

CLINICAL PRESENTATION OF PATIENTS WITH MUTATIONS IN MODERATE
PENETRANCE GENES: A CASE SERIES

By

AUDREY L. MORRISSETTE

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Jessica Joines

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ABSTRACT OF THE THESIS

Clinical presentation of patients with mutations in moderate penetrance genes: A case series

by AUDREY L. MORRISSETTE

Thesis Director: Jessica Joines

Although there is evidence in the literature of a connection between germline mutations in moderate penetrance genes and breast and/or ovarian cancer susceptibility, there is much to be discovered about genotype-phenotype correlations and cancer spectrums. This case series adds to the descriptive literature regarding clinical presentation and family history in patients with germline mutations in moderate penetrance breast and/or ovarian cancer genes. Eleven patients were included in this case series who were identified to have a pathogenic or likely pathogenic variant, in a moderate penetrance breast and/or ovarian cancer gene. Genes included *ATM* ($n = 4$), *BARD1* ($n = 1$), *CHEK2* ($n = 2$), *PALB2* ($n = 2$), and *RAD51C* ($n = 2$). Results from this case series illustrate areas for future research and add to the existing knowledge regarding phenotype seen in patients who harbor pathogenic variants in moderate penetrance genes.

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Introduction

Breast cancer is the most common cancer diagnosed in women (Tedaldi et al., 2017). In the United States, 1 in 8 women will develop breast cancer, leading to over 1 million newly diagnosed cases annually (Ataollahi, Sharifi, Paknahad, & Paknahad, 2015). Ovarian cancer is diagnosed less frequently, with about 20,000 new cases annually (Torre et al., 2018). The ability to do genetic testing for breast and ovarian cancer susceptibility has life-saving potential, as patients can undergo high-risk surveillance or opt for prophylactic, risk-reducing surgeries. Due to advances in genetic testing, identifying high-risk men and women is more possible today than ever before.

About 5-10% of patients diagnosed with breast cancer will have a hereditary form, caused by a single gene mutation (Carroll et al., 2008). Ovarian cancer has a slightly higher chance of being hereditary, with 15% of patients with serous ovarian cancer expected to have a germline mutation (Neff et al., 2017). The two main genes responsible for hereditary breast and ovarian cancer (HBOC) are *BRCA1* and *BRCA2*, which make up about two-thirds of these cases (Carroll et al., 2008). As these genes were the first to be discovered, they have been studied extensively, and clinical genetic testing for *BRCA1* and *BRCA2* has been available since the mid-1990s (Toland et al., 2018). The remaining one-third of HBOC is attributable to mutations in moderate and lower penetrance genes (Toland et al., 2018).

Unlike high-risk genes, which confer a relative risk for cancers great than five, moderate penetrance genes have a relative risk of 1.5-5 (Economopoulou, Dimitriadis, & Psyrris, 2015). Due to lower risks, the connection between moderate penetrance genes and cancer risk was discovered after high-risk genes, as it required different strategies to

identify these genes in families. The traditional linkage analysis that was used to identify many of the high-risk tumor-suppressor genes could not identify lower penetrance genes, as their risks are not high enough to cause a noteworthy presentation of cancer in a family (Hollestelle, Wasielewski, Martens, & Schutte, 2010). Case-control studies and genome-wide association studies later gave rise to the discovery of moderate-penetrance genes (Njiaju & Olopade, 2012) Despite the identification of additional genes that infer increased risks for cancer, there was initial reluctance to test patients for these genes, as their specific cancer risks and penetrance were not well-established, making clinical utility largely unknown (Tung et al., 2016)

As the price of next-generation sequencing declined, the availability of multi-gene panels increased, changing the landscape of clinical genetic testing, within the past ten years (Tung et al., 2016). Testing shifted from a focus on high-risk genes with well-established evidence for cancer risks and management recommendations, to the inclusion of genes on panels with limited information regarding risk and no subsequent management recommendations, for those who harbor pathogenic variants. It is estimated that approximately 2-5% of patients referred for genetic testing will carry a mutation in a moderate penetrance gene (Tung et al., 2016). Additionally, approximately 40% of patients tested with multi-gene panels will have a variant of uncertain significance (Tung et al., 2015). This shift in testing and number of patients testing positive for moderate penetrance genes, led to the need for a new framework on how to counsel positive patients and a need to better define the cancer spectrum and risks associated with mutations in these less-established genes.

The moderate penetrance breast and/or ovarian cancer genes that have been characterized in the literature include: *ATM*, *BARD1*, *BRIP1*, *CHEK2*, *NBN*, *PALB2*, and the *RAD51* paralogs (Ratajska et al., 2012). Although pathogenic variants in these genes are rare in the general population, founder mutations in some of these genes have been seen at higher frequencies in certain populations (Foulkes, 2008). For example, the first moderate-penetrance breast cancer susceptibility gene, *CHEK2*, was discovered in a family who carried the 1100delC pathogenic variant, later identified to be present in 1% of the Dutch and Finnish populations (Wendt & Margolin, 2019). The higher than expected frequency of some of these variants in moderate penetrance genes suggest that polygenic risk may play a role in who goes on to develop a cancer (Hollestelle et al., 2010).

Although cancer risks have been documented in the literature for patients harboring pathogenic variants in moderate penetrance genes, different variants in the same gene have been shown to infer different cancer risks and the strengths of association between genotype and cancer risk vary greatly (Hollestelle et al., 2010). This scenario illustrates the complexity in providing medical management recommendations at the gene level (Hollestelle et al., 2010). For example, in 2016, researchers at Memorial Sloan Kettering published on a novel germline mutation in the moderately penetrant breast cancer gene *PALB2*, found in a family with both breast and ovarian cancer (Yang et al., 2016). Researchers suggested through the use of segregation analysis, functional studies, and familial testing that *PALB2* may be associated with ovarian cancer predisposition (Yang et al., 2016). While this paper provides preliminary evidence that there may be an association between *PALB2* and ovarian cancer, more data is needed before a medical

management recommendation addressing ovarian cancer risk can be established. Until more is discovered about the clinical presentations, seen in patients with mutations in moderate penetrance breast and ovarian cancer genes, how to medically manage these patients and their families will have to rely heavily upon family history.

Medical management recommendations referenced in clinical cancer settings are devised by organizations such as the National Comprehensive Cancer Network (NCCN). The NCCN guidelines provide recommendations for management and risk reduction, including screening and prophylactic surgeries to decrease cancer risk, for patients who harbor pathogenic variants. The NCCN guidelines are reviewed and revised by experts in the field on a continual basis, with new guidelines produced at least annually. Genetic counselors, oncologists, and other health care professionals refer to these guidelines to appropriately counsel and manage patients based upon their risks. For patients with *BRCA1* and *BRCA2* mutations, these guidelines are well-established to aid in medical management. As defining the cancer spectrum and level of risk attributed to pathogenic variants in moderate penetrance genes remains a work in progress, guidelines are less-established for many of these genes.

Expansions and improvements in the NCCN guidelines, beyond *BRCA1* and *BRCA2*, are still a work in progress. Although the NCCN does list some associated cancers, with evidence-based risk levels, and subsequent medical management recommendations for individuals found to harbor pathogenic variants in moderate penetrance genes, certain cancer risks are listed as “unknown or insufficient evidence”, and therefore no management guidelines have been established (National Comprehensive Cancer Network, 2019). In addition to the challenges in appropriately counseling a

patient about his or her own risks and medical management, the utility of cascade testing of family members, when management recommendations may not exist, is debatable and may differ by gene (Tung et al., 2016).

With the overall lack of sufficient data regarding mutations in moderate penetrance genes, there is much to be discovered about genotype-phenotype correlations and cancer spectrums. Although patients are testing positive for these less-established genes on panels, finding enough positive patients to reach any sort of statistically significant conclusion regarding associated cancer risks takes ample time and collaboration.

The purpose of this case series is to contribute to the development of descriptive literature of patients with mutations in moderate penetrance genes. This case series will do so by describing the clinical presentation, genotype, and family history of patients seen for genetic counseling between December 2014 and April 2018, at the Rutgers Cancer Institute of New Jersey, who tested positive for a pathogenic or likely pathogenic variant in moderate penetrance breast and/or ovarian cancer gene. This case series will also examine the utility of current NCCN guidelines for these patients. Ultimately, the goal of this study is to, along with compilation of similar case series, contribute data on genotype and phenotype that could be incorporated into a meta-analysis that would have the statistical ability to draw larger conclusions about the clinical phenotype in patients with mutations in these genes.

Methods

This case series consisted of a retrospective chart review, utilizing the Familial Cancer Registry of the Rutgers, Cancer Institute of New Jersey (CINJ), housed in the

database Redcap, Patients in the registry were seen at CINJ for cancer genetic counseling between December 2014 and April 2018. Patients were consented, at the time of their genetic counseling appointments, to the registry thereby allowing for future research utilizing their deidentified information. Inclusion criteria for the study consisted of any patient who was found to have a pathogenic or likely pathogenic variant in a moderate penetrance breast or ovarian cancer gene. The genes of interest included: *ATM*, *BARD1*, *BRIP1*, *CHEK2*, *NBN*, *PALB2*, *RAD50*, *RAD51C*, and *RAD51D*. These genes were chosen based upon literature review and the lists of moderate penetrance breast and/or ovarian cancer genes included on multi-gene panels, at the time of the study.

