Implications of the germline variants of DNA damage response genes detected by cancer precision medicine for radiological risk communication and cancer therapy decisions

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ABSTRACT

Large-scale cancer-associated gene testing is now being rapidly incorporated into clinical settings, and is leading to incidental identification of the germline variants present in cancer patients. Because many cancer susceptibility genes are related to DNA damage response and repair, the variants may reflect not only the susceptibility to cancer but also the genetically defined radiation sensitivity of the patients and their relatives. When the presence of a certain germline variant increases the risk for developing radiation toxicity or radiation-induced secondary cancers, it will greatly influence the clinical decision-making. In order to achieve optimal radiological risk communication and to select the best cancer management for a given patient based on information from gene testing, healthcare professionals including genetic counselors, risk communicators and clinicians need to increase their knowledge of the health effects of various genetic variants. While germline loss-of-function mutations in both of the alleles of the DNA damage response genes cause rare hereditary diseases characterized by extreme hypersensitivity to radiation, the health effects of the carriers who have germline variants in one allele of such genes would be a matter of debate, especially when the significance of the variants is currently unknown. In this review, we describe the clinical significance of the genetic variants of the important DNA damage response genes, including ATM and TP53, and discuss how we can apply current knowledge to the management of cancer patients and their relatives from a radiological point of view.

Keywords: genetic variant; DNA damage response genes; ATM; TP53; radiological risk communication; cancer therapy

INTRODUCTION

Recent advances in DNA sequencing technology have led to the development of large-scale gene panels for cancer genetic testing. In Japan, the two cancer gene panels, the OncoGuide NCC Oncopanel and FoundationOne CDx test, were approved in May 2019 as diagnostic tests provided by the national health insurance system [1]. The former, developed by the National Cancer Center of Japan, can detect mutations and copy number alterations in 114 genes, gene fusions in 12 genes and the tumor mutational burden, using both tumor tissue and normal blood cells, which allows detection of both somatic mutations and germline mutations [2]. The latter can detect mutations and copy number changes in 324 genes, gene fusions in 36 genes, the tumor mutational burden and microsatellite instability, but it uses only tumor tissues; thus germline mutations cannot be identified, with the exception of rare, known germline-specific mutations [3].

Approximately 10% of all cancers are considered to have hereditary components, although there are variations across cancer types [4–6]. The best-known and most common forms of hereditary cancers are caused by mutations in the high-penetrance genes BRCA1, BRCA2 and TP53 [7–10]. Other genes including PALB2, ATM, CHEK2, BRIPI, RAD51C, RAD51D and FANCM have been described as moderate-penetrance cancer susceptibility genes [7, 11–21]. Among these genes, BRCA1, BRCA2, PALB2, BRIPI, RAD51C and RAD51D frequently exhibit germline mutations, whereas germline mutations in TP53, ATM, CHEK2 and FANCM are less frequent [22]. Since these genes are associated with response to DNA damage, they...
implicate defects in DNA damage response and repair as important causes of hereditary cancers. Cancer gene panels which are currently available actually include many DNA damage response genes to be examined. For example, the OncoGuide NCC Oncopanel includes ATM, CHEK2, TP53, SETD2, BRCA1, BRCA2, PALB2, BAP1, BARD1 and RAD51C, while the FoundationOne CDx test includes ATM, ATR, CHEK1, CHEK2, TP53, SETD2, CDK12, NBN, MRE11, BRCA1, BRCA2, PALB2, BAP1, BRIP1, BARD1, RAD51, RAD51B, RAD51C, RAD51D, XRCC2, RAD52, RAD54L, FANCA, FANCC, FANCN, FANCL and ERCC4.

Increased utilization of cancer gene panel testing in clinical settings has opened up the possibility for detecting germline variants incidentally [23–25]. Such germline variants have also been termed ‘secondary findings’ because their detection is secondary to the original purpose of identifying the cancer mutation profiles. The interpretation of a germline variant is sometimes very difficult, particularly when its functional effect or clinical significance is not fully understood. However, germline variants have the potential to provide valuable health-related information, such as susceptibility to other cancers or the genetically defined radiosensitivity of patients and their relatives, especially when the impacts of the variants on the functions of the DNA damage response are highly predicted or already established.

