RESEARCH ARTICLE

Cancer Genetics and Epigenetics

Germline pathogenic variants in the *MRE11*, *RAD50*, and *NBN* (MRN) genes in cancer predisposition: A systematic review and meta-analysis

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Abstract

The *MRE11*, *RAD50*, and *NBN* genes encode the MRN complex sensing DNA breaks and directing their repair. While carriers of biallelic germline pathogenic variants (gPV) develop rare chromosomal instability syndromes, the cancer risk in heterozygotes remains controversial. We performed a systematic review and meta-analysis of 53 studies in patients with different cancer diagnoses to better understand the cancer risk. We found an increased risk (odds ratio, 95% confidence interval) for gPV carriers in *NBN* for melanoma (7.14; 3.30–15.43), pancreatic cancer (4.03; 2.14–7.58), hematological tumors (3.42; 1.14–10.22), and prostate cancer (2.44, 1.84–3.24), but a low risk for breast cancer (1.29; 1.00–1.66) and an insignificant risk for ovarian cancer (1.53; 0.76–3.09). We found no increased breast cancer risk in carriers of gPV in *RAD50* (0.93; 0.74–1.16; except of c.687del carriers) and *MRE11* (0.87; 0.66–1.13). The secondary burden analysis compared the frequencies of gPV in MRN genes in patients from 150 studies with those in the gnomAD database. In *NBN* gPV carriers, this analysis additionally showed a high risk for brain tumors (5.06; 2.39–9.52), a low risk for colorectal (1.64; 1.26–2.10) and hepatobiliary (2.16; 1.02–4.06) cancers, and no risk for endometrial, and gastric cancer. The secondary burden analysis showed also a moderate risk for ovarian cancer (3.00; 1.27–6.08) in *MRE11* gPV carriers, and no risk for ovarian and hepatobiliary cancers in *RAD50* gPV carriers. These findings provide a robust clinical evidence of cancer risks to guide personalized clinical management in heterozygous carriers of gPV in the *MRE11*, *RAD50*, and *NBN* genes.

KEYWORDS
germline variants, meta-analysis, MRE11, NBN, RAD50

What's New?

Carriers of biallelic germline pathogenic variants in the MRN complex develop rare chromosomal instability syndromes. The cancer risks in heterozygotes however remain controversial.

This systematic study found that *NBN* variant carriers have increased but typically moderate risks of melanoma and pancreatic, hepatobiliary, prostate, hematological, and brain cancer. Their risk is negligible for breast and colorectal cancer, and insignificant for ovarian, endometrial, and gastric cancer. *RAD50* variant carriers show no cancer risk, and *MRE11* variant carriers have a moderate ovarian cancer risk. The findings provide robust clinical evidence to guide personalized clinical management in heterozygous carriers.

1 | INTRODUCTION

The *MRE11*, *RAD50*, and *NBN* genes code for the constituents of the nuclear heterotrimeric MRN protein complex sensing DNA double-strand breaks.[1] The MRN complex acts as a DNA damage sensor, aids in the selection of DNA repair strategies (facilitating homologous recombination repair) and participates in intracellular responses to DNA damage through multiple protein–protein interactions.[2] Carriers of bi-allelic germline pathogenic variants (gPV) develop rare autosomal recessive syndromes: Nijmegen breakage syndrome (NBS; OMIM: #251260), NBS-like disorder (NBSLD; OMIM:#613078) and Ataxia-telangiectasia-like disorder (ATLD; OMIM:#604391), caused by *NBN*, *RAD50* and *MRE11* deficiency, respectively.[3–5] Of these, NBS is by far the most common, especially in the Slavic populations of Central and Eastern Europe, where the founder *NBN* variant c.657_661del (c.657delACAAA, p.Lys219fs; described as c.657del5 in older publications and below) is enriched and causes 81% and 74% of NBS cases in the Czech/Slovak Republic and Poland, respectively.[6] Hereditary syndromes caused by biallelic defects in the *MRE11/RAD50/NBN* genes ("MRN genes") are characterized by different phenotypic features but chromosomal instability is present in all of them.[1] Because chromosomal instability has been recognized as one of the hallmarks of cancer,[7] numerous reports (with a significant number of studies originated from Central and Eastern European regions) have attempted to assess the involvement of heterozygous germline variants in susceptibility to various cancer types. Early studies mainly focused on founder *NBN* germline variants as those in *RAD50* and *MRE11* were considered much less common, with the exception of c.687del (c.687delT; p.Ser229fs) in *RAD50* in Finnish population.[8] The studies of c.657del5 in *NBN* suggested the increased risk for breast,[9,10] ovarian,[11] colorectal,[12] pancreatic,[13] brain,[14] prostate[15] cancer, melanoma,[12] and hematologic tumors[16]; however, with the conflicting evidence and imprecise estimation of the risk.[17] Similarly, the Finnish founder variant c.687del in *RAD50* has been described to increase breast cancer risk in Finns but not in other populations.[18] The implementation of NGS-based panel or exome analysis allowed the identification of rare pathogenic variants in MRN genes that may be included as a part of multi-gene testing in high-risk cancer individuals.[19,20] However, due to the low prevalence of heterozygous gPV in populations without founder variants, the precise estimation of cancer risk for specific cancer types in carriers of these gPV remains uncertain.[21]

The primary objective of this report was to assess the risk of various types of cancer in carriers of gPV in MRN genes based on a comprehensive systematic review and meta-analysis of case–control data. The secondary objective included the burden analysis of cancer risk considering all types of studies that identified carriers of gPV in the MRN complex genes in patients with various cancer diagnoses compared with carriers of these variants from the gnomAD database.

2 | MATERIALS AND METHODS

2.1 | Identification and eligibility of studies

We searched PubMed to identify studies reporting germline genetic testing of MRN complex genes in cancer patients published before April 1, 2023. The following search terms were used to identify relevant literature: (NBN OR NBS1 OR NBS-1 OR nibrin OR MRE11 OR MRE11A OR RAD50 OR "MRN complex") AND (alteration* OR variant* OR mutation*) AND (germline OR hereditary OR predispos*) AND (cancer*) AND (patient* OR women OR men OR male OR female) OR (657del*) NOT review[pt]; (breast OR colorectal OR ovarian OR endometrial OR melanoma OR lymphoma OR leukemia OR brain) AND (cancer AND controls AND (panel gene sequencing) AND (germline OR hereditary)) NOT ((review [pt]) OR (case reports [pt]) OR (case report [pt])). There were no language restrictions for eligible studies. Additional relevant studies were identified by a manual search.

