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ORIGINAL RESEARCH

An exploratory study of host polymorphisms in genes that clinically characterize breast cancer tumors and pretreatment cognitive performance in breast cancer survivors

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Purpose: Inspired by the hypothesis that heterogeneity in the biology of breast cancers at the cellular level may account for cognitive dysfunction symptom variability in survivors, the current study explored relationships between host single-nucleotide polymorphisms (SNPs) in 25 breast cancer-related candidate genes (*AURKA*, *BAG1*, *BCL2*, *BIRC5*, *CCNB1*, *CD68*, *CENPA*, *CMC2*, *CTSL2*, *DIAPH3*, *ERBB2*, *ESR1*, *GRB7*, *GSTM1*, *MELK*, *MKI67*, *MMP11*, *MYBL2*, *NDC80*, *ORC6*, *PGR*, *RACGAP1*, *RFC4*, *RRM2*, and *SCUBE2*), identified from clinically relevant prognostic multigene-expression profiles for breast cancer, and pretreatment cognitive performance.
Patients and methods: The sample (n=220) was comprised of 138 postmenopausal women newly diagnosed with early stage breast cancer and 82 postmenopausal age- and education-matched healthy controls without breast cancer. Cognitive performance was assessed after primary surgery but prior to initiation of adjuvant chemotherapy and/or hormonal therapy using a comprehensive battery of neuropsychological tests encompassing eight cognitive function composite domains: attention, concentration, executive function, mental flexibility, psychomotor speed, verbal memory, visual memory, and visual working memory. In total, 131 SNPs were included in the analysis. Standard and robust multiple linear regression modeling was used to examine relationships between each domain and the presence or absence of one or more minor alleles for each SNP. Genetic risk/protection scores (GRSs) were calculated for each domain to evaluate the collective effect of possession of multiple risk/protective alleles.
Results: With the exception of *CMC2*, *MMP11*, and *RACGAP1*, significant ($P<0.05$) SNP main effect and/or SNP by future prescribed treatment group interactions were observed for every gene between at least one domain and one or more SNPs. All GRSs were found to be significantly ($P<0.001$) associated with each respective domain score.
Conclusion: Associations between host SNPs and computed GRSs and variability in pretreatment cognitive function performance support the study hypothesis, and warrant further investigations to identify biomarkers for breast cancer-related cognitive dysfunction.
Keywords: breast neoplasms, genetics, cognition, biomarkers

Introduction

The recently published American Cancer Society/American Society of Clinical Oncology Breast Cancer Survivorship Care Guideline includes "assessment and management of physical and psychosocial long-term and late effects of breast cancer (BC) and treatment" as one of the five key areas of BC survivorship.[1] Cognitive impairment related to cancer and cancer treatments is included in the guideline as a common and detrimental symptom experienced by BC survivors that can result in

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"distress and impaired [quality of life]".[1] While assessment and management of cognitive dysfunction in BC survivors by clinicians are recommended, the guideline acknowledges that the causes of and treatment for cognitive dysfunction are not well established.[1] The guideline does not include recommendations for clinicians on how to predict which survivors will experience cognitive difficulties, the severity of the difficulties, or the duration of the impairment either.

The lack of biomarkers available to enhance precision survivorship care is in stark contrast to those that have been developed to refine outcome prediction and selection of optimal therapy for BC. Specifically, the introduction of advanced genetic technologies into patient care has greatly enriched the cellular-level characterization of breast neoplasms and led to the development of clinically relevant prognostic multigene-expression profiles for BC. Briefly, prognostic multigene-expression profiles for BC use tumor gene-expression algorithm-driven estimation to enrich prediction of long-term BC outcomes, including recurrence or metastasis, and/or benefit of adjuvant therapies.

Considering that many investigators theorize that cognitive difficulties, especially prior to adjuvant chemotherapy and/or hormonal therapy, are related to the cancer itself,[2–5] we propose the use of BC-related genetic biomarkers for cognitive dysfunction symptom prediction and hypothesize that heterogeneity in BCs at the cellular level may account for variability in cognitive performance within the context of BC. Because genes utilized in multigene-expression profiles for BC contribute to characterizing BCs at the cellular level in relation to aggressiveness and risk of progression, they represent ideal candidate genes for a biomarker study to test our hypothesis.

The potential use of BC-related genetic markers to account for cognitive difficulties among BC survivors is not without evidence. A growing number of studies are investigating associations between host genetic variability and alterations in cognitive performance in women diagnosed with and receiving treatment for BC. Four published investigations have reported relationships between *APOE* and cognitive performance in women with BC.[6–9] Associations with polymorphisms in genes involved in the dopamine and serotonin (*ANKK1*, *BDNF*, *COMT*, *MTHFR*, and *SLC64A*)[9–11] and DNA repair and oxidative stress (*CAT*, *ERCC2*, *ERCC3*, *ERCC5*, *GPX1*, *PARP1*, *SEPP1*, *SOD1*, and *SOD2*)[12] pathways have also been reported. However, to the best of our knowledge, no previous investigations have focused on BC-related genes as potential biomarkers for cognitive performance in women with BC.

To summarize, based on our hypothesis that heterogeneity in BCs at the cellular level may account for variability in cognitive performance within the context of BC, this study was conducted to explore the contribution of host polymorphisms within candidate genes and their regulatory regions known to differentiate BC heterogeneity at the cellular level to pretreatment (ie, postsurgery, preadjuvant therapy) cognitive performance in postmenopausal women diagnosed with BC.

Patients and methods

Participants

The sample (n=220) for this exploratory, genetic-association study was comprised of 138 postmenopausal women newly diagnosed with stage 1, 2, or 3A BC with no evidence of metastases and 82 postmenopausal age- and education-matched healthy controls (HCs) without BC. Participants were initially enrolled in a study examining the effects of the adjuvant antiestrogen therapy, anastrozole ± chemotherapy on cognitive function in postmenopausal women diagnosed with BC prior to, throughout, and following the antiestrogen-therapy regimen.[13] Women diagnosed with BC were recruited from the Comprehensive BC Program of the University of Pittsburgh Cancer Institute. HC participants were obtained via referral from participants diagnosed with BC, advertisements, and random-digit dialing through the University Center for Social and Urban Research. All study participants were 75 years of age or younger, able to speak and read English, and had completed a minimum of 8 years of education. Participants were excluded if they had a prior history of neurologic disease or cancer or had been hospitalized for psychiatric illness within the past 2 years. For this study, in order to account for the heterogeneity of BC tumors, women diagnosed with BC were further classified using prescribed future-treatment regimen as a surrogate for disease characteristics. Therefore, the analysis included two cohorts of women diagnosed with BC – those prescribed chemotherapy followed by anastrozole (prescribed C+A) (n=55) and those prescribed anastrozole only (prescribed AO) (n=83) – as well as a cohort of HC women (n=82). All participants provided written informed consent for study participation. Both the current genetic ancillary study and the parent study were approved by the University of Pittsburgh Institutional Review Board.

