university of groningen

University Medical Center Groningen

University of Groningen

# Multigene panel sequencing of established and candidate melanoma susceptibility genes in a large cohort of Dutch non-CDKN2A/CDK4 melanoma families.

Potjer, Thomas P; Bollen, S.; Grimbergen, Anneliese J.E.M.; van Doorn, Remco; Gruis, N. A.; Asperen, C.J.; Hes, F.J.; van der Stoep, Nienke; Dutch Working Group for Clinical Oncogenetics

*Published in:*
International Journal of Cancer

*DOI:*
10.1002/ijc.31984

**IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.**

*Document Version*
Publisher's PDF, also known as Version of record

*Publication date:*
2019

Link to publication in University of Groningen/UMCG research database

*Citation for published version (APA):*
Potjer, T. P., Bollen, S., Grimbergen, A. J. E. M., van Doorn, R., Gruis, N. A., Asperen, C. J., Hes, F. J., van der Stoep, N., & Dutch Working Group for Clinical Oncogenetics (2019). Multigene panel sequencing of established and candidate melanoma susceptibility genes in a large cohort of Dutch non-CDKN2A/CDK4 melanoma families. *International Journal of Cancer*, *144*(10), [31984]. https://doi.org/10.1002/ijc.31984

**Copyright**
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the “Taverne” license. More information can be found on the University of Groningen website: https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment.

**Take-down policy**
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

IJC
International Journal of Cancer

# Multigene panel sequencing of established and candidate melanoma susceptibility genes in a large cohort of Dutch non-*CDKN2A/CDK4* melanoma families

Thomas P. Potjer[1], Sander Bollen[1], Anneliese J.E.M. Grimbergen[1], Remco van Doorn[2], Nelleke A. Gruis[2], Christi J. van Asperen[1], Frederik J. Hes[1], Nienke van der Stoep[1], and on behalf of the Dutch Working Group for Clinical Oncogenetics[†]

[1]Department of Clinical Genetics, Leiden University Medical Centre, Leiden, the Netherlands
[2]Department of Dermatology, Leiden University Medical Centre, Leiden, the Netherlands

**Germline mutations in the major melanoma susceptibility gene *CDKN2A* explain genetic predisposition in only 10–40% of melanoma-prone families. In our study we comprehensively characterized 488 melanoma cases from 451 non-*CDKN2A/CDK4* families for mutations in 30 established and candidate melanoma susceptibility genes using a custom-designed targeted gene panel approach. We identified (likely) pathogenic variants in established melanoma susceptibility genes in 18 families ($n = 3$ *BAP1*, $n = 15$ *MITF* p.E318K; diagnostic yield 4.0%). Among the three identified *BAP1*-families, there were no reported diagnoses of uveal melanoma or malignant mesothelioma. We additionally identified two potentially deleterious missense variants in the telomere maintenance genes *ACD* and *TERF2IP*, but none in the *POT1* gene. *MC1R* risk variants were strongly enriched in our familial melanoma cohort compared to healthy controls (R variants: OR 3.67, 95% CI 2.88–4.68, $p < 0.001$). Several variants of interest were also identified in candidate melanoma susceptibility genes, in particular rare (pathogenic) variants in the albinism gene *OCA2* were repeatedly found. We conclude that multigene panel testing for familial melanoma is appropriate considering the additional 4% diagnostic yield in non-*CDKN2A/CDK4* families. Our study shows that *BAP1* and *MITF* are important genes to be included in such a diagnostic test.**

**Key words:** familial melanoma, genetic susceptibility, gene panel sequencing, *BAP1*, *MITF*, high-penetrance genes, candidate susceptibility genes, *OCA2*

**Abbreviations:** CM: cutaneous melanoma; MAF: minor allele frequency; UM: uveal melanoma

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

[†]Dutch Working Group for Clinical Oncogenetics: A. Wagner, L.E. van der Kolk, M.G. Ausems, T.A. Van Os, K.J, van Kaam, L. Spruijt, C.J. Dommering, P.C. van den Akker

**Conflict of interest**: None.

**Grant sponsor:** KWF Kankerbestrijding; **Grant numbers:** #UL 2015-7511 to TPP and NvdS and #UL 2012-5489 to RvD, NAG and NvdS

**DOI:** 10.1002/ijc.31984

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

**History:** Received 25 Aug 2018; Accepted 25 Oct 2018; Online 10 Nov 2018

**Correspondence to:** Nienke van der Stoep, PhD, Department of Clinical Genetics, Leiden University Medical Centre, Albinusdreef 2, 2333 ZA Leiden, the Netherlands, Tel.: 00-31-715269800, Fax: 00-31-715268276, E-mail: nvdstoep@lumc.nl

## Introduction

Cutaneous melanoma is the most aggressive type of common skin cancers and incidence has been increasing worldwide over the past decades.[1] With an age-standardized rate of 19.4 per 100.000, the Netherlands is among the countries with the highest incidence rates in the world, comparable to incidence rates in the northernmost European (Scandinavian) countries.[2] Well-established personal and environmental risk factors for melanoma include a fair skin type, having (many) atypical nevi, a high level of ultraviolet radiation exposure, and a history of sunburns in childhood.[3] A family history for the disease is also a significant risk factor and suggests a shared genetic predisposition among family members. This familial clustering occurs in approximately 5–10% of melanoma cases, and is referred to as familial melanoma.[4]

The major high-risk susceptibility gene for familial melanoma is *CDKN2A* and germline mutations are identified in 10–40% of familial cases.[5,6] In the Netherlands, a specific founder mutation in *CDKN2A*, known as p16-*Leiden* (c.225_243del, p.A76Cfs*64; RefSeq NM_000077.4), is the most frequent cause of familial melanoma (~80% of *CDKNA* mutations). Carriers of this mutation show not only a markedly increased risk for (multiple) cutaneous melanomas, but also for other cancers, especially pancreatic cancer and cancers of the upper respiratory tract (larynx, pharynx, oral cavity).[7,8] *CDKN2A* is an unusual gene in that it encodes two distinct

**What's new?**

**Germline mutations in *CDKN2A* are major contributors to familial melanoma. These mutations, however, are responsible for only 10 to 40 percent of genetic susceptibility in melanoma-prone families. In this study, 30 established and candidate melanoma susceptibility genes were investigated for associations with the disease in patients from 451 non-*CDKN2A*/*CDK4* melanoma families. From the candidate gene panel, (likely) pathogenic variants in *BAP1* and *MITF* were identified in several families, and potentially deleterious variants were identified in the shelterin complex genes *ACD* and *TERF2IP*. These genes appear to play a significant role in familial melanoma predisposition and are therefore promising candidates for incorporation into comprehensive genetic tests.**



proteins, p16INK4a and the alternatively spliced p14ARF, both of which are tumor-suppressors that act in two distinct pathways. The p16-retinoblastoma(Rb)-pathway controls cell-cycle G1-phase exit, while the p14ARF-p53 pathway induces cell cycle arrest or apoptosis.[9] Despite the major role of these pathways in melanoma susceptibility, only one other gene in the p16-retinoblastoma(Rb)-pathway, the *CDK4* gene, has been shown to be associated with familial melanoma, and only a small number of families with germline mutations in this gene have been identified to date.[10]

However, new melanoma susceptibility pathways have emerged in recent years.[5,6] Several high penetrance genes involved in telomere lengthening (*TERT*) or telomere maintenance (Shelterin complex: *POT1*, *ACD*, *TERF2IP*) have been identified, and mutations in these genes each account for approximately 1% of familial melanoma predisposition.[11–13] Furthermore, germline mutations in the BRCA1-associated protein (*BAP1*) gene cause a specific cancer predisposition syndrome mainly characterized by an increased susceptibility for uveal melanoma and malignant mesothelioma, but also including cutaneous melanoma, renal cancer, basal cell carcinoma and characteristic skin lesions called atypical Spitz tumors (AST) or melanocytic *BAP1*–mutated atypical intradermal tumors (MBAIT).[14] The *MITF* gene is a medium penetrance melanoma susceptibility gene and shows incomplete co-segregation with the phenotype. MITF is a basic-helix–loop–helix-leucine zipper transcription factor that has a key function in melanocyte homeostasis. Loss-of-function mutations in this gene cause auditory-pigmentary syndromes, such as Waardenburg syndrome type 2A (MIM #193510). However, a specific missense variant (c.952G>A, p.E318K; RefSeq NM_000248.3) located in a small-ubiquitin-like modifier (SUMO) consensus site impairs the SUMOylation of MITF, which results in a gain-of-function increase in *MITF* transcriptional activity. Carriers of this variant have an approximately three- to fourfold increased risk for melanoma and are more likely to develop multiple primary melanomas.[15] Several other cancers (renal cancer, pancreatic cancer) have also been reported in carriers of this variant.[16,17] In addition to these known high- and medium penetrance melanoma susceptibility genes, there are several well-established (common) variants in the lower penetrance *MC1R* gene that are associated with an increased risk for melanoma in the general population. *MC1R* encodes the receptor for α-melanocyte stimulating hormone (α-MSH), which plays an important role in skin pigmentation. Variants in *MC1R* that are most strongly associated with red hair color (RHC) confer an approximately twofold increased risk for melanoma (R variants), while other variants (r variants) show a weaker association with RHC (non-RHC) and confer a much smaller increase in risk for melanoma.[18] It has also been shown that both R and r variants in *MC1R* act as modifiers of melanoma risk in families with a *CDKN2A* germline mutation.[19] Furthermore, mutations in other cancer susceptibility genes have been recently reported in melanoma families in studies using mainly Whole Exome Sequencing (WES) technologies,[20–22] but the exact role of these and other candidate melanoma susceptibility genes in the familial setting remains unclear and requires further evaluation.

