MRN complex and cancer risk: old bottles, new wine

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Statement of translational relevance

Identifying genes that predispose to breast cancer has been a goal in oncogenetics since the identification of the BRCA1 locus in 1990. Genes of the MRN complex (*MRE11A*, *NBN*, *RAD50*) have been thought of as potential cancer predisposition genes in the context of breast cancer and consequently included in breast cancer multigene panels for a decade now. Here, we review data from all relevant studies, and based on the most recent data, recommend the exclusion of these genes from clinical breast cancer panels. On the other hand, unexpectedly, the MRN genes have been very recently linked to clonal hematopoiesis, that predisposes to hematological malignancies in addition to cardiovascular diseases. This should be considered carefully in oncology practice as the story of the MRN genes in cancer risk is going through a plot twist and there is more to come from this fascinating complex of genes.

Abstract

The MRN complex, composed of MRE11A, RAD50, and NBN, mediates vital molecular functions to maintain genomic stability and hence protect against related disorders. Germline mutations in the MRN genes predispose to three different syndromes: Ataxia-Telangiectasia-Like Disorder (MRE11A deficiency), Nijmegen Breakage Syndrome (NBS) (NBN deficiency) and NBS-Like Disorder (RAD50 deficiency). The potential cancer component of these syndromes in addition to the close physical and functional proximity of the MRN complex to BRCA1 have promoted the MRN genes as candidate risk genes for developing breast cancer. This notion has been challenged by independent large-scale population-based studies. Despite having their two-decade old candidacy as breast cancer genes close to being refuted, it has recently been reported that the MRN genes rise to have potential new roles in clonal hematopoiesis. In this article, we discuss the history and current status of MRN genes' clinical utility in breast cancer and then focus on their recently uncovered and less understood roles in clonal hematopoiesis that likely predispose to health-related disorders such as hematological malignancies and/or cardiovascular morbid events.

MRN genes as breast cancer susceptibility genes: how did it start?

MRE11A, RAD50, and NBN together compose the evolutionary-conserved MRN complex, that maintains genome integrity. Biallelic hypomorphic germline variants in the MRN genes predispose to 3 different, yet phenotypically related, autosomal recessive genetic syndromes that are associated with variable degrees of cancer risk.

In 1981, the Nijmegen Breakage Syndrome (NBS) was first reported and later linked to germline variants in the *NBN* gene, leading to establishing the first MRN-related genetic syndrome[1-3] (Figure 1). NBS patients present mainly with microcephaly, growth retardation, and immunodeficiency. Around 40% of NBS patients can develop a malignancy by the end of their second decade of life[4]. One major example is the c.657_661del5; p.Lys219fs (hereafter denoted as *NBN* 657del5) founder *NBN* germline variant, found predominantly in the Slavic population, which in homozygous carriers is associated with 45% incidence of malignancies, mainly Non-Hodgkin Lymphomas[5]. Although, the NBS-related malignancies are mainly hematological, tumors of different lineages have been reported in NBS patients[4,6,7]. These observations rationalized the possibility of *NBN* predisposing to solid tumors.

By 1999, biallelic germline mutations in *MRE11A* were shown to associate with Ataxia-Telangiectasia-Like Disorder (ATLD), a milder variant of the *ATM* deficiency-mediated Ataxia-Telangiectasia (A-T) in two different families[8]. ATLD patients suffer from cerebellar degeneration, wide range of neurological deficits that progress with age, in addition to chromosomal translocations that underlie irradiation sensitivity[8,9]. However, ATLD lacks the established cancer risk component that has been estimated to affect 25% of the A-T patients. Specifically, only two siblings with ATLD have been diagnosed with cancer so far[10]. The two siblings had stage 4 non-small lung cancer and died before the age of 10 and 16. Therefore, whether MRE11A-mediated ATLD is accompanied with a cancer risk has been an unanswered question.