Exclusion criteria consisted of any patient who had a second mutation in a high-risk breast and ovarian cancer gene, including *BRCA1* and *BRCA2*. Through the assistance of a third party honest broker, data from those patients who met inclusion criteria were selected and deidentified. The data available for review included ancestry, limited medical history centered around exposures and cancer screening, reproductive history, oncology history including type of cancer diagnosed, age of onset and treatment, genetic testing results including specific mutation, and family history.

The genotype and phenotype, for all patients in the case series, were compared to existing literature to see whether clinical presentations matched previously reported genotype phenotype correlations. To determine the utility of current NCCN guidelines, for each patient, the patient's genotype and phenotype were also compared to the Genetic/Familial High-Risk Assessment: Breast and Ovarian Version 3.2019 guidelines. For the patients with a personal history of cancer, comparison was focused on a) whether the patient's cancer history matched one of the cancers listed as "increased risk" for the

gene with the variant and b) whether any corresponding medical management guidelines existed for their type of cancer. For the unaffected patients, comparison was focused on whether a) the family history of cancer (first- and second-degree relatives) was consistent with the cancers listed as “increased risk” for the gene with the variant, suggesting an possible explanation for the family history of cancer and b) whether any corresponding medical management guidelines existed for the type of cancer seen in first or second degree relatives.

Following the comparison with the literature and the guidelines, patients were put into one of three categories—yes, no, or “gray zone”—corresponding to whether or not their presentation was consistent with the literature and current guidelines. The “yes” group consisted of any patient whose genotype and personal or family history of cancer was consistent with the literature and guidelines. The “no” group consisted of any patient whose personal or family history of cancer was inconsistent with the literature and guidelines. The “gray zone” group consisted of any patient whose personal or family history of cancer was reported in the literature, in associated with their genotype, but no corresponding management recommendations were listed on the guidelines.

Results

Between December 2014 and April 2018, 690 patients were consented to the Familial Cancer Registry of the Rutgers, Cancer Institute of New Jersey (CINJ). Twelve (1.74%) patients were identified to have a pathogenic or likely pathogenic variant, in a moderate penetrance breast and/or ovarian cancer gene. Moderate penetrance genes included *ATM* ($n = 4$), *BARD1* ($n = 1$), *CHEK2* ($n = 2$), *PALB2* ($n = 2$), and *RAD51C* ($n =$

3) (Table 1). All twelve patients were female (Appendix). Ten patients represented unique family kindreds, while two patients, come from the same family. To avoid bias, only those patients who are original probands will be discussed; therefore, the daughter of Patient #11 will not have a unique case in this series.

Of the 11 probands discussed in this case series, five (45.45%) had a diagnosis of breast cancer (*ATM* $n = 2$, *BARD1* $n = 1$, *CHEK2* $n = 2$). Personal history of ovarian cancer was present in one patient (9.09%) with a *RAD51C* likely pathogenic variant. Three patients (27.27%) had a personal history of a cancer other than breast or ovarian, including uterine and fallopian tube (*ATM*), osteosarcoma (*ATM*), and uterine (*PALB2*). The remaining two patients (18.18%) were unaffected (*RAD51C* $n = 1$ and *PALB2* $n = 1$) (Table 1).

Seven patients (63.64%) reported a family history of a first- or second- degree relative with breast and/or ovarian cancer (Table 2). Four (36.36%) patients reported no history of breast or ovarian cancer in first- or second- degree relatives (Table 2).

Genetic testing results revealed that five (45.45%) patients were found to carry likely pathogenic variants, while six (55.54%) had a known pathogenic variant (Table 3). The type of variants identified in the patients included missense ($n = 7$), deletion ($n = 2$), duplication ($n = 1$), and insertion ($n = 1$) (Table 3). All of the variants were searchable in ClinVar, with two variants having only a single entry listed. These variants included *BARD1* c.632dupT and *PALB2* c.1067_1068insTA (Table 3).

Table 1
Patient Characteristics

Patient	Gene	Sex	Cancer History	Age of onset	2nd Primary
1	ATM	F	Breast cancer, L	35	N/A
2	ATM	F	Uterine and fallopian tube	55	N/A
3	ATM	F	Osteosarcoma	14	N/A
4	ATM	F	DCIS, L	42	N/A
5	BARD1	F	DCIS, R	55	TNBC dx @ 56
6	CHEK2	F	DCIS, L	43	N/A
7	CHEK2	F	Breast cancer, R	52	Breast cancer, L @62
8	PALB2	F	Unaffected	N/A	N/A
9	PALB2	F	Uterine cancer	79	N/A
10	RAD51C	F	Unaffected	N/A	N/A
11	RAD51C	F	Ovarian cancer	57	N/A

Table 2
Family History

Patient	Gene	Ancestry	1st or 2nd degree relative w/ breast ca	Onset <50 years old	1st or 2nd degree relative w/ ovarian ca
1	ATM	Swedish, Irish, Polish			Y
2	ATM	Czech, Polish, Ukrainian			Y
3	ATM	European	Y	Y	
4	ATM	Turkish			
5	BARD1	Irish, Scottish	Y		
6	CHEK2	Greek			
7	CHEK2	Hungarian			
8	PALB2	Irish	Y	Y	Y
9	PALB2	European			
10	RAD51C	Unknown	Y	Y	Y
11	RAD51C	Puerto Rican	Y	Y	

Table 3
Variants

Patient	Gene	Pathogenic (P)/ Likely Pathogenic (LP)	Mutation	Type of mutation	# submissions in ClinVar
1	ATM	LP	c.6572+1G>A	Missense	3
2	ATM	P	c.5497-2A>C	Missense	5
3	ATM	P	c.1564_1565delGA	Deletion	9
4	ATM	P	c.3993+1G>A	Missense	5
5	BARD1	P	c.632dupT	Duplication	1
6	CHEK2	LP	c.470T>C	Missense	24
7	CHEK2	LP	c.470T>C	Missense	24
8	PALB2	LP	c.1067_1068insTA	Insertion	1
9	PALB2	P	c.172_175delTTGT	Deletion	13
10	RAD51C	P	c.709C>T	Missense	6
11	RAD51C	LP	c.837+1G>T	Missense	4

Case Reports

ATM.

Case 1. Personal history: A 39-year-old woman of mixed European ancestry presented for genetic counseling. She had a personal history of estrogen-receptor positive left breast cancer, diagnosed at 35 years old (Table 1). The patient's breast cancer was treated with adjuvant chemotherapy, lumpectomy and radiation. **Cancer screening:** The patient's cancer screenings included annual mammograms and gynecologic pelvic exams, as well as previous colonoscopies. It was reported that several polyps (<10), of unknown type, have been found on colonoscopy. **Environmental exposures:** The patient was a current smoker, at the time of her genetic counseling appointment, smoking less than 1 pack per day (PPD). No alcohol use was documented. **Reproductive history:** Patient was

a G5P2 with her first live birth at age 34. Menarche was documented at age 15 and the patient was premenopausal. She used birth control pills for a total of 10 years and had never taken hormone replacement therapy. Her ovaries and uterus were reportedly in-tact.

Family history: Family history included a mother with ovarian cancer, diagnosed at 50 years old (Appendix). Genetic testing: A multi-gene, Next-generation sequencing panel including *ATM*, *BRCA1*, *BRCA2*, *CDH1*, *CHEK2*, *PTEN*, *STK11*, and *TP53* returned positive for a likely pathogenic variant in the *ATM* gene (c.6572+1G>A) (Table 3).

Case 2: Personal history: A 57- year-old woman of mixed European ancestry presented for genetic counseling. She had a personal history of endometrioid uterine cancer and fallopian tube cancer, both diagnosed at 55 years old (Table 1). The patient's cancers were treated with neoadjuvant chemotherapy, radiation and a total abdominal hysterectomy and bilateral salpingo-oophorectomy (TAH-BSO). Cancer screening: The patient's cancer screenings included mammograms, gynecologic pelvic exams, and CA-125 testing, at unreported intervals. Environmental exposures: The patient was a prior smoker of 15 years with a reported usage of less than 1 PPD. Alcohol use of an unknown quantity was documented. Reproductive history: Patient was a G2P1 with her first live birth at age 22. Menarche was documented at age 13 and menopause at age 55. She used birth control pills for a total of 1 year and had never taken hormone replacement therapy. Family history: Family history included a sister with a brain tumor at 40, mother with ovarian cancer at 73, maternal uncle with throat cancer at 60, maternal aunt with lymphoma at 60, maternal cousin with breast cancer at 54, maternal cousin with breast cancer at 55, and a paternal aunt with brain cancer at 60 (Appendix). Genetic testing: A multi-gene, Next-generation sequencing panel including *ATM*, *BARD1*, *BRCA1*, *BRCA2*, *BRIP1*, *CDH1*, *CHEK2*, *MLH1*, *MRE11A*, *MSH2*, *MSH6*, *MUTYH*, *NBN*, *NF1*, *PALB2*,

PMS2, PTEN, RAD50, RAD51C, RAD51D, SMARCA4, STK11, and TP53 returned positive for a pathogenic variant in the *ATM* gene (c.5497-2A>C) (Table 3).