Although Dutch melanoma families are well characterized for *CDKN2A* and *CDK4* mutations,[23] no large scale investigation has yet been performed to identify (potential) deleterious variants in other established or candidate melanoma susceptibility genes. In the current study, we therefore sequenced a comprehensive panel of 30 (candidate) melanoma susceptibility genes in a large cohort of Dutch melanoma-prone families without a known *CDKN2A* or *CDK4* mutation. Our goal was to determine the frequency of pathogenic variants in established melanoma susceptibility genes and to investigate the role of a broad range of candidate susceptibility genes in familial melanoma.

## Patients and Methods

### Patient cohort

Both cutaneous melanoma (CM) and uveal melanoma (UM) patients were eligible for inclusion in the study if they had at least one other relative (up to third-degree) with CM and/or UM, and no previously identified pathogenic germline variant in the melanoma core genes *CDKN2A* or *CDK4*. Diagnostic sequencing of these two genes was performed at the Laboratory for Diagnostic Genome Analysis (LDGA) at the Department of Clinical Genetics of the Leiden University Medical Centre (LUMC), which has served as the primary sequencing facility for *CDKN2A* and *CDK4* in the Netherlands since 1998. In a small minority of referred families, the *CDKN2A* gene was only partly sequenced and/or the *CDK4* gene was not sequenced. Both genes were included in our research gene panel in order to exclude the presence of pathogenic variants in these genes. The study was approved by the LUMC Ethics Committee (#P15.341) and informed consent was obtained from all included individuals.

We initially selected 500 patients from 460 families for inclusion in the study. After critical re-evaluation of these

Table 1. Characteristics of the cohort

| Proband history | Family history | No. of families | No. of samples |
|---|---|---|---|
| Cutaneous melanoma (CM) | *Total no. of CM cases in family*[1] | | |
| | *1* | *4* | 5 |
| | *2* | *208* | 218 |
| | *3* | *182* | 198 |
| | *4+* | *52* | 62 |
| | Total | 446 | 483 |
| Uveal melanoma (UM) | *Total no. of UM cases in family*[2] | | |
| | *1* | *2* | 2 |
| | *2* | *3* | 3 |
| | Total | 5 | 5 |
| Total | | 451 | 488 |

[1]Uveal melanoma was present in all four single-case families (one additional sample included), six two-case families, one three-case family and six families with four or more cases.
[2]Cutaneous melanoma was present in both single-case families and in one two-case family.

families, 11 samples were excluded from the analysis based on failure to meet above mentioned inclusion criteria. In one of these samples, a pathogenic variant in the 5'UTR region of *CDKN2A* (c.-34G>T) was identified. Another sample was excluded because sequencing was unsuccessful. In total, 488 samples from 451 families remained for analysis (Table 1). Most families had a proband with CM ($n$ = 446) and the majority of these probands had at least one other relative with CM ($n$ = 442 families; $n$ = 478 samples). This 'familial CM' subgroup included 208 two-case families (83% of which consisted of first-degree relatives), 182 three-case families and 52 families with four or more melanoma cases. An additional four probands with CM had one or more relatives with UM, but no CM. The remaining five families had a proband with UM and one or more relatives with UM and/or CM. A control cohort consisted of a total of 449 adult individuals sequenced at the LUMC for a nonmelanoma, nononcogenic indication (MODY; MIM #606391). MODY is an autosomal dominant form of diabetes mellitus which manifests in young adults.

### Gene selection and sequencing

A total of 30 genes were selected by a multidisciplinary expert team (TP, RvD, NG, FH, NvdS; July 2016) and incorporated into a custom-designed targeted gene panel. This included nine established melanoma susceptibility genes and an additional 21 candidate genes identified in previous studies (Table 2). Sequencing of all coding exons, including exon-intron boundaries, was performed on the Illumina HiSeq4000 platform to yield 150 basepair, paired-end reads. Targets were captured using a custom-designed, gene panel-specific Agilent SureSelect $^{XT}$ Clearseq enrichment kit and sequenced using the 200 ng XT protocol. Capture, enrichment and sequencing were performed at the GenomeScan sequencing facility in Leiden (https://www.genomescan.nl/). Subsequent data analysis was performed using our in-house developed set-up for diagnostic next generation sequence (NGS) analysis. In brief, FastQ sequence data was analyzed using an in-house developed and stringent post-sequencing annotation pipeline (using BWA-GATK-VEP). Only variants that occurred with a minor allele frequency (MAF) of less than 5% in the 1,000 Genomes variant database were collected and annotated. Subsequent variant filtering and analysis was performed using a second in-house developed variant analysis tool called LOVDplus. Only variants that had an optimal Genotype Quality (GQ) score of 99 (range 0–99) were considered for further interpretation. The obtained sequencing data had an average depth of >1,000 (>99% at least 30x) with horizontal coverage >99%, and were aligned to human reference genome build GRCh37. Variants with an alternate read ratio of <0.2 were excluded.

### Variant selection and interpretation

We used Alamut® Visual (V.2.9.0, Interactive Biosoftware, Rouen, France) as an *in silico* tool for interpretation of the variants. In the primary filtering step, we selected exonic variants and intronic variants up to 10 nucleotides from the exon-intron junction with a MAF of less than 0.01 in the Exome Aggregation Consortium (ExAC; http://exac.broadinstitute.org/) and Genome of the Netherlands (GoNL; http://nlgenome.nl) public variant databases. Synonymous variants without a possible effect on splicing were excluded. The functional effect of missense variants was predicted by the *in silico* tools SIFT (http://sift.jcvi.org/), Align GVGD (http://agvgd.hci.utah.edu/), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) and the CADD score (http://cadd.gs.washington.edu/). A further selection of variants of interest (secondary filtering) was based on the following criteria: 1) known pathogenic variants in literature, 2) truncating variants, 3) missense variants with a CADD score >15 and at least two out of three *in silico* protein prediction tools predicting a possible functional effect, 4) in-frame indels, and 5) variants that likely affect splicing (predicted by SpliceSiteFinder-like, MaXEntScan, NNSPLICE, GeneSplicer and Human Splicing Finder, incorporated in Alamut®). Analysis of the *POLE* gene was confined to variants in the exonuclease domain (exon 9–14),[20] while analysis of *CDK4*, *TERT*, *MITF* and *MC1R* was restricted to specific variants known to be associated with an increased melanoma risk. This included the p.R24H and p.R24C variants in *CDK4*,[10] the c.-57T>G promoter variant in *TERT*,[13] the p.E318K variant in *MITF*,[15] and the R and r variants in *MC1R*.[18] Co-segregation analysis of the detected variants was possible for families in which more than one case was included in the study. Finally, all variants of interest were evaluated using a recently published *in silico* prediction tool, UMD-predictor (http://umd-predictor.eu/). This tool uses a combinatorial approach to predict pathogenicity of coding single nucleotide variants by pooling information at the nucleotide level, the protein level and at the mRNA level, and has an exceptionally good reported performance.[24]