Candidate-gene selection

A total of 25 biologically plausible candidate genes that are theorized to characterize the biology of BC at the cellular

level through utilization in prognostic multigene-expression profiles for BC were selected for investigation. Detailed rationale for selection and biological plausibility of candidate genes has been discussed previously.[5] Prognostic multigene-expression profiles for BC use tumor gene-expression algorithm-driven estimation to enrich prediction of long-term cancer outcomes (ie, recurrence or metastasis) and/or benefit of adjuvant therapy. A number of multigene-expression profiles for BC have been developed and include: the eleven-gene expression signature (Breast Cancer Index℠; Biotheranostics, San Diego, CA, USA),[14] the 14-gene prognostic expression signature (described in Tutt et al),[15] the 21-gene BC assay (Oncotype DX® Breast Cancer Assay; Genomic Health, Redwood City, CA, USA),[16,17] the 50-gene BC prognostic gene-signature assay (Prosigna® Breast Cancer Prognostic Gene Signature Assay; NanoString® Technologies Inc, Seattle, WA, USA) based on the PAM50 Breast Cancer Intrinsic Classifier,[18] and the 70-gene BC-recurrence assay (MammaPrint® 70-gene Breast Cancer Recurrence Assay; Agendia®, Irvine, CA, USA).[19,20] While the profiles vary in the number of genes utilized, patient-eligibility criteria, and specific prognostic goal, genes included in these profiles play an important role in characterizing the biology of BC at the cellular level to address aggressiveness and risk of progression, and thus point to ideal candidates for an initial investigation of the study hypothesis.

A total of 21 of the 25 candidate genes (*BAG1*, *BCL2*, *BIRC5*, *CCNB1*, *CENPA*, *CMC2*, *DIAPH3*, *ERBB2*, *ESR1*, *GRB7*, *MELK*, *MKI67*, *MMP11*, *MYBL2*, *NDC80*, *ORC6*, *PGR*, *RACGAP1*, *RFC4*, *RRM2*, and *SCUBE2*) were prioritized for this investigation, based on duplication in two or more of the previously named multigene-expression profiles.[12] Because the 21-gene BC assay is currently the most widely used profile in the US, the four remaining cancer genes used as part of this assay but not duplicated in another profile (*AURKA*, *CD68*, *CTSL2*, and *GSTM1*) were also prioritized.

Single-nucleotide polymorphism (SNP) selection

SNPs representing each candidate gene were selected. Functional or putatively functional (ie, known to influence expression levels, associated with BC, or associated with a cognitive phenotype) polymorphisms within or directly upstream of candidate genes were identified from the literature. When a functional polymorphism was not identified and/or did not fully represent the gene of interest, tagging SNPs were selected using the Phase III HapMap database. Because the profiles from which candidate genes were selected rely upon gene-expression data, evaluation of DNA variability was extended ±2,500 bps beyond the gene to capture the UTR5' and UTR3' regulatory regions. Initial criteria for selection of tagging SNPs were as follows: $R^2 \geq 0.8$, minor allele frequency (MAF) ≥0.2, and selected for Caucasian ancestry, which represented the majority of study participants. The MAF criterion was ultimately relaxed to identify tagging SNPs for *CTSL2*, *GRB7*, *MELK*, *MMP11*, and *RACGAP1*. In addition, select polymorphisms in *MIR125A*,[21,22] *CCDC170*,[23–27] and *NFE2L2*[28] were included to represent more fully *ERBB2*, *ESR1*, and *GSTM1*, respectively. In total, 163 functional and tagging SNPs were identified.

Genotype data collection and quality control

Samples (3 mL of whole blood or 2 mL of saliva) were obtained for genotyping. DNA was extracted from peripheral blood leukocytes using a simple salting-out procedure[29] or from saliva following the protocol and reagents supplied with Oragene® DNA-collection kits.[30] The iPlex® MassArray platform (Sequenom, San Diego, CA, USA) was used as the primary genotyping method for this study. SNPs not conducive to genotyping with the iPlex platform were genotyped using TaqMan® allelic discrimination with the ABI Prism 7000 Sequence Detection System (SDS) and SDS software version 1.2.3 (Thermo Fisher Scientific, Waltham, MA, USA) or using a restriction fragment-length polymorphism–polymerase chain reaction approach.

Negative controls were included with all analyses. Genotypes were double-called by individuals blinded to participant phenotypes, and discrepancies were addressed by reviewing raw data or regenotyping. Participant genotypes were classified for data analysis based on the presence or absence of the minor allele (MA) (homozygous wild type compared to the combination of heterozygotes and homozygous-variant genotypes).

SNPs with call rates less than 90% or MAFs of less than 0.05 were omitted. For SNPs not meeting the 90% call-rate threshold but deemed essential for inclusion in the study (due to functional consequence, location within a candidate gene, or lack of alternative SNPs available within a given gene), secondary genotyping approaches were attempted. Alternative SNPs in linkage disequilibrium were selected for essential SNPs in instances of multiple failed genotyping attempts and/or lack of availability of alternative genotyping methods. Each SNP was tested for Hardy–Weinberg equilibrium (HWE) using χ^2 goodness-of-fit or Fisher's exact tests to identify potential genotyping errors.

Pretreatment cognitive function evaluation

Cognitive performance was assessed using a comprehensive battery of neuropsychological tests encompassing eight cognitive function composite domains:

- attention – Cambridge Neuropsychological Test Automated Battery (CANTAB) Rapid Visual Information Processing Test[31]
- concentration – Digit Vigilance Test[32]
- executive function – CANTAB Stockings of Cambridge[31] and CANTAB Spatial Working Memory[31]
- mental flexibility – Delis Kaplan Executive Function System Color–Word Interference Test[33]
- psychomotor speed – Grooved Pegboard[34] and Digit Symbol Substitution Test[35]
- verbal memory – Rey Auditory Verbal Learning Test,[36] Verbal Fluency Test, and Rivermead Story Test[37]
- visual memory – CANTAB Paired Associates Learning[31] and Rey Complex Figure Test[38]
- visual working memory – CANTAB Stockings of Cambridge[31] and Rey Complex Figure Test.[38]

Women with BC completed the battery after surgery, but before initiation of prescribed C+A or AO adjuvant-therapy regimens. HCs completed the same neuropsychological test battery. Specifics related to the battery, creation of composite cognitive function domains, and *z*-score calculation have been reported previously.[13] Please note that more negative *z*-scores designate poorer performance. Age (in years), estimated verbal intelligence (National Adult Reading Test – revised),[39] depressive symptoms (Beck Depression Inventory II),[40] anxiety (Profile of Mood States Tension–Anxiety subscale),[41] fatigue (Profile of Mood States Fatigue–Inertia subscale),[41] and current pain at time of assessment (Brief Pain Inventory)[42] were also recorded.

Statistical analysis

Stata version 14.1 (StataCorp, College Station, TX, USA) and Statistical Package for the Social Sciences (SPSS) versions 23 and 24 (IBM Corporation, Armonk, NY, USA) were used to perform statistical analyses. Descriptive statistics were computed. Standard and robust multiple linear regression modeling was used to examine relationships between each domain and the presence (ie, homozygous-variant genotype plus heterozygous genotype) or absence (ie, homozygous wild-type genotype) of one or more MAs for each SNP. Both main SNP effects only and SNP–prescribed treatment group-interaction effect-regression models were fitted. In all models, HCs served as the reference group for the two prescribed treatment groups (ie, prescribed C+A or prescribed AO). Similarly, the wild-type genotype served as the reference group for possession of one or more MAs. All models were adjusted for age, estimated intelligence, and levels of depressive symptoms, anxiety, fatigue, and pain, and prescribed treatment group. Underlying assumptions were assessed. To lessen the impact of potentially influential points and adjust for heteroscedasticity, robust regression (generated using Huber weighting and biweighting iterations) model estimated regression coefficients and significance levels are reported.

Genetic risk/protection scores (GRSs) for each domain were then calculated to explore the influence of possession of multiple significant ($P<0.05$) genotypes on domain scores, as previously described.[12] SNP MAs that were significantly ($P<0.05$) negatively or positively associated with a domain by either SNP main effects and/or SNP–prescribed treatment interaction effects were used in GRS calculations. A weighted calculation method, in which unstandardized robust regression coefficients from the individual models were multiplied by 0 (absence) or 1 (presence), based on a participant's genotype and prescribed treatment-group membership and then summed, was used to assign greater risk/protection to MAs with stronger associations. A lower GRS conveys greater genetic risk for poorer cognitive function, and a higher GRS conveys greater genetic protection. GRSs were added as the final predictor to standard and robust multiple linear regression models adjusted for age, estimated verbal intelligence, levels of depressive symptoms, anxiety, fatigue, pain, and prescribed treatment group. Only participants with all genetic data necessary for calculation of a GRS were included in the GRS analysis.