In 2009, the third MRN-related genetic syndrome was reported by demonstrating that RAD50 biallelic variants can lead to an NBS-Like Disorder (NBSLD). The described patient was initially reported in 1991 as a 4-year-old girl with microcephaly, growth retardation, and a bird-like face[11]. She was diagnosed with NBS as the lack of ataxia and/or telangiectasia excluded the possible A-T diagnosis. However, genetic testing and molecular investigations in a follow up 18 years later suggested that compound heterozygous mutations in *RAD50*, rather than variants in *NBN*, lead to the clinical phenotype[12]. Moreover, as a distinction from NBS, the described NBSLD patient did not have immunodeficiency or any detectable malignancy. In 2020, another patient with NBSLD was reported to have overlapping clinical phenotypes with the previously described case due to a loss-of-function homozygous pathogenic variant in *RAD50*[13]. This very rare incidence or at least reporting of RAD50-mediated NBSLD hinders the assessment of whether RAD50-deficiency can lead to malignancies in humans.

The MRN complex acts mainly as a guardian of genomic stability specially for its role in the DNA damage response (DDR) following DNA double-strand breaks (DSBs). The DDR is orchestrated by different categories of proteins such as sensors, mediators/transducers, and effectors. Sensors identify the DSB site and are recruited immediately after inducing the break.

Mediators/transducers and effectors act on activating the cell cycle checkpoint inhibitors to prevent passing the errors to the following generation and eventually repairing the break.

The MRN complex plays key roles in this structured response at two different but yet connected fronts: sensing the DSB and executing the repair (Figure 2). Shortly after inducing the DSBs, the complex is recruited to the DSB site as one of the early steps in the DDR cascade to start a positive feedback loop with the mediator ATM kinase, the first domino of the signaling cascade involving multiple/hundreds of downstream effector proteins[14-16]. Furthermore, the complex also plays an integral role in executing the repair by mediating either the high fidelity/error-free homologous recombination (HR) exclusively during the G2 or S phase of cell cycle or error-prone non-homologous end joining (NHEJ) repair (reviewed in detail[17]). During initiating HR, the MRN complex, through the MRE11A enzymatic nuclease activity, along with the BRCA1-binding partner CtIP can resect the DSB ends to produce the ssDNA needed to fulfil the sequential steps of HR. This observation, in addition to multiple other reports, clearly showed the MRN complex can be found in a complex with the tumor suppressor BRCA1 to mediate the HR in a cell cycle-dependent manner[18-22] (Figure 2).

In summary, the reported cancer incidences with the MRN complex-related genetic syndromes presented the first clue that these genes might predispose to malignancies in general. Furthermore, the entangled molecular connection to BRCA1 have provided another rationale for the MRN genes as candidate breast cancer susceptibility genes (BCSGs) for about two decades now.

Clinical utility and validity of the MRN complex in breast cancer.

Current DNA sequencing technologies allow multiplexed screening for germline variants in more than 100 genes, including established BCSGs (i.e. *BRCA1/2* and *PALB2*) and candidate BCSGs, including the MRN genes. As informative these tests can be, the wide range of genes being tested can also be a source of confusion for clinical management, especially when variants in unestablished candidate BCSGs are defined. Consequently, clinical validity, defined by a proven or a highly likely risk of breast cancer that is associated with variants in a BCSG, has been proposed as a prerequisite for including a BCSG in a gene panel[23].

The initial rationale for including the MRN genes in multigene panels[23-25] (such as those listed in ref[23]), stemmed from clinical genetics studies that paralleled linking these genes to the BRCA1 complex (Figure 1). Bartkova et al. identified, for the first time, the *MRE11A* c.1897C>T; p.R633* nonsense variant, that has been initially linked to ATLD when in a homozygous state[8], in a breast cancer patient free from *BRCA* germline variants[26]. This patient's tumor cells showed a loss of MRE11A protein level in contrast to the surrounding non-cancerous stromal cells, further supporting a potential role for MRE11A in this tumor's pathogenesis. Since then, multiple studies have reported other truncating variants, in addition to missense variants, in *MRE11A* (reviewed in ref[27]). The initial suggestion of *RAD50* as a candidate BCSG goes back to identifying the *RAD50* c.687delT; p.Ser229fs variant (hereafter denoted as *RAD50* 687delT) as a founder allele in the Finnish population. Heikkinen et al. reported a frequency of 2.5% (8 carriers

