UV-radiation and *MC1R* germline mutations are risk factors for the development of conventional and spitzoid melanomas in children and adolescents

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Summary

Background Genomic characterisation has led to an improved understanding of adult melanoma. However, the aetiology of melanoma in children is still unclear and identifying the correct diagnosis and therapeutic strategies remains challenging.

Methods Exome sequencing of matched tumour-normal pairs from 26 paediatric patients was performed to study the mutational spectrum of melanomas. The cohort was grouped into different categories: spitzoid melanoma (SM), conventional melanoma (CM), and other melanomas (OT).

Findings In all patients with CM (n = 10) germline variants associated with melanoma were found in low to moderate melanoma risk genes: in 8 patients *MC1R* variants, in 2 patients variants in *MITF*, *PTEN* and *BRCA2*. Somatic *BRAF* mutations were detected in 60% of CMs, homozygous deletions of *CDKN2A* in 20%, *TERT*p mutations in 30%. In the SM group (n = 12), 5 patients carried at least one *MC1R* variant; somatic *BRAF* mutations were detected in 8.3%, fusions in 25% of the cases. No SM showed a homozygous *CDKN2A* deletion nor a *TERT*p mutation. In 81.8% of the CM/SM cases the UV damage signatures SBS7 and/or DBS1 were detected. The patient with melanoma arising in giant congenital nevus (CNM) demonstrated the characteristic *NRAS* Q61K mutation.

Interpretation UV-radiation and *MC1R* germline variants are risk factors in the development of conventional and spitzoid paediatric melanomas. Paediatric CMs share genomic similarities with adult CMs while the SMs differ genetically from the CM group. Consistent genetic characterization of all paediatric melanomas will potentially lead to better subtype differentiation, treatment, and prevention in the future.

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Research in context

Evidence before this study

The aetiology of paediatric melanoma is still poorly understood. Previous studies have shown that conventional paediatric melanomas (CM) have similar genetic features to adult melanomas with driver mutations in *BRAF* and *PTEN*, kinase fusions, *CDKN2A* deletions and mutational signatures associated with UV damage. Spitzoid melanomas (SM), the second-most important group of paediatric melanomas, are known to carry fusions involving *NTRK1*, *ROS1* and *MET*. While there has been some progress describing the somatic landscape of paediatric melanoma, germline variants in known high-risk melanoma genes like *CDKN2A* and *CDK4* are only rarely found. Other genetic factors like *MC1R* and *MITF* are discussed to contribute to the melanoma risk in children. Due to the rarity and histological heterogeneity, additional studies are needed to further characterise paediatric melanomas.

Added value of this study

We collected a paediatric melanoma cohort consisting of 26 cases grouped into spitzoid, conventional and other melanoma. Matched tumour-normal exome sequencing was used to detect genetic variants in germline cancer predisposition genes, somatic mutations and mutational signatures. We were able to confirm previous findings in tumour tissue and identified UV damage mutational signatures not only in conventional but also in spitzoid melanomas which has not been investigated so far. Moreover, we found germline variants in at least one of the moderate melanoma risk genes (*MC1R, MITF, BRCA, PTEN*) in all CM patients and 41.7% of SM patients carried at least one *MC1R* variant. This indicates that genetic risk factors may play a more important role than previously anticipated in this patient group.

Implications of all the available evidence

By discovering UV signatures and a high prevalence of germline variants in low and moderate risk genes that may play a role in the development of paediatric melanoma, we improve the overall understanding of genetic features in different paediatric melanoma subtypes. Our findings have the potential to be developed into combinatorial genetic models that might be used to establish prevention strategies for individuals at risk.

Introduction

While melanomas are among the most common malignant tumours in adults, they occur very rarely in childhood and adolescence. In children under the age of 15 years the incidence is only 1.3–1.6 per million, however, an exponential increase in incidence at adolescent age leads to an already 10-fold higher rate at the age of 15–19 years.[1] The increase in cases with age is presumably correlated with a greater cumulative exposure to sunlight.[2] However, because of their rarity, little is known about the aetiology, biology and behaviour of these tumours which are classified into three major groups according to the current WHO classification (4th edition): i) conventional adult-type melanomas (CM); ii) spitzoid melanomas (SM); and iii) melanomas arising in congenital nevi (CNM).[3] Diagnosis and differentiation between spitzoid and conventional melanomas can be difficult because benign lesions may have melanoma-like features or, in contrast, conventional melanomas may present as amelanotic or raised and nodular.[4,5] Additionally, paediatric melanomas present differently than their adult counterparts and there is evidence that they may be divergent entities.[6–8]