In this review, we first outline the mechanism of the DNA damage response by focusing on the key proteins associated with both the DNA damage response and cancer susceptibility. Next, we describe the severe radiosensitivity caused by germline mutations in both alleles of the DNA damage response genes and discuss potential radiation effects in carriers of germline variants who have mutations in only one of the two alleles of the genes. Finally, we discuss how we can apply the current knowledge of the clinical significance of the genetic variants to the management of cancer patients and their relatives from a radiological point of view.

**KEY PLAYERS IN THE DNA DAMAGE RESPONSE AND THEIR ASSOCIATION WITH CANCER SUSCEPTIBILITY**

The DNA double-strand break (DSB) is the most deleterious type of DNA damage produced in living cells. In order to prevent adverse effects, DSBs are recognized and repaired by proteins involved in the DNA damage response. In response to DSBs, the MRE11–RAD50–NBS1 (MRN) complex initially recognizes and binds the DSB sites, and recruits and activates the ataxia telangiectasia-mutated (ATM) kinase (Fig. 1) [26,27]. Then, ATM transmits the DNA damage signals by phosphorylating a large number of downstream proteins, including the MRN complex, the histone variant H2AX, the checkpoint kinase Chk2, the breast cancer susceptibility protein BRCA1, and p53, encoded by TP53 [28,29]. Phosphorylation of Chk2 can contribute to cell cycle arrest, and that of BRCA1 leads to homologous recombination (HR) or cell cycle arrest, whereas activation of p53 triggers cell cycle arrest or apoptotic cell death.

DSBs are repaired by error-prone pathways or a relatively error-free pathway [30]. Homologous recombination (HR) is the most accurate repair pathway for DSBs. It acts only during the S and G2 phases of the cell cycle, since it requires a non-damaged sister chromatid to serve as a template for repair. Following the recognition of the DSB ends by the MRN complex and activation of ATM, the DNA ends are resected by the MRN complex and CtIP, resulting in generation of 3′ single-stranded DNA (ssDNA) overhangs on both sides of the break. These overhangs are coated and stabilized by replication protein A (RPA). Next, BRCA1 facilitates numerous protein–protein interactions at the sites of the DNA break. The Fanconi anemia (FA) pathway, which plays a key role in the repair of interstrand cross-links (ICLs), also contributes to the activation of this step [31]. The FA core complex, consisting of FA proteins (FANCA/B/C/E/F/G/L/M/T) and associated proteins, is a multiprotein ubiquitin ligase which activates FANCD2 and FANCI by monoubiquitination in response to replication stress, which orchestrates the actions of downstream DSB repair proteins. The binding of BRIP1 to BRCA1 and formation of the BRCA1–PALB2–BRCA2 complex are critical for the early stage of HR repair. BRCA2 directly binds RAD51 and recruits it to the double-stranded DNA (dsDNA)–ssDNA junction. BRCA2 promotes the loading of RAD51 onto ssDNA and assembly of the RAD51–ssDNA filament, leading to strand invasion into an undamaged homologous DNA template, which is aided by RAD51 paralogs, including RAD51B, RAD51C, RAD51D, XRCC2 and XRCC3 [32].

Many of these genes encoding DNA damage response proteins are associated with cancer susceptibility. For breast cancer, the lifetime risk associated with a BRCA mutation carrier is 46–60%, whereas that for a BRCA2 mutation carrier is 49–55% [33–35]. For ovarian cancer, the risk for a BRCA1 mutation carrier is 12–59%, whereas that for a BRCA2 mutation carrier is 6–18% [33–35]. A carrier of a mutation in another high-penetration breast cancer susceptibility gene, TP53, has a high lifetime risk of 73–100% for any cancer [36]. The risk of breast cancer for a PALB2 mutation carrier is 35–45% [11,37]. The breast cancer risks associated with ATM and CHEK2 variants are lower, generally in the range of 25–30% [38]. For ovarian cancer, the ATM variants are reported to confer a 3-fold increase in risk, whereas the CHEK2 variants do not increase the risk at all. The carriers of mutations in BRIP1, RAD51C and RAD51D all have an elevated lifetime risk of ovarian cancer of 5.8, 5–15 and 5–15%, respectively [16–19]. In the FANCM gene, a nonsense mutation c.5101C>T (p.Q1701X) was significantly more frequent among breast cancer patients than among controls [odds ratio (OR) = 1.86], with particular enrichment in patients with triple-negative breast cancer (OR = 3.56) [20].

