RESEARCH ARTICLE

The Relationship between Common Genetic Markers of Breast Cancer Risk and Chemotherapy-Induced Toxicity: A Case-Control Study

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Abstract

Ninety-four common genetic variants are confirmed to be associated with breast cancer. This study tested the hypothesis that breast cancer susceptibility variants may also be associated with chemotherapy-induced toxicity through shared mechanistic pathways such as DNA damage response, an association that, to our knowledge, has not been previously investigated. The study included breast cancer patients who received neoadjuvant/adjuvant chemotherapy from the Pharmacogenetic SNPs (PGSNPS) study. For each patient, a breast cancer polygenic risk score was created from the 94 breast cancer risk variants, all of which were genotyped or successfully imputed in PGSNPS. Logistic regression was performed to test the association with two clinically important toxicities: taxane-related neuropathy (n = 1279) and chemotherapy-induced neutropenia (n = 1676). This study was well powered ($\geq$96%) to detect associations between polygenic risk score and chemotherapy toxicity. Patients with high breast cancer risk scores experienced less neutropenia compared to those with low risk scores (adjusted p-value = 0.06). Exploratory functional pathway analysis was performed and no functional pathways driving this trend were identified. Polygenic risk was not associated with taxane neuropathy (adjusted p-value = 0.48). These results suggest that breast cancer patients with high genetic risk of breast cancer, conferred by common variants, can safely receive standard chemotherapy without increased risk of taxane-related sensory neuropathy or chemotherapy-induced neutropenia and may experience less neutropenia. As neutropenia has previously been associated with improved

survival and may reflect drug efficacy, these patients may be less likely to benefit from standard chemotherapy treatment.

Introduction

Genome-wide association studies (GWAS) provide an empirical approach for identifying moderate risk alleles for a variety of widespread complex diseases and traits. Meta-analyses of 11 breast cancer GWAS (15,748 cases and 18,084 controls) and 41 studies in the Breast Cancer Association Consortium (BCAC) (46,785 cases and 42,892 controls) have confirmed 94 breast cancer susceptibility loci (p-value<5 x 10^{-8})[1–3]. Effect sizes of each genetic locus are generally modest (OR≤1.34), but together they explain approximately 16% of the excess familial risk of breast cancer. In a recent study by Mavaddat *et al*, a breast cancer polygenic risk score was created using 77 breast cancer risk variants. Women in the highest 1% of the risk score were 3.6 times more likely to develop breast cancer than women in the middle quintile [4].

We have hypothesised that genetic determinants of breast cancer incidence may be associated with the risk of chemotherapy-induced toxicity. This was based on the concept that variation in genes involved in pathways such as the repair of DNA damage may be important in both the mechanisms of tumour formation and proliferation and in the response to DNA damage induced by chemotherapy. For example, cyclophosphamide is an alkylating agent used to treat a variety of cancers including breast cancer. Cyclophosphamide interferes with DNA replication by forming intra-strand and inter-strand DNA cross-links, preventing tumour proliferation. The DNA cross-link repair 1B (*DCLRE1B*) gene is involved in repair of inter-strand cross-links and a common allele of this gene (rs11552449) is associated with increased risk of breast cancer [1]. The precise functional effect of this variant is unknown but patients with this mutation may also be less able to repair inter-strand cross-links induced in normal tissue by cyclophosphamide during treatment for breast cancer, resulting in increased toxicity.

Further, specific mutations that influence the risk of breast cancer developing may also affect genes in specific drug metabolism pathways. For example, it is known that polyadenosine 5'diphosphoribose polymerisation (PARP) enzymes play an important role in the repair of single strand breaks. PARP inhibitors (PARPi) target DNA homologous repair pathways, by preventing repair of single strand breaks leading to problems downstream with double strand repair. PARPi work, therefore, in synergy with DNA damaging agents like platinums, which also cause strand breaks. In patients with rare *BRCA1* or *BRCA2* mutations, the already compromised homologous repair pathways allow PARPi to work particularly effectively leading to "synthetic lethality" [5]. Thus, such genetic mutations both increase susceptibility to breast cancer and enable a better response to certain treatments, although the drug toxicity profile of patients carrying these mutations is, as yet, unclear. Variants of genes that play a role in drug metabolism may also lie in pathways unrelated to DNA repair.

To date, chemotherapy toxicity GWAS have had limited success in identifying common genetic variants that significantly influence a patient's risk of toxicity [6–11]. This is mainly due to lack of statistical power stemming from small samples and the requirement for stringent p-value thresholds for obtaining statistical significance. No single nucleotide polymorphisms (SNPs) reaching genome-wide significance have been independently replicated in validation samples to accepted GWAS levels of significance.