out of 317 screened cases of Finnish origin) for the 687delT variant that was found to be associated with a significant odds ratio (OR) of 4.3 in the investigated case-control study[28]. However, this high frequency was challenged by another study that reported lower frequency for the same variant (0.5%, 3/590 cases versus 0.2%, 1/560 controls of Finnish origins)[29]. Similar to the *MRE11A*, additional *RAD50* variants in breast cancer patients have been reported[30,31]. Notably, on the other hand, *NBN* held the highest possibility among the MRN genes of representing a true BCSG due to stronger associations with breast cancer incidences[23]. These associations were mainly with the Slavic founder allele *NBN* 657del5, that was initially reported in 2003[32]. A frequency of 3.7% was reported in the tested case series. Following reports suggested that this frequency and its associated breast cancer risk, (OR ~3.0), might be mainly confined to the Slavic population[33-35].

Although, the discussed studies supported the candidacy of the MRN genes as BCSGs, their true clinical validity and utility have remained questionable throughout the last decade. Perhaps two main reasons can be outlined. First, the rare incidences and/or reporting of the MRN-associated genetic syndromes. Second, the scarcity of the large-scale population-based studies that can refine the bias of studying series of cases selected for family history of breast cancer. Additionally, as the case of the NBN-associated breast cancer risk, almost all of the supporting data came from studies focusing on a specific population/ethnicity. However, the last two years have witnessed the emergence of relevant data that have helped cast an answer to the questionable MRN-associated breast cancer risk.

Using the ClinGen semiquantitative framework for assessing the gene-disease relationship[36], Lee et al. assessed the validity of 31 candidate susceptibility genes, including the MRN genes, in breast cancer[37]. The assessment depended on both genetic/clinical evidence (12 points) and experimental evidence (6 points). *MRE11A* candidacy was classified as "Disputed due to conflicting data", while that of both *RAD50* and *NBN* was classified as "Limited". Specifically, *RAD50* scored 3.5/12 and 2/6, while *NBN* scored 1/12 and 1.5/6, for genetic and experimental evidence, respectively. Shortly after, LaDuca et el. reported a retrospective study on 165,000 patients who underwent genetic testing for different tumor types[38]. They investigated the frequency of predicted pathogenic variants (PVs) in 32 genes, including the MRN complex, and their risk potential in around 90,000 breast cancer patients (Table 1). Predicted *MRE11A* and *RAD50* PVs were not associated with any increased risk for breast cancer. In contrast, the authors reported a statistically significant OR of 1.37 (95% CI: 1.01-1.86) for the *NBN* PVs in breast cancer (Table 1). These two studies suggested a lack of utility for at least two components of the MRN complex in familial/hereditary breast cancer.

Sequentially, as the third relevant investigation in the series, Hart et al. introduced an online open-access tool to determine the prevalence of mutations in the context of race, age, and breast cancer subtype based on about 150,000 multi-gene screening tests[39]. We interrogated this tool to define the prevalence of the MRN variants in different ethnicities/races and to test the possibility of a differential impact between ethnicities (Table 2). Notably, *MRE11A* variants showed highest prevalence (0.36% of tested subjects) in Black populations, which is a threefold higher to their frequency in Non-Hispanic Whites (0.12%), as similarly reported in a recent study[40]. Whether this difference will be maintained in larger studies and whether it can be translated into a higher risk potential in the Black population is still not established[40].

