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Alexandre Gaubert[1], Thibault Kervarrec[2,3], Henri Montaudié[4,5], Fanny Burel-Vandenbos[1], Nathalie Cardot-Leccia[1], Ilaria Di Mauro[6,7], Thibault Fabas[6], Anne Tallet[8], Valérie Kubiniek[6], Florence Pedeutour[6,7] and Bérengère Dadone-Montaudié[1,6,7]

Merkel cell carcinoma (MCC) is a rare and aggressive cutaneous neuroendocrine cancer. Management of advanced MCC is mainly based on immune-checkpoint inhibitors. The high failure rate warrants an investigation of new therapeutic targets. The recent identification of *BRCA1* or *BRCA2* (*BRCA1/2*) mutations in some MCC raises the issue of the use of poly-(ADP-Ribose)-polymerase inhibitors in selected advanced cases. The main objective of our study is to determine the accurate frequency of *BRCA1/2* pathogenic variants. We studied a series of 30 MCC and performed a meta-analysis of *BRCA1/2* variants of published cases in the literature. In our series, we detected only one *BRCA2* pathogenic variant. The low frequency of *BRCA1/2* pathogenic variants in our series of MCC (3%) was confirmed by the meta-analysis of *BRCA1/2* variants in the literature. Among the 915 MCC from 13 published series studied for molecular alterations of *BRCA1/2*, only 12 *BRCA1/2* pathogenic mutations were identified (1−2% of MCC), whereas many other *BRCA1/2* variants were variants of unknown significance or benign. *BRCA1/2* pathogenic variants are uncommon in MCC. However, in *BRCA*-mutated MCC, poly-(ADP-Ribose)-polymerase inhibitors might be a valuable therapeutic option requiring validation by clinical trials.

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INTRODUCTION

Over the last decade, the use of poly-(ADP-Ribose)-polymerase inhibitor (PARPi) treatment has considerably improved the survival and QOL of the subgroup of patients whose tumor cells present loss-of-function *BRCA1* or *BRCA2* mutations that hinder the homologous recombination repair (HRR) pathway (Audeh et al., 2010; González-Martín et al., 2022; Mirza et al., 2016). Although data mainly come from high-grade serous ovarian cancers, such treatments have been progressively extended to other tumors, such as breast, pancreatic, and prostate cancers (de Bono et al., 2020; Golan et al., 2019; Tutt et al., 2010). Other studies are needed to identify other tumors that remain resistant to standard therapy and could benefit from these treatments. Among them, Merkel cell carcinoma (MCC), a rare and aggressive skin tumor, has to be explored. Indeed, despite the evidence of *BRCA1/2* mutant cases, MCC is not listed as BRCA-related cancer (Becker et al., 2017).

At diagnosis, one third of patients with MCC present lymph node (26%) or visceral (8%) metastases, and 5-year overall survival rates are 35 and 14%, respectively (Harms et al., 2016; Walsh and Cerroni, 2021). In advanced MCC, systemic treatments are based primarily on immune-checkpoint inhibitors (PD-1/PD-L1 inhibitors). The objective response rates are 56% after first-line therapy and 32% in patients with chemorefractory advanced MCC (D'Angelo et al., 2018; Kaufman et al., 2016; Nghiem et al., 2016; Walsh and Cerroni, 2021). Since the discovery of the Merkel polyomavirus (MCPyV) in 2008, MCCs have been mainly divided into two groups: 80% of tumors result from MCPyV genome integration (MCPyV-positive MCCs), whereas approximately 20% are caused by UV exposure (MCPyV-negative MCCs)

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Abbreviations: aCGH, comparative genomic hybridization on array; Akt, protein kinase B; aSNP, SNP on array; CNA, copy number alteration; FFPE, formalin-fixed paraffin-embedded; HRR, homologous recombination repair; IHC, immunohistochemistry; LOH, loss of heterozygosity; MCC, Merkel cell carcinoma; MCPyV, Merkel cell polyomavirus; NGS, next-generation sequencing; PARPi, poly-(ADP-Ribose)-polymerase inhibitor; VUS, variant of unknown significance