Previous studies showed that CM has a similar genetic landscape compared to the adult melanoma with regards to driver mutations, mainly in *BRAF* and *PTEN*, kinase fusions, *CDKN2A* deletions, high mutational burden and UV-signature.[9–12] Data for SM is more sparse and it seems to be rather characterised by fusions including *NTRK1*, *ROS1*, and *MET*.[10] Due to its rarity, only data of few cases of the remaining subtypes (e.g., CNM) is available indicating a completely different pathogenesis including pathognomonic *NRAS* variants.[11] The causes for the occurrence of melanoma in children and adolescents remain elusive. The analysis of inherited genetic variants rarely reveals causative variants in known high-risk melanoma genes like *CDKN2A*, *CDK4* or in DNA repair pathways in sporadic paediatric melanoma.[13–15]

The aim of this study was to further characterise paediatric melanoma and to identify putative causative germline and somatic genetic alterations, which can be of relevance for differential diagnosis between SM and CM, as well as for treatment and surveillance.

Methods

Study cohort

The present study includes 26 childhood and adolescent patients who were diagnosed with paediatric melanoma and were registered at the German Registry for Rare Paediatric Tumours (STEP-Registry) or in the biobank of the Polish Paediatric Rare Tumours Study Group (PPRTSG) at the Medical University of Gdansk. The

STEP-Registry has registered 140 patients with diagnosis of SM, CM or OT between 2006 and 2021. Local pathologists of the treating hospitals were contacted; however, it was only possible to retrieve normal and tumour tissue from 24 patients out of them. The Polish Paediatric Rare Tumours Study Group (PPRTSG) contributed normal and tumour tissue from 5 patients. The histopathological diagnosis of the paediatric melanomas was established by the dermatopathologists of the cooperating centres. The samples were additionally evaluated histopathologically by a reference dermatopathologist of the STEP-Registry and manually assigned to one of three subgroups SM, CM, or OT following the current WHO classification (4th edition).[3] Inclusion criteria were: age ≤21 years, diagnosis of SM, CM or OT, reference pathology, availability of tumour tissue and normal tissue, written consent (exception see below). The databases of the STEP registry and the Polish Paediatric Rare Tumours Study Group (PPRTSG) contain patient and tumour characteristics as gender, age at diagnosis, histology, symptoms, localisation, TNM classification, Clark level, Breslow thickness, staging, therapy modalities, events, outcome, last follow-up, pre-existing diseases, history of sun burns, and family history. The data was documented by the treating hospitals; and the quality of the data was verified by comparison with physicians' letters and surgery, radiology and pathology reports. Events during therapy (progression, relapse, second malignancy or death) were documented. The status of patient and disease status were determined at the end of therapy and during follow-up appointments.

Ethics

Our study complies with the guidelines of the Declaration of Helsinki, and the local ethical board of the University of Tübingen approved the study (Project ID: 786/2018BO2). All patients and/or legal guardians of the patients had given written informed consent to conduct research on tumour and normal tissue samples, and to provide clinical data on patient and tumour characteristics. Written informed consent was waived for patients who already died or who were diagnosed over 5 years ago by the ethical committee due to the rarity of the disease.

Whole exome sequencing

The tumour was identified in a H&E stained slide and micro dissected. DNA of 29 tumour-normal pairs was isolated from 5 µm tumour and normal tissue paraffin sections using the Maxwell® RSC DNA FFPE Plus Kit (AS1720) and the Maxwell® RSC Instrument (Promega, Madison, WI, USA) according to the manufacturer's instructions. Three samples had to be excluded due to contamination. Sequencing libraries were prepared on a Beckman Coulter Biomek i7 using the Twist Library Preparation Enzymatic Fragmentation (EF) Kit and the Twist Hybridization and Wash Kit (Twist Bioscience) with an optimised manufacturer's protocol. In brief, 50 ng of genomic DNA, which was sheared enzymatically into 150–200 bp fragments, underwent end-repairing, A-tailing and ligation with indexed adapters sequentially. Finally, libraries were amplified by PCR and purified for target enrichment. Different libraries with unique indices were pooled together in desirable ratios for up to 2 µg of total library input. A custom Whole Exome Target Enrichment Panel (Twist Bioscience) was used for target capture that included additional regions for microsatellite instability detection, promoter regions of selected genes, oncoviral sequences and intronic regions for fusion detection (Supplementary Table S1). Subsequently, paired-end sequencing (2 × 100 bp) was performed on an Illumina NovaSeq6000 platform (Illlumina, San Diego, CA, USA) or on a DNBSEQ-G400 platform (MGI, Shenzen, China) as specified by the manufacturer.