The aim of this study was to look for associations between common genetic variants known to increase the risk of breast cancer and chemotherapy-induced toxicity using patient samples from the Pharmacogenetic SNPs (PGSNPS) study, one of the largest chemotherapy toxicity

GWAS to date. Common genetic variants have small individual effects on breast cancer so it is likely that they will also have small individual effects on chemotherapy toxicity. Thus, to increase the power to detect an association between genotype and toxicity, variants were combined in a polygenic risk score. Whilst for most chemotherapeutic agents there is a presumed mechanism of action, it is widely accepted that we do not have a complete understanding of all the mechanisms by which the majority of the agents function. Further, the precise impact of many of the breast cancer variants on the gene in which they lie and the mechanisms underlying the individual associations between each variant and breast cancer risk are as yet unknown. Thus, all breast cancer risk variants were included in our analyses.

Materials and Methods

Patients

The study cohort consisted of female breast cancer patients from PGSNPS, a large study that was set up to investigate the role of germline variants in chemotherapy toxicity [12]. The PGSNPS sample includes 2354 female patients from four UK breast cancer chemotherapy trials: NEAT [13], BR9601 [13], tAnGo [14] and Neo-tAnGo [15]. S1 Table and S1 Fig (see Supporting Information) summarise PGSNPS and the clinical trial regimens. In brief, patients in NEAT and BR9601 received either six or eight cycles of cyclophosphamide, methotrexate and 5-fluouracil (CMF) or four cycles of epirubicin (E) followed by four cycles of CMF, while patients in tAnGo and Neo-tAnGo received either four cycles of EC followed by four cycles of paclitaxel (T) with or without gemcitabine (±G) or four cycles of T±G followed by four cycles of T. DNA samples were collected along with demographic, tumour and treatment information, chemotherapy toxicity scores and relapse and survival times. An additional 56 patients who were not taking part in a clinical trial were recruited from the Cambridge University Hospitals NHS Foundation Trust breast unit using the same clinical response forms. These patients received four cycles of epirubicin (E) followed by four cycles of cyclophosphamide, methotrexate and 5-fluorouracil (CMF).

Ethics and data availability

PGSNPS [Pharmacogenetics of Early Breast Cancer Chemotherapy–reference number 05/Q0104/1] was approved by the NRES Committee East of England—Cambridge East. All participants provided informed consent to take part in PGSNPS.

The data used in this study is held by the Trial Management Group for PGSNPS, where the original concept for this analysis was designed. Any access requires appropriate ethical approvals and would be assessed by the Trial Management Group which includes the respective Chief Investigators of the clinical trials and PGSNPS. Transfer of data would require a specific Data Transfer Agreement.

Toxicity phenotypes

This study investigated two common and clinically important chemotherapy-induced toxicities: neutropenia and taxane-related sensory neuropathy (for the purposes of this study, this will be referred to as simply "neuropathy" from now on). For all patients, toxicity information was collected prospectively and graded using the National Cancer Institute Common Toxicity Criteria for Adverse Events (NCI CTCAE) version 2 or 3, depending on the clinical trial from which the patient was recruited into PGSNPS. Rates and grades of neutropenia were recorded in 1676 patients who received any of the trial chemotherapy regimens in NEAT, tAnGo and Neo-tAnGo (data for neutropenia was not available from BR9601) or were not in a trial and

received E-CMF. Rates and grades of neuropathy were recorded in 1279 patients who received a paclitaxel-containing regimen (tAnGo and Neo-tAnGo).

Genotyping, quality control and imputation

Samples were genotyped using the Affymetrix 6.0 SNP array. Quality control procedures were applied to remove variants that were missing in >5% of samples; had minor allele frequency (MAF) < 1%; or had MAF<5% and were missing in >1% samples. Variants were also removed if their genotype frequencies deviated from those expected under Hardy-Weinberg equilibrium (p-value < 10^{-5}). Samples were removed that had >10% of all variants missing. Principle components analysis (PCA) was used to identify and exclude individuals with non-European ancestry and control for population substructure. Genome coverage was increased by imputation using SHAPEIT [16] and IMPUTE v2 [17] with the 1000 Genomes reference panel [18]. Genotype dosages of the breast cancer risk alleles were extracted from the imputed data.