Case 3: Personal history: A 27-year-old woman of mixed European ancestry presented for genetic counseling. She had a personal history of osteosarcoma, diagnosed at 14 years old (Table 1). The patient's cancer was treated with adjuvant chemotherapy and surgery. **Cancer screening:** The patient's cancer screenings included gynecologic pelvic exams at unreported intervals. **Environmental exposures:** Smoking history and alcohol consumption were negative. **Reproductive history:** The patient had never been pregnant. Menarche was documented at age 11 and the patient is premenopausal. She used birth control pills for a total of 3 years and had never taken hormone replacement therapy. The patient's ovaries and uterus were reportedly in-tact. **Family history:** Family history included a maternal uncle with leukemia at 63, maternal aunt with DCIS at 64, maternal aunt with breast cancer at 40, maternal grandfather with a brain tumor at 50, maternal great uncle with stomach cancer at 50, two maternal great aunts with breast cancer at unknown ages, a paternal grandmother with breast cancer at 75, and a paternal grandfather with skin cancer at an unknown age (Appendix). **Genetic testing:** A multi-gene, Next-generation sequencing panel including *ATM, BRCA1, BRCA2, CDHI, CHEK2, NBN, PALB2, PTEN, and TP53* returned positive for a pathogenic variant in the *ATM* gene (c.1564_1565delGA) (Table 3).

Case 4: Personal history: A 44-year-old woman of Turkish ancestry presented for genetic counseling. She had a personal history of left ductal carcinoma in situ (DCIS) diagnosed at 42 years old (Table 1). Estrogen and progesterone receptors were positive. The patient's cancer was treated with a lumpectomy, radiation, and hormone replacement therapy. **Cancer screening:** The patient's cancer screenings included annual

mammograms and gynecologic pelvic exams. Environmental exposures: Smoking history was negative and alcohol consumption consisted of one drink per week. Reproductive history: The patient was a G2P3 with her first live birth at age 29. Menarche was documented at age 13 and menopause at age 43. She used birth control pills for a total of 5 years. Her ovaries and uterus were reportedly intact. Family history: Family history included a father with prostate cancer at 79, a maternal cousin with lung cancer at 40, and a maternal first cousin once-removed with Hodgkin lymphoma at 25(Appendix). Genetic testing: A multi-gene, Next-generation sequencing panel including *ATM*, *BRCAl*, *BRCA2*, *CDH1*, *CHEK2*, *PALB2*, *PTEN*, *STK11* and *TP53* returned positive for a pathogenic variant in the *ATM* gene (c.3993+1G>A) (Table 3).

BARDI.

Case 5. Personal history: A 56-year-old woman of Irish and Scottish ancestry presented for genetic counseling. She had a personal history of right DCIS diagnosed at 55 years old (Table 1). Estrogen and progesterone receptors were negative. The patient's cancer was treated with a lumpectomy. The patient had a second primary breast cancer diagnosed at 56 years old. This breast cancer was triple negative. This cancer was also treated with lumpectomy. Cancer screening: The patient's cancer screenings included annual mammograms and gynecologic pelvic exams, upper endoscopies every 3-5 years, and colonoscopies at unreported intervals. There was a history of less than 10 unspecified polyps reported. Environmental exposures: A positive smoking history was reported; however, number of years and quantity are unknown. Alcohol consumption was reported as less than one drink per week. Reproductive history: The patient was a G3P2 with her first live birth at age 27. Menarche was documented at age 14 and menopause at age 35.

She used birth control pills for a total of 15 years and had never taken hormone replacement therapy. Her ovaries and uterus were reportedly intact. Family history: Family history included a mother with breast cancer at 52, a maternal grandmother with breast cancer at 64, a father with melanoma at 60 and leukemia at 78, and a paternal aunt with lung cancer at 72 (Appendix). Genetic testing: Single site testing returned positive for a pathogenic variant in the *BARD1* gene (c.632dupT) (Table 3).

CHEK2.

Case 6. Personal history: A 43-year-old woman of Greek ancestry presented for genetic counseling. She had a personal history of left DCIS diagnosed at 43 years old (Table 1). The patient's cancer was treated with a unilateral mastectomy. Cancer screening: The patient's cancer screenings included annual mammograms, gynecologic pelvic exams, and dermatological exams. Colonoscopies were documented on a 3-5-year interval. Environmental exposures: Smoking history and alcohol consumption were negative. Reproductive history: The patient was a G5P3 with her first live birth at age 31. Menarche was documented at age 15 and menopause at age 50. The patient had never used birth control pills or hormone replacement therapy. Her ovaries and uterus were reportedly intact. Family history: Family history included a father with colon cancer at 67 and a maternal aunt with uterine cancer at 45 (Appendix). Genetic testing: A multi-gene, Next-generation sequencing panel including *ATM*, *BRCA1*, *BRCA2*, *CDH1*, *CHEK2*, *PALB2*, *PTEN*, and *TP53* returned positive for a likely pathogenic variant in the *CHEK2* gene (c.470T>C) (Table 3).

Case 7. Personal history: A 70-year-old woman of Hungarian ancestry presented for genetic counseling. She had a personal history of right breast cancer diagnosed at age

52 and left breast cancer diagnosed at age 62 (Table 1). The patient's cancer initial breast cancer was treated with chemotherapy, radiation and a right mastectomy. The patient's second breast cancer was treated with a left mastectomy. Cancer screening: The patient's cancer screenings included annual mammograms, gynecologic pelvic exams, and dermatological exams. Colonoscopies were documented on a 3-5 year interval.

Environmental exposures: The patient reported a prior smoking history for 16 years where she smoked less than 1 PPD. Alcohol consumption included 3-5 weeks per week.

Reproductive history: The patient was a G3P3 with her first live birth at age 23.

Menarche was documented at age 12 and menopause at age 50. The patient used birth control pills for a total of three years and hormone replacement therapy for two years.

The patient had a hysterectomy at age 62 and her ovaries were reportedly intact. Family

history: Family history included a sister with leukemia at 62, a mother with leukemia at 70, a maternal grandfather with jaw cancer at 48, a father with prostate cancer in his late 70s, a paternal aunt with an unknown cancer, and a paternal first cousin with breast cancer at 50 (Appendix). Genetic testing: A multi-gene, Next-generation sequencing panel including *ATM*, *BARD1*, *BRCA1*, *BRCA2*, *BRIP1*, *CDH1*, *CHEK2*, *EPCAM*, *FANCC*, *MLH1*, *MSH2*, *MSH6*, *NBN*, *PALB2*, *PMS2*, *PTEN*, *RAD51C*, *RAD51D*, *STK11*, *TP53*, and *XRCC2* returned positive for a likely pathogenic variant in the *CHEK2* gene (c.470T>C) (Table 3).

PALB2.

Case 8. Personal history: A 51-year-old woman of Greek ancestry presented for genetic counseling. She had no personal history of cancer (Table 1). Cancer screening: The patient's cancer screenings included annual mammograms, gynecologic pelvic

exams, and colonoscopies at unreported intervals. Environmental exposures: A prior smoking history for 4 years and an unspecified amount of alcohol consumption were reported. Reproductive history: The patient was a G1P1 with her first live birth at age 28. Menarche was documented at age 14 and menopause at age 48. The patient had used birth control pills in the past and had never been on hormone replacement therapy. Her ovaries and uterus were reportedly intact. Family history: Family history included a sister with triple negative breast cancer and melanoma at 39, a mother with breast cancer at 66 and ovarian cancer at 77, a maternal grandmother with ovarian cancer at an unknown age, a father with prostate cancer at 85, two paternal uncles with stomach cancer at unknown ages, a paternal cousin with bone cancer at an unknown age, a paternal cousin with liver cancer at an unknown age, and two paternal cousins once-removed with leukemia (Appendix). Genetic testing: A multi-gene, Next-generation sequencing panel including *ATM*, *BARD1*, *BRCA1*, *BRCA2*, *BRIP1*, *CDH1*, *CHEK2*, *EPCAM*, *FANCC*, *MLH1*, *MSH2*, *MSH6*, *NBN*, *PALB2*, *PMS2*, *PTEN*, *RAD51C*, *RAD51D*, *TP53*, and *XRCC2* returned positive for a likely pathogenic variant in the *PALB2* gene (c.1067_1068insTA) (Table 3).

