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Whole exome sequencing identifies candidate genes associated with hereditary predisposition to uveal melanoma

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Abstract

Objective—The aim of this study was to identify susceptibility genes associated with hereditary predisposition to uveal melanoma (UM) in patients with no detectable germline *BAP1* alterations.

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Design—Retrospective case series from academic referral centers.

Participants—Cohort of 154 UM patients with high risk of hereditary cancer defined as patients with one or more of the following: 1) familial UM; 2) young age (<35 years) at diagnosis; 3) personal history of other primary cancers; 4) family history of ≥2 primary cancers with no detectable mutation or deletion in *BAP1* gene.

Methods—Whole exome sequencing and/or cancer gene panel were carried out. Probands included 27 with familial UM, one with bilateral UM, one with congenital UM, and 125 UM patients with strong personal and/or family histories of cancer. Functional validation of variants was carried out by immunohistochemistry, RT-PCR and genotyping.

Main outcome and measures—Clinical characterization of UM patients with germline alterations in known cancer genes.

Results—We identified actionable pathogenic variants in eight known hereditary cancer predisposition genes *(PALB2, MLH1, MSH6, CHEK2, SMARCE1, ATM, BRCA1* and *CTNNA1)* in nine patients, including 3/27 (11%) with familial UM and 6/127 (4.7%) with high-risk for cancer. Two patients had pathogenic variants in *CHEK2* and *PALB2;* while variants in the other genes each occurred in one patient. Biallelic inactivation of *PALB2* and *MLH1* was observed in tumors from the respective patients. The frequencies of pathogenic variants in *PALB2, MLH1* and *SMARCE1* in UM patients were significantly higher than the observed frequencies in non-cancer controls [p=0.02, OR: 8.9 (1.5–30.6); p=0.04, OR: 25.4 (1.2–143); p= 0.001, OR: 2047 (52–4.5e15), respectively].

Conclusions—The study provides moderate evidence of gene/disease association of germline mutations in *PALB2* and *MLH1* with hereditary predisposition to UM. It also identifies several other candidate susceptibility genes. The results suggest locus heterogeneity in predisposition to UM. Genetic testing for hereditary predisposition to cancer is warranted in UM patients with strong personal and/or family history of cancers.

Precis

Genes associated with predisposition to cancer contribute to the etiology of uveal melanoma. Genetic testing for cancer genes is warranted in uveal melanoma patients with strong personal and/or family history of cancers.

Familial aggregation of uveal melanoma (familial UM) is rare [12] However, UM clusters in families with other cancers in up to 12% of cases [34] This, together with the rarity of the disease and lack of strong environmental causes suggests that genetic factors play an important role in UM etiology. Currently, BRCA-associated-protein 1 *(BAP1)* is the only gene with definitive evidence of association with hereditary predisposition to UM. Germline pathogenic variants in *BAP1* have been reported in at least 80 UM patients[5]. The frequency of *BAP1* pathogenic variants is 3–4% in UM with strong personal and family history of cancer [6–9] and about 20% in familial UM[10]. Germline pathogenic variants in *BRCA2* have been reported in a total of six UM patients [11–13], *MBD4* in four[14, 15], while only case reports of UM associated with several other genes, including *BRCA1*[11], *MLH1*[16], *CDKN2A*[17], *FLCN*[18, 19] and *MSH6*[20] are documented. This suggests the existence of other high penetrance drivers of UM which have yet to be identified. In this study we assessed coding

genetic alterations in UM patients with strong personal and/or family history of cancer and no detectable genetic alteration in *BAP1.*

MATERIALS AND METHODS

Samples

Patients were accrued from The Ohio State University Medical Center, Cleveland Clinic Foundation or referral to our program. Patients were included if they met one of the following criteria: 1) familial UM defined as a UM with 1st, 2nd or 3rd degree family member with UM;; 2) young age (<35 years) at diagnosis; 3) personal history of other primary cancers; 4) family history of ≥2 primary cancers with no detectable mutation or deletion in *BAP1* gene. The age cutoff of less than 35 years was chosen because it is 15–25 years younger than the median age of diagnosis in UM general population. Similar age cut off points have been used in clinical cancer genetic practice for germline genetic testing in other cancers[22–24]. Approval for this project was obtained from the Institutional Review Board at The Ohio State University (2006C0045) and Cole Eye Institute. Informed consents were obtained prior to testing.

DNA and RNA Extraction

Germline DNA was extracted from peripheral blood mononuclear cells (PBMCs) according to a published protocol [25] RNA was extracted using Trizol (Lifetechnology) from PBMCs and from tumor and non-tumor tissues in patients who underwent enucleation. DNA from tissue was extracted using Qiagen DNeasy or All prep RNA/DNA/miRNA kits (Qiagen, Valencia, CA).