Data analysis

Sequencing data was analysed for single-nucleotide variations (SNVs), small insertion/deletions (indels), structural variations (SVs) and copy number variations (CNVs). Raw data was processed using the megSAP data analysis pipeline (https://github.com/imgag/megSAP, version 2021_12) and the ngs-bits package (https://github.com/imgag/ngs-bits, version 2022_04). Briefly, sequencing reads were aligned to the human reference genome (GRCh38) with BWA-MEM2.[16] Somatic variants were called using Strelka2 and annotated with VEP and various other databases.[17,18] To obtain high-confidence variant lists, variants were required to have a tumour and normal depth-of-coverage of at least 20x, an allele frequency of 5% or more and a minimum of 3 reads showing the alternative allele. In addition, in cases with low tumour content the hot spot mutations in driver genes were analysed manually. Somatic variants were classified following published guidelines.[19] Mutations in *TERT* promoter (TERTp) hotspots were analysed independently. Tumour mutational burden (TMB) was calculated based on a publication of Johnson et al. and included the amount of coding variants in the exome per megabase. Reference values for different tumour entities have been published.[20,21] Germline variants were called with freebayes (https://github.com/freebayes/freebayes) and annotated with VEP (https://github.com/imgag/ClinCNV). Copy number alterations were detected using ClinCNV (https://github.com/imag/ClinCNV). Custom filters were used to reduce the variants to high-quality variants. Variants were further classified according to the ACMG guidelines.[22] Pathogenic or likely pathogenic germline variants (class 4 and 5) are reported and will be referred to as PGVs in the following. An overview of the selected 265 cancer predisposition genes can be found in the supplement (Supplementary Table S1). *MC1R* variants were

classified according to Duffy et al.[23] Mutational landscape plot was generated using matplotlib and pymaf based on manually selected genes. Mutational signatures were calculated using SigProfiler tools and the COSMIC Mutational Signatures (version 3.2) for single base substitutions (SBS) and doublet base substitutions (DBS) and include the UV signatures with a high prevalence of C > T and CC > TT mutations.[9]

Statistics

A descriptive analysis of tumour and patient characteristics was performed. To analyze survival rates, the Kaplan-Meier-Method was used within IBM® SPSS® Statistics. OAS was defined as the interval between the date of diagnosis (surgery) and death/last follow-up.

We tested for a significant enrichment of low-allele-frequency variants (1%–10% allele frequency in gnomAD or in-house controls) in MC1R identified in 26 childhood patients within a large cohort of adult melanoma compared to a non-cancer control cohort using Fisher's exact test. To this end we selected an in-house control cohort consisting of 1431 rare eye disease patients and an in-house cohort of 365 adult melanoma cases. In brief, we extracted allele counts and number of affected cases/controls for the three low-AF variants V92M, R151C and R160W from adult melanoma and eye-disease controls from our in-house diagnostic NGS database consisting of cases analysed with whole-exome or whole-genome sequencing. We recorded AFs for each variant in the three cohorts a) childhood melanoma, b) adult melanoma and c) eye disease controls, as well as in gnomAD in Supplementary Table S2. Finally, we used Fisher's exact test to calculate p-values for the enrichment of alternative allele vs. reference allele counts in the combined MEL cohort (combining childhood and adult melanoma cases) compared to the eye-disease controls (Supplementary Table S2). Furthermore, we obtained the number of cases affected by a variant in at least one of the three variants in each of the three cohorts and calculated the significance of the enrichment of affected cases in adult melanoma compared to controls, again using Fisher's exact test. As a side note, we removed the variant R163Q (previously rated 'r') from our association testing, as it is according to gnomAD found in 63% of the East Asian and ~23% in the Latino/Admixed American as well as the European (Finish) population (https://gnomad.broadinstitute.org/variant/16-89919746-G-A?dataset=gnomad_r3), i.e., this variant has extremely high allele frequency in populations without a wide distribution of red hair. We similarly consider *MC1R* V60E to be a benign population variant with ~30% AF in Ashkenazi Jewish and ~13% AF in the Middle Eastern population and excluded it from the association testing (https://gnomad.broadinstitute.org/variant/16-89919436-G-T?dataset=gnomad_r3).

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The funders had no role in designing this study, data acquisition, analysis and interpretation, and decision to publish or prepare this manuscript.