Table 2. List of genes included in the panel

| Gene | Full Name | Alt. Name | MIM no. | Refs. |
|---|---|---|---|---|
| *Established melanoma susceptibility genes* | | | | *Reviewed in: Aoude et al.,[5] Read et al.[6]* |
| *High to medium penetrance:* | | | | |
| *CDKN2A* | Cyclin-Dependent Kinase Inhibitor 2A | | 600160 | |
| *CDK4* | Cyclin-Dependent Kinase 4 | | 123829 | |
| *BAP1* | BRCA1-Associated Protein 1 | | 603089 | |
| *POT1* | Protection of Telomeres 1 | | 606478 | |
| *ACD* | Adrenocortical Dysplasia Homolog | *TPP1* | 609377 | |
| *TERF2IP* | TERF2-Interacting Protein | *RAP1* | 605061 | |
| *TERT* | Telomerase Reverse Transcriptase | | 187270 | |
| *MITF* | Microphthalmia-Associated Transcription Factor | | 156845 | |
| *Low to medium penetrance:* | | | | |
| *MC1R* | Melanocortin 1 receptor | | 155555 | |
| *Shelterin complex candidate genes* | | | | *Aoude et al.[12]* |
| *TERF1* | Telomeric Repeat-Binding Factor 1 | *TRF1* | 600951 | |
| *TERF2* | Telomeric Repeat-Binding Factor 2 | *TRF2* | 602027 | |
| *TINF2* | TERF1-Interacting Nuclear Factor 2 | *TIN2* | 604319 | |
| *Candidate genes from WES/WGS and GWA studies* | | | | |
| *BRIP1* | BRCA1-Interacting Protein 1 | | 605882 | *Tuominen et al.[22]* |
| *RAD51B* | RAD51 Paralog B | *RAD51L1* | 602948 | *Wadt et al.[21]* |
| *POLE* | DNA Polymerase Epsilon | | 174762 | *Aoude et al.[20]* |
| *NEK2* | NIMA-Related Kinase 2 | | 604043 | - |
| *NEK4* | NIMA-Related Kinase 4 | | 601959 | - |
| *NEK10* | NIMA-Related Kinase 10 | | - | - |
| *NEK11* | NIMA-Related Kinase 11 | | 609779 | - |
| *DOT1L* | DOT1-Like Histone Lysine Methyltransferase | | 607375 | - |
| *PARP1* | Poly (ADP-Ribose) Polymerase 1 | | 173870 | - |
| *CENPS* | Centromere Protein S | *APITD1* | 609130 | - |
| *CREB3L1* | CAMP Responsive Element Binding Protein 3 Like 1 | | 616215 | - |
| *MLLT6* | Mixed-Lineage Leukemia, Translocated to, 6 | | 600328 | - |
| *ERCC3* | ERCC Excision Repair 3 | | 133510 | - |
| *CBLB* | Cbl Proto-Oncogene B | | 604491 | - |
| *Other candidate genes* | | | | |
| *PTEN* | Phosphatase and Tensin Homolog | | 601728 | *Bubien et al.[48]* |
| *RASEF* | RAS and EF-Hand Domains-Containing Protein | | 611344 | *Maat et al.[49]* |
| *POLH* | DNA Polymerase Eta | | 603968 | *Di Lucca et al.* [50] |
| *OCA2* | OCA2 Melanosomal Transmembrane Protein | | 611409 | *Hawkes et al.* [45] |

Abbreviation: MIM, Mendelian Inheritance in Man (http://www.omim.org).

## Results

In our cohort of 488 samples (451 families), a total of 171 variants passed our primary filtering criteria (see Supporting Information). These included 151 exonic variants, of which eight were truncating (four frameshift, four nonsense), 138 missense, three in-frame indels, and two synonymous variants with a possible effect on splicing. The remaining 20 variants were intronic. Of the 171 variants, 44 were novel (not reported in the reference databases ExAC and GoNL), 41 were extremely rare (MAF < 0.0001), 29 were very rare (MAF < 0.001), and the remaining 57 variants were rare (MAF < 0.01). Subsequent filtering resulted in 60 variants of interest in 20 genes (Tables 3–5). These selected variants were only detected in probands with CM and in none of the probands with UM. The *MC1R* risk variants were separately analyzed (Table 6).

### Variants of interest in established melanoma susceptibility genes and shelterin complex genes

We detected two novel splice variants and one novel truncating variant in the *BAP1* gene in three probands (0.7% of families) (Table 3). The c.122+1G>T, p.? and c.1730-1G>A, p.? variants are both located in a canonical splice site and are

**Table 3.** Selected variants of interest in established melanoma susceptibility genes and shelterin complex candidate genes

| Gene | Variant | Type | Allele count | MAF (AN = 976) | MAF in ExAC[1] / GoNL | CADD | SIFT | Align GVGD[2] | PolyPhen-2[3] | UMD-Predictor | FD | CoS[4] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Established melanoma susceptibility genes* | | | | | | | | | | | | |
| *ACD* | c.871A>G, p.(Thr291Ala) | Missense | 1 | 0.0010025 | 0.0012/0.001 | 23.2 | Delet. | C55 | Prob. Damaging | Polymorphism | Y | |
| *BAP1* | c.122+1G>T, p.? | Splicing | 1 | 0.0010025 | −/− | | | | | | Y | |
| *BAP1* | c.1730-1G>A, p.? | Splicing | 1 | 0.0010025 | −/− | | | | | | Y | |
| *BAP1* | c.1936_1937insTT, p.(Tyr646Phefs*10) | Frameshift | 1 | 0.0010025 | −/− | | | | | | Y | |
| *MITF* | c.952G>A, p.(Glu318Lys) | Missense | 15 | 0.015369 | 0.0025/0.007 | 27.9 | Tol. | C0 | Prob. Damaging | Prob. Polymorphism | Y | |
| *TERF2IP* | c.398G>A, p.(Arg133Gln) | Missense | 1 | 0.0010025 | 0.00022/− | 23.4 | Delet. | C35 | Benign | Polymorphism | Y | |
| *Shelterin complex candidate genes* | | | | | | | | | | | | |
| *TERF1* | c.186_188dup, p.(Glu62dup) | In-frame Duplication | 2 | 0.002049 | 0.0005/−[5] | | | | | | Y | Y |
| *TERF1* | c.212_217dup, p.(Glu71_Ala72dup) | In-frame Duplication | 1 | 0.0010025 | 0.00014/− | | | | | | Y | |
| *TERF1* | c.1193A>G, p.(Tyr398Cys) | Missense | 1 | 0.0010025 | 0.000009/− | 24.7 | Delet. | C25 | Prob. Damaging | Pathogenic | Y | |
| *TERF2* | c.56A>G, p.(Asp19Gly) | Missense | 1 | 0.0010025 | 0.00012/− | 16.35 | Delet. | C0 | Pos. Damaging | N.a. | N | |
| *TERF2* | c.794G>A, p.(Arg265His) | Missense | 1 | 0.0010025 | 0.000027/− | 28.3 | Delet. | C0 | Pos. Damaging | Prob. Polymorphism | N | |
| *TERF2* | c.1492G>A, p.(Glu498Lys) | Missense | 4 | 0.004098 | 0.0022/0.003 | 34 | Delet. | C55 | Pos. Damaging | Prob. Polymorphism | Y | |
| *TINF2* | c.38G>T, p.(Arg13Leu) | Missense | 1 | 0.0010025 | −/− | 27 | Delet. | C0 | Prob. Damaging | Pathogenic | Y | |
| *TINF2* | c.734C>A, p.(Ser245Tyr) | Missense | 3 | 0.003074 | 0.00073/− | 22.7 | Delet. | C15 | Benign | Polymorphism | N | |

Gene reference sequences: *ACD*: NM_001082486.1, *BAP1*: NM_004656.3, *MITF*: NM_000248.3, *TERF2IP*: NM_018975.3, *TERF1*: NM_017489.2, *TERF2*: NM_005652.4, *TINF2*: NM_001099274.1.
Abbreviations: AN, allele number; MAF, minor allele frequency; CADD, Combined Annotation Dependent Depletion; FD, in known functional domain; CoS, co-segregation with melanoma in one or more families; Y, yes; N, no; Delet, deleterious; Pos, possibly; Prob, probably.
[1]In European (non-Finnish) population.
[2]Possible classifications in Align GVGD are C0, C15, C25, C35, C45, C55 and C65. Variants in class C0 have the least probability of being pathogenic, variants in class C65 have the highest probability of being pathogenic. See also http://agvgd.hci.utah.edu/classifiers.php
[3]HumVar trained PolyPhen-2 model used for prediction.
[4]Co-segregation analyses of variants with melanoma phenotype: *TERF1* p.E62dup: 2/2.
[5]Common variant (MAF › 1%) in one or more non-European populations.