Results

Participant characteristics

A total of 220 participants (n=55 prescribed C+A, n=83 prescribed AO, and n=82 HC) had genetic and complete covariate/confounder information and cognitive function scores available for one or more domains. A summary of overall demographic, covariate/confounder, and cognitive function data for participants included in this analysis can be found in Table 1.

Cohorts (ie, prescribed C+A, prescribed AO, and HC) differed statistically, yet not clinically meaningfully, by age and estimated verbal intelligence (Table 1). The groups also differed by level of anxiety ($P=0.003$), with women with BC prescribed C+A having higher mean pretreatment

Table 1 Participant characteristics

Characteristics	Total			By cohort			
	Mean ± SD or n (%)	Minimum	Maximum	Prescribed C+A, n=55	Prescribed AO, n=83	Healthy controls, n=82	*F*-test[a] or χ^2/Fisher's exact test[b]
Age (years)	60.02±6.086	43	75	58.76±5.467	62.47±5.964	58.39±5.858	*P*<0.001*
Education (years)	15.12±2.97	9	29	15.67±2.783	14.95±3.056	14.93±2.993	*P*=0.285
Estimated verbal intelligence (NART-R)	110.39±9.113	82.42	127.81	108.94±8.871	107.04±8.844	114.74±7.796	*P*<0.001*
Depressive symptoms (BDI-II)	4.83±4.957	0	29	5.24±4.615	4.6±4.65	4.79±5.495	*P*=0.757
Anxiety (POMS Tension-Anxiety subscale)	7.47±5.536	0	27	9.61±6.14	6.97±4.654	6.55±5.619	*P*=0.003*
Fatigue (POMS Fatigue-Inertia subscale)	5.61±5.942	0	27	5.11±5.329	5.84±6.352	5.72±5.955	*P*=0.763
Pain (BPI pain right now)	1.3±2.126	0	9	1.47±1.961	1.55±2.265	0.93±2.059	*P*=0.13
Marital status (currently married or living with significant other)	139 (63.2)	N/A	N/A	38 (69.1)	54 (65.1)	47 (57.3)	*P*=0.348
Number of children	2±1.403	0	8	1.75±1.22	2.05±1.387	2.13±1.522	*P*=0.266
Race (Caucasian)	209 (95)	N/A	N/A	52 (94.5)	81 (97.6)	76 (92.7)	*P*=0.305
Cognitive function composite Z-scores							
Attention, n=219	–0.107±0.94939	–4.02	1.7	–0.052±0.937	–0.202±1.017	–0.047±0.889	*P*=0.513
Concentration, n=219	–0.056±0.8317	–2.2	2.5	–0.204±0.667	–0.01±0.904	–0.005±0.85	*P*=0.322
Executive function, n=220	–0.2357±0.64539	–1.69	2.41	–0.218±0.599	–0.49±0.509	0.01±0.705	*P*<0.001*
Mental flexibility, n=219	0.0965±0.75203	–3.64	1.73	0.164±0.656	0.09±0.786	0.055±0.783	*P*=0.707
Psychomotor speed, n=220	–0.0548±0.88616	–3.67	1.22	0.071±0.845	–0.24±0.954	0.048±0.819	*P*=0.054
Verbal memory, n=220	–0.1087±0.72263	–1.77	1.67	0.018±0.662	–0.341±0.638	0.041±0.786	*P*=0.001*
Visual memory, n=220	0.0832±0.68602	–4.63	0.86	0.287±0.352	0.009±0.708	0.022±0.803	*P*=0.038*
Visual working memory, n=220	0.0358±0.77624	–3.02	1.33	0.299±0.514	–0.064±0.741	–0.039±0.913	*P*=0.014*

Notes: *P<0.05. [a]One-way ANOVAs utilized to compare mean values of continuous variables; [b]Pearson's χ^2 tests of independence, Fisher's exact test, or Fisher's exact test computed using two-sided Monte Carlo sampling based on 10,000 sampled tables used to examine associations between categorical variables. Only participants with complete confounder/covariate information were included in the participant-characteristic statistics.

Abbreviations: ANOVA, analyses of variance; AO, anastrozole only; BDI, Beck Depression Inventory; BPI, Brief Pain Inventory; C+A, chemotherapy plus anastrozole; NART-R, National Adult Reading Test – revised; POMS, Profile of Mood States; SD, standard deviation; N/A, not applicable.

anxiety levels (9.61±6.14) than women with BC prescribed AO (6.97±4.654) and HCs (6.55±5.619). Comparison of tumor features by prescribed treatment group confirmed expected differences in disease characteristics (Table 2). To summarize, women with BC prescribed C+A had higher frequencies of American Joint Committee on Cancer Stage 2A, 2B, and 3A BCs, larger mean tumor size, higher mean number of positive lymph nodes, higher mean Nottingham Score, greater frequency of lymphovascular invasion, lower ER H-score, greater frequency of HER2-positive cancer, higher mean Ki67 index, and higher mean Oncotype DX® BC Assay Recurrence Score® compared to women with BC prescribed AO.

No differences in covariates/confounders or pretreatment cognitive function *z*-scores were observed between HCs included in this ancillary genetic analysis and those enrolled in the parent study but not included in the genetic analysis (n=82). Women with BC prescribed AO included in the genetic analysis did have slightly lower (*P*=0.044) mean estimated verbal intelligence (107.04±8.844) than those enrolled in the parent study but not included in the genetic analysis (n=155, 109.42±8.542). Also, women with BC prescribed C+A included in the genetic analysis had higher mean pretreatment verbal (*P*=0.014, 0.02±0.662), visual (*P*=0.006, 0.29±0.352), and visual working (*P*=0.002, 0.3±0.514) memory performance *z*-scores compared to those enrolled in the parent study but not included in the genetic analysis (n=78; –0.28±0.697, 0.03±0.615, –0.07±0.746, respectively).

Candidate-gene analysis

Of the 163 SNPs originally identified, 32 SNPs that were not amenable to multiplexing, had call rates less than 90%, or study MAFs of less than 0.05 were excluded. Alternatives were selected for three essential SNPs. In total, 131 SNPs were included in the genetic analysis (Table 3). Genotyping call rates for these SNPs ranged from 90% to 100%. When considering all study participants, six SNPs were not