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(Becker et al., 2017; Feng et al., 2008). Both types are highly aggressive cancers. Older age and immunosuppression are also risk factors for MCCs. Because approximately half of the patients fail to respond to immunotherapy, other treatments urgently need to be investigated (Harms et al., 2018; Walsh and Cerroni, 2021), and molecular alterations in MCCs could provide new therapeutic options. The mutational landscape of MCCs is mainly represented by mutations in the tumor suppressor genes *RB1* and *TP53*, especially in MCPyV-negative MCC (Becker et al., 2017). Activation of the phosphoinositide 3-kinase/protein kinase B (Akt)/mTOR pathway either by activating mutations in *PIK3CA, AKT1, HRAS,* or *KRAS* genes or by *PTEN* deletions has been identified in MCPyV-negative MCC (Becker et al., 2017; Stachyra et al., 2021). In addition, inactivating mutations of chromatin-modifier genes (*KMT2A/C/D, ASXL1, ARID1A/B, SMARCA4*) and DNA damage repair pathway genes (*MSH2, ATM, BRCA1/2, BCOR*) have been described. The presence of *BRCA1/2* mutations in some cases may open the way for using PARPi in molecularly selected advanced cases of MCCs (Becker et al., 2017; Carter et al., 2018; Cohen et al., 2016; Cohen and Tsai, 2019; Donizy et al., 2021; Ferrarotto et al., 2018; Goh et al., 2016; González-Vela et al., 2017; Harms et al., 2021, 2015; Knepper et al., 2019; Shalhout et al., 2021; Stachyra et al., 2021; Starrett et al., 2020; Wong et al., 2015; Zehir et al., 2017). To the best of our knowledge, the only publication that focused on PARPi in MCCs described mutations of DNA damage repair genes in 64% of cases (Ferrarotto et al., 2018). Sensitivity to olaparib was observed in one MCC cell line, suggesting a potential use for conducting clinical trials using PARPis. However, data on pathogenic and targetable alterations of the *BRCA1/2* genes are scarce and remain to be established. The main issue is to distinguish, among published variants, those that are definitively or likely pathogenic and may therefore be efficiently targeted by PARPi from variants of unknown significance (VUSs) or benign. The individual classification of a variant implies a time-consuming task of database and literature screening. Moreover, it is noteworthy that variant status may change over time and need regular re-examination.

The aim of this study was to determine the accurate frequency of *BRCA1* and *BRCA2* pathogenic variants in MCCs. In this study, we explored *BRCA1/2* mutations as well as other mutations and copy number alterations (CNAs) in the HRR pathway in a series of 35 patients with MCC. We also performed a meta-analysis of *BRCA1/2* mutations reported in the literature. We aimed at retrospectively classifying these mutations according to their pathogenicity. The proportion of pathogenic variants would be a strong indicator of the proportion of MCC that could benefit from a PARPi treatment.

RESULTS

Clinicopathological characteristics in our series of 35 patients

The patients were 15 males and 20 females; the median age at diagnosis was 81 years (ranging from 56 to 95 years) (Table 1 and Supplementary Table S1). Primary cutaneous lesions were mostly located in photoexposed areas: limbs (49%) and head and neck region (31%). In three cases (9%), the disease was revealed by lymph node involvement, without a previous history of cutaneous MCCs. At diagnosis, 12 cases were metastatic (lymph node involvement, 35%), whereas six cases (18%) developed lymph node metastasis during the follow-up. In total, 53% of patients experienced metastatic disease. Nine patients died, of whom five had metastatic disease. The median duration of follow-up was 21.1 months (range = 1−120 months). All cases were diagnosed as MCC on the basis of morphological features and a combination of CK20 positivity (except cases #15 and #16, which showed a negative CK20 expression but no visceral tumor) and at least one neuroendocrine marker (synaptophysin and/or chromogranin). Immunohistochemistry (IHC) profile included positivity for CK20 (94%), neurofilament (dot expression) (82%), and neuroendocrine markers (100%) (Table 1 and Supplementary Table S1). The results of the detection of MCPyV were consistent using both qPCR and IHC large T antigen methods: 20 cases (57%) were MCPyV-positive MCCs, whereas 15 cases (43%) were MCPyV-negative MCCs (Table 1 and Supplementary Table S1).

Table 1. Patients' Clinicopathological Characteristics from Our Series of Merkel Cell Carcinomas (n = 35)

Characteristics	n (%)
Median age, y (n = 35)	81 (56–95)[1]
Gender (n = 35)	
Male	15 (43%)
Female	20 (57%)
Primary location (n = 35)	
Head and neck (skin)	11 (31%)
Limbs (skin)	17 (49%)
Other skin locations (not photoexposed areas)	4 (11%)
No primary skin location	3 (9%)
Size of the primary skin tumor (n = 22)	
Mean (cm)	3.2
Median (cm)	2.25 (0.9–12)[1]
Metastatic status (lymph node or visceral)	
At diagnosis (n = 34)	12 (35%)
During follow-up (n = 34)	6 (18%)
MCPyV status	
qPCR (n = 35)	
positive	20 (57%)
negative	15 (43%)
IHC (n = 34)	
positive	19 (56%)
negative	14 (41%)
not contributive	1 (3%)
IHC	
CK20 (n = 35)	33 (94%)
NF (n = 28)	23 (82%)
Synaptophysin and/or chromogranin and/or CD56 (n = 35)	35 (100%)

Abbreviations: MCPyV, Merkel cell polyomavirus; IHC, immunochemistry; NF, neurofilament
[1]Median (range).

