

MC1R variants in childhood and adolescent melanoma:

A pooled-analysis from a large worldwide multicenter cohort of patients

Running head: MC1R in childhood and adolescent melanoma

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Abstract

Background: Childhood/adolescent and adult melanoma share some, but not all, clinico-pathological characteristics suggesting that distinct factors may contribute to their respective development. Germline variants in *MC1R* may increase risk of childhood/adolescent melanoma, but a clear conclusion regarding this genetic association is challenging because of the limited number of studies and cases available. We aimed to evaluate the association of *MC1R* inherited variants and childhood/adolescent melanoma in a large study comparing the prevalence of *MC1R* variants of childhood/adolescent melanoma patients to that among adult melanoma cases and among unaffected controls.

Methods: Phenotypic and genetic data on 233 childhood/adolescent (age ≤ 20 years) and 932 adult melanoma patients, and 932 unaffected controls, were gathered through the M-SKIP Project, the Italian Melanoma Intergroup, and European centers. We calculated odds ratios (OR) for childhood/adolescent melanoma associated with *MC1R* variants by multivariable logistic regression. Subgroup analysis was done for children aged ≤ 18 and ≤ 14 years.

Findings: Children and adolescents had a higher odds of carrying *MC1R* *r* variants than adults (OR: 1.54; 95%CI: 1.02-2.33). In stratified analysis, ORs for *r* alleles were elevated also when restricted to cases ≤ 18 years (OR: 1.80; 95%CI: 1.06-3.07). All the investigated variants except R160W showed a higher frequency in childhood/adolescent melanoma compared to adult melanoma, with significant results obtained for V60L (OR: 1.60; 95%CI: 1.05-2.44) and D294H (OR: 2.15; 95%CI: 1.05-4.40). Compared to unaffected controls, childhood/adolescent melanoma patients had significantly higher frequencies of any *MC1R* variant, as well as of V60L, V92M, R151C, R163Q and D294H variants.

Interpretation: Our pooled-analysis of the largest worldwide multicenter cohort of childhood/adolescent patients with *MC1R* genetic data revealed that *MC1R* *r* variants were more prevalent in childhood/adolescent compared to adult melanoma especially in children ≤ 18 years. Our findings support the role of *MC1R* in childhood/adolescent melanoma

susceptibility and might have a potential clinical relevance in developing early melanoma detection and preventive strategies.

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Introduction

The incidence of cutaneous melanoma (CM), the most aggressive form of skin cancer, has increased over the past several decades.¹ CM currently accounts for 5% of all malignant tumors, and it mainly occurs in patients of adult age. It is rare in the pediatric population with only 2% of all CM cases diagnosed in patients younger than 20 years.²⁻⁴ In the childhood/adolescent population, the majority of CM are diagnosed among adolescents and only 8% occur in infancy and childhood.^{5,6}

Differences exist in the clinical aspects, histopathological features, and disease staging comparing childhood/adolescent CM to adult CM.^{2,7-8} CM in childhood are often amelanotic, show broad histopathological variability, and in some cases present with histologic uncertainty with ambiguous atypical characteristics that do not allow a definite classification as either a malignant or benign lesion.^{4,9} Children with CM present at a more advanced stage of disease with thicker lesions and higher rates of lymph node metastasis than their adult counterparts, leading to a worse prognosis.^{4,9} However, published studies report discordant data on survival rates.^{5,10}

It has long been debated whether adult and childhood/adolescent melanomas share a similar pathogenesis. Major risk factors for pediatric CM are giant congenital melanocytic nevi and hereditary conditions including xeroderma pigmentosum, immunodeficiency, and albinism.¹¹ Other known risk factors common to both pediatric and adult melanoma are family history of melanoma, dysplastic nevus syndrome, elevated number of acquired melanocytic nevi, red hair, sun-sensitive phenotype, and UV exposure.¹²⁻¹³

It is uncertain whether childhood/adolescent CM differs from adult CM with regard to predisposing genetic determinants. Pediatric CM are mostly sporadic cases, while adolescent CM are sometimes observed in melanoma-prone families. In general, there is a higher proportion of germline mutation carriers among young cancer patients,¹⁴ but whether this tendency holds true for CM is unclear due to the rarity of childhood/adolescent CM. Based on the few available studies, which by and large represent single-institution case series, childhood/adolescent patients have only rarely been found to carry germline mutations in the

two high-penetrance melanoma genes, *CDKN2A* (cyclin-dependent kinase inhibitor 2A) and *CDK4* (cyclin-dependent kinase 4)^{12,15-21} that are known to be significantly associated to melanoma only in a familial setting and not in a sporadic context.