Statistical methods

To quantify each patient's genetic risk of breast cancer, polygenic risk scores were created by summing the patient's risk allele dosages across all the variants. Two risk scores were calculated:

1. Non-weighted: $risk\ score_i = \sum_1^j G_i$

2. Weighted: $weighted\ risk\ score_i = \sum_1^j \beta_j G_i$

for patient i,
    where j = variant 1..94
    β_j = the per-allele log-odds ratio for risk of breast cancer associated with variant j
    G = risk allele dosage

The log-odds ratios used to weight the risk score were taken directly from the report by Mavaddat *et al* [4] who tested the association of each variant with breast cancer risk while adjusting for the effect of other variants (see Table 1). Seventeen variants have been identified since Mavaddat *et al* performed their analysis [2,3]. For these, the log-odds ratios used were those reported by Michailidou et al [3].

Neutropenia and neuropathy grades were dichotomised into cases (neutropenia grade ≥3, neuropathy grade ≥2) and controls (neutropenia grades 0–2, neuropathy grades 0–1) (see Table 2). Logistic regression was used to test the association between polygenic risk score and toxicity case status for neutropenia and neuropathy, respectively. Each of the 94 genetic variants was also tested separately for association with neutropenia and neuropathy. For multivariable analysis, pre-specified important non-genetic covariates were included in the models. The neutropenia analysis was adjusted for trial and patient age and the neuropathy analysis was adjusted for trial, pre-treatment body mass index (BMI) and the first two principle components to control for population substructure. Per-allele odds ratios (ORs) and 95% confidence intervals (CIs) are presented for the non-weighted polygenic risk score and individual variants. ORs and CIs corresponding to a one standard deviation (SD) increase in risk score are presented for the weighted polygenic risk score.

Pathway analysis. Interesting associations between polygenic risk and toxicity were followed up with exploratory pathway analysis to investigate whether a subset of the breast cancer variants, lying in a common pathway, were responsible for the observed association. The breast cancer variants were mapped to the genes in which they lay or to the nearest gene if they were intergenic. These variant-gene pairs were ranked using the p-value for association between

Table 1. Genetic variants known to influence risk of breast cancer.

Variant	Nearestgene	Chr	Position (build 37)	Breast cancer risk allele	Published odds ratio[a]	PGSNPS risk allele frequency	PGSNPS imputation $r^{2\ c}$
rs616488	PEX14	1	10566215	A	1.06	0.67	0.96
rs11552449	PTPN22-BCL2L15-AP4B1-DCLRE1B-HIPK1	1	114448389	T	1.08	0.18	0.94
rs11249433	None	1	121280613	G	1.1	0.43	0.81
rs12405132	RNF115	1	145644984	C	1.05[b]	0.63	1
rs12048493	OTUD7B	1	149927034	C	1.07[b]	0.34	0.52
rs6678914	LGR6	1	202187176	G	1.01	0.40	0.99
rs4245739	MDM4	1	204518842	C	1.03	0.28	1
rs72755295	EXO1	1	242034263	G	1.15[b]	0.04	0.72
rs12710696	OSR1	2	19320803	A	1.04	0.37	1
rs4849887	INHBB	2	121245122	C	1.09	0.92	1
rs2016394	METAP1D-DLX1-DLX2	2	172972971	G	1.05	0.55	0.87
rs1550623	CDCA7	2	174212894	A	1.06	0.85	1
rs1045485	CASP8[d]	2	202149589	G	1.04	0.87	0.99
rs13387042	IGFBP5[d]	2	217905832	A	1.14	0.51	1
rs16857609	DIRC3	2	218296508	T	1.07	0.28	0.99
rs6762644	ITPR1-EGOT	3	4742276	G	1.07	0.41	0.99
rs4973768	SLC4A7	3	27416013	T	1.09	0.49	0.99
rs12493607	TGFBR2	3	30682939	C	1.05	0.34	0.99
rs6796502	PRSS42	3	46866866	G	1.09[b]	0.91	0.96
rs1053338	ATXN7	3	63967900	G	1.08[b]	0.15	0.99
rs9790517	TET2	4	106084778	T	1.05	0.20	0.99
rs6828523	ADAM29	4	175846426	C	1.1	0.89	1
rs10069690	TERT[d]	5	1279790	T	1.02	0.25	0.66
rs7726159	TERT[d]	5	1282319	A	1.04	0.36	0.75
rs2736108	TERT[d]	5	1297488	C	1.07	0.73	0.78
rs13162653	MARCH11	5	16187528	G	1.05[b]	0.57	0.97
rs2012709	SUB1	5	32567732	T	1.05[b]	0.49	0.99
rs10941679	None	5	44706498	G	1.12	0.26	0.95
rs889312	MAP3K1[d]	5	56031884	C	1.12	0.30	0.99
rs10472076	RAB3C	5	58184061	C	1.04	0.38	0.94
rs1353747	PDE4D	5	58337481	T	1.09	0.89	0.99
rs7707921	ATG10	5	81538046	A	1.08[b]	0.75	0.99
rs1432679	EBF1	5	158244083	G	1.07	0.44	0.99
rs11242675	FOXQ1	6	1318878	T	1.06	0.63	0.99
rs204247	RANBP9	6	13722523	G	1.05	0.45	1
rs9257408	None	6	28926220	C	1.05[b]	0.37	0.98
rs17529111	None	6	82128386	G	1.05	0.22	0.98
rs12662670	ESR1[e]	6	151918856	G	1.14	0.09	0.99
rs2046210	ESR1[e]	6	151948366	A	1.05	0.38	1
rs6964587	AKAP9	7	91630620	T	1.05[b]	0.40	1
rs4593472	LINC-PINT	7	130667121	C	1.05[b]	0.64	1
rs720475	ARHGEF5-NOBOX	7	144074929	G	1.06	0.74	1
rs9693444	None	8	29509616	A	1.07	0.35	0.99
rs13365225	KCNU1	8	36858483	A	1.05[b]	0.84	1
rs6472903	CASC9	8	76230301	T	1.1	0.84	0.93
rs2943559	HNF4G	8	76417937	G	1.13	0.09	1

