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Germline Mutational Analysis of the *C19orf62* Gene in African-American Women with Breast Cancer

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Abstract

The past two decades have seen steady progress in the field of breast cancer genetics as we continually increase our understanding of how tumorigenesis results from damaged DNA. While *BRCA1* and *BRCA2* have taken center stage as indicators for breast cancer risk, we continue to identify other susceptibility genes and genomic regions, ever increasing our comprehension of breast cancer etiology. Through genome-wide association studies (GWAS), the 19p13 locus was found to contribute to breast and ovarian cancer risk. This region harbors a gene called *C19orf62* whose protein has been reported to be a pivotal component in DNA damage repair. In the present study, complete sequencing was performed to screen for deleterious mutations in the *C19orf62* gene. In our cohort of 110 familial and 144 non-familial African-American breast cancer female patients, we identified thirty-five DNA sequence variants, including four missense variants, which appeared unlikely to be disease-causing. We did not detect deleterious frameshift truncating or nonsense mutations in this cohort. Our findings suggest that germline deleterious mutations in *C19orf62* should be rare or absent in familial and non-familial breast cancer women. However, the possibility that mutations in *C19orf62* contribute to breast cancer risk warrant further studies in large diverse populations.

Keywords

C19orf62; germline mutation; breast cancer; African-American

INTRODUCTION

Breast cancer is a heterogeneous disease with genetic and lifestyle/environmental factors implicated in its etiology. The discovery of two breast cancer disease-causing genes, *BRCA1* and *BRCA2*, opened the gates and accelerated the field of genetic research leading to the identification of other breast cancer susceptibility genes, including *TP53*, *PTEN*, *CHEK2*, *ATM*, *BRIP1*, and *PALB2*, among others. In addition, mounting evidence from genome-wide association studies (GWAS) has suggested that some genomic loci may confer risk of breast cancer, such as 1p11.2, 2q35, 3p24, 5q11.2, 6q22.33, 6q25.1, 8q24.21, 10q26.13, 11p15.5, 16q12.1, 17q23, among others [1,2]. Recently, one GWAS identified that common variants on 19p13 were associated with ovarian cancer [3], while another

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GWAS pointed out that the same locus could modify the risk of breast cancer in *BRCA1* mutation carriers and was associated with susceptibility to ER-negative breast cancer [4].

The genomic region, 19p13, harbors an eight-exon gene *C19orf62* which is also known as *BABAM1/HSPC142/NBA1/MERIT40*. This gene spans about 12-kb in the human genome, and encodes a 329-residue protein MERIT40. MERIT40 was recently identified as playing an important role in DNA damage repair and checkpoint control by maintaining the integrity and stability of BRCA1-A complex [5–9]. The central region (amino acids 90–192) was predicted to possess a von Willebrand factor A (vWA) domain, and amino acids 93–320 was proposed to be an evolutionarily conserved, or unchanged, region over time. Additionally, it has been reported that residues 310–314 were essential for ionizing radiation-induced foci (IRIF) formation whereas amino acids 317–321 turned out to be dispensable [5]. Together with the aforementioned genetic findings, the biological evidence supports that *C19orf62* might be a plausible breast cancer susceptibility gene.

To evaluate the role of *C19orf62* in breast cancer risk, we performed germline mutational analysis by direct sequencing in 254 African-American breast cancer female patients and 262 African American non-cancer controls, with the purpose of looking for pathogenic mutations.

