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BRIEF REPORT

Non-RB1 germline cancer predisposing variants found in retinoblastoma patients

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ABSTRACT

Purpose: It is well known that individuals with hereditary retinoblastoma are at lifelong high risk for developing subsequent malignant neoplasms (SMN). However, the role that non-*RB1* germline variants play in tumorigenesis and SMN risk has not yet been studied. The purpose of this study is to report the frequency and spectrum of non-*RB1* germline cancer predisposing variants in individuals with retinoblastoma (RB).

Methods: Retrospective data collection from institutional electronic medical records of 94 individuals seen at our institution with personal history of retinoblastoma, who had undergone next-generation sequencing germline analysis.

Results: The prevalence of individuals with cancer predisposition was 57% (54/94). Of these individuals, 76% (41/54) had a pathogenic/likely pathogenic (P/LP) variant only in the *RB1* gene, 9% (5/54) harbored a P/LP variant only in a non-*RB1* gene, and 11% (6/54) had both. No difference was found between patients with and without non-*RB1* variants when comparing demographic and clinical characteristics, including time to SMN. Variants were found in 7 different genes, with only 1 variant repeating 3 times.

Conclusion: In this small cohort of patients with retinoblastoma, non-*RB1* variants did not appear to augment tumorigenesis or disease progression. Larger studies are required to determine associations between specific variants and development of SMN.

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Introduction

It is recognized that individuals with hereditary retinoblastoma (OMIM#180200) are at lifelong high risk for developing subsequent non-ocular malignancies.[1-4] Germline *RB1* variants,[4] radiation,[2,5] systemic chemotherapy,[1,5] and young age at treatment are important risk factors.[6] Pan-cancer studies have shown that between 8% to 18% of individuals with retinoblastoma harbor pathogenic or likely pathogenic (P/LP) variants in cancer predisposition genes and that 34% to 59% of those variants may be unrelated to the primary cancer, depending on the study population.[7–9] In fact, in an earlier report of individuals with retinoblastoma, we found 5 individuals who had pathogenic variants in cancer predisposing genes other than *RB1*.[10] If and how these non-*RB1* germline variants influence tumorigenesis, disease progression, and subsequent malignant neoplasms (SMN) has not yet been studied. The purpose of this study is to report the frequency and spectrum of non-*RB1* germline cancer predisposing variants from individuals with hereditary (HR) and non-hereditary retinoblastoma (nHR) seen at our institution.

Materials and Methods

Retrospective data collection from institutional electronic medical records of 94 individuals seen at Memorial Sloan Kettering Cancer Center (MSKCC) with a personal history of retinoblastoma (RB), who had previously undergone next-generation sequencing (NGS) germline analysis using the MSK-IMPACT (Integrated Mutation Profiling of Actionable Cancer Targets) platform (MSKCC; NCT01775072).[11] Testing was offered to all individuals undergoing enucleation and those who for clinical reasons were considered to benefit from broad germline testing. The study was approved by MSKCC's Institutional Review Board/Privacy Board.

MSK-IMPACT is an FDA approved, hybridization capture-based NGS panel designed to identify known P/LP germline variants in 90 cancer-associated genes[5-7,11] (the list of genes can be shared upon reasonable request). All the germline genes that are analyzed are clinically actionable. Germline variant classification follows the American College of Medical Genetics and Genomics guidelines.[12] Unless otherwise specified, only P/LP germline variants are reported.

Statistical Analysis

Patients' characteristics among HR, nHR, *RB1*, and non-*RB1* groups were analyzed using χ^2 test, Fisher's exact test, or Wilcoxon rank sum as appropriate.

Results

Description of the population

In this cohort of 94 individuals with retinoblastoma, 83 (88%) were ≤18 years and 11 (12%) were >18 years.

Participants' demographics are presented in Table 1. There was an even distribution of individuals with HR (52%; $n = 49$) and nHR (48%; $n = 45$).

The HR group was defined by those with a germline *RB1* variant and/or history of bilateral disease. This included 2 individuals with negative germline *RB1* testing and bilateral disease, 7 with positive germline *RB1* variants and unilateral disease, and 1 person with trilateral disease and a germline *RB1* pathogenic variant. All others were defined within the nHR group.

Of note, hereditary groups refer to the presence or absence of P/LP variants in the *RB1* gene. Two more groups are described based on the presence (non-*RB1* variant group) or absence (control group) of P/LP variants in genes other than *RB1*.

