Austin Journal of Cancer and Clinical Research

Open Access

Austin Publishing Group

Research Article

Mutational Spectrum Across 94 Cancer Predisposition Genes in Patients with TNBC

Lovejoy LA[1], Shriver CD[2] and Ellsworth RE[2]*

[1]Chan Soon-Shiong Institute of Molecular Medicine at Windber, USA

[2]Clinical Breast Care Project, Murtha Cancer Center, Walter Reed National Military Medical Center, USA

***Corresponding author:** Rachel E. Ellsworth, Clinical Breast Care Program, Murtha Cancer Center, Walter Reed National Military Medical Center, Windber, USA

Received: March 07, 2018; **Accepted:** April 11, 2018; **Published:** June 22, 2018

Abstract

Germline mutations in BRCA1 and BRCA2 have been detected in 8-14% and 5% of women with triple negative breast cancer (TNBC). The contribution of germline mutations in other cancer predisposition genes is, however, not well-studied, thus we employed panel testing of 94 known or predicted cancer predisposition genes in a cohort of 230 female patients with TNBC. Pathogenic mutations were classified as pathogenic, uncertain significance (VUS) or benign using ClinVar. Most patients were of European (66%) or African (31%) American ancestry and 26% had a family history. Forty women (17.4%) had pathogenic mutations in APC (n=1), BRCA1 (n=24), BRCA2 (n=4), BRIP1 (n=1), CHEK2 (n=1), MSH2 (n=1), MUTYH (n=3), PALB2 (n=3), RAD51C (n=1) and SDHB (n=1). An additional 33 women had VUS in 18 genes, including the high-risk breast cancer genes BRCA1, BRCA2, PALB2 and TP53.Although the majority of pathogenic mutations were in the BRCA1 and BRCA2 genes, panel testing allowed for the detection of mutations in an additional 5.2% of women in known breast, colon, ovarian and other cancer predisposition genes. Panel testing thus identifies genes other than BRCA1/2 associated with increased risk of TNBC and may incidentally identify women who would benefit from enhanced surveillance for other cancers.

Keywords: Triple negative breast cancer; Genetic predisposition; Germline; Panel testing

Introduction

Triple negative breast cancer (TNBC) is a clinical term used to describe breast tumors that do not express the estrogen (ER) or progesterone receptors (PR) or HER2. TNBC accounts for 15-20% of breast cancers diagnosed each year [1], is characterized by high-tumor grade, larger size, more frequent lymph node metastases, and distant metastasis, especially to lung and brain, and deaths are significantly higher in patients with TNBC during the first 5 years after diagnosis [2]. TNBC represents the predominant tumor type (71%) in patients with BRCA1 mutations compared to those in BRCA2 mutation carriers (25%) [3]. The frequency of BRCA1 or BRCA2 mutations in women with TNBC ranges from 9-100% and 2-12% in BRCA1 and BRCA2 depending on the selection criteria used [3] and being diagnosed with TNBC ≤60 years of age meets the National Comprehensive Cancer Network (NCCN) criteria for genetic testing for BRCA1 and BRCA2 mutations. The contribution of other cancer predisposition genes to TNBC is not well-understood, thus we employed panel testing to identify germline mutations in women with TNBC.

Subjects and Methods

All subjects voluntarily agreed to participate in the Clinical Breast Care Project (CBCP) and gave written informed consent. Blood samples were collected with approval from the Walter Reed National Military Medical Center (WRNMMC) Human Use Committee and Institutional Review Board. Patients for this study enrolled between 2001 and 2014 from WRNMMC, Bethesda, MD, Anne Arundel Medical Center, Annapolis, MD or the Joyce Murtha Breast Care Center, Windber, PA.

Tumors with <1% ER or PR positive staining cells were considered hormone receptor negative and tumors with HER2 IHC values of 0+, 1+ or 2+ with no amplification were defined as negative. Family history was determined using the NCCN Guidelines version 1.2017 for Familial Risk Assessment. Genomic DNA was isolated and sequenced across 94 cancer predisposition genes using next-generation sequencing technologies as previously described [4]. Data were filtered to include only misses or frame shift mutations, stop gains or losses, initiator codons, in-frame insertions or deletions and splice site alterations with a read depth of >10 and a minor allele frequency of >0.25. The predicted effect of all variants was evaluated using the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/) and Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php) and classified using American College of Medical Genetics and Genomics standards as pathogenic, likely pathogenic, uncertain significance (VUS), likely benign or benign [5].