Results

Twenty-six paediatric patients with melanoma were enrolled in our study. Twelve cases belonged to the SM group, ten to the CM group, and four cases to the OT group (1 melanoma arising in a giant congenital nevus (CNM), 1 pigment synthesizing melanoma, 1 melanoma arising in blue nevus and 1 intracerebral melanoma). The median age at diagnosis for the entire cohort was 12 (2–21) years. The median follow-up was 3.3 (0–15.6) years. There were more female patients (61.5%) than male. Most common sites were the extremities (50.0%) followed by head and neck (26.9%). Within the CM group, three patients presented with metastatic disease. Two of these patients had died and one patient was alive with disease one year after diagnosis at the time of the analysis. The 5-year overall-survival (OAS) in the CM group was 67%. All patients with SM were alive without disease (OAS 100% in 5 years). History of sun burns was either denied or unknown. The family history was positive for melanoma in 1 patient with SM (5 negative, 6 unknown) and 2 patients with CM (2 negative, 6 unknown). For more details on tumour and patient characteristics see Supplementary Table S2 in the supplement.

Genomic features of conventional melanoma

Of the ten patients with CMs, six (60%) harboured the somatic *BRAF* V600E mutation (Fig. 1). Three of the six CM patients with a *BRAF* mutation showed additional *BRAF* amplification. Those three patients were metastatic (PM044, PM062, PM061) and two of them have been treated with *BRAF*-inhibitors before, for the third metastatic patient we did not have any information on treatment. *PTEN* loss was also detected in one of the metastatic melanomas treated with *BRAF*-inhibitors (PM061). In these patients we sequenced the metastases, not the primary tumour.

A homozygous deletion of *CDKN2A* was seen in two (20%), and a heterozygous deletion of *CDKN2A* was seen in one advanced melanoma. *TERTp* mutations were detected in three (30%) cases; two also had an additional *BRAF* V600E mutation. An amplification of the *TERT* gene was detected in three cases (30%) and all of them had a coexistent *BRAF* V600E mutation. UV-induced DNA damage (Fig. 2) was found in all cases except two, one of those diagnosed with an acral subungual melanoma of the thumb and the other CM with predisposing germline variants in *BRCA2* and *PTEN*. The average tumour mutational burden in this patient group was 12.66 (0.37–49.72) Var/Mbp.