Table 2 Tumor characteristics by study cohort

Characteristics, mean ± SD or n (%)	Prescribed C+A, n=55	Prescribed AO, n=83	*F*-test[a] or χ^2/Fisher's exact test[b]
AJCC tumor stage, n=130			
Stage I	22 (44)	65 (81.3)	*P*<0.001*
Stage 2A	17 (34)	13 (16.3)	
Stage 2B	6 (12)	2 (2.5)	
Stage 3A	5 (10)	0	
Primary tumor size (cm), n=129	2.16±1.484	1.23±0.709	*P*<0.001*
Lymph-node status, n=129			
Positive	19 (38)	5 (6.3)	*P*<0.001*
Negative	31 (62)	74 (93.7)	
Number of positive nodes, n=130	0.94±1.789	0.06±0.244	*P*<0.001*
Invasive type, n=129			
Ductal	45 (90)	63 (79.7)	*P*=0.323
Lobular	5 (10)	14 (17.7)	
Ductal and lobular	0	2 (2.5)	
Nottingham score, n=125	6.60±1.370	5.72±1.122	*P*<0.001*
Nottingham grade, n=125			
Grade I	9 (18)	27 (36)	*P*<0.001*
Grade 2	26 (52)	44 (58.7)	
Grade 3	15 (30)	4 (5.3)	
ER status, n=130			
Positive	48 (96)	80 (100)	*P*=0.146
Negative	2 (4)	0	
ER H-score, n=124	240.08±73.684	265.87±44.592	*P*=0.017*
PR status, n=130			
Positive	38 (76)	71 (88.8)	*P*=0.055
Negative	12 (24)	9 (11.3)	
PR H-score, n=124	110.35±101.612	129.69±97.208	*P*=0.289
HER2 status, n=125			
Positive	9 (19.1)	4 (5.1)	*P*=0.017*
Negative	38 (80.9)	74 (94.9)	
LV invasion, n=127			
Present	21 (42.9)	6 (7.7)	*P*<0.001*
Absent	28 (57.1)	72 (92.3)	
Ki67 classification, n=68			
Low	10 (38.5)	18 (42.9)	*P*=0.114
Moderate	5 (19.2)	14 (33.3)	
High	6 (23.1)	9 (21.4)	
Very high	5 (19.2)	1 (2.4)	
Ki67 index, n=68	28.73±26.834	17.31±13.337	*P*=0.022*
Oncotype DX® Breast Cancer Assay Recurrence Score®, n=74	26.52±9.774	14.63±6.174	*P*<0.001*

Notes: *$P<0.05$. [a]One-way ANOVAs utilized to compare mean values of continuous variables; [b]Pearson's χ^2 tests of independence, Fisher's exact test, or Fisher's exact test computed using two-sided Monte Carlo sampling based on 10,000 sampled tables used to examine associations between categorical variables. Only participants with complete confounder/covariate information were included in the participant-characteristic statistics.

Abbreviations: ANOVA, analyses of variance; AO, anastrozole only; C+A, chemotherapy plus anastrozole; HER2, human epidermal growth factor receptor 2; AJCC, American Joint Committee on Cancer; ER, estrogen receptor; PR, progesterone receptor; IHC, immunohistochemistry; LV, lymphovascular; SD, standard deviation.

in HWE: *CTSL2* rs4361859 (*P*=0.0078), *ESR1* rs2234693 (*P*=0.0344), *ORC6* rs33994299 (*P*=0.0051), *PGR* rs1042838 (*P*=0.0466), *PGR* rs1042839 (*P*=0.0103), and *PGR* rs474320 (*P*=0.0434). In HC women alone, *PGR* rs1042838 (*P*=0.016), *PGR* rs1042839 (*P*=0.0027), and *PGR* rs474320 (*P*=0.0329) still did not meet HWE. We attributed the deviations from HWE to nonrandom sampling of study participants from the population leading to enrichment for BC in the cases and de-enrichment for BC in the controls for these genes known to be involved in BC.

Individual polymorphisms significantly ($P<0.05$) associated with a domain by either SNP main effects or SNP–prescribed treatment group-interaction effects are summarized by domain in Table 4. Overall, significant

Table 3 SNPs included in genetic regression analyses (n=220)

Gene SNP	Wild-type/ variant allele[a]	n	MAF	HWE[b]
AURKA				
rs1047972	G/A	219	0.148	*P*=1
rs16979877	A/G	207	0.08	*P*=0.3725
rs2273535	A/T	213	0.216	*P*=0.9788
rs6064389	G/T	219	0.445	*P*=0.2279
BAG1				
rs706118	T/G	214	0.248	*P*=0.1553
BCL2				
rs1564483	G/A	207	0.271	*P*=0.6855
rs17759659	A/G	219	0.425	*P*=0.8885
rs2279115	A/C	206	0.459	*P*=0.7047
rs4941195	C/A	218	0.429	*P*=0.423
rs4987852	A/G	218	0.078	*P*=1
rs4987853	A/G	217	0.189	*P*=0.5787
rs4987855	G/A	220	0.071	*P*=0.6073
rs956572	G/A	211	0.398	*P*=0.6542
rs9807663	T/A	218	0.108	*P*=0.7216
BIRC5				
rs1042489	T/C	217	0.359	*P*=0.5481
rs1508147	G/A	218	0.358	*P*=0.5738
rs17878467	C/T	217	0.111	*P*=1
rs2239680	T/C	214	0.299	*P*=0.71
rs3764383	A/G	213	0.305	*P*=0.9577
rs8073069	G/C	207	0.249	*P*=0.9445
rs8073903	T/C	212	0.366	*P*=0.6934
rs9904341	G/C	206	0.318	*P*=0.7061
CCNB1				
rs164390	G/T	214	0.371	*P*=0.52
rs350099	T/C	216	0.396	*P*=0.5396
rs350104	T/C	219	0.459	*P*=0.564
CD68				
rs8066665	G/A	220	0.457	*P*=0.1667
rs9901673	C/A	218	0.172	*P*=0.0915
CENPA				
rs3806517	A/G	215	0.34	*P*=0.8111
rs3806518	T/C	214	0.278	*P*=0.8532
CMC2				
rs1025065	C/A	209	0.361	*P*=0.8277
rs1981867	C/T	220	0.307	*P*=0.1739
rs9936489	T/G	215	0.319	*P*=0.7119
CTSL2				
rs16919034	A/G	213	0.169	*P*=0.3501
rs4361859	A/G	219	0.327	*P*=0.0078*; *P*=0.0695[HC]
DIAPH3				
rs1337652	G/A	217	0.212	*P*=0.9194
rs4547237	A/G	220	0.307	*P*=0.8219
ERBB2				
rs1058808	G/C	217	0.373	*P*=0.2746
rs1136201	A/G	220	0.232	*P*=0.9465
rs1476278	A/G	220	0.357	*P*=0.9976
rs1810132	T/C	212	0.318	*P*=0.1537
rs2517955	T/C	220	0.373	*P*=0.4823
rs4252596	C/A	220	0.121	*P*=0.7482
rs903501	G/A	211	0.332	*P*=0.3885
rs9303274	C/T	219	0.356	*P*=0.9485
rs12976445(*MIR125A*)	T/C	220	0.298	*P*=0.1467
ESR1				
rs10484919	C/T	204	0.088	*P*=0.6574
rs1062577	T/A	215	0.081	*P*=0.1495
rs1964281	C/T	214	0.075	*P*=1
rs12173570	C/T	219	0.132	*P*=0.3857
rs12665044	C/T	213	0.132	*P*=0.7665
rs1514348	A/C	220	0.468	*P*=0.9519
rs1801132	C/G	220	0.232	*P*=0.4098
rs1884051	A/G	207	0.336	*P*=0.917
rs2046210	C/T	213	0.357	*P*=0.352
rs2071454	T/G	213	0.11	*P*=1
rs2077647	G/A	217	0.484	*P*=0.0643
rs2228480	G/A	218	0.188	*P*=0.2292
rs2234693	C/T	206	0.481	*P*=0.0344*; *P*=0.9104[HC]
rs2347867	A/G	215	0.34	*P*=0.5008
rs2744677	A/C	215	0.249	*P*=0.6319
rs2813543	G/A	213	0.181	*P*=0.9848
rs2813544	A/G	215	0.235	*P*=0.0512
rs2941740	T/C	220	0.391	*P*=0.4585
rs3020314	T/C	208	0.358	*P*=0.4848
rs3778099	T/C	208	0.089	*P*=1
rs3798577	T/C	220	0.475	*P*=0.277
rs488133	C/T	209	0.285	*P*=0.5101
rs532010	T/C	212	0.434	*P*=0.7636
rs6557171	C/T	218	0.303	*P*=0.7528
rs77275268	C/T	217	0.083	*P*=0.1827
rs7761133	T/C	216	0.153	*P*=0.584
rs7761846	T/C	204	0.054	*P*=0.4501
rs7766585	T/G	218	0.154	*P*=0.1375
rs7767143	A/G	214	0.243	*P*=0.0849
rs827421	C/T	212	0.467	*P*=0.1489
rs851967	G/A	216	0.308	*P*=0.638
rs851971	G/A	216	0.313	*P*=0.5073
rs851982	T/C	217	0.362	*P*=0.3178
rs851998	C/T	219	0.313	*P*=0.654
rs910416	T/C	220	0.491	*P*=0.9961
rs9322331	C/T	215	0.381	*P*=0.8339
rs9340799	A/G	213	0.397	*P*=0.4781
rs9383938	G/T	218	0.083	*P*=0.3721
rs9397435	A/G	220	0.073	*P*=0.6108
rs9397456	G/A	203	0.217	*P*=0.1522
rs985694	C/T	218	0.12	*P*=0.7731
rs1038304(*CCDC170*)	G/A	218	0.456	*P*=0.1394
rs12662670(*CCDC170*)	T/G	217	0.069	*P*=0.274
rs3734805(*CCDC170*)	A/C	213	0.075	*P*=0.2359
rs3757318(*CCDC170*)	G/A	213	0.059	*P*=0.3629
rs6929137(*CCDC170*)	G/A	216	0.319	*P*=0.3411

