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: Germline variants in *MC1R* may increase risk of childhood/adolescent melanoma, but a clear conclusion is challenging because of the limited number of studies and cases. We evaluated the association of *MC1R* 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 unaffected controls.

Methods: Phenotypic and genetic data on 233 childhood/adolescent (≤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), also when analysis was 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 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* variants. .

Interpretation: Our pooled-analysis 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 with a potential clinical relevance in developing early melanoma detection and preventive strategies.

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Introduction

Cutaneous Melanoma (CM) mainly occurs in patients of adult age and is rare in the pediatric population, with only 2% of all CM cases diagnosed in patients younger than 20 years.[1-4] 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 clinical aspects, histopathological features and disease staging comparing childhood/adolescent CM to adult CM.[2,7-8] CM in childhood is often amelanotic, shows broad histopathological variability and may present with histologic uncertainty and ambiguous atypical characteristics that do not allow a definite malignant or benign classification.[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 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 genetic predisposition. Pediatric CM is mostly sporadic, while adolescent CM is 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, , childhood/adolescent patients have only rarely been found to carry germline mutations in the two high-penetrance melanoma genes, *CDKN2A* and *CDK4* [12,15-21] that are known to be significantly associated to melanoma only in a familial and not in a sporadic context.

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” (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 showed 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 pigmentation and UV-sensitivity.[22,23] In contrast, r variants confer normal or slightly impaired MC1R activity resulting in a low strength 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 for carriers of *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 in darkly pigmented individuals, highlighting the impact of *MC1R* through non-pigmentary pathways.[25,26] Moreover, *MC1R* genotype is associated with phenotypic characteristics of melanoma[27] and melanocytic nevi[28] and seems to influence the somatic mutational load in adult CM.[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 cases per study makes challenging to draw clear conclusions.[18-20]

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 terms of early melanoma detection and preventive strategies, we assessed 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 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 with sporadic single-primary CM 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 excluded melanoma cases diagnosed in the age range 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 pediatric 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. In addition, 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/MELanocytic Tumors of Uncertain Malignant Potential, ocular and mucosal melanomas.

Detailed information on recruitment 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 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 controls were selected from a study that was conducted in the nearest geographical proximity to the parent study of the childhood/adolescent case (Appendix, pp 1-2 and Appendix, pp 5-6). A geographical representation of the recruitment area of childhood/adolescent cases, adult cases and controls is shown in Figure 1.

Overall, we retrospectively collected data on 367 childhood/adolescent cases, 8,582 adult CM cases, and 5,770 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). For the remaining 50 childhood/adolescent patients from IMI/European centers who provided new blood or saliva samples, *MC1R* genotyping was centralized at the University of L'Aquila and performed as previously described.[33]

Statistical analysis

A complete description of the statistical analysis is presented in the Appendix, pp. 2-4. Briefly, the associations between risk factors and childhood/adolescent melanoma were analyzed 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 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) for *MC1R* variants 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 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 Green[35] 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 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.

Role of the funding source

No sponsor had role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Table 1 report populations' characteristics. Briefly, median age (interquartile range) was 18 years (15-19) in the children/adolescent case group, 55 years (45-67) in the adult case group and 50 years (43-59) in the control group. Among childhood/adolescent cases, 52 (22%) were aged ≤14 years, 96 (41%) between 15 and 18 and 85 (37%) >18. The total count of common melanocytic nevi was higher among childhood/adolescent patients [30 (range 15-64)] than among either adult patients [25 (10-45)] (P=0.0007) or controls [21 (5-30), P<0.0001]. 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). 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 than 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 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, 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 p 7). 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 and 0·45 mm (0·10-0·75) for the 21 cases in which the original diagnosis was not confirmed (*P*=0·0005, Appendix p 8). No other clinico-pathological features differed between the two groups (Appendix p 8).

The frequencies of *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) 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 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). 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) was found in the

primary analysis and after correction for possible misdiagnosis. Similar results were obtained in sensitivity analysis with conditional logistic regression models (Appendix p 8) and by excluding the 21 children/adolescents without centrally confirmed diagnosis (Appendix p 9). Finally, when we repeated the primary analysis on the subgroup of childhood/adolescent and adult cases arising from the same parental study, 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 p 11).

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 was even higher, but did not reach statistical significance because of the small number of subjects.

Appendix pp 12-13 show the ORs (95%CI) obtained for the case-control analysis comparing childhood/adolescent melanoma patients with 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. 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 *MC1R* variants are a genetic risk factor for childhood/adolescent CM and that the frequency of *r* variants is elevated in this young case group compared to adult CM cases. The impact of *r* alleles was confirmed in analyses limited to individuals ≤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] In line with two previous studies, we found that childhood/adolescent melanoma patients are 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, and developed melanomas mainly on the lower extremities and the trunk.[2,11,36] 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..'

The impact of *MC1R* alleles in childhood/adolescent melanoma was investigated in small series of patients.[18-20] *MC1R* variants were identified in 12/21 (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 10/23 patients (43%)[18] and in 4/6 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. 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.

Our findings demonstrate a stronger role of MC1R *r* variants in childhood/adolescent than in adult melanoma, suggesting the involvement of biological pathways other than pigmentation and UV-sensitivity such as antioxidant defenses, DNA repair and cell proliferation .[22,24,37] Indeed, MC1R signaling is crucial for melanocyte key processes [38], as suggested by the findings of Baron al (2014), demonstrating that *MC1R* variants combined with HERC2/ OCA2 alleles determine the number of nevi >2 mm in sunburned kids.[39]

Herein 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 populations.[40] A positive association of V60L with melanoma has been reported in the Mediterranean area, where this variant is the most frequent .[40] The D294H variant is common in individuals with the RHC phenotype. The association of D294H 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.[41]

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

children/adolescents with a histologically confirmed diagnosis and was helpful to calculate corrected risk estimates taking into account the issue of misdiagnosis.

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,] 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 decreased 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 more darkly pigmented cases from Southern European compared to Northern European origin, which may have resulted in relatively high frequencies of *r* variants, more common in Southern than in Northern Europe.[42] However, because childhood/adolescent cases were compared with adult cases and 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 did not include familial melanoma patients and the major susceptibility genes are rarely mutated in childhood/adolescent cases,[12,15,17,20] we did not analyze *CDKN2A* and *CDK4* in our

patients. It is possible that other major melanoma predisposition genes may influence the risk of disease in children/adolescents, but lack of genetic data on these genes, such as the *BAP1* gene prevented the analysis of possible gene–gene interactions. Finally, although we performed a relatively high number of statistical tests, we allowed unadjusted *P*-values to guide the interpretation of our results. Given the exploratory rather than confirmatory nature of this study, we believe that our approach of describing the tests of significance we performed, as advised by Perneger (1998),[43] 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 the *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.

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

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.

[a]OR calculated on the subgroup of subjects with confirmed diagnosis of melanoma after centralized pathological review of glass slides. [b]OR calculated on the whole sample of N=233 childhood/adolescent cases. [c]OR 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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