Ingle *Breast Cancer Research* 2010, **12(Suppl 4)**:S17
http://breast-cancer-research.com/supplements/12/S4/S17

Breast Cancer RESEARCH

SHORT COMMUNICATION

Genome-wide case-control study of musculoskeletal adverse events and functional genomics in women receiving aromatase inhibitors: going beyond associations

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Introduction

In postmenopausal women with early breast cancer, the third-generation aromatase inhibitors (AIs; anastrozole, exemestane, and letrozole) have been proven to be of value in multiple large well-conducted clinical trials as initial adjuvant endocrine therapy, after several years of tamoxifen, and as extended adjuvant endocrine therapy after about 5 years of tamoxifen [1-8]. A recent American Society of Clinical Oncology clinical practice guideline stated that an AI should be used in the adjuvant setting either initially or after some period of tamoxifen therapy [9].

Despite the proven value of the AIs, many women are not adherent [10]. About one-half of women treated with an AI have new or worsening joint complaints [11]. The importance of musculoskeletal complaints was identified in reviewing the experience with MA.27, a large phase III trial comparing the non-steroidal AI anastrozole with the steroidal AI exemestane as adjuvant therapy for early breast cancer. These musculoskeletal adverse events (MS-AEs) were the most common reason why patients discontinued AI therapy. We had previously demonstrated marked variability in metabolism and pharmacodynamics of one of the AIs, anastrozole [12]. We hypothesized that the variability seen with respect to MS-AEs in women treated with anastrozole or exemestane on MA.27 could be related to genetic variability of the patients. We proceeded to perform a genome-wide association study (GWAS) aimed at identifying SNPs associated with MS-AEs. The results of this GWAS and the functional genomic laboratory studies performed have recently been published [13]. This short communication summarizes the highlights of this work plus a commentary on future pharmacogenomic studies of anticancer agents.

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Methods

MA.27 is a randomized clinical trial conducted by the Breast Cancer Intergroup of North America that was coordinated by the NCIC Clinical Trials Group [13]. Eligible patients were those with a postmenopausal status and resected American Joint Committee on Cancer (version 6) stage I to III breast cancer that was hormone receptor positive. Patients were randomized to anastrozole or exemestane for a period of 5 years. A total of 6,827 women in North America were randomized and the majority provided DNA and consent for its use in genetic studies.

Cases were defined as those patients who developed a MS-AE, as previously defined [13], and each case was matched to two controls. The design utilized was a nested matched case-control study and the primary analyses were based on conditional logistic regression. The RIKEN Center for Genomic Medicine performed genotyping with the Illumina Human610-Quad platform. Imputation and fine mapping were performed in the region of interest on chromosome 14 containing the SNPs with the smallest *P*-values. Functional genomic studies, relating to the SNPs on chromosome 14, were performed using electrophoretic motility shift (EMS) assays, chromatin immunoprecipitation (ChIP) assays, and transfection studies.

Results

We studied 293 cases and 585 controls, and cases and controls were well balanced for all factors except prior hormone replacement therapy, which was significantly higher in cases than controls (66% versus 44%), and fractures within the past 10 years, which were slightly higher in cases than controls (13% versus 9%). A total of 551,395 SNPs were used in the association analyses after exclusion of genotype failures (n = 11,281), SNPs with a minor allele frequency <0.01 (n = 29,478), and SNPs with a departure (P < 1E-06) from Hardy-Weinberg equilibrium (n = 82). The conditional logistic regression analyses were adjusted for population stratification and revealed three SNPs (rs7158782, rs7159713, rs2369049) on chromosome

14 in high linkage disequilibrium with the smallest *P*-values (7.74E-07 to 2.23E-06), which approach the Bonferroni threshold of 1E-07. Subsequently, imputation and fine mapping revealed an additional SNP (rs11849538), also on chromosome 14 and in high linkage disequilibrium with the three genotyped SNPS, with the smallest *P*-value (6.67E-07). These four SNPs were all found to be close (that is, within 7,109 bp) of the 3' end of the T-cell leukemia 1A *(TCL1A*) gene with the closest SNP (rs11849538) being only 926 bp away.

Initially, we determined that *TCL1A* is variably expressed in 288 lymphoblastoid cell lines from three different ethnic groups for which we have expression array and genome-wide SNP data. Functional genomic studies were performed with the three genotyped SNPs and the one imputed/fine mapped SNP to examine for any functional significance. Lymphoblastoid cell nuclear extract used in EMS assays showed a 'shift', that is, protein binding, for three of the SNPs (rs11849538, rs7158782, rs7159713) with less binding by the variant than wild-type sequences. Of particular interest as it relates to a drug that lowers estrogen levels, a TRANSFAC database search predicted that the SNP (rs11849538) with the smallest *P*-value would create an estrogen response element and this was confirmed with a ChIP assay utilizing lymphoblastoid cells with known genotype for this SNP that had been transfected with estrogen receptor (ER)α. *TCL1A* expression was linked to estrogen by exposing U20S cells that had been stably transfected with ERα or ERβ to 0.1 nM E2, and demonstrating eight- and six-fold increases in *TCL1A* mRNA expression after 18 hours and 1 hour, respectively. To determine the effect of these four SNPs on estrogen-dependent *TCL1A* expression, lymphoblastoid cell lines with genotypes known for the four SNPs were transiently transfected with ERα and exposed to various concentrations of estradiol. All three ethnic groups showed greater *TCL1A* expression with the variant than the wild-type sequence. Because the clinical picture of the MS-AEs is reminiscent of a chronic inflammatory state, we utilized the same 288 lymphoblastoid cell lines noted above and determined the correlation between TCL1A expression and expression of IL17 and the IL17 receptor A (IL17RA). Remarkably, TCL1A and IL7RA expression were highly correlated ($P < 1.9E-10$). In studies using U20S cells transfected with either ERα or ERβ, small interfering RNA (siRNA) knockdown of TCL1A resulted in decreased expression of IL17RA but increased expression of IL17, whereas TCL1A overexpression resulted in increased IL17RA expression and decreased expression of IL17.