Int. J. Cancer: **144**, 2453–2464 (2019) © 2018 The Authors. *International Journal of Cancer* published by John Wiley & Sons Ltd on behalf of UICC

Table 4. Selected variants of interest in candidate melanoma susceptibility genes *BRIP1, POLE and OCA2*

| Gene | Variant | Type | Allele count | MAF (AN = 976) | MAF in ExAC[1] / GoNL | CADD | SIFT | Align GVGD[2] | PolyPhen-2[3] | UMD-Predictor | FD | CoS[4] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *BRIP1* | c.517C>T, p.(Arg173Cys) | Missense | 9 | 0.009221 | 0.0047/0.004 | 27.6 | Delet. | C55 | Prob. Damaging | Pathogenic | Y | |
| *BRIP1* | c.790C>T, p.(Arg264Trp) | Missense | 1 | 0.0010025 | 0.0012/0.003 | 32 | Delet. | C0 | Prob. Damaging | Pathogenic | Y | |
| *BRIP1* | c.894C>A, p.(Cys298*) | Nonsense | 1 | 0.0010025 | −/− | 36 | | | | Pathogenic | Y | |
| *BRIP1* | c.1198G>T, p.(Asp400Tyr) | Missense | 2 | 0.002049 | 0.000027/− | 33 | Delet. | C35 | Prob. Damaging | Pathogenic | Y | |
| *BRIP1* | c.1255C>T, p.(Arg419Trp) | Missense | 1 | 0.0010025 | 0.00046/0.001 | 33 | Delet. | C35 | Prob. Damaging | Pathogenic | Y | N |
| *BRIP1* | c.2069G>A, p.(Gly690Glu) | Missense | 1 | 0.0010025 | −/− | 32 | Delet. | C65 | Prob. Damaging | Pathogenic | Y | |
| *BRIP1* | c.2582C>G, p.(Ser861Cys) | Missense | 1 | 0.0010025 | 0.000027/− | 28.5 | Delet. | C65 | Prob. Damaging | Pathogenic | Y | |
| *BRIP1* | c.2593C>T, p.(Arg865Trp) | Missense | 1 | 0.0010025 | 0.000027/− | 34 | Delet. | C25 | Prob. Damaging | Pathogenic | Y | N |
| *POLE* | c.861T>A, p.(Asp287Glu) | Missense | 9 | 0.009221 | 0.0017/0.004 | 25.7 | Delet. | C35 | Prob. Damaging | Pathogenic | Y | |
| *POLE* | c.893A>G, p.(Tyr298Cys) | Missense | 1 | 0.0010025 | −/− | 28.3 | Delet. | C65 | Prob. Damaging | Pathogenic | Y | |
| *POLE* | c.1230G>A, p.(Trp410*) | Nonsense | 1 | 0.0010025 | −/− | 38 | | | | Pathogenic | Y | N |
| *OCA2* | c.163del, p.(Ala55Leufs*47)[5] | Frameshift | 1 | 0.0010025 | 0.000019/− | | | | | | N | |
| *OCA2* | c.796C>T, p.(Arg266Trp) | Missense | 1 | 0.0010025 | 0.0018/0.003[6] | 18.24 | Delet. | C0 | Pos. Damaging | Prob. Polymorphism | N | |
| *OCA2* | c.1255C>T, p.(Arg419Trp)[5] | Missense | 1 | 0.0010025 | 0.00011/− | 32 | Delet. | C0 | Prob. Damaging | Pathogenic | Y | |
| *OCA2* | c.1261C>T, p.(Arg421Trp) | Missense | 1 | 0.0010025 | 0.000065/− | 28 | Delet. | C0 | Prob. Damaging | Pathogenic | Y | N |
| *OCA2* | c.1327G>A, p.(Val443Ile)[5] | Missense | 18 | 0.018443 | 0.0051/0.008 | 34 | Tol. | C0 | Prob. Damaging | Polymorphism | Y | N |
| *OCA2* | c.1441G>A, p.(Ala481Thr)[5] | Missense | 1 | 0.0010025 | 0.0026/0.001[6] | 27.6 | Tol. | C0 | Pos. Damaging | Prob. Polymorphism | Y | |
| *OCA2* | c.1465A>G, p.(Asn489Asp)[5] | Missense | 7 | 0.007172 | 0.0007/0.003 | 28.2 | Delet. | C0 | Prob. Damaging | Pathogenic | Y | Y |
| *OCA2* | c.1592A>G, p.(Tyr531Cys) | Missense | 1 | 0.0010025 | 0.00011/0.001 | 25.3 | Delet. | C0 | Prob. Damaging | Pathogenic | Y | |
| *OCA2* | c.2037G>C, p.(Trp679Cys)[5] | Missense | 1 | 0.0010025 | 0.00015/− | 34 | Delet. | C0 | Prob. Damaging | Pathogenic | Y | |

Gene reference sequences: *BRIP1*: NM_032043.2, *POLE*: NM_006231.2, *OCA2*: NM_000275.2.
Abbreviations: AN, allele number; MAF, minor allele frequency; CADD, Combined Annotation Dependent Depletion; FD, in known functional domain; CoS, co-segregation with melanoma in one or more families; Y, yes; N, no; Delet, deleterious; Pos, possibly; Prob, probably.
[1]In European (non-Finnish) population.
[2]Possible classifications in Align GVGD are C0, C15, C25, C35, C45, C55 and C65. Variants in class C0 have the least probability of being pathogenic, variants in class C65 have the highest probability of being pathogenic. See also http://agvgd.hci.utah.edu/classifiers.php
[3]HumVar trained PolyPhen-2 model used for prediction.
[4]Co-segregation analyses of variants with melanoma phenotype: *BRIP1* p.R419W: 1/2, *BRIP1* p.R865W: 1/2, *OCA2* p.R421W: 1/2, *OCA2* p.V443I: 1/2 (two families), *OCA2* p.N489D: 3/3 (one family), *POLE* p.W410*: 1/2.
[5]Variants reported in patients with oculocutaneous albinism type 2.
[6]Common variant (MAF › 1%) in one or more non-European populations.

**Table 5.** Selected variants of interest in candidate melanoma susceptibility genes (excluding *BRIP1*, *POLE* and *OCA2*)

| Gene | Variant | Type | Allele count | MAF (AN = 976) | MAF in ExAC[1] / GoNL | CADD | SIFT | Align GVGD[2] | PolyPhen-2[3] | UMD-Predictor | FD | CoS[4] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *CBLB* | c.770A>T, p.(His257Leu) | Missense | 1 | 0.0010025 | –/– | 33 | Delet. | C0 | Prob. Damaging | Pathogenic | Y | |
| *CBLB* | c.1402C>G, p.(Arg468Gly) | Missense | 1 | 0.0010025 | 0.000018/– | 23.6 | Delet. | C0 | Pos. Damaging | Pathogenic | Y | |
| *ERCC3* | c.496G>A, p.(Val166Ile) | Missense | 1 | 0.0010025 | –/– | 24.6 | Delet. | C25 | Benign | Prob. Polymorphism | Y | |
| *ERCC3* | c.847C>T, p.(Arg283Cys) | Missense | 5 | 0.005123 | 0.0014/0.002 | 34 | Delet. | C65 | Benign | Pathogenic | Y | N |
| *ERCC3* | c.1421dup, p.(Asp474Glufs*2) | Frameshift | 1 | 0.0010025 | 0.00014/– | | | | | | Y | |
| *ERCC3* | c.1776T>G, p.(Ile592Met) | Missense | 1 | 0.0010025 | –/– | 24.9 | Delet. | C0 | Prob. Damaging | Prob. Pathogenic | Y | |
| *ERCC3* | c.2111C>T, p.(Ser704Leu) | Missense | 2 | 0.002049 | 0.0022/0.001 | 24 | Delet. | C15 | Benign | Pathogenic | N | N |
| *MLLT6* | c.655C>T, p.(Arg219Trp) | Missense | 1 | 0.0010025 | 0.000064/– | 25.2 | Delet. | C15 | Pos. Damaging | Pathogenic | N | |
| *MLLT6* | c.2195A>C, p.(Glu732Ala) | Missense | 1 | 0.0010025 | –/– | 24.6 | Delet. | C0 | Prob. Damaging | Pathogenic | Y | |
| *MLLT6* | c.2755G>A, p.(Gly919Arg) | Missense | 1 | 0.0010025 | –/– | 26.2 | Delet. | C0 | Pos. Damaging | Pathogenic | N | |
| *NEK2* | c.97-2A>G | Splicing | 1 | 0.0010025 | 0.00029/– | | | | | | Y | |
| *NEK2* | c.137A>G, p.(Glu46Gly) | Missense | 1 | 0.0010025 | –/– | 28 | Delet. | C0 | Prob. Damaging | Pathogenic | Y | N |
| *NEK2* | c.952C>T, p.(Arg318*) | Nonsense | 1 | 0.0010025 | 0.000018/– | 39 | | | | Pathogenic | Y | |
| *NEK4* | c.500T>C, p.(Ile167Thr) | Missense | 1 | 0.0010025 | 0.000009/– | 26.9 | Delet. | C25 | Prob. Damaging | Pathogenic | Y | |
| *NEK4* | c.1953_1955del, p.(Glu651del) | In-frame Deletion | 1 | 0.0010025 | 0.0011/0.002 | | | | | | N | |
| *NEK4* | c.2093+1G>C | Splicing | 1 | 0.0010025 | –/– | | | | | | N | |
| *NEK10* | c.1094G>A, p.(Arg365Gln) | Missense | 7 | 0.007172 | 0.0094/0.009 | 25.2 | Delet. | C0 | Pos. Damaging | Polymorphism | N | Y |
| *NEK11* | c.127G>C, p.(Val43Leu) | Missense | 2 | 0.002049 | 0.00016/–[5] | 27.5 | Delet. | C25 | Pos. Damaging | Prob. Polymorphism | Y | |
| *PARP1* | c.1814C>T, p.(Pro605Leu) | Missense | 1 | 0.0010025 | 0.000036/– | 22.6 | Delet. | C15 | Benign | Pathogenic | Y | |
| *PARP1* | c.2656G>A, p.(Val886Met) | Missense | 1 | 0.0010025 | 0.000027/– | 32 | Delet. | C0 | Prob. Damaging | Pathogenic | Y | |
| *POLH* | c.626G>T, p.(Gly209Val) | Missense | 2 | 0.002049 | 0.0032/0.003[5] | 28.1 | Delet. | C15 | Prob. Damaging | Prob. Polymorphism | Y | |
| *POLH* | c.890G>A, p.(Trp297*) | Nonsense | 1 | 0.0010025 | –/– | 40 | | | | Pathogenic | Y | Y[6] |
| *RASEF* | c.157C>T, p.(Arg53Trp) | Missense | 1 | 0.0010025 | 0.000049/– | 28.4 | Delet. | C0 | Prob. Damaging | Prob. Pathogenic | Y | |
| *RASEF* | c.1049_1050del, p.(His350Argfs*3) | Frameshift | 1 | 0.0010025 | 0.000063/– | | | | | | Y | |
| *RASEF* | c.2078A>G, p.(Asp693Gly) | Missense | 1 | 0.0010025 | 0.000018/– | 32 | Delet. | C0 | Pos. Damaging | Pathogenic | Y | |
| *RASEF* | c.2207A>T, p.(Asn736Ile) | Missense | 1 | 0.0010025 | 0.000027/– | 27.4 | Delet. | C0 | Pos. Damaging | Pathogenic | Y | |