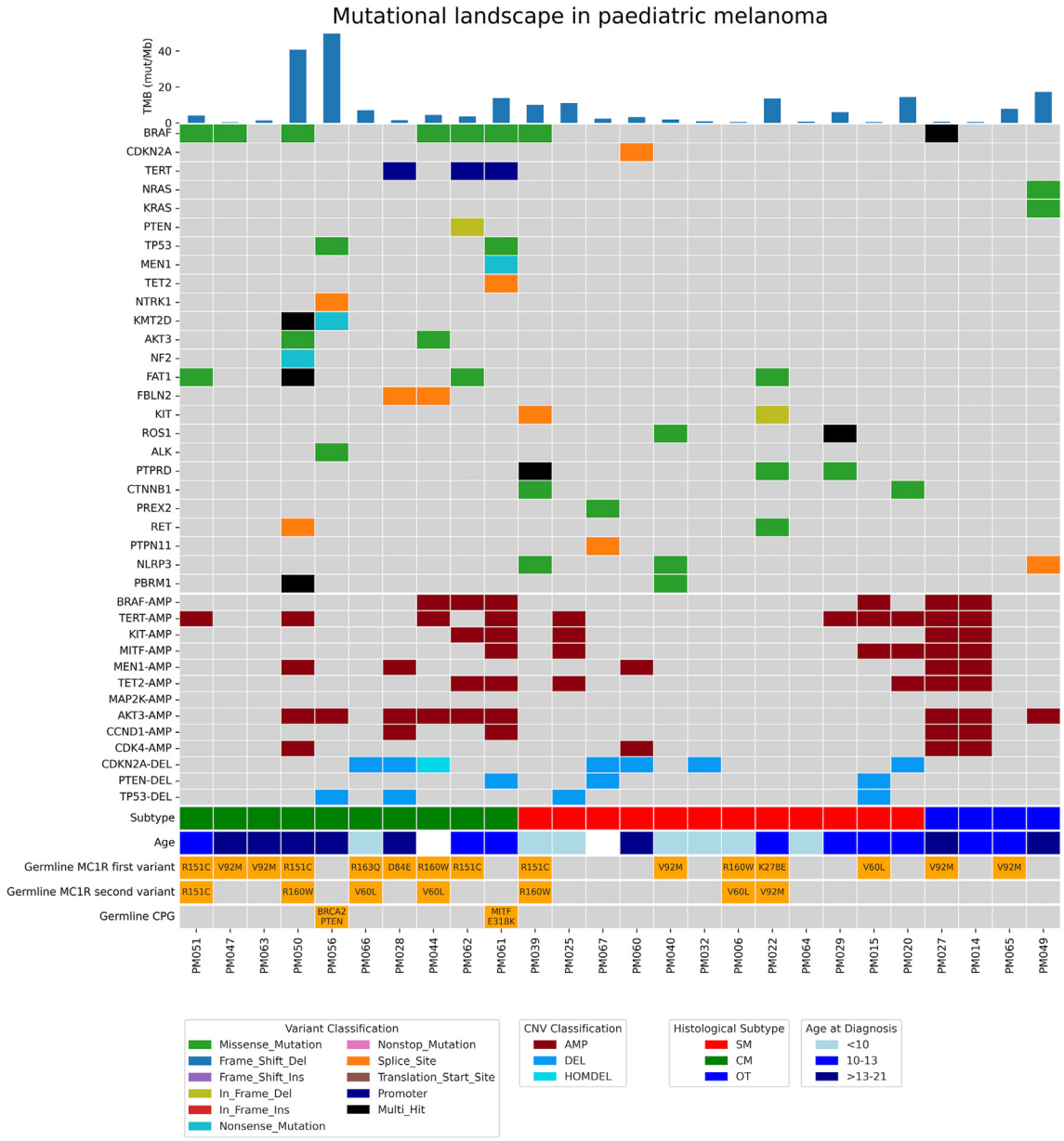

Fig. 1: **Whole exome sequencing analysis of genomic and clinical data of 26 paediatric melanomas: single nucleotide variations (SNV) and copy number variations (CNV) in selected cancer genes.** TMB = tumour mutational burden, AMP = amplification, DEL = deletion, LOH = loss of heterozygosity, SM = spitzoid melanoma, CM = conventional melanoma, OT = others, CPG = Cancer predisposition gene.

Genomic features of spitzoid melanoma

In our cohort of 12 SM patients none of the SM cases showed a homozygous *CDKN2A* deletion, 4 cases showed a heterozygous deletion of *CDKN2A*. No mutation of the *TERT*p was detected. The custom-designed enrichment allowed the detection of fusions in 3 cases ((*ALK-TPM4* and intergenic-*ALK* (PM006), *ROS1*-intergenic (PM025), and *MROH5*-intergenic (PM015)). In our SM cohort we identified signs of UV-induced DNA damage in all cases except for 2 (Fig. 2). The two cases that did not show signs of UV-induced DNA damage had both in common that the primary tumour site was the lower extremity whereas the primary tumour site of the cases with UV-signature was on the upper extremity and or head and neck. The average tumour mutational burden (5.74 Var/Mbp, range 0.44–14.42) was lower compared to the CM group. Of the 12 SM, one had the *BRAF* V600E mutation (7.6%). Previously described

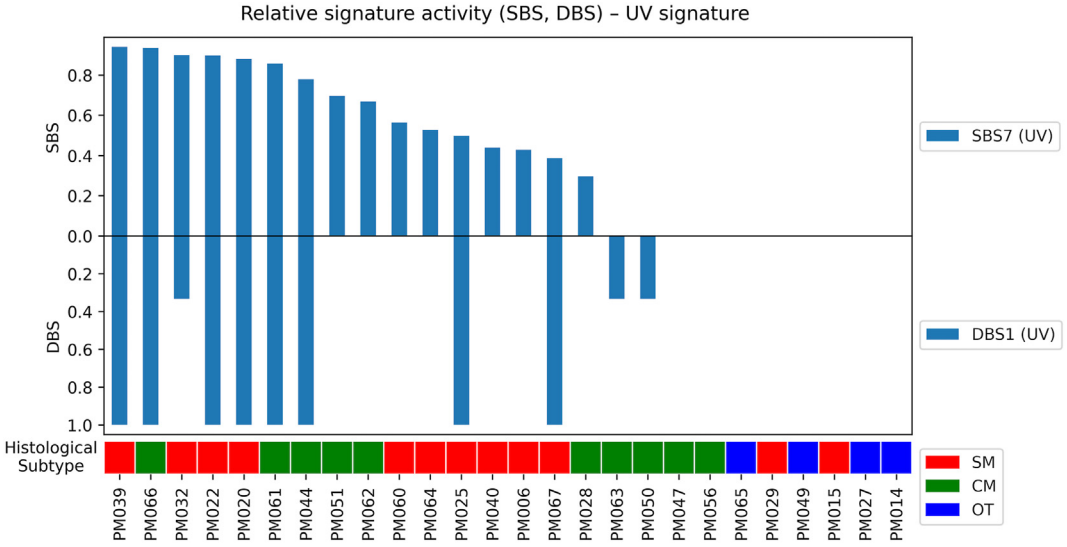

***Fig. 2:* Mutational signatures of paediatric melanomas.** Relative signature activity of COSMIC SBS 7 and DBS 1 UV signatures. SM = spitzoid melanoma, CM = conventional melanoma, OT = others.

hotspot mutations in *CTNNB1* (*S37F, S45F*) associated with adult melanoma were detected in two SM cases (16.7%).