Whole Exome Sequencing (WES) and Cancer Gene Panel

WES was carried out for six UM probands, two relatives and one UM tumor at the Genetic Resources Core Facility, Johns Hopkins Institute of Genetic Medicine, Baltimore, MD, according to their published protocol[26] utilizing Human All Exon V5 (Agilent) and HiSeq 2000 sequencer (Illumina) at a depth of 20X. The remaining 23 probands, seven relatives and three tumors were sequenced to an average depth of 104X at Nationwide Children's Hospital (Columbus, OH) utilizing the SureSelect Human All Exon V6 plus COSMIC (Agilent) and the HiSeq 4000 sequencer (Illumina) to produce paired-end 150bp reads. One sample was sequenced on the two platform for validation. The analysis of the sequencing data was conducted using the Churchill pipeline [27], in which the data was aligned to GRCh37 using Burrows-Wheeler Aligner-MEM[28], deduplicated using SAMBLASTER, and variants were jointly called across all samples using GATK's HaplotypeCaller. Variants with a maximum frequency >0.005 as observed in the ExAC (Exome Aggregation Consortium) without TCGA (The Cancer Genome Atlas), the 1000 Genome Project and the EVS (Exome Variant Server) were excluded. Furthermore, any variants that did not segregate in other affected family members were removed.

We focused our primary analysis on pathogenic or likely pathogenic alterations defined as null variants (stop gain, start loss, frame shift, canonical splice site ≤2 base pairs from exons) and missense variants reported as pathogenic in ClinVar[29].

For missense variants of uncertain significance we prioritized those with multiple lines of computational evidence supporting pathogenicity (SIFT [30]: ≤0.05, a Genomic Evolutionary Rate Profiling (GERP) score of >2 and Polyphen 2[31]: ≥0.453) in genes with established associations with hereditary cancer from the COSMIC cancer genes census[32], as well as genes involved in DNA repair[33, 34]

The BROCA multi-gene panel testing of 30 DNA repair/hereditary predisposition genes assay sequences all exons and flanking intronic sequences of tested genes[35, 36], Supplement Table 1 Large deletions and duplications were detected using methods described by Nord et al. 2011[37]. Confirmation of the variants and assessment of segregation in families were carried out by direct (Sanger) sequencing.

Quantitative RT-PCR

TaqMan 5' nuclease quantitative (real-time) RT-PCR assays were carried out using pre-developed assays from Applied Biosystems according to the manufacturer's protocol. Reactions were carried out in triplicate. The endogenous controls *GUSB* and/or *PP1A* were tested in separate reactions. Relative expression levels were assessed by the comparative threshold cycle (CT) method.

Microsatellite Instability Assay

A custom panel of ten microsatellite markers, labeled with either FAM or HEX dyes, was used (BAT25, BAT26, BAT40, NR27, NR21, NR22, D2S123, D3S1260, D17S250 and D5S346). This included the five NCI panel[38].

Immunohistochemistry for Assessment of Candidate Gene Product Expression

Immunostaining for MLH1 and MSH6 was carried out at the Department of Pathology (OSU) Clinical Laboratory according to standard practices. Immunostaining for other candidate gene products was carried out in the laboratory of M. Abdel-Rahman using published protocols[39–41] Supplement Table 2..

Statistical Analysis

GnomAD Loss-of-Function Intolerance Metric was obtained from gnomAD v2.1.1 at https://gnomad.broadinstitute.org on June 28, 2019. The metric is ratio of the observed number of loss-of-function variants in the gene in gnomAD to what would be expected if such variants were selectively neutral. The conditional maximum likelihood estimate of the odds ratio and Fisher's exact test two-sided mid-p value for the null hypothesis that the odds ratio equals 1 were obtained using the exact2×2 package in R [42, 43] The two-sided mid-p value was calculated by doubling the smaller of the one-sided p-values. The 95% confidence interval (CI) was obtained by inverting the test based on the two-sided mid-p value using this same package [42, 43]

RESULTS

Patient Population

163 unrelated UM patients were accrued. WES was carried out on germline DNA of 29 unrelated UM patients, nine of their relatives and available UM tissues of four patients. The probands for WES included 25 with familial UM, one with bilateral UM, one with congenital UM,[21] and two patients with strong family history of cancers, (Table 1). The relatives included six with UM, one with ovarian cancer and the unaffected parents of the congenital UM. Two UM relatives were first degree, while the remaining four were third degree. The cancer gene panel was assessed on 125 additional UM patients with personal and/or family histories suggestive of high-risk hereditary predisposition to cancer, (Table 1).