We first screened the titles of all retrieved studies; and potentially relevant articles were retrieved for full-text reading. Studies were included in the meta-analysis if they met the following criteria: (i) studies used a case–control study design, (ii) studies estimated the association between *NBN*, *MRE11* or *RAD50* truncating variants and cancer risk, (iii) there was sufficient information describing the source of cases and controls. Abstracts without full text, cell lines and animal studies, case reports, case series, meta-analyses, or review articles were not considered. If studies reported on (partially) overlapping patient populations, we included only the most recent or complete study (Supplementary Table S1). Data were extracted by one reviewer (B.S.) and controlled by three independent reviewers (T.D., K.M., B.N.).

2.2 | Data extraction

The following data were carefully extracted from each study: first author, year of publication, country of origin, cancer type, sample size, source of controls and source of cases, number of truncating variants

(nonsense, frameshift, and splice-site pathogenic/likely pathogenic variants) in cases and controls (Supplementary Table S2). In the case of the *NBN* gene, we investigated the proportion of the recurrent founder variant c.657del5 in patients and controls. Data were extracted separately for studies that included subjects of different ethnicities, from different countries, and cancer types. At least three independent studies were considered for the meta-analysis.

2.3 | Statistical analysis

The meta-analysis was performed in a random effects model (assuming the diverse effect size caused by differences in patient ascertainment, age, disease severity, or treatment characteristics) using the "meta" package in R 4.2.2 software.[22] The association between variants in individual MRN complex genes and cancer risk was measured by odds ratios (ORs) with a 95% confidence interval (CI) and a *p*-value<.05 was considered significant. The Cochran's *Q*-test and *Q*-statistic was used to test for heterogeneity between studies. Heterogeneity was quantified by I^2 metric ($I^2 < 25\%$ no heterogeneity; $I^2 = 25\%$–50% moderate heterogeneity; $I^2 > 75\%$ extreme heterogeneity) and *p*-value ($p > .1$ no heterogeneity). Publication bias was assessed graphically by the funnel plot asymmetry and statistically by Egger's linear regression test where *p*-value<.05 was considered a significant publication bias, *t* describes a *t*-statistic for the intercept test, and *df* is the degrees of freedom.[23]

2.4 | Secondary analysis of case-only studies with gnomAD database

For the secondary analysis of the effect of the *MRE11*, *RAD50*, and *NBN* truncating gPV on cancer risk, we also considered relevant publications that included only patients' data (i.e., patient studies without corresponding control data). Except for the number and source of controls, data were assessed and extracted identically as described in Section 2.2 (Supplementary Table S3). Data from the Genome Aggregation database (gnomAD database v2.1.1 unrestricted for population or ethnic subgroups; broadinstitute.org) were used as a control group for the secondary burden analysis using Fisher's Exact Test.[24] The calculation for unselected controls (gnomAD v2.1.1) and non-cancer controls (excluding cancer patients datasets; gnomAD v2.1.1 non-cancer) were performed in parallel. All protein truncating variants (nonsense, frameshift, and splice-site variants) in *MRE11*, *RAD50* and *NBN* were retrieved from gnomAD when classified in ClinVar or LOVD as pathogenic or likely pathogenic.

For each gene separately, the gnomAD database provided the number of variant alleles in slightly different sizes of analyzed individuals. To unify the number of carriers of pathogenic or likely pathogenic variants, we calculated the median of allele numbers (divided by two as all carriers were heterozygotes). The overall frequency of the pathogenic or likely pathogenic variant carriers were finally obtained as a sum of pathogenic or likely pathogenic variant allele frequencies

multiplied by the median allele number (provided in detail in Supplementary Tables S4–S9).

3 | RESULTS

3.1 | Characteristics of published studies

The PRISMA diagram describes the selection of the relevant studies investigating germline variants in the *MRE11*, *RAD50*, and *NBN* genes (Figure 1). We retrieved a total of 758 publications, however, only 53 were case–control studies (Supplementary Table S2) that met our inclusion criteria.

3.2 | Cancer risk associated with germline pathogenic variants in *NBN*

A total of 47 *NBN* studies met the inclusion criteria, and the requirement of at least three studies per cancer diagnosis. This allowed meta-analysis of cancer risk for carriers of gPV in breast cancer, ovarian cancer, prostate cancer, pancreatic cancer, melanoma, and hematologic tumors.

The risk of breast cancer was determined in 24 studies (170,523 cases and 212,648 controls).[10,12,21,25–45] We found a marginally significant but low breast cancer risk in carriers of *NBN* variants with OR 1.29 (95%CI: 1.00–1.66; $p = .047$; Figure 2). In this analysis, we combined patient data for studies by Couch[33] and Shimelis,[34] and by Steffen et al.[12] and Steffen et al.,[44] respectively, because each of these pairs used the same control datasets. Conversely, we calculated German and Belarusian populations separately from the study by Bogdanova.[41] No evidence of heterogeneity or publication bias was observed between the studies.

Four independent studies were available for risk calculation of ovarian cancer (13,833 cases and 75,055 controls)[11,21,46,47] in which we found no statistically significant risk for the *NBN* gPV carriers (OR = 1.53; 95%CI: 0.76–3.09; $p = .238$; Figure 3A).

Nine studies (21,292 cases and 32,178 controls) were available for prostate cancer risk calculation[48–56] showing that males carrying a *NBN* gPV had a significantly increased moderate prostate cancer risk (OR = 2.44; 95%CI: 1.84–3.24; $p = 6.00 \times 10^{-10}$; Figure 3B).

For pancreatic cancer, four studies (1,927 cases and 31,882 controls) were eligible.[13,57–59] The results of the meta-analysis revealed a significantly increased risk for carriers of *NBN* gPV (OR = 4.03; 95% CI: 2.14–7.58; $p = 1.56 \times 10^{-5}$; Figure 3C). The Czech and Belgian populations were calculated separately in the study by Wieme.[59]

Melanoma risk was assessed in three studies (449 cases and 3,629 controls)[12,60,61] showing a significant risk for carriers of *NBN* gPV (OR = 7.14; 95%CI: 3.30–15.43; $p = 5.72 \times 10^{-7}$; Figure 3D).