The *MC1R* (melanocortin-1 receptor) gene is a key determinant of human pigmentation involved in melanin production.²² *MC1R* is highly polymorphic in the general population and specific variants can be defined as “R” (D84E, R142H, R151C, I155T, R160W, D294H) or ‘r’ (V60L, V92M, R163Q) alleles according to the strength of association with the red hair color (RHC) phenotype.²³ Extensive *in vitro* and *in vivo* evidence has shown that both R and r alleles produce hypomorphic proteins with compromised activity compared with native *MC1R* function.²² The R alleles are reported to have major impact on the pigmentation, and UV-sensitivity.^{22,23} In contrast, r variants confer normal or slightly impaired *MC1R* activity resulting in, a low strength of association with the fair skin phenotype.²³

Natural variation at *MC1R* is an established risk factor for CM across multiple populations worldwide.²⁴ Risk of CM is higher among *MC1R* variant carriers than among individuals with wild-type *MC1R*, with the strongest association observed among carriers of R alleles and carriers of multiple variants.²⁴ *MC1R* variants confer a significant increased risk in darkly pigmented individuals, highlighting the impact of *MC1R* through non-pigmentary pathways, including DNA repair mechanisms.^{25,26} Moreover, *MC1R* genotype is associated with phenotypic characteristics of melanomas²⁷ and of melanocytic nevi²⁸. In addition, *MC1R* variants have been shown to influence the somatic mutational load in adult CM, thus highlighting the interplay between germline and somatic genetics.²⁹

Childhood/adolescent CM patients have an elevated prevalence of *MC1R* variants, but the limited number of available studies coupled to the small number of case subjects per study make it challenging to draw clear conclusions regarding the role of *MC1R* in childhood/adolescent CM.¹⁸⁻²⁰ Thus, large-scale association studies are needed to investigate the importance of the *MC1R* gene in childhood/adolescent melanoma.

To help elucidate the role of *MC1R* in childhood/adolescent CM and to better understand the genetic and clinical diversity of childhood/adolescent and adult CM with

potential clinical impact in term of early melanoma detection and preventive strategies, we assess these tumors in a large multicenter pooled dataset established from the Melanocortin 1 receptor SKin cancer and Phenotypic characteristics (M-SKIP) Project, the Italian Melanoma Intergroup (IMI), and other European melanoma groups. The endpoints of our study were: (1) to compare the prevalence of *MC1R* variants between childhood/adolescent cases and unaffected controls with a case-control study design and (2) between childhood/adolescent and adult CM patients using a case-case study design.

Material and Methods

Study population

Our analysis included children and adolescents diagnosed with sporadic single primary CM at age ≤ 20 years, adult cases diagnosed with sporadic single primary melanoma at age ≥ 35 years, and unaffected adult controls. Since age is a continuous variable and an exact age cut-off between adolescents and adults would not be expected, we decided to exclude melanoma cases diagnosed in the age range 21-34 years, in order to avoid a possible overlap between categories and thus enable comparison between groups likely to have distinct clinical and genetic characteristics.

Because of the known challenges in diagnosing pediatric melanoma³⁰⁻³² and to decrease misdiagnosis in our analysis, participating investigators were asked to provide the original histopathological reports and representative glass slides for central and independent review by a dermatopathologist (D.M., F.F.). Only patients for whom the original histopathological report containing standard pathological information was available were eligible. In addition, we restricted the study to cases with completed *MC1R* genotyping. We excluded familial melanoma cases, defined as subjects with at least one first-degree relative or at least two second-degree relatives with melanoma, multiple melanoma cases, cases with a history of cancer at any site other than non-melanoma skin cancer, atypical spitzoid neoplasms/MELanocytic Tumors of Uncertain Malignant Potential (MELTUMP), ocular and mucosal melanomas.

