Published OnlineFirst March 14, 2016; DOI: 10.1158/0008-5472.CAN-15-1410

Prevention and Epidemiology

Cancer Research

Cytochrome P450 Allele *CYP3A7*1C* Associates with Adverse Outcomes in Chronic Lymphocytic Leukemia, Breast, and Lung Cancer

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Abstract

CYP3A enzymes metabolize endogenous hormones and chemotherapeutic agents used to treat cancer, thereby potentially affecting drug effectiveness. Here, we refined the genetic basis underlying the functional effects of a *CYP3A* haplotype on urinary estrone glucuronide (E1G) levels and tested for an association between *CYP3A* genotype and outcome in patients with chronic lymphocytic leukemia (CLL), breast, or lung cancers. The most significantly associated SNP was rs45446698, an SNP that tags the *CYP3A7*1C* allele; this SNP was associated with a 54% decrease in urinary E1G levels. Genotyping this SNP in 1,008 breast cancer, 1,128 lung cancer, and 347 CLL patients, we found that rs45446698 was associated with breast cancer mortality (HR, 1.74; $P = 0.03$), all-cause mortality in lung cancer patients (HR, 1.43; $P = 0.009$), and CLL progression (HR, 1.62; $P = 0.03$). We also found borderline evidence of a statistical interaction between the *CYP3A7*1C* allele, treatment of patients with a cytotoxic agent that is a CYP3A substrate, and clinical outcome ($P_{interaction} = 0.06$). The *CYP3A7*1C* allele, which results in adult expression of the fetal *CYP3A7* gene, is likely to be the functional allele influencing levels of circulating endogenous sex hormones and outcome in these various malignancies. Further studies confirming these associations and determining the mechanism by which *CYP3A7*1C* influences outcome are required. One possibility is that standard chemotherapy regimens that include CYP3A substrates may not be optimal for the approximately 8% of cancer patients who are *CYP3A7*1C* carriers. *Cancer Res; 76(6); 1485–93. ©2016 AACR.*

Introduction

The *CYP3A5*, *CYP3A7*, and *CYP3A4* genes, which form the cytochrome P450 family 3 subfamily A (*CYP3A*) gene cluster at 7q22.1, encode enzymes that metabolize a diverse range of substrates (1). Specifically, in addition to a role in the oxidative metabolism of endogenous hormones, CYP3A enzymes metabolize around 50% of all clinically used drugs including many of the agents used in treating cancer (2). Of particular relevance to breast cancer, the hormonal agent tamoxifen, the alkylating agent cyclophosphamide, the taxanes, paclitaxel and docetaxel, and the topoisomerase II inhibitor, mitoxantrone are all CYP3A substrates (1, 3). *CYP3A* genes are differentially regulated and substantial interindividual differences in expression have been reported for all three genes (4). *CYP3A4*, the major isoform in adults, is predominantly expressed in the liver, where it is the most abundant P450, accounting for 30% of total CYP450 protein (5). *CYP3A5* is "polymorphically" expressed, with approximately 33% of Europeans and 60% of African Americans expressing detectable levels in the adult liver (4). *CYP3A7*, the major isoform in the fetus, is generally silenced shortly after birth (6).

We have previously screened 642 SNPs tagging 42 genes involved in sex steroid synthesis or metabolism, and tested for association with premenopausal urinary estrone glucuronide (E1G) levels, measured in serial urine samples collected at prespecified days of the woman's menstrual cycle (7). We demonstrated that a rare haplotype, defined by two SNPs spanning the *CYP3A* gene cluster (rs10273424 and rs680055), was associated with a highly significant 32% difference in urinary E1G (7). Predicated on the assumption that genetically determined effects on metabolism may impact on patient outcome, we have (i) refined the genetic basis for this association and (ii) examined the association between genotype and outcome in three cancers—breast and lung cancer and chronic lymphocytic leukemia (CLL).

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Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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doi: 10.1158/0008-5472.CAN-15-1410

Published OnlineFirst March 14, 2016; DOI: 10.1158/0008-5472.CAN-15-1410

Patients and Methods

Ethics

The study was conducted in accordance with the tenets of the Declaration of Helsinki and all patients provided written informed consent. Ethical approval for the study was obtained from the Royal Marsden NHS Trust.

Study subjects—fine mapping of the *CYP3A* locus

Full details of the 371 women from the British Breast Cancer (BBC; ref. 8) and the 358 women from the Mammography Oestrogens and Growth Factors (MOG; ref. 9) studies genotyped for this analysis have been published previously (7). Briefly, they comprised premenopausal women who were first-degree relatives and friends of breast cancer cases (BBC study) or participants in the intervention arm of a trial of annual mammographic screening in young women (10) conducted in Britain (MOG study). To be eligible, women had to be having regular menstrual cycles, not using hormone replacement therapy or oral contraceptives and not to have been diagnosed with breast cancer at recruitment to the study. All women had self-reported Northern European ancestry. To be included in the original analysis and this fine-mapping analysis women had to have provided serial urine samples, at pre-specified days of their menstrual cycle for measurement of creatinine-adjusted urinary E1G. E1G was measured using an in-house ELISA (7).