Gene reference sequences: *CBLB*: NM_170662.4, *ERCC3*: NM_000122.1, *MLLT6*: NM_005937.3, *NEK2*: NM_002497.3, *NEK4*: NM_003157.5, *NEK10*: NM_152534.4, *NEK11*: NM_024800.4, *PARP1*: NM_001618.3, *POLH*: NM_006502.2, *RASEF*: NM_152573.3.

Abbreviations: AN, allele number; MAF, minor allele frequency; CADD, Combined Annotation Dependent Depletion, FD; in known functional domain; CoS, co-segregation with melanoma in one or more families; Y, yes; N, no; Delet, deleterious; Pos, possibly; Prob, probably.

[1]In European (non-Finnish) population.

[2]Possible classifications in Align GVGD are C0, C15, C25, C35, C45, C55 and C65. Variants in class C0 have the least probability of being pathogenic, variants in class C65 have the highest probability of being pathogenic. See also http://agvgd.hci.utah.edu/classifiers.php

[3]HumVar trained PolyPhen-2 model used for prediction.

[4]Co-segregation analyses of variants with melanoma phenotype: *ERCC3* p.R283C: 1/2 (one family), *ERCC3* p.S704L: 1/2 (one family), *NEK2* p.E46G: 1/2, *NEK10* p.R365Q: 2/2 (one family).

[5]Common variant (MAF › 1%) in one or more non-European populations.

[6]The proband with the *POLH* p.W297* variant had a father with the recessively inherited disease xeroderma pigmentosum (MIM #278750) and he is therefore highly likely to have carried this variant as well.

Table 6. Association of *MC1R* risk variants with familial cutaneous melanoma

| | Familial CM cohort[1] (AN = 956) | Control cohort[1] (AN = 898) | OR | 95% CI | *p* value[2] |
|---|---|---|---|---|---|
| No. of individuals | 478 | 449 | | | |
| Reference sequence[3] | 388 | 549 | *Ref.* | *Ref.* | *Ref.* |
| **All R variants** | **0.342** | **0.140** | **3.67** | **2.88–4.68** | **<0.001** |
| c.252C>A, p.D84E | 0.017 | 0.004 | 5.66 | 1.88–17.06 | 0.001 |
| c.425G>A, p.R142H | 0.008 | 0.008 | 1.62 | 0.58–4.50 | 0.431 |
| c.451C>T, p.R151C | 0.145 | 0.058 | 3.78 | 2.68–5.34 | <0.001 |
| c.478C>T, p.R160W | 0.150 | 0.059 | 3.82 | 2.72–5.37 | <0.001 |
| c.880G>C, p.D294H | 0.022 | 0.011 | 2.79 | 1.38–6.38 | 0.005 |
| **All r variants** | **0.252** | **0.248** | **1.53** | **1.22–1.91** | **<0.001** |
| c.178G>T, p.V60L | 0.105 | 0.104 | 1.52 | 1.12–2.08 | 0.008 |
| c.274G>A, p.V92M | 0.082 | 0.081 | 1.51 | 1.07–2.13 | 0.021 |
| c.464T>C, p.I155T | 0.006 | 0.006 | 1.70 | 0.52–5.60 | 0.540 |
| c.488G>A, p.R163Q | 0.060 | 0.058 | 1.55 | 1.04–2.31 | 0.032 |

*MC1R* reference sequence: NM_002386.3.
Abbreviation: AN, allele number.
[1]Minor allele frequency (MAF).
[2]Using Fisher's exact test (two-sided).
[3]Number of alleles without any R or r variant.

predicted to inactivate the splice donor site of intron 3 and splice acceptor site of intron 13, respectively, likely resulting in a prematurely truncated protein. The c.1936_1937insTT, p.(Y646Ffs*10) frameshift variant is also predicted to cause a truncated protein due to a premature stop codon. All three families had multiple members with CM (see Supporting Information). In two families, possible *BAP1*-associated nevi (Spitz nevi) were reported in first-degree relatives, and in one of these families, multiple relatives were also diagnosed with (one or several) basal cell carcinomas. No other *BAP1*-specific tumors, such as UM, malignant mesothelioma or renal cell carcinoma, were reported in these families. Interestingly, in the proband who carried the *BAP1* c.122+1G>T, p.? variant we also identified a novel nonsense variant in the *BRIP1* gene (c.894C>A, p.(C298*)). Ovarian cancer was not reported in this family.

The *MITF* p.E318K risk variant was detected in a total of fifteen probands (3.3%), a frequency more than twice that of the Dutch reference population (MAF 0.015; GoNL: 0.007) (Table 3). All *MITF* p.E318K families had at least two members with CM ('familial CM'; seven two-case families, six three-case families, and two families with four or more cases). The median age of probands at melanoma diagnosis was 41 years (range 27–74). One proband had multiple primary melanomas, a feature also present in two additional families. Renal cancer and pancreatic cancer were present in two families and in one family, respectively.

In the three shelterin complex subunits that have been reported as high penetrance melanoma susceptibility genes (*POT1*, *ACD*, *TERF2IP*), we identified two potentially deleterious variants (Table 3). A rare missense variant in the *ACD* gene (c.871A>G, p.(T291A)), detected in a proband from a two-case family, is located in the POT1 binding domain in which previously reported pathogenic variants seem to cluster.[12] A very rare missense variant in the *TERF2IP* gene (c.398G>A, p.(R133Q)), located in the MyB DNA binding domain, was detected in a proband of another two-case family. These variants had a CADD score >20 and were predicted to be damaging by at least two *in silico* tools, although UMD-predictor classified both variants as polymorphisms. Remarkably, we did not detect any potentially deleterious variants in the *POT1* gene. In the other shelterin complex subunit genes *TERF1*, *TERF2* and *TINF2*, we identified eight potentially deleterious variants (six missense, two in-frame dups) (Table 3). These included a novel variant in the ACD/TERF2 binding motif domain of the *TINF2* gene (c.38G>T, p.(R13L)) and two extremely rare variants in the *TERF1* gene (c.1193A>G, p.(Y398C); MyB DNA binding domain) and the *TERF2* gene (c.794G>A, p.(R265H)). An in-frame duplication in the *TERF1* gene (c.186_188dup, p.(E62dup); telomeric repeat binding factor homology domain) was shared among two third-degree relatives with CM in one family, but as this is a common variant in Asian and African populations (MAF ~2% in ExAC) it is unlikely to be pathogenic. None of the patients in our cohort carried the known melanoma susceptibly variant in the *TERT* promoter region (c.-57T>G).

Since we were particularly interested in the frequency of *MC1R* risk variants in *familial* CM cases, we only analyzed the *MC1R* gene in the 'familial CM' subgroup ($n$ = 478 individuals). In this cohort, we observed a substantial enrichment of R variants compared to controls (OR 3.67, 95% CI 2.88–4.68, $p < 0.001$) (Table 6). The frequency of p.D84E was most strikingly increased in our cohort (OR 5.66, 95% CI

1.88–17.06, $p = 0.001$), followed by p.R160W (OR 3.82, 95% CI 2.72–5.37, $p < 0.001$) and p.R151C (OR 3.78, 95% CI 2.68–5.34, $p < 0.001$). Although less prominent, r variants were also enriched in familial CM cases (any r variant: OR 1.53, 95% CI 1.22–1.91, $p < 0.001$).

