Association Between Genes in the Nuclear Factor E2–Related Factor 2 Antioxidative Response Elements Pathway and Cancer-Related Fatigue in Women With Early-Stage Breast Cancer

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OBJECTIVES: To explore genes in the nuclear factor E2–related factor 2 antioxidative response elements (Nrf2-ARE) signaling pathway using a multiomics approach for associations with variability of cancer-related fatigue (CRF) in postmenopausal women with early-stage hormone receptor–positive breast cancer.

SAMPLE & SETTING: Postmenopausal women (N = 116) with early-stage hormone receptor–positive breast cancer were recruited from western Pennsylvania.

METHODS & VARIABLES: Candidate genes from the Nrf2-ARE pathway were investigated for associations with CRF occurrence and severity. Associations were evaluated using logistic regression for occurrence and linear regression for severity.

RESULTS: The rs2706110 TT genotype in *NFE2L2* was associated with a 3.5-fold increase in odds of CRF occurrence. The cytosine-phosphate-guanine (CpG) site cg22820568 in *PRDX1* was associated with CRF occurrence and severity.

IMPLICATIONS FOR NURSING: Biomarkers based on Nrf2-ARE genes may help to identify women at increased risk for more severe CRF and to develop targeted interventions.

KEYWORDS antioxidants; breast neoplasms; DNA methylation; fatigue; polymorphism
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More than 3.8 million women in the United States are breast cancer survivors (Giaquinto et al., 2022). Cancer-related fatigue (CRF) is among the most common symptoms associated with breast cancer and its treatments (Berger et al., 2012; Mao et al., 2018; Sanft et al., 2023). One in four women with breast cancer will experience CRF at some point (Maass et al., 2021). The National Comprehensive Cancer Network defines CRF as "a distressing, persistent, subjective sense of physical, emotional, and/or cognitive tiredness or exhaustion related to cancer or cancer treatment that is not proportional to recent activity and interferes with usual functioning" (Sanft et al., 2023, p. 797). CRF is different from general fatigue because it is not likely to improve with adequate rest, may be more severe, and is more likely to cause interference in daily activities (Berger et al., 2015). Patients with breast tumors report among the highest prevalence of moderate to severe CRF (Kang et al., 2023). A growing body of evidence suggests that early identification and treatment of CRF across the trajectory of cancer treatment may decrease its occurrence and severity (Bower et al., 2019). The burden associated with CRF includes interference with activity and physical functioning, inability to work (Schmidt et al., 2019) and socialize, greater financial stress, and increased healthcare utilization (Behringer et al., 2016). These effects result in decrements in quality of life (Bower et al., 2000). In addition, CRF is associated with worse disease trajectories and reductions in survival (Groenvold et al., 2007).

Despite the high prevalence of CRF and its debilitating effects, the mechanisms that underlie CRF are not fully understood. A multitude of factors, including genomic factors (e.g., DNA methylation variation [Xiao et al., 2021], single nucleotide polymorphisms [SNPs] [Kober et al., 2016]), are likely to be involved in the occurrence and severity of CRF. Inflammatory mechanisms are among the most studied mechanisms for CRF (Saligan et al., 2015; Wright et al., 2017). Of note, oxidative stress regulation, one of many inflammatory mechanisms, plays an important role in the development and progression of different cancers, including breast cancer (Hayes et al., 2020; Lee et al., 2017).

One potential pathway involved in oxidative stress regulation that may help to explain the complexity of CRF in women with breast cancer is the nuclear factor E2–related factor 2 antioxidative response elements (Nrf2-ARE) signaling pathway. The Nrf2-ARE pathway maintains redox homeostasis through the regulation of intracellular antioxidant response and mediates anti-inflammatory processes for various conditions, such as neurodegenerative diseases (George et al., 2022) and aging (Liguori et al., 2018). In the context of cancer, Nrf2-ARE has tumor-suppressing and tumor-promoting properties (Rojo de la Vega et al., 2018). In general, when reactive oxygen species levels exceed antioxidant capacity in the cell, they cause destruction of a number of cell components (e.g., lipids, proteins, DNA) and subsequently lead to apoptosis or necrosis (Rojo de la Vega et al., 2018). However, a more aggressive cellular response can occur that initiates a significantly higher level of inflammation (Rojo de la Vega et al., 2018), which may result in CRF. On the other hand, in some situations, Nrf2-ARE protects cells from destruction (Schmidlin et al., 2021); this protective role may prevent the development of cancer and may decrease the occurrence and/or severity of CRF. However, pathways involved in antioxidative stress are not well studied and warrant additional investigation (García-González et al., 2023). To the current research team's understanding, only three studies, including one done by the current team, have investigated associations between oxidative stress mechanisms and CRF (Davis et al., 2023; Dickinson et al., 2020; Repka & Hayward, 2018). It is hypothesized that antioxidative stress cellular responses to cellular death and tumor growth or suppression may contribute to CRF. Therefore, the aim of this study was to use a multiomics approach to explore the role of genes in the Nrf2-ARE pathway in the occurrence and severity

