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Germline genetic variants in *ABCB1*, *ABCC1*, and *ALDH1A1* and risk of hematological and gastrointestinal toxicities in a SWOG Phase III trial S0221 for breast cancer

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Abstract

Hematological and gastrointestinal toxicities are common among patients treated with cyclophosphamide and doxorubicin for breast cancer. To examine whether single nucleotide polymorphisms (SNPs) in key pharmacokinetic genes were associated with risk of hematological

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or gastrointestinal toxicity, we analyzed 78 SNPs in *ABCB1*, *ABCC1* and *ALDH1A1* in 882 breast cancer patients enrolled in the SWOG trial S0221 and treated with cyclophosphamide and doxorubicin. A two-SNP haplotype in *ALDH1A1* was associated with an increased risk of grade 3 and 4 hematological toxicity (odds ratio [OR]=1.44, 95% confidence interval [CI]=1.16-1.78), which remained significant after correction for multiple comparisons. In addition, 4 SNPs in *ABCC1* were associated with gastrointestinal toxicity. Our findings provide evidence that SNPs in pharmacokinetic genes may have an impact on the development of chemotherapy-related toxicities. This is a necessary first step towards building a clinical tool that will help assess risk of adverse outcomes prior to administration of chemotherapy.

Keywords

breast cancer; chemotherapy; toxicity; pharmacokinetics; pharmacogenetics; *ALDH1A1*

Introduction

Systematically administered breast cancer chemotherapeutic drugs, including cyclophosphamide (C), doxorubicin (A) and taxanes (T), are cytotoxic compounds which target not only rapidly dividing cancer cells, but also normal cells in other exposed organs, resulting in debilitating and sometimes life-threatening toxicities¹. Hematological toxicities, such as neutropenia and leucopenia, and gastrointestinal toxicities, such as nausea, vomiting, stomatitis and diarrhea, are commonly seen in patients treated with cyclophosphamide and doxorubicin. In addition to adversely affecting patients' quality-of-life, these acute side effects can lead to delay, alteration, or discontinuation of treatment, which may subsequently lead to cancer recurrence or premature mortality². It is thus important to assess the relative risk/benefit ratio of adjuvant chemotherapy, which may facilitate physicians' and patients' decision-making in selection of chemotherapy regimens.

In the search for genetic biomarkers that are predictive of chemotherapy toxicity in breast cancer patients, genes in drug pharmacokinetic pathways, which may have direct impact on the effective drug dose reaching target cells and the rate of drug disposal, are excellent candidates^{3, 4}. An important gene for cyclophosphamide pharmacokinetics is aldehyde dehydrogenase 1 family, member A1 (*ALDH1A1*), which is a major enzyme responsible for the detoxification of active cyclophosphamide metabolites⁵. In a small study of cancer patients (primarily breast) treated with high dose cyclophosphamide, patients heterozygous for *ALDH1A1**2 had an increased risk of liver toxicity⁶. For doxorubicin, two major efflux transporters, ATP-binding cassette, subfamily B, member 1 (*ABCB1*), also known as multidrug resistance protein 1 (*MDR1*), and ATP-binding cassette, subfamily C, member 1 (*ABCC1*), known as multidrug resistance associated protein 1 (*MRP1*), have been implicated in cellular transport of doxorubicin⁷. Although germline genetic variants in *ABCB1* and *ABCC1* have been examined with survival outcomes after chemotherapy for breast cancer, few studies have examined them in relation to chemotherapy-induced toxicity.

In this study, we analyzed 78 tagSNPs in *ABCB1*, *ABCC1* and *ALDH1A1* which were selected to comprehensively capture germline genetic variations in these key

pharmacokinetic genes for cyclophosphamide and doxorubicin, among 882 breast cancer patients enrolled in a cooperative group clinical trial SWOG S0221. We examined potential associations of single SNPs and haplotypes with risk of high grade hematological and gastrointestinal toxicities induced by doxorubicin and cyclophosphamide.