**RADIATION HYPERSENSITIVITY SYNDROMES CAUSED BY GERMLINE DEFECTS IN THE DNA DAMAGE RESPONSE**

Germline pathogenic mutations in both of the alleles of the DNA damage response and repair genes lead to hereditary diseases which share common clinical features, including hypersensitivity to DNA damage and increased risk of cancer development.

Ataxia telangiectasia (AT) is a rare autosomal recessive disease caused by germline mutations in both alleles of the ATM gene [39]. It is characterized by progressive neurodegeneration, immunodeficiency, hypersensitivity to radiation, telangiectasia and cancer predisposition [40]. Similarly, defects in one of the components of the MRN complex also lead to hereditary radiosensitive syndromes such as Nijmegen breakage syndrome, AT-like disorder and Nijmegen...
Fig. 1. Schematic representation showing the involvement of products of cancer susceptibility genes in DNA damage response and repair. The products of major high-penetrance cancer susceptibility genes are colored in pink and those of other moderate-penetrance cancer susceptibility genes are colored in blue. The proteins colored in gray are not currently established as products of cancer susceptibility genes, but include the products of the genes in which rare hereditary mutations in cancer are reported. In response to double-strand breaks (DSBs), the MRN complex initially recognizes and binds the DSB sites, and recruits and activates the ATM kinase, which transmits the DNA damage signals by phosphorylating a large number of downstream proteins, leading to DNA repair, cell cycle arrest and apoptotic cell death. Homologous recombination (HR) is the most accurate DNA repair pathway for DSBs, and the Fanconi anemia (FA) pathway, which plays a key role in the repair of interstrand cross-links (ICLs), also contributes to the activation of HR.

breakage syndrome-like disorder, which are caused by germline pathogenic mutations in both alleles of the NBS1, MRE11 and RAD50 genes, respectively [41–43]. Germline mutations in genes involved in non-homologous end-joining, an error-prone DNA repair pathway for DSBs which directly rejoins DSB ends by ligation and takes place throughout the cell cycle, also cause severe combined immunodeficiency associated with hypersensitivity to radiation; the genes responsible are DNA-PKcs, Artemis, LIG4 (DNA ligase IV) and XLF [44–48]. Because these hereditary diseases confer extreme hypersensitivity to radiation, radiation is generally contraindicated in patients with these diseases.

Germline defects in the components of the FA pathway cause an autosomal recessive disorder characterized by multiple congenital abnormalities, bone marrow failure, hypersensitivity to DNA cross-linking agents and increased susceptibility to cancer [49]. Since patients with FA show extreme hypersensitivity to DNA cross-linking agents such as cisplatin and mitomycin C, these drugs are contraindicated in these patients. While patients with FA are more sensitive to radiation than the general population, radiation can be delivered if clinically indicated.

DIFFICULTIES IN THE INTERPRETATION OF THE CLINICAL SIGNIFICANCE OF GERMLINE VARIANTS

Given the severe hypersensitivity to radiation in patients with hereditary diseases with biallelic germline mutations in the DNA damage response genes, as described above, the question arises of whether the carriers of germline variants in DNA damage response genes will develop increased clinical toxicities or second cancers after exposure to radiation. Because it has been suggested that exposure to environmental carcinogens including radiation may worsen the cancer incidence of the carriers who have a pathogenic germline variant in one allele of the DNA damage response genes [50], the answer should greatly influence the clinical decision-making for the given patient.