TABLE 1. Patient Demographics and Clinical Characteristics at Enrollment (N = 116)

Characteristic	$\bar{X}$	SD
Age (years)	62.4	7.1
Body mass index (kg/m^2)	29.8	6.7
Maximal oxygen consumption (ml/kg/minute)	21.9	4.6
National Area Deprivation Index percentile	42.3	22.3
State Area Deprivation Index decile	3.6	2.5
Characteristic	**n**	**%**
Race		
Asian	1	1
Black	8	7
White	104	90
Other[a]	3	3
Ethnicity		
Hispanic/Latina	2	2
Non-Hispanic/Latina	114	98
Marital or partner status		
Married or partnered	79	68
Not married or partnered	37	32
Parental status		
Parent	101	87
Not a parent	15	13
Employment status		
Employed	71	61
Not employed	45	39
Cancer stage		
Ductal carcinoma in situ	18	16
Stage I	70	60
Stage IIa	17	15
Stage IIb	6	5
Stage IIIa	5	4
Aromatase inhibitor therapy		
No	114	98
Yes	2	2
Chemotherapy		
No	98	84
Yes	18	16
Radiation therapy		
Yes	97	84
No	19	16

[a] Includes 2 individuals identifying as Native American and White, and 1 identifying as Native American and Black.
Note. Because of rounding, percentages may not total 100.

TABLE 2. Candidate Genes, SNPs, and DNA Methylation Extraction Information

Gene	Name	SNPs	chr: Extraction Window[a]	Number of CpG Sites
CAT	Catalase	■ rs511895 ■ rs525938 ■ rs566979 ■ rs1001179[b] ■ rs769214[b]	chr11: 34458472–34495607	25
KEAP1	Kelch-like ECH-associated protein 1	■ rs9676881 ■ rs1048290	chr19: 10594796–10616054	25
NFE2L2	Nuclear factor erythroid 2–like 2	■ rs2706110	chr2: 178093031–178131859	34
PRDX1	Peroxiredoxin 1	■ rs945179 ■ rs7522705 ■ rs1041740	chr1: 45974707–45990562	24
SOD1	Superoxide dismutase 1	■ rs1041740 ■ rs10432782	chr21: 33029935–33043243	18
SOD2	Superoxide dismutase 2	■ rs4880 ■ rs5746136 ■ rs8031	chr6: 160098149–160116353	6
TXN	Thioredoxin reductase 1	■ rs2301241 ■ rs4135225	chr9: 113004092–113020920	10

[a] Build 37/hg19 in the University of California, Santa Cruz, Genomics Institute Genome Browser
[b] SNP failed quality control.
chr—chromosome; CpG—cytosine-phosphate-guanine; SNP—single nucleotide polymorphism

of CRF in postmenopausal women with early-stage hormone receptor–positive breast cancer. Hormone receptor–positive breast cancer cells have receptor proteins for estrogen, progesterone, or both.

Methods and Variables

Study Design

This exploratory study employed a cross-sectional observational design that capitalized on existing data and biospecimens from postmenopausal women with early-stage breast cancer who participated in the Exercise Program in Cancer and Cognition (Gentry et al., 2018) and the Epigenomics of Neurocognitive Function in Breast Cancer studies (together referred to as "the parent study" in this article). The parent study's aim was to investigate the effects of moderate intensity aerobic exercise on cognitive function in women with early-stage postmenopausal breast cancer within the first six months of an aromatase inhibitor treatment. For this study, data from the prerandomization time point were used. Extensive demographic, clinical, and CRF-related data, as well as stored biosamples and whole-genome methylation data, were available from the parent study. In the current study, SNP genotypes were generated and used with DNA methylation data to investigate targeted genes from the Nrf2-ARE pathway.

Sample and Setting

A total of 116 women were included in this analysis. Women were included if they had hormone receptor–positive breast cancer, were aged younger than 80 years, were diagnosed with stage 0, I, II, or IIIa breast cancer, and had available blood samples. Women were excluded if they had prior cancer diagnoses (except for some skin cancers), metastases, self-reported hospitalization for psychiatric illness within the past two years, history of neurologic illness, breast cancer surgery complications, reconstructive surgery within the study period, or history of chronic fatigue. This study was approved by the University of Pittsburgh Institutional Review Board.