(*Continued*)

(*Continued*)

Table 3 (*Continued*)

Gene SNP	Wild-type/ variant allele[a]	n	MAF	HWE[b]
GRB7				
rs9910678	T/C	218	0.053	*P*=0.1079
GSTM1				
rs1065411	C/G	209	0.194	*P*=0.0884
rs412543	G/C	216	0.081	*P*=0.3714
rs35652124(*NFE2L2*)	T/C	218	0.298	*P*=0.2739
rs6721961(*NFE2L2*)	G/T	212	0.101	*P*=1
MELK				
rs10973007	C/G	209	0.189	*P*=0.8337
rs2250340	C/T	220	0.075	*P*=1
rs3780350	C/T	213	0.155	*P*=0.6424
MKI67				
rs10732438	A/G	211	0.367	*P*=0.1859
rs10764751	A/C	220	0.239	*P*=0.5706
MMP11				
rs131451	T/C	216	0.107	*P*=1
MYBL2				
rs11556379	C/G	220	0.05	*P*=0.4243
rs2070235	A/G	220	0.093	*P*=1
rs619289	C/T	216	0.197	*P*=0.7837
rs826943	T/C	213	0.146	*P*=0.7823
rs826944	C/T	219	0.142	*P*=1
NDC80				
rs12408485	A/G	203	0.382	*P*=0.4731
rs2292274	T/C	207	0.268	*P*=0.5054
ORC6				
rs33994299	T/C	220	0.475	*P*=0.0051*; *P*=0.1405^{HC}
PGR				
rs1042838	G/T	216	0.141	*P*=0.0466*; *P*=0.016^{HC,*}
rs1042839	C/T	208	0.13	*P*=0.0103*; *P*=0.0027^{HC,*}
rs10895068	G/A	214	0.063	*P*=1
rs11224561	C/T	214	0.119	*P*=0.746
rs1893505	C/T	220	0.382	*P*=0.7593
rs1942836	T/C	217	0.201	*P*=0.7604
rs471767	A/G	216	0.313	*P*=0.3574
rs474320	T/A	197	0.147	*P*=0.0434*; *P*=0.0329^{HC,*}
rs4754732	T/C	220	0.334	*P*=0.178
rs484389	T/C	212	0.217	*P*=0.6804
rs568157	A/G	219	0.493	*P*=0.312
rs590688	C/G	215	0.463	*P*=0.1662
rs608995	A/T	218	0.22	*P*=0.5724
RACGAP1				
rs7303531	G/A	214	0.058	*P*=1
RFC4				
rs1354091	A/C	214	0.238	*P*=0.9537
RRM2				
rs1138729	A/G	202	0.136	*P*=0.3821
rs4309551	C/T	218	0.452	*P*=0.8925
rs4668664	G/A	215	0.263	*P*=0.685
SCUBE2				
rs1136966	T/G	213	0.211	*P*=0.8348
rs4910440	C/T	219	0.47	*P*=0.4879
rs6486125	A/G	207	0.266	*P*=0.198

Notes: *$P<0.05$. [a]Wild-type and variant alleles based on study sample; [b]χ^2 goodness-of-fit or exact-test *P*-value.
Abbreviations: HC, healthy control; HWE, Hardy–Weinberg equilibrium; MAF, minor allele frequency; SNP, single-nucleotide polymorphism.

relationships were noted between at least one domain and one or more polymorphisms of all candidate genes, except *CMC2*, *MMP11*, and *RACGAP1*. Comprehensive results from the individual SNP and cognitive function regression analyses are located in Table S1.

Therefore, one or more polymorphisms from the following genes, through either main SNP effects or SNP–prescribed treatment group-interaction effects, were included in GRSs: attention – *ERBB2–MIR125A*, *ESR1*, *MYBL2*, and *SCUBE2*; concentration – *AURKA*, *BCL2*, *CCNB1*, *CENPA*, *DIAPH3*, *ESR1*, *ESR1–CCDC170*, *GRB7*, *MELK*, and *PGR*; executive function – *BAG1*, *BCL2*, *CCNB1*, *CTSL2*, *DIAPH3*, *ESR1*, *GSTM1*, *MELK*, *MYBL2*, *PGR*, and *SCUBE2*; mental flexibility – *BCL2*, *DIAPH3*, *ERBB2–MIR125A*, *ESR1*, *GSTM1–NFE2L2*, *MKI67*, *NDC80*, *RFC4*, *RRM2*, and *SCUBE2*; psychomotor speed – *BCL2*, *CENPA*, *ESR1*, *MKI67*, and *PGR*; verbal memory – *AURKA*, *BCL2*, *CCNB1*, *CD68*, *CENPA*, *CTSL2*, *DIAPH3*, *ESR1*, *ESR1–CCDC170*, *GSTM1*, *MYBL2*, *NDC80*, *ORC6*, and *PGR*; visual memory – *BAG1*, *BCL2*, *CCNB1*, *DIAPH3*, *ESR1*, *GSTM1*, *MYBL2*, *PGR*, and *RRM2*; and visual working memory – *AURKA*, *BAG1*, *BIRC5*, *CCNB1*, *CD68*, *DIAPH3*, *ESR1*, *GRB7*, *GSTM1*, *MELK*, *MYBL2*, and *PGR*. All GRSs were found to be significantly ($P<0.001$) related to the respective domain score (Table 4). Reported associations were all positive, such that as GRS increased (ie, protection), cognitive function performance score improved (Figure 1).