MATERIAL AND METHODS

Study subjects

A total of 273 African-American women with breast cancer, diagnosed at the Cancer Risk Clinic at the University of Chicago, Chicago, from 1993 to 2008, participated in this study. Their family history of breast and/or ovarian cancer, age of onset, along with other clinical characteristics was documented in detail by physicians. We did not base our selection of participants on whether or not they had a family history, or early age onset, of breast and/or ovarian cancer, so as not to introduce bias with the purpose of achieving a broader understanding of the mutation spectrum in both familial and non-familial breast cancer patients. Nineteen breast cancer cases who previously tested positive for *BRCA1/2* were excluded; the remaining 254 breast cancer patients consisted of 19 patients with strong family history (more than two first- and/or second-degree relatives affected by breast and/or ovarian cancer, FDR + SDR $n = 3$; age of onset = 45.8 ± 10.9 years [mean $\pm$ SD]); 25 patients with moderate family history (FDR + SDR $n = 2$; age of onset = 44.6 ± 7.4 years); 66 patients with weak family history (FDR + SDR $n = 1$; age of onset = 44.8 ± 9.7 years); and 144 non-familial or sporadic breast cancer cases (age of onset = 43.7 ± 10.0 years). In an attempt to explore the frequencies of nonsynonymous/missense variants in the cancer-free normal population, a set of 262 African-American female controls who did not have breast cancer were further sequenced. Informed consents were obtained from all the participants before collecting blood samples. This study was approved by the Institutional Review Board at the University of Chicago.

Mutation identification by sequencing

Genomic DNA was isolated from peripheral blood leukocytes by standard methods and stored at -20°C . All the coding exons, exon/intron boundaries, 5'UTR and 3'UTR of the *C19orf62/MERIT40* gene were targeted and covered by oligonucleotide primers designed by Primer3 v0.4.0 (<http://primer3.sourceforge.net/>). PCR products were cleaned by ExoSap-IT (USB, USA) subsequently sequenced using BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) on an Applied Biosystems 3730xl DNA Analyzer according to the manufacturer's instructions. Mutational analysis based on the sequenced traces was successfully performed in SeqScape v2.6.0 (Applied Biosystems, USA). Due to the four

missense variants found in three amplicons (see below), exon 1 and partial 5'UTR, exon 2, as well as exons 6 and 7, we sequenced these amplicons in the normal control subjects. The average sequencing success rates for all amplicons were 99.18% in cases and 99.13% in controls, respectively.

***In silico* analysis of nonsynonymous variants**

All variants detected in the present study were classified according to their variant type as nonsynonymous, synonymous, untranslated-5'UTR, untranslated-3'UTR, and intronic. Five different computational programs, SIFT (<http://sift.jcvi.org/>), PolyPhen (<http://genetics.bwh.harvard.edu/pph/>), Align GVGD (<http://agvgd.iarc.fr/>), PMUT (<http://mmb2.pcb.ub.es:8080/PMut/>), and PANTHER (<http://www.pantherdb.org/tools/csnpscoreform.jsp>) were carried out for functional predictions of nonsynonymous variants. Furthermore, it is suggested that *in silico* assessments might tend to be more accurate when concordance between predictions are obtained. From our work, we determined a missense variant to be deleterious, in the circumstance of at least four functional prediction algorithms providing pathological implications.

Nomenclature

The variants found in this study were named according to the GenBank *C19orf62* reference sequence NM_001033549 and NP_001028721, following the instructions provided by the HGVS (<http://www.hgvs.org>). We also checked the nomenclature by Mutalyzer v2.0 (<http://www.mutalyzer.nl/2.0/>).