Patients and Methods

Patient population

Data and DNA samples for this study were from a North American Breast Cancer Intergroup clinical trial S0221 led by SWOG. S0221 is a Phase III trial to test if a continuous “metronomic” schedule of AC (doxorubicin and cyclophosphamide) for 15 weeks was superior to a more conventional Q2 week schedule of AC for 6 cycles, followed by either weekly T (paclitaxel) for 12 weeks or Q2 week T for 6 cycles. All patients received subcutaneous filgrastim at 5 mcg/kg or pegfilgrastim at 6 mg/kg starting 24 hours after chemotherapy injection. The randomization schema is shown in Figure 1. Details of the trial design and patients included in this pharmacogenetic study have been described previously⁸. In brief, patients of age ≥ 18 with a histologically confirmed operable Stage II or III high risk invasive breast cancer with known estrogen or progesterone receptor status and no previous malignancies were eligible to participate in the trial. Blood samples were drawn at the time of registration and shipped to the Roswell Park Cancer Institute (RPCI) laboratory for processing and DNA extraction. Because blood collection was not instituted until after the trial had begun, and there were delays because of the need for the multiple institutions involved to get IRB approval for the amendment, blood specimens were not collected from the approximately 1,000 patients enrolled on the trial before approval for specimen collection. At the time of the genotyping performed in March 2009, 2,314 patients had been enrolled in the trial and 2,062 had toxicity evaluation completed. Among them, a total of 905 patients with available DNA samples were included in this study. The enrollment of new patients in S0221 was closed in January 2012 with continued follow up ongoing. Because the main findings from S0221 have not been published, we cannot disclose the treatment arm assignment, examine toxicities by treatment arm, or evaluate genotypes with survival outcomes in this study.

Collection of toxicity data

In S0221, toxicities were monitored and reported according to the NCI Common Toxicity Criteria for Adverse Events (CTCAE) Version 3.0⁹. Each adverse event was graded based on the severity, where grade 3 toxicities interfere with activities of daily living, and grade 4 toxicities are life-threatening and usually require hospitalization. In this analysis, grade 3 and 4 hematological toxicities and grade 3 gastrointestinal toxicities that occurred during the AC segment of chemotherapy before the T segment treatment, two major side effects related to cyclophosphamide and doxorubicin, were used as the major analytical endpoints. Toxicity data were recoded as occurring or not during AC treatment, and timing relative to the treatment period was not collected in this study.

SNP selection and genotyping

To systematically interrogate common genetic variations in *ABCB1*, *ABCC1* and *ALDH1A1*, tagSNPs were selected using the SNAGGER program¹⁰ based on the HapMap genotype data from the CEU population of European ancestry and the YRI population of African ancestry¹¹. The minimum allele frequency (MAF) was set at 0.05 and r^2 threshold of 0.80, with a consideration of position relative to functional genomic regions and Illumina design scores when prioritizing selections. A total of 16 SNPs in *ABCB1*, 48 SNPs in *ABCC1* and 15 SNPs in *ALDH1A1* were selected and genotyped using the Illumina GoldenGate platform in the Genomics Core Facility at Roswell Park Cancer Institute. To control for population admixture bias, 70 uncorrelated SNPs were selected and genotyped as ancestry informative markers to estimate individual ancestry using the STRUCTURE program¹². The average call rate was 97.1%, and the concordance rate among 5% blind duplicates was 100%. A total of 23 patients were excluded from the analysis for genotyping call rates lower than 90%, or discordance between self-reported race and estimated genetic ancestry. None of the SNPs in the three selected genes were excluded due to call rate below 90%. One SNP in *ABCC1* was removed for violation of Hardy-Weinberg Equilibrium in patients of European ancestry, leaving a total of 78 SNPs and 882 patients in the final analysis.