Genomic features of rare melanoma subtypes

CNM is a very aggressive form of melanoma. We identified the typical NRAS Q61K mutation in tumour and the surrounding non-tumorous tissue at a lower frequency of 30%. A second KRAS D154N variation (uncertain significance) was identified. Many different large CNVs distributed over the genome were found in the patient. The TMB was high with 17.3 Var/Mbp (PM049). The tumour of the patient with an intracerebral melanoma showed two BRAF mutations on the same allele (BRAF V600E and W604L) and additionally an amplification of this BRAF-mutant allele. This patient received BRAF-inhibitor treatment. The TMB was 0.57 Var/Mbp (PM027). In a patient with pigment synthesizing melanoma no oncogenic mutation or fusion was detected. There was a copy number gain of the p-arm of chromosome 10. The TMB was 0.4 Var/Mbp (PM014). The case with melanoma arising in blue nevus did not show any of the known mutations in genes associated with melanoma. The TMB was 7.79 Var/Mbp (PM065). None of the OT melanomas showed signs of UV-induced DNA damage (Fig. 2).

Germline variants in cancer susceptibility genes

No pathogenic or likely pathogenic variants were found in high-risk melanoma genes, e.g., *CDK4, CDKN2A*. In our study, *MC1R* risk alleles were identified in 8 out of 10 patients of the CM group, four patients had two *MC1R* variants. Those two patients (PM056 and PM061) without *MC1R* variants were the patients with (likely) pathogenic germline variants in other (candidate) melanoma predisposition genes such as *BRCA2* (ENST00000380152.8, c.9253dup, p.Thr3085Asn*26), *PTEN* (ENST00000371953.8, c.800del, p.Lys267Arg*9), and *MITF* (E318K). In the SM group, 5 out of 12 patients carried at least one *MC1R* variant and 3 out of those 5 patients carried even two *MC1R* variants, respectively. The patient with the melanoma arising in blue nevus and the patient with the intracerebral melanoma carried the V92M *MC1R* variant each. In total, 15 out of all 26 (57.7%) melanoma patients carried at least one *MC1R* variant. In the CM subgroup, 80% of patients carried at least one *MC1R* variant (Fig. 1).

In total we identified seven variant loci in *MC1R* in our cohort, of which 2 are (ultra-) rare (gnomAD: K278E = 0.04% and D84E = 0.52%), and two are population variants with more than 10% allele frequency (AF) in the population variant database gnomAD and/or a large inhouse control cohort. Interestingly, the remaining three low-AF germline variants, V92M, R151C and R160W, show strongly increased AF in our patients (11.54%, 11,36% and 9.09%) compared to gnomAD (7.81%, 4.63% and 4.72%), i.e., up to 2.5-fold change. As our paediatric cohort is relatively small, we aimed at independently replicating this finding in an inhouse cohort of 365 adult melanoma patients, which we compared to an inhouse control cohort consisting of 1431 cases with non-cancer rare eye disease. We indeed found a highly significant enrichment of R151C in melanoma cases compared to non-cancer controls ($p = 0.002$, Fisher's exact test, Supplementary Table S3) and borderline significant enrichment of V92M

(p = 0.055, Fisher's exact test) and R160W (p = 0.053, Fisher's exact test). Taken together, 54% of paediatric melanoma and 49% of adult melanoma cases are affected by at least one of the three variants V92M, R151C and R160W, compared to only 37% of the controls (p = 0.0001, Fisher's exact test).

Discussion

To date, the aetiology and biology of paediatric melanomas are not fully understood, and it remains a challenge to correctly diagnose and treat them. In this study, we used exome sequencing for matched tumour-normal samples to obtain a comprehensive understanding of distinct genomic features and melanoma risk factors in the different groups of paediatric melanomas.