Molecular features in our series

Next-generation sequencing (NGS) analysis failed in five cases (Figure 1 and Supplementary Table S1). Comparative genomic hybridization on array (aCGH)/SNP on array (aSNP) analyses failed in one case (case number #12) and could not

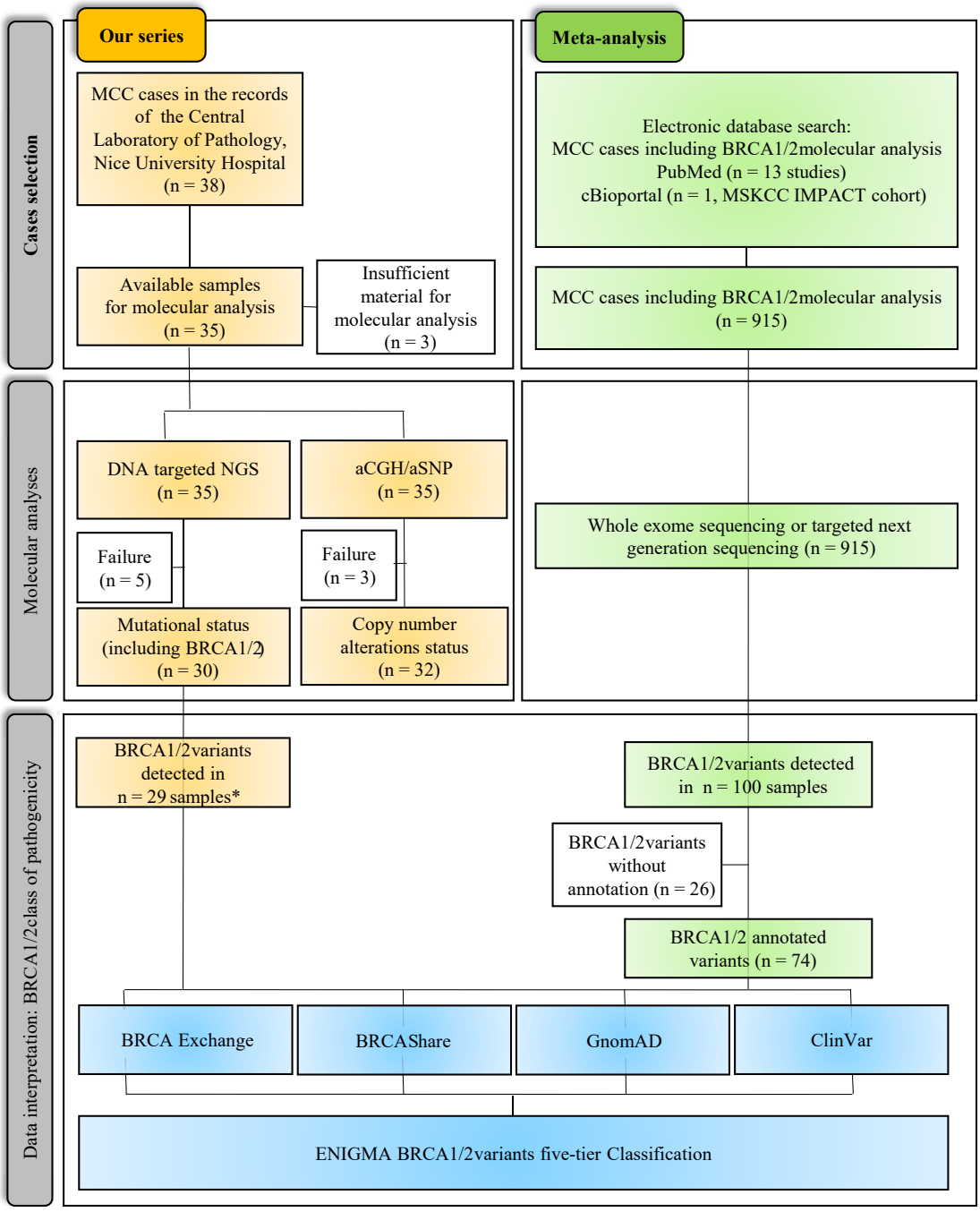

Figure 1. Flowchart showing two cohorts: our series of MCC and the meta-analysis of MCC cases harboring *BRCA1/2* variants. MCC, Merkel cell carcinoma.

be performed in case numbers #9 and #25 because of an insufficient amount of genomic DNA (Figure 1 and Supplementary Table S1).

The frequency of pathogenic *BRCA1/2* variants in our series was 3% (Supplementary Tables S1 and S2). We only found one *BRCA2* pathogenic variant: a nonsense mutation of *BRCA2* in case number #34, with a variant allele frequency of 10% classified as pathogenic, according to all databases (class 5 variant) (Figure 2 and Table 2). In case number #4, we detected a missense mutation of *BRCA2*, with a variant allele frequency of 45% classified as VUS because this variant was not yet reviewed in BRCA Exchange and BRCAshare databases and unknown in the ClinVar and gnomAD databases (class 3 variant) (Figure 2 and Table 2). In both cases, we could not perform a matched germline control. Other *BRCA1/2* variants (classified as benign or likely benign) were identified in all but one case (case number #26), including many silent or intronic mutations (data not shown) (Figure 1).