Pathogenic Alterations in Established Cancer Genes

We identified actionable pathogenic variants in eight known hereditary cancer predisposition genes *(PALB2, MLH1, MSH6, CHEK2, SMARCE1, ATM, BRCA1* and *CTNNA1)* accross nine patients, including 3/27 (11.1%) patients with familial UM and 6/127 (4.7%) UM patients with strong personal and/or family history of cancer, (Table 2 and 3).

Prioritized Variants in other Potential Cancer Genes

Given the rarity of UM, for the 29 UM patients studied by WES we focused our analysis on rare variants (≤ 0.005 minor allele frequency (MAF) in the general population) with strong evidence of pathogenesis (stop gain, start loss, frame shift, canonical splice site ≤2 base pairs from exons) and missense variants reported as pathogenic in ClinVar[29]. Of the 527 variants identified, 493 were null and 34 missense. In addition to pathogenic variants in four known cancer predisposition genes, *CHEK2, MLH1, PALB2* and *SMARCE1* (Table 2), pathogenic variants in five other genes were detected, Table 4. This included three *(RECQL4, MMS19* and *POLI)* genes associated with DNA damage repair, one *(DLEC1)* reported as a tumor suppressor in UNIPROT (http://www.uniprot.org/keywords/KW-0043), and *TP53AIP1,* which has been suggested as a cutaneous melanoma (CM) susceptibility gene [44]

In addition, a total of 1969 unique missense variants predicted as deleterious by multiple computational tools were identified by WES. Out of these, 47 were in cancer associated genes in 21 patients including: *RET, MSH3, MSH6, FANCD2, FANCM, RB1* and *WT1*, with established associations with hereditary cancer predisposition. The remaining genes are associated with DNA damage repair and/or are proposed as tumor suppressors, (Supplemental Table 3). In six patients, no variants in suspected cancer genes were detected, while in 16 patients, more than one variant (range 2–6) was detected (Supplementary Table 3). Variants in *DAPK1* and *MSH3* were each seen in two patients, variants in LZSTS1 in three patients, while variants in *SRRM2* were seen in four patients, including one with two different variants. Variants in all the other genes were seen once, Supplement Table 3.

Validation of Potential Candidate Genes

i- PALB2—A canonical splice site variant, c.3201+1G>C, in *PALB2* was detected in a patient with a personal history of UM and RCC, both diagnosed at age 67 years. Family

history was positive for a maternal relative with UM. WES of the tumor identified biallelic inactivation of *PALB2,* with a somatic truncating mutation, c.3279delT:p.Ile1093fs. A 75–80% decrease in the expression of *PALB2* was detected in the UM compared to the non-tumor choroid of the same patient, confirming the biallelic inactivation of *PALB2* (Figure 1).

A different canonical splice site variant, c.49–1G>A, was identified in another patient in the replication cohort. The 63year-old male presented with UM at age 51 years with no personal history of other cancers but a family history of breast and pancreatic cancers in his mother, breast cancer in his maternal aunt and a maternal first cousin, and colon cancer in a maternal aunt. In addition to *PALB2,* this patient had a likely pathogenic non-synonymous variant, c.T470C:p.Ile157Thr (rs17879961) in *CHEK2.* No tumor tissue was available for the assessment of biallelic inactivation.

One additional patient, a female presenting with UM at age 65 years, breast cancer at age 67 years and multiple non-melanoma skin cancers (ages 62–67 years), had a missense variant of uncertain significance (VUS), c.3418T>G :p.Trp1140Gly (rs62625283) in *PALB2.* Family history was positive for non-melanoma skin cancers, leukemia and prostate cancer. Recent, biochemical studies of this variant suggests that it has a deleterious effect on the DNA damage repair function of PALB2 and its interaction with BRCA2[45]. The patient was treated by brachytherapy and tumor tissue was not available for evaluation of biallelic inactivation.

We further surveyed a database of patients with germline mutations in *PALB2* for cases of UM. We identified one additional proband, a 65 year-old female who was diagnosed with bilateral breast cancer at 36 and 56 years. She was subsequently diagnosed with a UM at 62 years, which was managed by enucleation. The family history was notable for a male breast cancer in her brother at 67 years. Genetic testing identified a pathogenic germline *PALB2* c.3113G>A variant, which results in the generation of a premature stop codon and has been previously reported on multiple occasions[46]. To check for somatic pathogenic variants elsewhere in *PALB2* and in other genes previously associated with cancer, DNA was extracted from tumor tissue and analyzed using the Illumina TruSight Cancer panel, but no additional pathogenic variant in *PALB2* was detected and there was no evidence of loss of heterozygosity.

