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

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Summary

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

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

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

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

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Research in context

Evidence before this study

The development of melanoma in children and adolescents has been hypothesised to have a stronger genetic component than that of melanoma in adults. We searched PubMed for studies published up to July 31, 2018, on melanoma susceptibility in paediatric patients, without language or date restrictions. We used the search terms “pediatric melanoma” OR “childhood melanoma” AND “genetics”. We found that genetic predisposition for melanoma has been poorly investigated in childhood and adolescence because of the rarity of the disease. Most published research included few cases, mainly from single-institution cohorts, investigating the main susceptibility genes for melanoma CDKN2A (cyclin-dependent kinase inhibitor 2A), CDK4 (cyclin-dependent kinase 4), and MCIR (melanocortin 1 receptor). The overall findings reported a marginal role in paediatric patients of the two major melanoma susceptibility genes, CDKN2A and CDK4. By contrast, a high frequency of germline variants has been identified in the intermediate-penetrance MCIR gene, but the very low number of paediatric cases of melanoma made any significant conclusions impossible. Therefore, we hypothesised that a large-scale association study could explore the importance of the MCIR gene in paediatric melanoma predisposition.

Added value of this study

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

Implications of all the available evidence

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

Whether adult melanoma and childhood and adolescent melanoma share a similar pathogenesis has long been a subject of debate. Major risk factors for paediatric cutaneous melanoma include giant congenital melanocytic naevi and hereditary conditions such as xeroderma pigmentosum, immunodeficiency, and albinism. Other known risk factors common to paediatric and adult melanoma are family history of melanoma, dysplastic naevus syndrome, elevated number of acquired melanocytic naevi, red hair, sun-sensitive phenotype, and ultraviolet radiation (UV) exposure.11,12 It is uncertain whether childhood and adolescent cutaneous melanoma differs from the adult melanoma regarding genetic predisposition. The paediatric form of the disease is mostly sporadic, whereas adolescent cutaneous melanoma is sometimes observed in melanoma-prone families. In general, there is a higher proportion of germline mutation carriers among young patients with cancer than among older patients,5 but whether this tendency holds true for cutaneous melanoma is unclear because of the rarity of this disease occurring in children or adolescents. On the basis of the few available studies,13,15-20 child and adolescent patients have only rarely been found to carry germline mutations in the two high-penetrance melanoma genes, CDKN2A and CDK4, which are known to be significantly associated with melanoma in a familial context alone. The MCIR (melanocortin 1 receptor) gene is a key determinant of human pigmentation.21 MCIR is highly polymorphic in the general population, and specific variants were defined as R (Asp84Glu, Arg142His, Arg151Cys, Ile155Thr, Arg160Trp, Asp294His) or r (Val60Leu, Val92Met, Arg163Gln) alleles, according to their strength of association with the red hair colour phenotype.22 Extensive in vitro and in vivo evidence showed that both R and r alleles produce hypomorphic proteins with compromised activity compared with native MCIR function.23 The R alleles have been found to have a major effect on pigmentation and UV sensitivity.22,23 By contrast, r alleles confer normal or slightly impaired MCIR activity, resulting in a low-strength association with the fair skin phenotype.23 Natural MCIR variation is an established risk factor for cutaneous melanoma across multiple populations worldwide.24 The risk of cutaneous melanoma is higher for carriers of an MCIR variant than for wild-type individuals, with the strongest association among carriers of R alleles and multiple variants.25 MCIR variants confer a significant increased risk of cutaneous melanoma in darkly pigmented individuals, highlighting the effect of MCIR through non-pigmentary pathways.25,26 More recently, MCIR variant genotypes are associated with phenotypic characteristics of melanoma27 and melanocytic naevi28 and seem to influence the somatic mutational load in adult cutaneous melanoma.29 Young patients (aged 20 years or younger) with cutaneous melanoma have an elevated prevalence of MCIR variants, but the low number of available studies, coupled with the small
number of cases per study, makes drawing clear conclusions a challenge.16–20

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

Methods

Study design and participants

We analysed a large, multicentre cohort pooled from the M-SKIP Project, the IMI, and other European groups (appendix), including participants from 11 countries (Australia, Canada, France, Greece, Italy, the Netherlands, Serbia, Spain, Sweden, Turkey, and the USA; figure 1). Our analysis included children and adolescents diagnosed with sporadic single-primary cutaneous melanoma at age 20 years or younger, adult patients with sporadic single-primary cutaneous melanoma diagnosed at age 35 years or older, and healthy adult individuals as controls. Because age is a continuous variable, and an exact age cutoff between adolescents and adults would not be expected, we excluded melanoma cases diagnosed in the age range of 21–34 years to avoid a possible overlap between categories, and thus enable comparison between groups with distinct clinical and genetic characteristics. Because of the known challenges in diagnosing paediatric melanoma16–19 and to decrease misdiagnosis, participating investigators were asked to provide the original histopathological reports and representative glass slides for central review. Only patients for whom the original histopathological report was available were eligible. Additionally, we restricted the study to cases with complete MC1R genotyping. We excluded familial melanoma cases, cases with a history of cancer at any site other than non-melanoma skin cancer, atypical spitzoid neoplasms or melanocytic tumours of uncertain malignant potential, and ocular and mucosal melanomas.