Results

TNBC were diagnosed in 16.9% (305/1,801) of women with ER, PR and HER2 status available in the database. Genomic DNA was available for 230 of the women with TNBC. The majority of patients were of European (66%) or African (31%) American ancestry and 33% had a family history. Average age at diagnosis was 53.5 years (range 23-83 years) and 12% died of disease with an average time from diagnosis to death from disease of 2.81 years.

Forty women harbored pathogenic mutations, with the majority

Citation: Lovejoy LA, Shriver CD and Ellsworth RE. Mutational Spectrum Across 94 Cancer Predisposition Genes in Patients with TNBC. Austin J Cancer Clin Res. 2018; 5(1): 1082.

Table 1: Pathogenic or likely pathogenic mutations in known breast cancer genes identified in 40/230 women diagnosed with TNBC.

Patient	Gene	Mutation	Ethnicity	Family history
3[a]	APC	NM_000038.5(APC):c.3920T>A (p.Ile1307Lys)	European American	
17	BRCA1	NM_007294.3(BRCA1):c.250G>T (p.Glu84Ter)	African American	
19	BRCA1	NM_007294.3(BRCA1):c.68_69delAG (p.Glu23Valfs)	European American	√
31	BRCA1	NM_007294.3(BRCA1):c.3937C>T (p.Gln1313Ter)	European American	√
33	BRCA1	NM_007294.3(BRCA1):c.815_824dupAGCCATGTGG (p.Thr276Alafs)	African American	√
37	BRCA1	NM_007294.3(BRCA1):c.4603G>T (p.Glu1535Ter)	African American	√
40	BRCA1	NM_007294.3(BRCA1):c.962G>A (p.Trp321Ter)	European American	
59	BRIP1	NM_032043.2(BRIP1):c.2392C>T (p.Arg798Ter)	European American	√
106	BRCA1	NM_007294.3(BRCA1):c.191G>A (p.Cys64Tyr)	African American	√
123	CHEK2	NM_007194.3(CHEK2):c.349A>G (p.Arg117Gly)	African American	
138	BRCA2	NM_000059.3(BRCA2):c.4876_4877delAA (p.Asn1626Serfs)	European American	√
236	BRCA1	NM_007294.3(BRCA1):c.181T>G (p.Cys61Gly)	European American	
360	MUTYH	NM_001128425.1(MUTYH):c.536A>G (p.Tyr179Cys)	European American	
426	BRCA2	NM_000059.3(BRCA2):c.6944_6947delTAAA (p.Ile2315Lysfs)	European American	√
499[a]	RAD51C	NM_058216.2(RAD51C):c.790G>A (p.Gly264Ser)	African American	
506	BRCA1	NM_007294.3(BRCA1):c.5319dupC (p.Asn1774Glnfs)	European American	
537	BRCA1	NM_007294.3(BRCA1):c.4524G>A (p.Trp1508Ter)	European American	√
644	BRCA1	NM_007294.3(BRCA1):c.5137delG (p.Val1713Terfs)	European American	√
656	BRCA1/CHEK2	BRCA1, 6-KB DUP, EX13 NM_007194.3(CHEK2):c.470T>C (p.Ile157Thr)	European American	√
685	BRCA1	NM_007294.3(BRCA1):c.2679_2682delGAAA (p.Lys893Asnfs)	European American	√
746[a]	PALB2	NM_024675.3(PALB2):c.298C>T (p.Leu100Phe)	European American	
768	BRCA1	NM_007294.3(BRCA1):c.5467+1G>A	African American	√
785	PALB2	NM_024675.3(PALB2):c.3114G>A (p.Trp1038Ter)	African American	√
819	BRCA1	NM_007294.3(BRCA1):c.2722G>T (p.Glu908Ter)	European American	√
822	MUTYH	NM_001128425.1(MUTYH):c.1187G>A (p.Gly396Asp)	African American	√
849	BRCA1	NM_007294.3(BRCA1):c.5266dupC (p.Gln1756Profs)	European American	√
856[a]	MUTYH	NM_001128425.1(MUTYH):c.821G>A (p.Arg274Gln)	European American	
894[b]	BRCA2	NM_000059.3(BRCA2):c.3599_3600delGT (p.Cys1200Terfs)	European American	
895[b]	BRCA2	NM_000059.3(BRCA2):c.2842dupG (p.Val948Glyfs)	European American	
896[c]	SDHB	NM_003000.2(SDHB):c.268C>T (p.Arg90Ter)	African American	
901[a]	MSH2	NM_000251.2(MSH2):c.815C>T (p.Ala272Val)	European American	
920	BRCA1	NM_007294.3(BRCA1):c.5193+2delT	European American	√
1040	BRCA1	NM_007294.3(BRCA1):c.5266dupC (p.Gln1756Profs)	European American	√
1041	BRCA1	NM_007294.3(BRCA1):c.4964_4982del19 (p.Ser1655Tyrfs)	European American	√
1045	BRCA1	NM_007294.3(BRCA1):c.4986+6T>G	European American	√
1049	BRCA1	NM_007294.3(BRCA1):c.5062_5064delGTT (p.Val1688del)	African American	√
1052	BRCA1	NM_007294.3(BRCA1):c.4986+3G>C	European American	√
1061	BRCA1	NM_007294.3(BRCA1):c.68_69delAG (p.Glu23Valfs)	Ashkenazi	√
1062	PALB2	NM_024675.3(PALB2):c.3549C>A (p.Tyr1183Ter)	European American	√
1063	BRCA1	NM_007294.3(BRCA1):c.135-1G>T	European American	√

