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Familial Melanoma and Pancreatic Cancer: studies on genotype, phenotype and surveillance

Potjer, T.P.

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

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

ABSTRACT

Germline mutations in the major melanoma susceptibility gene *CDKN2A* explain genetic predisposition in only 10-40% of melanoma-prone families. In this 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 multi-gene 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.

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 proteins, p16INK4a and the alternatively spliced p14ARF, both of which are tumour-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 tumours (AST) or melanocytic *BAP1*–mutated atypical intradermal tumours (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 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 non-melanoma, non-oncogenic indication (MODY; MIM #606391). MODY is an autosomal dominant form of diabetes mellitus which manifests in young adults.

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*[a]		
	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*[b]		
	1	*2*	2
	2	*3*	3
	Total	**5**	**5**
Total		**451**	**488**

[a] 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

[b] Cutaneous melanoma was present in both single-case families and in one two-case family

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 1000 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 >1000 (>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®).

TABLE 2. List of genes included in the panel

Gene	Full Name	Alt. Name	MIM no.	Refs.
Established melanoma susceptibility genes				
High to medium penetrance:				
CDKN2A	Cyclin-Dependent Kinase Inhibitor 2A		600160	
CDK4	Cyclin-Dependent Kinase 4		123829	
BAP1	BRCA1-Associated Protein 1		603089	*Reviewed in: Aoude et al.,*[5] *Read et al.*[6]
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				
TERF1	Telomeric Repeat-Binding Factor 1	*TRF1*	600951	
TERF2	Telomeric Repeat-Binding Factor 2	*TRF2*	602027	*Aoude et al.*[12]
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]

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

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]

RESULTS

In our cohort of 488 samples (451 families), a total of 171 variants passed our primary filtering criteria (see *supplementary table S1*). 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 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 *supplementary figure S1*). 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 tumours, 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).

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[a] / GoNL
Established melanoma susceptibility genes					
ACD	c.871A>G, p.(Thr291Ala)	missense	1	0.0010025	0.0012/0.001
BAP1	c.122+1G>T, p.?	splicing	1	0.0010025	-/-
BAP1	c.1730-1G>A, p.?	splicing	1	0.0010025	-/-
BAP1	c.1936_1937insTT, p.(Tyr646Phefs*10)	frameshift	1	0.0010025	-/-
MITF	c.952G>A, p.(Glu318Lys)	missense	15	0.015369	0.0025/0.007
TERF2IP	c.398G>A, p.(Arg133Gln)	missense	1	0.0010025	0.00022/-
Shelterin complex candidate genes					
TERF1	c.186_188dup, p.(Glu62dup)	in-frame duplication	2	0.002049	0.0005/-[d]
TERF1	c.212_217dup, p.(Glu71_Ala72dup)	in-frame duplication	1	0.0010025	0.00014/-
TERF1	c.1193A>G, p.(Tyr398Cys)	missense	1	0.0010025	0.000009/-
TERF2	c.56A>G, p.(Asp19Gly)	missense	1	0.0010025	0.00012/-
TERF2	c.794G>A, p.(Arg265His)	missense	1	0.0010025	0.000027/-
TERF2	c.1492G>A, p.(Glu498Lys)	missense	4	0.004098	0.0022/0.003
TINF2	c.38G>T, p.(Arg13Leu)	missense	1	0.0010025	-/-
TINF2	c.734C>A, p.(Ser245Tyr)	missense	3	0.003074	0.00073/-

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
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
[a] In European (Non-Finnish) population
[b] 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
[c] HumVar trained PolyPhen-2 model used for prediction
[d] Common variant (MAF>1%) in one or more non-European populations
[e] Co-segregation analyses of variants with melanoma phenotype: *TERF1* p.E62dup: 2/2

CADD	SIFT	Align GVGD[b]	PolyPhen-2[c]	UMD-Predictor	FD	CoS[e]
23.2	delet.	C55	prob. damaging	polymorphism	Y	
					Y	
					Y	
					Y	
27.9	tol.	C0	prob. damaging	prob. polymorphism	Y	
23.4	delet.	C35	benign	polymorphism	Y	
					Y	Y
					Y	
24.7	delet.	C25	prob. damaging	pathogenic	Y	
16.35	delet.	C0	pos. damaging	n.a.	N	
28.3	delet.	C0	pos. damaging	prob. polymorphism	N	
34	delet.	C55	pos. damaging	prob. polymorphism	Y	
27	delet.	C0	prob. damaging	pathogenic	Y	
22.7	delet.	C15	benign	polymorphism	N	

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[a] / GoNL
BRIP1	c.517C>T, p.(Arg173Cys)	missense	9	0.009221	0.0047/0.004
BRIP1	c.790C>T, p.(Arg264Trp)	missense	1	0.0010025	0.0012/0.003
BRIP1	c.894C>A, p.(Cys298*)	nonsense	1	0.0010025	-/-
BRIP1	c.1198G>T, p.(Asp400Tyr)	missense	2	0.002049	0.000027/-
BRIP1	c.1255C>T, p.(Arg419Trp)	missense	1	0.0010025	0.00046/0.001
BRIP1	c.2069G>A, p.(Gly690Glu)	missense	1	0.0010025	-/-
BRIP1	c.2582C>G, p.(Ser861Cys)	missense	1	0.0010025	0.000027/-
BRIP1	c.2593C>T, p.(Arg865Trp)	missense	1	0.0010025	0.000027/-
POLE	c.861T>A, p.(Asp287Glu)	missense	9	0.009221	0.0017/0.004
POLE	c.893A>G, p.(Tyr298Cys)	missense	1	0.0010025	-/-
POLE	c.1230G>A, p.(Trp410*)	nonsense	1	0.0010025	-/-
OCA2	c.163del, p.(Ala55Leufs*47)[f]	frameshift	1	0.0010025	0.000019/-
OCA2	c.796C>T, p.(Arg266Trp)	missense	1	0.0010025	0.0018/0.003[d]
OCA2	c.1255C>T, p.(Arg419Trp)[f]	missense	1	0.0010025	0.00011/-
OCA2	c.1261C>T, p.(Arg421Trp)	missense	1	0.0010025	0.000065/-
OCA2	c.1327G>A, p.(Val443Ile)[f]	missense	18	0.018443	0.0051/0.008
OCA2	c.1441G>A, p.(Ala481Thr)[f]	missense	1	0.0010025	0.0026/0.001[d]
OCA2	c.1465A>G, p.(Asn489Asp)[f]	missense	7	0.007172	0.0007/0.003
OCA2	c.1592A>G, p.(Tyr531Cys)	missense	1	0.0010025	0.00011/0.001
OCA2	c.2037G>C, p.(Trp679Cys)[f]	missense	1	0.0010025	0.00015/-

Gene reference sequences: *BRIP1*: NM_032043.2, *POLE*: NM_006231.2, *OCA2*: NM_000275.2
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