Melanoma risk and surveillance

The development of a melanoma involves a chain of mutational steps and it usually does not appear after the first sunburns as a child, but after years or decades. Nevertheless, in rare cases a melanoma occurs in early years, which makes it suspicious of tumour predisposition. In this sense, it is surprising that adult melanoma high-risk genes such as *CDKN2A* and *CDK4* seemingly do not play an important role in paediatric melanoma.[24] Our study confirmed this finding. Although no high-risk variants were identified, we could show a significant association for *MC1R* germline variants as well as UV-radiation in the development of paediatric melanoma.

The spectrum of pathogenic germline variants identified in our cohort differs from familial melanoma cases and is similar to those in mainly sporadic adult melanoma patients.[25,26] Other groups described variants in *MC1R* in up to 66% of individuals with melanoma while *MC1R* variants are more prevalent in childhood and adolescent melanoma than in adult melanoma.[27–29] *MC1R* variants are highly prevalent 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 colour (RHC) phenotype. Those nine *MC1R* variants were assigned as higher-risk based on elevated odds (OR ≥ 1.8) of melanoma among individuals with limited risk phenotypes and they are the most common *MC1R* variants and range in frequency between 0.5% and 11% in the general population.[29] In our study, the high rate of *MC1R* variants was especially pronounced for CM patients. Similar to a previous study,[14] we found the R151C variant to be enriched in paediatric melanoma cases (11.36% compared to 4.63% in gnomAD) and to be strongly associated with melanoma risk in an independent adult melanoma cohort (365 cases) in comparison to an inhouse control cohort of (p = 0.002, Fisher's exact test). In addition, we observe close to significant enrichments of V92M and R160W and found the rare variant D84E (gnomAD: 0.5%) in our small cohort of 26 children. Considered together, 54% of paediatric melanoma and 49% of adult melanoma cases are affected by at least one of the three variants V92M, R151C and R160W, compared to only 37% of the controls (p = 0.0001, Fisher's exact test). Based on this independent replication of the variant enrichment observed in paediatric melanoma in an independent adult melanoma cohort, we hypothesize that *MC1R* variants V92M, R151C and R160W are associated with risk (possibly in combination with UV-damage) for paediatric and adult melanoma. Although we observed slightly higher AFs for all three variants in paediatric compared to adult melanoma cases, these differences are not significant. However, the strongest enrichment of *MC1R* variants was found in paediatric CM with 80% affected patients, indicating an even higher impact of *MC1R* variants in early onset CM that calls for evaluation in a large paediatric CM cohort. This result supports that frequent and rare *MC1R* missense variants can be an independent risk factor for the development of early-onset melanoma, however, even larger scale genetic studies are required to accurately estimate the effect sizes and associated risks. Supporting the idea of a combinatory effect of genetic variants, *CDKN2A* carriers with more than one *MC1R* variant seem to have a higher melanoma risk than those with only a single *MC1R* variant.[30] Additionally, in one patient (PM061) with metastatic melanoma we identified the likely pathogenic *MITF* E318K variant, which has been associated with a 3- to 5-fold risk of developing melanoma, depending on personal or family history this risk can even be higher.[31] In another patient (PM056) with nodular melanoma a pathogenic *BRCA2* as well as a pathogenic *PTEN* variant was detected. Both genes are associated with melanoma-subordinate syndromes meaning that melanoma risk is elevated but lower than the risk of other associated cancers.[32]

Constitutional risk factors and environmental influences that can increase the risk of developing melanoma in childhood have been little studied. While the role of exposure to ultraviolet light causing DNA damage has already been described for CM, this study reported UV-induced DNA damage in SM patients.[33] Strouse et al. were able to describe constitutional factors that correlate with an increased risk of melanoma: the presence of numerous melanocytic nevi or dysplastic nevi, the inability to tan, fair skin, hair, and eyes, and severe freckling of the face.[34] These factors could not be investigated in our cohort; however, it should be noted that *MC1R* plays a central role in human pigmentation and thus may provide an explanation for the described correlation. The role of other constitutional factors and environmental influences needs further investigation.