Statistical methods

Unconditional logistic regression was used to compute odds ratios (OR) and 95% confidence intervals (CI) for both single SNP and haplotype analysis, with adjustment for age, proportion of genetic ancestry, and treatment arm. Codominant genetic models, i.e. independent effects of all three genotypes, and dominant models, i.e. identical effect of the rare homozygote and heterozygote, were both tested. The R package *snp.plotter*¹³ was used to generate the linkage disequilibrium (LD) map with p-values from single SNP analysis. The criteria by Gabriel et al¹⁴ were used to determine haplotype blocks and to estimate haplotype frequency for each gene. Each haplotype was tested in comparison to all other haplotypes combined. In addition to adjusting for genetic ancestry estimates, subgroup analyses were also performed within patients of European ancestry only, the majority of the study population (83%), to control for potential population admixture bias. In addition to combining grade 3 and 4 hematological toxicity as one endpoint, grade 4 hematological toxicity versus grade 0-2 toxicity was also tested as a separate endpoint, and ordinal logistic regression model was also used, which treated hematological toxicity grade 2, grade 3 and grade 4 as an ordinal response variable. Multiple testing was controlled using 10,000 permutation using the PLINK program¹⁵. The results are reported in accordance with the REMARK criteria¹⁶.

Results

For purposes of this study, only the AC segment of the trial was examined for associations between genotypes and toxicity, since paclitaxel rarely causes either Grade 3/4 hematological or gastrointestinal toxicity, especially in the setting of routine growth factor administration. Descriptive characteristics of the patient population included in this study are summarized in Table 1. The average age of breast cancer diagnosis was 50 years, with 83% of patients self-reported as European American, 8% as African American, 5% as Asian

American, and 4% as other race or ethnicity. Data are shown for patients included in this study as well as those for whom DNA was not available in Table 1; characteristics and outcomes did not differ between those with and without DNA. According to the CTC/AE, in the AC segment of the chemotherapy, 19% and 14% of the patients developed grade 3 and grade 4 hematological toxicities, respectively; 14% of patients developed grade 3 gastrointestinal toxicity. No grade 4 gastrointestinal toxicities were observed in the AC segment for those with DNA and only four Grade 4 events were observed in those without DNA.

P-values for the associations of the 78 SNPs in *ABCB1*, *ABCC1* and *ALDH1A1* with hematological and gastrointestinal toxicity plotted with LD map for each gene are shown in Figure 2, and ORs and 95% CIs in Supplementary Tables S2 and S3. Using an uncorrected $p < 0.05$ for statistical significance, we observed an association between grade 3 and 4 hematological toxicity and three SNPs in *ABCC1* (rs903880, rs16967126 and rs4148350) and three SNPs in *ALDH1A1* (rs8187996, rs3764435 and rs63319) (Table 2). Except for two SNPs (rs4148350 in *ABCC1* and rs63319 in *ALDH1A1*), these associations remained significant when the analysis was restricted to European American women only. However, none of the four SNPs was significant after correction for multiple comparisons (Table 2). For the most significant SNP rs3764435 in *ALDH1A1*, the AA genotype was associated with a 76% increased risk of grade 3 and 4 hematological toxicity (codominant model, OR=1.76, 95% CI=1.18-2.61, uncorrected $p=0.004$, and corrected $p=0.21$). A similar increased risk was found in patients carrying the AA genotype when the analysis was restricted to European American women only (OR=1.84, 95% CI=1.22-2.78), or when grade 4 hematological toxicity was compared to grade 0-2 toxicity (OR=2.00, 95% CI=1.11-3.58). In the ordinal logistic regression model treating grade 2, grade 3 and grade 4 hematological toxicity as an ordinal outcome, patients carrying the AA genotype were also at an increased risk of higher toxicity (OR=1.73, 95% CI=1.17-2.55, $p=0.005$). Consistent with the single SNP analysis, a two-SNP haplotype consisting of the A allele of rs3764435 and a neighboring SNP rs168351 was associated with an increased risk of grade 3 and 4 hematological toxicity (OR=1.44, 95% CI=1.16-1.78, uncorrected $p=0.0008$), which remained significant after correction for multiple comparisons (corrected $p=0.03$) (Table 4), or when the analysis was restricted to European American women only (OR=1.48, 95% CI=1.19-1.85, uncorrected $p=0.0005$, corrected $p=0.02$). None of the SNPs in *ABCB1* was associated with hematological toxicity.