### Variants of interest in candidate melanoma susceptibility genes

In addition to the novel, truncating variant in the *BRIP1* gene (c.894C>A, p.(C298*)) found in one of the *BAP1*-families, an additional seven potentially deleterious missense variants were identified in *BRIP1* (Table 4). This included one novel variant (c.2069G>A, p.(G690E)) and two extremely rare variants (c.2582C>G, p.(S861C) and c.2593C>T, p.(R865W)) located in the DNA helicase domain and predicted to be damaging by all *in silico* tools including UMD-predictor. However, the latter variant did not co-segregate with the phenotype in a two-case family. In this same domain, a different missense variant was previously reported to co-segregate in a three-case melanoma family.[22] The remaining four variants were located in the ATPase/helicase core domain, and included an extremely rare variant (c.1198G>T, p.(D400Y)) in two probands and a very rare variant (c.1255C>T, p.(R419W)) in one proband. Currently, little is known from literature about the effect of these missense variants and no functional testing has been performed.

We further identified two missense variants in the exonuclease domain of the *POLE* gene: one novel variant (c.893A>G, p.(Y298C)) in a single proband and a rare variant (c.861T>A, p.(D287E)) in nine other probands (Table 4). Both variants were predicted to be damaging by all *in silico* tools including UMD-predictor. In another proband, we identified a novel truncating variant in *POLE* (c.1230G>A, p.(W410*)), but this variant did not co-segregate with the phenotype in a two-case family.

In the *OCA2* gene, we identified nine (potentially) deleterious variants, of which six were previously reported in patients with the recessively inherited condition oculocutaneous albinism type 2 (MIM #203200) (Table 4). Two of these established pathogenic variants, c.1327G>A, p.(V443I) and c.1465A>G, p.(N489D), were detected in multiple individuals ($n = 17$ and 7, respectively) and the frequency of these variants was more than twice that found in the Dutch GoNL reference database (MAF: 0.018 and 0.0071; GoNL: 0.008 and 0.003, respectively). Co-segregation analysis was, however, ambiguous: the c.1465A>G, p.(N489D) variant co-segregated with the phenotype in a three-case family (all first-degree relatives), but the c.1327G>A, p.(V443I) variant did not co-segregate in two two-case families. Interestingly, one proband was homozygous for the c.1327G>A, p.(V443I) variant. This proband had a medical history of three primary melanomas from age 57 and a first-degree relative (sibling) with melanoma. Although the proband was reported to have a fair skin type and reddish hair, no other physical signs of albinism were reported. Another proband, with a medical history of three primary melanomas from age 48 and a first-degree relative (child) with melanoma at age 32, carried two pathogenic variants in the *OCA2* gene (c.1327G>A, p.(V443I) and c.2037G>C, p.(W679C)). Since physical signs of albinism were not reported in the proband, it is possible that these variants are located on the same allele, but this could not be confirmed because co-segregation data was unavailable.

In the other included candidate melanoma susceptibility genes, largely derived from whole exome/genome sequencing studies by both our own research group and other research groups, we detected four truncating variants (in *ERCC3*, *NEK2*, *POLH*, *RASEF*), two canonical splice site variants (in *NEK2*, *NEK4*) and several potentially deleterious missense variants (in *CBLB*, *ERCC3*, *MLLT6*, *NEK2*, *NEK4*, *NEK10*, *NEK11*, *PARP1*, *POLH*, *RASEF*) (Table 5). All of these variants occurred in only one proband and co-segregation data was only occasionally available. UMD-predictor classified the majority of these variants as (probably) pathogenic.

## Discussion

In our study, we performed multigene panel testing of 30 (candidate) melanoma susceptibility genes in 451 Dutch melanoma-prone families without a *CDKN2A* or *CDK4* mutation. We identified (likely) pathogenic variants in established high- and medium penetrance melanoma susceptibility genes in 4.0% of these families (18/451; $n = 3$ *BAP1*, $n = 15$ *MITF*). In addition, two potentially deleterious missense variants were detected in important functional domains of the *ACD* and *TERF2IP* genes (0.4%) and, surprisingly, none of the 451 families carried a variant of interest in the *POT1* gene.

The frequency of *BAP1* mutations in our cohort ($n = 3$; 0.7%) is in line with a reported frequency of ~1% among melanoma-prone families worldwide.[25] *BAP1* is a deubiquitinating hydrolase that acts as a tumor suppressor and is involved in the regulation of key pathways including cell proliferation, cell differentiation, cell survival and the DNA damage response. Germline *BAP1* mutations have been reported in patients with several types of tumors, but particularly in UM and malignant mesothelioma.[14] Interestingly, these two major cancers were not present in our three families. Although CM itself is relatively common in *BAP1* mutation carriers (13–18%),[14,26] *BAP1* mutations are rarely reported in CM families without these other cancers: a study by Njauw *et al.*[27] detected only one *BAP1* mutation in 193 CM families (0.5%), and a study by Wadt *et al.*[28] found no *BAP1* mutations in 133 high-risk CM patients (of which 94 CM *families*). By contrast, Gerami *et al.*[29] found a *BAP1* mutation in a single case with multiple primary cutaneous melanomas and a dysplastic nevus phenotype, with no family history for either CM or UM or any other *BAP1*-associated cancers. A recent population-based study reported only three loss-of-function *BAP1* mutations in CM cases (<0.2%), and all these cases had relatives with *BAP1*-associated cancers, although none had

UM.[30] Our study demonstrates that *BAP1* mutations can indeed be detected in some CM families without UM or malignant mesothelioma and it is therefore important to incorporate the *BAP1* gene in a diagnostic (cutaneous) melanoma gene panel test. However, it should be noted that basal cell carcinoma and (atypical) Spitz nevi, features also associated with *BAP1* mutations, were reported in two of the families.

Fifteen probands in our familial CM cohort (15/442; 3.4%) carried the *MITF* p.E318K risk variant, which is among the highest frequencies reported in familial non-*CDKN2A* cases. Only one small study from Switzerland reported a higher frequency, 7.7% (2/26), in melanoma-prone families.[31] A similar frequency, 3.4% (19/558) in familial cases, was found in a study from the United States, although it is unclear if these patients were all pre-screened for *CDKN2A* mutations.[32] Frequencies in various other cohorts range from 0 to 3%,[16,28,33–35] with the lowest frequency (<1%) reported in familial cases from Italy.[17,36] In the Netherlands, diagnostic testing for the *MITF* p.E318K risk variant is now included in the default genetic work-up for familial CM and all carriers are offered regular dermatologic surveillance (regardless of the familial burden for CM). This regular surveillance is recommended because carriers are at increased risk for developing subsequent (multiple primary) melanomas[15] that might also be fast-growing[35] and/or amelanotic,[37] a subtype less easily recognized by the patient and/or the dermatologist. Hence, knowledge about *MITF* p.E318K mutation status can be relevant for both the patient and the dermatologist. Surveillance for other cancers such as renal- or pancreatic cancer is not (yet) offered because the actual risk for these cancers is insufficiently established and surveillance methods are more challenging.

Germline mutations in the telomere maintenance pathway genes in melanoma families have been described in several studies.[11–13] The present study demonstrates that mutations in these genes are probably very rare in the Dutch familial melanoma population. We identified only two potentially deleterious missense variants in *ACD* and *TERF2IP* (0.4%) and none in *POT1* or the promoter region of *TERT*. In the *ACD* and *TERF2IP* genes, both nonsense and pathogenic missense variants have been previously reported in familial melanoma kindreds.[12] Interestingly, the *TERF2IP* p.(R133Q) variant that we detected in a two-case melanoma family was previously reported in a three-case chronic lymphocytic leukemia (CLL) family (without melanoma).[38] Because the variant co-segregated with only two of the cases, the authors concluded that this is a medium penetrance variant for CLL. Leukemia was not reported in relatives of the proband in our cohort. Of the eight potentially deleterious missense variants detected in the *TERF1*, *TERF2* and *TINF2* genes, co-segregation analysis was only possible for one of these variants. There is no additional evidence for pathogenicity of these missense variants, and as yet no protein truncating variants have been reported in these latter genes. Therefore, their role in melanoma susceptibility remains uncertain.