Detailed information on recruitment of childhood/adolescent CM patients is reported in the Appendix pp 1-2. Ethics Committee approval was obtained at each institution in which new blood samples were drawn. For each childhood/adolescent CM case, four adult CM cases and four unaffected controls were randomly selected from the same parent study that gave rise to the childhood/adolescent case. When this was not possible, adult cases and unaffected controls were selected from a study that was conducted in nearest geographical proximity to the parent study that gave rise to the childhood/adolescent case (Appendix pp 1-2 and Appendix Table 1). A geographical representation of the recruitment area of childhood/adolescent cases, adult cases, and unaffected controls is shown in Figure 1.

Overall, we collected data on 367 childhood/adolescent cases, 8,582 adult CM cases, and 5,770 unaffected controls (Figure 1). For 59 childhood/adolescent patients, information on *MC1R* was not available either because of patients' death (N=2) or refusal to participate in the study (N=57). Among the remaining 308 patients, 75 had no original histopathological report available, leaving 233 children/adolescent cases for inclusion in the statistical analysis. For the selected 932 adult cases, 474 arose from the same parent study as the childhood/adolescent case and 458 came from a geographically close study population. For the selected 932 controls, 354 arose from the same parent study as the childhood/adolescent cases and 578 came from a geographically close study population.

Molecular analysis

For 135 childhood/adolescent patients from M-SKIP and 48 from IMI/European centers, *MC1R* sequencing had already been performed in study-specific laboratories (Appendix pp 5-6) all of these cases also had an available histopathological report. For the remaining 50 childhood/adolescent patients from IMI/European centers who provided new blood or saliva samples, *MC1R* genotyping efforts were centralized at the University of L'Aquila. DNA extraction and the mutational screening of *MC1R* gene (NCBI accession NG_012026.1) were performed as previous described.³³

Statistical analysis

A complete description of the statistical analysis is presented in the Supplemental Appendix pp. 2-4. Briefly, the associations between risk factors and childhood/adolescent melanoma were analysed by logistic regression in comparison with two reference groups, (1) adult cases and (2) unaffected controls, with adjustment for study/geographical location.

The frequency of any *MC1R* variants among children/adolescents was compared to that among adults and controls by logistic regression analysis with adjustment for study/geographical location. These comparisons were repeated for any *MC1R* R variant, any r variant, a score calculated by summing across the *MC1R* alleles giving a value of 1 to “r” and 2 to “R” variants, as previously proposed,³⁴ and for each of the nine most prevalent *MC1R* variants and of any rare *MC1R* variants (presence/absence). We then used multivariable unconditional logistic regression models to calculate the odds ratio (OR) and corresponding 95% confidence interval (CI) for *MC1R* variants using *MC1R* wild-type as the reference category after adjusting for study/geographical location and other covariables (as available), including sex, melanoma body site, histopathological subtype, hair color, and skin type. Sensitivity analysis with multivariable conditional logistic regression models was also performed.

The primary analysis compared the entire sample of childhood/adolescent cases to unaffected controls and adult cases. In order to take into account the possible misdiagnosis in childhood/adolescent cases, we repeated the primary analysis including only the subgroup of childhood/adolescent patients with CM diagnosis confirmed after central slide review; and then we calculated a modified ORs, applying the method proposed by Green³⁵ that incorporates adjustment based on the predictive value of a positive test.

Sensitivity analysis on the subgroup of childhood/adolescent and adult cases arising from the same parental study, and after the exclusion of patients without confirmed diagnosis from the main analysis were also conducted. Subgroup analyses were done according to age at diagnosis of childhood/adolescent cases.

Generally, p-values <0.05 were considered statistically significant. However, we also calculated False Discovery Rate (FDR) corrected p-values to take into account multiple comparisons. The analysis was carried out by using the software SAS (version 9.4) and STATA (version 13).