To further establish the risk associated with different BCSGs, including the MRN genes, two independent studies undertook an unbiased population-based approach to investigate around 71,000 breast cancer patients in addition to about 83,000 controls, collectively[41,42] (Table 3 for detailed numbers). The two studies were not enriched for familial breast cancer patients or for those with early onset cancer, and hence complementing LaDuca's report (~90% of the studied persons in all cancer types had a history of cancer in the first- and second-degree relatives). Similarly, they reported a nonsignificant OR of 0.88 and 0.69 for *MRE11A* and 1.08 and 0.73 for *RAD50* predicted pathogenic variants (Table 3). However, in a disagreement with LaDuca's analyses, *NBN* scored a nonsignificant OR of 0.9 and 1.05. Furthermore, the Slavic founder *NBN* 657del5 allele was associated with an OR of 0.93 (95% CI = 0.52-1.68), suggesting a lack of universal validity for this variant, beyond the Slavic populations.

One of these two studies[41] further included rare missense variants in the MRN genes within their analysis pipeline (as shown in Table 3). None of the MRN genes showed any significance or potential risk in such analysis. Finally, none of the MRN genes associated with an increased risk for any breast cancer subtype in the two studies.

In summary, all these studies together provide strong evidence for the lack of utility for including the MRN genes on multi-gene testing panels for breast cancer patients, especially among the White populations which represented the majority of the studied subjects.

Clonal hematopoiesis: new chapter in the MRN cancer risk story

In parallel to the reported possible null risk for the MRN genes in breast cancer, a striking observation has linked this complex to the less understood clonal hematopoiesis (CH) phenomenon[43,44]. CH is the process of generating an expanded mutant clone due to a post zygotic mutation occurring in the hematopoietic system. Such mutation provides the cell with a proliferation advantage and hence the clonal expansion. Despite the fact that expanded clones are not malignant due to the lack of transformation beyond expansion, CH might predispose to hematological malignancies[45]. Consequently, the term "CH of indeterminate potential" (CHIP) was introduced to define harboring a cancer (hematological malignancy)-associated mutation, but without an overt hematological malignancy (i.e. normal blood counts)[46,47]. CHIP was, surprisingly, shown to predispose to coronary heart diseases[45,48], where, CHIP mutations were found to multiply the risk for developing early-onset myocardial infarction by 4 times[48]. Furthermore, CHIP-associated mutations are predicted to increase the risk for developing acute myeloid leukemia[49]. These important studies and others established the clinical consequences of CHIP and built the rationale for the hunt for the genetic causes and molecular mechanisms behind it [45,46,50].

Two back-to-back *Nature* reports investigated the inherited genetic factors predisposing to CH[43,44]. In particular, they investigated inherited variants that when found *in cis* with mosaic chromosomal alterations (mCA) can potentially predispose to CH and confer a proliferation advantage for the clone. Strikingly, the two independent studies defined heterozygous variants in *MRE11A* (rs587781384; rs762019591, and the intronic 11:94160189 variant) and *NBN*

(rs1187082186; rs777460725; rs756831345) as susceptible to copy-neutral loss of heterozygosity (CN-LOH). This CN-LOH leads to the loss of the wild type allele and presumably provides the mutated cells with the proliferative advantage. Also, interestingly, variants in *ATM* were pinpointed, highlighting the potential role for the MRN-ATM pathway in CH[43].

Multiple insights can be gleaned from these studies. First, the two studies compiled data from different population: European and Japanese/Asian. It was shown that different populations probably have different distributions of mCAs in addition to preferential tendency for different lineages of CH (i.e. B vs T cell lineages). Indeed, despite the similarity in the approach and goals of the two studies, the identified *MRE11A* and *NBN* variants were exclusive between the two studies. Would that be translated into a differential MRN-associated CH risk and consequential clinical risk for hematological cancers and/or cardiovascular disorders among different populations? These questions remain open and pressing for future investigations to tackle, given that biallelic PVs in *NBN* as part of the NBS disorder can predispose to lymphoma, as discussed before. In addition, certain variants of the functionally related gene *ATM* were also found to predispose to CH, and *ATM* is linked to predisposition to hematological cancers[51]. Intriguingly, the *MRE11A* rs587781384 variant, that we recently found not to be associated with a breast cancer risk[27], was found in strong linkage disequilibrium with the lead identified CH-associated *MRE11A* variant (rs762019591; OR 130). This suggests a possible role for this variant in CH and its associated disorders, rather than developing breast cancer and warrants further future investigations.