RESULTS

All the eight exons, exon/intron boundaries, 5'UTR and 3'UTR of the *C19orf62* gene were successfully sequenced. The details and occurrence of the genetic variants identified by sequencing were listed in Table 1. We did not detect any truncating or nonsense mutation in the gene in 254 African-American breast cancer patients. Our mutation screening revealed a total of 35 nucleotide variants in the *C19orf62* gene, including 34.3% (12/35) exonic, 57.1% (20/35) intronic, as well as 8.6% (3/35) regulatory variants: four nonsynonymous, eight synonymous, twenty intronic variants, one variant in the 5'UTR and two variants in the 3'UTR of the gene. Of these 35 variants, 23 (65.7%) were novel while 12 (34.3%) were previously uncovered and deposited in the NCBI SNP database (dbSNP 132, <http://www.ncbi.nlm.nih.gov/SNP/>). A Majority (28/35, 80.0%) of the variants found were rare (minor allele frequency, MAF < 0.05), and of them, 18 (64.3%) were singletons in the breast cancer patients, meaning, detected only once. We observed four nucleotide alteration resulting in an amino acid change, c.133G>A (p.Val45Met), c.320T>G (p.Leu107Arg), c.343G>A (p.Gly115Ser), and c.590C>T (p.Pro197Leu). We also investigated their occurrences in control subjects. Except for c.320T>G, the remaining three were detectable in normal controls. We performed *in silico* functional predictions for these four nonsynonymous variants utilizing SIFT, PolyPhen, Align GVGD, PMUT and PANTHER. We established an evaluation method for determining deleterious variants, namely, a missense variant was defined as deleterious when pathological outputs from at least four prediction algorithms were obtained. Based on this classification setting, c.133G>A and c.320T>G were predicted to be benign, whereas c.343G>A and c.590C>T were likely to be deleterious. However, there was no difference in the prevalence of these four variants between the case and control groups. The Fisher exact test showed a two-sided p=0.64 (8/254 vs. 11/262) for c.343G>A and c.590C>T only; and a two-sided p=0.83 (10/254 vs. 12/262) for all the four missense variants combined.

DISCUSSION

One in eight women in the United States will develop breast cancer during her lifetime and one of thirty-five will die because of it. It is well known that deleterious mutations within the *BRCA1* and *BRCA2* genes confer high risks of breast and ovarian cancer, while some moderate-penetrance genes like *PTEN*, *CHEK2*, *ATM*, *BRIP1*, and *PALB2* may contribute to a limited portion of breast cancer risk. Genome-wide association studies from recent years have significantly broadened our current views of breast cancer susceptibility, however, the inherited risks of breast cancer remain unclear for the more than 70% of the breast cancer patients, especially for the non-familial/sporadic cases [10,11].

The *C19orf62* gene is mapped to 19p13, a region recently found to predispose one to breast and/or ovarian cancer. Antoniou *et al.* performed a GWAS that found five single nucleotide polymorphisms (SNPs) on 19p13 to be associated with breast cancer and in follow-up replication found association in 4,176 affected and 4,202 unaffected *BRCA1* mutation carriers [4]. Further investigation on these SNPs also suggested an association with ER-negative and triple-negative breast cancer, where tumors lack expression of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 Her2/neu. These five SNPs were located in a 200-kb linkage disequilibrium (LD) block harboring *NR2F6*, *USHBP1*, *C19orf62*, *ANKLE1* and *ABHD8*. In addition, two of the SNPs, rs8170 (in *C19orf62*) and rs2363956 (in *ANKLE1*), showed no evidence of association with breast cancer risk for *BRCA2* mutation carriers (n = 2,486). Bolton *et al.* conducted a three-phase GWAS of epithelial ovarian cancer (EOC) in more than 10,000 cases and 13,000 controls [3] where rs8170 and rs2363956 were associated with EOC survival and the risk of serous subtype EOCs. Their array comparative genomic hybridization (aCGH) data demonstrated amplification (copy number gain) of the associated region and the expression analysis suggested that *C19orf62* but not *ANKLE1* was remarkably over-expressed in ovarian cancer cell lines.

The gene product of *C19orf62*, MERIT40, together with other proteins such as BRCA1, RAP80/UIMC1, ABRAXA/CCDC98/, BRCC36, and BRCC45/BRE, form the BRCA1-A macrocomplex which was reported to play a crucial role in G2-M checkpoint control, cellular resistance to ionizing radiation, and BRCA1 retention at damage-induced foci [8]. MERIT40 is responsible for the integrity and stability of BRCA1-A complex in order to successfully undertake DNA double-strand breaks (DSBs) recognition and (de)ubiquitination regulation upon DNA damage response [5–7,9].