Results

Table 1 reports population characteristics of childhood/adolescent cases, adult cases, and unaffected controls. Median age (interquartile range) was 18 years (15-19 years) in the children/adolescents, 55 years (45-67 years) in the adult case group, and 50 years (43-59 years) in the unaffected control group. Among childhood/adolescent cases, 52 (22%) were aged ≤ 14 years, 96 (41%) were between 15 and 18 years old, and 85 (37%) were >18 years. Females accounted for 59% of the childhood/adolescent group, 51% of the adult group ($P=0.03$), and 46% of the control group ($P=0.0003$). Comparing the childhood/adolescent cases to adult cases and to controls, the total count of common melanocytic nevi was higher among childhood/adolescent patients [30 nevi (range 15-64)] than among either adult patients [25 nevi (10-45)] ($P=0.0007$) or controls [21 nevi (5-30), $P<0.0001$] groups. A higher proportion (43%) of children/adolescents cases had atypical melanocytic nevi than did adult cases (32%) and controls (9%) ($P=0.01$ and $P<0.0001$, respectively). Five percent and 11% of melanomas occurred on the upper limbs and 34% and 29% on the lower limbs in children/adolescents and adults, respectively ($P=0.04$). No lentigo maligna melanoma (LMM) subtype was diagnosed in the children/adolescent group, whereas 7% of adults had LMM. A spitzoid melanomas was identified in 13 (7%) childhood/adolescent cases compared to 2 (0%) adult cases; 21 (11%) children/adolescents had other specified types of melanoma compared with 8 (1%) adult cases ($P<0.0001$). Children/adolescents less frequently (36%) had blue eyes compared to adults (50%; $P=0.01$) or controls (47%, $P<0.0001$), and they were less likely (15%) to have solar lentigines compared to adults (75%, $P<0.0001$) and controls (68%, $P<0.0001$).

Table 2 shows frequencies of any *MC1R* variants, any R variants, any r variants, *MC1R* score, and any of the nine most prevalent *MC1R* variants in 233 childhood/adolescent cases, 932 adult cases, and 932 controls. In univariable analysis, no significant differences were observed in frequency of *MC1R* variants between childhood/adolescent and adult cases. However, childhood/adolescent cases had significantly higher frequency of any variants, R variants, r variants, and *MC1R* score than unaffected controls, confirming the role of *MC1R* in melanoma susceptibility. Eight rare *MC1R* variants (i.e. other than the nine most prevalent

variants separately analyzed) were found in childhood/adolescent patients: 86insA (N=2), V51A, T95M, V122M, R151H, A218T, F258L, K278E, (N=1 each). No association was found between childhood/adolescent melanoma and any *MC1R* rare variant (data not shown).

Among the 233 childhood/adolescent cases, the representative histopathological slides of the tumor were available for 85 patients and were centrally reviewed for quality control by one dermatopathologist (D.M.). The group of 85 patients had similar clinico-pathological characteristics compared to the 148 for whom glass slides were not reviewed (Appendix Table 2). The original diagnosis of melanoma was confirmed in 64/85 (75%) cases. The remaining slides from 21/85 (25%) cases were deemed as not being representative or difficult to interpret for technical reasons, or were reclassified as atypical melanocytic nevi, atypical junctional melanocytic proliferations, pagetoid melanocytosis overlying congenital nevi, or ambiguous atypical melanocytic proliferations with spitzoid features. In the latter cases, serial unstained slides or paraffin blocks were not available and so additional immunohistochemical and/or molecular analyses which would have clarified interpretation were precluded. Such doubtful cases were independently reviewed by a second dermatopathologist (F.F.); the conflicting discrepancy with the original diagnosis remained unresolved. The median Breslow thickness (interquartile range) was 1.00 mm (0.50-1.90) for the 64 cases with a confirmed diagnosis on histopathological review and 0.45 mm (0.10-0.75) for the 21 cases in which the original diagnosis was not confirmed ($P=0.0005$, Appendix Table 3). No other clinico-pathological features differed between the two groups (Appendix Table 3).

The frequencies of any *MC1R* variants, any R variants, any r variants, *MC1R* score, and any of the nine most studied *MC1R* variants in the subgroup of 64 children/adolescents with a confirmed diagnosis after histopathological review, 254 adults, and 254 controls are shown in Table 2 and are similar to those reported for the primary analysis (Table 2).