We identified five studies (2,800 cases and 47,643 controls) to calculate the risk of hematologic tumors that included leukemia and lymphoma patients.[12,16,44,62,63] We noticed a significant risk in carriers of *NBN* gPV (OR = 3.42; 95%CI: 1.14–10.22; $p = .027$; Figure 3E).
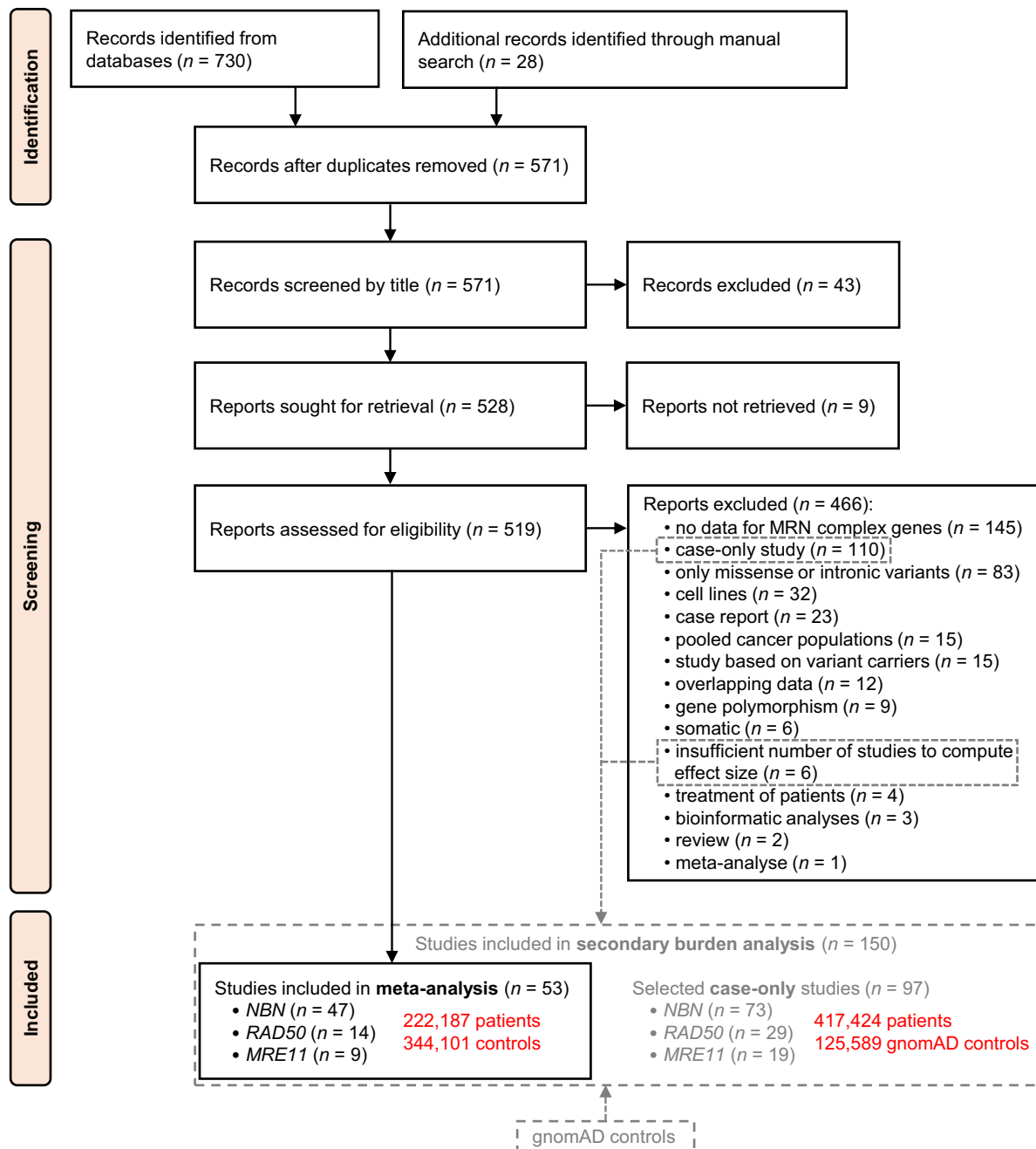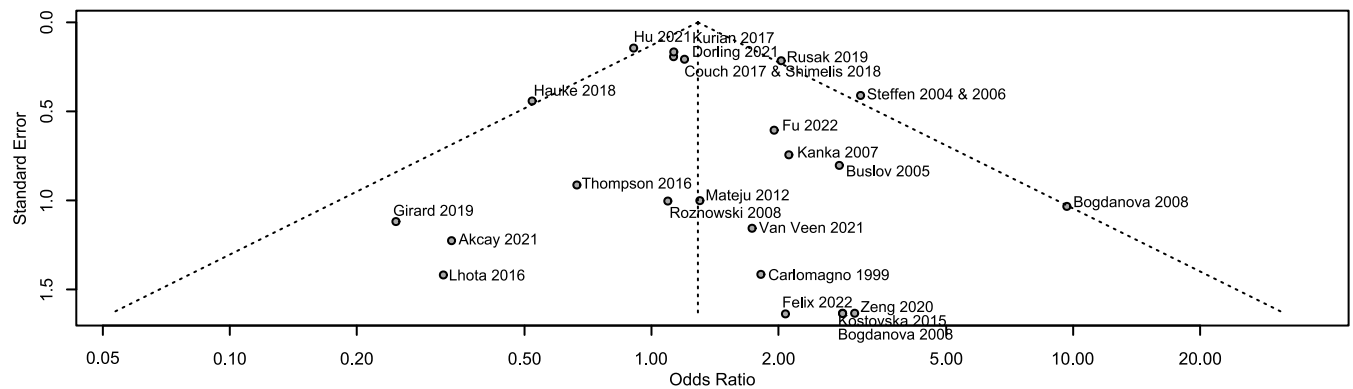
FIGURE 1 PRISMA flow diagram of systematic review and meta-analysis.

We combined data for cases from two studies by Steffen et al.[12] and Steffen et al.[44] that used the same control dataset.

Except for evidence of moderate heterogeneity in the ovarian cancer studies ($I^2 = 59\%$) and hematologic tumors ($I^2 = 54\%$), heterogeneity was not observed in the meta-analyses of any of the above mentioned *NBN* studies (Figure 3A–E), and no publication bias was observed in any of these studies (Supplementary Figure S1A–E).

The most common germline variant c.657del5 in *NBN* has been described as a functional hypomorphic alteration.[64] To investigate whether its effect differs from that of other *NBN* gPV, we performed an independent meta-analysis that included solely c.657del5 carriers

(33% of all *NBN* variant carriers) and other *NBN* gPV (9% of all *NBN* variant carriers) separately (summarized in Supplementary Table S2; with corresponding forest and funnel plots provided in Supplementary Figures S2A–E and Supplementary Figure S3). We found the comparable risk in carriers of c.657del5 variant and carriers of other *NBN* gPV for breast and prostate cancer patients; however, the extremely low prevalence of non-c.657del5 variants precluded to reach the statistically significant conclusive results. A fundamental effect of c.657del5 in meta-analyses considering all *NBN* gPV can explain similar risk found in c.657del5 carriers (compare the results in Figures 2 and 3 with Supplementary Table S10).