(Continued)

Table 1. (*Continued*)

Variant	Nearestgene	Chr	Position (build 37)	Breast cancer risk allele	Published odds ratio[a]	PGSNPS risk allele frequency	PGSNPS imputation r[2c]
rs13267382	None	8	117209548	A	1.05[b]	0.36	0.93
rs13281615	MYC[d]	8	128355618	G	1.1	0.43	0.99
rs11780156	MYC[d]	8	129194641	T	1.07	0.19	1
rs1011970	CDKN2A/B	9	22062134	T	1.05	0.18	0.99
rs10759243	KLF4[d]	9	110306115	A	1.05	0.29	1
rs865686	KLF4[d]	9	110888478	T	1.11	0.64	0.99
rs2380205	ANKRD16	10	5886734	C	1.02	0.57	1
rs7072776	MLLT10-DNAJC1	10	22032942	A	1.06	0.30	1
rs11814448	DNAJC1	10	22315843	C	1.22	0.02	0.98
rs10995190	NRBF2[d]	10	64278682	G	1.17	0.87	0.99
rs704010	ZMIZ1	10	80841148	T	1.07	0.42	1
rs7904519	TCF7L2	10	114773927	G	1.06	0.48	0.99
rs11199914	FGFR2[d]	10	123093901	C	1.06	0.70	0.99
rs2981579	FGFR2[d]	10	123337335	A	1.25	0.44	0.99
rs3817198	LSP1	11	1909006	C	1.07	0.34	1
rs3903072	DKFZp761e198-OVOLI-SNX32-CFL1-MUS81	11	65583066	G	1.06	0.56	1
rs78540526	CCND1[d]	11	69331418	T	1.18	0.08	0.98
rs554219	CCND1[d]	11	69331642	G	1.12	0.13	0.99
rs75915166	CCND1[d]	11	69379161	A	1.024	0.07	0.95
rs11820646	BARX2	11	129461171	C	1.05	0.60	0.99
rs12422552	None	12	14413931	C	1.03	0.29	0.91
rs10771399	PTHLH	12	28155080	A	1.16	0.89	0.99
rs17356907	NTN4	12	96027759	A	1.1	0.72	1
rs1292011	None	12	115836522	A	1.08	0.58	1
rs11571833	BRCA2-N4BP2LI-N4BP2L2	13	32972626	T	1.26	0.01	0.99
rs2236007	PAX9-SLO25A21	14	37132769	G	1.09	0.81	0.98
rs2588809	RAD51L1	14	68660428	T	1.07	0.17	1
rs999737	RAD51L1	14	69034682	C	1.08	0.76	0.99
rs941764	CCDC88C	14	91841069	G	1.06	0.35	0.99
rs11627032	RIN3	14	93104072	T	1.06[b]	0.76	0.99
rs3803662	TOX3	16	52586341	A	1.23	0.30	1
rs17817449	MIRI972-2-FTO	16	53813367	T	1.08	0.61	0.99
rs11075995	FTO	16	53855291	T	1.04	0.24	0.99
rs13329835	CDYL2	16	80650805	G	1.08	0.23	0.99
rs146699004	TEFM	17	29230520	GGT	1.08[b]	0.81	0.87
rs6504950	COX11[e]	17	53056471	G	1.07	0.72	1
rs745570	CBX8	17	77781725	A	1.05[b]	0.50	1
rs527616	None	18	24337424	G	1.04	0.66	0.94
rs1436904	CHST9	18	24570667	T	1.06	0.60	1
rs6507583	SETBP1	18	42399590	A	1.10[b]	0.93	0.99
rs8170	ABHD8/ANKLE1[e]	19	17389704	A	1.03	0.20	0.99
rs2363956	ABHD8/ANKLE1[e]	19	17394124	T	1.03	0.51	0.96
rs4808801	SSBP4-ISYNA1-ELL	19	18571141	A	1.07	0.66	1
rs3760982	C19orf61-KCNN4-LYPD5-ZNF283	19	44286513	A	1.06	0.49	0.99
rs2823093	NRIP1	21	16520832	G	1.08	0.75	0.97