Case 9. Personal history: A 79-year-old woman of European ancestry presented for genetic counseling. She had a personal history of papillary serous uterine cancer diagnosed at age 79 (Table 1). The patient's cancer was treated with adjuvant chemotherapy and a TAH-BSO. Cancer screening: The patient's cancer screenings included annual mammograms, gynecologic pelvic exams, colonoscopies, CA-125 testing, and dermatologic exams at unreported intervals. Environmental exposures: A prior smoking history included 1-2 PPD for 30 years. Alcohol consumption was documented as less than 1 drink per week. Reproductive history: The patient was a G3P3

with her first live birth at age 23. Menarche was documented at age 10 and menopause at age 50. The patient had used birth control pills for less than 1 year. A prior history of hormone replacement therapy was documented for an unspecified amount of time.

Family history: Family history included a son with leukemia at 3, a daughter with basal cell carcinoma at 38, a maternal aunt with stomach cancer at 70, three maternal cousins with breast cancer in their 60s-70s, and a paternal aunt with uterine cancer at 49

(Appendix). Genetic testing: A multi-gene, Next-generation sequencing panel including *ATM*, *BARD1*, *BRCA1*, *BRCA2*, *BRIP1*, *CDH1*, *CHEK2*, *EPCAM*, *MLH1*, *MRE11A*, *MSH2*, *MSH6*, *MUTYH*, *NBN*, *NF1*, *PALB2*, *PIK3CA*, *PMS2*, *PTEN*, *RAD50*, *RAD51C*, *SMARCA4*, *STK11*, and *TP53* returned positive for a pathogenic variant in the *PALB2* gene (c.172_175delTTGT) (Table 3).

RAD51C.

Case 10. A 42-year-old woman of unknown ancestry presented for genetic counseling. She had no personal history of cancer (Table 1). Cancer screening: The patient's cancer screenings included annual mammograms and gynecologic pelvic exams.

Environmental exposures: The patient was a current smoker, smoking less than 1 PPD.

Alcohol consumption was documented as less than 1 drink per week. Reproductive

history: The patient was a G4P2 with her first live birth at age 32. Menarche was

documented at age 10 and the patient was pre-menopausal. The patient had used birth control pills for a total of five years and had never taken hormone replacement therapy.

Her ovaries and uterus were reportedly intact. Family history: Family history included a mother with breast cancer at 45 and a grandmother with ovarian cancer at 50. Her mother was reportedly *BRCA*-negative (Appendix). Genetic testing: A multi-gene, Next-

generation sequencing panel including *APC*, *ATM*, *BARD1*, *BMPRIA*, *BRCA1*, *BRCA2*, *BRIP1*, *CDH1*, *CDK4*, *CDKN2A*, *CHEK2*, *EPCAM*, *MLH1*, *MSH2*, *MSH6*, *MUTYH*, *NBN*, *PALB2*, *PMS2*, *PTEN*, *RAD51C*, *RAD51D*, *SMAD4*, *STK11*, and *TP53* returned positive for a pathogenic variant in the *RAD51C* gene (c.709C>T) (Table 3).

Case 11. A 58-year-old woman of Puerto Rican ancestry presented for genetic counseling. She had a personal history of serous ovarian cancer diagnosed at age 57 (Table 1). The patient's cancer was treated with bilateral salpingo-oophorectomy. Cancer screening: The patient's cancer screenings included biannual mammograms and colonoscopies every 3-5 years. In addition, the patient was being screening via gynecologic pelvic exams, transvaginal ultrasounds, and CA-125 testing at undocumented intervals. Environmental exposures: Smoking history and alcohol consumption were negative. Reproductive history: The patient was a G3P3 with her first live birth at age 18. Menarche was documented at age 11 and surgical menopause at 34, following a TAH-BSO. The patient had never used birth control pills or hormone replacement therapy. Family history: Family history included a maternal half-brother with bilateral breast cancer at 40, who was reportedly *BRCA*-negative, a maternal half-sister with breast cancer at 45, and a maternal half-aunt with stomach cancer at 58. Additionally, this patient had a 38-year-old unaffected daughter who tested positive for *RAD51C* on single site testing, after Patient 11 was found to be positive (Appendix). Genetic testing: A multi-gene, Next-generation sequencing panel including *ATM*, *BARD1*, *BRCA1*, *BRCA2*, *BRIP1*, *CDH1*, *CHEK2*, *EPCAM*, *MLH1*, *MRE11A*, *MSH2*, *MSH6*, *MUTYH*, *NBN*, *NF1*, *PALB2*, *PMS2*, *PTEN*, *RAD50*, *RAD51C*, *RAD51D*, *SMARCA4*, *STK11*, and *TP53* returned positive for a likely pathogenic variant in the *RAD51C* gene (c.837+1G>T) (Table 3).

Categorization of Patients

A review of the literature and NCCN guidelines allowed for categorization of patients into three categories- yes, no, and “gray zone”- depending upon on how patients’ clinical presentation and genotype compared to what has been published. Three patients (27.27%) were categorized in the “yes” category, given that their personal or family history of cancer had been reported in connection to the gene in which they were found to harbor a pathogenic variant and a management recommendation addressing that cancer risk was present (Table 4). This includes patients #1 and #4 who have personal histories of breast cancer and carry an *ATM* variant, and patient #11 with a personal history of ovarian cancer and a *RAD51C* variant (Table 4). Two patients (18.18%) were categorized in the “no” category, as their genotype and phenotype had not been reported in the literature and therefore did not have an applicable management guideline. These patients included patient #3 with a personal history of osteosarcoma and an *ATM* variant and patient #9 with uterine cancer and a *PALB2* variant (Table 4). The remaining six patients (54.54%), (Patients #2, #5-8, and #10) were categorized as the gray zone (Table 4). These patients fell into the gray zone as their personal or family history of cancer had been reported in connection to the gene in which they were found to harbor a pathogenic variant, but no guidelines were present.

Table 4
Presentation Consistent with Literature and NCCN Management Guidelines

Patient	Cancer History	Gene	Yes	No	Gray zone
1	Breast cancer, L	ATM	✓		
2	Uterine and fallopian tube	ATM			✓
3	Osteosarcoma	ATM		✓	
4	DCIS, L	ATM	✓		
5	DCIS & TNBC	BARD1			✓
6	DCIS, L	CHEK2			✓
7	Bilateral breast cancer	CHEK2			✓
8	Unaffected	PALB2			✓
9	Uterine cancer	PALB2		✓	
10	Unaffected	RAD51C			✓
11	Ovarian cancer	RAD51C	✓		

Discussion

This case series described the clinical presentation and family history of patients who underwent genetic testing at the Rutgers, Cancer Institute of New Jersey between December 2014 and April 2018 and were found to carry a pathogenic or likely pathogenic variant in a moderate penetrance breast and/or ovarian cancer gene. Although this study lacked functional data to illustrate whether patients' genotype contributed to their phenotype, the genotypes and phenotypes were compared to the literature to see whether they had been reported previously and whether guidelines for medical management addressed the cancer risks seen in the patient and/or family.

Cancer Spectrum with Moderate Penetrance Genes

ATM. Four patients (36.36%) were found to carry pathogenic or likely pathogenic variants in *ATM*. Personal histories included breast ($n=2$), osteosarcoma, a dual diagnosis of uterine and fallopian tube cancer (Table 1). Two patients reported a family history of ovarian cancer, one patient reported a family history of breast cancer, and one patient reported no family history of breast or ovarian cancer, in first- or second- degree relatives (Table 1).

It has long been established that biallelic mutations in *ATM* cause ataxia telangiectasia, with heterozygous carriers at an increased risk to develop breast and possibly other cancers (Tavtigian et al., 2009). It is also well-known that different types of mutations lead to different phenotypes, as some are more damaging than others. For example, ataxia telangiectasia patients most commonly have truncating mutations and missense mutations are associated with a milder phenotype (Scott et al., 2002). For heterozygous carriers, the same is true for many missense mutations, which are associated with milder risks (Scott et al., 2002). Furthermore, it is also known that *ATM* is a large gene and therefore there are more opportunities for pathogenic variants, as well as benign changes (Wright et al., 1996). The size of the gene may explain why *ATM* was the most frequently mutated gene in this case series.

Three of the four patients found to harbor pathogenic variants in *ATM* had missense mutations, while one had a deletion within the gene (Table 3). The *ATM* c.6572+1G>A likely pathogenic variant identified in Patient #1 was reported in a patient of Russian descent who had a diagnosis of ataxia telangiectasia; however, this patient's family history was not reported in this case report (Birrell et al., 2005). The pathogenic

variant, *ATM* c.5497-2A>C, identified in Patient #2, with a history of uterine and fallopian tube cancer, was reported in 32-year-old woman with a history of invasive breast cancer and a family history of a relative with ataxia telangiectasia (Renault et al., 2018). The pathogenic variant, *ATM* c.1564_1565delGA, identified in Patient #3, was reported in a patient with pancreatic ductal adenocarcinoma, diagnosed at an unknown age (Peters et al., 2017). The last *ATM* variant, c.3993+1G>A, identified in Patient #4 has not been published in the literature.