Second, defining CH-associated MRN variants raises technical and biological points regarding assessing the MRN complex in patients affected with solid cancers in general and breast cancer in particular. Peripheral blood samples are routinely used to test for germline variants by broad clinical genetic panels. In this context, it could be difficult to distinguish whether the defined MRN variant is a germline variant or simply an acquired somatic mutation that led to selecting a detectable clone by sequencing. Indeed, this notion was highlighted in a recent report, where *ATM* CHIP-associated variants were defined by analyzing cell-free DNA from advanced prostate cancer patients[52]. Furthermore, CH mutations frequency increases significantly during aging[53] and as the clinical practice might be directed towards generalized testing for germline variants in breast cancer patients regardless the age/onset[54,55], it will be important to perform matched analysis of the patients' tumors along with peripheral blood samples in order to differentiate a germline variant from a somatic CH mutation. Such a pipeline has recently been used to define candidate CH driver genes[56].

Conclusions and future perspectives

The studies discussed here provide a strong evidence for the lack of validity and hence clinical utility for the MRN genes in breast cancer. This notion calls for excluding these genes from the BCSGs panels. As this conclusion marks a closure and an end for the two-decade old chapter of the MRN complex story, only two minor questions, that are unlikely to change the current conclusion, are left without a certain answer. One concerns the NBS-associated founder *NBN* 657del5 allele and its associated breast cancer risk in Slavic populations that might not be high enough for affecting the clinical management. Second is the *MRE11A* associated breast cancer risk in Black populations that is still unestablished due to lack of large-scale studies focusing on these populations. On the other hand, linking *MRE11A* and *NBN* to clonal hematopoiesis marks the beginning of understanding of MRN genes' role in disease. The latter finding highlights the importance of considering the CH phenomenon in the technical design of assessing the disease associated MRN susceptibility alleles and opens multiple questions for these genes. Is there a connection between these variants and hematological malignancies? Can variants in these genes be associated with risk to develop cardiovascular disorders through CHIP promoted mechanism? Studies will be needed to start exploring the next chapter in the MRN story.

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Tables

Table 1. Prevalence of the MRN genes predicted pathogenic variants and their associated odds ratio as reported by LaDuca et al.[38]

		MRE11A	*RAD50*	*NBN*
Cases	Total N	75,818	75,818	75,818
	PVs (frequency)	36 (0.05%)	92 (0.12%)	81 (0.11%)
Controls	Total N	111,326	111,109	111,166
	PVs (frequency)	53 (0.05%)	129 (0.12%)	87 (0.08%)
Odds Ratio (95% CI)		1 (0.65-1.54)	1.05 (0.8-1.38)	1.37 (1.01-1.86)
P Value		1	0.784	0.0491

Table 2. Frequency of the MRN genes variants in different ethnicities (generated by the online tool from Hart et al[39])

Race/ethnicity		*MRE11A*	*RAD50*	*NBN*
No filter	Tested	59,375	59,375	59,375
	Positive (%)	79 (0.13%)	164 (0.28%)	120 (0.2%)
Non-Hispanic White	Tested	43,958	43,958	43,958
	Positive (%)	53 (0.12%)	129 (0.29%)	104 (0.24%)
Black	Tested	4,731	4,731	NA
	Positive (%)	17 (0.36%)	12 (0.25%)	NA
Ashkenazi Jewish	Tested	NA	3,985	3,985
	Positive (%)	NA	6 (0.15%)	6 (0.15%)
Asian	Tested	3,085	3,085	NA
	Positive (%)	5 (0.16%)	8 (0.26%)	NA
Hispanic	Tested	NA	3,616	3,616
	Positive (%)	NA	9 (0.25%)	4 (0.11%)