(*Continued*)

Table 1. (*Continued*)

Variant	Nearestgene	Chr	Position (build 37)	Breast cancer risk allele	Published odds ratio[a]	PGSNPS risk allele frequency	PGSNPS imputation r[2][c]
rs17879961	*CHEK2*	22	29121087	G	1.36	0.001	0.86
rs132390	*EMID1-RHBDD3-EWSR1*	22	29621477	C	1.11	0.04	0.78
rs6001930	*MKL1*	22	40876234	C	1.13	0.10	1

[a]Adjusted breast cancer odds ratios from Mavaddat *et al* (4)

[b]Unadjusted odds ratios from BCAC meta-analysis (1–3)

[c]Mean imputation r^2 from IMPUTE2 ($r^2 = 1$ for genotyped SNPs)

[d]published target gene

[e]known target gene, not yet published

Chr: chromosome.

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each variant and toxicity, from most strongly to least strongly associated, regardless of the direction of effect on toxicity. Where more than one variant mapped to the same gene, the most significant toxicity-associated variant was used for ranking. The top 50% of the ranked genes were entered into the Database for Annotation, Visualization and Integrated Discovery (DAVID) version 6.7 functional annotation tool [19,20]. DAVID draws functional annotations from various online databases to group genes that are in the same biological pathway and performs a Fisher's exact test to determine whether genes from any particular pathway are overrepresented in the user's list of genes. A Fisher exact p-value≤0.05 identified pathways that were significantly enriched among the top genes for toxicity.

Statistical Power. This study was well powered to detect significant associations between breast cancer polygenic risk score and the toxicity endpoints examined. Assuming a 30% prevalence of moderate-severe toxicity (neutropenia ≥3, neuropathy grade ≥2) in breast cancer patients, the power to detect a small difference of 0.1 in mean risk score between patients with moderate-severe toxicity and patients with no or mild toxicity, at p-value<0.05, would be 96% in the neuropathy sample and 99% in the neutropenia sample. This difference in mean risk score is equivalent to a relative risk of moderate-severe toxicity of 1.1 for patients with a higher polygenic risk score.

Results

The total number of patients included in this study was 1677. Patient characteristics are summarised in S2 Table (see Supporting Information). All 94 genetic variants known to increase the risk of breast cancer were genotyped or successfully imputed (IMPUTE2 info metric>0.5)

Table 2. Distribution of chemotherapy-induced neutropenia and taxane-related sensory neuropathy in the PGSNPS sample according to the National Cancer Institute Common Toxicity Criteria for Adverse Events (NCI CTCAE) version 2/3.

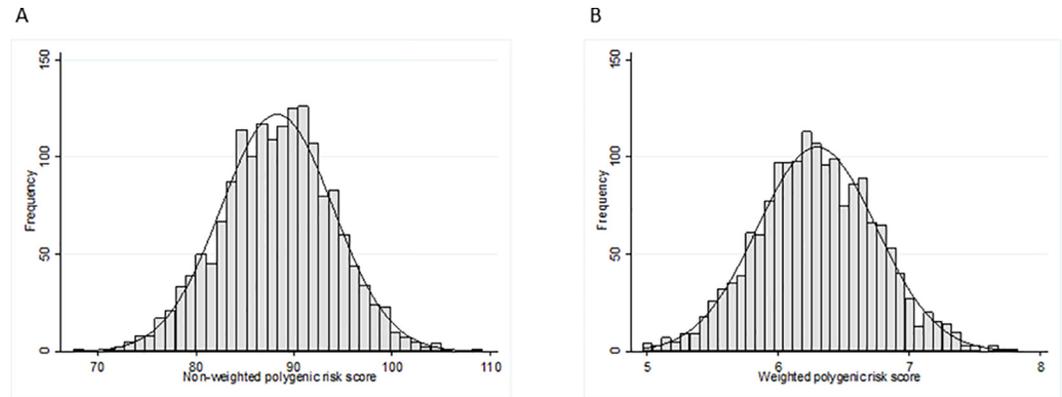| NCI CTCAE grade	Neutropenia; total N = 1676 n (%)	Neuropathy; total N = 1279 n (%)
0	733 (43.7)	271 (21.2)
1	199 (11.9)	648 (50.7)
2	245 (14.6)	304 (23.7)
3	293 (17.5)	56 (4.4)
4	206 (12.3)	0 (0)
Toxicity cases (moderate-severe toxicity)	grade ≥3	grade ≥2
	499 (29.8)	360 (28.1)