ii. MLHI—A missense pathogenic variant, c.200G>A, p.Gly67Glu (rs63749939), in *MLH1* was detected in a female diagnosed with UM at age 49 years, breast cancer at age 39 and a sebaceous adenocarcinoma. Her family cancer history was significant, with a brother diagnosed with UM at age 45 and Hodgkin's lymphoma at age 32, the mother diagnosed with colon cancer at age 39, the father diagnosed with prostate, urinary bladder and skin cancers, and a maternal and paternal grandfathers with colon cancers. All family members with cancers were deceased, with no tissue samples available. Archival tumor tissue was available from the UM for the proband. Sequencing of the tumor showed a relative decrease in the allele frequency of the wild type allele. Genotyping with markers in the vicinity of *MLH1* (D3S3135) showed loss of heterozygosity, and immunostaining showed loss of MLH1 protein expression in the tumor (Figure 2) but no microsatellite instability was observed. Taken together, these results confirm somatic biallelic inactivation of *MLH1* in the UM.

Two additional germline variants in *MLH1* were identified in our cohort. One, c.1039–8T>A (rs193922367) was reported benign in ClinVar, while the other one, c.277A>G:p.Ser93Gly (rs41295282) is rare in the general population (MAF 0.00003 in ExAC and 0.00008 in GO-ESP)but recently reported as likely benign by multiple clinical laboratories. Tumor tissue was not available from that patient for assessment of biallelic inactivation. One additional VUS in *MLH1* was identified in one of the sporadic UM patients[47].

iii. SMARCE1—A novel truncating pathogenic variant, c.373G>T:p.Glu125*, was detected in a female patient who was diagnosed with UM at age 46 and endometrial carcinoma at age 51. The patient had a family history of multiple non-melanoma skin cancer in mother and a maternal aunt and UM in a maternal aunt. An unreported missense VUS in *MSH6,* c.2589C>G:p.Cys863Trp, was also identified in the proband. Immunohistochemistry for SMARCE1 showed strong nuclear expression in the endometrial cancer and UM tumor cells. MSH6 loss of expression was observed in the endometrial cancer and only weak expression was observed in the UM tumor cells. The tumor showed areas of necrosis and there was insufficient material to study somatic mutation and copy number alteration.

DISCUSSION

Using WES and a cancer gene panel we identified actionable pathogenic variants in eight known hereditary cancer predisposition genes: *PALB2, MLH1, MSH6, CHEK2, SMARCE1, ATM, BRCA1* and *CTNNA1* in nine UM patients, including three (11%) with familial UM and six with strong personal and/or family history of cancer (4.7%). Combined with our previous study of germline mutation in *BAP1* in the same cohort[48], pathogenic variants in established hereditary predisposition genes were detected in 9/33 (27.3%) familial UM patients and 8/129 (6.1%) of UM patients with strong personal and/or family history of cancer[48]. This suggests the importance of referring these patients and their family members for cancer genetic counseling and genetic testing for proper management of risk of other cancers.

One of the major challenges in the field of cancer genetics is to establish evidence of gene-disease associations. Among the eight cancer predisposition genes with pathogenic variants observed in our cohort only two *(PALB2* and *MLH1)* showed moderate evidence of an association with hereditary predisposition to UM[49]. The evidence included the higher frequency of pathogenic variants in the UM cohort compared to the general population, the biallelic inactivation of the gene product in tumors and the observation of pathogenic variants in more than one unrelated UM patient. For *MLH1,* evidence included a previously reported UM case with a truncating mutation[16] in addition to the case identified in this study. For *PALB2* three unrelated cases were identified with pathogenic variants, two identified by screening a UM cohort and the third by screening a *PALB2* cohort. One additional case had a VUS which may be pathogenic based on recent experimental evidence [45]. The personal and family history of the patient with a pathogenic variant in *MLH1* was consistent with Lynch syndrome[50] and the personal and/or family histories of the three patients with *PALB2* were consistent with the reported cancer phenotype for the gene[51]. It is not clear whether UM hereditary risk is unique to *MLH1* or could be associated with other mismatch repair genes especially that germline pathogenic variant in *MSH6* was observed in

one UM patient in our cohort and has been reported in another patient[20]. Validation studies in larger cohorts will be needed. One potential explanation of the rarity of UM in subjects with germline pathogenic variant in *MLH1* and *PALB2* is the 4–6/million general population prevalence of UM so even a 100-fold increase in the odds ratio would still be observed as a rare phenotype.