In MCPyV-positive MCC, missense mutations of *HRAS*, *POLD1*, *PIK3CA*, or *RAD51B* were observed in case numbers #1, #2, #10, and #19, respectively. We did not detect any

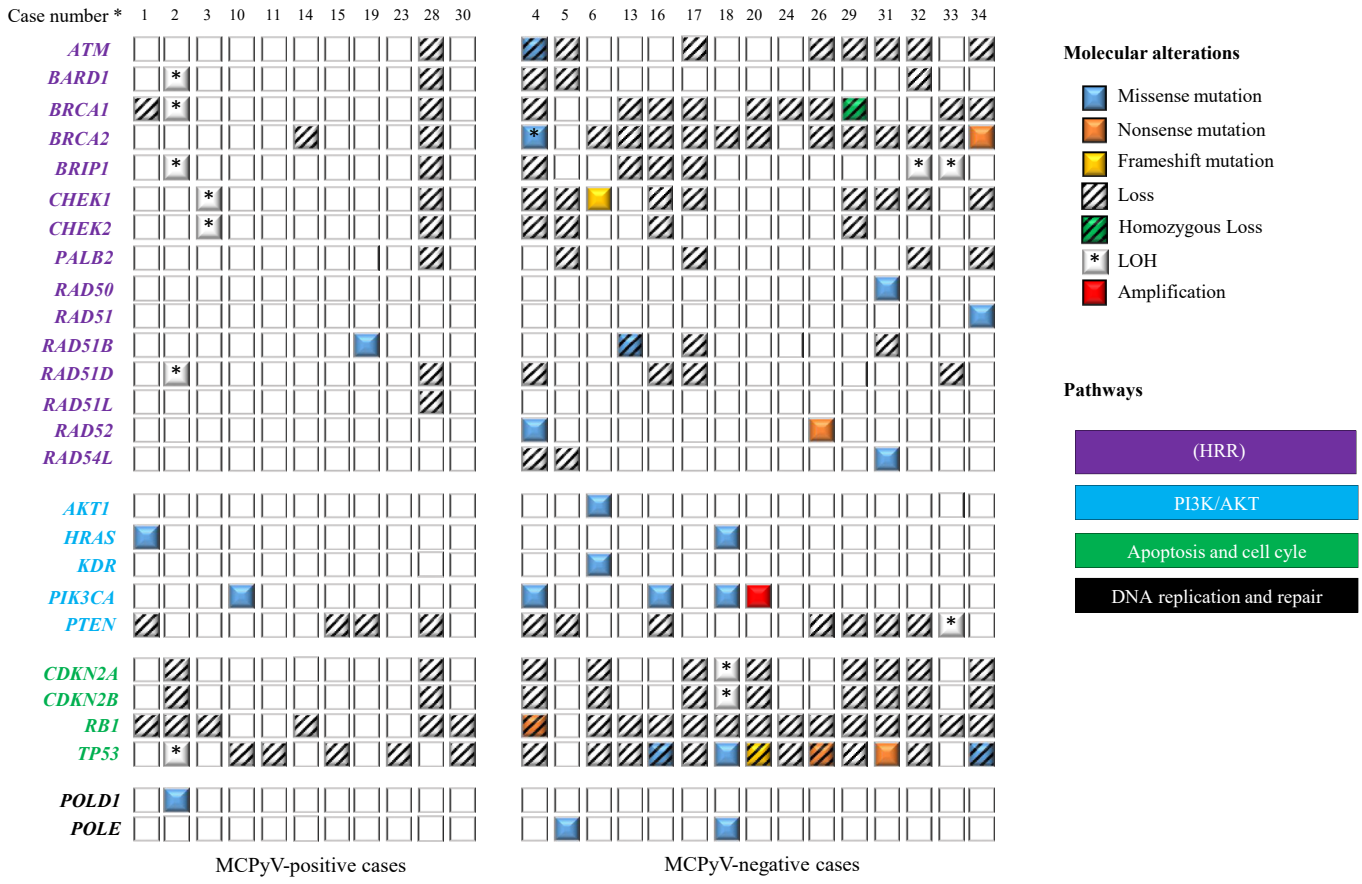

Figure 2. Distribution of molecular alterations in our series of 35 patients with MCC. Only cases with molecular alterations are represented. Cases are divided into two groups according to their MCPyV status. Molecular alterations include single nucleotide variations and CNAs such as losses, amplifications, and LOHs. Akt, protein kinase B; CNA, copy number alteration; LOH, losses of heterozygosity; MCC, Merkel cell carcinoma; MCPyV, Merkel cell polyomavirus; PI3K, phosphoinositide 3-kinase.

point mutations in the other 14 MCPyV-positive MCC cases (77%). Notably, no pathogenic mutation was detected in the *RB1*, *TP53*, *BRCA1,* and *BRCA2* genes (Figure 2 and Supplementary Table S1). Using aCGH/aSNP, two cases showed no CNA (case numbers #7 and #27), 14 cases presented only a few CNAs (ranging from 1 to 14), and two cases harbored a complex quantitative profile (i.e., several CNAs and notably more than 10 segmental chromosomal alterations). We observed losses of tumor suppressor genes in the following cases: *RB1* in six cases (32%), *TP53* in six cases (32%), *PTEN* in four cases (21%), and *CDKN2A/CDKN2B* in two cases (11%) (Figure 2). Loss of *BRCA1* was detected in three cases (16%; case numbers #1, #2, and #28) and loss of *BRCA2* in two cases (11%: case numbers #14 and #28) (Figure 2).