Study	Population	Carriers	Patients Total	%	Carriers	Control Total	%	Odds Ratio	OR	95%-CI	P-value	Weight
Girard 2019	FR	1	1207	0.08	4	1199	0.33		0.25	[0.03; 2.22]	.212	1.2%
Lhota 2016	CZ	1	325	0.31	1	105	0.95		0.32	[0.02; 5.18]	.423	0.8%
Akcay 2021	TR	1	728	0.14	2	490	0.41		0.34	[0.03; 3.71]	.373	1.0%
Hauke 2018	DE	12	5589	0.21	9	2189	0.41		0.52	[0.22; 1.24]	.140	5.7%
Thompson 2016	AU	2	2000	0.10	3	1997	0.15		0.67	[0.11; 3.99]	.656	1.8%
Dorling 2021	EU	90	48826	0.18	103	50703	0.20		0.91	[0.68; 1.20]	.500	14.8%
Roznowski 2008	PL	2	270	0.74	2	295	0.68		1.09	[0.15; 7.82]	.929	1.5%
Hu 2021	US	57	32247	0.18	51	32544	0.16		1.13	[0.77; 1.65]	.532	12.9%
Kurian 2017	US	53	26384	0.20	115	64649	0.18		1.13	[0.82; 1.56]	.464	14.0%
Couch 2017 & Shimelis 2018	US	58	32626	0.18	39	26264	0.15		1.20	[0.80; 1.80]	.384	12.4%
Mateju 2012	CZ	2	703	0.28	2	915	0.22		1.30	[0.18; 9.27]	.792	1.5%
Van Veen 2021	GB	1	302	0.33	3	1567	0.19		1.73	[0.18; 16.71]	.635	1.1%
Carlomagno 1999	DE	1	477	0.21	1	866	0.12		1.82	[0.11; 29.12]	.673	0.8%
Fu 2022	CN	6	8067	0.07	5	13129	0.04		1.95	[0.60; 6.40]	.269	3.6%
Rusak 2019	PL	57	4964	1.15	35	6152	0.57		2.03	[1.33; 3.10]	1.20e-03	12.0%
Felix 2022	BR	1	173	0.58	0	119	0.00		2.08	[0.08; 51.45]	.655	0.6%
Kanka 2007	PL	2	181	1.10	21	4000	0.53		2.12	[0.49; 9.10]	.313	2.5%
Buslov 2005	RU	7	873	0.80	2	692	0.29		2.79	[0.58; 13.47]	.202	2.2%
Bogdanova 2008	DE	1	1076	0.09	0	1017	0.00		2.84	[0.12; 69.75]	.523	0.6%
Kostovska 2015	MK	1	300	0.33	0	283	0.00		2.84	[0.12; 70.00]	.523	0.6%
Zeng 2020	CN	1	831	0.12	0	839	0.00		3.03	[0.12; 74.55]	.497	0.6%
Steffen 2004 & 2006	PL	15	786	1.91	10	1620	0.62		3.13	[1.40; 7.00]	5.42e-03	6.3%
Bogdanova 2008	BY	15	1588	0.94	1	1014	0.10		9.66	[1.27; 73.24]	2.82e-02	1.4%
Random effects model		**387**	**170523**	**0.23**	**409**	**212648**	**0.19**		**1.29**	**[1.00; 1.66]**	**4.73e-02**	**100.0%**

Heterogeneity: $I^2 = 29\%$, $\tau^2 = 0.0894$, $P = 0.098$
Test for overall effect: $z = 1.98$ ($P = 4.73e-02$)

0.1 0.5 1 2 10

FIGURE 2 Forest plot illustrating the impact of gPV in *NBN* on the risk of breast cancer (upper panel) and funnel plot showing low heterogeneity between studies (lower panel). No study bias was observed in Egger's test ($t = 0.93$; $df = 21$; $p = .365$).

3.3 | Cancer risk associated with germline pathogenic variants in *RAD50* and *MRE11*

Compared to *NBN*, fewer studies analyzing gPV in *RAD50* and *MRE11* were published (details provided in Supplementary Table S2).

Thirteen studies[10,18,25–27,29–31,33,34,37,65,66] (134,791 cases and 134,095 controls) met the inclusion criteria for the *RAD50* meta-analysis. However, only the risk of breast cancer could be estimated due to the insufficient number of studies in other cancer types (Figure 4A). The patient data from the studies by Couch[33] and Shimelis[34] were pooled together, as they both used the same control dataset. The results show no breast cancer risk (OR = 0.93; 95%CI: 0.74–1.16; $p = .502$) in heterozygote carriers of gPV in *RAD50* (we found no evidence for heterogeneity or publication bias among these studies; Supplementary Figure S4A). Due to the high prevalence of the Finnish germline founder variant c.687del, we excluded two Finnish studies[8,18] (907 breast cancer patients and 1560 controls) from this analysis. Their independent analysis (Supplementary Figure S5) suggested that *RAD50* variants are associated with increased breast

cancer risk in their carriers (OR = 4.42; 95%CI: 1.71–11.37; $p = .002$) compared to other European or non-European populations.

The fewest studies were eligible for *MRE11*, with only nine studies meeting the inclusion criteria for the meta-analysis.[25,27,29–31,33,34,67,68] The risk calculation could only be performed for breast cancer (Figure 4B) due to the lack of multiple studies for other cancer types. The patient data from the studies by Couch[33] and Shimelis[34] were pooled together, as they both used the same control dataset. The result of the random effect model showed no significant risk observed in breast cancer (OR = 0.87; 95%CI: 0.66–1.13, $p = .297$), with no heterogeneity or publication bias between these studies (Supplementary Figure S4B).

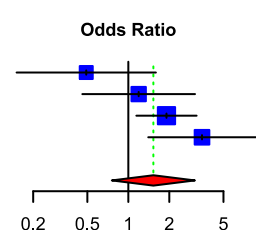## 3.4 | Secondary analysis of case-only studies with gnomAD database

While only 53 case–control studies out of 758 publications met the inclusion criteria for the meta-analysis, additional 97 case-only

(observational) studies reported the frequencies of gPV in *MRE11*, *RAD50*, and *NBN* in patients with various cancer diagnoses (Supplemen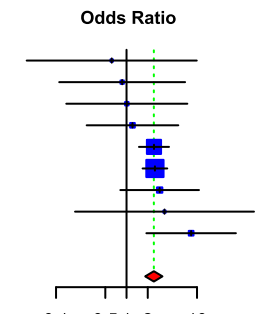tary Table S3). To test the feasibility of gene-centered burden analysis using the overall gnomAD controls (unrestricted to non-cancer population), we first re-analyzed cancer risks calculated from 53 case–control studies (eligible for the meta-analysis described

FIGURE 3 Forest plot describing the effect of germline truncating *NBN* pathogenic variants on the risk of (A) ovarian, (B) prostate, (C) pancreatic cancer, (D) melanoma, and (E) hematologic tumors. Funnel plots for individual meta-analyses are provided in Supplementary Figure S1.