Patients were recruited during their initial post-operative appointment, which is typically two to three weeks following breast surgery and three

TABLE 3. Significant CpG Sites Associated With CRF Occurrence Adjusted Logistic Regression Results

Gene	CpG Site	Position	Region Location[a]	Relationship to Island[b]	Odds Ratio	95% CI	p	FDR
PRDX1	cg09131901	chr1: 45980096	Body	South Shore	0.0121	[0, 0.48]	0.028	0.998
SOD2	cg10002977	chr6: 160113557	TSS 1500	Island	15.807	[1.59, 204.14]	0.023	0.998
NFE2L2	cg12647821	chr2: 178128273	TSS 1500, TSS 200	South Shore	8.816	[1.15, 93.64]	0.049	0.998

[a] Identified from Infinium Methylation EPIC, version 1.0, Manifest from Illumina product files Build GRCh 37/hg19; TSS 200 = 0–200 bases upstream of the TSS, TSS 1500 = 200–1500 bases upstream of the TSS, Body = between the ATG start site and stop codon (irrespective of the presence of introns, exons, TSS, or promoters)
[b] Identified from Infinium Methylation EPIC, version 1.0, Manifest from Illumina product files or the University of California, Santa Cruz, Genomics Institute Genome Browser Build GRCh 37/hg19; location of the CpG site relative to the CpG Island: South Shore = downstream (3′) of CpG Island
chr—chromosome; CI—confidence interval; CpG—cytosine-phosphate-guanine; CRF—cancer-related fatigue; FDR—false discovery rate; *NFE2L2*—nuclear factor erythroid 2–like 2; *PRDX1*—peroxiredoxin 1; *SOD2*—superoxide dismutase 2; TSS—transcriptional start site

to four weeks before beginning radiation therapy. Recruitment took place at the Comprehensive Breast Care Program of the University of Pittsburgh Cancer Institute and University of Pittsburgh Medical Center cancer treatment centers from October 2016 to September 2021. All patients provided written informed consent (Gentry et al., 2018).

CRF Measures

The Patient-Reported Outcomes Measurement Information System (PROMIS) Fatigue Short Form 8a is a valid and reliable self-report measure of fatigue in individuals with chronic disease including cancer (Cella et al., 2016). This measure is an eight-item, five-point Likert-type scale with responses ranging from 1 (not at all) to 5 (very much). T scores are calculated and standardized to a mean of 50 and an SD of 10 in reference to the U.S. general population. Higher T scores indicate greater fatigue. Two different CRF outcomes were evaluated, namely CRF occurrence (PROMIS Fatigue T score of 50 or greater) and CRF severity as a continuous score (Cella et al., 2014).

Additional measures that were evaluated as potential covariates included age, employment status, marital or partner status, parental status, State and National Area Deprivation Indices (state index measured by decile, national index measured by percentile) (Kind & Buckingham, 2018), body mass index (BMI), estimated maximal oxygen consumption (VO_2 max), radiation therapy, chemotherapy, pain severity (Brief Pain Inventory–Short Form, range = 0–10), pain interference (Brief Pain Inventory–Short Form, range = 0–10) (Cleeland & Ryan, 1994), anxiety (PROMIS Emotional Distress–Anxiety Short Form, T score range = 35–75) (Pilkonis et al., 2011), depression (Beck Depression Inventory–II, range = 0–63) (Beck et al., 1996), daytime sleepiness (Epworth Sleepiness Scale, range = 0–24) (Johns, 1991), and self-reported cognitive function (Patient Assessment of Own Functioning Inventory, range = 0–155) (Chelune et al., 1986). Because of the relatively small sample size, all covariates that were significantly correlated with CRF could not be included in the regression models. Therefore, a priori and data-driven covariate identification methods were used (Greenland & Pearce, 2015).

Descriptive Statistical Analysis

Analyses were completed using R statistical software, version 4.0.4 (R Core Team, 2021), and PLINK, version 1.9 (Purcell et al., 2007). Univariate statistics and visualization of dependent, independent, and potential covariate variables were performed based on level of measurement for each variable. Additional variables known to influence CRF were identified using a priori and data-driven bivariate analysis methods. Continuous covariates were analyzed for associations with CRF using Pearson correlation. Nominal variables were evaluated using the point biserial correlation test. Bivariate comparison analyses, consisting of two-sample t tests, chi-square tests of independence, and Fisher's exact tests, were performed to evaluate equality of group means for continuous variables and group proportions for categorical variables. Using

PLINK, allele frequencies were calculated and Hardy–Weinberg Equilibrium was assessed for each SNP. Additional descriptive and quality control analyses are described in the following sections.