Study subjects—risk analysis

Royal Marsden Hospital Lifestyle and Family History study. The Royal Marsden Hospital (RMH) Lifestyle and Family History study comprises 1,786 consecutive breast cancer patients who attended the RMH Breast Unit (RMH, Chelsea, London, UK) between June 2000 and January 2007. Women were invited to participate in the study by completing a questionnaire, consenting to access to medical records and providing a blood sample. We identified all women with self-reported White ethnicity for whom DNA was available (n = 1,536). We excluded: women born outside the British Isles (n = 173, 11.3%), women not followed up at the RMH (n = 96, 6.3%), secondary referrals where the pathology report from the referring hospital was missing or incomplete (n = 97, 6.3%), cases presenting with metastatic disease (n = 13, 0.8%), complex disease histories (n = 8), atypical histology (n = 6), women who had a prophylactic mastectomy (n = 2), and cases unable to be traced (n = 13). Finally, we excluded 118 (7.7%) cases with noninvasive cancer leaving 1,010 cases for analysis. Two hundred and twenty-one patients died during follow-up; for 159 (71.9%) cause of death was recorded as breast cancer.

GELCAPS lung cancer cases. Patients with lung cancer were ascertained through the Genetic Lung Cancer Predisposition Study (GELCAPS), a population-based study of lung cancer. Full details of the design and conduct of the study have been described previously (11). The current analysis is based on 1,142 patients from whom detailed clinicopathologic data and follow-up information had been collected using a standardized proforma. All cases were of self-reported White ethnicity and were United Kingdom residents.

The UK Leukemia Research Fund CLL4 trial. We studied CLL patients entered in the UK Leukemia Research Fund CLL4 trial. Comprehensive details about the design and conduct of the trial have been published elsewhere (12). Briefly, CLL4 was a randomized phase III trial established to compare the efficacy of fludarabine, chlorambucil, and the combination of fludarabine plus cyclophosphamide as a first-line treatment for Binet stages B, C, and A-progressive CLL. Age was not a criterion for entry into the study. Of the 777 patients entered into the trial, the current analysis is based on a random subset of 356 patients of White Caucasian ethnicity who had blood samples taken for clinical diagnostic purposes and cell marker studies at participating centers.

SNP selection, imputation, and genotyping. To fine map the 7q22.1 association signal for urinary E1G levels (7), we used SNAP (13)

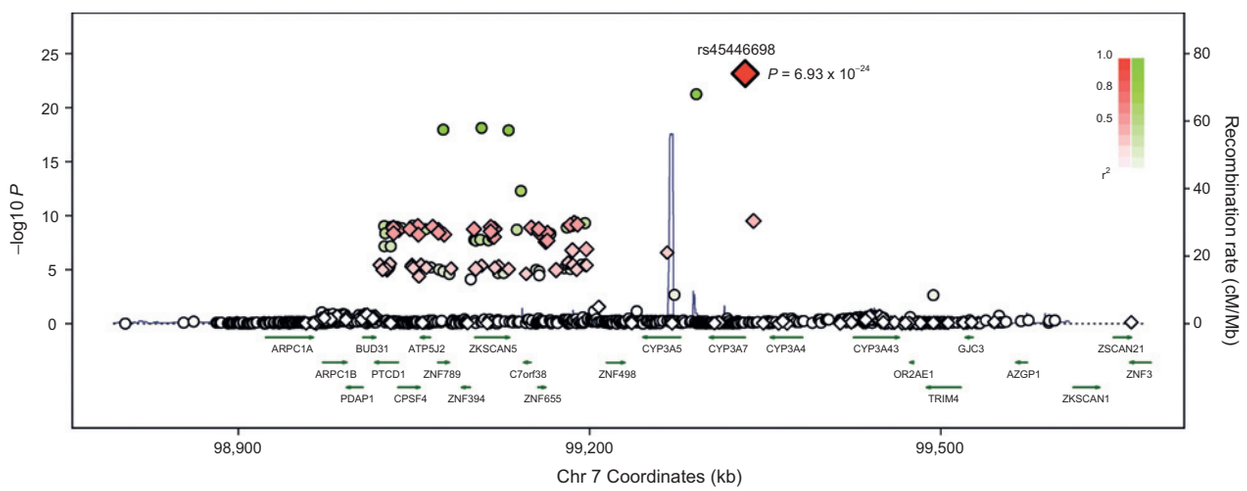

Figure 1.
Regional association plot of the *CYP3A* locus at 7q22.1 (98,803,430–99,662,733). Chromosome position is indicated on the x-axis and $-\log_{10} P$ value on the y-axis. Directly genotyped SNPs are represented as red diamonds, with the most significant SNP (rs45446698) indicated by a large red diamond. Imputed SNPs/indels are represented as green circles. The color intensity of each diamond/circle reflects the extent of linkage disequilibrium with rs45446698—red/green ($r^2 > 0.8$) through to white ($r^2 < 0.2$). The local recombination rate is plotted in blue. Physical positions are based on hg19.

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to identify 184 SNPs that were correlated ($r^2 \geq 0.1$) with rs10273424 and rs680055, based on the CEU 1000 genomes (1KG) pilot data. We were able to design Sequenom plexes (Sequenom Inc.) for 154 of these; we also included 7 SNPs genotyped as part of the original study (7) for monitoring of quality control. Post genotyping, we excluded 19 SNPs that failed genotyping and 4 SNPs for which the call rate was <95%, leaving 138 SNPs for analysis. The mean call rate for these 138 SNPs was 99.4% and genotypes of all SNPS were in accordance with Hardy–Weinberg equilibrium (i.e., $P > 0.05$). On the basis of the 25 (3.4%) duplicate samples, concordance was 100% across the 138 SNPs.

To increase the density of our fine mapping, we imputed untyped genotypes using IMPUTE version 2.2 software, with 1KG as the reference. Thresholding at an *INFO* score of ≥0.8, 725 additional SNPs and indels were successfully imputed, resulting in a total of 863 variants for analysis in 727 samples.