(A) RAD50 - Breast cancer

Study	Population	Carriers	Patients Total	%	Carriers	Control Total	%	Odds Ratio	OR	95%-CI	P-value	Weight
Thompson 2016	AU	2	2000	0.10	6	1997	0.30		0.33	[0.07; 1.65]	1.77e-01	1.9%
Girard 2019	FR	2	1207	0.17	3	1199	0.25		0.66	[0.11; 3.97]	6.51e-01	1.5%
Hu 2021	US	57	32247	0.18	82	32544	0.25		0.70	[0.50; 0.98]	3.96e-01	23.3%
Couch 2017 & Shimelis 2018	US	52	32626	0.16	54	26474	0.20		0.78	[0.53; 1.14]	2.04e-01	20.3%
Van Veen 2021	GB	1	302	0.33	6	1567	0.38		0.86	[0.10; 7.21]	8.93e-01	1.1%
Lhota 2016	CZ	1	325	0.31	0	105	0.00		0.98	[0.04; 24.12]	9.88e-01	0.5%
Dorling 2021	EU	120	48826	0.25	121	50703	0.24		1.03	[0.80; 1.33]	8.19e-01	30.5%
Fu 2022	CN	21	8067	0.26	31	13129	0.24		1.10	[0.63; 1.92]	7.30e-01	12.2%
Fan 2018	CN	26	7657	0.34	9	5000	0.18		1.89	[0.88; 4.04]	1.00e-01	7.3%
Akcay 2021	TR	1	728	0.14	0	490	0.00		2.02	[0.08; 49.75]	6.66e-01	0.5%
Tommiska 2006	GB	1	702	0.14	0	786	0.00		3.36	[0.14; 82.70]	4.58e-01	0.5%
Aloraifi 2015	IE	2	104	1.92	0	101	0.00		4.95	[0.23; 104.42]	3.04e-01	0.5%
Random effects model		**286**	**134791**	**0.21**	**312**	**134095**	**0.23**		**0.93**	**[0.74; 1.16]**	**5.02e-01**	**100.0%**

Heterogeneity: $I^2 = 5\%$, $\tau^2 = 0.0254$, $P = 3.99$e-01
Test for overall effect: $z = -0.67$ ($P = 5.02$e-01)

0.01 0.1 1 10 100

(B) MRE11 - Breast cancer

Study	Population	Carriers	Patients Total	%	Carriers	Control Total	%	Odds Ratio	OR	95%-CI	P-value	Weight
Elkholi 2021	CA	0	1920	0.00	2	945	0.00		0.10	[0.00; 2.05]	1.34e-01	0.8%
Girard 2019	FR	2	1207	0.17	3	1199	0.25		0.66	[0.11; 3.97]	6.51e-01	2.2%
Hu 2021	US	25	32247	0.08	32	32544	0.10		0.79	[0.47; 1.33]	3.73e-01	26.1%
Couch 2017 & Shimelis 2018	US	23	32626	0.07	23	26767	0.09		0.82	[0.46; 1.46]	5.02e-01	21.4%
Dorling 2021	EU	47	48826	0.10	55	50703	0.11		0.89	[0.60; 1.31]	5.47e-01	47.1%
Akcay 2021	TR	1	728	0.14	0	490	0.00		2.02	[0.08; 49.75]	6.66e-01	0.7%
Thompson 2016	AU	4	2000	0.20	0	1997	0.00		9.00	[0.48; 167.36]	1.40e-01	0.8%
Sepahi 2019	DE	7	73	9.59	0	60	0.00		13.65	[0.76; 244.03]	7.57e-02	0.9%
Random effects model		**109**	**119627**	**0.09**	**115**	**114705**	**0.10**		**0.87**	**[0.66; 1.13]**	**2.97e-01**	**100.0%**

Heterogeneity: $I^2 = 17\%$, $\tau^2 < 0.0001$, $P = 2.92$e-01
Test for overall effect: $z = -1.04$ ($P = 2.97$e-01)

0.01 0.1 1 10 100

FIGURE 4   Forest plot of ORs and 95%CI describing the effect of (A) *RAD50* gPV on breast cancer risk and (B) *MRE11* gPV on breast cancer risk.
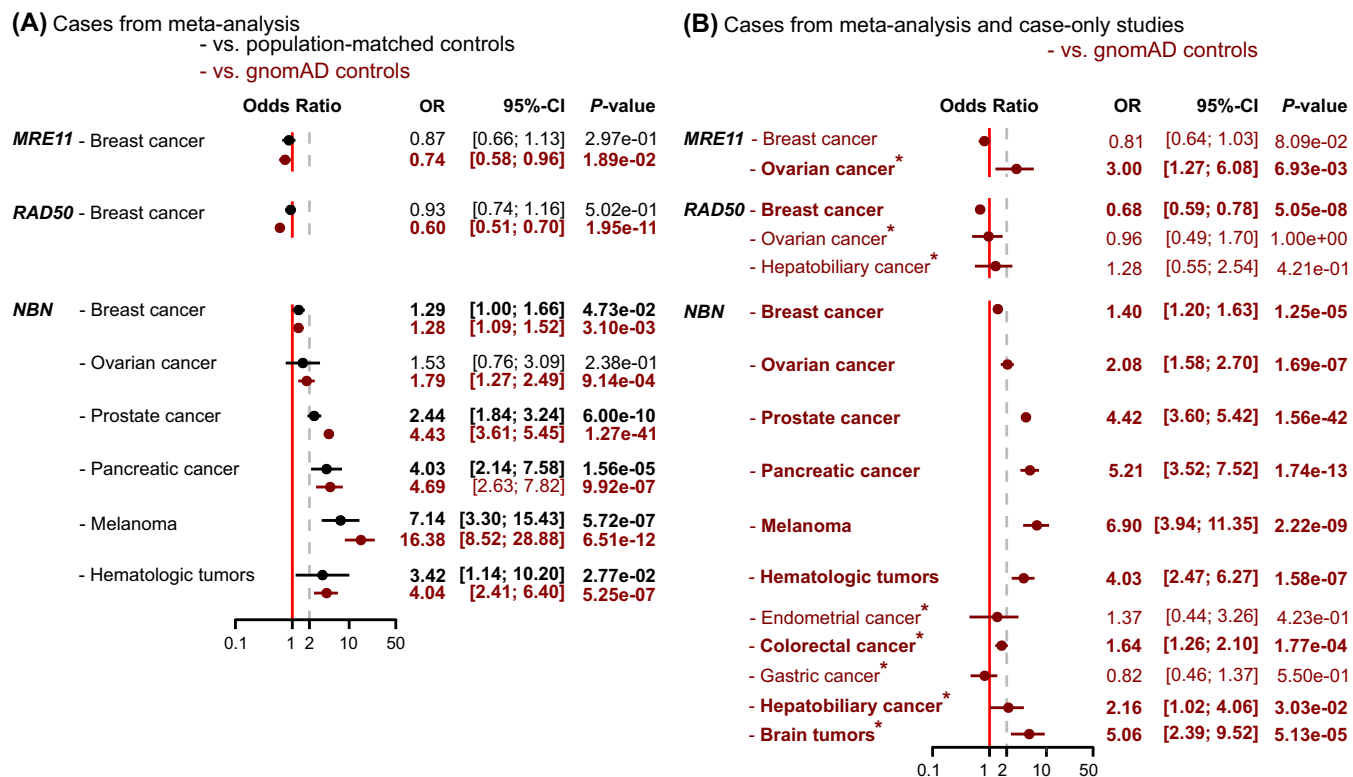
in the previous sections) by including data from patients and replacing data from population-matched controls with overall gnomAD controls. Comparison of the results from the meta-analysis and the secondary burden analysis showed similar outcomes (Figure 5A). An increased risk of prostate cancer and melanoma in carriers of *NBN* gPV was probably influenced by population differences between case and control datasets with patients from Slavic European populations (with founder variant c.657del5) which were largely absent in gnomAD controls.[69] As the discrepancies between the results of the meta-analysis and the secondary burden analysis with gnomAD controls were modest, we decided to perform a secondary burden analysis with pooled data from all the 150 studies (Supplementary Table S3), which allowed us to analyze the cancer risk of germline MRN variants in an expanded set of cancer diagnoses (Figure 5B).