SMARCE1 is another potential candidate identified in our cohort. Our data provide only limited evidence for its potential association with UM predisposition based on the significantly higher frequency of truncating mutation observed in UM patients compared to general population. So far seven probands/families have been reported with germline mutations in *SMARCE1:* six with meningiomas[52, 53] and one with Coffin-Siris Syndrome and anaplastic astrocytoma[54]. It is noteworthy that evidence of biallelic inactivation of SMARCE1 was observed in some but not all of these patients. Further studies of the potential role of *SMARCE1* in UM tumorigenesis is warranted.

A germline truncating variant in *TP53AIP1* was identified in one of our patients. This variant was the same variant detected in a report suggesting *TP53AIP1* as a predisposition gene to CM[44]. Although a significant difference was observed between cases and controls in that study, the high frequency (2%), of *TP53AIP1* truncating variants in the non-cancer general population largely preclude it as a predisposition gene to UM or CM.

It is worth noting that VUS in two genes with suggested role in hereditary predisposition to cancer, *DAPK1*[55] and *SRRM2*[56], were observed in more than one proband in our cohort; however, we did not identify additional supporting evidence of their role in predisposition to UM.

Pathogenic and likely pathogenic variants in more than one cancer associated gene were observed in several patients most notably *PALB2/CHEK2* in one and *SMARCE1/MSH6* in another, supplement Table 1. Also VUS in established and/or potential cancer genes were observed in several of UM probands suggesting multi-gene etiology in a subset of UM patients.

Combined with previous reports of germline pathogenic variants of several known cancer genes such as *BRCA2*[11–13], *BRCA1*[11], *MLH1*[16] *CDKN2A*[17], *FLCN*[18, 19] and *MSH6*[20], our study suggests that in UM patients with strong personal and/or family history of cancer a panel testing of at least the known-cancer genes is warranted. WES can be used as a research tool. Given the high frequency of somatic *BAP1* pathogenic alterations in UM, IHC screening of the tumors for BAP1 has very limited role in predicting patients with germline mutation.

We selected 35 year-old as cut off point for patients with early age of onset of their tumors which represents 15–25 years younger than the reported median age of onset of UM[57]. Other investigators have used younger than 18 or 21 year-old to define pediatric and young UM, respectively[58, 59] but no assessment of germline pathogenic variants were carried out. The median age of onset of patients with pathogenic variants in this study, 51 years (range 39–89), was slightly lower than the median age of patients with no pathogenic variants, median 58 years (range 3 months-87 years), but the difference was not statistically

significant. Younger age of onset has been observed in patients with germline *BAP1* pathogenic variants [5] and the frequency of pathogenic variants is much higher (19%) in subjects with young age of onset ≤ 30 of their tumors compared to general UM population (1–2%). We recommend screening UM patients developing their tumors at young age for at least germline pathogenic variants in *BAP1.*

In conclusion, our results suggest locus heterogeneity in predisposition to UM. Actionable pathogenic variants in established cancer genes, other than *BAP1,* were identified in 3/27 (11%) familial UM and 6/129 (4.7%) of UM with high-risk for hereditary cancer. In addition, possibly deleterious variants were detected in several other genes in the DNA-damage pathway. Our results provide moderate evidence of association of *PALB2* and *MLH1* with predisposition to UM. Genetic testing for hereditary predisposition to cancer is warranted in UM patients with strong personal and/or family history of cancers. Future studies to explore multigenic factors, gene rearrangements and noncoding elements using a whole genome approach are warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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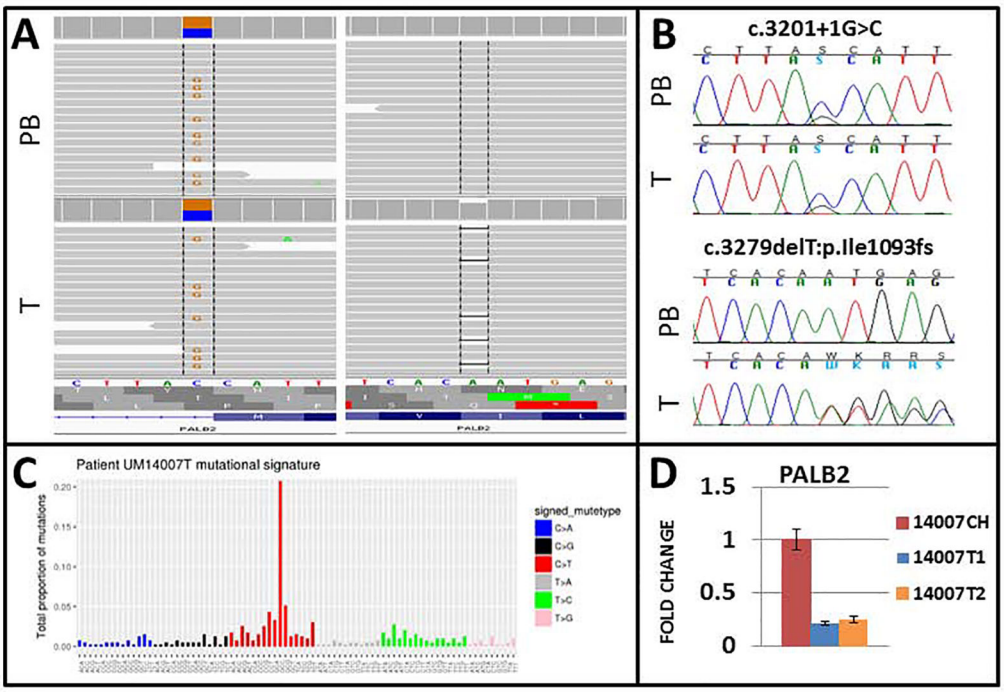