DNA Methylation Data Collection and Statistical Analysis

Genes from the Nrf2-ARE pathway involved in the reduction of oxidative damage and/or breast cancer were chosen using Ingenuity Pathway Analysis, version 21.0.1. Transporter genes for exogenous chemicals that were not known to be associated with aromatase inhibitor drugs were excluded. Whole-methylome data were generated using the Infinium MethylationEPIC, version 1.0, BeadChip, and were put through a substantial quality control pathway. Quality control procedures included calculating beta values from the ratio of the intensity of methylated probes to the intensity from total probes and correcting for background and dye bias. Beta values were normalized using the preprocessFunnorm function from the minfi package, version 1.40.0, in Bioconductor. Normalization was verified with cluster analysis on technical replicates. Probes were removed if they were cross-reactive, located on the Y chromosome, or located near a known SNP. Cell type heterogeneity was not corrected for in this study. Cytosine-phosphate-guanine (CpG) site data were extracted for each candidate gene within a +/- 2,000 base pair (cis) window to allow for evaluation of proximal regulatory regions. M values were used because of their statistically valid properties for differential analysis of methylation levels (Xie et al., 2019) in the context of the outcome of interest (i.e., CRF).

Regression analyses were used to identify methylated regions associated with CRF occurrence

TABLE 4. Significant CpG Sites Associated With CRF Severity Adjusted Linear Regression Results

Gene	CpG Site	Position	Region Location[a]	Relationship to Island[b]	b	95% CI	p	FDR
PRDX1	cg15627031	chr1: 45987803	1st Exon, 5' UTR	Island	4.813	[1.38, 8.24]	0.006	0.54
PRDX1	cg22820568	chr1: 45987803	TSS 1500	South Shore	12.529	[3.45, 21.61]	0.008	0.54
NFE2L2	cg15956152	chr2: 178129847	TSS 1500, TSS 200	South Shore	5.622	[1.09, 10.15]	0.016	0.575
TXN	cg14509895	chr9: 113019297	Body	Island	4.695	[0.8, 8.59]	0.019	0.575
NFE2L2	cg21382890	chr2: 178104794	Body, 5' UTR	North Shore	4.489	[0.68, 8.3]	0.022	0.575
KEAP1	cg12095186	chr19: 10614280	TSS 1500	South Shore	-8.84	[-16.49, 1.19]	0.024	0.575
SOD2	cg14515483	chr6: 160114863	Body	Island	3.553	[0.18, 6.92]	0.039	0.742
PRDX1	cg09131901	chr1: 45980096	Body	South Shore	-8.255	[0.18, 6.92]	0.042	0.742

[a] Identified from Infinium Methylation EPIC, version 1.0, Manifest from Illumina product files Build GRCh 37/hg19; TSS 200 = 0–200 bases upstream of the TSS, TSS 1500 = 200–1500 bases upstream of the TSS, 5' UTR = within the 5' UTR, between the TSS and the ATG start site, Body = between the ATG start site and stop codon (irrespective of the presence of introns, exons, TSS, or promoters)
[b] Identified from Infinium Methylation EPIC, version 1.0, Manifest from Illumina product files or the University of California, Santa Cruz, Genomics Institute Genome Browser Build GRCh 37/hg19; location of the CpG site relative to the CpG Island: South Shore = downstream (3') of CpG Island; North Shore = upstream (5') of CpG Island
b—beta coefficient; chr—chromosome; CI—confidence interval; CpG—cytosine-phosphate-guanine; CRF—cancer-related fatigue; FDR—false discovery rate; *KEAP1*—Kelch-like ECH-associated protein 1; *NFE2L2*—nuclear factor erythroid 2–like 2; *PRDX1*—peroxiredoxin 1; *SOD2*—superoxide dismutase 2; TSS—transcriptional start site; *TXN*—thioredoxin reductase 1; UTR—untranslated region
Note. Table rows are sorted by smallest p value.

TABLE 5. Significant SNPs Associated With CRF Occurrence Adjusted Logistic Regression Results

Gene	SNP	Position	Odds Ratio	95% CI	p	FDR
NFE2L2	rs2706110	chr2: 178092162	3.54	[1.28, 11.07]	0.02	0.245
SOD1	rs10432782	chr21: 33036391	0.318	[0.19, 1.69]	0.038	0.245

chr—chromosome; CI—confidence interval; CRF—cancer-related fatigue; FDR—false discovery rate; *NFE2L2*—nuclear factor erythroid 2–like 2; SNP—single nucleotide polymorphism; *SOD1*—superoxide dismutase 1

(logistic regression) and severity (linear regression). Regression models were adjusted for covariates identified in this study. Unadjusted and adjusted p values were calculated using the Benjamini–Hochberg procedure (Benjamini & Hochberg, 1995). A false discovery rate of 0.05 was applied to control for multiple testing.

SNP Data Collection and Statistical Analysis

From the genes identified in the Nrf2-ARE pathway, functional polymorphisms were selected using the University of California, Santa Cruz, Genomics Institute (n.d.), Genome Browser and through a literature search using the terms *functional polymorphism* AND the full name of each gene. SNPs known to influence fatigue and/or breast cancer were prioritized.