For grade 3 gastrointestinal toxicity, SNPs with an uncorrected $p < 0.05$ are shown in Table 3, which included four SNPs in *ABCC1* (rs35596, rs4148354, rs2889517 and rs11861115). Associations remained significant when the analysis was restricted to European American women only; however, none of the four SNPs was significant after correction for multiple comparisons. Women carrying the A allele of rs2889517 had a decreased risk of gastrointestinal toxicity as compared to those homozygous for the G allele (GA genotype: OR=0.57, 95% CI=0.37-0.86; AA genotype: OR=0.62, 95% CI=0.32-1.20). The association was confirmed in haplotype analysis, where a two-SNP haplotype consisting of the A allele of rs2889517 and the G allele of a neighboring SNP rs2074087 was associated with a decreased risk of gastrointestinal toxicity (OR=0.54, 95% CI=0.34-0.84, uncorrected

p=0.006). However, the association became non-significant after correction for multiple comparisons (corrected p=0.24). None of the SNPs in *ABCB1* or *ALDH1A1* was associated with gastrointestinal toxicity.

Discussion

In this study, we examined tagSNPs in *ABCB1*, *ABCC1* and *ALDH1A1* in relation to risk of high grade hematological and gastrointestinal toxicities after treatment for breast cancer with doxorubicin and cyclophosphamide in a SWOG clinical trial. None of the *ABCB1* SNPs were associated with either toxicity, but 3 SNPs in *ABCC1* and 3 SNPs and 1 haplotype in *ALDH1A1* were associated with grade 3 and 4 hematological toxicity. Four SNPs and 1 haplotype in *ABCC1* were associated with grade 3 gastrointestinal toxicity. The only association that remained significant after correction for multiple comparison was a haplotype in *ALDH1A1* consisting of rs3764435 and a neighboring SNP. The A-A haplotype of *ALDH1A1* was associated with a 44% increased risk of grade 3 and 4 hematological toxicity as compared to the other haplotypes. Our findings suggest that common genetic variations in *ALDH1A1*, which plays a critical role in the detoxification of active cyclophosphamide metabolites, may affect the risk of severe hematological toxicity caused by chemotherapy, despite the prophylactic use of granulocyte colony-stimulating factors (CSFs).

Neutropenia, characterized by markedly low absolute neutrophil count (<500 cells/ul), is one of the most common hematological toxicities occurring among patients treated with myelosuppressive chemotherapy drugs¹⁷. Profound and prolonged neutropenia often leads to hospitalization due to fever or infection, which sometimes can be life-threatening. The American Society of Clinical Oncology (ASCO) recommends the use of primary prophylaxis with hematopoietic CSFs when the risk of febrile neutropenia from a specific regimen exceeds 20%¹⁸. In S0221, all patients received either filgrastim (G) or pegfilgrastim (PEGG) as prophylaxis following AC therapy. Nevertheless, 19% and 14% of patients still experienced grade 3 and 4 adverse hematological events, respectively, during the AC segment of the trial, indicating either that these patients were extremely susceptible to myelosuppressive drugs, and/or that growth factors were not adequately efficacious for them. Our findings that a haplotype in *ALDH1A1* was associated with risk of grade 3 and 4 hematological toxicities, indicates that genetic variants may be useful biomarkers for identifying a subgroup of patients at high risk of neutropenia caused by myelosuppressive chemotherapy, even when given growth factors.

Thus, if confirmed, these results could have clinical relevance. One might consider a number of strategies directed towards patients who are at higher risk for myelosuppression, such as increased dose of growth factor, reduction of chemotherapy dose, or administration of prophylactic antibiotics. However, each of these strategies is associated with potential risks and costs. Therefore, such strategies, following replication, need to be tested in properly designed, prospective trials.