Discussion

We identified four SNPs on chromosome 14 that were associated with MS-AEs in women receiving AIs as adjuvant therapy for early-stage breast cancer. Given that AIs perturb estrogen levels in these women, we examined the relationship between the SNPs and estrogen action. Of particular note is that the SNP with the smallest *P*-value (rs11849538) created an estrogen response element that was shown by ChIP assay to be functional. Estrogens induced TCL1A expression that was significantly higher in cells with the variant SNPs than those with the wild-type sequence. Expression of TCL1A was directly associated with IL17RA expression in that siRNA knockdown of *TCL1A* resulted in decreased expression of IL17RA and increased expression of IL17 whereas overexpression of TCL1A resulted in increased expression of IL17RA and decreased expression of IL17.

The identification of a relationship between TCL1A expression and the inflammatory cytokine IL17 is particularly noteworthy given the recent identification of a third lineage of T-helper cells (in addition to Th1 and Th2), that is, Th17, that is associated with chronic inflammation [14], and in which IL17 plays a central role. IL17 and IL17RA represent potential targets and efforts to target these factors in a variety of inflammatory diseases are in progress (reviewed in [14]).

The study reported here demonstrates the value of GWASs. A GWAS is, by definition, a 'hypothesis-free' interrogation of the entire genome and, in our study, identified a gene, *TCL1A*, which was totally unexpected. However, it was clearly necessary to perform the functional genomic studies to begin to understand the potential biologic implications of these findings. Clearly, replication is an important consideration in the performance of a GWAS because of the problem of false positive associations [15]. The replication of genome-wide pharmacogenomic studies of anti-cancer agents will likely be found to be substantially more problematic than genome-wide studies examining risk of developing a cancer. For example, in a recent study to identify breast cancer susceptibility alleles, a GWAS was performed in 3,659 cases and 4,897 controls, and then promising associations were then studied in 12,576 cases and 12,223 controls [16]. In the case of pharmacogenomic studies of anti-cancer agents, a GWAS will likely be best conducted utilizing patients entered into a prospectively conducted clinical trial. MA.27, the study from which the patients in our GWAS were obtained, is such a trial but is the largest study conducted of AIs alone, taking 8 years to complete at a cost of tens of millions of dollars. Replication will not be possible utilizing a larger cohort of patients as it simply does not exist. Although the conduct of rigorous and insightful functional genomic studies can be viewed as important in pursuing leads from any GWAS, such studies may have greater importance in the case of anti-cancer agents because of limitations imposed by a relative paucity of patient cohorts, at least compared to studies

examining risk, that is, genetic predisposition, of developing cancer.

In conclusion, our GWAS identified four SNPs related to MS-AEs in women receiving AIs as adjuvant therapy for their early breast cancer. These SNPs were related to *TCL1A*, which in turn was related to the inflammatory cytokine IL17. Further work is ongoing in our laboratory to expand our understanding of the mechanisms involved in the *TCL1A*-IL17 relationship. Ultimately, this new knowledge should help identify means to ameliorate the AI-related MS-AEs in order to allow more women with early breast cancer to take these potentially life-saving drugs.

Abbreviations

AI, aromatase inhibitor; bp, base pair; ChIP, chromatin immunoprecipitation; EMS, electrophoretic motility shift; ER, estrogen receptor; GWAS, genome-wide association study; IL, interleukin; IL17RA, IL17 receptor A; MS-AE, musculoskeletal adverse event; siRNA, small interfering RNA; SNP, single nucleotide polymorphism.

Competing interests

The author declares that he has no competing interests.

Acknowledgments

Supported in part by NIH grants U01GM61388, U01GM63173, P50CA116201, U10CA77202, CCS 015469 from the Canadian Cancer Society, the Biobank Japan Project funded by the Ministry of Education, Culture, Sports, Science and Technology, Japan, and the Breast Cancer Research Foundation. The MA.27 trial, from which the patients in this study were obtained, was supported in part by Pfizer, Inc.

This article has been published as part of *Breast Cancer Research* Volume 12 Supplement 4, 2010: Controversies in Breast Cancer 2010. The full contents of the supplement are available online at http://breast-cancer-research.com/supplements/12/S4

Published: 20 December 2010

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doi:10.1186/bcr2746
Cite this article as: Ingle JN: Genome-wide case-control study of musculoskeletal adverse events and functional genomics in women receiving aromatase inhibitors: going beyond associations. *Breast Cancer Research* 2010, **12(Suppl 4):**S17.