Though a small sample size, when looking at the personal and family histories of the *ATM*+ patients in this case series, it is noteworthy that two patients (Patients #1 and #2) reported a mother with ovarian cancer and one of those (Patient #2) patients reported a personal history of fallopian tube cancer, thought to be the site of origin of many cases of ovarian cancer (Reade et al., 2014). Upon further review of the literature, there is some evidence that *ATM* may be linked to an increased risk for ovarian cancer. It is for this reason that patient #2, with a personal history of uterine and fallopian tube cancer, was placed into the gray zone.

The *ATM* gene lies within the homologous recombination (HR) pathway, known to play a role in the development of ovarian cancer (Minion et al., 2015). Due to its involvement in the HR pathway and established breast cancer risks, *ATM* has been studied in regard to whether or not germline mutations infer a higher risk for ovarian cancer. In a study looking at hereditary predisposition to ovarian cancer in *BRCA*-negative women, *ATM* was the second most commonly mutated gene in the HR pathway, following *BRIP1*, for patients with ovarian and fallopian tube cancer (Minion et al., 2015). Another study also pointed to a potential link between *ATM* and ovarian cancer, stating that findings could rule out a possible connection, although noting the risk may

not be dramatically elevated (Norquist et al., 2016). A third study, looking at reanalysis of 48 previously *BRCA*-negative ovarian cancer patients, found that 2 had known pathogenic variants in *ATM* and an additional 6 had likely pathogenic variants with *ATM* (Stafford et al., 2017). Despite these proposed connections between ovarian cancer and *ATM* in the literature, there are several other publications denying any association between ovarian cancer and *ATM*. It is likely for this inconsistency in the literature that *ATM* is listed as having “potential increase in ovarian cancer risk” in the NCCN guideline (National Comprehensive Cancer Network, 2019).

BARD1. One patient (#5) was found to harbor a pathogenic variant in *BARD1* (Table 1). This variant, c.632dupT, has not yet been reported on in the literature; therefore, this is the first case report on patient with this variant. The patient was diagnosed with DCIS at 55, triple negative breast cancer at 56, and reported a family history of a mother with breast cancer at 52 and a maternal grandmother with breast cancer at 64 (See Appendix).

BARD1 stands for *BRCA1*-associated ring domain 1 and plays a crucial role in the tumor-suppressing function of *BRCA1* (Irminger-Finger & Jefford, 2006). *BARD1* also is involved in regulation of cell apoptosis and DNA repair (Irminger-Finger & Jefford, 2006). Overtime, research has concluded that germline mutations in *BARD1* play a role in breast cancer susceptibility. In addition to breast cancer risk in general, there is emerging evidence, that *BARD1* may be associated more specifically to triple negative breast cancer (De Brakeleer et al., 2016).

Germline mutations, in genes such as *BRCA1*, are known to be present more often in those with triple negative breast cancer (TNBC), compared to other types of

breast cancer (Couch et al., 2015). It is for that reason that triple negative breast cancer is an indication for genetic counseling (Couch et al., 2015). Although the association between *BRCA1* and TNBC is well-defined, studies have shown that additional genes, such as *PALB2* and *BARD1*, also infer a higher risk for TNBC (Couch et al., 2015). In a study of 8,753 TNBC patients, results indicated that mutations in *BARD1* were associated with a greater than 20% lifetime risk of breast cancer (Shimelis et al., 2018). Given this information, the patient's presentation is consistent with previous literature regarding *BARD1* and triple negative breast cancer risks. Although the connection in the literature between *BARD1* and TNBC is emerging, researchers point to the need for future research to better define risks, in order to establish medical management guidelines (Shimelis et al., 2018). Due to the absent medical management recommendations regarding breast cancer risk in those who harbor pathogenic variants in *BARD1*, patient #5 was categorized as gray zone.

CHEK2. The same likely pathogenic variant, Ile157Thr (c.470T>C), was detected in both patients who were positive for *CHEK2* (Table 2). Upon literature review, this variant, also known as Ile157Thr, is a well-documented founder mutation in the *CHEK2* gene, likely explaining why it detected in two unrelated patients (Leedom et al., 2016). Patient #6 was diagnosed with DCIS at 43 and patient #7 was diagnosed with right-sided breast cancer at 52 and left-sided breast cancer at 62 (Table 1). *CHEK2* is a tumor suppressor gene involved in the DNA repair pathway (Apostolou & Papanotiriou, 2017). Pathogenic variants in *CHEK2* have been associated with an increased risk for breast and colon cancer (Leedom et al., 2016). Consequently, the NCCN guidelines list respective management recommendations, such a timing of mammography and colonoscopy.

Although an increased risk for breast cancer and management recommendations are listed for *CHEK2*, a comment within the NCCN guidelines specific to Ile157Thr variant placed these two patients in the gray zone. The comment on the guidelines states, “The risks for most missense variants are unclear but for some pathogenic/likely pathogenic variants, such as Ile157Thr, the risk for breast cancer appears to be lower. Management should be based on best estimates of cancer risk for the specific pathogenic/likely pathogenic variant (National Comprehensive Cancer Network, 2019)”. This comment makes it unclear just how guidelines should be followed for patients with variants in Ile157Thr. In addition, unlike when guidelines are straightforward, this comment opens up the possibility that two patients with this same variant could be managed very differently, based on who is overseeing their care. The presentation seen in patient #7, who has this variant and a history of bilateral breast cancer, also contradicts that this variant is lower penetrance. For these reasons, patient #6 and #7 were categorized as the gray zone.

According to the literature, the Ile157Thr founder mutation has a high carrier frequency of 5 per 100 people, in Europe (Kaczmarek-Ryś et al., 2015). This variant has been associated with an increased risk for developing multiple cancers, including breast, colon, kidney, prostate and thyroid (Leedom et al., 2016). The risks for breast cancer, in females, is considered to be 1.5x greater than the general population risk (Leedom et al., 2016). This increase in risk is less than that of other *CHEK2* variants, which generally have a 2-fold increase; thus, the risks associated with this specific mutation are considered to be attenuated (Leedom et al., 2016). Other researchers have also claimed that the Ile157Thr variant may lead to an increased risk for lobular breast cancer, or that this variant may only play a role in increasing breast cancer risk if another pathogenic

CHEK2 variant is present (Apostolou & Papatirou, 2017).

Both patients had a personal history of breast cancer, one patient having bilateral breast cancer (Table 1). Despite other documented cancer risks with this variant, neither patient reported a striking family history of these cancers. Patient #6 reported a father with colon cancer diagnosed at 67 and Patient #7 reported a father diagnosed with prostate cancer in his late 70s (See Appendix). The remaining questions, from these results, is how much of a role this variant played in the development of two separate primary breast cancers in Patient #7, as well as whether these variants were paternally inherited and possibly involved in the development of the colon and prostate cancer seen in the family histories. As this variant has a high frequency in the European population, which both of these patients report ancestries consistent with, it points to the theory that other factors must be involved in order for someone with this variant to go on to develop a cancer. *CHEK2* is a gene that has been described to possibly need another genetic change, in order to actually lead to an increased cancer risk (Jalilvand et al., 2017). The history of cancer in these patients may perhaps be explained by other gene-gene interactions, or environmental exposures.

This variant has also been associated with increased risks to develop thyroid cancer, a cancer not addressed by the NCCN guidelines. In a study specifically looking at the presence of the Ile157Thr variant in Polish patients with differentiated thyroid cancer, researchers found that the variant is associated with a 1.81 odds ratio for risk of developing differentiated thyroid cancer. (Kaczmarek-Ryś et al., 2015). In patients who are homozygous for the allele, researchers noted a 12.81 odds ratio for risk of developing papillary thyroid cancer (Kaczmarek-Ryś et al., 2015). These results illustrate that the risk for thyroid cancer, with this variant, is higher than the risk for breast cancer,

highlighting the importance of genetic testing for select thyroid cancer patients. In addition, the specific cancer risks with this variant bring up the question of whether managing at the specific genotype level would be more beneficial for *CHEK2*⁺ patients due to evidence surrounding genotype phenotype correlations. Of note, neither of the *CHEK2*⁺ families in the case series reported a family history of thyroid cancer.

PALB2. Two patients were found to harbor a pathogenic/likely pathogenic variant in *PALB2*. The first was Patient #8 who was 51-years-old and unaffected, with a strong family history of breast and ovarian cancer, including a mother with breast cancer at 66 and papillary serous ovarian cancer at 77, a sister with triple negative breast cancer and melanoma at 39, and a maternal grandmother with ovarian cancer at an unknown age (See Appendix). The likely pathogenic variant identified in patient #8, *PALB2* c.1067_1068insTA, has not been published in the literature; therefore, this is the first case report on a patient with this variant. Patient #9 also tested positive for a pathogenic variant in *PALB2*. She had a personal history of uterine cancer diagnosed at 79 and reported no family history of breast or ovarian cancer, in first- or second- degree relatives (Table 1). The pathogenic variant detected in Patient #9, *PALB2* c.172_175delTTGT, is a Polish Founder mutation, reported in several publications (Lener et al., 2016).