Prevalence of non-*RB1* P/LP variants

Eleven individuals (12%) had variants in a non-*RB1* gene; 6 had HR and 5 had nHR. These represent 12% of all individuals with HR and 11% of individuals with nHR. The rate of non-*RB1* variants was not significantly different between the 2 groups (Table 1).

Supplemental Table 1 compares clinical and demographic characteristics of patients with and without non-*RB1* variants. No statistically significant difference was found between the 2 groups from the variables analyzed.

Development of secondary cancers

In an attempt to evaluate any impact on long-term outcomes, we looked for any association between having P/LP variants in *RB1* and/or non-*RB1* genes with the development of SMN (Figure 1). Only 13 individuals had developed a SMN at the time of our study, and 5 developed a third cancer. All individuals who developed SMN except 1 had HR; 3 individuals also had a non-*RB1* variant.

The mean age of SMN diagnosis was 13 years (12 and 24 years in the HR and non-HR groups, respectively).

Frequency of variants in *RB1* and non-*RB1* genes

The overall prevalence of individuals with cancer predisposition (individuals with HR and/or those with a non-*RB1* variant) was 57% ($n = 54$), of which 11% ($n = 6$) had 2 different cancer predisposing variants, 42 (45%) had only an *RB1* P/LP variant, and 5 (5%) had only a non-*RB1* P/LP variant.

Table 1 Demographics and Rate of non-*RB1* variants in the HR and non-HR groups

Characteristic	Hereditary, $n = 49$[a]	Non-hereditary, $n = 45$[a]	Overall, $N = 94$[a]
Sex			
Female	22 (45%)	21 (47%)	43 (46%)
Male	27 (55%)	24 (53%)	51 (54%)
Age at time of study (years)	7 (1-45)[b]	6 (0-24)[b]	7 (0-45)[b]
Age at RB diagnosis (years)	1 (0-4)[b]	1 (0-9)[b]	1 (0-9)[b]
Unknown	1	0	1
Race description			
Asian-Far East, Indian Subcontinent	7 (16%)	9 (21%)	16 (18%)
Black or African American	6 (14%)	6 (14%)	12 (14%)
Other	4 (9%)	4 (9%)	8 (9%)
White	27 (61%)	24 (56%)	51 (59%)
Unknown	5	2	7
Ethnicity description			
Non-Spanish; Non-Hispanic	40 (83%)	35 (80%)	75 (82%)
Spanish NOS; Hispanic NOS, Latino NOS	8 (17%)	9 (20%)	17 (18%)
Unknown	1	1	2
Laterality			
Bilateral	41 (84%)	0 (0%)	41 (44%)
Trilateral	1 (2%)	0 (0%)	1 (1%)
Unilateral	7 (14%)	45 (100%)	52 (55%)
RB1 germline variant			
Negative	2 (4%)	45 (100%)	47 (50%)
Positive	47 (96%)	0 (0%)	47 (50%)
Germline variant in gene other than *RB1*	6 12% (95%CI: 5%, 25%)	5 11% (95%CI: 4%, 25%)	$P = .86$[c]
CHEK2	1	0	
ERCC3	0	1	
FANCC	0	1	
MITF	1	0	
MSH3	1	1	
MUTYH	3	0	
NTHL1	0	2	
Second Germline variant in gene other than *RB1*: *TSC2* c.4570-1G>A	0 (0%)	1 (2%)	

CI, confidence Interval; *NOS*, not otherwise specified.
[a]*n* (%).
[b]Median (Range).
[c]Pearson's χ^2 test.

The frequencies of variants in *RB1* and non-*RB1* genes are presented in Table 1. Only 1 patient had reported variants in 2 non-*RB1* genes: a LP variant in *NTHL1* and a *TSC2* variant, which was later reclassified as variant of uncertain significance.

Spectrum of variants in the non-*RB1* group

A total of 9 non-*RB1* heterozygous P/LP variants were found in 11 individuals; no one had biallelic variants. The variants were found in 7 different genes, *MUTYH, MSH3, NTHL1, ERCC3, FANCC, CHEK2,* and *MITF*, with 1 variant identified in each gene except for *MSH3* and *NTHL1,* in which 2 different variants were found (Supplemental Table 3).