ALDH1A1 is a major enzyme responsible for the detoxification of the cytotoxic cyclophosphamide metabolites, aldophosphamide and acrolein⁵. Interestingly, *ALDH1A1*

was proposed as a biomarker of stem cells, and in hematopoietic stem cells, deficient expression of *Aldh1a1* was related to hyper-sensitivity to cyclophosphamide in mice¹⁹, consistent with its role in the detoxification of this drug. In a recent study of 513 breast cancer patients, expression of *ALDH1A1* was inversely related to disease-free survival and overall survival after neoadjuvant chemotherapy containing cyclophosphamide²⁰. To date, only a 17-bp deletion polymorphism (*ALDH1A1**2 or rs615103) has been examined with pharmacokinetics and toxicity of cyclophosphamide. In 113 patients with various cancers (including breast cancer) who were treated with cyclophosphamide, patients heterozygous for *ALDH1A1**2 had increased risk of hemorrhagic cystitis⁶. However, in another study from the same group, this variant allele was not associated with the clearance of cyclophosphamide or its 4-hydroxyl metabolite²¹. In S0221, we selected and genotyped 15 SNPs in *ALDH1A1*, which comprehensively represents common variations in this gene. We found that SNP rs3764435 was associated with grade 3 and 4, as well as grade 4 alone, hematological toxicity after AC treatment. The association was confirmed by a haplotype consisting of rs3764435 and a neighboring SNP, with the association remaining significant after correction for multiple comparison. This SNP is located in the intronic region, underneath 2 transcription factor binding sites in human ductal breast epithelial tumor cell lines (T47d), including GATA3. GATA3 yields luminal epithelial cell differentiation in the mammary gland and EP300, playing an important role in regulating cell growth and division. Furthermore, in these T47d cell lines, this SNP resides in a DNase hypersensitivity site, and has been associated with the expression of at least 27 genes in lymphoblastoid cell lines²², indicating that rs3764435 may have an impact on gene expression regulation. Because *ALDH1A1* also plays a role in the conversion of acetaldehyde to acetate in the alcohol metabolism pathway²³, SNPs in this gene have been previously examined with alcohol dependence, and rs3764435 was identified as the driving variant for a risk haplotype in a European population²⁴. Although it is unclear whether this SNP has a similar impact on the metabolism of cyclophosphamide, this finding and ours call for future study to characterize the functionality of this SNP.

ABCB1 and *ABCC1* belong to the ATC-binding cassette family, and are among the best characterized cellular efflux transporters implicated in resistance to multiple drugs, including doxorubicin⁷. The majority of the previous pharmacogenetic studies of genetic variants in *ABCB1* and *ABCC1* focused on survival outcomes after doxorubicin-containing chemotherapy, while studies on drug toxicity are scarce. In a small study of 62 breast cancer patients, SNPs in *ABCB1* were associated with plasma concentration of doxorubicin; however, toxicity was not evaluated in that study²⁵. A large pharmacogenetic study of 1,697 aggressive non-Hodgkin's lymphoma patients examined SNPs in a large number of genes, including *ABCB1* and *ABCC1*, in relation to doxorubicin-induced cardiotoxicity²⁶. A non-synonymous coding SNP rs45511401 (Gly671Val) in *ABCC1* was associated with markedly increased risk of cardiotoxicity. In S0221, genetic variants in *ABCC1* were associated with hematological toxicity and gastrointestinal toxicity in breast cancer patients after AC treatment, although the associations did not remain significant after controlling for multiple comparisons.

There are some limitations in the study that need to be considered when interpreting our results. First, we selected three genes that are central in the pharmacokinetic pathways of cyclophosphamide and doxorubicin to be included in this study. However, this is not a complete coverage of the important genes in these pathways. Considering the relatively moderate effect associated with each genetic variant found in our study, it may be important to consider multiple SNPs and genes and examine their combinational effects on treatment outcomes in future studies. Second, considering the sample size available in this study, we chose to focus on common variants with an MAF > 0.05 , which is likely to miss rare variants and their potential impact on chemotherapy-induced toxicity. Next-generation sequencing has now been widely used to interrogate rare variants. However, the immediate pharmacogenetic significance of rare variants is still not apparent, as screening for rare variants among a large number of patients may not be cost-effective. Third, the observed associations in our study are somewhat marginal, especially after correction for multiple comparisons by permutation. However, our study is more of exploratory nature, since pharmacogenetic studies on toxicities are still sparse, which is a clinically important research area for patients' quality-of-life and survivorship. The patient population from a cooperative group clinical trial, the vigorous and systematic evaluation and collection of toxicity data, and the consistency between single SNP analysis and haplotype analysis are all strengths making our results less likely to be spurious. Although we still cannot exclude the possibility of false positivity for the lack of replication cohort, we feel that our report will likely stimulate others' attempt to validate our findings.