[a] In European (Non-Finnish) population

[b] 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

[c] HumVar trained PolyPhen-2 model used for prediction

[d] Common variant (MAF>1%) in one or more non-European populations

[e] 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

[f] Variants reported in patients with oculocutaneous albinism type 2

CADD	SIFT	Align GVGD[b]	PolyPhen-2[c]	UMD-Predictor	FD	CoS[e]
27.6	delet.	C55	prob. damaging	pathogenic	Y	
32	delet.	C0	prob. damaging	pathogenic	Y	
36				pathogenic	Y	
33	delet.	C35	prob. damaging	pathogenic	Y	
33	delet.	C35	prob. damaging	pathogenic	Y	N
32	delet.	C65	prob. damaging	pathogenic	Y	
28.5	delet.	C65	prob. damaging	pathogenic	Y	
34	delet.	C25	prob. damaging	pathogenic	Y	N
25.7	delet.	C35	prob. damaging	pathogenic	Y	
28.3	delet.	C65	prob. damaging	pathogenic	Y	
38				pathogenic	Y	N
					N	
18.24	delet.	C0	pos. damaging	prob. polymorphism	N	
32	delet.	C0	prob. damaging	pathogenic	Y	
28	delet.	C0	prob. damaging	pathogenic	Y	N
34	tol.	C0	prob. damaging	polymorphism	Y	N
27.6	tol.	C0	pos. damaging	prob. polymorphism	Y	
28.2	delet.	C0	prob. damaging	pathogenic	Y	Y
25.3	delet.	C0	prob. damaging	pathogenic	Y	
34	delet.	C0	prob. damaging	pathogenic	Y	

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[a] / GoNL
CBLB	c.770A>T, p.(His257Leu)	missense	1	0.0010025	-/-
CBLB	c.1402C>G, p.(Arg468Gly)	missense	1	0.0010025	0.000018/-
ERCC3	c.496G>A, p.(Val166Ile)	missense	1	0.0010025	-/-
ERCC3	c.847C>T, p.(Arg283Cys)	missense	5	0.005123	0.0014/0.002
ERCC3	c.1421dup, p.(Asp474Glufs*2)	frameshift	1	0.0010025	0.00014/-
ERCC3	c.1776T>G, p.(Ile592Met)	missense	1	0.0010025	-/-
ERCC3	c.2111C>T, p.(Ser704Leu)	missense	2	0.002049	0.0022/0.001
MLLT6	c.655C>T, p.(Arg219Trp)	missense	1	0.0010025	0.000064/-
MLLT6	c.2195A>C, p.(Glu732Ala)	missense	1	0.0010025	-/-
MLLT6	c.2755G>A, p.(Gly919Arg)	missense	1	0.0010025	-/-
NEK2	c.97-2A>G	splicing	1	0.0010025	0.00029/-
NEK2	c.137A>G, p.(Glu46Gly)	missense	1	0.0010025	-/-
NEK2	c.952C>T, p.(Arg318*)	nonsense	1	0.0010025	0.000018/-
NEK4	c.500T>C, p.(Ile167Thr)	missense	1	0.0010025	0.000009/-
NEK4	c.1953_1955del, p.(Glu651del)	in-frame deletion	1	0.0010025	0.0011/0.002
NEK4	c.2093+1G>C	splicing	1	0.0010025	-/-
NEK10	c.1094G>A, p.(Arg365Gln)	missense	7	0.007172	0.0094/0.009
NEK11	c.127G>C, p.(Val43Leu)	missense	2	0.002049	0.00016/-[d]
PARP1	c.1814C>T, p.(Pro605Leu)	missense	1	0.0010025	0.000036/-
PARP1	c.2656G>A, p.(Val886Met)	missense	1	0.0010025	0.000027/-
POLH	c.626G>T, p.(Gly209Val)	missense	2	0.002049	0.0032/0.003[d]
POLH	c.890G>A, p.(Trp297*)	nonsense	1	0.0010025	-/-
RASEF	c.157C>T, p.(Arg53Trp)	missense	1	0.0010025	0.000049/-
RASEF	c.1049_1050del, p.(His350Argfs*3)	frameshift	1	0.0010025	0.000063/-
RASEF	c.2078A>G, p.(Asp693Gly)	missense	1	0.0010025	0.000018/-
RASEF	c.2207A>T, p.(Asn736Ile)	missense	1	0.0010025	0.000027/-

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

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

[a] In European (Non-Finnish) population

[b] 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

[c] HumVar trained PolyPhen-2 model used for prediction

[d] Common variant (MAF>1%) in one or more non-European populations

CADD	SIFT	Align GVGD[b]	PolyPhen-2[c]	UMD-Predictor	FD	CoS[e]
33	delet.	C0	prob. damaging	pathogenic	Y	
23.6	delet.	C0	pos. damaging	pathogenic	Y	
24.6	delet.	C25	benign	prob. polymorphism	Y	
34	delet.	C65	benign	pathogenic	Y	N
					Y	
24.9	delet.	C0	prob. damaging	prob. pathogenic	Y	
24	delet.	C15	benign	pathogenic	N	N
25.2	delet.	C15	pos. damaging	pathogenic	N	
24.6	delet.	C0	prob. damaging	pathogenic	Y	
26.2	delet.	C0	pos. damaging	pathogenic	N	
					Y	
28	delet.	C0	prob. damaging	pathogenic	Y	N
39				pathogenic	Y	
26.9	delet.	C25	prob. damaging	pathogenic	Y	
					N	
					N	
25.2	delet.	C0	pos. damaging	polymorphism	N	Y
27.5	delet.	C25	pos. damaging	prob. polymorphism	Y	
22.6	delet.	C15	benign	pathogenic	Y	
32	delet.	C0	prob. damaging	pathogenic	Y	
28.1	delet.	C15	prob. damaging	prob. polymorphism	Y	
40				pathogenic	Y	Y[f]
28.4	delet.	C0	prob. damaging	prob. pathogenic	Y	
					Y	
32	delet.	C0	pos. damaging	pathogenic	Y	
27.4	delet.	C0	pos. damaging	pathogenic	Y	

[e] 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)
[f] 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

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).

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

	familial CM cohort[a] (AN=956)	control cohort[a] (AN=898)	OR	95% CI	p value[b]
No. of individuals	478	449			
Reference sequence[c]	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

AN = allele number

[a] minor allele frequency (MAF)

[b] using Fisher's exact test (two-sided)

[c] number of alleles without any R or r variant

8

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 this study, we performed multi-gene 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 tumour 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 tumours, 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 amongst 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 0-3% [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 this 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 tumours 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 multi-gene 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 non-established 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 multi-gene 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 tumour risk assessment in melanoma families.

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COLLABORATORS

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.