Discussion

Individual candidate genes

In this first study exploring relationships among polymorphisms in biologically plausible BC-related candidate genes, we report significant relationships between performance on at least one cognitive function composite domain and one or more polymorphisms of all genes evaluated, with the exception of

Table 4 GRS and cognitive performance results

Cognitive function composite domain	Gene SNP used in GRS calculation	b_{GRS} *P*-value[a]	R^2	R^2 change for GRS
Attention (n=201)	*MIR125A* rs12976445	0.4665 *P*<0.001	0.2593	0.066
	ESR1 rs2347867			
	ESR1 rs3020314	0.48		
	ESR1 rs6557171	*P*<0.001		
	ESR1 rs985694			
	MYBL2 rs2070235			
	SCUBE2 rs6486125			
Concentration (n=177)	*AURKA* rs1047972	0.5098	0.2495	0.189
	BCL2 rs9807663	*P*<0.001		
	CCNB1 rs164390			
	CCNB1 rs350099	0.5358		
	CENPA rs3806517	*P*<0.001		
	DIAPH3 rs4547237			
	ESR1 rs488133			
	ESR1 rs7767143			
	ESR1 rs910416			
	ESR1 rs9397456			
	CCDC170 rs12662670			
	CCDC170 rs3734805			
	CCDC170 rs3757318			
	CCDC170 rs6929137			
	GRB7 rs9910678			
	MELK rs10973007			
	PGR rs10895068			
Executive function (n=137)	*BAG1* rs706118	0.3526	0.4296	0.204
	BCL2 rs1564483	*P*<0.001		
	BCL2 rs4987853			
	CCNB1 rs164390	0.3589		
	CCNB1 rs350099	*P*<0.001		
	CCNB1 rs350104			
	CTSL2 rs4361859			
	DIAPH3 rs1337652			
	DIAPH3 rs4547237			
	ESR1 rs2234693			
	ESR1 rs488133			
	ESR1 rs7761846			
	ESR1 rs827421			
	CCDC170 rs3757318			
	GSTM1 rs412543			
	MELK rs10973007			
	MELK rs2250340			
	MYBL2 rs11556379			
	PGR rs1042838			
	PGR rs474320			
	PGR rs484389			
	PGR rs608995			
	SCUBE2 rs6486125			
Mental flexibility (n=154)	*BCL2* rs1564483	0.504	0.4712	0.224
	BCL2 rs4987853	*P*<0.001		
	DIAPH3 rs1337652			
	MIR125A rs12976445	0.5383 *P*<0.001		
	ESR1 rs2347867			
	ESR1 rs6557171			
	ESR1 rs985694			
	NFE2L2 rs35652124			
	MKI67 rs10732438			
	MYBL2 rs11556379			
	NDC80 rs12408485			
	NDC80 rs2292274			
	RFC4 rs1354091			
	RRM2 rs1138729			
	SCUBE2 rs6486125			
Psychomotor speed (n=181)	*BCL2* rs4941195	0.7265	0.2527	0.093
	BCL2 rs956572	*P*<0.001		
	CENPA rs3806518			
	ESR1 rs2347867	0.6674		
	ESR1 rs488133	*P*<0.001		
	ESR1 rs9322331			
	ESR1 rs9340799			
	MKI67 rs10732438			
	PGR rs568157			
Verbal memory (n=146)	*AURKA* rs16979877	0.3406	0.5048	0.209
	BCL2 rs2279115	*P*<0.001		
	BCL2 rs4987852			
	BIRC5 rs3764383	0.3401		
	CCNB1 rs164390	*P*<0.001		
	CCNB1 rs350099			
	CCNB1 rs350104			
	CD68 rs9901673			
	CENPA rs3806518			
	CTSL2 rs16919034			
	DIAPH3 rs4547237			
	ESR1 rs10484919			
	ESR1 rs12665044			
	ESR1 rs2941740			
	ESR1 rs488133			
	ESR1 rs77275268			
	ESR1 rs7767143			
	ESR1 rs9383938			
	ESR1 rs9397435			
	CCDC170 rs3734805			
	CCDC170 rs3757318			
	GSTM1 rs412543			
	MYBL2 rs2070235			
	MYBL2 rs619289			
	NDC80 rs2292274			
	ORC6 rs33994299			
	PGR rs484389			
	PGR rs568157			
Visual memory (n=165)	*BAG1* rs706118	0.7477	0.3167	0.148
	BCL2 rs1564483	*P*<0.001		
	CCNB1 rs350104			

(*Continued*)

Table 4 (*Continued*)

Cognitive function composite domain	Gene SNP used in GRS calculation	b_{GRS} *P*-value[a]	R^2	R^2 change for GRS
	DIAPH3 rs1337652	0.6078		
	DIAPH3 rs4547237	*P*<0.001		
	ESR1 rs2077647			
	ESR1 rs2813544			
	ESR1 rs488133			
	ESR1 rs7761846			
	ESR1 rs7767143			
	CCDC170 rs3757318			
	GSTM1 rs412543			
	MYBL2 rs2070235			
	PGR rs11224561			
	PGR rs1942836			
	RRM2 rs4309551			
Visual working memory (n=154)	*AURKA* rs2273535	0.4198	0.47	0.241
	BAG1 rs706118	*P*<0.001		
	BIRC5 rs1508147			
	BIRC5 rs9904341	0.4131		
	CCNB1 rs164390	*P*<0.001		
	CCNB1 rs350099			
	CCNB1 rs350104			
	CD68 rs9901673			
	DIAPH3 rs1337652			
	DIAPH3 rs4547237			
	ESR1 rs2941740			
	ESR1 rs488133			
	ESR1 rs7761846			
	ESR1 rs910416			
	ESR1 rs9397456			
	GRB7 rs9910678			
	GSTM1 rs412543			
	MELK rs2250340			
	MYBL2 rs2070235			
	MYBL2 rs619289			
	PGR rs11224561			
	PGR rs608995			

Notes: [a]Standard multiple linear regression coefficient and *P*-value listed first, robust (generated using Huber weighting and biweighting iterations) multiple linear regression coefficient and *P*-value listed subsequently. Model R^2 and R^2 change reported from standard multiple linear regression models. Participants missing genetic data necessary for completion of a GRS calculation were not included in the GRS analysis. All regression models adjusted for age, estimated verbal intelligence, levels of depressive symptoms, anxiety, fatigue, pain, and prescribed treatment group.
Abbreviations: GRS, genetic risk/protection score; SNP, single-nucleotide polymorphism.

CMC2, *MMP11*, and *RACGAP1*. Significant findings related to the candidate genes found most broadly to impact cognitive function performance across multiple domains, specifically *ESR1*, *CCDC170*, *PGR*, *CCNB1*, *MYBL2*, *BCL2*, *GSTM1*, and *DIAPH3*, are discussed in detail in the following sections.

ESR1 and *CCDC170*

The *ESR1* gene encodes an estrogen receptor. Polymorphisms in *ESR1* have been previously associated with cognitive outcomes, including functioning, impairment, and Alzheimer's disease.[43] We found that performance on every cognitive domain was related to *ESR1* polymorphisms through either main effects and/or interaction effects. The most global associations with a single *ESR1* polymorphism occurred with an intronic upstream variant – rs488133. The effects of this polymorphism on cognitive function performance were different by domain and study cohort: rs488133-CT+TT contributed positively to executive function and psychomotor speed performance in all study participants. rs488133-CT+TT negatively impacted concentration performance in HCs, but positively impacted concentration performance in women with BC prescribed AO. In contrast, rs488133-CT+TT positively impacted memory performance in HCs, but negatively impacted memory performance in women with BC prescribed AO. In addition, while reported in other investigations of middle-aged and older women, we did not observe global cognitive impairment trends or memory deficits related to two well-studied polymorphisms in exon 1 of *ESR1* named for the respective restriction enzyme-recognition sites: PvuII (rs2234693) and Xbal (rs9340799).[44–47]

Polymorphisms in *CCDC170*, the upstream neighbor of *ESR1*, were included in this study to represent more fully variability in *ESR1*. Associations between *CCDC170* polymorphisms and BC susceptibility, progression, and survival have been reported.[25,26,48–50] In addition, *ESR1–CCDC170* chromosomal rearrangements have been associated with more aggressive estrogen receptor-positive BCs.[51] While the function of *CCDC170* is unknown, and no studies to date have investigated associations between *CCDC170* polymorphisms and cognitive phenotypes, results from this analysis, in which possession of one or more *CCDC170* MAs in four (rs12662670, rs3734805, rs3757318, and rs6929137) of the five SNPs evaluated was related to poorer concentration performance in all study participants, suggest that variation in *CCDC170* plays an important role in concentration.