The OR (95%CI) estimated by unconditional multivariable logistic regression for the 233 children/adolescent CM cases and 932 adult CM cases (OR all patients), for the subgroup of 64 children/adolescent CM cases with a confirmed diagnosis after histopathological review and 256 adult CM cases (OR confirmed diagnosis) and after correction by the estimated

outcome misclassification rate (corrected OR) are shown in Figures 2 and 3. We found that children/adolescent melanoma had a significantly higher odds of carrying any *r* variants compared to adult cases (OR: 1.54; 95%CI: 1.02-2.33, FDR-corrected $P=0.17$, Figure 2). Concerning specific *MC1R* variants, we found a positive association for all *MC1R* variants with childhood/adolescent melanoma, except for the R160W variant (Figure 3). We found a statistically significant association for V60L and D294H variants (OR: 1.60; 95%CI: 1.05-2.44, FDR-corrected $P=0.17$, and OR: 2.15; 95%CI: 1.05-4.40, FDR-corrected $P=0.17$) in the primary analysis and after correction for possible misdiagnosis. Similar results were obtained in sensitivity analysis with conditional logistic regression models (Appendix Table 3) and by excluding the 21 children/adolescents without centrally confirmed diagnosis of melanoma (Appendix Table 4). Finally, when we repeated the primary analysis on the subgroup of childhood/adolescent and adult cases with an exact matching by study population, we obtained even stronger associations for carriers of any *MC1R* variant (OR: 2.04 95% CI: 1.19-3.50), *r* variants (OR: 2.61 95% CI: 1.43-4.73), V60L (OR: 2.67 95% CI: (1.44-4.95), and D294H variants (OR: 3.12 95% CI: 1.08-9.03) (Appendix Table 5).

Table 3 lists OR (95%CI) calculated for childhood/adolescent cases ≤ 18 and ≤ 14 years of age. A statistically significant higher frequency of *r* variants was observed in cases ≤ 18 years of age compared to adults (OR: 1.80; 95%CI: 1.06-3.07, FDR-corrected $P=0.61$). The corresponding OR for cases ≤ 14 years of age was even higher, but did not reach statistical significance because of the small number of subjects.

Appendix Figures 1 and 2 show the ORs (95%CI) obtained for the case-control analysis comparing childhood/adolescent melanoma patients with unaffected controls. Regarding OR obtained from the primary analysis, we found a significantly higher risk of childhood/adolescent melanoma for carriers of any *MC1R*, *R*, *r* and the most common *MC1R* V60L, V92M, R151C, R163Q and D294H variants. The results remained statistically significant after correction for multiple comparison except for the V92M variant (FDR-corrected $P=0.07$).

Discussion

Our pooled-analysis showed that natural variation at *MC1R* is a genetic risk factor for childhood/adolescent CM and that the frequency of *MC1R* *r* variants is elevated in this younger case group compared to adult CM cases. The impact of *r* alleles was confirmed in analyses limited to individuals aged ≤ 18 years and was even stronger for children ≤ 14 years, although this difference was not statistically significant. The *MC1R* V60L and D294H variants showed the most robust association with melanoma in childhood and adolescence, even after correction for possible misdiagnosis.

Childhood/adolescent melanoma has been reported to occur most commonly in whites and in females.^{2,10,13} According to two previous Australian studies, we found that childhood/adolescent melanoma patients are phenotypically characterized by a fairer phenotype compared to healthy controls,^{12,13} including traits such as red hair and skin type. In contrast, when compared to location-matched adult cases, childhood/adolescent patients presented with more darkly pigmented characteristics such as brown eyes, skin type III-IV and a lower prevalence of freckles. Consistent with the majority of published studies, our childhood/adolescent patients showed a high number of melanocytic nevi, both common and atypical lesions, and developed melanomas mainly on the lower extremities and the trunk.^{2,11,36} Among our cases, childhood/adolescent melanoma was more commonly diagnosed as nodular melanoma compared to the adult counterpart. Spitzoid melanomas were more frequently identified in childhood/adolescent patients, while LMM were only seen in adulthood. A higher prevalence of nodular and spitzoid subtypes have been previously reported mainly in pre-pubertal cases.^{2,4,37}

The impact of *MC1R* alleles in childhood/adolescent melanoma has been investigated in small series of patients.¹⁸⁻²⁰ *MC1R* variants were identified in 12 of 21 (57%) patients, with a higher frequency of *r* compared to *R* allele by Daniotti et al. (2009).¹⁹ More recently, two case series reported *MC1R* variants in 10 of 23 patients (43%)¹⁸ and in 4 of 6 patients (67%).²⁰ In our pooled analysis, *MC1R* variants were detected in 75% of childhood/adolescent patients.