All non-*RB1* variants in our study were unique, except for a *MUTYH (NM_001128425)* variant, c.1187G>A p.(Gly396Asp), which was found in 3 different individuals, all of whom had HR. Among them, 2 developed SMN (osteosarcoma and meningioma at 12 and 32 years, respectively). Only 1 other individual with a non-*RB1* variant developed a SMN, a 23-year-old female with HR and a *MSH3* variant who developed meningioma at age 20. None of the SMNs seemed to have any correlation with the respective non-*RB1* gene identified on each individual, but they all have been previously reported as SMN in individuals with HR (Supplemental Tables 2 and 3).

Similarly, all *RB1* variants were different when looking at the subgroup of individuals with HR in the non-*RB1* variant group. The spectrum of *RB1* variants within that group include 2 frameshift, 1 nonsense, 1 splice site, 1 exon-level deletion (all truncating variants), and 1 missense variant (believed to retain some residual activity and be

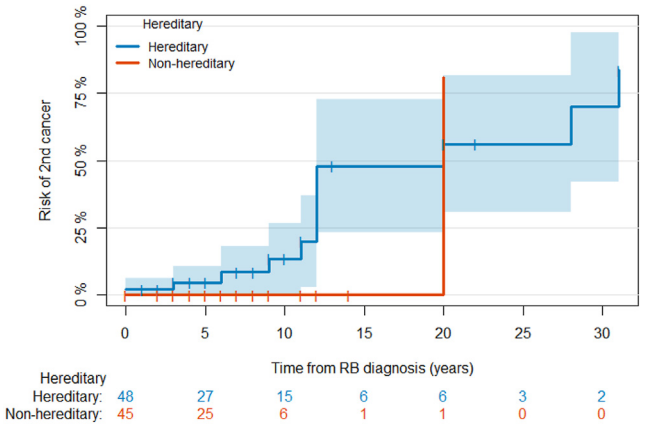

Figure 1 Cumulative incidence of second cancers. Because the age at second cancer is a censored observation, we used survival analysis to estimate the rate of SMN diagnosis in each of the groups and analyze any associations with presence of a non-*RB1* variant. The cumulative incidence of SMN in the overall study population is presented in Figure 1. The cumulative incidence of SMN was 48% at 15 years in the hereditary group versus 0% in the non-hereditary group, although the difference was not statistically significant ($P = .07$). Likewise, there was no evidence of a significant difference in the time to second cancer between patients with and without non-*RB1* variants in the overall study population nor within the subgroup of patients with hereditary disease. Tick marks represent censoring times. The subgroup of patients with non-hereditary disease was not analyzed because of the small number of second cancers in that group. Time to SMN was defined as the time from the age at RB diagnosis to the age at the first SMN diagnosis. Patients without SMN diagnoses were censored at the current age. Deaths before the occurrence of SMN were treated as competing risks. The cumulative incidence of SMN was estimated using an Aalen-Johansen estimator,[13] and the curves were compared using a Gray's test.[14] Analyses were done using R v.3.6.3.

associated with decreased penetrance[15-17]). All variants are spread along the *RB1* gene from exon 4 to exon 20.[18]

Clinical, demographic, and molecular information of individuals with non-*RB1* variants can be found in Supplemental Table 2.

Discussion

This represents the largest cohort of individuals with retinoblastoma in which the prevalence of germline non-*RB1* P/LP variants has been reported to date. We found that 12% of individuals with retinoblastoma harbored a P/LP variant in a non-*RB1* cancer predisposing gene, independent of their underlying germline predisposition to retinoblastoma. There was no significant difference of prevalence between individuals with HR and nHR (12% and 11%, respectively). Furthermore, our study has an ascertainment bias given by the nature of the IMPACT test, which was designed to be performed in individuals with available tumor sample; therefore, our study population is mainly composed by those who required enucleation (representing fewer than 5% of all individuals with retinoblastoma in our institution). No other treatment modalities or exposures were found to be over-represented in our study population].