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SUPPLEMENTARY MATERIAL

SUPPLEMENTARY TABLE S1. All variants with a MAF <1% (after primary filtering)

Gene	mRNA refSeq	Chr. Position (GRCh37)	cDNA change	Exon	Protein change	Variant type	dbSNP
ACD	NM_001082486.1	chr16: 67694102	c.280G>A	1	p.(Val94Ile)	ms	rs149365469
ACD	NM_001082486.1	chr16: 67694044	c.338G>A	1	p.(Arg113Gln)	ms	rs142507451
ACD	NM_001082486.1	chr16: 67692863	c.871A>G	7	p.(Thr291Ala)	ms	rs139438549
ACD	NM_001082486.1	chr16: 67691917	c.1436G>A	10	p.(Arg479Lys)	ms	rs531580930
BAP1	NM_004656.3	chr3: 52443569	c.122+1G>T	-	p.?	intron	-
BAP1	NM_004656.3	chr3: 52439834	c.878C>T	10	p.(Pro293Leu)	ms	rs777664260
BAP1	NM_004656.3	chr3: 52437424	c.1729+8T>C	-	p.?	intron	rs150945583
BAP1	NM_004656.3	chr3: 52437315	c.1730-1G>A	-	p.?	intron	-
BAP1	NM_004656.3	chr3: 52436841	c.1936_1937in-sTT	15	p.(Tyr-646Phefs*10)	fs	-
BRIP1	NM_032043.2	chr17: 59924572	c.517C>T	6	p.(Arg173Cys)	ms	rs4988345
BRIP1	NM_032043.2	chr17: 59924512	c.577G>A	6	p.(Val193Ile)	ms	rs4988346
BRIP1	NM_032043.2	chr17: 59924505	c.584T>C	6	p.(Leu195Pro)	ms	rs4988347
BRIP1	NM_032043.2	chr17: 59924502	c.587A>G	6	p.(Asn196Ser)	ms	rs550707862
BRIP1	NM_032043.2	chr17: 59885956	c.790C>T	7	p.(Arg264Trp)	ms	rs28997569
BRIP1	NM_032043.2	chr17: 59885852	c.894C>A	7	p.(Cys298*)	ns	-
BRIP1	NM_032043.2	chr17: 59876603	c.1198G>T	9	p.(Asp400Tyr)	ms	rs764711572
BRIP1	NM_032043.2	chr17: 59876546	c.1255C>T	9	p.(Arg419Trp)	ms	rs150624408
BRIP1	NM_032043.2	chr17: 59853928	c.1936-5T>A	-	p.?	intron	-
BRIP1	NM_032043.2	chr17: 59853790	c.2069G>A	14	p.(Gly690Glu)	ms	-
BRIP1	NM_032043.2	chr17: 59821955	c.2098-3T>C	-	p.?	intron	-
BRIP1	NM_032043.2	chr17: 59821830	c.2220G>T	15	p.(Gln740His)	ms	rs45589637
BRIP1	NM_032043.2	chr17: 59763520	c.2582C>G	19	p.(Ser861Cys)	ms	rs774415723
BRIP1	NM_032043.2	chr17: 59763509	c.2593C>T	19	p.(Arg865Trp)	ms	rs578022079
CBLB	NM_170662.4	chr3: 105572408	c.269G>A	3	p.(Ser90Asn)	ms	-

	Minor Allele Frequencies			Evolutionary Conservation / Distance			In-Silico Prediction				
Allele count	MAF (AN=976)	MAF ExAC[a]	MAF GoNL	NT	AA	GD	CADD Phred	SIFT	Align GVGD[b]	Poly Phen-2[c]	Spl
1	0.001025	0.00076	-	W	M	S	12.74	T	C0	B	
3	0.003074	0.0022	0.002	N	W	S	18.17	T	C0	B	
1	0.001025	0.0012	0.001	M	H	S	23.2	D	C55	D	
1	0.001025	0.000099	0.001	N	W	S	0.006	T	C0	B	
1	0.001025	-	-	-	-	-	-	-	-	-	+
1	0.001025	0	-	M	M	M	23.8	D	C0	B	
5	0.005123	0.006	0.007	-	-	-	-	-	-	-	
1	0.001025	-	-	-	-	-	-	-	-	-	+
1	0.001025	-	-	-	-	-	-	-	-	-	
9	0.009221	0.0047	0.004	M	H	L	27.6	D	C55	D	
4	0.004098	0.0054	0.007	N	W	S	0.002	T	C0	B	
1	0.001025	0.002	0.002	W	W	M	4.462	T	C0	B	
1	0.001025	0.000018	-	W	W	S	0.003	T	C0	B	
1	0.001025	0.0012	0.003	M	M	M	32	D	C0	D	
1	0.001025	-	-	-	-	-	36	-	-	-	
2	0.002049	0.000027	-	H	H	L	33	D	C35	D	
1	0.001025	0.00046	0.001	W	H	M	33	D	C35	D	
1	0.001025	-	-	-	-	-	-	-	-	-	
1	0.001025	-	-	H	H	M	32	D	C65	D	
2	0.002049	-	-	-	-	-	-	-	-	-	
1	0.001025	0.00065	0.001	W	M	S	25.7	T	C0	P	
1	0.001025	0.000027	-	M	H	M	28.5	D	C65	D	
1	0.001025	0.000027	-	W	H	M	34	D	C25	D	
1	0.001025	0	-	M	M	S	21.2	T	C0	B	

SUPPLEMENTARY TABLE S1 CONTINUED.

Gene	mRNA refSeq	Chr. Position (GRCh37)	cDNA change	Exon	Protein change	Variant type	dbSNP
CBLB	NM_170662.4	chr3: 105572313	c.364A>G	3	p.(Ile122Val)	ms	rs748358316
CBLB	NM_170662.4	chr3: 105464836	c.770A>T	6	p.(His257Leu)	ms	-
CBLB	NM_170662.4	chr3: 105438947	c.1351G>A	10	p.(Asp451Asn)	ms	rs377118360
CBLB	NM_170662.4	chr3: 105438896	c.1402C>G	10	p.(Arg468Gly)	ms	-
CBLB	NM_170662.4	chr3: 105421032	c.1865G>C	12	p.(Ser622Thr)	ms	rs41302192
CBLB	NM_170662.4	chr3: 105421025	c.1872T>G	12	p.(Asn624Lys)	ms	-
CBLB	NM_170662.4	chr3: 105400624	c.2240A>T	15	p.(His747Leu)	ms	rs149189614
CBLB	NM_170662.4	chr3: 105397298	c.2546A>T	17	p.(Gln849Leu)	ms	-
CENPS	NM_199294.2	chr1: 10494754	c.209+7A>G	-	p.?	intron	rs760512781
CENPS	NM_199294.2	chr1: 10502454	c.409G>A	5	p.(Glu137Lys)	ms	rs146240548
CREB3L1	NM_052854.3	chr11: 46329489	c.454G>A	3	p.(Ala152Thr)	ms	rs199951144
CREB3L1	NM_052854.3	chr11: 46332586	c.599A>T	5	p.(Asp200Val)	ms	rs187725533
CREB3L1	NM_052854.3	chr11: 46337910	c.1105G>A	9	p.(Ala369Thr)	ms	rs201046043
DOT1L	NM_032482.2	chr19: 2191003	c.265-8G>A	-	p.?	intron	rs374436091
DOT1L	NM_032482.2	chr19: 2191226	c.480G>A	5	p.(=)	syn	-
DOT1L	NM_032482.2	chr19: 2210451	c.1058C>G	13	p.(Ala353Gly)	ms	rs138206172
DOT1L	NM_032482.2	chr19: 2211098	c.1352A>G	15	p.(Asp451Gly)	ms	rs377185393
DOT1L	NM_032482.2	chr19: 2214564	c.1892C>T	19	p.(Ser631Leu)	ms	rs200661860
DOT1L	NM_032482.2	chr19: 2216470	c.2114G>C	20	p.(Ser705Thr)	ms	-
DOT1L	NM_032482.2	chr19: 2216545	c.2189C>T	20	p.(Ser730Leu)	ms	rs750873331
DOT1L	NM_032482.2	chr19: 2216610	c.2254C>T	20	p.(Pro752Ser)	ms	rs370203392
DOT1L	NM_032482.2	chr19: 2216658	c.2302G>C	20	p.(Ala768Pro)	ms	rs758184437
DOT1L	NM_032482.2	chr19: 2217034	c.2489C>T	21	p.(Pro830Leu)	ms	rs368118931
DOT1L	NM_032482.2	chr19: 2217094	c.2544+5G>A	-	p.?	intron	rs202211033
DOT1L	NM_032482.2	chr19: 2217786	c.2560G>A	22	p.(Ala854Thr)	ms	rs201843576
DOT1L	NM_032482.2	chr19: 2217801	c.2575G>A	22	p.(Gly859Arg)	ms	rs753001418
DOT1L	NM_032482.2	chr19: 2222325	c.3157G>A	24	p.(Ala1053Thr)	ms	rs144165419