Among MCPyV-negative MCC, all but one case showed point mutations (91%), with a mean of 2.6 mutations per sample (ranging from 1 to 5) (Supplementary Table S1) using targeted NGS. Among genes frequently altered in MCC, six cases harbored *TP53* mutations—three missense, two nonsense, and one frameshift mutation—and one case harbored an *RB1* nonsense mutation (Figure 2). Other mutations occurred in the *PIK3CA* (three cases), *POLE* (two cases), *HRAS* (one case), *AKT1* (one case), and *KDR* (one case) genes (Figure 2). The HRR pathway was altered in six cases (Figure 2) (case numbers #4, #6, #13, #26, #31, and #34). In case number #4, three different mutations in the HRR pathway were observed (*BRCA2*, *ATM,* and *RAD52*). In case number #34, two different mutations in the HRR pathway were detected (*BRCA2* and *RAD51*). In the four remaining cases, mutations in the HRR pathway were missense mutations of *RAD54L* and *RAD50* (case number #31), a frameshift mutation of *CHEK1* (case number #6), a missense mutation of *RAD51B* (case number #13), and a nonsense mutation of *RAD52* (case number #26). Whole-genome copy-number analyses revealed complex profiles in all but one case (93%). *RB1* (n = 13), *TP53* (n = 11), *CDKN2A* and *CDKN2B* (n = 9), and *PTEN* (n = 8) were tumor suppressor genes that were frequently deleted or had a loss of heterozygosity (Figure 2). *BRCA2* was deleted (or with loss of heterozygosity) in 12 cases, and *BRCA1* was deleted in 10, including one case with homozygous *BRCA1* deletion (case number #29) (Figure 2).

Meta-analysis of *BRCA1/2* variants reported in the literature

To the best of our knowledge, 13 molecular studies of MCC that included reports of *BRCA1/2* variants (Figure 1) are available in the literature. Among all MCC cases (n = 915) studied by whole-exome sequencing or targeted NGS, including *BRCA1* and *BRCA2*, we have listed 100 *BRCA1/2* variants reported, and we have collected data about genomic

Table 2. List of *BRCA1* and *BRCA2* Pathogenic Variants in Our Series and in Reported Cases of Literature

BRCA1/2 Gene	Change in Coding DNA (or Genomic) Sequence	Consequence at DNA Level	Change in Protein Sequence	Consequence at Protein Level	VAF	Class of Pathogenicity[1]	Reference
BRCA2	c.4904_4905 delTGinsAGT	Frameshift insertion	p.Leu1635Ter (L1635*)	Nonsense	10%	Pathogenic (class 5)	Case #34 in our series
BRCA2	c.8524C>T	Substitution	p.Arg2842Cys (R2842C)	Missense	NA	Conflicting interpretations: pathogenic; likely pathogenic; VUS	Sample P-0003235 in Zehir et al. (2017)
BRCA2	c.(2805_2808del)	Frameshift deletion	p.Ala938fs (A938fs)	Frameshift	NA	Pathogenic (class 5)	Sample 42/ME00778 in Harms et al. (2021)
BRCA2	c.(9139C>T)	Substitution	p.Gln3047Ter (Q3047*)	Nonsense	NA	Pathogenic (class 5)	Sample 99/ME00411 in Harms et al. (2021)
BRCA2	c.(7516C>T)	Substitution	p.Gln2506Ter (Q2506*)	Nonsense	NA	Pathogenic (class 5)	Sample 117/ME00462 in Harms et al. (2021)
BRCA2	c.(8760T>G)	Substitution	p.Tyr2920Ter (Y2920*)	Nonsense	NA	Pathogenic (class 5)	Sample 221/ME00751 in Harms et al. (2021)
BRCA1	c.(5266C>T)	Substitution	p.Gln1756Ter (Q1756*)	Nonsense	NA	Pathogenic (class 5)	Case #13 in Cohen et al. (2016)
BRCA1	c.5265_5266 delinsTT	Frameshift insertion	p.Gln1756Ter (Q1756*)	Nonsense	NA	Pathogenic (class 5)	Sample P-0010462 in Zehir et al. (2017)
BRCA1	c.(4462C>T)	Substitution	p.Gln1488Ter (Q1488*)	Nonsense	NA	Pathogenic (class 5)	Sample 24/ME00512 in Harms et al. (2021)
BRCA1	c.(5302C>T)	Substitution	p.Gln1768Ter (Q1768*)	Nonsense	NA	Pathogenic (class 5)	Sample 25/ME00471 in Harms et al. (2021)
BRCA1	c.(5137G>A)	Substitution	p.Asp1713Asn (D1713N)	Missense	NA	Conflicting interpretations: pathogenic; likely pathogenic; VUS	Sample 43/ME00516 in Harms et al. (2021)
BRCA1	c.(1209dup)	Frameshift insertion	p.Glu404Ter (E404*)	Nonsense	NA	Pathogenic (class 5)	Sample 47/ME00614 in Harms et al. (2021)
BRCA1	c.(1551delT)	Frameshift deletion	p.Phe517fs (F517fs)	Frameshift	NA	Pathogenic (class 5)	Sample 83/ME00426 in Harms et al. (2021)

Abbreviations: NA, not available; ND, not done; VAF, variant allele frequency.