Regarding the clinical significance of germline variants detected in cancer genetic testing, the most crucial stage may be to determine whether the detected germline variants are pathogenic. In 2015, the American College of Medical Genetics and Genomics-Association for Molecular Pathology (ACMG-AMP) provided guidelines for the interpretation of sequence variants for genetic disorders [51]. This report recommended that the variants should be classified into five categories—pathogenic, likely pathogenic, uncertain significance
Fig. 2. The relationship among protein function, type of mutation and classification based on clinical significance of a variant. The degree of protein function, type of mutation and clinical significance are indicated in the upper panel, the middle panel and the lower panel, respectively. Variants are classified into five categories on a range from pathogenic (left) to benign (right) based on their likelihood of affecting the protein functions (upper panel) and their potential clinical significance (lower panel). Since a DNA damage response gene product mostly acts as a tumor suppressor, loss of function of the gene product will be a critical determinant for pathogenicity. The variant which results in a loss, frameshift or nonsense mutation of the gene can be pathogenic or likely pathogenic (left), whereas a variant which results in a silent mutation can be benign or likely benign because it will not affect the function of the gene product (right). A variant which results in a non-truncating missense mutation is usually difficult to interpret, and is classified as a ‘variant of unknown significance (VUS)’. 

The updated information on VUSs is available on the ClinVar website (https://www.ncbi.nlm.nih.gov/clinvar/), a freely accessible archival database that aggregates information about genetic variants and their relationship to human disease. As of January 2021, ~20,000 VUSs are listed for the high/moderate penetrance DNA damage response genes in ClinVar (Table 1). Germline VUSs in genes involved in the DNA damage response often catch clinicians unaware. While most of the VUSs are currently evaluated by referring to the personal and family history of cancer, prediction tools and co-occurrence with already established pathogenic variants, numerous groups are currently working to identify tests that can be easily used to accurately interpret the functional effects of VUSs.

Identification of the pathogenicity of unknown BRCA VUSs will be of particular medical value, since pathogenic germline BRCA variants may trigger several important clinical management actions. These actions include increased surveillance or prophylactic surgery for healthy carriers of germline pathogenic BRCA variants, and poly(ADP-ribose) polymerase (PARP) inhibitor treatment for the carriers of pathogenic BRCA variants newly diagnosed as having breast cancer, the efficacy of which is mediated through synthetic lethality in cancer cells with loss of function of BRCA [54]. Ikegami et al. recently developed a method for high-throughput functional evaluation of unknown BRCA2 VUSs by assessing the effects of BRCA2 variants on HR
with germline mutations in breast cancer susceptibility genes based on a systematic review of the literature and a formal consensus process [66]. They recommend that previously healthy carriers of BRCA mutations who are newly diagnosed with breast cancers may be considered for breast-conserving therapy, whereas bilateral mastectomy should be considered in cases at significant risk of developing a contralateral breast cancer, especially in young women, and those at high risk of new cancers in the ipsilateral breast. Radiation exposure should not have to be avoided, if clinically indicated. They also recommend that PARP inhibitors or platinum agents are preferable to non-platinum single-agent chemotherapy for treatment of advanced breast cancer in BRCA1/2 carriers.

**RISKS OF RADIATION EXPOSURE IN THE CARRIERS OF GERMLINE VARIANTS OF ATM**

The AT carriers (heterozygotes for the ATM variants) constitute ~1% of the general population [67]. Although they appear clinically normal, epidemiological studies have demonstrated that they have a 3- to 5-fold increased risk of developing breast cancer. Cells heterozygous for the ATM variants show moderate radiation hypersensitivity in vitro [68]. In an in vivo mouse model, the AT heterozygosity increased the susceptibility to radiation-induced breast cancer [69].

However, it is unclear to what degree the observations of the preclinical studies correlate with those in clinical phenotypes. In terms of increased clinical toxicity to normal tissues, studies regarding the differences between ATM variant carriers and non-carriers are limited, variable and inconsistent [70–72]. Regarding increased risks for contralateral breast cancer, Bernstein et al. report that deleterious ATM mutations may confer an increased risk of contralateral breast cancer specifically among patients treated by radiation therapy [73]. However, in most patients, this risk is likely to be low, since other studies showed no significant increase [62, 70]. Since ATM variants are likely to cause various differential effects in radiation response according to the types of gene alterations, it will be necessary to distinguish deleterious and neutral ATM alterations in order to properly define the clinical guidelines for the management of ATM carriers and their relatives.