	Minor Allele Frequencies			Evolutionary Conservation / Distance			In-Silico Prediction				
Allele count	MAF (AN=976)	MAF ExAC[a]	MAF GoNL	NT	AA	GD	CADD Phred	SIFT	Align GVGD[b]	Poly Phen-2[c]	Spl
1	0.001025	0	-	M	H	S	13.69	T	C0	B	±
1	0.001025	-	-	H	H	M	33	D	C0	D	
1	0.001025	0	-	H	M	S	27.7	T	C0	D	
1	0.001025	0.000018	-	W	M	M	23.6	D	C0	P	
9	0.009221	0.0083	0.007	W	W	S	5.998	T	C0	B	
1	0.001025	-	-	W	W	M	0.007	T	C0	B	
2	0.002049	0.000045	-	M	M	M	19.56	T	C0	B	
1	0.001025	0.000018	-	M	W	M	20.7	T	C0	B	
1	0.001025	0.000046	-	-	-	-	-	-	-	-	
1	0.001025	0.0014	0.002	M	M	S	10.71	T	C0	B	
1	0.001025	0.0065[d]	0.006	N	M	S	3.186	T	C0	B	
7	0.007172	0.0059	0.006	M	H	L	26.9	D	C0	B	
1	0.001025	0.00033	0.001	W	M	S	22.7	D	C0	B	
4	0.004098	0.00034	0.001	-	-	-	-	-	-	-	
1	0.001025	0.000018	-	N	-	-	-	-	-	-	±
1	0.001025	0.0024	0.004	M	W	S	19.18	T	C0	B	
1	0.001025	0.00025	0.001	M	M	M	23.3	T	C0	D	±
1	0.001025	0.00055	0.001	H	H	L	34	T	C0	D	
1	0.001025	-	-	M	M	S	23	T	C0	B	
1	0.001025	0.000027	-	M	W	L	25.9	T	C0	B	
1	0.001025	0.000027	-	M	M	M	25.8	T	C0	B	
1	0.001025	0.0001	-	N	W	S	22.2	T	C0	B	
3	0.003074	0.000018	-	W	M	M	26.4	T	C0	B	
2	0.002049	0.0015	0.003	-	-	-	-	-	-	-	±
2	0.002049	0.0017	0.004	N	W	S	3.499	T	C0	B	
1	0.001025	0.000009973	-	M	M	M	25.8	T	C0	P	
1	0.001025	0.0018	-	N	W	S	0.136	T	C0	B	

SUPPLEMENTARY TABLE S1 CONTINUED.

Gene	mRNA refSeq	Chr. Position (GRCh37)	cDNA change	Exon	Protein change	Variant type	dbSNP
DOT1L	NM_032482.2	chr19: 2223364	c.3475G>C	25	p.(Asp1159His)	ms	rs377512955
DOT1L	NM_032482.2	chr19: 2226219	c.3699C>T	27	p.(=)	syn	rs771189396
DOT1L	NM_032482.2	chr19: 2226539	c.4019A>G	27	p.(Lys1340Arg)	ms	-
DOT1L	NM_032482.2	chr19: 2226592	c.4072G>A	27	p.(Gly1358Ser)	ms	rs376766280
DOT1L	NM_032482.2	chr19: 2226694	c.4174G>A	27	p.(Gly1392Ser)	ms	rs375002753
DOT1L	NM_032482.2	chr19: 2226728	c.4208C>A	27	p.(Thr1403Asn)	ms	rs200561588
DOT1L	NM_032482.2	chr19: 2226833	c.4313T>G	27	p.(Leu1438Arg)	ms	rs371610616
DOT1L	NM_032482.2	chr19: 2226929	c.4409C>T	27	p.(Pro1470Leu)	ms	-
DOT1L	NM_032482.2	chr19: 2226935	c.4415C>G	27	p.(Pro1472Arg)	ms	-
DOT1L	NM_032482.2	chr19: 2227081	c.4561C>T	27	p.(His1521Tyr)	ms	-
ERCC3	NM_000122.1	chr2: 128047825	c.496G>A	4	p.(Val166Ile)	ms	-
ERCC3	NM_000122.1	chr2: 128047311	c.611G>A	5	p.(Gly204Glu)	ms	rs751705179
ERCC3	NM_000122.1	chr2: 128046416	c.847C>T	7	p.(Arg283Cys)	ms	rs145201970
ERCC3	NM_000122.1	chr2: 128044468	c.1153G>A	8	p.(Asp385Asn)	ms	-
ERCC3	NM_000122.1	chr2: 128038129	c.1421dup	9	p.(Asp474Glufs*2)	fs	rs587778281
ERCC3	NM_000122.1	chr2: 128030492	c.1776T>G	11	p.(Ile592Met)	ms	-
ERCC3	NM_000122.1	chr2: 128016978	c.2111C>T	14	p.(Ser704Leu)	ms	rs4150521
MITF	NM_000248.3	chr3: 70014091	c.952G>A	9	p.(Glu318Lys)	ms	rs149617956
MLLT6	NM_005937.3	chr17: 36864133	c.354+8G>A	-	p.?	intron	rs113618401
MLLT6	NM_005937.3	chr17: 36868139	c.592G>A	7	p.(Ala198Thr)	ms	rs2241012
MLLT6	NM_005937.3	chr17: 36868202	c.655C>T	7	p.(Arg219Trp)	ms	rs369771793
MLLT6	NM_005937.3	chr17: 36869017	c.794C>T	8	p.(Pro265Leu)	ms	rs754493479
MLLT6	NM_005937.3	chr17: 36872024	c.979G>A	9	p.(Ala327Thr)	ms	rs146278240
MLLT6	NM_005937.3	chr17: 36872922	c.1339G>T	10	p.(Ala447Ser)	ms	rs145966494
MLLT6	NM_005937.3	chr17: 36876664	c.2195A>C	15	p.(Glu732Ala)	ms	-
MLLT6	NM_005937.3	chr17: 36878443	c.2755G>A	17	p.(Gly919Arg)	ms	-