[1]Only class 4 (likely pathogenic) or class 5 (pathogenic) *BRCA1/2* variants were listed in this table. The pathogenicity was established according to public databases (BRCA Exchange, BRCAShare, ClinVar, and gnomAD). Full data of *BRCA1/2* alterations in our series and in the literature are available in Supplementary Table S2.

and protein variations as well as copy-number deletions of *BRCA1/2* and MCPyV status, if available (Figure 1 and Supplementary Table S2). Before classification according to their pathogenicity, the frequency of *BRCA1/2* mutations ranged from 4−5% (Carter et al., 2018; Harms et al., 2021, 2015; Knepper et al., 2019) to 25−40% (González-Vela et al., 2017; Shalhout et al., 2021; Wong et al., 2015). In three published series (Carter et al., 2018; Goh et al., 2016; Knepper et al., 2019), *BRCA1/2* variants were not annotated; authors only mentioned *BRCA1/2* mutations as missense, nonsense, or frameshift. This did not make it possible to classify them according to their pathogenicity. In the remaining cases of MCC with available genomic and/or protein data, we were able to determine a class of pathogenicity, with concordant classes most of the time according to the four public databases. We only identified 10 pathogenic (class 5) *BRCA1/2* nonsense or frameshift mutations (Table 2). We also found two missense mutations subject to conflicting interpretations of pathogenicity (at least one interpretation as pathogenic). In fact, the numerous remaining *BRCA1/2* variants reported in the literature were VUS as well as benign variants and mainly corresponded to missense mutations. After classification, the frequency of pathogenic *BRCA1/2* variants in the literature decreased to 1−2%. These 12 pathogenic or likely pathogenic variants mostly occurred in MCPyV-negative MCC (89%, two missing data) (Harms et al., 2021). They were not associated with copy-number deletions of *BRCA1/2* (one missing data) (Harms et al., 2021; Zehir et al., 2017). The variant allele frequency, available in two cases, was 28% for one missense mutation and 21% for one pathogenic nonsense mutation (Zehir et al., 2017).

DISCUSSION

The poor outcome of patients with advanced MCCs and the low efficiency of standard treatments imply the necessity of finding therapeutic targets. Among the molecular alterations of MCC reported in the literature, *BRCA1/2* mutations were mentioned. However, precisions about their frequency and, more importantly, about their pathogenicity and actionability were not provided.

In our series of 30 molecularly analyzed MCCs, we found *BRCA1/2* pathogenic variants with a frequency of 3%. This low frequency was confirmed by our meta-analysis of 13 published studies (n = 915 MCC cases). Interestingly, we have identified two large series of MCC reporting a few *BRCA1/2* variants: in the study by Harms et al. (2021), most of the *BRCA1/2* variants were confirmed to be pathogenic, whereas in the study by Knepper et al. (2019), the annotations about genomic variations and their pathogenicity were not provided. Altogether, both studies reported a frequency of

BRCA1/2 variants of about 4–5% (Harms et al., 2021; Knepper et al., 2019). Our results regarding the low frequency of *BRCA1/2* pathogenic variants have highlighted discrepancies from those of studies reporting the whole ensemble of *BRCA1/2* mutations. In these studies, the overestimated frequencies of *BRCA1/2* mutations ranged from 10 to 40% (Cohen et al., 2016; Donizy et al., 2021; Ferrarotto et al., 2018; Goh et al., 2016; González-Vela et al., 2017; Shalhout et al., 2021; Wong et al., 2015; Zehir et al., 2017). The observation that a majority of variants in MCC are benign or of unknown significance does not breach the usual molecular landscape of *BRCA1/2*: germline or somatic *BRCA1/2* variants are mainly nonpathogenic in all types of tumors. The pathogenicity of germline *BRCA1/2* variants is notably based on criteria established by the ENIGMA (Evidence-based Network for the Interpretation of Germline Mutant Alleles) consortium (https://enigmaconsortium.org/). These criteria include clinical data (cosegregation with disease, co-occurrence with a pathogenic variant in the same gene, reported family history, breast tumor histology) as well as in silico prediction of pathogenicity. For somatic mutations, the pathogenicity is determined by querying public databases: BRCAExchange and BRCAShare, which are specific to *BRCA1/2* variants, and other databases such as ClinVar and gnomAD. The type of mutations is also important because many pathogenic variants are nonsense or frameshift mutations, leading to premature protein truncation, whereas most of the missense and intronic variants of *BRCA1/2* (except in splice sites) are benign variants or VUS (in 90% of cases according to Parsons et al. [2019]). However, classification is a dynamic process, and it cannot be excluded that some VUS might be further reclassified as pathogenic, potentially some of the numerous *BRCA1/2* VUS identified in our meta-analysis. Altogether, these benign or VUS variants have no value from the perspective of personalized treatment by PARPi (Pujol et al., 2021).