[a]Conflicting interpretations of pathogenicity or uncertain significance in ClinVar, associated with ovarian, breast, colon cancer in HMGD.
[b]Patients 894 and 985 were diagnosed with TNBC at ages 63 and 75 years, respectively.
[c]Patient 896 also harbors a VUS in the PMS2 gene, NM_000535.6(PMS2):c.2186_2187delTC (p.Leu729Glnfs).

in BRCA1 (10.4%) or BRCA2 gene (1.7%). Two women with BRCA2 mutations were diagnosed with TNBC >60 years of age. Pathogenic mutations in seven other genes were found in 5.2% of the cohort (Table 1). An additional 33 women had VUS in 18 genes (Table 2).

Table 2: VUS identified in 33/202 women diagnosed with TNBC.

Patient	Gene	Mutation	Ethnicity	Family history	Family cancer types
32	BRIP1	NM_032043.2(BRIP1):c.550G>T (p.Asp184Tyr)	White		
72	CHEK2	NM_007194.3(CHEK2):c.246_260del CCAAGAACCTGAGGA (p.Asp82_Glu86del)	African American		
89	PMS2	NM_000535.6(PMS2):c.2186_2187delTC(p.Leu729Glnfs)	African American		
91	TP53	NM_000546.5(TP53):c.88_90delAAC (p.Asn30del)	European American	Yes	Mother breast (postmenopausal); maternal cousin breast (premenopausal); brother esophagus
132	APC	NM_000038.5(APC):c.7471A>G (p.Met2491Val)	African American		Mother lung, kidney
150	CDK4	NM_000075.3(CDK4):c.776C>T (p.Ser259Leu)	European American		Mother colon
293	PALB2	NM_024675.3(PALB2):c.1970_1976delAGGAACT (p.Glu657GlyfsTer13)[a]	European American		Father prostate, paternal grandfather colon
309	MSH2 MSH6	NM_000251.2(MSH2):c.820A>G (p.Ile274Val) NM_000179.2(MSH6):c.1474A>G (p.Met492Val)	European American	Yes	Sister ovarian, paternal grandfather stomach
324	MSH6	NM_000179.2(MSH6):c.831A>C (p.Glu277Asp)	African American	Yes	Paternal aunt and cousin breast (postmenopausal), sister colon
385	ATM	NM_000051.3(ATM):c.8921C>T (p.Pro2974Leu)	European American	Yes	Sister breast (premenopausal); Father lung
389	CDKN2A	NM_000077.4(CDKN2A):c.415G>A (p.Gly139Ser)	European American	Yes	Maternal grandmother breast (premenopausal)
442	CHEK2	NM_007194.3(CHEK2):c.1420C>T (p.Arg474Cys)	European American	Yes	Father breast; Sister stomach
462	BRCA2	NM_000059.3(BRCA2):c.8368A>T (p.Thr2790Ser) NM_000059.3(BRCA2):c.8378G>A(p.Gly2793Glu)	European American	Yes	Sister ovarian
525	APC	NM_000038.5(APC):c.2218G>C (p.Ala740Pro) NM_000038.5(APC):c.6779G>A (p.Ser2260Asn)	European American		Mother and maternal aunt breast (postmenopausal); maternal grandmother stomach
559	BRCA2 CHEK2	NM_000059.3(BRCA2):c.7769C>G (p.Ser2590Cys) NM_007194.3(CHEK2):c.1130A>G (p.Glu377Gly)	African American	Yes	2 maternal aunts breast (premenopausal)
607	PMS2	NM_000535.6(PMS2):c.572A>G (p.Tyr191Cys)	African American		Mother uterine
625	PMS2	NM_000535.6(PMS2):c.2186_2187delTC(p.Leu729Glnfs)	European American		Mother stomach
626	ATM	NM_000051.3(ATM):c.1073A>G (p.Asn358Ser)	African American		
662	ATM	NM_000051.3(ATM):c.6543G>T (p.Glu2181Asp)	African American		Sister colon
678	BRCA2	NM_000059.3(BRCA2):c.9875C>T (p.Pro3292Leu)	European American		