In the case of *MRE11*, we additionally found moderate ovarian cancer risk (OR = 3.00; 95%CI: 1.27–6.08; p = .007). For *RAD50*, the secondary burden analysis indicated that heterozygotes carrying gPV did not have an increased risk of ovarian and hepatobiliary cancer. For the *NBN* gene, the secondary burden analysis confirmed all the risk associations from the meta-analysis and additionally showed a moderate risk for hepatobiliary cancer and a high risk for brain tumors, and a significant but small and clinically negligible risk for colorectal cancer. A parallel secondary burden analysis including gnomAD non-cancer controls (excluding cancer patient datasets) yielded very similar data that differed slightly numerically, but retained all significant/non-significant associations found in analysis of unselected gnomAD population (Supplementary Figure S6A–C).

4 | DISCUSSION

Carriers of biallelic germline pathogenic variants in the MRN genes develop severe rare recessive syndromes that share a genomic instability feature resulting from defects in the MRN complex; however, the cancer risk in heterozygotes is much less understood.[17] To fill this gap, we conducted this systematic review and meta-analysis. To expand the range of different cancers for which we can conclusively analyze the risk, we performed a secondary analysis, which included case-only studies, into a gene-based burden analysis using data from a large population database (gnomAD) as a control.

Germline *NBN* gene variants have been investigated in the largest number of case–control studies, most of which focused on breast cancer risk (Figure 5C). This allowed us to convincingly determine that the risk of breast cancer in carriers of pathogenic germline *NBN* variants is low, with a marginal statistical significance (OR = 1.29; OR 1.00–1.66; 24 studies; 170,523 patients). The inclusion of 36 case-only studies (+101,924 patients) in the secondary burden analysis (Figure 5B) confirmed our observation, which is similar to the results of the two largest breast cancer studies by BCAC and by Hu et al. who demonstrated no association with breast cancer risk (OR = 0.90; 95%CI: 0.67–1.20 and OR = 1.05; 95%CI: 0.71–1.56, respectively).[30,31] Lack of association with breast cancer in *NBN* pathogenic variant carriers was also found in the meta-analysis of cancer predisposition in breast cancer patients using gnomAD controls for risk calculation by Suszynska et al. (OR = 1.18; 95%CI: 0.94–1.48) which was twice smaller than our meta-analysis in the breast cancer patients

(A) Cases from meta-analysis
- vs. population-matched controls
- vs. gnomAD controls

	Odds Ratio	OR	95%-CI	P-value
MRE11 - Breast cancer		0.87	[0.66; 1.13]	2.97e-01
		0.74	[0.58; 0.96]	1.89e-02
RAD50 - Breast cancer		0.93	[0.74; 1.16]	5.02e-01
		0.60	[0.51; 0.70]	1.95e-11
NBN - Breast cancer		1.29	[1.00; 1.66]	4.73e-02
		1.28	[1.09; 1.52]	3.10e-03
- Ovarian cancer		1.53	[0.76; 3.09]	2.38e-01
		1.79	[1.27; 2.49]	9.14e-04
- Prostate cancer		2.44	[1.84; 3.24]	6.00e-10
		4.43	[3.61; 5.45]	1.27e-41
- Pancreatic cancer		4.03	[2.14; 7.58]	1.56e-05
		4.69	[2.63; 7.82]	9.92e-07
- Melanoma		7.14	[3.30; 15.43]	5.72e-07
		16.38	[8.52; 28.88]	6.51e-12
- Hematologic tumors		3.42	[1.14; 10.20]	2.77e-02
		4.04	[2.41; 6.40]	5.25e-07

0.1 1 2 10 50

(B) Cases from meta-analysis and case-only studies
- vs. gnomAD controls

	Odds Ratio	OR	95%-CI	P-value
MRE11 - Breast cancer		0.81	[0.64; 1.03]	8.09e-02
- Ovarian cancer*		3.00	[1.27; 6.08]	6.93e-03
RAD50 - Breast cancer		0.68	[0.59; 0.78]	5.05e-08
- Ovarian cancer*		0.96	[0.49; 1.70]	1.00e+00
- Hepatobiliary cancer*		1.28	[0.55; 2.54]	4.21e-01
NBN - Breast cancer		1.40	[1.20; 1.63]	1.25e-05
- Ovarian cancer		2.08	[1.58; 2.70]	1.69e-07
- Prostate cancer		4.42	[3.60; 5.42]	1.56e-42
- Pancreatic cancer		5.21	[3.52; 7.52]	1.74e-13
- Melanoma		6.90	[3.94; 11.35]	2.22e-09
- Hematologic tumors		4.03	[2.47; 6.27]	1.58e-07
- Endometrial cancer*		1.37	[0.44; 3.26]	4.23e-01
- Colorectal cancer*		1.64	[1.26; 2.10]	1.77e-04
- Gastric cancer*		0.82	[0.46; 1.37]	5.50e-01
- Hepatobiliary cancer*		2.16	[1.02; 4.06]	3.03e-02
- Brain tumors*		5.06	[2.39; 9.52]	5.13e-05