At present, it would be reasonable to offer radiation therapy for patients with breast cancer who are carriers of ATM variants, with the exception of young patients who carry deleterious heterozygous ATM mutations [66, 74]. For these patients, radiation therapy must be carefully considered. In such cases, it would be preferable to avoid optional radiation and instead emphasize follow-up screening for second cancers when the indicated radiation therapy was delivered.

**RISKS OF RADIATION EXPOSURE IN THE CARRIERS OF GERMLINE VARIANTS OF TP53**

Germline variants of the high-penetrance cancer susceptibility gene TP53 have important clinical significance. A family harboring germline TP53 alterations is considered to have a cancer predisposition disorder referred to as Li–Fraumeni syndrome (LFS), characterized by early onset of any types of cancer, including sarcoma, adrenocortical carcinoma, breast cancer, glioblastoma and leukemia [75, 76].

As suspected from the critical role of p53 as a tumor suppressor, the carriers of a TP53 mutation would be expected to be at high

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**Table 1. Number of variants of unknown significance (VUSs) in the high-/moderate-penetrance DNA damage response genes**

| Gene   | VUSs  | Submitted variants |
|--------|-------|--------------------|
| BRCA1  | 2850  | 11533              |
| BRCA2  | 5068  | 12740              |
| ATM    | 4742  | 8945               |
| TP53   | 734   | 2042               |
| PALB2  | 1797  | 3506               |
| CHEK2  | 1227  | 2261               |
| BRIP1  | 1763  | 3052               |
| RAD51C | 619   | 1132               |
| RAD51D | 492   | 975                |
| FANCM  | 479   | 698                |

Updated data are available on the ClinVar website: https://www.ncbi.nlm.nih.gov/clinvar/
Table 2. Clinical evidence of risk of radiation therapy and clinical management recommended for the carriers of germline variants in high-/moderate-penetrance DNA damage response genes

| Gene      | Clinical evidence of risk of radiation therapy | Recommendations for clinical management |
|-----------|-----------------------------------------------|----------------------------------------|
| BRCA1/BRCA2 | No direct evidence                             | No need to avoid radiation exposure     |
| ATM       | Only limited, variable and inconsistent evidence | In most patients, radiation therapy can be offered |
|           | Low risk in most patients                      | Necessary to distinguish deleterious and neutral mutations (Exceptions) For carriers of deleterious mutations, radiation therapy must be carefully considered Follow-up screening for secondary cancer when indicated radiation therapy was delivered Avoid optional radiation |
| TPS3      | High risk of secondary cancers                 | Radiation therapy is contraindicated Avoid high-dose radiological examination and treatment Mastectomy should be a reasonable therapeutic option using magnetic resonance imaging from a young age For carriers of CHEK2 1100delC, radiation therapy should be avoided |
| CHEK2     | No direct evidence with the exception of carriers of CHEK2 1100delC Increased risk of contralateral breast cancer in carriers of CHEK2 1100delC | Therapeutic decisions should not be influenced by mutation status |
| Others    | No direct evidence                              |                                        |
| PALB2     |                                              |                                        |
| BRIP1     |                                              |                                        |
| RAD51C    |                                              |                                        |
| RAD51D    |                                              |                                        |
| FANCM     |                                              |                                        |

Risk for significant radiation-associated effects. An in vivo mouse model shows that radiation dramatically increased tumor development in p53 heterozygous mice [77]. Although there is limited clinical evidence showing the risk of developing new primary tumors after radiation therapy in patients with LFS, Heymann et al. studied the clinical outcomes of such patients with breast cancer [78]. In a group of six patients, there were three contralateral breast cancers, two radiation-induced cancers and three new primary cancers. In contrast, among the patients who had not received radiation therapy, only one showed a contralateral breast cancer recurrence. Thus, in LFS, radiation therapy is contraindicated. Replacement of high-dose radiological examination and treatment with other alternatives is generally recommended [66]. For cases of breast cancer in LFS, mastectomy should be considered as a reasonable therapeutic option. For the relatives of the patients who are healthy carriers of the TP53 germline mutation, extensive cancer surveillance using magnetic resonance imaging from a young age should be recommended.