733	CDH1	NM_004360.4(CDH1):c.1697T>C (p.Ile566Thr)	European American		
735	PALB2	NM_024675.3(PALB2):c.1564C>T (p.Pro522Ser)	African American		
738	BRCA1	NM_007294.3(BRCA1):c.3560G>A (p.Ser1187Asn)	European American		2 paternal aunts breast (postmenopausal)
744	PALB2	NM_024675.3(PALB2):c.2507_2509delTCG(p.Val836del)	African American		Paternal grandmother pancreatic
787	BRCA1	NM_007294.3(BRCA1):c.1581G>C (p.Lys527Asn)	European American	Yes	Sister breast (postmenopausal); Mother pancreatic; brother kidney and bladder
897	ATM RET	NM_000051.3(ATM):c.4505G>T (p.Cys1502Phe) NM_020975.4(RET):c.1946C>T (p.Ser649Leu)	European American		
898	BRCA2	NM_000059.3(BRCA2):c.4068G>T (p.Leu1356Phe)	European American		
899	CDH1	NM_004360.4(CDH1):c.2336G>A (p.Arg779Gln)	European American		Brother and 2 sisters colon
900	CHEK2	NM_007194.3(CHEK2):c.1451C>T (p.Pro484Leu)	European American		Father prostate
902	MSH6	NM_000179.2(MSH6):c.335A>G (p.Asn112Ser)	African American		Sister and paternal aunt breast (postmenopausal)
903	NBN	NM_002485.4(NBN):c.1405G>T (p.Asp469Tyr)	African American		Mother breast (postmenopausal)
905	RAD51C	NM_058216.2(RAD51C):c.890T>C (p.Leu297Pro)	African American		
906	RAD51D	NM_002878.3(RAD51D):c.433C>T (p.Arg145Cys)	European American		Sister colon

[a]Variant not reported in ClinVar

Women with pathogenic mutations (65%) were significantly more likely (P<0.001) to have a family history compared to those without (27.0%) and a younger age at diagnosis (P=0.007, 49 and 54 years, in carriers and non-carriers, respectively); ethnicity (P=0.775) and survival (P=0.181) did not differ significantly between carriers and non-carriers. Age at diagnosis was significantly lower (P=0.0013) in women with BRCA1 mutations (average 43 years) compared to those with BRCA2 mutations (62.0 years).

Discussion

The contribution of germline mutations in cancer predisposition genes to TNBC in this study is similar to that in a recent study that evaluated 17 DNA breast cancer susceptibility genes in 1,824 women with TNBC unselected for family history [6], in which 11.2% of women harbored mutations in BRCA1 (8.5%) and BRCA2 (2.7%). Mutations in breast cancer genes, including PALB2 (1.2%), BRIP1 (0.4%) and RAD51C (0.3%), accounted for another 3.7% of women. Similar mutation frequencies for PALB2 (1.3%), BRIP1 (0.45%) and RAD51C (0.4%) were detected in our study.

In contrast to the study by Couch, et al. who detected no germline mutations in CHEK2, we identified two women who carried CHEK2 mutations classified as pathogenic or likely pathogenic. Patient 656 harbored both BRCA1 6-KB DUP, EX13 and CHEK2 I157T mutations; as CHEK2 I157T has been classified as a low-risk allele, it is likely that the TNBC in this patient was associated primarily with the BRCA1 mutation. Patient 123, with a CHEK2 R117G mutation, had ER+/HER2- tumor diagnosed in her right breast in 1999 and a TNBC in her left breast in 2004, thus the effect of the CHEK2 R117G mutation in this woman is not clear.