DNA was extracted from banked blood samples taken from the patients at enrollment. A simple salting out procedure was used to extract DNA. Allelic discrimination was performed using the Applied Biosystems QuantStudio™ 3 Real-Time PCR System and TaqMan™ allelic discrimination assays. To ensure rigor of the data, genotypes were blindly double-called, compared, reconciled, or rerun.

The quality control pathway for the genotype data was completed using the PLINK toolset (Purcell et al., 2007) to identify per-marker missingness (greater than 5%), per-individual missingness (greater than 5%), and per-marker minor allele frequency (less than 5%), as well as to calculate per-marker deviation from Hardy–Weinberg Equilibrium. Genetic associations were tested under an additive model for which SNPs were treated as ordinal variables based on genotype (SNP genotype was coded as 0 for homozygous wild type, 1 for heterozygous, and 2 for homozygous minor allele).

To investigate the effect of the prioritized SNPs on CRF outcomes, regression models were employed. The CRF occurrence and CRF severity outcomes were evaluated using logistic regression and linear regression modeling, respectively. Models were adjusted for covariates and a Benjamini–Hochberg procedure, (Benjamini & Hochberg, 1995), and a false discovery rate correction of 0.05 was applied to control for multiple testing.

Genetically Driven Methylation Variation Measurement and Statistical Analysis

To understand whether genetic variation influenced DNA methylation variation, the associations between genetic and local methylation variations (i.e., methylation quantitative trait loci [meQTLs]) were analyzed. The meQTL analyses were limited to significant SNPs that were associated with CRF occurrence and severity and located within a +/- 2,000 base pair (cis) window of each CpG site. Unadjusted linear regression modeling was used to examine the associations between the identified SNPs and DNA methylation M value levels. A significant meQTL was identified if a CpG site was regulated by at least one SNP (Gaunt et al., 2016). Using the Benjamini–Hochberg procedure (Benjamini & Hochberg, 1995), a false discovery rate correction of 0.05 was applied to control for multiple testing. The mQTLdb database was used to interpret significant meQTLs (Gaunt et al., 2016).

Results

Patient Characteristics

As shown in Table 1, the 116 participants had a mean age of 62.4 years (SD = 7.1); 90% self-reported their race as White; 98% self-reported their ethnicity as non-Hispanic/Latina; 61% were currently employed; 68% were married and/or partnered; and 87% were parents. Participants had a mean BMI of 29.8 kg/m^2 (SD = 6.7) and a mean VO_2 max of 21.9 ml/kg/minute (SD = 4.6). Most patients had stage I hormone receptor–positive breast cancer (60%), and 84% had received radiation therapy prior to surgery. Because the parent study was conducted during the COVID-19 pandemic, relaxation of the initial study inclusion and exclusion criteria was allowed, resulting in about 16% of the

patients having received chemotherapy and less than 2% having received aromatase inhibitor therapy prior to enrollment. Additional analyses were performed to compare the entire cohort to those who did not receive chemotherapy or aromatase inhibitor therapy prior to study entry (n = 96). These results are summarized in Supplemental Tables 1–3 online, with minor differences noted.

Additional Measures Evaluated as Potential Covariates

A summary of the correlated and a priori variables that were included in regression models is presented in Supplemental Table 4 online. In the bivariate analyses, although estimated VO_2 max ($p = 0.02$), BMI ($p = 0.04$), pain interference ($p \leq 0.001$), pain severity ($p = 0.02$), anxiety ($p \leq 0.001$), depression ($p \leq 0.001$), daytime sleepiness ($p \leq 0.001$), and self-reported cognitive function ($p < 0.001$) were positively correlated with CRF severity, being married or partnered ($p = 0.01$) was negatively correlated with CRF severity. No correlations were found between age, employment status, parental status, radiation therapy, chemotherapy, or State or National Area Deprivation Indices and CRF severity. However, because it is well established that age influences the regulation of oxidative stress, age was included in all the regression models. Because pain severity was correlated with pain interference, pain severity was not included in the models. Although self-reported cognitive function was correlated with CRF severity, because of the small sample size, this characteristic was not included in the analyses. The characteristics used as covariates in the adjusted analyses were age, BMI, VO_2 max, pain interference, anxiety, depression, and daytime sleepiness.

Genetic Extraction Information and Quality Control Procedures

A total of 142 CpG sites and 18 SNPs were evaluated across seven candidate genes and are summarized in Table 2. After applying a variety of quality control procedures, all 142 CpG sites and 16 SNPs from 116 patients were analyzed.

Following identification of per-marker missingness of greater than 5%, two SNPs were excluded. Although one SNP deviated from Hardy–Weinberg Equilibrium ($p \leq 0.001$), this SNP was not eliminated from analyses on the basis that this study's cohort does not represent the general population because it is enriched for breast cancer. Therefore, a total of 16 SNPs from 116 women who provided blood samples passed all the genotype quality control procedures and were analyzed (see Supplemental Figure 1 online).