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Fig 1. Distribution of polygenic risk scores in the PGSNPS cohort. A) Non-weighted polygenic risk score. B) Weighted polygenic risk score.

in the PGSNPS sample. The variants and information about MAF and imputation certainty can be found in Table 1. Fig 1 shows the approximately normal distribution of the two polygenic risk scores in the PGSNPS patients.

The non-weighted risk score was significantly associated with a decreased risk of neutropenia (grade ≥3) on univariable analysis (per-allele OR = 0.98; 95% CI = (0.96, 0.99); p = 0.04) (Table 3). This finding was not nominally significant when adjusted for age and trial (p = 0.06) but the effect size was the same. The weighted risk score was not significantly associated with neutropenia (grade ≥3) but the effect was in the same direction as with the non-weighted score. Neither the non-weighted nor the weighted risk score was associated with neuropathy (OR = 0.99 (0.97, 1.01); p = 0.48 and OR = 0.99 (0.95, 1.02); p = 0.47, respectively). None of the individual genetic variants were significantly associated with neuropathy or neutropenia at the $p < 5 \times 10^{-4}$ level.

Pathway analysis

Pathway analysis was performed to investigate whether a specific molecular pathway was driving the trend between increased breast cancer risk and reduced risk of neutropenia. The individual breast cancer risk variants were ranked according to their p-value for association with neutropenia and the ranked variant-gene pairs were compiled to create a list of 76 genes. Table 4 shows the top 50% (n = 38) of genes in the ranked list. The DAVID functional overrepresentation tool [19,20] was used to annotate the top 38 genes and identified the p53 signalling pathway as the most strongly enriched pathway (Fisher exact p-value = 0.004). Three genes (*CCND1*, *CHEK2*, *MDM4*) in the p53 signalling pathway appeared in the top 38 genes. However, this observed enrichment did not remain significant following Bonferroni correction for the multiple pathways tested by the DAVID tool (corrected p53 signalling pathway p-value = 0.13).

Table 3. Association of polygenic risk scores with chemotherapy-related neutropenia and taxane-related sensory neuropathy.

	Odds ratio (95% confidence interval) & p-value[a]			
	Non-weighted risk score		**Weighted risk score**	
	unadjusted	**adjusted[b]**	**unadjusted**	**adjusted[b]**
neutropenia (n = 1676)	0.98 (0.96, 0.99) p = 0.04	0.98 (0.96, 1.00) p = 0.06	0.98 (0.95, 1.01) p = 0.16	0.98 (0.95, 1.01) p = 0.20
neuropathy (n = 1279)	0.99 (0.97, 1.01) p = 0.41	0.99 (0.97, 1.01) p = 0.48	0.98 (0.95, 1.02) p = 0.37	0.99 (0.95, 1.02) p = 0.47

[a]Non-weighted risk score: per-allele odds ratio and confidence interval; Weighted risk score: per-standard deviation odds ratio and confidence interval
[b]Neutropenia: adjusted for age and trial; neuropathy: adjusted for body mass index, trial and first two principle components

Discussion

The hypothesis behind this study was that common genetic variants known to increase the risk of breast cancer may also increase the likelihood of developing treatment-related toxicity

Table 4. Top 38 genes according to p-value for association between highest-ranking breast cancer risk variant and neutropenia.