Heterozygous mutations in *PALB2* are associated with an increased risk for breast cancer, while biallelic mutations are associated with Fanconi Anemia (Rahman et al., 2006). NCCN guidelines list an increased risk for breast cancer with those who harbor pathogenic variants in *PALB2* and provide subsequent medical management guidelines. For ovarian cancer risk and management, evidence is listed as “unknown” or “insufficient” (National Comprehensive Cancer Network, 2019). For Patient #8, with the

family history of breast and ovarian cancer, the lack of genetic testing results of the affected family members makes it undeterminable whether these cases were sporadic, linked to a mutation in an established ovarian cancer gene such as the *BRCA* or *RAD51* genes, or if *PALB2* may have played a role in the development of their ovarian cancer, which has been demonstrated in another family (Yang et al., 2016). As this mutation has not been reported previously in the literature, a potential ovarian cancer risk linked to this genotype cannot be excluded. As a result, Patient #8 was categorized as gray zone.

The c.172_175delTTGT variant, found in Patient #9, has been linked to an increased risk for breast cancer in multiple studies, primarily looking at patients in Poland. In one study that genotyped 12,529 Polish women with breast cancer, it was determined that the *PALB2* c.172_175delTTGT variant was present in 76 breast cancer patients and 7 controls, with a calculated 5.02 odds ratio for risk of developing breast cancer (Cybulski et al., 2015). This variant has also been studied in the context of pancreatic cancer and has been noted to infer an increased risk (Lener et al., 2016). To date, this variant has not been linked to uterine cancer, in the literature.

The case series is the first to report a patient with uterine cancer and the *PALB2* c.172_175delTTGT variant. Interestingly, this patient also reported a paternal half aunt with uterine cancer, prior to her death at age 49. Though the patient reports three maternal cousins with breast cancer in their 60's and 70's, there were no closer relatives with breast cancer and no pancreatic cancer reported in the family history (See Appendix). Based on the personal and family history alone, it cannot be determined whether this variant played a role in the development of uterine cancer, or whether it was maternally or paternally inherited and potentially involved in the development of other cancers in the family.

RAD51C. Two patients were positive for pathogenic/likely pathogenic variants in *RAD51C*. The first was Patient #10 who was a 42-year-old unaffected female found to be positive for the pathogenic c.709C > T variant (Table 2). Patient #10 reported a mother with breast cancer at 45, who was reportedly *BRCA* negative, and a maternal grandmother with ovarian cancer in her 50's (See Appendix). Patient #11 was found to harbor the *RAD51C* c.837 +1 G > T likely pathogenic variant (Table 2). This variant has not been published in the literature, previously. Patient #11 reported a maternal half-brother with bilateral breast cancer in his 40s, who was reportedly *BRCA* negative, a maternal half-sister with breast cancer in her late 40s, and a maternal half-aunt with stomach cancer at 58 (See Appendix).

The *RAD51C* gene is part of the DNA repair pathway (Goldmard et al., 2017). Biallelic pathogenic variants are known to lead to Fanconi Anemia whereas single variants infer an increased risk for cancer (Somyajit et al., 2010). *RAD51C* is considered to be a moderate penetrance ovarian cancer susceptibility gene that has also reported to play role in breast cancer susceptibility (Coulet et al., 2013; Song et al., 2015). Emerging evidence of *RAD51C*'s role in breast cancer has led to some researchers to conclude that this gene should be included on panels for women undergoing genetic testing for a personal or family history of breast cancer (Somyajit et al., 2010). The NCCN guidelines document an increased risk for ovarian cancer and "unknown or insufficient evidence for breast cancer risk" (National Comprehensive Cancer Network, 2019).

Upon literature review, the *RAD51C* c.709C > T variant has been previously reported. The first report was in a patient with both ovarian cancer diagnosed at 54 and follicular thyroid cancer diagnosed at 50 (Blanco et al., 2014). Though the mutation was

not confirmed in other relatives, similar to Patient #10, this patient reported a family history of both breast and ovarian cancer (Blanco et al., 2014). Other pathogenic *RAD51C* variants have also been detected in patients with personal and family histories of breast cancer, suggesting further research into if and how much *RAD51C* variants increase the risk to develop breast cancer (Blanco et al., 2014). Despite the connection between *RAD51C* and breast cancer still developing, researchers have supported the idea that the *RAD51* genes be included on panels for patients seeking genetic testing for both breast and ovarian cancer indications (Golmard et al., 2017).

Based on Patient #10's family history of a grandmother with ovarian cancer, it warrants further investigation into whether this variant was maternally inherited, as *RAD51C* is associated with ovarian cancer. Since the patient's mother had breast cancer at 45, and is *BRCA*-negative, this family history also poses the question of whether the variant is maternally inherited and possibly involved in the development of the mother's breast cancer. Although genetic testing and further research would be needed to illustrate whether the variant is tracking with the cancers in the family, the family history suggests that this is a possibility for this family. As there are no corresponding medical management recommendation for breast cancer risk in *RAD51C*+ patients, this patient was categorized as in the gray zone.

In addition to breast and ovarian cancer, the c.709C > T variant was also reported, in the literature, after being detected in a patient with diffuse gastric cancer diagnosed at 73 years old. This particular patient also met the hereditary diffuse gastric cancer criteria (Sahasrabudhe et al., 2017). Results from that study suggested further research into *RAD51C* as a candidate gene for gastric cancer (Sahasrabudhe et al., 2017).

Limitations

This case series focused on a small sample of patients and their clinical presentation and family history. By nature of this type of study, one main limitation was the small sample size. As the data used for analysis was retrieved from a registry, through a third party, complete medical records were not available for review, which limited the analysis. In regard to genotype-phenotype correlations, this case series simply reported what was seen in the patients. Functional data or loss of heterozygosity studies in the tumor were not completed; therefore, causality could not be determined. Additionally, lack of genetic testing results for additional family members is a limitation of this study. Without cascade testing, it cannot be determined which other family members harbor the same pathogenic variant. Furthermore, as family member's pathology was not part of the review, family history information is by patient report only, which is a limitation of the study. With such a small sample size and limited information, it is impossible to draw larger conclusions, however, this case series adds to existing literature regarding moderate penetrance genes and highlights areas where future research is needed.

Genetic Counseling Considerations

This case series points to some important considerations for genetic counselors seeing patients for personal or family histories of cancer. First is in regard to the utility of multi-gene panel testing. The potential downside of multi-gene panel testing is well-described in the literature, with possible challenges including the increased potential to identify variants of uncertain significance or true pathogenic variants with no established guidelines. Despite those challenges, this case series demonstrates the benefits of multi-

gene testing in identifying genetic changes that may not have been detected had testing been directed at phenotype only. For example, Patient #3 with osteosarcoma at 14 may not have discovered she has an increased breast cancer risk from an *ATM* variant, if her testing would have been more narrowly selected, based only on phenotype.

This testing also contributes to a better understanding of the cancer spectrum by identifying those who are positive for pathogenic variants. By offering large gene panels to patients, there is a greater likelihood of findings changes that may be incidental, as well as ones that may not currently have guidelines but are later linked to the patient's phenotype. In either scenario, there is an added benefit to both the patient and the family members, who may be able to be managed differently for risks they would have otherwise been unaware of. Additionally, through cascade testing, it may be possible to determine which cancers are related to the mutation, better defining the cancer risks and spectrums seen with moderate penetrance genes.

Another consideration for counselors when working with patients who are identified to have a pathogenic variant in a moderate penetrance gene is the implications of environmental and gene-gene interactions. As these genes are known to have lower cancer risks, pathogenic variants may not solely explain a personal or family history of cancer; therefore, genetic counselors should be careful not to over interpret positive results. Environmental exposures, reproductive history, and gene-gene interactions are likely to play a larger role in leading to a cancer in those with moderate penetrance genes, as opposed to those with *BRCA* mutations, and should be taken into consideration.

Future Directions

Suggestions for future research include a greater number of cancer institutes to perform similar chart reviews, in order to publish phenotypic and family history data for their patients who test positive for pathogenic variants for moderate penetrance breast and/or ovarian cancer genes. Future case reports and case series should include functional data, pathology, genetic testing results for patients and family members, environmental exposures, reproductive histories, medical history and as much relevant information about the patient and family as possible. More robust case reports and case series would ultimately assist in better establishing important information surrounding gene mutations such as penetrance, cancer risks, recurrence risks, and how medical management should be tailored.

Furthermore, genetic counselors and healthcare providers should encourage patients to enroll in studies like the Prospective Registry Of MultiPlex Testing (PROMPT) to help obtain more data regarding patients with pathogenic variants in moderate penetrance genes. In addition to further research to better define the cancer spectrum, longitudinal studies would be beneficial to look at the impact of the current guidelines on positive patients, specifically in regard to the reduction and early detection of cancer. In addition to research at outside hospital and institutions, this case series also identified families who may be good candidates for additional research and follow-up testing. These families include those in the gray zone category.