DNA Methylation Analysis Results

Three CpG sites were significantly associated with CRF occurrence (see Table 3). Eight CpG sites were significantly associated with CRF severity (see Table 4). All of the DNA methylation results for CRF occurrence and severity are presented in Supplemental Tables 5 and 6 online, respectively.

SNP Analysis Results

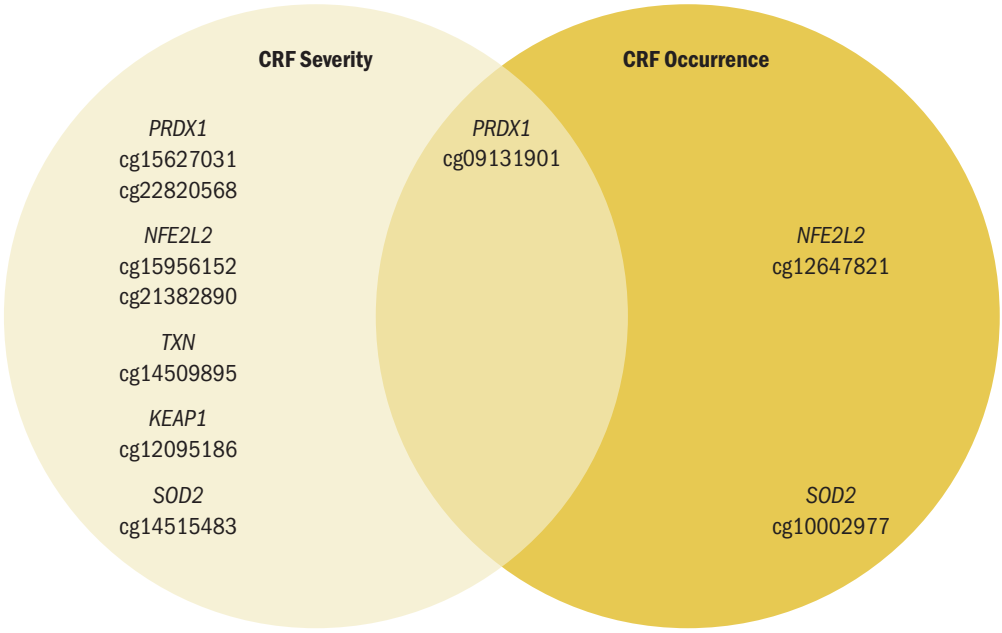
Although no associations were found with CRF severity, significant associations were found for two SNPs (rs2706110 in nuclear factor erythroid 2–like 2 [*NFE2L2*] and rs10432782 in superoxide dismutase 1 [*SOD1*]) with CRF occurrence (see Table 5). All of the SNP results for CRF occurrence and severity are presented in Supplemental Tables 7 and 8 online, respectively.

TABLE 6. Significant meQTL (SNP rs2706110 in *NFE2L2*) and Associated CpG Sites Unadjusted Linear Regression Results

CpG Site	Position	b	95% CI	p	FDR
cg08334199	178129847	–0.082	[–0.15, –0.02]	0.017	0.488
cg09248506	178097269	0.103	[0.02, 0.19]	0.023	0.488
cg05303734	178101031	0.079	[0, 0.15]	0.029	0.488
cg03988329	178128667	–0.139	[–0.27, –0.01]	0.038	0.488
cg01417537	178093274	0.12	[0.00, 0.24]	0.047	0.488

b—beta coefficient; CI—confidence interval; CpG—cytosine-phosphate-guanine; FDR—false discovery rate; meQTL—methylation quantitative trait locus; *NFE2L2*—nuclear factor erythroid 2–like 2; SNP—single nucleotide polymorphism

FIGURE 1. Significant CpG Sites Associated With CRF Severity and Occurrence



CpG—cytosine-phosphate-guanine; CRF—cancer-reated fatigue; *KEAP1*—Kelch-like ECH-associated protein 1; *NFE2L2*—nuclear factor erythroid 2–like 2; *PRDX1*—peroxiredoxin 1; *SOD2*—superoxide dismutase 2; *TXN*—thioredoxin reductase 1

meQTL Analysis Results

The two SNPs that were significantly associated with CRF occurrence (rs2706110 in *NFE2L2* and rs10432782 in *SOD1*) were tested for local CpG sites. Local CpG sites were any CpG sites within 2,000 base pairs up- and downstream from the gene. *NFE2L2* rs2706110 was identified to be an meQTL for the following five local *NFE2L2* CpG sites: cg01417537, cg09248506, cg08334199, cg05303734, and cg03988329 (see Table 6). No meQTLs were found for *SOD1* rs10432782.