Figure 1. UM patient with germline PALB2 mutation with evidence of biallelic inactivation of PALB2 in the tumor

A) Whole exome sequencing of germline DNA (PB) and tumor tissue (T) identified a germline canonical splice site variant c.3201+1G>C with a somatic frameshift mutationc.3279delT:p.Ile1093fs in PALB2. B) Variants were confirmed by direct sequencing. C), Mutation signatures from the patient's tumor were significant for SBS39 and SBS1 which are commonly reported in sporadic UM. D) The expression of PALB2 mRNA was significantly lower in the tumor tissue from the patient compared to the matching non-tumor choroid.

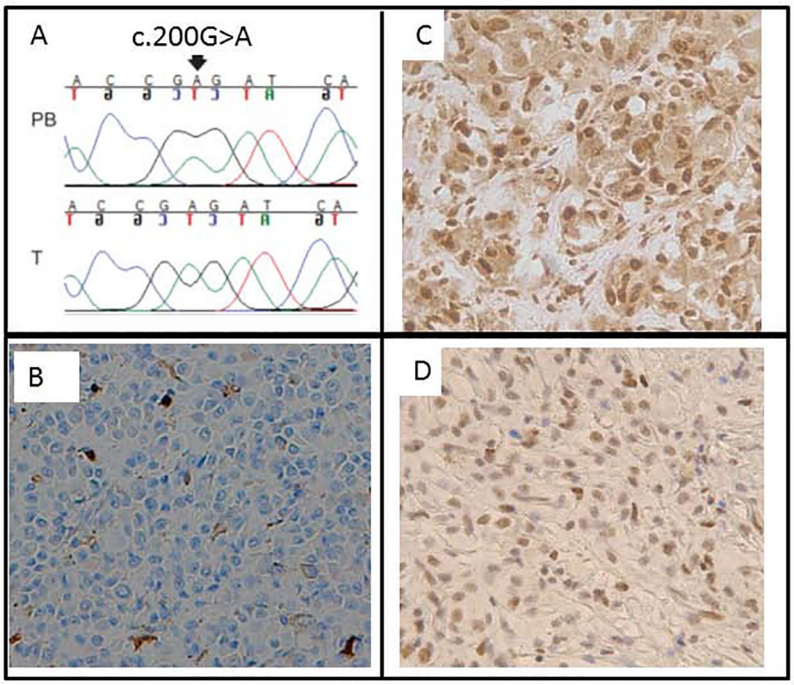

Figure 2: Assessment of biallelic inactivation in tumors from patients with germline variants in MLH1, SMARCE1 and MSH6

A) Direct sequencing confirmedthe germline variant c.200G>A, p.Gly67Glu and showed allelic imbalance in the tumor tissue. The variant is reported as pathogenic in ClinVar (rs63749939). B) Immunohistochemistry of tumor and tumor with adjacent non-tumor tissue show loss of nuclear expression of MLH1 protein in all tumor cells with preserved expression in the nuclei in non-tumor tissue. C) and D) A patient showed a null variant in SMARCE1 with variant of uncertain significance in MSH6. Immunohistochemistry showed preserved nuclear expression of SMARCE1 (C) and MSH6 (D) in the tumor.

Table 1.