PGR

Progesterone receptors, encoded by *PGR*, are expressed throughout the brain in every neural cell type.[52] Henderson et al found that progesterone concentrations were significantly and positively related to global cognition and verbal memory performance in healthy women less than 6 years since menopause.[53] Moreover, Voytko et al found that estrogen plus progesterone improved executive function and attention performance in surgically menopausal monkeys.[54] For executive function performance, we observed significant interactions between multiple *PGR* polymorphisms and study cohorts. In all instances, possession of *PGR* rs1042838–GT+TT,

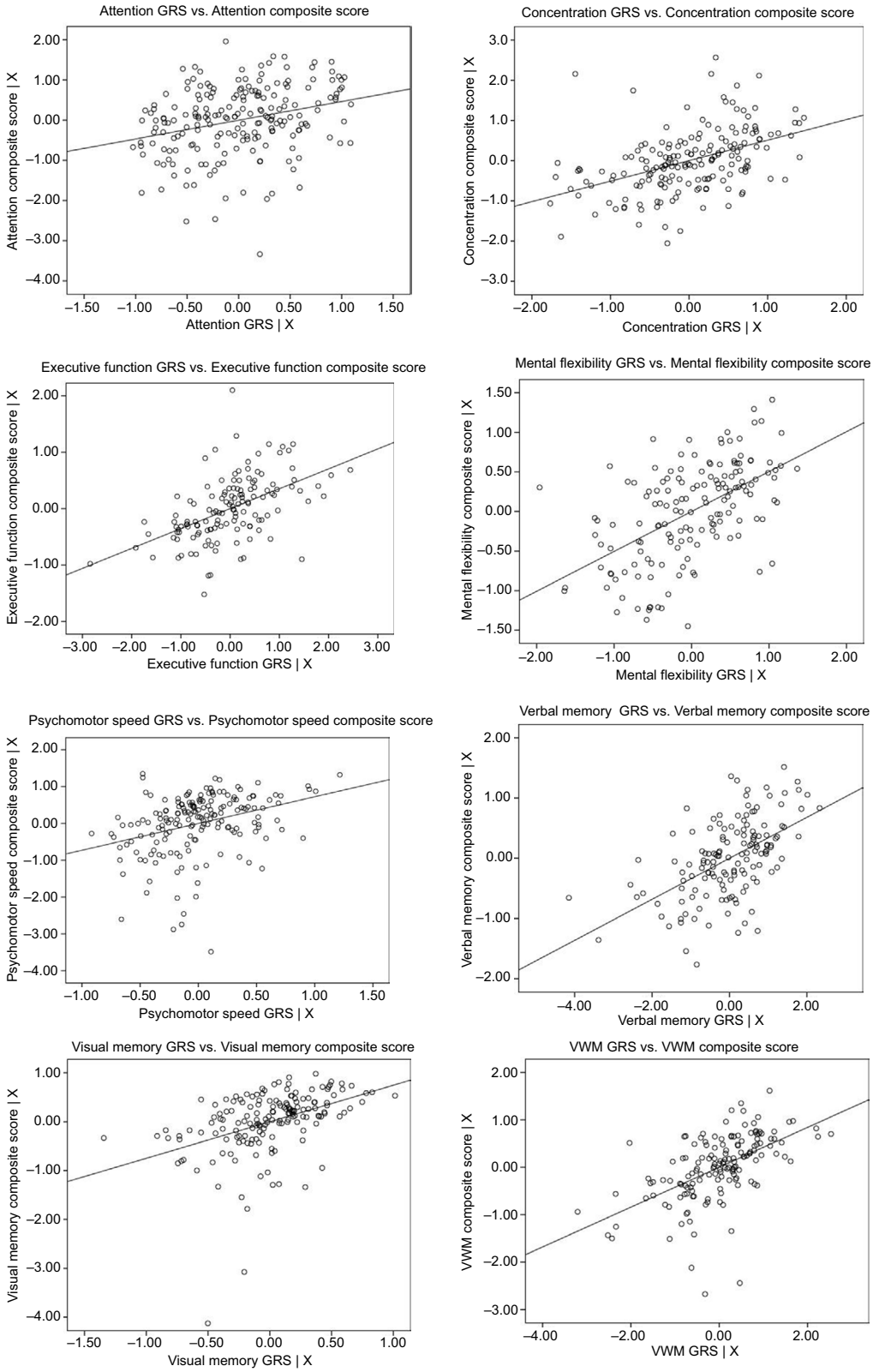

Figure 1 GRS by cognitive function composite score-partial regression plots.
Notes: X = age, estimated verbal intelligence, levels of depressive symptoms, anxiety, fatigue, pain, and prescribed treatment. Figure generated using SPSS version 24 (IBM Corporation, Armonk, NY, USA).
Abbreviations: GRS, genetic risk/protection score; VWM, Visual Working Memory.

PGR rs474320–TA+AA, *PGR* rs484389–TC+CC, or *PGR* rs608995–AT+TT genotypes contributed positively to executive function-performance scores in HCs. When we looked at the interaction of these MAs within the context of BC, we saw the opposite effect: the combination of possession of one or more MAs and membership in a BC cohort was found to impact scores negatively, offsetting the positive SNP main effects and contributing an overall negative input to executive function performance in multiple instances.

The first SNP, rs1042838 (Val660Leu, G>T), is a missense polymorphism in exon 4 that is in linkage disequilibrium with rs1042839 (His770His, C>T), a silent polymorphism in exon 5, and a 320 bp Alu-element insertion at intron G; collectively, these polymorphisms form a variant haplotype called PROGINS. While the functional consequences remain unclear, the PROGINS allele has been associated with increased breast and ovarian cancer risk.[55–59] Also evaluated in this study was rs474320, an intronic variant reported to be in tight linkage with PROGINS,[60] and rs1042839, which is tightly linked to rs1042838. Both SNPs were found to be significant and as expected: rs1042839 generated very similar results to rs1042838; discrepancies in call rate may account for the differences in significance. The remaining significant SNPs, rs484389 and rs608995, are located in the UTR3' of *PGR*. Taken together, these findings indicate that variation in regulation of progesterone receptors may be associated with executive function performance, and furthermore that the polymorphic impact on performance may vary in the systemic environment of a healthy individual compared to that of an individual diagnosed with BC.

CCNB1

CCNB1 encodes a cell-cycle regulatory protein important in mitosis.[61] Because expression levels from this gene are used in three of five of the prognostic multigene-expression profiles for BC from which candidate genes were identified, *CCNB1* was one of our top candidates for investigation of study hypotheses.[5] Significant interactions were reported with study cohorts for three functional polymorphisms – rs164390 (102G>T), rs350099 (–957C>T), and rs350104 (–457C>T) – located in the promotor region of *CCNB1* and memory and executive function performance. In general, we found that possession of rs164390GT+TT or rs350099CT+CC genotypes contributed positively to performance scores in HCs but close to zero or negatively in women with BC. The opposite contribution was observed for rs350104CT+CC genotypes. The genotypes associated with poorer cognitive performance in the cohorts of women with BC, rs164390–GT+TT, rs350099–CT+CC, and rs350104–TT, are all hypothesized to lead to lower levels of *CCNB1* expression via reduced recruitment of transcription factors to the promotor region of the gene.[62] This result is contradictory to anticipated findings, as higher cyclin B levels in breast tissue are associated with more severe cancer phenotypes.[63,64] In addition, cyclin B levels were reported to be upregulated in autopsy hippocampal tissue in individuals with neuropathological Alzheimer's disease and clinical dementia compared to individuals with normal aging.[65] Nevertheless, the consistency of findings across three variants all theorized to impact expression in the same direction lends support to these associations. We would like to point out that one or more polymorphisms in the four other genes represented in three prognostic multigene-expression profiles for BC – *CENPA*, *MELK*, *MYBL2*, and *ORC6* – were associated with performance on at least one domain.