Discussion

To the knowledge of this research team, this study is the first to evaluate for associations between Nrf2-ARE genes and CRF in women with early-stage postmenopausal hormone receptor–positive breast cancer. The most notable finding is the association between CRF and *NFE2L2*. In particular, rs2706110 in *NFE2L2* was associated with CRF occurrence. In addition, this SNP was identified as an meQTL for the following five CpG sites in this gene: cg01417537, cg09248506, cg08334199, cg5303734, and cg03988329. In addition, the CpG site *PRDX1* (cg09131901) was found to be significantly associated with both CRF occurrence and severity (see Figure 1).

NFE2L2

The SNP rs2706110 in *NFE2L2* was significantly associated with CRF occurrence and was found to be an meQTL for five CpG sites. For CRF occurrence, three of these CpG sites were hypermethylated (cg01417537, cg09248506, cg05303734) and two were hypomethylated (cg08334199 and cg03988329). These CpG sites are visualized in a map of *NFE2L2* that demonstrates the distribution of these sites within the gene (see Figure 2).

Nuclear factor erythroid 2–related factor 2 (Nrf2), encoded by *NFE2L2*, is an important transcription factor that binds to antioxidant response elements to regulate various detoxifying and antioxidant defense genes (Moon & Giaccia, 2015). Nrf2 is a master regulator for several genes that contain antioxidant response element enzymes involved in the redox cycle (e.g., peroxiredoxin, thioredoxin, catalase, superoxide dismutase). These enzymes are responsible for mediating the reduction of reactive oxygen species to prevent oxidative stress–induced cell damage (Moon & Giaccia, 2015; Yamamoto et al., 2018). When reactive oxygen species are not regulated by the redox cycle, oxidative stress may occur and lead to DNA damage and, in some cases, tumorigenesis. In contrast, when Nrf2 is upregulated, it

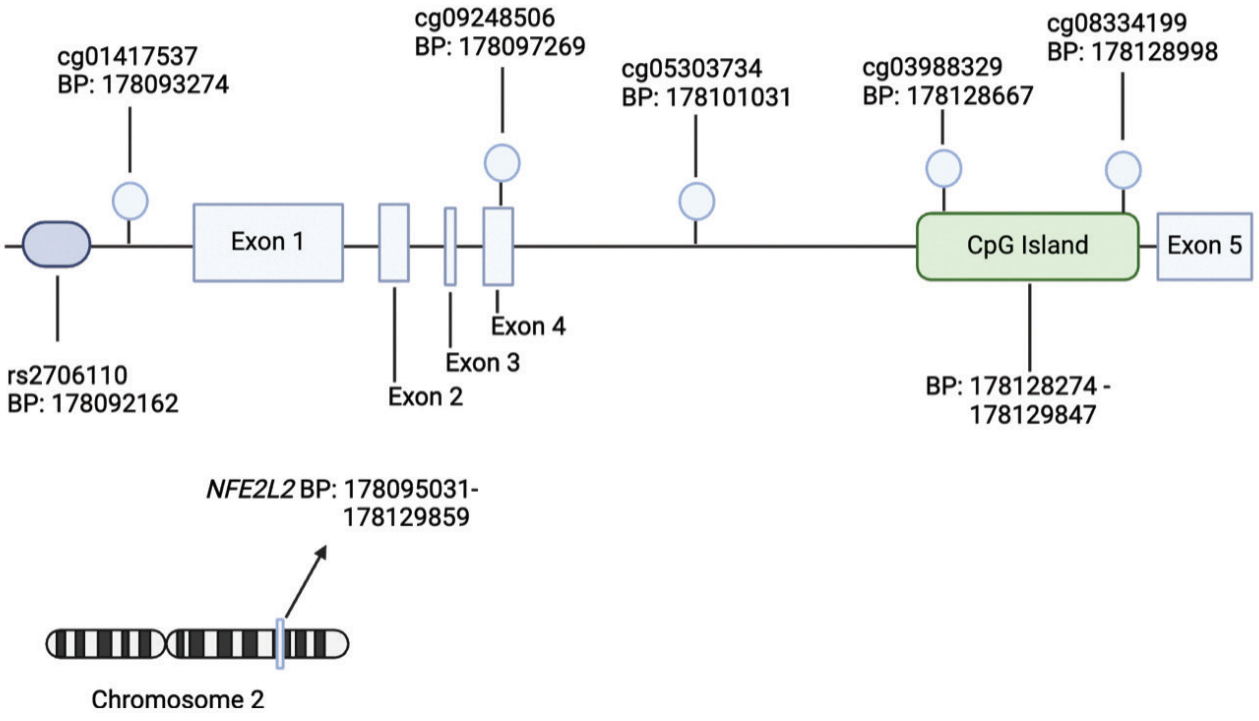
has protective effects and may prevent the initiation of tumor development caused by oxidative stress–induced injury in healthy cells (Hayes et al., 2020; Wu et al., 2019). On the other hand, upregulation of Nrf2 in malignant cells may be protective for these cells and promote tumor progression. In previous studies (Hartikainen et al., 2012; Karihtala & Soini, 2007), higher levels of antioxidative enzymes were found to be present in tumor tissue compared to nontumor tissue. These findings suggest that depending on the tissue of origin, Nrf2 may be pro- or antitumorigenic.