To confirm imputed genotypes for the most significant SNP (rs45446698) and to test for association with patient outcome we genotyped rs45446698 by TaqMan (Life Technologies). Call rates were 96.9% (fine-mapping and RMH Lifestyle and Family History study), 98.8% (GELCAPS), and 97.5% (CLL4). Concordance with imputed genotypes (fine-mapping) and between duplicate samples (cancer cases) was 100%.

Sequencing. We confirmed that rs45446698 was serving as a proxy for the *CYP3A7*1C* allele by Sanger sequencing a 370 bp PCR fragment, including the 60 bp region that defines this allele in four common homozygotes, three heterozygotes, and one rare homozygote (primer sequences available on request).

Statistical analysis

Fine-mapping. The percentage of change in hormone level per allele of each SNP was estimated by linear regression models of $\log_e$-transformed hormone levels. We used *t* tests of the regression coefficient to calculate *P* values for linear trend.

Risk analysis. We used Cox regression to test for an association between rs45446698 genotype and breast cancer specific survival (BCSS; RMH Lifestyle and Family History study) or progression-free survival (PFS; CLL4). For lung cancer (GELCAPS), where the prognosis is poor (5-year survival <10%), we used overall survival as a proxy for a disease-specific outcome. In the breast cancer series, to allow for differences in ascertainment between incident and prevalent cases, the time at risk began on the date of diagnosis, but the time under observation began on the date of entry to the study (defined as receipt of blood sample; refs. 14, 15). Time at risk ended on the date of death from breast cancer, or censoring (defined as date of last follow up or death from other causes). All cases were censored at 15 years after diagnosis on the basis that follow-up was likely to be unreliable after this period. For GELCAPS cases, time at risk began on the date of diagnosis and ended on the date of death from any cause, last follow-up or censoring. For the CLL4 cases, time at risk began at randomization to the trial and ended on date of progression, date of last follow-up or censoring. Because rs45446698 has a minor allele frequency of 0.04, we combined rare homozygotes with heterozygotes and used a one degree of freedom (1df) test. To test for statistical interaction between genotype and each stratifying variable, we compared models with and without interaction terms using likelihood ratio tests. The proportional hazards assumption was tested using Schoenfeld residuals. Statistical analyses were performed using R software, version 2.11 (R Foundation for Statistical Computing) and STATA software, version 11.0. All reported *P* values are two-sided.

Results

The most significantly associated SNP was rs45446698, which was associated with a 54% reduction in urinary E1G [95% confidence interval (CI), −61.0% to −47.7%; $P = 6.9 \times 10^{-24}$; Fig. 1; Supplementary Table S1]. rs45446698 is one of seven highly correlated SNPs (rs11568824, rs45494802, rs45575938, rs45467892, rs11568825, rs11568826, and rs45446698) that cluster within the *CYP3A7* promoter and comprise the *CYP3A7*1C* allele (4). Sequencing of the *CYP3A7* promoter in four carriers of the rare rs45446698-C allele and four rs45446698-A common homozygotes confirmed that rs45446698 tags all seven base

Table 1. Characteristics of 1,008[a] breast cancer cases according to rs45446698 genotype

	rs45446698 A:C + C:C *n* = 73	rs45446698 A:A *n* = 935	*P*[b]
Mean age	55.0	56.1	
Age range	27–82	24–89	0.44
Tumor size group (cm)			
<2	44 (60.3)	521 (55.7)	
2–5	23 (31.5)	363 (38.8)	
5+	6 (8.2)	51 (5.5)	0.35
Grade			
1	11 (15.1)	155 (16.6)	
2	36 (49.3)	435 (46.5)	
3	26 (35.6)	345 (36.9)	0.89
ER status			
Positive	64 (87.7)	789 (84.4)	
Negative	9 (12.3)	146 (15.6)	0.45
Positive nodes			
0	30 (41.1)	498 (53.3)	
1–3	17 (23.3)	239 (25.6)	
4+	19 (26.0)	115 (12.3)	
N/A	7 (9.6)	83 (8.9)	0.003
Vascular invasion			
Positive	29 (39.7)	315 (33.7)	
Negative	41 (56.2)	598 (64.0)	
N/A	3 (4.1)	22 (2.3)	0.24
Surgery			
Yes	72 (98.6)	923 (98.7)	
No	1 (1.4)	12 (1.3)	0.95
Radiotherapy			
Yes	68 (93.1)	808 (86.4)	
No	5 (6.9)	124 (13.3)	
N/A	0 (0)	3 (0.3)	0.11
Chemotherapy			
Yes	43 (58.9)	481 (51.4)	
No	30 (41.1)	452 (48.3)	
N/A	0 (0)	2 (0.2)	0.23
Tamoxifen			
Yes	58 (79.5)	736 (78.7)	
No	15 (20.5)	195 (20.9)	
N/A	0 (0)	4 (0.4)	0.94
Pyrs	447	5625	
Events	19	140	
Rate (per 1,000 pyrs)	42.5 (27.1–66.6)	24.9 (21.1–29.4)	0.02

[a]Excludes two women with missing genotype.
[b]Two-sided *t* test (age), χ^2 test (size, grade, ER status, positive nodes, vascular invasion, surgery, radiotherapy, chemotherapy, and tamoxifen), and log-rank test (events).

Published OnlineFirst March 14, 2016; DOI: 10.1158/0008-5472.CAN-15-1410

changes that define the *CYP3A7*1C* allele in these Northern European women (data not shown).