MYBL2 and *BCL2*

MYBL2 encodes a nuclear protein, B-Myb, involved in cell-cycle progression and promotion of cell survival through activation of antiapoptotic genes.[61,66] However, overexpression of B-Myb in certain settings induces apoptosis, and has been reported to contribute to neuronal cell death.[66–69] We found significant relationships with two missense polymorphisms in *MYBL2*: rs11556379 (Ile624Met, C>G) and rs2070235 (Ser427Gly, A>G). The MAs of these polymorphisms have been reported to alter protein conformation, impair regulation of downstream targets, decrease antiapoptotic activity, and reduce cancer risk.[70] Interestingly, for all study participants, rs2070235–AG+GG genotypes contributed positively to attention and negatively to memory-performance scores, while rs11556379–CG+GG genotypes contributed positively to mental flexibility-performance scores. We also reported a significant interaction related to executive function, where rs11556379–CG+GG genotypes had the opposite impact on performance in HCs (positive contribution to scores) and women with BC (negative contribution to scores).

Additionally, we report associations between polymorphisms in a gene regulated by *MYBL2* that is also involved in apoptosis, *BCL2*, and concentration, executive function, mental flexibility, psychomotor speed, verbal memory, and visual memory performance. *BCL2* expression has been associated with prognostication of disease-free survival, overall survival, and recurrence in BC.[71–78] Moreover, normal breast tissue from women with BC was reported to display higher levels of *BCL2* expression than breast tissue from women with no evidence of cancer.[79] In relation to neurologic

phenotypes, polymorphisms in *BCL2* have been found to impact outcomes after traumatic brain injury and have been associated with hippocampal volume.[80,81]

GSTM1

One of the functional polymorphisms located in the promoter region of *GSTM1*, rs412543(–498C>G), was found to be important for memory and executive function performance. *GSTM1* encodes an enzyme with antioxidant properties that detoxifies electrophilic compounds, including carcinogens, drugs, and environmental toxins, throughout the body.[61] By decreasing the binding capability of the transcription factor AP2 to the *GSTM1*-promoter region, the G allele has been reported to decrease *GSTM1* transcription by 30%–40% compared to the C allele.[82] Both decreased and enhanced (attributed to counterproductive depletion of glutathione) *GSTM1* expression has been associated with increased BC risk.[82–84] We found that rs412543–GG+CG and hypothesized decreased *GSTM1* expression contributed negatively to executive function and memory performance in all study participants. However, we also found positive interaction effects between rs412543–GG+CG and BC cohort related to verbal and visual working memory. While the mechanism is unclear, the paradoxical quality of *GSTM1* under- and overexpression combined with study results suggests that decreased or moderate *GSTM1* expression may be beneficial to certain aspects of cognitive function in women with BC. Considering the detoxification properties of *GSTM1*, further evaluation of cognitive decline over time in women with BC receiving adjuvant chemotherapy and/or antiestrogen therapy is recommended.

DIAPH3

Variation in the two upstream intronic polymorphisms selected to represent *DIAPH3*, rs1337652 and rs4547237, were associated with performance for multiple domains as well. *DIAPH3* is involved in actin remodeling and regulation of cell movement and adhesion.[61] *DIAPH3* downregulation and silencing has been associated with metastatic disease due to loss of normal gene function and acquisition of an amoeboid cancer-cell phenotype.[85] Evidence also suggests that *DIAPH3* is critical to brain development and is involved in cell migration, the formation of dendrites and axons, axon guidance, and synaptic activity.[86]

CMC2, *MMP11*, and *RACGAP1*

Three candidate genes were not significantly associated with pretreatment cognitive performance in this study. While genes with significant findings from our analysis are represented by multiple functional and/or tagging SNPs and are well described in the literature, it is notable that the three genes not found to be significant are less well represented in the literature and the HapMap database. Single SNPs, rs131451 and rs7303531, were included in the analysis for *MMP11* and *RACGAP1*, respectively. Both SNPs are upstream variants. No associations have been reported between *MMP11* or *RACGAP1* and cognitive phenotypes in the literature. *CMC2* is an even more poorly described and studied gene, with reported involvement in cytochrome C oxidase activity.[87] Two upstream (rs1025065 and rs1981867) polymorphisms and one downstream (rs9936489) polymorphism were identified using the Phase III HapMap database based on National Center for Biotechnology Information gene location (Chr16: 80975802…81006897), as *CMC2* is not a displayed gene in HapMap. We must be mindful that our analysis is limited to current information known about these genes and polymorphisms, and thus these genes cannot be ruled out as important to understanding cognitive function within the context of BC.

Genetic risk/protection scores

Because of the complexity of BC as a disease and cognitive function as a phenotype, we calculated weighted GRSs for each domain to evaluate the collective effect of possession of multiple risk or protective MAs of genes used to clinically evaluate the biology of BC. Every GRS was significantly ($P<0.001$) and positively associated with its respective domain. When the GRSs were added as predictors to regression models, including age, estimated verbal intelligence, levels of depressive symptoms, anxiety, fatigue, pain, and prescribed treatment group, the explained variance (R^2) increased by 0.066 to 0.244 for each domain. This substantial increase in R^2 speaks to both the importance of host variation in genes used to evaluate clinically the biology of BC to pretreatment cognitive performance and the use of multiple common variants, plus personal and environmental factors, to model a complex phenotype.

Limitations and future directions

Small sample sizes limited our ability to conduct genetic analyses by genotype, rather than by the presence or absence of one or more MAs; therefore, we were unable to evaluate gene-dosage effects. In addition, the sample was comprised of postmenopausal women with hormone receptor-positive, early stage BC who were primarily Caucasian; therefore, the generalizability of study findings to premenopausal women, hormone-negative, different-stage BCs, or more diverse

patient populations is unknown. The number of statistical tests completed as part of this exploratory study and possible inflation of type I error should also be acknowledged; all reported results will need to be confirmed in future independent studies. Limitations related to the prioritization and inclusion of select candidate genes has been discussed previously.[5]

While biomarkers of host DNA and cognitive performance are advantageous for a number of reasons, including the stability and tissue nonspecificity of DNA polymorphisms, associations with gene-expression and protein levels should also be conducted, as some of the most prominent findings from this study were related to polymorphisms with known functional consequences or located in regulatory regions. We postulate that cognitive performance variability in women with BC may be at least partially driven by tumor-gene expression and corresponding protein levels. Longitudinal studies that include cognitive assessment prior to primary surgery would be ideal for evaluation of the effect of tumor-gene expression, as well as changes in gene expression due to tumor removal and treatment of primary and secondary cancer sites, on variability in cognitive performance. Significant relationships from tumor gene-expression studies are advantageous for different reasons; namely, they could directly expand the clinical utility of currently marketed prognostic multigene-expression profiles for BC. Future analyses should also investigate the effect of polymorphisms in genes used to clinically evaluate the biology of BC and tumor-expression levels on cognitive function throughout and following adjuvant chemotherapy and/or antiestrogen-therapy regimens.

Conclusion

In summary, the objective of this study was to explore the hypothesis that host variation in candidate genes involved in BC development and prognosis is associated with variability in the presence and/or severity of alterations in pretreatment cognitive performance among postmenopausal women diagnosed with early stage BC. Significant associations between host polymorphisms representing 25 candidate genes used to clinically evaluate the biology of BC and computed GRSs and variability in pretreatment cognitive function performance support this hypothesis and merit independent replication and further investigation into the identification of clinically relevant biomarkers for BC-related cognitive dysfunction.

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Disclosure

The authors report no conflicts of interest in this work.

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