Randomized clinical trials with archived biospecimens have unique strengths in pharmacogenetic research. Although trials are typically designed to randomize patients on treatment but not genotypes, the randomization and the prospective nature minimizes most other potential biases, such as heterogeneous disease characteristics, treatment modality, incomplete report of clinical outcomes and follow-up. It has been proposed that findings of retrospective analysis of biospecimens from prospective trials can reach a high level of evidence in biomarker studies, provided that a large number of representative samples are included, there is a well-established assay for biomarker measurement, a pre-specified analytical plan of the biomarker of interest, and validation of the initial findings in a similar but independent cohort²⁷. As far as the above criteria are concerned, our study included a large proportion of patients enrolled in S0221 who also had banked blood samples, we selected tagSNPs to comprehensively capture common genetic variations, and the genotyping quality was high as demonstrated by very high call rate and concordance rate. In addition, the genotyped population was representative of those not genotyped (Table 1). However, our study still lacks a validation cohort to test whether the findings can be repeatedly observed in a different patient population, and these findings need to be followed up in other similar cohorts.

In conclusion, among 882 breast cancer patients from a cooperative group randomized trial S0221, we found evidence of associations between genetic variants in *ABCC1* and *ALDH1A1* with risk of hematological and gastrointestinal toxicities induced by AC treatment. The finding that patients carrying the risk allele of the *ALDH1A1* SNP were more likely to have hematological toxicity, despite the use of prophylactic growth factors,

indicates that pharmacogenetic markers may be useful in identifying a subgroup of patients at high risk of neutropenia induced by myelosuppressive chemotherapy. Future studies are warranted to replicate our findings and to examine other genes in relation to chemotherapy-induced toxicities.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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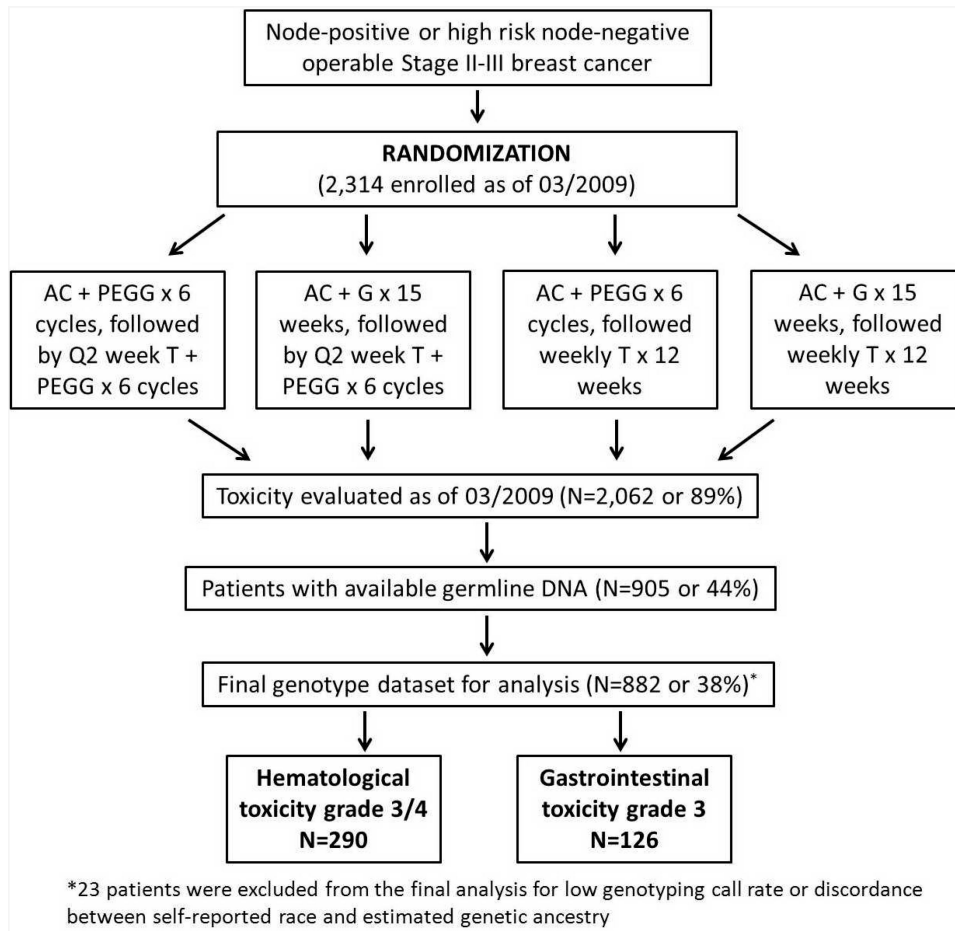