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

Procedures

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

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

Statistical analysis

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

We compared the frequency of any MCIR variants among children or adolescents with that of adult patients and controls by logistic regression, with adjustment for study or geographical location. These comparisons were repeated for any MCIR R variant, for any r variant, for a score calculated by summing across the MCIR alleles, which gives a value of 1 to r and 2 to R variants (as proposed elsewhere), and for each of the nine most prevalent MCIR variants and any rare MCIR variants (presence or absence).
We then used multivariable unconditional logistic regression models to calculate the odds ratio (OR) for MC1R variants after adjusting for study or geographical location and other covariables (as available) including sex, melanoma body site, histopathological subtype, hair colour, and skin type. We also did a sensitivity analysis with multivariable conditional logistic regression models. Because of the retrospective and multicentre nature of the study, information on covariables was not available for all the patients. Covariables with more than 30% of missing data were not included in the models, whereas multiple imputation models were done for variables with less than 30% of missing data (appendix).

The primary analysis compared the entire sample of young patients with adult controls and adult patients. Considering the possible misdiagnosis in young patients, we repeated the primary analysis including only the young patients with adult controls and adult patients.

We generally, p values lower than 0.05 were considered statistically significant. However, we also calculated p values corrected for false discovery rate (FDR) to take into account multiple comparisons. We used SAS software (version 9.4) and STATA (version 15) for our analyses.

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

### Results
We retrospectively collected data up to Dec 31, 2016, of 367 young patients, 8582 adult patients, and 5770 adult controls (figure 2). For 59 young patients, information on MC1R was not available either because of patients’ death (two patients) or refusal to participate in the study (n=57). Among the remaining 308 patients, 75 had no original histopathological report available, leaving 233 young patients for inclusion in the statistical analysis. For the selected 932 adult patients, 474 were from the same parent study as the young patients and 458 came from a geographically close study population. For the selected 932 adult controls, 345 were from the same parent study

<table>
<thead>
<tr>
<th>All studied patients</th>
<th>Patients with centralised confirmed melanoma diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Young patients</strong></td>
<td><strong>Adult patients</strong></td>
</tr>
<tr>
<td>(n=233)</td>
<td>(n=932)</td>
</tr>
<tr>
<td>MC1R variants</td>
<td></td>
</tr>
<tr>
<td>Any MC1R variants</td>
<td>173 (74%)</td>
</tr>
<tr>
<td>Any R variants</td>
<td>86 (37%)</td>
</tr>
<tr>
<td><strong>Score</strong></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>60 (26%)</td>
</tr>
<tr>
<td>1</td>
<td>71 (30%)</td>
</tr>
<tr>
<td>2</td>
<td>64 (27%)</td>
</tr>
<tr>
<td>3</td>
<td>28 (12%)</td>
</tr>
<tr>
<td>≥4</td>
<td>10 (4%)</td>
</tr>
<tr>
<td>Val50Leu variants</td>
<td>77 (33%)</td>
</tr>
<tr>
<td>Asp84Glu variants</td>
<td>3 (1%)</td>
</tr>
<tr>
<td>Val92Met variants</td>
<td>30 (13%)</td>
</tr>
<tr>
<td>Arg142His variants</td>
<td>7 (3%)</td>
</tr>
<tr>
<td>Arg151Cys variants</td>
<td>30 (13%)</td>
</tr>
<tr>
<td>Ile155Thr variants</td>
<td>4 (2%)</td>
</tr>
<tr>
<td>Arg160Trp variants</td>
<td>21 (9%)</td>
</tr>
<tr>
<td>Arg189Gln variants</td>
<td>13 (6%)</td>
</tr>
<tr>
<td>Asp294His variants</td>
<td>19 (8%)</td>
</tr>
</tbody>
</table>
Articles

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

<table>
<thead>
<tr>
<th>n</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Any MC1R variants</strong></td>
<td></td>
</tr>
<tr>
<td>Confirmed diagnosis*</td>
<td>0.93 (0.46–1.90)</td>
</tr>
<tr>
<td>All patients†</td>
<td>1.38 (0.96–2.00)</td>
</tr>
<tr>
<td>Corrected†</td>
<td>1.53 (0.98–2.29)</td>
</tr>
<tr>
<td><strong>Any R variants</strong></td>
<td></td>
</tr>
<tr>
<td>Confirmed diagnosis*</td>
<td>0.90 (0.36–2.24)</td>
</tr>
<tr>
<td>All patients†</td>
<td>1.15 (0.72–1.86)</td>
</tr>
<tr>
<td>Corrected†</td>
<td>1.21 (0.70–2.37)</td>
</tr>
<tr>
<td><strong>Any r variants</strong></td>
<td></td>
</tr>
<tr>
<td>Confirmed diagnosis*</td>
<td>0.88 (0.39–2.01)</td>
</tr>
<tr>
<td>All patients†</td>
<td>1.54 (1.02–2.33)</td>
</tr>
<tr>
<td>Corrected†</td>
<td>1.75 (1.02–3.42)</td>
</tr>
</tbody>
</table>

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

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See Online for appendix
melanoma than those of the primary analysis for carriers of any MCIR variant (OR 2.04, 95% CI 1.19–3.50), r variants (2.61, 1.43–4.73), and Val60Leu (2.67, 1.44–4.95) and Asp294His variants (3.12, 1.08–9.03; appendix).