From these genetic and biological findings identifying *C19orf62* as a candidate breast cancer susceptibility gene, we initiated a direct sequencing study to investigate whether mutations in the *C19orf62* gene could predispose one to the breast cancer risk. We sequenced all the exons, exon/intron boundaries, 5'UTR and 3'UTR of *C19orf62* in 254 African-American women with breast cancer, whether or not they had a positive family history or early age of onset of breast and/or ovarian cancer. Although no frameshift truncating or nonsense mutation was detected, we were able to identify thirty-five sequence variants in which four were missense variants (c.133G>A, c.320T>G, c.343G>A, and c.590C>T). Five *in silico* functional predictions indicated that c.133G>A and c.320T>G could be benign, whereas c.343G>A and c.590C>T might be pathogenic. Furthermore, our examination showed that these variants also occurred in the normal controls, except c.320T>G. It appears no difference in prevalence of these nonsynonymous variants between the case and control groups. It is notable that Solyom *et al.* recently sequenced the same gene in 125 familial breast cancer patients in Finland but failed to detect disease-related germline variants in the *C19orf62* gene [12]. In line with Solyom *et al.*, our findings support that germline deleterious coding mutations in *C19orf62* are rare or absent in familial and non-familial

breast cancer women. Our study has sufficient power (87%) to detect a deleterious mutation with minor allele frequency of 0.004. Therefore, a frameshift truncating or nonsense mutation, if exist in this gene, may be rarer than 0.004. To capture very rare mutations (<0.001), it will be necessary to carry out sequencing study using a larger sample size. Alternatively, regulatory elements which have generally subtle effect may account for the risk of breast cancer. As of February 2011, there are over 800 GWAS on human complex diseases/traits, but most of the signals have not directly linked to causal variants that lead to amino acid changes. It therefore remains to be seen whether common regulatory variants and/or rare coding mutations in *C19orf62* or other genes within the same LD block on 19p13 contribute to the breast cancer risk. Furthermore, it will be interesting to explore the possibility of *C19orf62* mutations predisposing to other cancers, for instance, ovarian cancer. To date, there exists only two studies including ours reporting the mutational screening results of *C19orf62* in breast cancer patients. To fully appreciate the role of *C19orf62* in breast cancer susceptibility, mutation spectrum analyses requires further investigation in diverse populations including Asians, Africans, Latinos, among others.