Five women in our study had mutations in non-breast cancer predisposition genes, including four women with mutations in colon cancer genes. Three women had monoallelic (heterozygous) mutations in the MUTYH that have been associated with an increased risk of breast cancer (HR, 1.4; 95% CI, 1.0-2.0) [7] and one had a mutation in MSH2 which was been linked to increased risk for TNBC through genome-wide association studies [8]. In addition to increased risk of gastrointestinal stromal, and renal cell carcinomas, germline mutations in SDHB have been associated with increased risk of breast cancer in patients with features of Cowden Syndrome [9] and mitochondrial oxidative phosphorylation is the primary metabolic pathway in TNBC [10].

In conclusion, the data demonstrate that 12% of women unselected for age or family history have germline mutations in BRCA1 and BRCA2 including two women with BRCA2 mutations who were not eligible for genetic testing using the NCCN 1.2017 criteria. In addition, 5% of the women in this cohort had germline mutations in genes other than BRCA1 and BRCA2, including women with pathogenic variants in BRIP1, MSH2, MUTYH and SDHB who may be at increased risk for secondary tumors in non-breast organs and thus may benefit from increased surveillance. Use of panel testing in women with TNBC can detect 30% more women with a hereditary component compared to testing for BRCA1 and BRCA2 alone, which may have a significant effect on clinical management of the patient and risk assessment within the family.

Funding

This research was supported by a grant from the Office of the Congressionally Directed Medical Research Programs (Department of Defence Breast Cancer Research Program W81XWH-11-2-0135).

Ethical Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Acknowledgement

The views expressed in this article are those of the authors and do not reflect the official policy of the Department of Defense or U.S. Government. This research was supported by a grant from the Office of the Congressionally Directed Medical Research Programs (Department of Defence Breast Cancer Research Program W81XWH-11-2-0135).

References

1. Bauer KR, Brown M, Cress RD, Parise CA, and Caggiano V. Descriptive analysis of estrogen receptor (ER)-negative, progesterone receptor (PR)-negative, and HER2-negative invasive breast cancer, the so-called triple-negative phenotype: a population-based study from the California cancer Registry. Cancer. 2007; 109: 1721-1728.
2. Dent R, Trudeau M, Pritchard KI, Hanna WM, Kahn HK, Sawka CA, et al. Triple-negative breast cancer: clinical features and patterns of recurrence. Clin Cancer Res. 2007; 13: 4420-4434.
3. Peshkin BN, Alabek ML, Isaacs C. BRCA1/2 mutations and triple negative breast cancers. Breast Dis. 2010; 32: 25-33.
4. Rummel SK, Lovejoy L, Shriver CD, Ellsworth RE. Contribution of germline mutations in cancer predisposition genes to tumor etiology in young women diagnosed with invasive breast cancer. Breast Cancer Res Treat. 2017; 164: 593-601.
5. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015; 17: 405-424.
6. Couch FJ, Hart SN, Sharma P, Toland AE, Wang X, Miron P, et al. Inherited mutations in 17 breast cancer susceptibility genes among a large triple-negative breast cancer cohort unselected for family history of breast cancer. J Clin Oncol. 2015; 33: 304-311.
7. Win AK, Reece JC, Dowty JG, Buchanan DD, Clendenning M, Rosty C, et al. Risk of extracolonic cancers for people with biallelic and monoallelic mutations in MUTYH. Int J Cancer. 2016; 139: 1557-1563.
8. Hicks C, Kumar R, Pannuti A, Backus K, Brown A, Monico J, et al. An Integrative Genomics Approach for Associating GWAS Information with Triple-Negative Breast Cancer. Cancer Inform. 2013. 12: 1-20.
9. Ni Y, Zbuk KM, Sadler T, Patocs A, Lobo G, Edelman E, et al. Germline mutations and variants in the succinate dehydrogenase genes in Cowden and Cowden-like syndromes. Am J Hum Genet. 2008; 83: 261-268.
10. Kim MJ, Kim DH, Jung WH, Koo JS. Expression of metabolism-related proteins in triple-negative breast cancer. Int J Clin Exp Pathol. 2014; 7: 301-312.

Citation: Lovejoy LA, Shriver CD and Ellsworth RE. Mutational Spectrum Across 94 Cancer Predisposition Genes in Patients with TNBC. Austin J Cancer Clin Res. 2018; 5(1): 1082.