We identified several variants of interest in the known cancer susceptibility genes *BRIP1* and *POLE*, including a nonsense variant in *BRIP1*. BRIP1 (BRCA1-interacting protein C-terminal helicase 1) is a Fanconi anemia group protein and is required for the double-strand break repair function of BRCA1. Heterozygous protein truncating variants in *BRIP1* have mainly been associated with an increased susceptibility for ovarian cancer,[39] but there were no diagnoses of ovarian cancer in family members of the proband with the nonsense *BRIP1* variant in our study. Interestingly, this variant co-occurred with a canonical splice site variant in *BAP1* in the same proband, the latter presumably being the predominant melanoma susceptibility factor in this family. We additionally identified several potentially deleterious missense variants in *BRIP1,* some novel or extremely rare, and most of which were predicted to be damaging by all *in silico* tools used. In a recent study from Sweden, an extremely rare missense variant in the DNA helicase domain of *BRIP1* was found to co-segregate in a three-case melanoma family.[22] Three missense variants in our cohort were located in this same functional domain. Based on these findings, the *BRIP1* gene might be involved in melanoma susceptibility, but more research is needed to clarify this, in particular replication studies in other melanoma cohorts and functional studies to address the pathogenicity of missense variants. The *POLE* gene is a polymerase gene involved in DNA repair and replication and is primarily associated with colorectal cancer. It appears that only missense variants in the exonuclease domain confer an increased susceptibility for cancer through impaired proofreading, which results in tumors with a high mutation burden.[40] Therefore, we restricted our analysis of variants to this specific exonuclease domain and, consequently, all reported variants in *POLE* are located within this domain. Recently, a novel missense variant in the exonuclease domain of *POLE* was reported in a seven-case melanoma family and showed near-complete co-segregation.[20] Although we were not able to perform co-segregation analysis for the novel missense variant (c.893A>G, p.(Y298C)) detected in our cohort, functional analysis of melanoma tissue (mutation burden test) might provide more insight. Of note, colorectal cancer was not reported in this family.

Biallelic germline mutations in *OCA2* cause oculocutaneous albinism type 2 (MIM #203200). *OCA2* encodes the P-protein which has multiple functions in the biosynthesis of melanin. Loss-of-function of the P-protein results in hypopigmentation of the skin, hair and iris and an increased risk for sun-induced skin cancers, in particular basal cell carcinoma and squamous cell carcinoma.[41] Although melanoma is not known to be a common cancer type in patients with *OCA2*-related albinism, families with multiple members with albinism and melanoma have been reported.[42] In our cohort, one proband with a possible subclinical phenotype of albinism carried a homozygous pathogenic *OCA2* variant. Additionally, we observed an increased frequency of rare heterozygous variants in the *OCA2* gene, in particular the known pathogenic variants c.1327G>A, p.(V443I) and c.1465A>G, p.(N489D).[43,44] The association with

melanoma predisposition of the c.1327G>A, p.(V443I) variant in combination with another *OCA2* variant was also studied by Hawkes *et al*.[45] in one albinism-melanoma family. They concluded that these variants might be high penetrance loci for melanoma in this family (OR 6.5). In a recent study by Goldstein *et al*.,[46] the *OCA2* gene was included in a multigene panel test of 42 (candidate) melanoma susceptibly genes that were sequenced in 144 melanoma cases from 76 American families. Comparable to our study, numerous rare variants in *OCA2* were found. The frequency of rare variants in other albinism genes (*TYR, TYRP1*) was also significantly increased in the Goldstein study. Interestingly, a nonsense variant in *TYR* showed near-complete co-segregation in a large family with six melanoma cases. The precise role of *OCA2* (and other albinism genes) in melanoma predisposition remains to be determined, but based on these findings a medium penetrance or modifier effect can be hypothesized. The albinism genes are therefore good candidates for further investigation.

There is extensive literature on the association between *MC1R* R and r variants and sporadic melanoma in population-based cohorts.[18] In our 'familial CM' cases, we observed a high frequency of *MC1R* R variants in particular, a finding comparable to the results of a Danish high-risk melanoma cohort.[28] This suggests that these common risk variants also play a significant role in the familial setting. Since some of the familial occurrence of melanoma might be explained by the aggregation of common risk variants in a family, we are currently incorporating all *MC1R* R and r variants in a polygenic risk score (PRS) model that also includes approximately 40 other common risk variants derived from large melanoma GWAS. PRS models have already been shown to improve risk stratification in other familial cancer cohorts, in particular familial breast cancer.[47]

A major strength of our study is cohort size. With the inclusion of 451 families lacking a mutation in the *CDKN2A* or *CDK4* genes, of which 442 families had at least two cases of CM, to our knowledge this is the largest melanoma gene panel study to date. Although our inclusion criteria were not highly stringent, most families had at least two close relatives with melanoma (for instance, 83% of the two-case families consisted of first-degree relatives). Furthermore, our panel included all eight currently known high- and medium penetrance melanoma susceptibility genes and therefore our reported 4% diagnostic yield for these genes (excluding *CDKN2A* and *CDK4*) is probably very accurate. As a custom-designed targeted gene panel was used, filtering of variants was less strict compared to most reported WES studies. It is therefore very unlikely that potential pathogenic variants in the selected genes were missed in our study. A limitation is that co-segregation analysis of variants was not possible in many families. This was primarily due to Ethics Committee restrictions that prohibited us from re-contacting patients when variants of uncertain significance (VUS) or variants in nonestablished genes were detected. However, co-segregation analysis of (likely) pathogenic variants in known cancer susceptibility genes (*BAP1, MITF, BRIP1*) is currently being initiated.

To conclude, we demonstrate that multigene panel testing for familial melanoma results in an additional 4% diagnostic yield in non-*CDKN2A/CDK4* families. The identification of several families with pathogenic variants in the *BAP1* and *MITF* genes suggests a significant role of these genes in melanoma predisposition and it is therefore important to include these in a diagnostic test. Conversely, variants in the telomere maintenance genes, especially *POT1*, seem to be (very) rare in the Dutch population. When including these genes in a panel test, one should be aware of identifying variants of uncertain significance, as we did in the current study. In view of the relatively high frequency of (potential) pathogenic variants in the *OCA2* gene in both our own and in a recently published American familial melanoma cohort, further elucidation of the role of heterozygous *OCA2* variants in melanoma predisposition appears to be of particular interest. In the future, candidate susceptibility genes such as *OCA2* could potentially be added to routine germline diagnostics, given sufficient evidence for their pathogenicity in melanoma predisposition. This will in turn enhance the diagnostic yield of the panel and improve tumor risk assessment in melanoma families.



## Acknowledgements

We gratefully thank the patients and family members for their participation in this project. We further thank Michiel van der Wielen and Golan Vegter from the LDGA for their assistance in the lab work. This work was supported by the Dutch Cancer Society (#UL 2015-7511 to TPP and NvdS and #UL 2012-5489 to RvD, NAG and NvdS).

## References

1. Erdmann F, Lortet-Tieulent J, Schuz J, et al. International trends in the incidence of malignant melanoma 1953-2008--are recent generations at higher or lower risk? *Int J Cancer* 2013;132:385–400.
2. Ferlay J, Soerjomataram I, Ervik M, et al. *v1.0, cancer incidence and mortality worldwide: IARC CancerBase no. 11 [internet]*. Lyon, France: International Agency for Research on Cancer, 2012. 2013.
3. Bataille V, de Vries E. Melanoma--part 1: epidemiology, risk factors, and prevention. *BMJ (Clinical Research ed)* 2008;a2249:337.
4. Olsen CM, Carroll HJ, Whiteman DC. Familial melanoma: a meta-analysis and estimates of attributable fraction. *Cancer Epidemiol Biomarkers Prevent* 2010;19:65–73.
5. Aoude LG, Wadt KA, Pritchard AL, et al. Genetics of familial melanoma: 20 years after CDKN2A. *Pigment Cell Melanoma Res* 2015;28:148–60.
6. Read J, Wadt KA, Hayward NK. Melanoma genetics. *J Med Genet* 2016;53:1–14.
7. de Snoo FA, Bishop DT, Bergman W, et al. Increased risk of cancer other than melanoma in CDKN2A founder mutation (p16-Leiden)-positive melanoma families. *Clin Cancer Res* 2008;14:7151–7.
8. Potjer TP, Kranenburg HE, Bergman W. de Vos tot Nederveen Cappel WH, van Monsjou HS, barge-Schaapveld DQ, Vasen HF. Prospective risk of cancer and the influence of tobacco use in carriers of the p16-Leiden germline variant. *Eur J Hum Genetics* 2015;23:711–4.
9. Sherr CJ. The INK4a/ARF network in tumour suppression. *Nat Rev Mol Cell Biol* 2001;2:731–7.
10. Puntervoll HE, Yang XR, Vetti HH, et al. Melanoma prone families with CDK4 germline