Overall, multivariable analysis suggested that childhood/adolescent cases had greater odds to carry any *MC1R* variant and a significantly greater odds to carry *r* variants compared to adult cases. Our findings demonstrate a stronger role of *r* variants in childhood/adolescent than in adult melanoma, suggesting that *MC1R* might influence childhood/adolescent melanoma through biological pathways other than pigmentation and UV-sensitivity such as antioxidant defenses, DNA repair and melanocyte proliferation/differentiation.^{22,24,39} Interestingly, the odds of carrying *r* alleles increased in subgroup analysis limited to adolescents ≤ 18 years old, and was stronger still (although not statistically significant) among cases ≤ 14 years old, suggesting a higher prevalence of the *MC1R* variants in childhood melanoma.

In the present study, the *MC1R* variants V60L and D294H showed significantly higher prevalence in childhood/adolescent compared to adult melanoma. The role of V60L in adult melanoma is controversial and the magnitude of risk varies across the populations.³⁷ A positive association of V60L with melanoma status has been reported in the Mediterranean area, where this variant is the most frequent *MC1R* allele.³⁷ The D294H variant is common in individuals with the RHC phenotype. The association of this variant with melanoma risk demonstrates heterogeneity between Northern versus Southern European populations, where individuals who are more darkly pigmented are at higher risk of melanoma associated with D294H than Northern populations.⁴⁰

To the best of our knowledge, our current series of childhood/adolescent melanoma patients is the largest worldwide multicenter cohort published so far with available *MC1R* genetic data. The large numbers of childhood/adolescent and comparable adult melanoma subjects provide powerful estimates of the association between *MC1R* variants and childhood/adolescent melanoma within different populations. A further strength of our study was our centralized data quality control and statistical analysis pipeline that provided consistency across the numerous parent studies in defining and adjusting for important covariates. Histopathological centralized review of one third of the included subjects allowed us to calculate association estimates in a subset of children/adolescents with a histologically

confirmed diagnosis of melanoma and was helpful to calculate corrected risk estimates taking into account the issue of misdiagnosis.

In our main analysis, we grouped all childhood/adolescent patients into a single category to increase the statistical power. However, childhood/adolescent melanoma patients represent a heterogeneous group, including neonates, children and adolescents, with a variety of distinct presentations.⁹ Childhood melanoma may indeed differ from adolescent melanoma and both may differ from adult melanoma.⁴ Some studies on childhood/adolescent melanoma used standard age categorization,^{2,3,41} whereas others classified childhood/adolescent patients into pre- and post-pubertal cases.^{7,11,42} To further address heterogeneity between melanomas developed at different ages, we performed a stratified analysis for patients ≤ 14 and ≤ 18 years. Our non-significant findings among cases ≤ 14 years may have resulted from decrease power related to the small sample size (N=59) of this group, while a separate multivariable analysis limited to children ≤ 10 years of age was not possible due to the limited number of patients (N=23). In our childhood/adolescent sample we had an oversampling of more darkly pigmented cases from Southern European compared to Northern European origin, which may have resulted in relatively high frequencies of *r* variants, which tend to be more common in the Southern than in the Northern Europe.⁴³ However, because childhood/adolescent cases were compared with adult cases and unaffected controls from the same geographical areas, we do not believe this affected our results. Indeed, sensitivity analysis conducted in the subgroup of childhood/adolescent cases with adult cases sampled from the same parent study provided similar results. A centralized review of all melanomas would be desirable, but unfortunately it was not feasible due to the retrospective nature of the study. In order to limit disease misclassification, we excluded from the analysis patients whose histopathological reports were not available. We also provided risk estimates corrected for our observed misclassification rate among patients with histopathological centralized review, a group that was representative of the entire cohort of childhood/adolescent patients. Nevertheless, it should be noted that this correction could not be able to provide an exact