Another interesting issue is the copy-number deletions of *BRCA1/2*. We used pangenomic copy-number analyses (aCGH/aSNP) (Figure 2) and observed a high frequency of copy-number deletions of *BRCA1/2* (of one copy of the gene, probably heterozygous deletions) in the MCPyV-negative subgroup. This could reflect the high level of chromosomal instability or aneuploidy classically described in this subgroup (Becker et al., 2017). Although copy-number deletions of *BRCA1/2* were common, homozygous deletions were rare in our series (3%). Besides the mutational status, the quantitative status of *BRCA1/2* might become a valuable predictive biomarker for the use of PARPis. Notably, it has been shown that it is better for patients treated with PARPi for metastatic prostate cancer with a homozygous deletion of *BRCA2* (Carreira et al., 2021). Indeed, tumors with *BRCA2* homozygous deletion are not able to produce secondary resistance mutations (reversion mutations that restore the DNA repair function) (Carreira et al., 2021; Li et al., 2020).

It is well-known that some *BRCA1/2* germline mutations are more common in some populations (Felix et al., 2018). In our study, we did not identify any of those mutations. Concerning our two mutated cases, we could not determine whether they were germline or somatic. This germline/somatic status was not investigated in the MCC studies reported in the literature. Nevertheless, because pathogenic germline mutations of *BRCA1/2* may not only be predisposed to cancer but also determine the use of PARPi in advanced cancers (such as breast and pancreatic cancers), this point should be addressed in further studies.

Our results raise the issue of the pertinence of BRCA testing in MCC samples. It would be useful in a few cases. Those few patients might greatly benefit from this testing, leading to PARPi treatment. Moreover, the effectiveness of PARPi in the context of advanced or metastatic MCCs needs to be investigated in clinical trials (e.g., in pan-solid tumors basket trials). In our study, we have focused on pathogenic *BRCA1/2* variants because, to date, the approval of PARPi for the treatment of patients with ovarian, breast, or pancreatic cancers is restricted to BRCA-mutated cases (gBRCA or tBRCA variants) (https://www.fda.gov/drugs/resources-information-approved-drugs). In the United States, for treatment of metastatic castration-resistant prostate cancers, the approval of PARPi is extended to patients with deleterious alterations in HRR genes (not limited to *BRCA*-mutated cases) (https://www.fda.gov/drugs/resources-information-approved-drugs). In our series, mutations in the HRR pathway were more frequent than those in *BRCA1* and *BRCA2*, and they represented 33% of MCC. Another predictive marker of PARPi sensitivity proposed in ovarian cancers is the homologous recombination deficiency status combining BRCA and genomic instability status (Hodgson et al., 2018).

Our study has confirmed that signaling pathways other than BRCA1/2 were involved in MCC and could be worth considering as potential targets. The phosphoinositide 3-kinase/Akt pathway as well as its upstream or downstream targets, such as *HRAS* or *PTEN*, were altered: missense mutations and/or amplification of *PIK3CA* in 17% of MCC and missense mutations of *HRAS* or *AKT1* in 10% of MCC. Our results were consistent with previous reports from the large series of Knepper et al. (2019) and Harms et al. (2021) in which *PIK3CA* mutations were identified in 21% and 14% of cases, respectively. Other therapeutics targeting mutations in the *TP53* or *RB1* genes might become an option because these genes are frequently altered in MCC. Surprisingly, we identified lower rates of *TP53* and *RB1* mutations in our series of MCPyV-negative MCCs (40 and 7%, respectively) than in those previously reported (94 and 64%, respectively, in the study by Harms et al. [2021] and 97 and 80%, respectively, in the tumor mutation burden–high subgroup, associated with the absence of MCPyV, in the study by Knepper et al. [2019]). Mutational heterogeneity and clonal evolution already described in MCC (Harms et al., 2017), *TP53/RB1* inactivation by other mechanisms such as copy-number deletions (frequent in our series), as well as the use of different techniques and panels of NGS might explain these differences.

In conclusion, *BRCA1/2* pathogenic variants are uncommon in MCC. However, in *BRCA*-mutated MCC, PARPi might be a valuable therapeutic option requiring validation by clinical trials.

MATERIALS AND METHODS

Patients

We identified 38 patients with a diagnosis of MCC in the records of the Central Laboratory of Pathology of the Nice University Hospital

(Nice, France) from 2010 to 2021 (Figure 1). Formalin-fixed paraffin-embedded (FFPE) tissue was available to perform molecular analyses in 35 cases. Written, informed consent was not required in agreement with the French law (number 2018-155; May 3, 2018) regarding research not involving the human person and according to the Reference Methodology MR004. All patients were informed about the use of their biological samples and the associated clinical data through a nonobjection form and were free to express their opposition to their inclusion in this project. The design of the study and the protection of the patient's data were in accordance with the local institutional rules, the current French legislation, and the European Union 2016/679 General Data Protection Regulation on the protection of natural persons with regard to the processing of personal data. Approval was obtained (reference number 458; date of approval: September 17, 2021) by the approving institution Direction de l'Innovation Numérique et du Système d'Information of the University Hospital of Nice.