Patient Demographics and Cancer Phenotype

	Patients studied by WES	**Pathogenic variants[1]**	**Patients studied by multi-gene panel**	**Pathogenic variants[1]**
Total	29	4	125	5
Male/Female	12/17	0/4	54/71	4/1
Age at UM diagnosis (median, range)	55 (0.25–79)	49 (46–67)	58 (15–87)	58 (50–89)
Patients diagnosed UM at young age (<35)	2	0	12	0
- Age of patients (years)	0.25, 25		15, 19, 23, 27 (x4), 28 (x2), 29, 31, 33	
Treatment				
- Enucleation	5	2	37	2
TNM Stage				
- pT1	4	0	13	1
- pT2	5	2	26	0
- pT3	5	0	43	1
- pT4	1	0	8	0
- Unknown	14	2	37	3
Clinical outcome				
- Average Follow Up (months)	78	68	102	90.8
- Alive	22	3	89	4
- Deceased	6	1	36	1
- Unknown	1	0	0	0
- Metastatic UM	5	1	29	2
Bilateral or multiple UM	1	0	4	0
Personal /family history of cancer*:	29	4	125	6
- BAP1-TPDS	18	2	31	0
- Lynch Syndrome	0	0	2	1
- Breast Ovarian	3	1	14	2
- Familial Melanoma	0	0	3	0
- Nonspecific	10	1	83	3
Family History of UM +	25	3	2	0
- BAP1-TPDS	15	2	1	0
- Lynch Syndrome	0	0	0	0
- Breast Ovarian	1	0	0	0
- Nonspecific	9	1	1	0

1) Pathogenic variants (null or confirmed pathogenic missense) in established hereditary cancer predisposition gene

WES: whole exome sequencing

BAP1-TPDS: BAP1 tumor predisposition syndrome

* Cancer syndrome classification is based on clinical presentation. Numbers are greater than total because more than one individual may be at risk for more than one syndrome.

Table 2.

Pathogenic and Likely Pathogenic Variants Detected in Known Cancer Predisposition Genes

Gene	Transcript	Variant (dbSNP)	Effect	Proband cancer	Cancer in Family	Heterozygous Cancer Risk[1]
A- Whole Exome Sequencing						
CHEK2	NM_007194.3	c.1100delC:p.Thr367Metfs (rs555607708)	Frameshift	UM, RCC, basal cell carcinoma	CM, breast	Breast
PALB2	NM_024675.3	c.3201+1G>C (rs587776423)	Splice site	UM, RCC	UM	Breast, Pancreatic
SMARCE1	NM_003079.4	c.373G>T, p.Glu125* (not reported)	Stopgain	UM, uterine	UM, Skin,	Meningioma
MLH1	NM_001258271.1	c.200G>A, p.Gly67Glu (rs63749939)	Non-synonymous	UM, breast (sebaceous adenocarcinoma)	UM, colon, skin, bladder, prostate, Hodgkins	Colon, ovarian, endometrial
B- BROCA Multi-Gene Panel						
MSH6	NM_000179.2	c.C2731T: p.Arg911* (rs63751017)	Non-synonymous	UM, endometrial, BCC, SCC	Lynch syndrome in family	Colon, endometrial
BRCA1	NM_007300.3	c.C2603G: pSer868* (rs80356925)	Non-synonymous	UM, prostate	Breast × 3, stomach, mycosis fungoides, lymphoma, cervical, Skin, Liver	Breast, ovarian
ATM	NM_000051.3	c.2251(−10)T>G (rs730881346)	Splice site	UM, lung	None	Breast, pancreatic
CHEK2	NM_007194.3	c.T470C:p.Ile157Thr (rs17879961)	Non-synonymous	UM	Pancreatic, breast × 2, colon	Breast
PALB2	NM_024675.3	c.49–1G>A (not reported)	Splice site			Breast, pancreatic
CTNNA1	NM_001903	c.1072_1075del AAAG: Arg360Valfs*8 (not reported)	Frameshift	UM	None	Gastric

Pathogenic and likely pathogenic is based on ClinVar

Total of 156 unrelated proband with UM

[1]Only the most commonly associated cancer types are listed.

BCC: Basel Cell Carcinoma; CM: Cutaneous Melanoma; RCC: Renal Cell Carcinoma; UM: Uveal Melanoma

Table 3.

Frequencies of null variants in the cancer genes in UM cohort and controls from public database