0.1 1 2 10 50

(C) Cases from meta-analysis and cases and gnomAD controls from secondary burden analysis

Gene	Tumor type	Meta–analysis			Secondary burden analysis			gnomAD	
		Studies; N	Cases; N	Carriers; N (%)	Studies; N	Cases; N	Carriers; N (%)	Cases; N	Carriers; N (%)
MRE11	Breast cancer	9	119627	109 (0.09)	24	143131	143 (0.09)	125518	154 (0.12)
	Ovarian cancer	–	–	–	7	2171	8 (0.36)		
RAD50	Breast cancer	13	134791	286 (0.21)	34	168038	406 (0.24)	114729	407 (0.35)
	Ovarian cancer	–	–	–	7	1767	8 (0.45)		
	Hepatobiliary cancer	–	–	–	4	3526	12 (0.34)		
NBN	Breast cancer	24	170523	387 (0.22)	60	272447	672 (0.24)	125589	222 (0.18)
	Ovarian cancer	4	13833	44 (0.31)	17	21260	78 (0.36)		
	Prostate cancer	9	21292	167 (0.78)	12	22139	173 (0.78)		
	Pancreatic cancer	4	1927	16 (0.83)	12	3689	34 (0.92)		
	Melanoma	3	449	13 (2.89)	6	1393	17 (1.22)		
	Hematologic tumors	6	2800	20 (0.71)	8	3085	22 (0.71)		
	Endometrial cancer	–	–	–	4	2060	5 (0.24)		
	Colorectal cancer	–	–	–	9	30440	88 (0.28)		
	Gastric cancer	–	–	–	6	2614	10 (0.38)		
	Hepatobiliary cancer	–	–	–	3	1118	10 (0.89)		
	Brain tumors	–	–	–	4	10986	16 (0.14)		

FIGURE 5 Risk of various cancer types in carriers of gPV in *MRE11*, *RAD50*, and *NBN* calculated in the meta-analysis (black symbols and letters) and the secondary burden analysis (dark red symbols and letters). (A) Comparison of risk calculated from this meta-analysis (Figures 2–4; using case–control data) and risk calculated using the same case data but gnomAD control data. (B) Cancer risk in carriers of gPV in MRN complex genes calculated using all available cancer data from cancer patients (gathering data from case–control and case-only studies) compared with gnomAD controls for *MRE11*, *RAD50*, and *NBN*, respectively (Supplementary Tables S4–S6; at least three studies were required for the burden analysis; * indicates additional cancer types added by the burden analysis; significant associations are highlighted in bold). (C) Number of studies and individuals included in each analysis (cases from meta-analysis, and cases and gnomAD controls from secondary burden analysis).

(93,123 vs. 170,523).[70] Similarly, the recent large *NBN* study by Belhadj et al. unveiled no association with breast cancer but suggested *NBN* as a pan-cancer predisposing gene, which was confirmed by our analysis.[71] Regarding ovarian cancer, our meta-analysis did not show a significant association (OR = 1.53; 95%CI: 0.76–3.09; four studies; 13,833 patients); however, a significantly increased but clinically low risk of ovarian cancer was observed in the secondary burden analysis (OR = 2.08; 95%CI:1.58–2.70; +13 studies; +7427 patients). A similar risk was described in the meta-analysis by Suszynska et al. (OR = 2.17; 95%CI: 1.35–3.49) including 7,150 ovarian cancer patients (in comparison with 21,260 in our study).[70]

Our study confirmed the previous suggestion that carriers of germline *NBN* alterations have a significantly increased moderate risk of prostate cancer. Our meta-analysis (OR = 2.44; 95%CI: 1.84–3.2; 9 studies; 21,292 patients) likely provided more realistic estimates compared to the secondary burden analysis that described almost a

doubling of the risk after the addition of three studies (+3 studies; +847 patients). The prostate cancer risk found in our study was comparable to that described by Consortium of Investigators of Modifiers of BRCA1/2 (CIMBA) study in carriers of known prostate cancer predisposition gene BRCA2 (RR = 2.22; 95%CI: 1.63–3.03).[72] The presence of NBN gPV has been shown to increase the aggressiveness of prostate cancer in a large study of prostate cancer patients of European ancestry by Darst et al.[73] who confirmed previous analysis by Mijuskovic et al. that had shown enrichment of NBN carriers in British prostate cancer patients with aggressive phenotype with increased susceptibility to develop metastases.[74] The association NBN with prostate cancer progression (defined as either having metastases or prostate cancer-specific mortality) was found in the meta-analysis by Shi et al. (OR = 6.38; 95%CI: 2.25–18.05).[75]

Carriers of pathogenic germline variants in NBN showed a significantly increased risk of pancreatic cancer (OR = 4.03; 95%CI: 2.14–7.58) in our meta-analysis (4 studies; 1927 patients), which was confirmed in the secondary analysis (+8 studies; +1762 patients; OR = 5.21; 95%CI: 3.52–7.52). The increased rate of NBN gPV in pancreatic cancer patients with a higher rate of somatic loss of the wild-type allele in the tumors was observed in study by Belhadj et al.[71] The pancreatic cancer risk associated with NBN in our study was comparable to that of established pancreatic cancer genes BRCA1 (OR = 2.58; 95%CI: 1.54–4.05), BRCA2 (OR = 6.20; 95%CI: 4.62–8.17), ATM (OR = 5.71; 95%CI: 4.38–7.33)[76] or PALB2 (RR = 2.37; 95%CI: 1.24–4.50).[77] Carriers of gPV in these genes with one or more first-degree relatives with pancreatic cancer should be considered as high-risk individuals for pancreatic cancer screening.[78]

The increased melanoma risk was observed in carriers of NBN gPV (OR = 7.14; 95%CI: 3.30–15.43) but this result was based on a limited number of 449 patients from 3 studies. The observed melanoma risk was further increased (OR = 6.90; 95%CI: 3.94–11.35) in secondary burden analysis (+3 studies; +944 patients) but this result should be interpreted with caution due to the regional/ethnical differences between patients (mostly from Slavic, Central European populations enriched in c.657del5 founder variant) and gnomAD controls (with underrepresented Slavic populations).[69] Moreover, it remains to be established how this risk can be modified by a skin phototype and UV exposure as a significant melanoma predisposing factor.[79]