To assess rs45446698 as a potential marker of disease outcome, we genotyped 1,008 breast cancer patients participating in the RMH Lifestyle and Family History study (Materials and Methods; Table 1). For the majority of patient and tumor characteristics, there was no association with rs45446698 genotype. The exception was the number of positive lymph nodes; rs45446698-C carriers were more likely to have four or more positive nodes than rs45446698-A homozygotes (Table 1; $P = 0.003$). In an unadjusted analysis, carrier status for rs45446698-C was associated with a 74% increase in breast cancer mortality (HR, 1.74; 95% CI, 1.08–2.82; $P = 0.02$; Fig. 2A). Restricting the analysis to the 889 individuals for whom we had complete data, and adjusting for established prognostic factors (age, tumor size, grade, positive nodes, and vascular invasion) and radiotherapy did not alter this result (HR, 1.74; 95% CI, 1.05–2.90; $P = 0.03$). Stratifying on estrogen receptor (ER) status showed no evidence that the association differed by ER status (HR, 1.86; 95% CI, 1.03–3.34; $P = 0.04$ and HR, 1.70; 95% CI, 0.55–5.24; $P = 0.35$ for ER-positive and ER-negative disease respectively; $P_{\text{heterogeneity}} = 0.92$).

In this cohort of breast cancer patients, median time from diagnosis to enrolment was 1.7 years, but the range was wide (0–12.3); excluding the 65 (7.3%) cases diagnosed more than 5 years before enrolment did not alter the HR estimate (HR, 1.82; 95% CI, 1.09–3.04; $P = 0.02$) and stratifying on time from diagnosis to enrolment, we found no evidence that the HR was biased by the inclusion of prevalent cases (HR, 1.72; 95% CI, 0.72–4.08; HR, 2.03; 95% CI, 1.06–3.89, for cases diagnosed <1 or ≥1 year before entry to the study, $P_{\text{heterogeneity}} = 0.76$). There was, however, some evidence that the risk associated with rs45446698-C carrier status varied with time since diagnosis (test of the assumption of proportional hazards, $P = 0.01$); stratifying the analysis into two time periods, the HRs were HR, 1.14 (95% CI, 0.53–2.47; $P = 0.74$) and HR, 4.46 (95% CI, 1.98–10.04; $P = 0.0003$) for the first ($t < 7.5$ year since diagnosis) and second ($t \geq 7.5$ years since diagnosis) halves of the study, respectively.

To determine whether rs45446698 was associated with outcome for other site-specific cancers, we genotyped the GELCAPS lung cancer cases (Table 2; ref. 11) and the CLL4 trial series (Table 3; ref. 12). There was no evidence that rs45446698 was associated with patient or disease characteristics. In GELCAPS, rs45446698-C carrier status was associated with a 26% increase in all causes mortality (HR, 1.26; 95% CI, 0.96–1.64; $P = 0.09$; Fig. 2B). After adjusting for standard prognostic factors (age, gender, stage, and smoking status), surgery, and radiotherapy the HR was 1.43 (95% CI, 1.09–1.87; $P = 0.009$) with no evidence that the association differed between small-cell lung cancer (SCLC) and non–small cell lung cancer (NCLSC; HR, 1.93; 95% CI, 1.18–3.16; $P = 0.009$ and HR, 1.25; 95% CI, 0.90–1.74; $P = 0.19$ for SCLC and NSCLC respectively, $P_{\text{heterogeneity}} = 0.16$). In this cohort of lung cancer cases, there was no evidence that the association varied with time since diagnosis (test of non-proportional hazards $P = 0.93$). In the CLL series, carrying the variant allele of rs45446698-C was associated with a 54% increased risk of progression (HR, 1.54; 95% CI, 1.00–2.36; $P = 0.05$; Fig. 2C). Adjusting for standard prognostic factors (age, gender, and stage) altered this result only marginally (HR, 1.62; 95% CI, 1.05–2.50;