Gene	Breast cancer risk variant	Variant association with neutropenia	
		Odds Ratio	P-value
ZMIZ1	rs704010	1.20	0.02
KCNN4	rs3760982	0.85	0.03
MAP3K1	rs889312	1.19	0.04
DCLRE1B	rs11552449	0.81	0.05
KCNU1	rs13365225	0.81	0.05
DLX2	rs2016394	0.86	0.06
ITPR1	rs6762644	1.15	0.08
MYC	rs13281615	1.14	0.10
RANBP9	rs204247	1.13	0.10
CASC9[a]	rs6472903	0.84	0.10
CBX8	rs745570	0.89	0.13
SNX32	rs3903072	0.90	0.17
SLC4A7	rs4973768	1.11	0.18
TET2	rs9790517	0.89	0.22
KLF4	rs10759243	1.10	0.23
TERT	rs2736108	0.89	0.26
ANKRD16	rs2380205	0.92	0.27
CDYL2	rs13329835	0.90	0.27
NRIP1	rs2823093	0.91	0.29
CHST9	rs1436904	0.93	0.34
CCND1	**rs78540526**	**1.14**	**0.37**
FGFR2	rs2981579	1.07	0.37
DNAJC1	rs11814448	0.74	0.37
HNF4G	rs2943559	0.88	0.38
ATXN7	rs1053338	0.91	0.38
AKAP9	rs6964587	1.07	0.38
MKL1	rs6001930	0.90	0.41
LGR6	rs6678914	1.06	0.45
FOXQ1	rs11242675	0.94	0.46
ANKLE1	rs8170	1.07	0.50
RNF115	rs12405132	0.95	0.51
SETBP1	rs6507583	1.10	0.52
ADAM29	rs6828523	0.92	0.52
NRBF2	rs10995190	0.93	0.53
ESR1	rs12662670	0.92	0.53
CHEK2	**rs17879961[b]**	**1.36×10^{-19}**	**0.54**
PTHLH	rs10771399	0.93	0.56
IGFBP5	rs13387042	0.96	0.59
MDM4	**rs4245739**	**0.96**	**0.60**

[a]CASC9 not mapped by DAVID tool so excluded from pathway analysis

[b]variant frequency in PGSNPS = 0.001

p53 signalling pathway genes highlighted in bold.

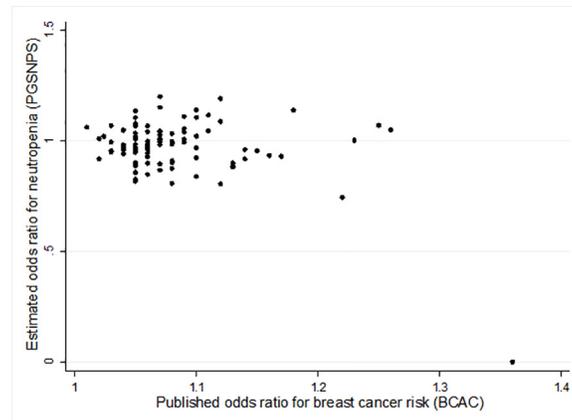doi:10.1371/journal.pone.0158984.t004

Fig 2. Scatter plot comparing variant-associated odds ratios for breast cancer risk, published by the Breast Cancer Association Consortium (BCAC), with odds ratios for neutropenia risk estimated in the Pharmacogenetic SNPs (PGSNPS) study.

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following chemotherapy for breast cancer. In this well powered study, no evidence was found for an association between common variants known to increase breast cancer risk and taxane-related sensory neuropathy in the PGSNPS cohort. Interestingly, and contrary to our hypothesis, there was some evidence of a relationship between carrying an increased number of breast cancer risk alleles and decreased risk of experiencing chemotherapy-induced neutropenia grade ≥ 3 (OR = 0.98; 95% CI = (0.96, 1.00)). Weighting the alleles by the estimate of their effect on breast cancer risk reduced the strength of this association. This suggests that the magnitude of effect that these variants have on risk of neutropenia is not equal to their magnitude of effect on risk of breast cancer. This is demonstrated in Fig 2, which shows the effects that the individual variants have on breast cancer risk (as reported by Mavaddat *et al* 2015) plotted against their effects on neutropenia in the PGSNPS sample; there is no visible relationship between the effects. Pathway analysis did not identify any significant pathway enrichment in the genes representing the top-ranked breast cancer risk variants.

None of the individual variants were significantly associated with chemotherapy-induced toxicity. The original work that confirmed the association of the 94 genetic variants with breast cancer risk was performed by BCAC and based on samples of over 100,000 patients. In contrast, the PGSNPS breast cohort studied in the current analysis has fewer than 2,000 patients. Therefore, the power to detect a true association at genome-wide significance is much lower than that of the breast cancer susceptibility studies. With an increased sample size, there would be greater power to detect strong associations between individual variants and chemotherapy toxicity.