Figure 1. Randomization schema of SWOG S0221 clinical trial

Abbreviations: A, doxorubicin; C, cyclophosphamide; T, paclitaxel; G, filgrastim; PEGG, pegfilgrastim

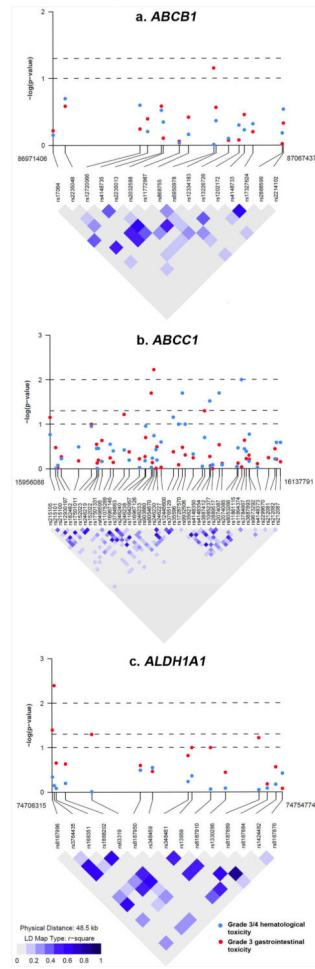


Figure 2. Linkage disequilibrium map and single SNP associations with grade 3 and 4 hematological toxicity and grade 3 gastrointestinal toxicity

Table 1
Descriptive characteristics of breast cancer patients enrolled in the S0221 trial with assessed toxicity by the availability of DNA at the time of genotyping

Variable	Patients with DNA available (n=882)	Patients without DNA available (n=1,180)
Age, years, mean \pm standard deviation	50.0 \pm 10.2	50.4 \pm 9.9
Self-reported race, N (%)		
European American	734 (83)	921 (78)
African American	69 (8)	149 (13)
Asian	41 (5)	61 (5)
Other	38 (4)	49 (4)
AC Treatment arm, N (%)		
Arm 1	446 (51)	619 (52)
Arm 2	436 (49)	561 (48)
CTCAE Grade of hematological toxicity, N (%)		
Grade 0-2	592 (67)	830 (70)
Grade 3	165 (19)	175 (15)
Grade 4	125 (14)	175 (15)
CTCAE Grade of gastrointestinal toxicity, N (%)		
Grade 0-2	756 (86)	1004 (85)
Grade 3	126 (14)	172 (15)
Grade 4	0 (0)	4 (0)

Table 2
SNPs in significant association with grade 3 and 4 hematological toxicity in S0221 trial