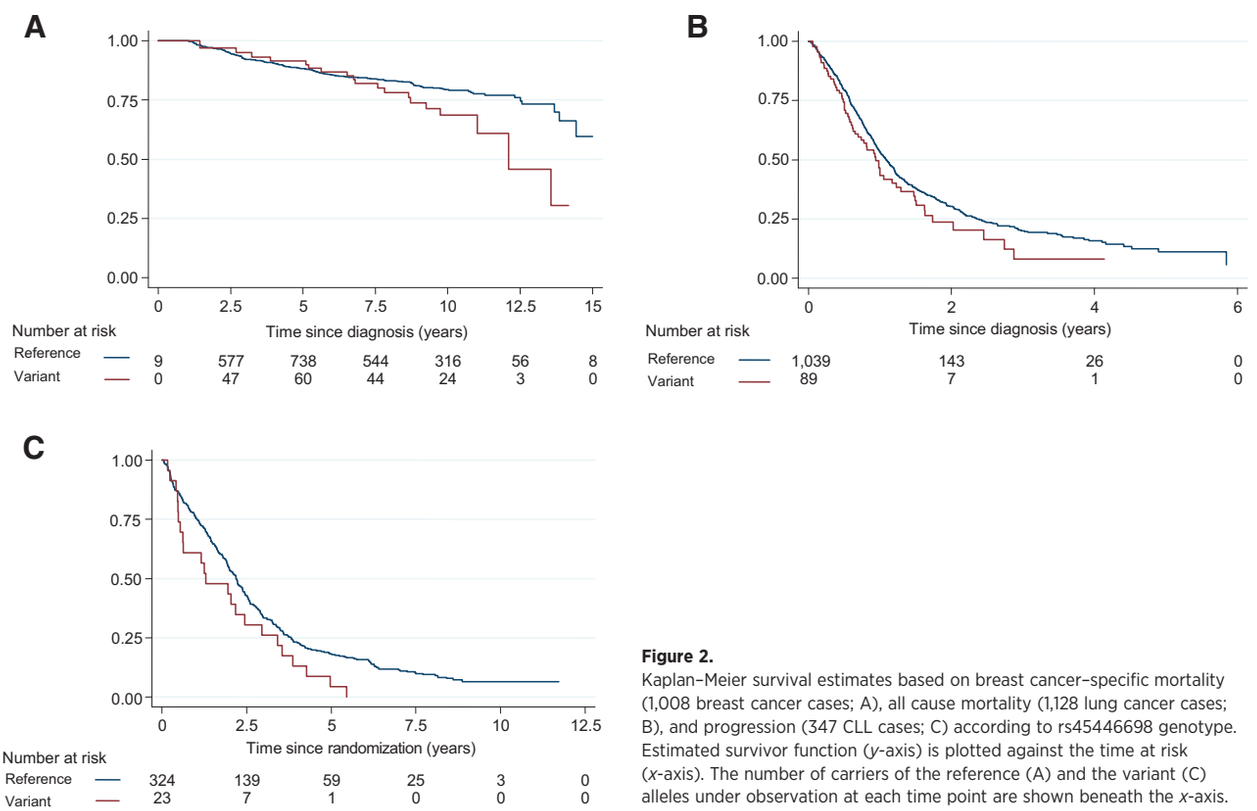

Figure 2.
Kaplan–Meier survival estimates based on breast cancer–specific mortality (1,008 breast cancer cases; A), all cause mortality (1,128 lung cancer cases; B), and progression (347 CLL cases; C) according to rs45446698 genotype. Estimated survivor function (*y*-axis) is plotted against the time at risk (*x*-axis). The number of carriers of the reference (A) and the variant (C) alleles under observation at each time point are shown beneath the *x*-axis.

Table 2. Characteristics of 1,128[a] lung cancer cases according to rs45446698 genotype

	rs45446698 A:C + C:C $n = 89$	rs45446698 A:A $n = 1{,}039$	P^b
Mean age	62.8	64.8	
Age range	32–81	26–88	0.08
Gender			
Male	41 (46.1)	435 (41.9)	
Female	48 (53.9)	604 (58.1)	0.44
Smoking status			
Never	7 (7.9)	72 (6.9)	
Ever	82 (92.1)	967 (93.1)	0.74
Diagnosis			
SCLC	28 (31.5)	245 (23.6)	
NSCLC (squamous)	30 (33.7)	383 (36.9)	
NSCLC (adeno)	23 (25.8)	242 (23.3)	
NCSLC (other)	8 (9.0)	169 (16.3)	0.15
Stage			
1	16 (18.0)	223 (21.5)	
2	10 (11.2)	110 (10.6)	
3	32 (36.0)	335 (32.2)	
4	31 (34.8)	371 (35.7)	0.83
Surgery			
Yes	14 (15.7)	179 (17.2)	
No	75 (84.3)	860 (82.8)	0.72
Radiotherapy			
Yes	24 (27.0)	265 (25.5)	
No	65 (73.0)	774 (74.5)	0.76
Chemotherapy			
Yes	67 (75.3)	745 (71.7)	
No	22 (24.7)	294 (28.3)	0.47
Pyrs follow-up	824	1151	
Events	59	644	
Rate (per 1,000 pyrs)	715.8 (554.6–923.8)	559.7 (518.1–604.7)	0.09

[a]Excludes 14 women with missing genotype.
[b]Two-sided *t* test (age), χ^2 test (gender, smoking status, diagnosis, stage, surgery, radiotherapy, and chemotherapy), and log-rank test (events).

$P = 0.03$). In the 283 cases for whom 13q deletion and *IGHV* mutation status were available (HR, 1.46; 95% CI, 0.89–2.39; $P = 0.13$), adjusting for 13q deletion (HR, 1.46; 95% CI, 0.89–2.38; $P = 0.13$) or mutation status (HR, 1.36; 95% CI, 0.83–2.22; $P = 0.23$) altered the result only marginally. In this cohort of CLL cases, there was no evidence that the association between genotype and disease-free progression varied with time since diagnosis (test of non-proportional hazards $P = 0.78$).