Hematologic tumors, especially early-onset lymphomas, are common in NBS patients carrying germline biallelic NBN pathogenic variants. Interestingly, our meta-analysis including six studies (2800 patients) showed a moderate risk of hematologic tumors (leukemia and lymphoma patients in pediatric and adult individuals; OR = 3.42; 95%CI: 1.14–10.20) in NBN heterozygotes, which was confirmed by the secondary burden analysis (+2 studies; +285 patients). The study by Tomasik et al. in Polish pediatric patients showed that heterozygous c.657del5 carriers have an increased risk of relapsing B-cell acute lymphoblastic leukemia.[80]

The secondary gene-centered burden analysis exploiting the gnomAD controls allowed us to calculate the risk of other cancer types (Figure 5). The results showed that heterozygous carriers of gPV in NBN have no increased risk of endometrial and gastric cancer, have a low increase in colorectal (OR = 1.64; 95%CI: 1.26–2.10) and hepatobiliary (OR = 2.16; 95%CI: 1.02–4.06) cancer risks, and a high risk of brain tumors (OR = 5.06; 95%CI: 2.39–9.52).

While the NBS patients carrying biallelic NBN germline alterations exhibit increased frequencies of multiple cancer types,[81] the increased cancer frequency does not characterize patients with NBS-like disease caused by biallelic RAD50 variants.[1] In this context, we found no association with breast and ovarian cancer risk in meta-analysis and no risk for hepatobiliary cancer in secondary burden analysis for the heterozygous carriers of gPV in RAD50. Moreover, our secondary burden analysis and the meta-analysis by Suszynska et al. showed that female carriers of gPV in RAD50 conferred a significantly moderately reduced risk of breast cancer (OR = 0.68; 95%CI: 0.59–0.78 and OR = 0.51; 95%CI: 0.40–0.64).[70] On the other hand, the Finnish founder pathogenic variant RAD50 c.687del may be associated with increased breast cancer risk in a variant-specific manner (Supplementary Figure S5).[8] Whether this phenomenon reflects a hypomorphic behavior of c.687del and full pathogenic effect of other germline RAD50 truncations remains to be clarified.[82] The information about a presence of germline RAD50 alteration has potential prognostic or predictive importance. Fan et al. found no cancer risk association with germline RAD50 variants in 7657 Chinese female BRCA1/BRCA2-negative breast cancer patients but observed that RAD50 carriers had significantly worsened recurrence-free survival (HR = 2.66; 95%CI: 1.18–5.98).[66] Ramos et al. found an increased sensitivity to PARP inhibitors in a RAD50-deficient model in vitro.[83]

The rarest MRE11 gPV were not associated with breast cancer risk in the meta-analysis; the secondary burden analysis revealed only a moderate increase in ovarian cancer risk (OR = 3.00; 95%CI: 1.27–6.08). Rebbeck et al. found an increased risk of ovarian cancer in selected MRE11 haplotypes[84] and a study by Darst et al. in prostate cancer patients indicated that the presence of MRE11 gPV increased the aggressiveness of the disease.[73] However, the evidence in the literature is conflicting.[68]

Although this study represents the largest analysis examining the association of heterozygous germline pathogenic variants in MRN genes with cancer susceptibility, we are aware of several limitations. First, the meta-analysis and the secondary burden analysis pooled studies that were unified by one cancer type but differed in diagnostic approaches and clinicopathologic characteristics (including methods of germline variant analysis, age, disease severity, histopathologic subtypes, family history of cancer), which may biased the study results, particularly for cancer diagnoses for which only few studies were available. We also cannot exclude or confirm risks for other cancers that have only been studied in few studies, and we completely neglect other gPV beyond truncations, nonsense variants, variants classified as pathogenic/likely pathogenic in ClinVar, or spliceogenic alterations at canonical splicing sites. Furthermore, the secondary burden analysis exploited the gnomAD controls of mixed ethnicity and thus certain analyses considering dominantly patients from populations with frequent founder gPV (e.g., NBN:c.657del5 carriers from Central Europe, which are largely missing in the gnomAD dataset) might overestimate the calculated risks.[69] The limitations regarding the discrepant ethnicities of cases and controls would be overcome by the index-test

method estimating the cancer risk for heterozygotes in NBS families, as demonstrated by Seemanova et al. in a small study including 344 relatives from 24 NBS families.[81] Larger index-test based studies from founder populations would improve the conclusions of our current study. The secondary analyses performed in parallel, considering the total unselected population and the non-cancer gnomAD population of controls separately, showed very similar data, but the earlier analysis would better reflect the real population context including a considerable proportion of cancer cases in adult populations.

In conclusion, when considering the most conservative lowest risks revealed by our study for particular cancer types, the carriers of gPV in *NBN* have moderately increased risk of prostate cancer (OR = 2.44), hematologic tumors (OR = 3.42) and pancreatic cancer (OR = 4.03), and a high risk of melanoma (OR = 7.14). In addition, our analysis showed that the risk of breast cancer is clinically negligible (OR = 1.29), and the risk of ovarian cancer is low if any (OR = 1.53). The clinical management of gPV carriers in *NBN* needs to be justified. The secondary burden analysis suggested that carriers of gPV in *NBN* have no risk of endometrial cancer, colorectal cancer, gastric cancer, and low risk of hepatobiliary cancer but may have an increased risk of brain tumors; however, the risks in these cancers need to be further reckoned by large multi-cancer studies using appropriate population-matched control datasets. Carriers of heterozygous gPV in *RAD50* have no evidence of increased risk of breast, ovarian, and hepatobiliary cancers. Carriers of gPV in *MRE11* are very rare and have no breast cancer risk. The moderate risk of ovarian cancer observed in the small group of ovarian cancer patients in the secondary burden analysis warrants further examination.

AUTHOR CONTRIBUTIONS

Barbora Stastna (BS): Conceptualization; methodology; data curation; formal analysis; investigation; writing—original draft; writing—review & editing. **Tatana Dolezalova (TD)**: Investigation; writing—review & editing. **Katerina Matejkova (KM)**: Investigation; writing—review & editing. **Barbora Nemcova (BN)**: Investigation; writing—review & editing. **Petra Zemankova (PZ)**: Formal analysis; writing—review and editing. **Marketa Janatova (MJ)**: Writing—review & editing. **Petra Kleiblova (PK)**: Writing—review & editing. **Jana Soukupova (JS)**: Writing—review & editing. **Zdenek Kleibl (ZK)**: Writing—original draft; writing—review & editing; supervision; funding acquisition; conceptualization. The work reported in the paper has been performed by the authors, unless clearly specified in the text.

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CONFLICT OF INTEREST STATEMENT

All authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

All data are available within the manuscript or as Supplementary Material. Further information is available from the corresponding author upon request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.