	Minor Allele Frequencies			Evolutionary Conservation / Distance			In-Silico Prediction				
Allele count	MAF (AN=976)	MAF ExAC[a]	MAF GoNL	NT	AA	GD	CADD Phred	SIFT	Align GVGD[b]	Poly Phen-2[c]	Spl
2	0.002049	0.00022	0.001	H	M	M	28.9	T	C0	D	
1	0.001025	0.00018	-	W	-	-	-	-	-	-	±
1	0.001025	-	-	W	W	S	25.2	T	C0	D	
1	0.001025	0.000059	-	W	W	S	8.724	T	C0	B	
1	0.001025	0.00052	0.002	N	W	S	2.832	T	C0	B	
1	0.001025	0.00024	-	N	W	S	12.39	T	C0	B	
1	0.001025	0.00011	-	W	W	M	11.78	T	C0	D	
1	0.001025	-	-	M	W	M	25.3	D	C0	B	
1	0.001025	-	-	M	W	M	23.3	T	C0	B	
1	0.001025	-	-	M	W	M	25.4	T	C0	D	
1	0.001025	-	-	H	H	S	24.6	D	C25	B	
1	0.001025	0.000009	-	M	H	M	18.35	T	C0	B	
5	0.005123	0.0014	0.002	H	H	L	34	D	C65	B	
2	0.002049	-	-	H	H	S	21.8	T	C0	B	
1	0.001025	0.00014	-	-	-	-	-	-	-	-	
1	0.001025	-	-	W	H	S	24.9	D	C0	D	
2	0.002049	0.0022	0.001	H	M	L	24	D	C15	B	
15	0.015369	0.002527	0.007	H	H	S	27.9	T	C0	D	
1	0.001025	0.00031[d]	-	-	-	-	-	-	-	-	
1	0.001025	0.00001[d]	-	N	W	S	0.011	T	C0	B	
1	0.001025	0.000064	-	W	H	M	25.2	D	C15	P	
1	0.001025	0.000063	-	M	M	M	25	D	C0	B	
9	0.009221	0.0067	0.008	W	M	S	18.24	D	C0	B	
9	0.009221	0.0069	0.008	N	W	M	6.782	T	C0	B	
1	0.001025	-	-	M	H	M	24.6	D	C0	D	
1	0.001025	-	-	M	H	M	26.2	D	C0	P	

SUPPLEMENTARY TABLE S1 CONTINUED.

Gene	mRNA refSeq	Chr. Position (GRCh37)	cDNA change	Exon	Protein change	Variant type	dbSNP
MLLT6	NM_005937.3	chr17: 36881009	c.3020C>T	19	p.(Ala1007Val)	ms	rs150198262
NEK2	NM_002497.3	chr1: 211847857	c.97-2A>G	-	p.?	intron	rs201869074
NEK2	NM_002497.3	chr1: 211847815	c.137A>G	2	p.(Glu46Gly)	ms	-
NEK2	NM_002497.3	chr1: 211842488	c.952C>T	6	p.(Arg318*)	ns	rs146817802
NEK4	NM_003157.5	chr3: 52800252	c.500T>C	3	p.(Ile167Thr)	ms	-
NEK4	NM_003157.5	chr3: 52786252	c.1064A>G	7	p.(Asn355Ser)	ms	-
NEK4	NM_003157.5	chr3: 52783745	c.1469G>A	8	p.(Arg490Gln)	ms	rs189287859
NEK4	NM_003157.5	chr3: 52780883	c.1544G>T	9	p.(Gly515Val)	ms	-
NEK4	NM_003157.5	chr3: 52777417	c.1953_1955del	12	p.(Glu651del)	del	rs534558039
NEK4	NM_003157.5	chr3: 52775426	c.2093+1G>C	-	p.?	intron	-
NEK10	NM_152534.4	chr3: 27343261	c.1094G>A	14	p.(Arg365Gln)	ms	rs75891446
NEK10	NM_152534.4	chr3: 27233631	c.2394G>T	27	p.(Gln798His)	ms	rs766212798
NEK10	NM_152534.4	chr3: 27216236	c.2594C>A	28	p.(Pro865His)	ms	rs140958685
NEK10	NM_152534.4	chr3: 27216215	c.2615A>G	28	p.(Tyr872Cys)	ms	rs141326474
NEK10	NM_152534.4	chr3: 27203966	c.2996A>G	32	p.(Asn999Ser)	ms	-
NEK10	NM_152534.4	chr3: 27182990	c.3124A>G	34	p.(Ile1042Val)	ms	rs41487750
NEK10	NM_152534.4	chr3: 27161337	c.3275C>T	36	p.(Pro1092Leu)	ms	rs34545563
NEK11	NM_024800.4	chr3: 130748679	c.127G>C	3	p.(Val43Leu)	ms	rs140058289
NEK11	NM_024800.4	chr3: 130947497	c.1525G>C	15	p.(Glu509Gln)	ms	-
OCA2	NM_000275.2	chr15: 28326992	c.29G>A	2	p.(Arg10Gln)	ms	rs199752361
OCA2	NM_000275.2	chr15: 28326984	c.37G>A	2	p.(Gly13Ser)	ms	rs201554429
OCA2	NM_000275.2	chr15: 28326977	c.44C>G	2	p.(Pro15Arg)	ms	-
OCA2	NM_000275.2	chr15: 28326858	c.163del	2	p.(Ala55Leufs*47)	fs	-
OCA2	NM_000275.2	chr15: 28263599	c.751G>A	7	p.(Val251Met)	ms	rs147432138
OCA2	NM_000275.2	chr15: 28263554	c.796C>T	7	p.(Arg266Trp)	ms	rs33929465
OCA2	NM_000275.2	chr15: 28261316	c.824C>T	8	p.(Thr275Met)	ms	rs369750458