To determine whether the association between rs45446698 genotype and outcome was influenced by chemotherapy, specifically treatment with an agent that is a CYP3A substrate (Table 4), we carried out stratified analyses and tested for statistical interaction. Stratifying on treatment with tamoxifen, in the breast cancer series, there was no evidence that the association differed between strata; HRs were 1.68 (95% CI, 0.90–3.13; $P = 0.10$) and 1.89 (95% CI, 0.69–5.17; $P = 0.21$) for treated and non-treated, respectively ($P_{\text{heterogeneity}} = 0.89$). Stratifying on treatment with a cytotoxic agent that is metabolized by a CYP3A enzyme (Table 4), the association between genotype and outcome appeared to be specific to patients who were treated with a CYP3A substrate (HR, 1.96; 95% CI, 1.11–3.45; $P = 0.02$) compared with those who were not (HR, 0.98; 95% CI, 0.28–3.49; $P = 0.98$), but this difference was not statistically significant ($P_{\text{heterogeneity}} = 0.57$). Similarly, in the lung cancer study, the HR in cases who were treated with a CYP3A substrate was more extreme (HR, 1.71; 95% CI, 1.21–2.41; $P = 0.003$) than in those who were not (HR, 1.11; 95% CI, 0.71–1.74; $P = 0.64$), but with no evidence of statistical interaction ($P_{\text{heterogeneity}} = 0.24$). In the CLL trial, the only chemotherapeutic agent that was a CYP3A substrate was cyclophosphamide (Table 4). Comparing cases who were or were not treated with cyclophosphamide, respective HRs for rs45446698-C carriers were HR, 2.15 (95% CI, 0.82–5.64; $P = 0.12$) and HR, 1.36 (95% CI, 0.83–2.22; $P = 0.22$; $P_{\text{heterogeneity}} = 0.52$). Combining data across all three studies, the HR for rs45446698-C carriers who were treated with a cytotoxic agent that is a CYP3A substrate was 1.80 (95% CI, 1.36–2.39; $P = 4.1 \times 10^{-5}$) compared with an HR of 1.20 (95% CI, 0.87–1.65; $P = 0.26$; Fig. 3) for those who were not, with borderline evidence of statistical interaction between treated and non-treated ($P_{\text{heterogeneity}} = 0.06$), but no evidence of heterogeneity between studies ($P_{\text{heterogeneity}} = 0.79$ and 0.87 for non-treated and treated, respectively).

Discussion

Our data support the *CYP3A7*1C* allele, tagged by rs45446698 as being the likely genetic basis for the association between the rs10273424-A rs680055-G haplotype and urinary E1G levels (7). This allele has previously been associated with significantly reduced serum dehydroepiandrosterone sulfate (DHEAS) and estrone (E_1) levels in men, providing independent support for our findings (16).

The *CYP3A7*1C* allele arose from a gene conversion event in which an approximately 60 bp region within the fetal *CYP3A7* promoter was replaced with the equivalent region from the adult *CYP3A4* gene (17). Comparing the variant *CYP3A7*1C* allele with the reference *CYP3A7* allele, there are seven, highly correlated, single base changes, all of which map to the *CYP3A7* promoter and which result in expression of *CYP3A7* in adult carriers of the *CYP3A7*1C* allele. Functional analyses demonstrated that two of these SNPs (rs11568825 and rs11568826) are necessary and sufficient for determining pregnane-X-receptor (PXR)–dependent activation of *CYP3A7* and four of the

Table 3. Characteristics of 347[a] CLL cases according to rs45446698 genotype

	rs45446698 A:C + C:C $n = 23$	rs45446698 A:A $n = 324$	P^b
Median age	63.4	64.8	
Age range	42–83	46–84	0.44
Gender			
Male	18 (78.3)	239 (73.8)	
Female	5 (21.7)	85 (26.3)	0.64
Stage			
A	2 (8.7)	88 (27.2)	
B	12 (52.2)	139 (42.9)	
C	9 (39.1)	97 (29.9)	0.15
13q deletion			
Deletion	12 (52.2)	186 (57.4)	
No deletion	10 (43.5)	115 (35.5)	
N/A	1 (4.4)	23 (7.10)	0.50
IGHV mutation status			
Mutation	6 (26.1)	111 (34.3)	
No mutation	12 (52.2)	169 (52.2)	
N/A	5 (21.8)	44 (13.6)	0.60
Pyrs follow-up	45	933	
Events	23	298	
Rate (per 1,000 pyrs)	514.3 (341.8–773.9)	319.4 (285.1–357.8)	0.05

[a]Excludes 9 patients with missing genotype.
[b]Two-sided *t* test (age), χ^2 test (gender, stage, 13q deletion, and mutation), and log-rank test (events).

Published OnlineFirst March 14, 2016; DOI: 10.1158/0008-5472.CAN-15-1410

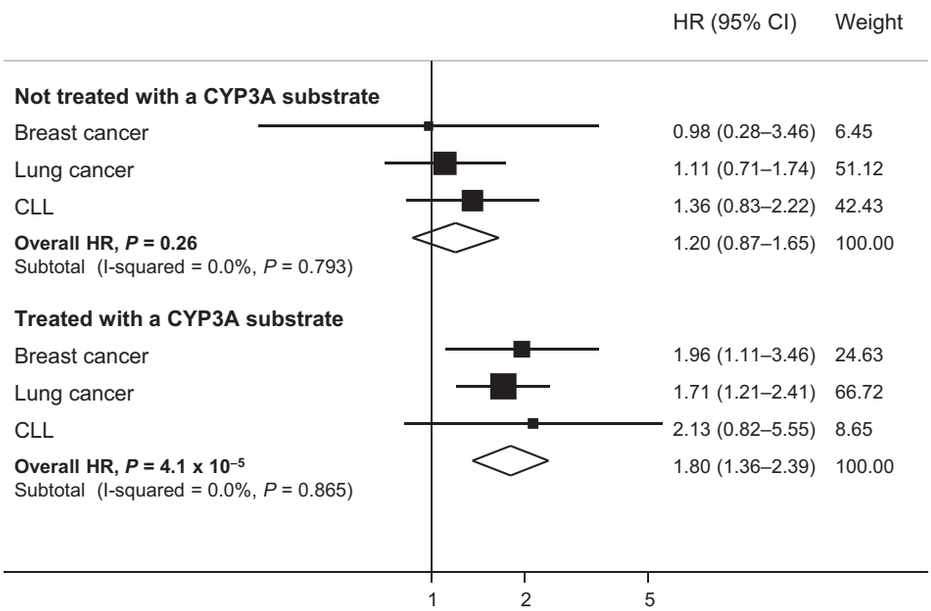

Figure 3.
Association of rs45446698 with disease-specific mortality (breast cancer), all cause mortality (lung cancer), and progression (CLL), stratified by whether the patient's treatment regimen included a cytotoxic agent that is metabolized by a CYP3A enzyme. Horizontal lines, 95% CIs. Square boxes, cancer-specific fixed-effects estimates. Diamonds, the combined, fixed-effects estimates of the HRs and 95% CIs in each stratum. Vertical line, the null effect (HR, 1.0).

other five SNPs (rs11568824, rs45494802, rs45575938, and rs45467892) influence constitutively activated receptor (CAR)–mediated activation (18).