Conclusions

This case series was the first to report on two novel mutations: *BARD1* c.632dupT and *PALB2* c.1067_1068insTA. Information from this case series, particularly on these two novel mutations, has the potential to allow for comparison to future patients who may be identified with the same genetic changes. Furthermore, findings from this case series add to the existing descriptive literature on the clinical presentation seen in patients with pathogenic variants in moderate penetrance genes, as well as illustrate areas for future research. By researchers continuing to publish data on moderate penetrance genes, the understanding of these genes will improve overtime. With better understanding, improved management recommendations can eventually be incorporated into clinical practice to better aid the prevention and early detection of cancers in patients who harbor pathogenic variants in moderate penetrance genes.

References

- Apostolou, P., & Papasotiriou, I. (2017). Current perspectives on CHEK2 mutations in breast cancer. *Breast cancer (Dove Medical Press)*, *9*, 331-335. doi:10.2147/BCTT.S111394
- Ataollahi, M. R., Sharifi, J., Paknahad, M. R., & Paknahad, A. (2015). Breast cancer and associated factors: a review. *J Med Life*, *8*(Spec Iss 4), 6-11.
- Birrell, G. W., Kneebone, K., Nefedov, M., Nefedova, E., Jartsev, M. N., Mitsui, M., . . . Lavin, M. F. (2005). ATM mutations, haplotype analysis, and immunological status of Russian patients with ataxia telangiectasia. *25*(6), 593-593. doi:doi:10.1002/humu.9341
- Blanco, A., Gutiérrez-Enríquez, S., Santamariña, M., Montalban, G., Bonache, S., Balmaña, J., . . . Treatment. (2014). RAD51C germline mutations found in Spanish site-specific breast cancer and breast-ovarian cancer families. *147*(1), 133-143. doi:10.1007/s10549-014-3078-4
- Carroll, J. C., Cremin, C., Allanson, J., Blaine, S. M., Dorman, H., Gibbons, C. A., . . . Wilson, B. J. (2008). Hereditary breast and ovarian cancers. *Canadian family physician Medecin de famille canadien*, *54*(12), 1691-1692.
- Couch, F. J., Hart, S. N., Sharma, P., Toland, A. E., Wang, X., Miron, P., . . . Fasching, P. A. (2015). Inherited mutations in 17 breast cancer susceptibility genes among a large triple-negative breast cancer cohort unselected for family history of breast cancer. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*, *33*(4), 304-311. doi:10.1200/JCO.2014.57.1414
- Coulet, F., Fajac, A., Colas, C., Eyries, M., Dion-Minière, A., Rouzier, R., . . . Soubrier, F. (2013). Germline RAD51C mutations in ovarian cancer susceptibility. *83*(4), 332-336. doi:10.1111/j.1399-0004.2012.01917.x
- Cybulski, C., Kluźniak, W., Huzarski, T., Wokołorczyk, D., Kashyap, A., Jakubowska, A., . . . Lubiński, J. (2015). Clinical outcomes in women with breast cancer and a PALB2 mutation: a prospective cohort analysis. *The Lancet Oncology*, *16*(6), 638-644. doi:[https://doi.org/10.1016/S1470-2045\(15\)70142-7](https://doi.org/10.1016/S1470-2045(15)70142-7)
- De Brakeleer, S., De Grève, J., Desmedt, C., Joris, S., Sotiriou, C., Piccart, M., . . . Teugels, E. (2016). Frequent incidence of BARD1-truncating mutations in germline DNA from triple-negative breast cancer patients. *89*(3), 336-340. doi:10.1111/cge.12620
- Economopoulou, P., Dimitriadis, G., & Psyrris, A. (2015). Beyond BRCA: New hereditary breast cancer susceptibility genes. *Cancer Treatment Reviews*, *41*(1), 1-8. doi:<https://doi.org/10.1016/j.ctrv.2014.10.008>

- Foulkes, W. D. (2008). Inherited Susceptibility to Common Cancers. *359*(20), 2143-2153. doi:10.1056/NEJMra0802968
- Golmard, L., Castéra, L., Krieger, S., Moncoutier, V., Abidallah, K., Tenreiro, H., . . . Houdayer, C. (2017). Contribution of germline deleterious variants in the RAD51 paralogs to breast and ovarian cancers. *European Journal of Human Genetics*, *25*(12), 1345-1353. doi:10.1038/s41431-017-0021-2
- Hollestelle, A., Wasielewski, M., Martens, J. W. M., & Schutte, M. (2010). Discovering moderate-risk breast cancer susceptibility genes. *Current Opinion in Genetics & Development*, *20*(3), 268-276. doi:<https://doi.org/10.1016/j.gde.2010.02.009>
- Irminger-Finger, I., & Jefford, C. E. (2006). Is there more to BARD1 than BRCA1? *Nature Reviews Cancer*, *6*, 382. doi:10.1038/nrc1878
- Jalilvand, M., Oloomi, M., Najafipour, R., Alizadeh, S. A., Saki, N., Rad, F. S., & Shekari, M. (2017). An association study between CHEK2 gene mutations and susceptibility to breast cancer. *Comparative clinical pathology*, *26*(4), 837–845. doi:10.1007/s00580-017-2455-x
- Kaczmarek-Ryś, M., Ziemnicka, K., Hryhorowicz, S. T., Górczak, K., Hoppe-Gołębiewska, J., Skrzypczak-Zielińska, M., . . . Pławski, A. (2015). The c.470 T > C CHEK2 missense variant increases the risk of differentiated thyroid carcinoma in the Great Poland population. *Hereditary cancer in clinical practice*, *13*(1), 8-8. doi:10.1186/s13053-015-0030-5
- Leedom, T. P., LaDuca, H., McFarland, R., Li, S., Dolinsky, J. S., & Chao, E. C. (2016). Breast cancer risk is similar for CHEK2 founder and non-founder mutation carriers. *Cancer Genetics*, *209*(9), 403-407. doi:10.1016/j.cancergen.2016.08.005
- Lener, M. R., Scott, R. J., Kluźniak, W., Baszuk, P., Cybulski, C., Wiechowska-Kozłowska, A., . . . Lubiński, J. (2016). Do founder mutations characteristic of some cancer sites also predispose to pancreatic cancer? , *139*(3), 601-606. doi:doi:10.1002/ijc.30116
- Minion, L. E., Dolinsky, J. S., Chase, D. M., Dunlop, C. L., Chao, E. C., & Monk, B. J. (2015). Hereditary predisposition to ovarian cancer, looking beyond BRCA1/BRCA2. *Gynecologic Oncology*, *137*(1), 86-92. doi:10.1016/j.ygyno.2015.01.537
- Njiaju, U. O., & Olopade, O. I. (2012). Genetic Determinants of Breast Cancer Risk: A Review of Current Literature and Issues Pertaining to Clinical Application. *18*(5), 436-442. doi:10.1111/j.1524-4741.2012.01274.x
- National Comprehensive Cancer Network. (2019). Genetic/Familial High-Risk Assessment: Breast and Ovarian Version 3.2019

- Neff, R. T., Senter, L., & Salani, R. (2017). *BRCA* mutation in ovarian cancer: testing, implications and treatment considerations. *Therapeutic advances in medical oncology*, 9(8), 519-531.
- Norquist, B. M., Harrell, M. I., Brady, M. F., Walsh, T., Lee, M. K., Gulsuner, S., . . . Birrer, M. J. (2016). Inherited Mutations in Women With Ovarian Carcinoma. *JAMA oncology*, 2(4), 482-490. doi:10.1001/jamaoncol.2015.5495
- Peters, M. L. B., Brand, R., Borazanci, E. H., Stobie, L., Dudley, B., Karloski, E., . . . Tung, N. M. (2017). Germline genetic testing in unselected pancreatic ductal adenocarcinoma (PDAC) patients. 35(15_suppl), 1501-1501. doi:10.1200/JCO.2017.35.15_suppl.1501
- Rahman, N., Seal, S., Thompson, D., Kelly, P., Renwick, A., Elliott, A., . . . Stratton, M. R. (2006). PALB2, which encodes a BRCA2-interacting protein, is a breast cancer susceptibility gene. *Nature Genetics*, 39, 165. doi:10.1038/ng1959 <https://www.nature.com/articles/ng1959#supplementary-information>
- Ratajska, M., Antoszewska, E., Piskorz, A., Brozek, I., Borg, Å., Kusmierek, H., . . . Treatment. (2012). Cancer predisposing BARD1 mutations in breast-ovarian cancer families. *131*(1), 89-97. doi:10.1007/s10549-011-1403-8
- Reade, C. J., McVey, R. M., Tone, A. A., Finlayson, S. J., McAlpine, J. N., Fung-Kee-Fung, M., & Ferguson, S. E. (2014). The Fallopian Tube as the Origin of High Grade Serous Ovarian Cancer: Review of a Paradigm Shift. *Journal of Obstetrics and Gynaecology Canada*, 36(2), 133-140. doi:10.1016/S1701-2163(15)30659-9
- Renault, A.-L., Mebirouk, N., Fuhrmann, L., Bataillon, G., Cavaciuti, E., Le Gal, D., . . . kConFab. (2018). Morphology and genomic hallmarks of breast tumours developed by ATM deleterious variant carriers. *Breast Cancer Research*, 20(1), 28. doi:10.1186/s13058-018-0951-9
- Sahasrabudhe, R., Lott, P., Bohorquez, M., Toal, T., Estrada, A. P., Suarez, J. J., . . . Carvajal-Carmona, L. G. (2017). Germline Mutations in PALB2, BRCA1, and RAD51C, Which Regulate DNA Recombination Repair, in Patients With Gastric Cancer. *Gastroenterology*, 152(5), 983-986.e986. doi:10.1053/j.gastro.2016.12.010
- Scott, S. P., Bendix, R., Chen, P., Clark, R., Dork, T., & Lavin, M. F. (2002). Missense mutations but not allelic variants alter the function of ATM by dominant interference in patients with breast cancer. *Proceedings of the National Academy of Sciences of the United States of America*, 99(2), 925-930. doi:10.1073/pnas.012329699
- Shimelis, H., LaDuca, H., Hu, C., Hart, S. N., Na, J., Thomas, A., . . . Couch, F. J. (2018). Triple-Negative Breast Cancer Risk Genes Identified by Multigene Hereditary Cancer Panel Testing. *Journal of the National Cancer Institute*, 110(8), 855-862. doi:10.1093/jnci/djy106