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Table 1

Genetic variants found in the *C19orf62* gene

Location	Nucleotide/protein change	rs# (dbSNP132)	Variant Type	Computational prediction					Allele frequency	
				SIFT	PolyPhen	Align GVGd	PMUT	PANTHER	Case	Control
intron between 5'UTRs	c.-1279G>T	rs113704445	intrinsic	-	-	-	-	-	1/506 (0.002)	
intron between 5'UTRs	c.-142G>C	-	intrinsic	-	-	-	-	-	1/504 (0.002)	0/514 (0)
intron between 5'UTRs	c.-82G>A	rs116873439	intrinsic	-	-	-	-	-	6/504 (0.012)	6/514 (0.012)
intron between 5'UTRs	c.-58C>T	rs10424178	intrinsic	-	-	-	-	-	172/506 (0.340)	185/514 (0.360)
5'UTR	c.-22T>C	-	untranslated-5'UTR	-	-	-	-	-	1/506 (0.002)	1/516 (0.002)
exon 1	c.15G>A/p.=	-	synonymous	-	-	-	-	-	1/506 (0.002)	0/516 (0)
exon 1	c.78C>T/p.=	-	synonymous	-	-	-	-	-	2/504 (0.004)	1/516 (0.002)
exon 1	c.133G>A/p.Val45Met	-	nonsynonymous	affect (0.00)	benign (1.100)	benign (C0)	neutral (0.321)	deleterious (-3.096)	1/504 (0.002)	1/516 (0.002)
exon 1	c.204C>T/p.=	-	synonymous	-	-	-	-	-	1/504 (0.002)	0/516 (0)
intron 1	c.285+26T>C	-	intrinsic	-	-	-	-	-	3/504 (0.006)	1/512 (0.002)
intron 1	c.286-160A>G	rs111324512	intrinsic	-	-	-	-	-	7/502 (0.014)	2/518 (0.004)
exon 2	c.320T>G/p.Leu107Arg	-	nonsynonymous	tolerated (0.53)	benign (0.007)	benign (C0)	pathological (0.809)	neutral (-1.171)	1/502 (0.002)	0/518 (0)
exon 2	c.343G>A/p.Gly115Ser	rs115366940	nonsynonymous	affect (0.03)	possibly damaging (1.722)	benign (C0)	pathological (0.833)	deleterious (-3.486)	7/502 (0.014)	10/518 (0.019)
intron 2	c.344+41A>T	rs10420922	intrinsic	-	-	-	-	-	325/502 (0.647)	359/518 (0.693)
exon 3	c.408C>T/p.=	-	synonymous	-	-	-	-	-	1/508 (0.002)	-
exon 4	c.483C>T/p.=	-	synonymous	-	-	-	-	-	1/508 (0.002)	-
exon 4	c.507C>T/p.=	-	synonymous	-	-	-	-	-	1/508 (0.002)	-
intron 4	c.544+46_48delTCT	-	intrinsic	-	-	-	-	-	1/508 (0.002)	-
intron 4	c.545-118C>G	rs75262583	intrinsic	-	-	-	-	-	23/506 (0.046)	-
intron 5	c.569+126G>A	rs73931066	intrinsic	-	-	-	-	-	45/506 (0.089)	-
intron 5	c.570-50G>A	rs61634114	intrinsic	-	-	-	-	-	69/506 (0.136)	75/524 (0.143)
exon 6	c.590C>T/p.Prol197Leu	-	nonsynonymous	affect (0.04)	probably damaging (2.724)	benign (C0)	pathological (0.952)	deleterious (-4.163)	1/506 (0.002)	1/524 (0.002)
exon 6	c.687G>A/p.=	-	synonymous	-	-	-	-	-	1/506 (0.002)	3/524 (0.006)
intron 6	c.700-86G>A	-	intrinsic	-	-	-	-	-	4/506 (0.008)	2/524 (0.004)
intron 6	c.700-84C>T	-	intrinsic	-	-	-	-	-	1/506 (0.002)	0/524 (0)

Location	Nucleotide/protein change	rs# (dbSNP132)	Variant Type	Computational prediction					Allele frequency	
				SIFT	PolyPhen	Align GVG D	PMUT	PANTHER	Case	Control
intron 6	c.700-47G>A	rs10416551	intronic	-	-	-	-	-	44/506 (0.087)	43/524 (0.082)
intron 7	c.786+34G>A	-	intronic	-	-	-	-	-	1/506 (0.002)	1/524 (0.002)
intron 7	c.786+47delT	-	intronic	-	-	-	-	-	1/506 (0.002)	1/524 (0.002)
intron 7	c.786+59C>T	-	intronic	-	-	-	-	-	2/504 (0.004)	2/522 (0.004)
intron 7	c.786+65C>G	-	intronic	-	-	-	-	-	2/504 (0.004)	0/522 (0)
intron 7	c.787-13G>A	-	intronic	-	-	-	-	-	1/496 (0.002)	-
intron 7	c.787-6C>T	rs10406920	intronic	-	-	-	-	-	87/496 (0.175)	-
exon 8	c.837G>A/p.=	rs8170	synonymous	-	-	-	-	-	91/496 (0.184)	-
3'UTR	c.*79G>C	-	untranslated-3'UTR	-	-	-	-	-	2/496 (0.004)	-
3'UTR	c.*242T>C	-	untranslated-3'UTR	-	-	-	-	-	1/494 (0.002)	-