To our knowledge, this is the first study to test for an association between the *CYP3A7*1C* allele and outcome in cancer patients. Genome-wide association studies of breast and lung cancer survival have been published (19–30); although none has reported an association with variants at the *CYP3A* locus only one, a recent meta-analysis from the Breast Cancer Association Consortium (BCAC), that combined data from nine breast cancer studies, has had power to detect moderate effects for variants with MAF <0.05 at genome-wide significance. The lack of association between rs45446698 and outcome in the BCAC meta-analysis (30) may reflect a relatively short mean duration of follow-up with censoring of cases at 10 years after diagnosis. In our breast cancer data, we found evidence of non-proportional hazards such that the association between the *CYP3A7*1C* allele and outcome varied with time since diagnosis with HRs of 1.14 and 4.46 at <7.5 and ≥7.5 years after diagnosis, respectively. Replication of this finding in additional studies will be needed to determine whether this time dependence is a chance finding, whether any such effect is specific to breast cancer, and whether it is influenced by the doses and combinations of chemotherapeutic agents that the patients received.

Because of the wide diversity of exogenous and endogenous substrates that are metabolized by the CYP3A enzymes, there are many different potential mechanisms by which *CYP3A* expression could influence disease outcome and we cannot at this juncture confirm or refute any particular mechanism. Possibilities include the *CYP3A7*1C* allele (i) associating with markers of disease prognosis (e.g., stage, grade, or lymph node involvement) dependent on, or independent of, endogenous hormone levels or (ii) by influencing plasma clearance of chemotherapeutic agents that are CYP3A substrates. In support of a *CYP3A* allele–influencing outcome through association with prognostic markers, association of the *CYP3A4*1B* allele with higher tumor-lymph node-metastasis and Gleason score has been reported for prostate cancer (31); and in a study of Ewing's sarcomas, high expression of *CYP3A4* was significantly associated with distant metastases (32). In this analysis, we observed an association between the *CYP3A7*1C* allele and lymph node metastasis (≥4 positive nodes) in breast cancer cases. There was, however, no association between carrier status and disease stage for lung cancer or CLL and the association of *CYP3A7*1C* with adverse outcome for all three cancers remained after adjusting for established prognostic markers.

In support of the *CYP3A7*1C* allele–influencing plasma clearance of chemotherapeutic agents, this allele was associated with adverse outcome across three site-specific cancers and, although these cancers have differing etiologies and prognoses, the treatment regimens for all three include CYP3A substrates (Table 4). Consistent with an extensive body of evidence demonstrating that the efficacy of tamoxifen therapy cannot be predicted by *CYP2D6* genotype (33, 34), we found no evidence of statistical interaction between the *CYP3A7*1C* allele, outcome and treatment with tamoxifen. For cytotoxic cancer drugs, there was consistency across the three studies; HRs in *CYP3A7*1C* carriers were more extreme in patients treated with a drug that was a CYP3A substrate, and in the combined data there was some evidence of statistical interaction between the *CYP3A7*1C* allele, treatment with a cytotoxic agent that was a CYP3A substrate and outcome ($P_{\text{heterogeneity}} = 0.06$). Further studies confirming the association of the *CYP3A7*1C* allele with adverse outcome and investigating the mechanism by which this allele may influence outcome are required.

There are several limitations to this analysis; the endpoints that we analyzed varied across the three different malignancies (disease-specific mortality, breast cancer), all-cause mortality (lung cancer), and disease progression (CLL). Although genotypes are effectively randomized at birth (35) and there was no association between being a carrier of the *CYP3A7*1C* allele and receiving chemotherapy (Table 1, $P = 0.23$ and Table 2, $P = 0.47$ for breast and lung cancers respectively), treatment was not randomized in the two observational studies. The

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Table 4. Chemotherapy regimens used in the treatment of breast cancer, lung cancer, and CLL cases

Breast cancer (cytotoxic treatment)[a]	**N treated (%)**
Doxorubicin, **cyclophosphamide**	173 (17.2)[b]
Cyclophosphamide, methotrexate, fluorouracil	18 (1.8)
Epirubicin, cisplatin, fluorouracil	13 (1.3)[c]
Fluorouracil, epirubicin, **cyclophosphamide**	202 (20.0)[d]
Methotrexate, **mitoxantrone**	93 (9.2)[e]
Vinorelbine, epirubicin	21 (2.1)
Other/not known	6 (0.6)
No cytotoxic treatment	482 (47.8)
Total	1,008 (100)
Breast cancer (hormonal treatment)[a]	
Tamoxifen	495 (49.1)
Tamoxifen, anastrozole	128 (12.7)
Tamoxifen, letrozole	171 (17.0)
Other/not known	75 (7.4)[f]
No hormonal treatment	139 (13.8)
Total	1,008 (100)
Lung cancer	
Cisplatin/carboplatin, **etoposide**	206 (18.3)[g]
Cisplatin/carboplatin, **vinorelbine/vincristin**	180 (16.0)[h]
Cisplatin/carboplatin, **vinorelbine/vincristin**, mitomycin	133 (11.8)
Cisplatin/carboplatin, gemcitabine	194 (17.2)
Cisplatin/carboplatin, **taxane**	28 (2.5)
Doxorubicin, **cyclophosphamide, vinorelbine/vincristin**	33 (2.9)
Doxorubicin, **cyclophosphamide, etoposide**	11 (1.0)
Other/not known	27 (2.4)
No cytotoxic treatment	316 (28.0)
Total	1,128 (100)
CLL	
Chlorambucil	166 (47.8)
Fludarabine	88 (25.4)
Fludarabine, **cyclophosphamide**	93 (26.8)
No cytotoxic treatment	0 (0)
Total	347 (100)