RISKS OF RADIATION EXPOSURE IN THE CARRIERS OF GERMLINE VARIANTS IN OTHER GENES

As described above, carriers with a mutated allele of moderate-penetrance cancer susceptibility genes including PALB2, CHEK2, BRIP1, RAD51C, RAD51D and FANCM have an elevated risk of cancer. Moreover, some reports suggest the possibility that variants of other DNA damage response genes, including NBS1, RAD50, MRE11, XRCC2, FANCA and FANCC, may also contribute to cancer susceptibility, although the reported cases are still limited [79–82]. Because all of these genes are implicated in the responses to radiation, it has been hypothesized that heterozygotes for these genes might also be more sensitive to radiation than non-carriers, even at low doses. For example, mice heterozygous for the Nbn gene, a mouse homolog of the human NBS1 gene, showed a dramatically increased occurrence of spontaneous tumors by radiation exposure, revealing a clear relationship between NBS1 heterozygosity, radiation sensitivity and increased cancer risk [83]. The pathogenic mechanism for this is presumed to be ‘haploinsufficiency’, which is defined as a moderate loss-of-function phenotype in diploid organisms due to inactivation of one of the two alleles.

Regarding the significance of these moderate- or low-penetrance genes in terms of clinical radiation effects, there is no evidence of a significant increase in toxicity or development of secondary cancer related to radiation exposure, with the exception of breast cancers carrying the specific variant CHEK2 1100delC, which confers increased risks of contralateral breast cancer [84]. Furthermore, for patients with breast cancer with mutations in moderate-penetrance genes, there are currently no robust data to support the use of PARP inhibitors or platinum agents. Thus, for cancer patients carrying germline variants in one allele of the moderate- or low-penetrance cancer susceptibility genes, the therapy decisions should not be influenced by mutation status alone.
CONCLUSIONS AND FUTURE PERSPECTIVES

To summarize, based on the current information about genetic variants in the DNA damage response genes, radiation therapy should not be withheld because of the existence of such variants, except for the pathogenic variants of the high-penetrance cancer susceptibility gene TP53 (Table 2). While pathogenic germline mutations in two other high-penetrance cancer susceptibility genes BRCA1 and BRCA2 or other moderate-penetrance genes involved in DNA damage response and repair also increase lifetime cancer risk, there is currently no evidence that they increase radiation toxicity or development of radiation-induced cancers.

It is important to note that not every heterozygous germline variant of a specific gene that is identified by cancer genetic testing impacts the function of the gene product to result in increased radiosensitivity or increased cancer susceptibility risk. As in the case of VUSs, the functional effects and the clinical significance of many variants are still unknown. At present, VUSs are considered non-deleterious until functional genomic data emerge to demonstrate otherwise, and possession of germline alterations in a single copy of a gene critical for the DNA damage response does not necessarily equate to increased risk of radiation-induced toxicity or secondary cancers, except for TP53, some deleterious ATM mutations and the specific variant CHEK2 1100delC.

In order to more effectively apply the information of germline variants detected by cancer precision medicine to the management of the patients and their relatives, the following should be achieved in the future. First, functional validation of VUSs potentially associated with radiosensitivity is required. Secondly, more epidemiological studies on the association of secondary cancers and adverse effects with germline variants should be performed. Thirdly, education on radiosensitivity and medical genetics is required for those who are involved in the care of the patients and their relatives. These educative efforts should be targeted to a broad range of healthcare professionals, including medical oncologists, pathologists, clinical geneticists, genetic counselors and bioinformaticians, as well as radiological risk communicators and radiation oncologists.

Progress toward these goals will result in better understanding of the clinical significance of the germline variants and will bring novel insights in terms of personalized risk assessment, cancer prevention, and diagnostic and therapeutic options for patients and their relatives.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

PRESENTATION AT A CONFERENCE

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