Gene	gnomAD Loss-of-Function Intolerance Metric *(90% CI)**	UM Cohort**	gnomAD Control Cohort***	Odds Ratio (95% CI)****	P-value****
		# Pathogenic Variant Carriers/# Individuals (%)			
BAP1	**0.12 (0.06 – 0.28)**	**7/172 (4.1%)**	**2/59095 (0.0034%)**	**1255, 95% CI: 277 – 8192**	**<2.2e-16**
BRCA1	0.73 (0.59 – 0.92)	1/156 (0.64%)	153/59095 (0.26%)	2.5 (0.1 – 12.6)	0.40
PALB2	**0.76 (0.58 – 1.01)**	**2/156 (1.3%)**	**86/59095 (0.15%)**	**8.9 (1.5 – 30.6)**	**0.02**
MLH1	**0.37 (0.25 – 0.57)**	**1/156 (0.64%)**	**15/59095 (0.025%)**	**25.4 (1.2 – 143.2)**	**0.04**
MSH6	0.34 (0.23 – 0.50)	1/156 (0.64%)	65/59095 (0.11%)	5.9 (0.3 – 30.1)	0.17
CHEK2	1.15 (0.87 – 1.53)	2/156 (1.3%)	409/59095 (0.69%)	1.9 (0.3 – 6.3)	0.39
CTNNA1	**0.17 (0.10 – 0.31)**	**1/156 (0.64%)**	**17/59095 (0.029%)**	**22.4 (1.1 – 124.8)**	**0.05**
ATM	0.60 (0.51 – 0.71)	1/156 (0.64%)	198/59095 (0.34%)	1.9 (0.1 – 9.7)	0.51
RECQL4	0.96 (0.77 – 1.21)	1/29 (3.4%)	232/59095 (0.39%)	9.1 (0.4 – 48.2)	0.11
SMARCE1	**0.04 (0.01 – 0.19)**	**1/29 (3.4%)**	**1/59095 (0.0017%)**	**2047 (52 – 4.5e15)**	**0.001**
TP53AIP1	1.73 (0.82 – 1.95)	1/29 (3.4%)	987/59095 (1.7%)	2.1 (0.1 – 11.1)	0.47
DLEC1	0.74 (0.61 – 0.91)	1/29 (3.4%)	844/59095 (1.4%)	2.4 (0.1 – 13.0)	0.41
MMS19	**0.27 (0.18 – 0.42)**	**1/29 (3.4%)**	**33/59095 (0.056%)**	**63.8 (3.0 – 354.2)**	**0.02**
POLI	**0.61 (0.41 – 0.93)**	**1/29 (3.4%)**	**60/59095 (0.10%)**	**35.1 (1.7 – 191.2)**	**0.03**

* This metric is the ratio of the observed number of loss-of-function variants in the gene in gnomAD to what would be expected if such variants were selectively neutral. Values closer to zero indicate loss-of-function intolerance (fewer than expected). Obtained from gnomAD v2.1.1 at https://gnomad.broadinstitute.org on June 28, 2019.

** One UM patient with large germline deletion of *BAP1* was not counted in order to make a valid comparison with gnomAD.

*** The gnomAD non-Finnish European non-cancer cohort comprised 59,095 unrelated individuals with data (51,377 with exomes and 7,718 with whole genomes). The number of carriers of pathogenic variants meeting the same criteria used in the UM cohort was estimated for each gene by the number of variant alleles at missense variants reported as pathogenic or likely pathogenic in ClinVar and null (stop, large deletion, frameshift, canonical splicing) variants not reported as benign and/or variants of uncertain significance in ClinVar. This approximation assumes that multiple pathogenic variant alleles do not appear on the same haplotype and that there are no individuals who are compound heterozygotes or homozygotes for pathogenic variants. To the extent that these assumptions are not met, the frequency of carriers in the control cohort would be overestimated, which would bias associations toward the null. No pathogenic or likely pathogenic missense variants were reported in ClinVar for for *DLEC1, MMS19, POLI,* and *TP53AIP1.*

**** Carrying pathogenic variants in genes highlighted in bold was associated with higher odds of UM ($P \leq 0.05$).

Table 4.

Null Variants in Genes Associated with Cancer

Gene	Transcript	Variant (dbSNP)	Effect	Proband Cancer	Cancer in Family (1st and 2nd degree)	Heterozygous Cancer Risk[1]
DLEC1	NM_007335.2	c.5068_5071dupAACA:p.Ser1691fs (rs577236284)	frameshift	UM	UM, CM, SCC, BCC, thyroid	-
MMS19	NM_022362.4	c.733_734delCT:p.Leu245fs	frameshift	UM, CM	UM, skin	-
POLI	NM_007195.2	c.729_732delAACA:p.Thr244fs (rs747005866)	frameshift	UM	UM, CASU, Leukemia, Hodgkins, Lung, colon, stomach, prostate	-
TP53AIP1	NM_022112.2	c.63dupG:p.Gln22fs (rs141395772)	frameshift	UM	UM, CM, breast, ovarian	Cutaneous melanoma
RECQL4	NM_004260.3	c.1573delT, p.Cys525fs (rs386833845)	frameshift	UM, RCC	UM, colon, cervical, lung	Osteosarcoma

Total of 29 unrelated proband with UM assessed by whole exome sequencing

[1]Only the most commonly associated cancer types are listed.

BCC: Basel Cell Carcinoma; CASU: Cancer Site Unknown; CM: Cutaneous Melanoma; RCC: Renal Cell Carcinoma; SCC: Squamous Cell Carcinoma; UM: Uveal Melanoma