NOTE: Agents that are metabolized by a CYP3A enzyme are in bold.

[a]For breast cancer, treatment with a cytotoxic agent and a hormonal agent were not mutually exclusive; 415 (41.2%) of the 1,008 cases were treated with both a cytotoxic agent and a hormonal agent.

[b]Includes 19 women also treated with a taxane.

[c]Includes one woman also treated with a taxane.

[d]Includes 20 women also treated with a taxane.

[e]Includes 14 women also treated with a taxane.

[f]Comprises 41 women who were treated with anastrozole, 29 who were treated with letrozole, 1 who was treated with both and 4 for whom treatment details are not known.

[g]Includes 4 individuals also treated with ifosfamide and 1 who was also treated with both ifosfamide and vincristine.

[h]Includes 2 individuals also treated with ifosfamide.

frequency of *CYP3A7*1C* allele is just 4% (8% carriers); accordingly, we were unable to analyze cases who were heterozygous or homozygous for the variant allele separately. The relative rarity of this allele and the lack of detailed information on drug doses and numbers of treatment cycles limited our ability to carry out meaningful subgroup analyses. We could not investigate whether the time dependence of the association between the *CYP3A7*1C* allele and outcome that we observed in the breast cancer study depended on particular combinations of cancer drugs and we were unable to test for statistically significant interaction between outcome, the *CYP3A7*1C* allele and individual chemotherapeutic agents or specific treatment regimens. The pooled estimate of the increased risk in carriers of the *CYP3A7*1C* allele, treated with one or more cytotoxic agents that are CYP3A substrates (HR, 1.80; $P = 4.1 \times 10^{-5}$) represents a "weighted average," which may vary substantially between different treatment regimens and across cancer types. Finally, even in the combined data from all three of these retrospective studies, the evidence for statistical interaction between outcome, the *CYP3A7*1C* allele and the cytotoxic agents that are CYP3A substrates was weak ($P = 0.06$). Although this may reflect the heterogeneity of treatment regimens and a lack of power, we cannot exclude the possibility that the association between *CYP3A7*1C* and disease outcome is mediated by some other mechanism.

In conclusion, we have shown that the *CYP3A7*1C* allele, which results in the adult expression of the fetal *CYP3A7* gene, is likely to be the functional allele that is associated with both lower levels of circulating endogenous sex hormones and adverse outcome in breast cancer, lung cancer, and CLL. Our results require independent replication in larger studies, preferably with more detailed information on chemotherapy schedules and dosages, and across other cancer sites. However, one implication of our findings is that the doses and regimens of chemotherapeutic agents that provide optimal benefit for the average patient may not be optimal for the approximately 8% of cancer patients who are *CYP3A7*1C* carriers.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Published OnlineFirst March 14, 2016; DOI: 10.1158/0008-5472.CAN-15-1410

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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): G. Migliorini, N. Orr, F. Dudbridge, I. dos-Santos-Silva, O. Fletcher
Writing, review, and/or revision of the manuscript: N. Johnson, P. De Ieso, G. Migliorini, D. Catovsky, A. Matakidou, T. Eisen, C. Goldsmith, F. Dudbridge, J. Peto, I. dos-Santos-Silva, A. Ashworth, G. Ross, R.S. Houlston, O. Fletcher
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): P. Broderick, C. Goldsmith, J. Peto

Acknowledgments

The authors are grateful to all the patients and control subjects for their participation. The authors thank the clinicians and other hospital staff, cancer registries, and study staff who contributed to the blood sample and data collection for the BBC study, CLL4, GELCAPS, the Royal Marsden Lifestyle and Family History study and the MOG Factors study.

Grant Support

O. Fletcher, N. Orr, and A. Ashworth received funding from Breakthrough Breast Cancer (recently merged with Breast Cancer Campaign forming Breast Cancer Now); R.S. Houlston and D. Catovsky received funding from Leukemia and Lymphoma Research (LRF05001, LRF06002, and LRF13044) and Cancer Research UK (C1298/A8780 and C1298/A8362). R.S. Houlston, A. Matakidou, and T. Eisen received funding from HEAL; T. Eisen received funding from Sanofi-Avensis; J. Peto and I. dos-Santos-Silva received funding from Cancer Research UK (C150/A5660 and C1178/A3947); F. Dudbridge received funding from the MRC (G1000718 and K006215); G. Ross received funding from the Cridlan Trust. All the authors acknowledge National Health Service funding to the NIHR Biomedical Research Centre and the National Cancer Research Network (NCRN).

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Received May 22, 2015; revised November 5, 2015; accepted November 23, 2015; published online March 15, 2016.

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Published OnlineFirst March 14, 2016; DOI: 10.1158/0008-5472.CAN-15-1410

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Cancer Res 2016;76:1485-1493. Published OnlineFirst March 14, 2016.