- Somyajit, K., Subramanya, S., & Nagaraju, G. (2010). RAD51C: a novel cancer susceptibility gene is linked to Fanconi anemia and breast cancer. *Carcinogenesis*, *31*(12), 2031-8.
- Song, H., Dicks, E., Ramus, S. J., Tyrer, J. P., Intermaggio, M. P., Hayward, J., . . . Pharoah, P. D. P. (2015). Contribution of Germline Mutations in the RAD51B, RAD51C, and RAD51D Genes to Ovarian Cancer in the Population. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*, *33*(26), 2901-2907. doi:10.1200/JCO.2015.61.2408
- Stafford, J. L., Dyson, G., Levin, N. K., Chaudhry, S., Rosati, R., Kalpage, H., . . . Tainsky, M. A. (2017). Reanalysis of BRCA1/2 negative high risk ovarian cancer patients reveals novel germline risk loci and insights into missing heritability. *PLoS one*, *12*(6), e0178450-e0178450. doi:10.1371/journal.pone.0178450
- Tavtigian, S. V., Oefner, P. J., Babikyan, D., Hartmann, A., Healey, S., Le Calvez-Kelm, F., . . . Chenevix-Trench, G. (2009). Rare, evolutionarily unlikely missense substitutions in ATM confer increased risk of breast cancer. *Am J Hum Genet*, *85*(4), 427-446. doi:10.1016/j.ajhg.2009.08.018
- Tedaldi, G., Tebaldi, M., Zampiga, V., Danesi, R., Arcangeli, V., Ravegnani, M., . . . Calistri, D. (2017). Multiple-gene panel analysis in a case series of 255 women with hereditary breast and ovarian cancer. *Oncotarget*, *8*(29), 47064-47075. doi:10.18632/oncotarget.16791
- Toland, A. E., Forman, A., Couch, F. J., Culver, J. O., Eccles, D. M., Foulkes, W. D., . . . on behalf of the, B. I. C. S. C. (2018). Clinical testing of BRCA1 and BRCA2: a worldwide snapshot of technological practices. *npj Genomic Medicine*, *3*(1), 7. doi:10.1038/s41525-018-0046-7
- Torre, L. A., Trabert, B., DeSantis, C. E., Miller, K. D., Samimi, G., Runowicz, C. D., . . . Siegel, R. L. (2018). Ovarian cancer statistics, 2018. *68*(4), 284-296. doi:doi:10.3322/caac.21456
- Tung, N., Battelli, C., Allen, B., Kaldate, R., Bhatnagar, S., Bowles, K., . . . Hartman, A.-R. (2015). Frequency of mutations in individuals with breast cancer referred for BRCA1 and BRCA2 testing using next-generation sequencing with a 25-gene panel. *121*(1), 25-33. doi:10.1002/cncr.29010
- Tung, N., Domchek, S. M., Stadler, Z., Nathanson, K. L., Couch, F., Garber, J. E., . . . Robson, M. E. (2016). Counselling framework for moderate-penetrance cancer-susceptibility mutations. *Nature reviews. Clinical oncology*, *13*(9), 581-588. doi:10.1038/nrclinonc.2016.90
- Wendt, C., & Margolin, S. (2019). Identifying breast cancer susceptibility genes – a review of the genetic background in familial breast cancer. *Acta Oncologica*, *58*(2), 135-146. doi:10.1080/0284186X.2018.1529428

- Wright, J., Teraoka, S., Onengut, S., Tolun, A., Gatti, R. A., Ochs, H. D., & Concannon, P. (1996). A high frequency of distinct ATM gene mutations in ataxia-telangiectasia. *American journal of human genetics*, *59*(4), 839–846.
- Yang, C., Arnold, A. G., Trottier, M., Sonoda, Y., Abu-Rustum, N. R., Zivanovic, O., . . . Zhang, L. (2016). Characterization of a novel germline PALB2 duplication in a hereditary breast and ovarian cancer family. *Breast cancer research and treatment*, *160*(3), 447-456. doi:10.1007/s10549-016-4021-7

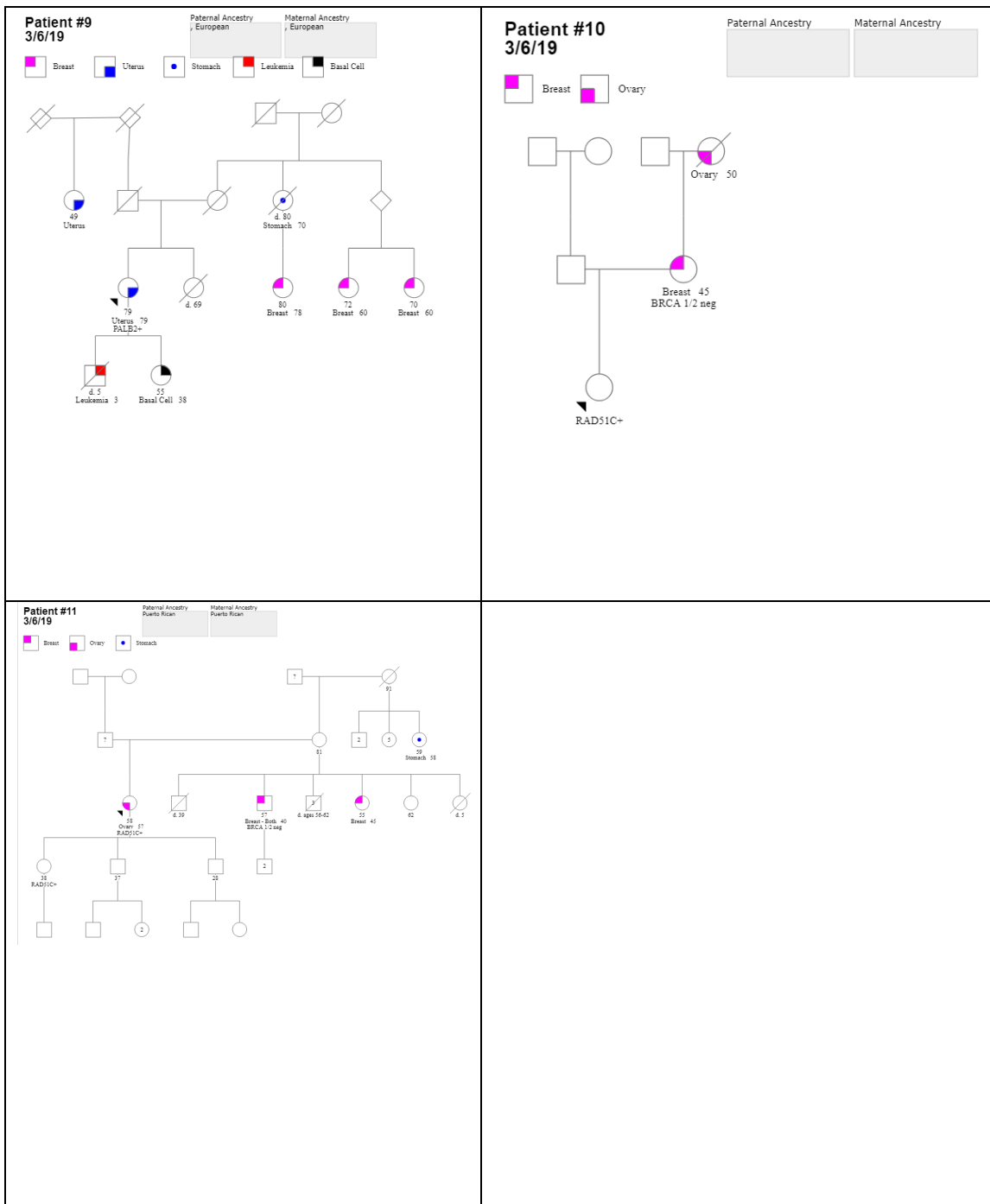


Figure 1A: Patient Pedigrees