Genetic features of different melanoma subtypes

The conventional melanoma group of our study is genetically very similar to the conventional melanoma in adulthood. In both groups, *BRAF* mutations and UV-induced DNA damage are frequent.[11,35] Interestingly, UV-signatures have been explained by the accumulation of UV-induced DNA damage over the lifetime. Few studies have looked at UV-induced DNA damage in childhood or adolescent CM cases[11,12,33] and surprisingly in most of the young CM patients UV-signatures were detected. Until now it remains unclear why those young patients are so susceptible to UV-induced damage and have a comparable high TMB. Additional prognostic markers in adult conventional melanoma like loss of *CDKN2A* or mutations and amplifications in *TERT*p can be found in advanced paediatric and adolescent CM patients as well.[12,36–40] In our cohort we found two patients with metastatic melanomas and mutations in *TERTp* and *BRAF* which in combination is known to be associated with a poor prognosis in conventional adult melanoma.[41] Those two metastatic cases have been treated with *BRAF*-inhibitors before which could explain the detected *BRAF* amplifications as a sign of developing *BRAF*-inhibitor resistance in the sequenced metastases. Another mode of *BRAF*-inhibitor resistance is *PTEN* loss that was also detected in one of the metastatic melanomas treated with *BRAF*-inhibitors (PM061). *BRAF*-amplifications and *PTEN*-loss have been described as one potential acquired resistance mechanism in adult conventional melanoma and were found in our study in childhood patients treated with *BRAF*-inhibitors.[42–45]

The SM subgroup in our cohort is not characterised by adult-type driver mutations except for two *CTNNB1* hotspot variants (S37F, S45F) that have been associated with different histological subtypes of adult melanoma.[46–48] Contrary to previous reports, we were not able to detect homozygous *CDKN2A* deletions or identify a *TERT*p mutation.[49] As described by other authors before, we found fusions to be relevant affecting the well-described genes *ROS1* and *ALK* and a new gene *MROH5*.[50] Finally, in this study we were able to report UV-induced DNA damage signatures in SM patients which is surprising in this young group of patients.

Other rare melanoma subtypes have very different genetic landscapes. In one patient with CNM the typical Q61K mutation in *NRAS* was found not only in tumour but also the surrounding presumably non-tumourous tissue[11,51–53] Possible postzygotic mosaicism has been discussed in a similar case.[53] The intracerebral melanoma case showed two different *BRAF* mutations but no signs of UV-induced DNA damage. Opposed to the other cases, in our patients with pigment synthesizing melanoma or melanoma arising in blue nevus we did not detect any characteristic findings to discriminate them from other subentities.

The added value of genetic characterisation is illustrated in a case that was histologically classified as atypical spitzoid tumour (PM039). In this patient, a *BRAF* V600E mutation, a very high UV signature activity and two *MC1R* variants were found. Fusions were absent. In accordance with the WHO classification and previous studies, the molecular test results would lead to a reclassification of the tumour as a low-CSD (cumulative sun damage) melanoma.[3,54] The differentiation between CM and SM is crucial, though, as a different therapeutic approach is needed.

Our findings suggest that conventional paediatric melanomas show genomic similarities with conventional adult melanomas including prognostic and predictive markers. UV-induced DNA damage seems to play a role in CM but also in the other entities of melanomas such as SM as shown in this study. Germline mutations in high-risk CPGs are rare in paediatric melanoma but variants in low to moderate melanoma risk genes are common and future studies will have to show if combinatorial genetic models will be helpful to establish prevention strategies. In conclusion, matched tumour-normal sequencing helps to identify different melanoma subtypes and supports treatment as well as preventive decisions. Consistent genetic characterisation of all paediatric melanomas will lead to better subtype differentiation, treatment stratification, and prevention in the future.

Contributors

A.L., investigation, data curation, formal analysis, project administration, writing-original draft. J.A., software, methodology, data curation, visualisation, writing-review and editing. S.A.E, formal analysis, investigation, writing-review and editing. H.W.: project administration, data curation, resources. A.G., software, methodology, data curation. O.S.K., methodology, formal analysis. C.Se., M.A., M.Sc., M.P., M.C., M.Ku., data curation. G.D., M.St., formal analysis, software. I.B., formal analysis, data curation, resources. O.R., resources, supervision, writing-review and editing. E.B., M.Kr., E.P., A.F., U.L.S., resources and data curation. E.J., resources. S.F., supervision, data curation, writing review-and editing. T.M., resources and supervision. T.K.E., D.T.S., resources, writing-review and editing. C.Sc., conceptualization, data curation, formal analysis, visualisation, supervision, writing review and editing, funding acquisition. S.O., conceptualization, funding acquisition, software, methodology, supervision, resources, writing-review and editing. I.B.B., conceptualization, resources, data curation, formal analysis, funding acquisition, project administration, visualisation, supervision, writing-review and editing. A.L., I.B.B., C.Sc. and S.O. reviewed the manuscript critically and conducted editing and rewriting. A.L., J.A., S.A.E., C.Sc., S.O., I.B.B., verified the underlying data. All authors have read and approved the final version of the manuscript.

Data sharing statement

The data obtained in this study are not publicly available due to the strict data protection regulations that apply to germline mutations of patients, but they can be obtained from the corresponding author on reasonable request.

Declaration of interests

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2023.104797.

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