	Minor Allele Frequencies			Evolutionary Conservation / Distance			In-Silico Prediction				
Allele count	MAF (AN=976)	MAF ExAC[a]	MAF GoNL	NT	AA	GD	CADD Phred	SIFT	Align GVGD[b]	Poly Phen-2[c]	Spl
4	0.004098	0.0021	0.002	M	M	S	33	D	C0	B	
1	0.001025	0.00029	-	-	-	-	-	-	-	-	+
1	0.001025	-	-	H	H	M	28	D	C0	D	
1	0.001025	0.000018	-	-	-	-	39	-	-	-	
1	0.001025	0.000009	-	H	H	M	26.9	D	C25	D	
1	0.001025	-	-	N	W	S	0.001	T	C0	B	
2	0.002049	0.00088	-	W	W	S	6.179	T	C0	B	
1	0.001025	-	-	N	W	M	11.36	T	C0	B	±
1	0.001025	0.0011	0.002	W	M	-	-	-	-	-	
1	0.001025	-	-	-	-	-	-	-	-	-	+
7	0.007172	0.0094	0.009	M	M	S	25.2	D	C0	P	
2	0.002049	0	-	N	W	S	8.073	T	C0	B	
2	0.002049	0.00065	-	W	W	M	21	D	C0	B	
5	0.005123	0.0016	0.006	W	W	L	1.542	T	C0	B	
1	0.001025	0.000018	-	W	W	S	2.977	T	C0	B	
3	0.003074	0.0063[d]	0.004	W	W	S	13.03	T	C0	B	
1	0.001025	0.000064	-	W	W	M	12.07	T	C0	B	
2	0.002049	0.00016[d]	-	H	H	S	27.5	D	C25	P	
1	0.001025	0	-	W	W	S	5.17	T	C0	B	
1	0.001025	0.00051	0.002	W	W	S	11.75	T	C0	B	
1	0.001025	0.000038	-	W	W	S	10.86	T	C0	B	
1	0.001025	-	-	W	W	M	11.18	T	C0	B	
1	0.001025	0.000019	-	-	-	-	-	-	-	-	
1	0.001025	0.00017[d]	-	N	W	S	4.551	T	C0	B	
1	0.001025	0.0018[d]	0.003	W	H	M	18.24	D	C0	P	
1	0.001025	0.000036	-	M	M	M	25.7	T	C0	P	

SUPPLEMENTARY TABLE S1 CONTINUED.

Gene	mRNA refSeq	Chr. Position (GRCh37)	cDNA change	Exon	Protein change	Variant type	dbSNP
OCA2	NM_000275.2	chr15: 28230319	c.1255C>T	13	p.(Arg419Trp)	ms	rs143218168
OCA2	NM_000275.2	chr15: 28230313	c.1261C>T	13	p.(Arg421Trp)	ms	rs372899234
OCA2	NM_000275.2	chr15: 28230247	c.1327G>A	13	p.(Val443Ile)	ms	rs121918166
OCA2	NM_000275.2	chr15: 28230238	c.1336A>G	13	p.(Met446Val)	ms	rs140566426
OCA2	NM_000275.2	chr15: 28228553	c.1441G>A	14	p.(Ala481Thr)	ms	rs74653330
OCA2	NM_000275.2	chr15: 28228529	c.1465A>G	14	p.(Asn489Asp)	ms	rs121918170
OCA2	NM_000275.2	chr15: 28211880	c.1592A>G	15	p.(Tyr531Cys)	ms	rs143699063
OCA2	NM_000275.2	chr15: 28202728	c.1784+6G>A	-	p.?	intron	rs779188429
OCA2	NM_000275.2	chr15: 28171315	c.2037G>C	19	p.(Trp679Cys)	ms	rs121918169
OCA2	NM_000275.2	chr15: 28116379	c.2165T>C	21	p.(Ile722Thr)	ms	rs1800417
PARP1	NM_001618.3	chr1: 226595491	c.120+9_120+20 del	-	p.?	intron	-
PARP1	NM_001618.3	chr1: 226578278	c.450G>T	4	p.(Gln150His)	ms	rs142376976
PARP1	NM_001618.3	chr1: 226573230	c.986A>G	7	p.(Asn329Ser)	ms	-
PARP1	NM_001618.3	chr1: 226570767	c.1129C>T	8	p.(Pro377Ser)	ms	rs2230484
PARP1	NM_001618.3	chr1: 226570748	c.1148C>A	8	p.(Ser383Tyr)	ms	rs3219062
PARP1	NM_001618.3	chr1: 226567661	c.1505C>T	10	p.(Ala502Val)	ms	rs183533639
PARP1	NM_001618.3	chr1: 226564936	c.1814C>T	13	p.(Pro605Leu)	ms	rs369900729
PARP1	NM_001618.3	chr1: 226564855	c.1895C>T	13	p.(Thr632Met)	ms	rs138228205
PARP1	NM_001618.3	chr1: 226552705	c.2656G>A	19	p.(Val886Met)	ms	rs776746526
PARP1	NM_001618.3	chr1: 226549169	c.3037C>A	23	p.(Leu1013Met)	ms	rs138906127
POLE	NM_006231.2	chr12: 133253180	c.861T>A	9	p.(Asp287Glu)	ms	rs139075637
POLE	NM_006231.2	chr12: 133253148	c.893A>G	9	p.(Tyr298Cys)	ms	-
POLE	NM_006231.2	chr12: 133250290	c.1230G>A	13	p.(Trp410*)	ns	-
POLH	NM_006502.2	chr6: 43550882	c.272+4A>G	-	p.?	intron	rs373430329
POLH	NM_006502.2	chr6: 43555032	c.296T>C	4	p.(Val99Ala)	ms	rs750026446
POLH	NM_006502.2	chr6: 43555151	c.415G>A	4	p.(Ala139Thr)	ms	rs554936509

	Minor Allele Frequencies			Evolutionary Conservation / Distance			In-Silico Prediction				
Allele count	MAF (AN=976)	MAF ExAC[a]	MAF GoNL	NT	AA	GD	CADD Phred	SIFT	Align GVGD[b]	Poly Phen-2[c]	Spl
1	0.001025	0.00011	-	N	H	M	32	D	C0	D	
1	0.001025	0.000065	-	W	M	M	28	D	C0	D	
18	0.018443	0.0051	0.008	H	H	S	34	T	C0	D	
2	0.002049	0.00025	0.001	W	M	S	1.045	T	C0	B	
1	0.001025	0.0026[d]	0.001	H	H	S	27.6	T	C0	P	
7	0.007172	0.0007	0.003	M	H	S	28.2	D	C0	D	
1	0.001025	0.00011	0.001	W	M	L	25.3	D	C0	D	
1	0.001025	0.000019	-	-	-	-	-	-	-	-	
1	0.001025	0.00015	-	H	H	L	34	D	C0	D	
1	0.001025	0.00091[d]	-	W	W	M	0.342	T	C0	B	
1	0.001025	-	-	-	-	-	-	-	-	-	
3	0.003074	0.00051	0.001	W	H	S	23.4	T	C0	D	
1	0.001025	-	-	W	W	S	7.255	T	C0	B	
3	0.003074	0.0068	0.004	M	H	M	13.58	T	C0	B	
9	0.009221	0.0027	0.002	H	M	L	24.6	D	C0	B	
3	0.003074	0.00082	-	W	M	S	10.01	T	C0	B	
1	0.001025	0.000036	-	M	M	M	22.6	D	C15	B	
2	0.002049	0.00034	-	M	M	M	26.2	T	C0	B	
1	0.001025	0.000027	-	H	H	S	32	D	C0	D	
2	0.002049	0.00095	-	W	H	S	18.79	T	C0	B	
9	0.009221	0.0017	0.004	W	H	S	25.7	D	C35	D	
1	0.001025	-	-	H	H	L	28.3	D	C65	D	
1	0.001025	-	-	-	-	-	38	-	-	-	
1	0.001025	0.00041	-	-	-	-	-	-	-	-	±
1	0.001025	0.000036	-	H	H	S	27.4	T	C0	D	
2	0.002049	-	0.001	M	M	S	23	T	C0	P	

SUPPLEMENTARY TABLE S1 CONTINUED.

