls if they had a relatively high number of nevi.

A major limitation of our study is the different proportion of children and adolescents in the cohort, which might have led us to underestimate the clinico-pathological and genetic differences between childhood and adolescence melanoma. In addition, due to the small sample size in some categories, it was not possible to fully evaluate the impact of *CDKN2A* and *MC1R* in children. However, these proportions are representative of the occurrence of melanoma in these age classes and may therefore give a more realistic and complete picture of the disease. Furthermore, the differential diagnosis between spitzoid melanoma and atypical Spitz tumours/MELTUMP may be challenging due to conflicting histopathological criteria and low interobserver reproducibility. Only one of four spitzoid melanomas was centrally reviewed and confirmed. A centralized histopathological review of all melanomas would have been desirable considering the diagnostic difficulties of childhood lesions, but unfortunately, it was only feasible for a minority of sporadic patients. Furthermore, we did not screen for all known melanoma susceptibility genes but only for those with a known impact in the Italian population. Nonetheless, this remains the largest multicentre cohort of paediatric melanoma patients with available genetic data on a panel of high- and medium-risk susceptibility genes. A further strength of our study is that we first analysed the genetic inheritance differences between childhood and adolescence.

In conclusion, we confirm the scarce involvement of established high-risk susceptibility genes in paediatric melanoma, with *CDKN2A* being implicated only in a familial context. In addition, we support a major role of *MC1R* gene, and the influence of r variants in the development of melanoma in children.

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References

1 Wood BA. Paediatric melanoma. *Pathology* 2016; **48**: 155–165.
2 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**: 2207–2213.
3 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**: 614–624.
4 Merkel EA, Mohan LS, Shi K, Panah E, Zhang B, Gerami P. Paediatric melanoma: clinical update, genetic basis, and advances in diagnosis. *Lancet Child Adolesc Health* 2019; **3**: 646–654.
5 Bartenstein DW, Kelleher CM, Friedmann AM *et al.* Contrasting features of childhood and adolescent melanomas. *Pediatr Dermatol* 2018; **35**: 354–360.
6 Stefanaki C, Chardalias L, Soura E, Katsarou A, Stratigos A. Paediatric melanoma. *J Eur Acad Dermatol Venereol* 2017; **31**: 1604–1615.
7 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.
8 Goldstein AM, Stidd KC, Yang XR, Fraser MC, Tucker MA. Pediatric melanoma in melanoma-prone families. *Cancer* 2018; **124**: 3715–3723.
9 Fargnoli MC, Argenziano G, Zalaudek I, Peris K. High- and low-penetrance cutaneous melanoma susceptibility genes. *Expert Rev Anticancer Ther* 2006; **6**: 657–670.
10 Goldstein AM, Chan M, Harland M *et al.* Features associated with germline CDKN2A mutations: a GenoMEL study of melanoma-prone families from three continents. *J Med Genet* 2007; **44**: 99–106.
11 Dalmasso B, Ghiorzo P. Evolution of approaches to identify melanoma missing heritability. *Expert Rev Mol Diagn* 2020; **20**: 523–531.
12 Shi J, Yang XR, Ballew B *et al.* Rare missense variants in POT1 predispose to familial cutaneous malignant melanoma. *Nat Genet* 2014; **46**: 482–486.
13 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.
14 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**: 618–631.
15 Tagliabue E, Gandini S, García-Borrón JC *et al.* Association of melanocortin-1 Receptor variants with pigmentary traits in humans: a pooled analysis from the M-skip project. *J Invest Dermatol* 2016; **136**: 1914–1917.
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 Berg P, Wennberg AM, Sander B, Rozell BL, Platz A, Hansson J. Germline CDKN2A mutations are rare in child and adolescent cutaneous melanoma. *Melanoma Res* 2004; **14**: 251–255.
18 Lu C, Zhang J, Nagahawatte P *et al.* The genomic landscape of childhood and adolescent melanoma. *J Invest Dermatol* 2015; **135**: 816–823.
19 Daniotti M, Ferrari A, Frigerio S *et al.* Cutaneous melanoma in childhood and adolescence shows frequent loss of INK4A and gain of KIT. *J Invest Dermatol* 2009; **129**: 1759–1768.
20 Rabbie R, Rashid M, Arance AM *et al.* Genomic analysis and clinical management of adolescent cutaneous melanoma. *Pigment Cell Melanoma Res* 2017; **30**: 307–316.
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**: 1049–1060.
22 Pellegrini C, Botta F, Massi D *et al.* MC1R variants in childhood and adolescent melanoma: a retrospective pooled analysis of a multicentre cohort. *Lancet Child Adolesc Health* 2019; **3**: 332–342.
23 Paradela S, Fonseca E, Prieto VG. Melanoma in children. *Arch Pathol Lab Med* 2011; **135**: 307–316.
24 Carrera C, Scope A, Dusza SW *et al.* Clinical and dermoscopic characterization of pediatric and adolescent melanomas: multicenter study of 52 cases. *J Am Acad Dermatol* 2018; **78**: 278–288.