estimate of the associations as in a sample with only centrally confirmed diagnosed cases, and a certain imprecision of estimates could therefore not be ruled out. Because our cohort not include patients recruited from melanoma-prone families and the major familial melanoma genes are rarely mutated in childhood/adolescent cases,¹² we did not analyze *CDKN2A* and *CDK4* in our patients. Indeed, in cases of melanoma, *CDKN2A* mutations have been reported to be rare and no *CDK4* mutations have been reported.^{12,15,17,20} It is possible that other major melanoma predisposition genes may influence the risk of disease in children/adolescents, but our lack of genetic data on these genes, such as the *BAP1* (BRCA1 associated protein 1) gene prevented the analysis of possible gene–gene interactions. Finally, although we performed a relatively high number of statistical tests in univariable analysis, we allowed unadjusted *P*-values to guide the interpretation of our results. Given the exploratory rather than confirmatory nature of the present study, we believe that our approach of describing the tests of significance we performed, as advised by Perneger (1998),⁴¹ is appropriate. However, to directly address the issue of multiple testing, we also present FDR-corrected *P*-values.

In conclusion, our pooled analysis showed that natural variation at *MC1R* is a genetic risk factor for childhood/adolescent CM as well as for adult CM. A major role of *MC1R* variants, mainly *r* alleles, was suggested in childhood/adolescent compared to adult melanoma, possibly through a pigmentation-independent pathway. In addition, we observed a stronger effect of these *r* alleles when the analysis was restricted to melanoma patients aged less than 18 years.

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FIGURE LEGENDS

Figure 1. Flow chart of melanoma cases included in the analysis and their geographical area of recruitment. A, adult melanoma patients; Ch/Ad, children/adolescents melanoma patients; CM, cutaneous melanoma; Co, unaffected controls; *MC1R*, melanocortin-1 receptor gene.

Figure 2. Covariable-adjusted OR (95%CI) for the association between any *MC1R* variants, R and r variants and childhood/adolescent melanoma compared to adulthood melanoma.

All the OR were adjusted by sex, matching stratum variable, melanoma body site and histopathological subtype, hair color and skin type. For each OR, the comparison groups included childhood/adolescent patients frequency matched 4:1 with adult cases by study/geographical area. The reference category for OR were *MC1R* wild-type (WT) subjects. Number of children/adolescents and adults reported here are the total number of subjects included in each analysis, independently by *MC1R* status. Note that for the analysis on any R variant vs WT, subjects carrying only r variants were excluded, and vice versa for the analysis on any r variant vs WT.

^aOR calculated on the subgroup of subjects with confirmed diagnosis of melanoma after centralized pathological review of glass slides. ^bOR calculated on the whole sample of N=233 childhood/adolescent cases. ^cOR corrected by probability of misdiagnosis combining information from OR(a) and OR(b) as previously suggested (51).

MC1R, melanocortin-1 receptor; CI, Confidence Intervals; OR, Odds Ratio. R variants include the D84E, R142H, R151C, I155T, R160W, D294H and other rare variants classified as R according to the algorithm proposed by Davies et al (2012);³⁴ r variants include the V60L, V92M, R163Q and other rare variants classified as r according to the algorithm proposed by Davies et al (2012).³⁴

Figure 3. Covariable-adjusted OR (95%CI) for the association between the nine most prevalent *MC1R* variants and childhood/adolescent melanoma compared to adulthood melanoma.

All the OR were adjusted by sex, matching stratum variable, cancer body site and histological type, hair color and skin type. For each OR, the comparison groups included childhood/adolescent patients frequency matched 4:1 with adult cases by study/geographical area. The reference category for OR were *MC1R* wild-type (WT) subjects. Number of children/adolescents and adults reported here are the total number of subjects included in each analysis, independently by *MC1R* status. Note that for the analysis on each variant vs WT, subjects carrying only other *MC1R* variants were excluded.

^aOR calculated on the subgroup of subjects with confirmed diagnosis of melanoma after centralized pathological review of glass slides. ^bOR calculated on the whole sample of N=233 childhood/adolescent cases. ^cOR corrected by probability of misdiagnosis combining information from OR(a) and OR(b) as previously suggested.³⁵

MC1R, melanocortin-1 receptor; CI, Confidence Intervals; NC, not calculable; OR, Odds Ratio. R variants include the D84E, R142H, R151C, I155T, R160W, D294H and other rare variants classified as R according to the algorithm proposed by Davies et al (2012); r variants include the V60L, V92M, R163Q and other rare variants classified as r according to the algorithm proposed by Davies et al (2012).³⁴

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