Gene	SNP	Genotype	# Grade 3/4 vs. grade 2	Adjusted OR (95% CI)*	P _{raw}	P _{permutation}
<i>ABCC1</i>	rs903880	CC	148/355	1.00	0.006	0.23
		CA	108/191	1.38 (1.02-1.88)		
		AA	34/46	1.86 (1.10-3.15)		
<i>ABCC1</i>	rs16967126	AA+AC	142/237	1.46 (1.09-1.95)	0.01	0.39
		AA	229/507	1.00	0.03	0.65
		AG	59/79	1.63 (1.12-2.37)		
<i>ABCC1</i>	rs4148350	GG	2/6	0.63 (0.12-3.22)		
		AG/GG	61/85	1.55 (1.08-2.25)	0.02	0.52
		CC	244/527	1.00	0.12	0.72
<i>ABCC1</i>	rs4148350	CA	46/64	1.54 (1.02-2.33)		
		AA	0/1	-		
		CA/AA	46/65	1.52 (1.01-2.29)	0.05	0.61
<i>ALDH1A1</i>	rs8187996	GG	253/543	1.00	0.10	0.65
		GA	36/48	1.63 (1.02-2.58)		
		AA	1/1	2.05 (0.13-33.87)		
<i>ALDH1A1</i>	rs3764435	GA/AA	49/37	1.64 (1.04-2.58)	0.04	0.61
		CC	63/160	1.00	0.004	0.21
		AC	127/286	1.11 (0.77-1.56)		
<i>ALDH1A1</i>	rs63319	AA	100/145	1.76 (1.18-2.61)		
		AC+AA	227/431	1.64 (1.20-2.25)	0.002	0.16
		CC	71/176	1.00	0.05	0.65
<i>ALDH1A1</i>	rs63319	CA	135/277	1.19 (0.84-1.68)		
		AA	84/139	1.48 (1.00-2.18)		
		CA+AA	219/416	1.28 (0.93-1.77)	0.13	0.78

Footnote:

* Logistic regression model adjusted for age of diagnosis, treatment arm and genetic ancestry.

Table 3
SNPs in significant association with grade 3 gastrointestinal toxicity in S0221 trial

Gene	SNP	Genotype	# Grade 3 vs. grade 2	Adjusted OR (95% CI)*	P _{raw}	P _{permutation}
<i>ABCC1</i>	rs35596	AA	58/420	1.00	0.02	0.52
		AG	57/277	1.58 (1.05-2.36)		
		GG	11/56	1.75 (0.84-3.63)		
<i>ABCC1</i>	rs4148354	AG+GG	68/333	1.60 (1.08-2.36)	0.02	0.39
		AA	49/192	1.00	0.03	0.59
		AG	47/355	0.53 (0.34-0.83)		
<i>ABCC1</i>	rs2889517	GG	30/208	0.60 (0.36-1.00)		
		AG+GG	77/563	0.55 (0.37-0.83)	0.003	0.23
		GG	76/351	1.00	0.02	0.52
<i>ABCC1</i>	rs11861115	GA	38/315	0.57 (0.37-0.86)		
		AA	12/90	0.62 (0.32-1.20)		
		GA+AA	50/405	0.58 (0.39-0.85)	0.006	0.24
<i>ABCC1</i>	rs11861115	GG	87/431	1.00	0.10	0.95
		GA	35/275	0.64 (0.42-0.98)		
		AA	4/48	0.41 (0.15-1.18)		
		GA/AA	39/323	0.61 (0.40-0.91)	0.01	0.26

Footnote:

* Logistic regression model adjusted for age of diagnosis, treatment arm and genetic ancestry.

Table 4
Haplotypes in significant associations with high grade hematological and gastrointestinal toxicity in S0221 trial

Gene	SNPs	Haplotype	MHF in high grade toxicity	MHF in low grade toxicity	Adjusted OR (95% CI)*	P _{raw}	P _{permutation}
ALDH1A1	rs3764435-rs168351	A-A	<i>Grade 3 and 4 hematological toxicity</i>				
			0.46	0.37	1.44 (1.16-1.78)	0.0008	0.03
ABCC1	rs2889517-rs2074087	A-G	<i>Grade 3 gastrointestinal toxicity</i>				
			0.09	0.16	0.54 (0.34-0.84)	0.006	0.24

Footnote:

* Logistic regression model adjusted for age of diagnosis, treatment arm and genetic ancestry. Odds ratio (OR) and 95% confidence interval (CI) were derived by comparing the specified haplotype with all other haplotypes combined.