Gene	mRNA refSeq	Chr. Position (GRCh37)	cDNA change	Exon	Protein change	Variant type	dbSNP
POLH	NM_006502.2	chr6: 43565568	c.626G>T	5	p.(Gly209Val)	ms	rs2307456
POLH	NM_006502.2	chr6: 43572357	c.890G>A	8	p.(Trp297*)	ns	-
POLH	NM_006502.2	chr6: 43573062	c.1074+6A>G	-	p.?	intron	-
POLH	NM_006502.2	chr6: 43581662	c.1510C>T	11	p.(Pro504Ser)	ms	-
POLH	NM_006502.2	chr6: 43581782	c.1630C>A	11	p.(Leu544Ile)	ms	-
POLH	NM_006502.2	chr6: 43581915	c.1763C>T	11	p.(Ser588Phe)	ms	-
POT1	NM_015450.2	chr7: 124491972	c.903G>T	11	p.(Gln301His)	ms	rs116916706
POT1	NM_015450.2	chr7: 124465356	c.1742A>G	20	p.(Lys581Arg)	ms	rs201023336
PTEN	NM_000314.4	chr10: 89690796	c.210-7_210-3del	-	p.?	intron	rs587780544
RAD51B	NM_133509.3	chr14: 68301816	c.218A>G	4	p.(Gln73Arg)	ms	rs774570772
RAD51B	NM_133509.3	chr14: 68331840	c.436G>A	5	p.(Ala146Thr)	ms	rs200741476
RAD51B	NM_133509.3	chr14: 68352579	c.453-7C>T	-	p.?	intron	rs201722637
RAD51B	NM_133509.3	chr14: 68352672	c.539A>G	6	p.(Tyr180Cys)	ms	rs28910275
RAD51B	NM_133509.3	chr14: 68353784	c.619G>T	7	p.(Val207Leu)	ms	rs28908168
RAD51B	NM_133509.3	chr14: 69061228	c.1063G>A	11	p.(Ala355Thr)	ms	rs61758785
RASEF	NM_152573.3	chr9: 85677626	c.157C>T	1	p.(Arg53Trp)	ms	rs766102616
RASEF	NM_152573.3	chr9: 85640842	c.432-9_432-6dup	-	p.?	intron	-
RASEF	NM_152573.3	chr9: 85622429	c.960-9A>G	-	p.?	intron	rs375961814
RASEF	NM_152573.3	chr9: 85620394	c.1049_1050del	8	p.(His350Argfs*3)	fs	rs755447494
RASEF	NM_152573.3	chr9: 85619464	c.1151G>A	9	p.(Arg384Lys)	ms	rs138418572
RASEF	NM_152573.3	chr9: 85613340	c.1745C>A	13	p.(Thr582Asn)	ms	rs143848788
RASEF	NM_152573.3	chr9: 85605345	c.2078A>G	16	p.(Asp693Gly)	ms	-
RASEF	NM_152573.3	chr9: 85597608	c.2207A>T	17	p.(Asn736Ile)	ms	rs762067279
TERF1	NM_017489.2	chr8: 73921307	c.186_188dup	1	p.(Glu62dup)	dup	rs149294115
TERF1	NM_017489.2	chr8: 73921333	c.212_217dup	1	p.(Glu71_Ala72dup)	dup	rs755588334
TERF1	NM_017489.2	chr8: 73939257	c.857A>G	6	p.(Glu286Gly)	ms	-

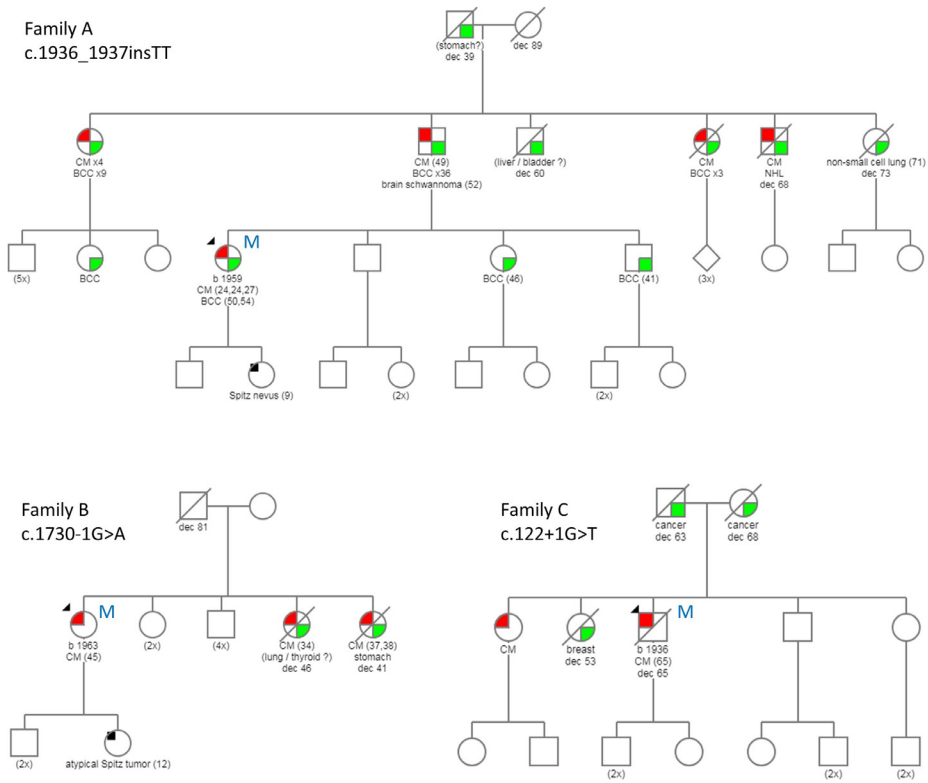
	Minor Allele Frequencies			Evolutionary Conservation / Distance			In-Silico Prediction				
Allele count	MAF (AN=976)	MAF ExAC[a]	MAF GoNL	NT	AA	GD	CADD Phred	SIFT	Align GVGD[b]	Poly Phen-2[c]	Spl
2	0.002049	0.0032[d]	0.003	M	H	M	28.1	D	C15	D	
1	0.001025	-	-	-	-	-	40	-	-	-	
1	0.001025	-	-	-	-	-	-	-	-	-	
1	0.001025	0.000009	-	W	M	M	0.001	T	C0	B	
1	0.001025	-	-	W	M	S	9.276	T	C0	B	
1	0.001025	-	-	W	M	L	17.46	T	C0	B	
1	0.001025	0.0042	0.003	N	H	S	15.65	T	C0	P	
3	0.003074	0.000045	-	W	W	S	10.18	T	C0	B	
1	0.001025	0.0003	-	-	-	-	-	-	-	-	±
1	0.001025	0.000018	-	W	W	S	4.787	T	C0	B	
1	0.001025	0.00027	0.002	H	H	S	25.3	T	C0	P	
1	0.001025	0.0004	0.001	-	-	-	-	-	-	-	
8	0.008197	0.0039[d]	-	W	M	L	18.52	T	C0	B	
4	0.004098	0.0029	0.004	W	H	S	15.32	D	C0	B	
9	0.009221	0.0041	0.004	W	W	S	7.995	T	C0	B	
1	0.001025	0.000049	-	W	M	M	28.4	D	C0	D	
1	0.001025	-	-	-	-	-	-	-	-	-	
3	0.003074	0.000027	-	-	-	-	-	-	-	-	±
1	0.001025	0.000063	-	-	-	-	-	-	-	-	±
1	0.001025	0.0005	0.001	H	M	S	15.71	T	C0	B	
1	0.001025	0.00017	-	M	H	S	22.6	D	C0	B	
1	0.001025	0.000018	-	H	H	M	32	D	C0	P	±
1	0.001025	0.000027	-	W	M	L	27.4	D	C0	P	
2	0.002049	0.0005[d]	-	W	W	-	-	-	-	-	
1	0.001025	0.00014	-	M	H	-	-	-	-	-	
1	0.001025	-	-	W	M	M	28.4	D	C0	B	