Histopathological and IHC analyses

Histological and IHC analyses were performed on 2-μm sections from FFPE biopsies or surgical excision samples. Sections were stained with H&E for standard histological examination. IHC was performed using the following primary antibodies from Dako-Agilent (Santa Clara, CA): CK20 (clone IT.Ks208 Dako, prediluted, pH9), chromogranin (clone DAK-A3 Dako, dilution 1/400, pH9), synaptophysin (clone 2F11 Dako, dilution 1/1,000, pH6), neurofilament (clone 2F11 Dako, dilution 1/1,000, pH6), and CD56 (clone 123C3 Dako, prediluted, pH9). Immunolabeling and detection were performed using the Dako Autostainer, according to the manufacturer's instructions. The diagnosis of MCC was based on morphological features and a combination of CK20 positivity and at least one neuroendocrine marker (synaptophysin and/or chromogranin and/or CD56) and no visceral tumor (on the basis of computed tomography scan or positron emission tomography scan) (Elder et al., 2018).

Tumor DNA extraction

Genomic DNA was isolated from FFPE samples using the Maxwell 16 FFPE Plus LEV DNA purification kit (Promega, Madison, MI).

MCPyV detection and quantification

IHC detection of MCPyV was performed manually from 2-μm sections of FFPE samples targeting the large T antigen (clone Ab3, at a dilution of 1/1,600, Abcam, Cambridge, United Kingdom). A semi-quantitative Allred score was used for quantification as previously described (Kervarrec et al., 2019). The molecular detection of MCPyV was performed by qPCR. Large T antigen real-time PCR assay was performed as described (Kervarrec et al., 2019). The ΔCt method was used for quantification, with results expressed as the number of MCPyVcopies/cells (Kervarrec et al., 2019).

DNA-targeted NGS

We used both the Ion AmpliSeq Cancer Hotspot Panel, version 2, designed to amplify and sequence 207 amplicons covering 2,800 COSMIC (Catalogue of Somatic Mutations in Cancer) mutations in 50 genes (Thermo Fisher Scientific, Waltham, MA) (full list of genes is available in the Supplementary Table S3) and the Oncomine HRR pathway predesigned panel to amplify and sequence 1,471 amplicons in *BRCA1*, *BRCA2,* and 26 other genes involved in the HRR pathway (Thermo Fisher Scientific) (full list of genes is also in the Supplementary Table S3). Of note, this panel was designed to cover the entire coding sequence and a few adjacent nucleotides. Emulsion PCR was performed on the Ion Chef System (Thermo Fisher Scientific), and sequencing was performed on the Ion GeneStudio System S5 using the semiconductor-based technology (Thermo Fisher Scientific). Base calling, barcode sorting and trimming, alignment to the human reference genome (hg19, Genome Reference Consortium Human Build 37), and variant calling were achieved using the Torrent Suite, version 5.16, and the Torrent Variant Caller plugin with the Somatic-Low stringency default parameters. Mutations were annotated using Ion Reporter, version 5.16 (Thermo Fisher Scientific).

Classification of pathogenicity of *BRCA1/2* variants

Every *BRCA1* and *BRCA2* variant was classified according to their pathogenicity using public databases such as BRCAExchange (https://brcaexchange.org), ClinVar (https://www.ncbi.nlm.nih.gov/clinvar), GnomAD (https://gnomad.broadinstitute.org), and BRCA-Share (formerly known as the UMD-BRCA mutations database) (Figure 1). Only *BRCA1/2* variants of class 5 (pathogenic), class 4 (likely pathogenic), and class 3 (VUS or not yet reviewed) were listed in the Results section, Supplementary Table S2, and Figure 2. Polymorphisms and silent/intronic (except at splicing sites) variants were not listed in the Results section.

aCGH and aSNP

We performed aCGH/aSNP on 80 ng DNA/sample to detect whole-genome CNAs and losses of heterozygosity using the OncoScan CNV Assay (Thermo Fisher Scientific) according to the manufacturer's instructions. Data were analyzed using the Chromosome Analysis Suite (ChAS 3.3) software (Thermo Fisher Scientific). Annotations were based on the human reference hg19 (Genome Reference Consortium Human Build 37). Raw data have been submitted to the Gene Expression Omnibus database with the accession number GSE210602.

Meta-analysis of *BCRA1* and *BRCA2* variants reported in the literature

The PubMed database was searched for studies published in English from January 2000 to November 2022, using the following terms: BRCA1 AND Merkel cell carcinoma, BRCA2 AND Merkel cell carcinoma, sequencing AND Merkel cell carcinoma, molecular AND Merkel cell carcinoma, and gene AND Merkel cell carcinoma. We have also interrogated large-scale molecular, pan-cancer cohorts available in the cbioportal database (https://www.cbioportal.org) to identify molecular analyses in MCC. We collected data when *BRCA1* and/or *BRCA2* variants were found (Figure 1). We used the same procedure previously described to classify these variants according to their pathogenicity.

Data availability statement

Datasets related to this article can be found at https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE210602, hosted at Gene Expression Omnibus with the reference number GSE210602.

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CONFLICT OF INTEREST

The authors state no conflict of interest.

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AUTHOR CONTRIBUTIONS

Conceptualization: BDM; Data Curation: AG, TK, BDM, NCL, VK; Investigation: TF, AT; Methodology: IDM, BDM; Resources: AG, NCL, HM; Writing - Original Draft Preparation: AG, BDM; Writing - Review and Editing: BDM, FP, TK, HM, FBV

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2023.01.014

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