Table 3. Summary of the MRN genes variants frequencies and associated odds ratio from the most recent large-scale population-based studies[41,42]

	BCAC 2021				Hu 2021			
Study Population	Patients (N = 48,826) Controls (N= 50,703)				Patients (N=32, 247) Controls (N= 32,544)			
	Cases	***Controls***	***Odds Ratio (95% CI)***	***P Value***	***Cases***	***Controls***	***Odds Ratio (95% CI)***	***P Value***
MRE11A	***Protein-truncating Variants***				25	32	0.69 (0.38-1.20)	0.19
	48	55	0.88 (0.59-1.32)	0.54				
	Rare Missense Variants							
	552	611	0.94 (0.84-1.06)	0.33				
RAD50	***Protein-truncating Variants***				57	82	0.73 (0.51-1.04)	0.08
	120	121	1.08 (0.83-1.40)	0.57				
	Rare Missense Variants							
	1046	1089	0.99 (0.91-1.08)	0.83				
NBN	***Protein-truncating Variants***				57	51	1.05 (0.71-1.56)	0.81
	90	103	0.90 (0.67-1.20)	0.48				
	Rare Missense Variants							
	665	725	0.95 (0.85-1.06)	0.37				

Figures Legends

Figure 1. A timeline for the highlights of the MRN genes' research contributing to their candidacy as BCSGs. The history of the MRN genes candidacy as BCSGs can be split into three chapters. First is identifying the associated genetic syndromes. Second is revealing the connection with BRCA1, the *bona fide* BCSG that paralleled the initial reports of cancer association with germline variants in the MRN genes. Third, after including these genes in the multigene panels, large-scale studies have suggested a lack of utility and validity for these genes in breast cancer. Finally, most recently, *MRE11A* and *NBN* have been linked to clonal hematopoiesis, marking a potential new chapter for the MRN genes beyond breast cancer. *The MRN genes are still included on different panels for breast cancer susceptibility (for example, ARUP laboratories: https://ltd.aruplab.com/Tests/Pub/2012026 ; InVitae: https://www.invitae.com/en/physician/tests/01202/ ; Fulgent: https://www.fulgentgenetics.com/focuscancer-breast)

Figure 2. A simplistic molecular view of how the MRN complex is functionally linked to the core and other candidate BCSGs. Immediately after the induction of a double strand break (DSB), the MRN complex will sense and be recruited to the DSB site. This will lead to establishing a positive feedback circuit with the kinase ATM, the master regulator of DNA damage response (DDR), that can stimulate hundreds of downstream proteins to mediate the DDR and define the fate of the cell. Importantly, ATM can activate the kinase CHK2 that will contribute to cell cycle arrest in addition to stabilizing TP53 that can contribute to cell cycle arrest and/or stimulating programmed cell death. In the meantime, the MRN complex in collaboration with the BRCA1-CtIP complex will resect the DSB and mediate the production of the single-stranded DNA (ssDNA). Then, the BRCA1-PALB2-BRCA2 complex will help the loading of RAD51 and consequently the formation of RAD51 nucleofilaments, a key step to complete the homology-directed repair (HDR). Among multiple other key players in this tangled landscape, BARD1 along with BRCA1 are suggested to contribute to the earlier step of end resection in addition to the RAD51 recruitment. Finally, RAD51C and RAD51D, two out of five RAD51 paralogs, contribute to the accumulation of RAD51 at the break sites to ensure an efficient HDR process.
The BCSGs (*BRCA1*, *BRCA2*, *PALB2*, *ATM*, *CHEK2*, *BARD1*, *RAD51C*, *RAD51D* and *TP53**) that were associated with a statistically significant risk for developing breast cancer in the recent two large-scale population-based studies[41,42] are in black. *The main association between *TP53* and risk of breast cancer has been established from breast cancer cases in the context of Li–Fraumeni syndrome.