mutation: phenotypic profile and associations with MC1R variants. *J Med Genet* 2013;50:264–70.
11. Robles-Espinoza CD, Harland M, Ramsay AJ, et al. POT1 loss-of-function variants predispose to familial melanoma. *Nat Genet* 2014;46:478–81.
12. Aoude LG, Pritchard AL, Robles-Espinoza CD, et al. Nonsense mutations in the shelterin complex genes ACD and TERF2IP in familial melanoma. *J Natl Cancer Inst* 2015;107. https://academic.oup.com/jnci/article/107/2/dju408/901198
13. Harland M, Petljak M, Robles-Espinoza CD, et al. Germline TERT promoter mutations are rare in familial melanoma. *Fam Cancer* 2016;15:139–44.
14. Rai K, Pilarski R, Cebulla CM, et al. Comprehensive review of BAP1 tumor predisposition syndrome with report of two new cases. *Clin Genet* 2016;89:285–94.
15. Paillerets BB, Lesueur F, Bertolotto C. A germline oncogenic MITF mutation and tumor susceptibility. *Eur J Cell Biol* 2014;93:71–5.
16. Bertolotto C, Lesueur F, Giuliano S, et al. A SUMOylation-defective MITF germline mutation predisposes to melanoma and renal carcinoma. *Nature* 2011;480:94–8.
17. Ghiorzo P, Pastorino L, Queirolo P, et al. Prevalence of the E318K MITF germline mutation in Italian melanoma patients: associations with histological subtypes and family cancer history. *Pigment Cell Melanoma Res* 2013;26:259–62.
18. Raimondi S, Sera F, Gandini S, et al. MC1R variants, melanoma and red hair color phenotype: a meta-analysis. *Int J Cancer* 2008;122:2753–60.
19. Demenais F, Mohamdi H, Chaudru V, et al. Association of MC1R variants and host phenotypes with melanoma risk in CDKN2A mutation carriers: a GenoMEL study. *J Natl Cancer Inst* 2010;102:1568–83.
20. Aoude LG, Heitzer E, Johansson P, et al. POLE mutations in families predisposed to cutaneous melanoma. *Fam Cancer* 2015;14:621–8.
21. Wadt KA, Aoude LG, Golmard L, et al. Germline RAD51B truncating mutation in a family with cutaneous melanoma. *Fam Cancer* 2015;14:337–40.
22. Tuominen R, Engstrom PG, Helgadottir H, et al. The role of germline alterations in the DNA damage response genes BRIP1 and BRCA2 in melanoma susceptibility. *Genes Chromosomes Cancer* 2016;55:601–11.
23. Potjer TP, Helgadottir H, Leenheer M, et al. CM-score: a validated scoring system to predict CDKN2A germline mutations in melanoma families from northern Europe. *J Med Genet* 2018;55:661–8.
24. Salgado D, Desvignes JP, Rai G, et al. UMD-predictor: a high-throughput sequencing compliant system for pathogenicity prediction of any human cDNA substitution. *Hum Mutat* 2016;37:439–46.
25. Potrony M, Badenas C, Aguilera P, et al. Update in genetic susceptibility in melanoma. *Ann Transl Med* 2015;3:210.
26. Haugh AM, Njauw CN, Bubley JA, et al. Genotypic and phenotypic features of BAP1 cancer syndrome: a report of 8 new families and review of cases in the literature. *JAMA Dermatol* 2017;153:999–1006.
27. Njauw CN, Kim I, Piris A, et al. Germline BAP1 inactivation is preferentially associated with metastatic ocular melanoma and cutaneous-ocular melanoma families. *PLoS one* 2012;7:e35295.
28. Wadt KA, Aoude LG, Krogh L, et al. Molecular characterization of melanoma cases in Denmark suspected of genetic predisposition. *PLoS one* 2015;10:e0122662.
29. Gerami P, Yelamos O, Lee CY, et al. Multiple cutaneous melanomas and clinically atypical moles in a patient with a novel Germline BAP1 mutation. *JAMA Dermatol* 2015;151:1235–9.
30. O'Shea SJ, Robles-Espinoza CD, McLellan L, et al. A population-based analysis of germline BAP1 mutations in melanoma. *Hum Mol Genet* 2017;26:717–28.
31. Mangas C, Potrony M, Mainetti C, et al. Genetic susceptibility to cutaneous melanoma in southern Switzerland: role of CDKN2A, MC1R and MITF. *Br J Dermatol* 2016;175:1030–7.
32. Berwick M, MacArthur J, Orlow I, et al. MITF E318K's effect on melanoma risk independent of, but modified by, other risk factors. *Pigment Cell Melanoma Res* 2014;27:485–8.
33. Muller C, Wendt J, Rauscher S, et al. Characterization of patients at high risk of melanoma in Austria. *Br J Dermatol* 2016;174:1308–17.
34. Yokoyama S, Woods SL, Boyle GM, et al. A novel recurrent mutation in MITF predisposes to familial and sporadic melanoma. *Nature* 2011;480:99–103.
35. Potrony M, Puig-Butille JA, Aguilera P, et al. Prevalence of MITF p.E318K in patients with melanoma independent of the presence of CDKN2A causative mutations. *JAMA Dermatol* 2016;152:405–12.
36. Pellegrini C, Maturo MG, Martorelli C, et al. Characterization of melanoma susceptibility genes in high-risk patients from Central Italy. *Melanoma Res* 2017;27:258–67.
37. Sturm RA, Fox C, McClenahan P, et al. Phenotypic characterization of nevus and tumor patterns in MITF E318K mutation carrier melanoma patients. *J Invest Dermatol* 2014;134:141–9.
38. Speedy HE, Kinnersley B, Chubb D, et al. Germ line mutations in shelterin complex genes are associated with familial chronic lymphocytic leukemia. *Blood* 2016;128:2319–26.
39. Rafnar T, Gudbjartsson DF, Sulem P, et al. Mutations in BRIP1 confer high risk of ovarian cancer. *Nat Genet* 2011;43:1104–7.
40. Palles C, Cazier JB, Howarth KM, et al. Germline mutations affecting the proofreading domains of POLE and POLD1 predispose to colorectal adenomas and carcinomas. *Nat Genet* 2013;45:136–44.
41. Simeonov DR, Wang X, Wang C, et al. DNA variations in oculocutaneous albinism: an updated mutation list and current outstanding issues in molecular diagnostics. *Hum Mutat* 2013;34:827–35.
42. De Summa S, Guida M, Tommasi S, et al. Genetic profiling of a rare condition: co-occurrence of albinism and multiple primary melanoma in a Caucasian family. *Oncotarget* 2017;8:29751–9.
43. Passmore LA, Kaesmann-Kellner B, Weber BH. Novel and recurrent mutations in the tyrosinase gene and the P gene in the German albino population. *Hum Genet* 1999;105:200–10.
44. Hutton SM, Spritz RA. Comprehensive analysis of oculocutaneous albinism among non-Hispanic caucasians shows that OCA1 is the most prevalent OCA type. *J Invest Dermatol* 2008;128:2442–50.
45. Hawkes JE, Cassidy PB, Manga P, et al. Report of a novel OCA2 gene mutation and an investigation of OCA2 variants on melanoma risk in a familial melanoma pedigree. *J Dermatol Sci* 2013;69:30–7.
46. Goldstein AM, Xiao Y, Sampson J, et al. Rare germline variants in known melanoma susceptibility genes in familial melanoma. *Hum Mol Genet* 2017;26:4886–95.
47. Mavaddat N, Pharoah PD, Michailidou K, et al. Prediction of breast cancer risk based on profiling with common genetic variants. *J Natl Cancer Inst* 2015;107. https://academic.oup.com/jnci/article/107/5/djv036/891009?searchresult=1
48. Bubien V, Bonnet F, Brouste V, et al. High cumulative risks of cancer in patients with PTEN hamartoma tumour syndrome. *J Med Genet* 2013;50:255–63.
49. Maat W, Beiboer SH, Jager MJ, et al. Epigenetic regulation identifies RASEF as a tumor-suppressor gene in uveal melanoma. *Invest Ophthalmol Vis Sci* 2008;49:1291–8.
50. Di Lucca J, Guedj M, Lacapere JJ, et al. Variants of the xeroderma pigmentosum variant gene (POLH) are associated with melanoma risk. *Eur J Cancer (Oxford, England : 1990)* 2009;45:3228–36.


# THE DIFFERENCE OF BREAKTHROUGH MOMENTS

**WITH COMPLETE SOLUTIONS FOR GROUNDBREAKING DISCOVERIES FROM A TRUSTED PARTNER.**

Your next breakthrough could be closer than you imagine, especially with the right resources to help you advance your research. At BD, we are dedicated to helping you get the data you need, when, where and how you need it. Our integrated solutions in instrumentation, software and reagents are optimized to work together to bring you closer to your next breakthrough moment. And you can depend on us for world-class training, service and support to help you get the most from the results your research depends on. Discover a range of optimized solutions that open endless possibilities for your future research. **Discover the new BD.**

Learn how you can advance your research >

BD