The observed association between polygenic breast cancer risk and decreased neutropenia suggests that breast cancer patients who present with a high genetic risk of breast cancer, conferred by common variants, can safely receive standard chemotherapy and may experience less neutropenia compared to patients with low genetic risk of breast cancer. There is strong evidence to support the relationship between neutropenia or leukopenia and improved survival [21–23]. Abraham *et al* have shown that in a cohort of over 6000 early breast cancer patients from randomised clinical trials, those who achieved neutropenia grade ≥ 3 during their treatment had statistically significant improved relapse-free survival (hazard ratio = 0·86; 95% CI = (0·76–0·97); p = 0·02) [23]. In the current study population, expanded clinical and survival data was available for 1450 patients. After adjusting for non-genetic predictors of survival, the 29% of breast cancer patients who experienced neutropenia grade ≥ 3 had longer relapse-free

survival compared to the 71% who did not experience neutropenia grade ≥3 (HR = 0.71; 95% CI = (0.54–0.94); p = 0.02). Neutropenia may therefore be a surrogate marker of efficacy, although the mechanisms underlying the association between neutropenia and survival are unclear. The hypothesis that neutropenia may reflect efficacy is supported by a recent prospective randomised phase III trial of tailored and dose-dense versus standard tri-weekly adjuvant chemotherapy for high risk breast cancer. In the tailored and dose-dense therapy arm of the trial, where a patient had a toxicity of grade 2 or less, the chemotherapy dose was escalated. The results of the trial showed that the tailored approach resulted in an improvement in all studied efficacy endpoints [24].

Given the potential relationship between neutropenia and clinical outcome, the finding that patients with high polygenic risk of breast cancer experience less neutropenia may, firstly, reflect the fact that for some patients standard chemotherapy regimens are sub-optimal and, secondly, suggests that genetic risk of cancer may potentially distinguish these patients, who may tolerate more intense chemotherapy that could improve survival. If this is the case, common breast cancer risk variants may be a useful tool for predicting which patients are likely to have poorer prognosis. We evaluated the relationship between the breast cancer risk polygenic score and relapse-free survival in the same cohort of patients. The polygenic risk score was predictive of relapse-free survival such that patients who have an increased risk of breast cancer (and therefore lower risk of neutropenia) tended to have shorter relapse-free survival (HR = 1.02; 95% CI = (1.00–1.04); p = 0.06). This equates to a 23% increase in risk of relapse or death for every 10 extra risk alleles that a patient carries (HR = 1.23; 95% CI = (0.99–1.51); p = 0.06). This difference in hazards is illustrated in a Kaplan-Meier plot in Fig 3. After adjusting for neutropenia case-control status, this relationship was weakened slightly; patients carrying an extra 10 risk alleles had 21% increase in risk of relapse or death (HR = 1.216; 95% CI = (0.98–1.49); p = 0.08). These results support the hypothesis that neutropenia is a marker of efficacy of chemotherapy and that efficacy could be predicted by breast cancer polygenic risk. However, a large study with more power to detect subtle survival effects is required to confirm these results.

In conclusion, for breast cancer patients who are carrying common genetic variants known to increase the risk of breast cancer, standard chemotherapy for breast cancer, although safe, may not be adequately effective. It is likely that there are less common variants and rare mutations that have large effects on toxicity response to chemotherapy and these may prove more useful for predicting patient drug response in the clinic. Thus, targeted sequencing of candidate
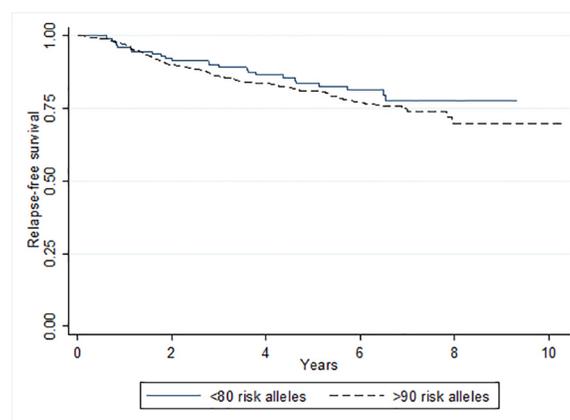

Fig 3. Kaplan-Meier plot comparing relapse-free survival in patients carrying >90 risk alleles to those carrying <80 risk alleles.

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genes or whole-exome/genome sequencing in large patient samples should be a next step in the search for pharmacogenetic determinants of chemotherapy toxicity.

Supporting Information

S1 Fig. Summary of PGSNPS clinical trials regimens.
(DOCX)

S1 Table. Summary of Clinical Trials contributing to PGSNPS.
(DOCX)

S2 Table. Patient Characteristics.
(DOCX)

Acknowledgments

Author Contributions

Conceived and designed the experiments: LD SK KM PDPP JEA. Performed the experiments: LD SK. Analyzed the data: LD. Contributed reagents/materials/analysis tools: LD SK. Wrote the paper: LD SK KM LH CT HME PDPP JEA. Chief investigators of trials/studies in PGSNPS: HME CJP CT JEA. Co-ordination of trials and collection of data: JEA LH SB RH SI ALV CT CJP HME JAD. Overall co-ordination of PGSNPS: JEA PDPP CC HME.

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