Although no studies have evaluated the relationship between rs2706110 and CRF, Hartikainen et al. (2012) investigated its effect in the context of breast cancer. In this study, survivors with the rs2706110 AA genotype had an increased risk of breast cancer (Hartikainen et al., 2012). In the current study, the rs2706110 TT genotype was associated with a 3.5-fold increase in odds of CRF occurrence (odds ratio = 3.54, 95% confidence interval [1.28, 11.07], p = 0.02). This finding suggests that this SNP may play a role in the occurrence of CRF in patients with breast cancer.

PRDX1 cg09131901

cg09131901 in peroxiredoxin 1 (*PRDX1*) is a CpG site that was associated with CRF occurrence and severity. Peroxiredoxin enzymes, including peroxiredoxin 1, which is encoded by *PRDX1* (Neumann et al., 2003), are responsible for more than 90% of the detoxification of the cellular reactive oxygen species, particularly hydrogen peroxide (H_2O_2) (Hayes et al., 2020). Peroxiredoxin 1 is primarily involved in scavenging for H_2O_2 and converting it into water and oxygen, blocking the circulation of reactive oxygen species (Hayes et al., 2020). When H_2O_2 is at extreme levels, the system becomes overwhelmed and inactivates *PRDX1*. This action results in the inability of cells to regulate oxidative stress efficiently (Wood et al., 2003). This process creates chronic levels of H_2O_2, and results in the progression and metastasis of breast cancer (Ding et al., 2017). This finding in the current study suggests the importance of the protective role

FIGURE 2. Significant meQTL (SNP rs2706110 in *NFE2L2*) and Associated CpG Sites



BP—base pair; CpG—cytosine-phosphate-guanine; meQTL—methylation quantitative trait locus; *NFE2L2*—nuclear factor erythroid 2–like 2; SNP—single nucleotide polymorphism
Note. Image created by authors using BioRender.com.

of the *PRDX1* gene and may also be important for understanding the occurrence and severity of CRF in patients with breast cancer.

Oxidative Stress and Fatigue

A study by Richards et al. (2000) suggests that oxidative stress overabundance may contribute to myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) and its symptom presentation. Like CRF, ME/CFS is a complex condition that involves multiple symptoms. In addition to fatigue, some patients with CRF and ME/CFS report sleep disturbance, pain, cognitive dysfunction, and mood disturbance (Aoun Sebaiti et al., 2022). In a study that compared patients with and without chronic fatigue (Lee et al., 2018), serum levels of antioxidative proteins regulated by *NFE2L2* (i.e., catalase and superoxide dismutase) were significantly lower in patients with idiopathic chronic fatigue. This study suggests that *NFE2L2* (Nrf2) and downstream targeted proteins (e.g., peroxiredoxin, thioredoxin, superoxide dismutase) have a role in fatigue.

Strengths and Limitations

Some strengths of this study include capitalizing on data from a single-blinded randomized clinical trial, as well as having access to existing whole-genome methylation data and banked serial blood samples to generate additional omics data (i.e., SNPs). Also, the data and methods used in this study were novel in the context of CRF. Although the current study has many strengths, it does have limitations. The sample size was relatively small, primarily comprised postmenopausal women who reported their race as White, and consisted of women recruited from western Pennsylvania. Therefore, the generalizability of these findings is limited. Replication of these findings is warranted in a larger and more diverse sample.

Conclusion and Implications for Nursing

The findings from this study add to the understanding of the biologic mechanisms underlying variability in CRF occurrence and severity among patients with breast cancer. This study sheds light on the importance of the genes involved in the Nrf2-ARE pathway and patient-reported CRF, which is a novel finding. If replicated, these results have the potential to identify patients who are most at risk for the development of CRF early in the disease trajectory. Knowing which patients with cancer are at risk for development of CRF, particularly severe CRF, and knowing what intervention could target its underlying mechanism(s) could assist with clinical management and improved quality of life for patients with cancer.

KNOWLEDGE TRANSLATION

- Understanding the biology of cancer-related fatigue (CRF) in the early stages of breast cancer or within the context of its treatment may influence future nursing practice, including symptom assessments and patient education about how to manage CRF.
- These results may contribute to future targeted and early interventions for CRF, including regular exercises (e.g., aerobic exercise, strength training, mindfulness) that are known to mitigate CRF in patients with cancer.
- Genes in the nuclear factor E2–related factor 2 antioxidative response elements signaling pathway may be important to the biology of CRF in patients with breast cancer.

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