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Figure 1

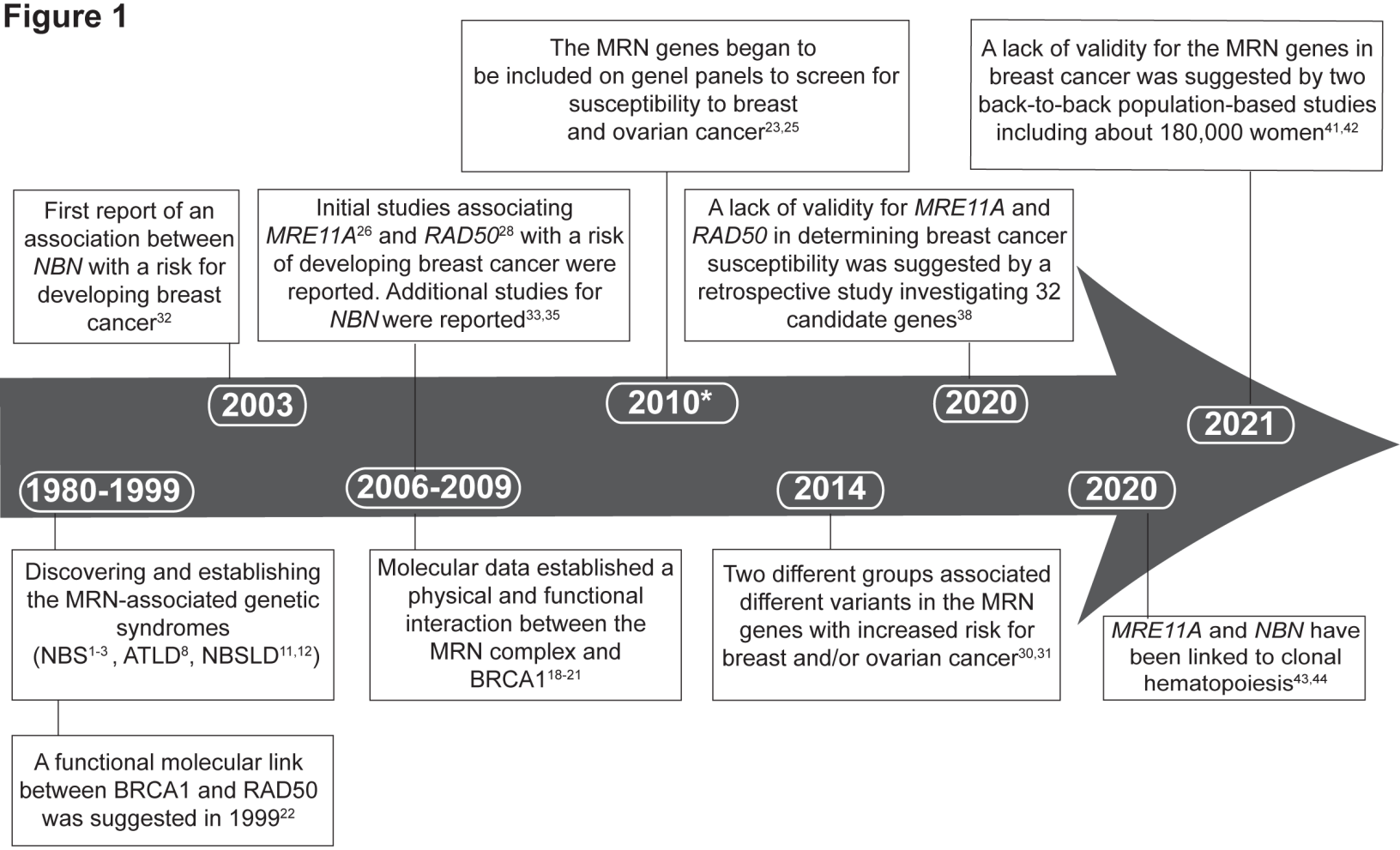
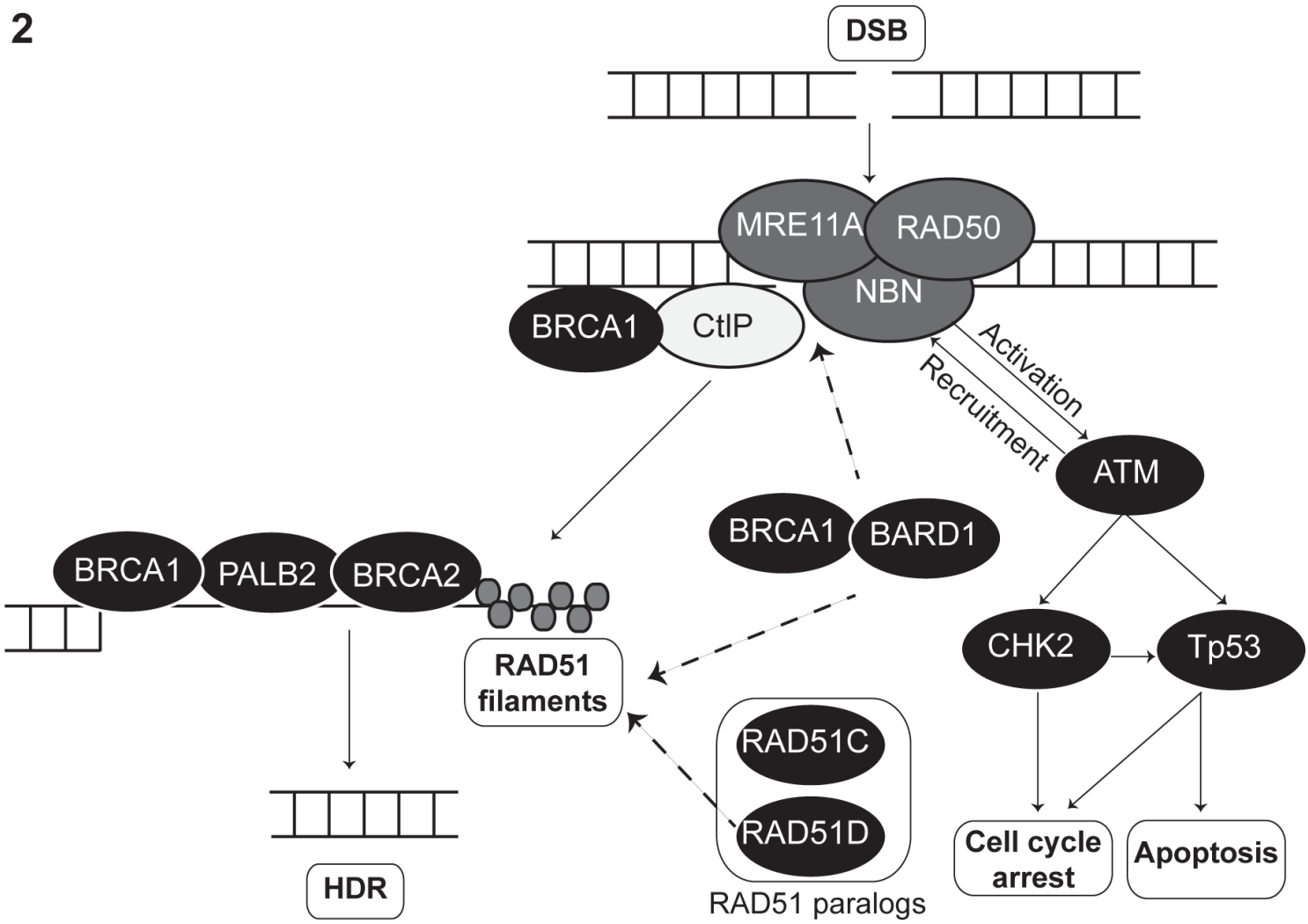


Figure 2



AACR American Association for Cancer Research

Clinical Cancer Research

MRN complex and cancer risk: old bottles, new wine

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