SUPPLEMENTARY TABLE S1 CONTINUED.

Gene	mRNA refSeq	Chr. Position (GRCh37)	cDNA change	Exon	Protein change	Variant type	dbSNP
TERF1	NM_017489.2	chr8: 73958245	c.1193A>G	10	p.(Tyr398Cys)	ms	rs760966818
TERF2	NM_005652.4	chr16: 69419852	c.23C>G	1	p.(Ala8Gly)	ms	-
TERF2	NM_005652.4	chr16: 69419819	c.56A>G	1	p.(Asp19Gly)	ms	rs773981277
TERF2	NM_005652.4	chr16: 69419801	c.74C>T	1	p.(Pro25Leu)	ms	rs749171225
TERF2	NM_005652.4	chr16: 69406163	c.693+9G>A	-	p.?	intron	rs191776266
TERF2	NM_005652.4	chr16: 69404432	c.794G>A	5	p.(Arg265His)	ms	rs763347805
TERF2	NM_005652.4	chr16: 69401088	c.962C>T	7	p.(Pro321Leu)	ms	-
TERF2	NM_005652.4	chr16: 69395387	c.1346C>T	8	p.(Pro449Leu)	ms	-
TERF2	NM_005652.4	chr16: 69390938	c.1492G>A	10	p.(Glu498Lys)	ms	rs150757154
TERF2IP	NM_018975.3	chr16: 75682178	c.398G>A	1	p.(Arg133Gln)	ms	-
TINF2	NM_001099274.1	chr14: 24711501	c.38G>T	1	p.(Arg13Leu)	ms	-
TINF2	NM_001099274.1	chr14: 24711477	c.62A>G	1	p.(Gln21Arg)	ms	rs367835995
TINF2	NM_001099274.1	chr14: 24711465	c.74G>C	1	p.(Gly25Ala)	ms	rs202093758
TINF2	NM_001099274.1	chr14: 24709976	c.710G>A	6	p.(Gly237Asp)	ms	rs17102313
TINF2	NM_001099274.1	chr14: 24709965	c.721C>T	6	p.(Pro241Ser)	ms	rs17102311
TINF2	NM_001099274.1	chr14: 24709952	c.734C>A	6	p.(Ser245Tyr)	ms	rs142777869

AN = allele number, NT = nucleotide (PhyloP score), AA = amino acid, GD = Grantham distance, Spl = splicing effect
Variant type: ms = missense, fs = frameshift, ns = nonsense, syn = synonymous, del = in-frame deletion, dup = in-frame duplication
Evolutionary Conservation/Distance: N = not, W = weak, M = moderate, H = high, S = small, L = large
In-Silico Prediction Tools: T = tolerated, D = deleterious (SIFT), B = benign, P = possibly damaging, D = probably damaging (PolyPhen-2), + = likely affects splicing, ± = possibly affects splicing
[a] in European (Non-Finnish) population
[b] 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
[b] HumVar trained PolyPhen-2 model used for prediction
[d] Common variant (MAF>1%) in one or more non-European populations

	Minor Allele Frequencies			Evolutionary Conservation / Distance			In-Silico Prediction				
Allele count	**MAF (AN=976)**	**MAF ExAC[a]**	**MAF GoNL**	**NT**	**AA**	**GD**	**CADD Phred**	**SIFT**	**Align GVGD[b]**	**Poly Phen-2[c]**	**Spl**
1	0.001025	0.000009	-	W	H	L	24.7	D	C25	D	
1	0.001025	-	-	W	W	S	12.75	D	C0	-	
1	0.001025	0.00012	-	W	W	M	16.35	D	C0	P	
2	0.002049	0.00043	-	M	W	M	19.54	D	C0	B	
4	0.004098	0.0037	0.002	-	-	-	-	-	-	-	
1	0.001025	0.000027	-	M	M	S	28.3	D	C0	P	
1	0.001025	-	-	W	M	M	21.2	D	C0	B	
1	0.001025	-	-	W	W	M	20.5	T	C0	B	
4	0.004098	0.0022	0.003	M	H	S	34	D	C55	P	
1	0.001025	0.00022	-	W	H	S	23.4	D	C35	B	
1	0.001025	-	-	M	H	M	27	D	C0	D	
1	0.001025	0.00023	-	N	M	S	0.001	T	C0	B	
1	0.001025	0.0024	0.001	W	M	S	12.31	T	C0	B	
1	0.001025	0.00015[d]	-	W	H	M	7.836	T	C0	B	
1	0.001025	0.000063[d]	-	W	H	M	23.4	T	C0	D	
3	0.003074	0.00073	-	N	W	L	22.7	D	C15	B	



SUPPLEMENTARY FIGURE S1. Families with a (likely) pathogenic variant in *BAP1*.

Symbols quarter-filled in upper left corner (red) represent melanoma, symbols quarter-filled in lower right corner (green) represent other cancers, symbols filled with a square in upper left corner (black) represent benign skin lesions. Age of onset in years is shown in parentheses. Unconfirmed cancer diagnoses are also shown in parentheses with "?". Probands are indicated with an arrow point and M (=mutation).

CM = cutaneous melanoma, BCC = basal cell carcinoma, NHL = Non-Hodgkin lymphoma, b = year of birth, dec = age deceased

Note: the proband in family C also carried a (likely) pathogenic variant in BRIP: c.894C>A, p.(C298)*