25 Hawryluk EB, Moustafa D, Bartenstein D *et al*. A retrospective multicenter study of fatal pediatric melanoma. *J Am Acad Dermatol* 2020; **83**: 1274–1281.
26 Raimondi S, Gandini S, Fargnoli MC *et al*. Melanocortin-1 receptor, skin cancer and phenotypic characteristics (M-SKIP) project: study design and methods for pooling results of genetic epidemiological studies. *BMC Med Res Methodol* 2012; **12**: 116.
27 Della Torre G, Pasini B, Frigerio S *et al*. CDKN2A and CDK4 mutation analysis in Italian melanoma-prone families: functional characterization of a novel CDKN2A germ line mutation. *Br J Cancer* 2001; **85**: 836–844.
28 Ghiorzo P, Gargiulo S, Pastorino L *et al*. Impact of E27X, a novel CDKN2A germ line mutation, on p16 and p14ARF expression in Italian melanoma families displaying pancreatic cancer and neuroblastoma. *Hum Mol Genet* 2006; **15**: 2682–2689.
29 Menin C, Vecchiato A, Scaini MC *et al*. Contribution of susceptibility gene variants to melanoma risk in families from the Veneto region of Italy. *Pigment Cell Melanoma Res* 2011; **24**: 728–730.
30 Casula C, Muggiano A, Cossu A *et al*. Role of key-regulator genes in melanoma susceptibility and pathogenesis among patients from South Italy. *BMC Cancer* 2009; **9**: 352.
31 Pellegrini C, Maturo MG, Martorelli C *et al*. Characterization of melanoma susceptibility genes in high-risk patients from Central Italy. *Melanoma Res* 2017; **27**: 258–267.
32 Dessinioti C, Antoniou C, Katsambas A, Stratigos AJ. Melanocortin 1 receptor variants: functional role and pigmentary associations. *Photochem Photobiol* 2011; **87**: 978–987.
33 Fargnoli MC, Gandini S, Peris K, Maisonneuve P, Raimondi S. MC1R variants increase melanoma risk in families with CDKN2A mutations: a meta-analysis. *Eur J Cancer* 2010; **46**: 1413–1420.
34 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**: 110–122.
35 Kalani N, Guidry JA, Farahi JM, Stewart SB, Dellavalle RP, Dunnick CA. Pediatric melanoma: characterizing 256 cases from the Colorado Central Cancer Registry. *Pediatr Dermatol* 2019; **36**: 219–222.
36 Berk DR, LaBuz E, Dadras SS, Johnson DL, Swetter SM. Melanoma and melanocytic tumors of uncertain malignant potential in children, adolescents and young adults–the Stanford experience 1995–2008. *Pediatr Dermatol* 2010; **27**: 244–254.
37 Cordoro KM, Gupta D, Frieden IJ, McCalmont T, Kashani-Sabet M. Pediatric melanoma: results of a large cohort study and proposal for modified ABCD detection criteria for children. *J Am Acad Dermatol* 2013; **68**: 913–925.
38 Ferrari A, Bisogno G, Cecchetto G *et al*. Cutaneous melanoma in children and adolescents: the Italian rare tumors in pediatric age project experience. *J Pediatr* 2014; **164**: 376–82.e1-2.
39 Mantelli M, Barile M, Ciotti P *et al*. High prevalence of the G101W germline mutation in the CDKN2A (P16(ink4a)) gene in 62 Italian malignant melanoma families. *Am J Med Genet* 2002; **107**: 214–221.
40 Mantelli M, Pastorino L, Ghiorzo P *et al*. Early onset may predict G101W CDKN2A founder mutation carrier status in Ligurian melanoma patients. *Melanoma Res* 2004; **14**: 443–448.
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 Kinsler VA, Abu-Amero S, Budd P *et al*. Germline melanocortin-1-receptor genotype is associated with severity of cutaneous phenotype in congenital melanocytic nevi: a role for MC1R in human fetal development. *J Invest Dermatol* 2012; **132**: 2026–2032.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1. P-values for the association between genetic variants* in *MC1R* gene and characteristics of the study population
Table S2. P-values for the association between genetic alterations* in *CDKN2A* gene and characteristics of the study populatio