We did a subgroup analysis by age at diagnosis (patients who were 18 years or younger and patients who were 14 years or younger at diagnosis), and we observed a significantly higher frequency of r variants in patients aged 18 years or younger than in adult patients (OR 1.80, 95% CI 1.06–3.07; FDR-corrected p=0.07).

By contrast, a fairer phenotype than that of healthy controls, including that young patients with melanoma are characterised by a small number of patients.

We also did a case-control analysis comparing young patients with melanoma with healthy adult controls (appendix). We found a significantly higher risk of childhood and adolescent melanoma in carriers of any MCIR, R, and r variants, as well as for the most common MCIR Val60Leu, Val92Met, Arg151Cys, Arg163Gln, and Asp294His variants compared with that in healthy controls. Results remained significant after correction for multiple comparison, except for the Val92Met variant (FDR-corrected p=0.07).

Discussion

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

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

![Image](https://via.placeholder.com/150)

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

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

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

Our findings showed a stronger role of MC1R \(r\) variants in childhood and adolescent melanoma than in adult melanoma, suggesting an involvement of biological pathways other than pigmentation and UV-sensitivity, such as antioxidant defences, DNA repair, and cell proliferation.\(^ {22,24,37}\) Indeed, MC1R signalling was found to be crucial for melanocyte key processes,\(^ {39}\) showing that MC1R variants, combined with HERC2/OCAR2 alleles, can determine the number of naevi bigger than 2 mm in sunburned children.\(^ {18}\)

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

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

Young patients with melanoma represent a heterogeneous group, including neonates, children, and adolescents, with various distinct presentations.\(^ {9}\) Childhood melanoma might indeed differ from adolescent melanoma, and both might differ from adult melanoma.\(^ {4}\) To further address heterogeneity between melanomas developed at different ages, we did a stratified analysis for patients aged 14 years or younger and 18 years or younger. Our non-significant findings from the younger subgroup might have resulted from decreased power related to the small sample size (59 patients) of this subgroup, whereas a separate multivariable analysis limited to children aged 10 years or younger was not possible because of the low number of patients (23 patients). In our child and adolescent sample, we had more darkly pigmented patients from southern European countries than from northern European origin, which might have resulted in high frequencies of \(r\) variants, more common in southern Europe than in northern Europe.\(^ {4}\) However, because young patients were compared with adult patients and controls from the same geographical areas, we do not believe this affected our results. Indeed, a sensitivity analysis done in the subgroup of young patients with adult patients sampled from the same parent study provided similar results. A centralised review of all melanomas would be desirable but, unfortunately, it was not feasible because of the retrospective nature of the study. To reduce disease misclassification, we excluded from the analysis patients whose histopathological reports were not available. We also provided risk estimates corrected for our observed
misclassification prevalence among patients with histopathological centralised review, a group that was representative of the entire cohort of young patients. Nevertheless, we should note that this correction could not provide an exact estimate of the associations, as in a sample with only centrally confirmed diagnosed cases, and some imprecision of estimates could therefore not be ruled out. Because our cohort did not include patients with familial melanoma and the major susceptibility genes are rarely mutated in young patients,\textsuperscript{12,15,17,20} we did not analyse \textit{CDKN2A} and \textit{CDK4} genes in our patients. It is possible that other major melanoma predisposition genes might influence the risk of disease in children and adolescents, but the absence of genetic data on these genes, such as \textit{BAP1}, prevented the analysis of possible gene–gene interactions. Finally, although we did a high number of statistical tests, we allowed unadjusted \( p \) values to guide the interpretation of our results.

Because of the exploratory, rather than confirmatory, nature of this study, we believe that our approach of describing the tests of significance we did, as advised by Thomas V Perneger,\textsuperscript{44} is appropriate. However, to directly address the issue of multiple testing, we also presented FDR-corrected \( p \) values.

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

\textbf{Contributors}

CP and SR did the literature search. SR, SG, PM, PAK, JCG–B, HN, MTL, JL, IN–B, FS, and MCF contributed to study design. M–FA, FD, BB–dp, VH, AEC, HAC–C, SBG, RPG, LM, RZ, TD, NET, CBB, MB, SP, MP, EN, PG, CMe, AMM, MR, SB, EP, LKS, FBl, GG, AS, FO, FA, RF–d–M, PQ, GR, AR, EM, IS, PAK, MAP, and MCF contributed to data collection. DM and FF did the histopathological review. CP and CMa did the molecular analysis of \textit{MCIR} for new samples. FBs and SR analysed the data. CP, DM, SG, PAK, MTL, JL, MCF, and SR contributed to data interpretation. CP, FBs, and CMa wrote the manuscript. FB did the figures.

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Declaration of interests
We declare no competing interests.

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