The overall prevalence of individuals with a cancer predisposition in our study is 57%, which, when compared with the prevalence of HR (52%), shows that a small but significant percentage (5%) of individuals with cancer predisposition can be missed if only *RB1* directed testing is performed in persons with retinoblastoma. Considering that IMPACT only tests for actionable genes, some of these individuals and their families could be missing from surveillance interventions recommended based on each gene. These percentage is probably even higher given that (1) IMPACT only reports variants classified as P/LP and does not report variant of uncertain significance that may eventually be upgraded to pathogenic, and (2) testing was limited to the 90 gene version of IMPACT, but the current number of known cancer predisposition genes is significantly higher and continues to expand. Overall, this represents a significant number of persons being missed, considering that our cohort only represents 5% of the individuals with retinoblastoma seen at our institution. This supports the benefit of using broader gene panels for RB patients in the research setting, to facilitate for future studies to determine any gene variants that might be enriched in the RB population that could be considered for clinical testing in patients who would benefit from a diagnosis (ie, diagnosis would modify screening and surveillance recommendations).

We found that 11% of individuals with HR (6% of all individuals) harbored a second P/LP variant in a cancer predisposition gene. We have yet to determine the impact that non-*RB1* variants can have in individuals with HR to understand if they confer an independently added risk of cancer or if they interact through common pathways and have disease modifying roles that could help predict disease outcomes and prognosis. We did not find any change in the mean age of retinoblastoma diagnosis when comparing individuals with and without non-*RB1* variants that would suggest a modifying effect in the development of the primary tumor. Similarly, there was no significant difference between the same groups in the time to second cancer diagnosis among the overall study population nor within individuals with HR. However, these findings were not statistically significant, likely because of the low rate of second cancers in our study given a primarily pediatric population (mean age of 7 years), with second malignancies typically developing later in life (reported median range of age is 13-17 years).[18-21] Nonetheless, it should be noted that, among the 6 individuals with HR and a non-*RB1* variant, 3 individuals are older than 10 years (all females) and all have developed a second cancer (Supplemental Table 3).[22–29]

All of the genes in which a non-*RB1* variant was identified have a different role in cell regulation. Only 1 is an oncogene (*MITF*), and the others are tumor suppressors, each involved in different mechanisms. Supplemental Table 2 provides information on each of the genes, including their role in carcinogenesis, and the known associated cancer risks. Although most of the genes in which variants were found are associated with

autosomal recessive disease, these results provide valuable information for future family planning. Furthermore, screening recommendations also changed for some of the individuals (and their families) in whom the second variant was found in genes associated with autosomal dominant inheritance.

Given that *RB1* not only initiates cancer in the retina but is also an important mechanism of cancer development in non-ocular cancers of later development,[14,30–34] functional studies can help determine if germline variants in genes with specific roles within the cell could somehow play a role in the pathogenesis leading to the second somatic hit in *RB1,* facilitating the development of second cancers. A possible role of germline *MUTYH* variants in the pathogenesis of retinoblastoma has been recently suggested by Newman et al[35] and Akdeniz et al,[34] from results of functional studies from tumor samples of individuals with hereditary retinoblastoma and germline *MUTYH* variants, that suggest an important role of the loss of *MUTYH* function in the tumorigenesis of retinoblastoma.[33,35] These findings along with the increased frequency of *MUTYH* variants in our cohort (found in half of the individuals with HR and a non-*RB1* variant, with 2 of them who also developed a second cancer), could suggest a possible interaction between *MUTYH* and *RB1* with an impact in tumorigenesis.

Further studies are required to identify those genes in which germline variants are more frequently found in persons with HR to determine good candidate genes for functional assessment, which might provide new insights into unknown biological mechanisms contributing to disease, which might help guide management and surveillance strategies in the future.

Conclusion

We found that 12% of individuals with a personal history of retinoblastoma harbored a P/LP variant in a non-*RB1* cancer predisposing gene, and 6% of them had 2 different cancer predisposition syndromes. Although the size of our cohort limited the analysis in our study, there were no findings suggesting any association between having HR or non-HR and the presence of a non-*RB1* variant and they likely represent 2 completely independent factors. However, further studies are required to determine possible associations with the presence of specific variants and the development of second cancers that could provide a better understanding of tumorigenesis in RB individuals and influence future management, screening, and surveillance recommendations.

Data Availability

All data relevant to the study are included in the article or uploaded as supplementary information. All data were deidentified for use in this study.

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Ethics Declaration

Memorial Sloan Kettering Institutional Review Board (IRB) approval was obtained for the study, and all parents/guardians provided written consent for germline genetic testing under IRB # 12-245.

Conflict of Interest

All authors declare no conflicts of interest.

Additional Information

The online version of this article (https://doi.org/10.1016/j.gimo.2024.101836) contains supplemental material, which is available to authorized users.

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