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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 impacting 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 single nucleotide polymorphism (SNP) was rs45446698, a 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 (hazard ratio [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.

INTRODUCTION

The *CYP3A5*, *CYP3A7* and *CYP3A4* genes, which form the cytochrome P450 3A (*CYP3A*) gene cluster at 7q22.1, encode enzymes that metabolise a diverse range of substrates (1). Specifically, in addition to a role in the oxidative metabolism of endogenous hormones, *CYP3A* enzymes metabolise 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 inter-individual 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 single nucleotide polymorphisms (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 pre-specified 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 leukaemia (CLL).

MATERIALS 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 (8)) and the 358 women from the Mammography Oestrogens and Growth Factors (MOG (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 enzyme-linked immunosorbent assay (ELISA; (7)).

Study Subjects - Risk analysis

Royal Marsden Hospital (RMH) Lifestyle and Family History study

The RMH Lifestyle and Family History study comprises 1,786 consecutive breast cancer patients who attended the RMH Breast Unit (Royal Marsden Hospital, 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=1536). 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 non-invasive 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 clinico-pathological data and follow-up information had been collected using a standardised proforma. All cases were of self-reported White ethnicity and were United Kingdom residents.

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 centres.

SNP selection, imputation and genotyping

To fine map the 7q22.1 association signal for urinary estrone glucuronide levels(7) we used SNAP (13) 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, San Diego, USA) for 154 of these; we also included 7 SNPs genotyped as part of the original study(7) for monitoring of QC. 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. $P > 0.05$). Based on 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, Paisley, UK). 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 percent 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) (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. Since 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, Vienna, Austria) and STATA software, version 11.0 (College Station, TX, USA). All reported *P*-values are two-sided.

RESULTS

The most significantly associated SNP was rs45446698, which was associated with a 54% reduction in urinary estrone glucuronide (95% CI -61.0% to -47.7%, $P=6.9 \times 10^{-24}$; Figure 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 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 (Methods; Table 1). For the majority of patient and tumour 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, $P=0.02$; Figure 2A). Restricting the analysis to the 889 individuals for whom we had complete data, and adjusting for

established prognostic factors (age, tumour size, grade, positive nodes, 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; $P_{heterogeneity}=0.92$).

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 prior to 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, $P_{heterogeneity}=0.76$). 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 hazard ratios 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 (11) (Table 2) and the CLL4 trial series (12) (Table 3). 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, $P=0.09$; Figure 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 (NSCLC) (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, $P_{heterogeneity}=0.16$). 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, $P=0.05$; Figure 2C). Adjusting for standard prognostic factors (age, gender and stage) altered this result only marginally (HR=1.62, 95% CI 1.05-2.50, $P=0.03$). 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 ($P_{heterogeneity}=0.89$). Stratifying on treatment with a cytotoxic agent that is metabolised 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 to those who were not (HR=0.98, 95% CI 0.28-3.49, $P=0.98$) but this difference was not statistically significant ($P_{heterogeneity}=0.57$). 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 ($P_{heterogeneity}=0.24$). 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, $P=0.22$; $P_{heterogeneity}=0.52$). 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 to an HR of 1.20 (95% CI 0.87-1.65; $P=0.26$; Figure 3) for those who were not, with borderline evidence of statistical interaction between treated and non-treated ($P_{heterogeneity}=0.06$) but no evidence of heterogeneity between studies ($P_{heterogeneity}=0.79$ and 0.87 for non-treated and treated, respectively).

DISCUSSION

Our data supports 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 estrone glucuronide 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 other five SNPs (rs11568824, rs45494802, rs45575938, 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); while 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.

Due to 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 (eg 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, while these cancers have differing aetiologies 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)). While 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 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 ($P=0.06$). While 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.

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Table 1: Characteristics of 1,008* breast cancer cases according to rs45446698 genotype

		rs45446698 A:C + C:C n = 73	rs45446698 A:A n = 935	P-value [#]
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)	0.35
	2-5	23 (31.5)	363 (38.8)	
	5+	6 (8.2)	51 (5.5)	
Grade	1	11 (15.1)	155 (16.6)	0.89
	2	36 (49.3)	435 (46.5)	
	3	26 (35.6)	345 (36.9)	
ER status	Positive	64 (87.7)	789 (84.4)	0.45
	Negative	9 (12.3)	146 (15.6)	
Positive nodes	0	30 (41.1)	498 (53.3)	0.003
	1-3	17 (23.3)	239 (25.6)	
	4+	19 (26.0)	115 (12.3)	
	N/A	7 (9.6)	83 (8.9)	
Vascular invasion	Positive	29 (39.7)	315 (33.7)	0.24
	Negative	41 (56.2)	598 (64.0)	
	N/A	3 (4.1)	22 (2.3)	
Surgery	Yes	72 (98.6)	923 (98.7)	0.95
	No	1 (1.4)	12 (1.3)	
Radiotherapy	Yes	68 (93.1)	808 (86.4)	0.11
	No	5 (6.9)	124 (13.3)	
	N/A	0 (0)	3 (0.3)	
Chemotherapy	Yes	43 (58.9)	481 (51.4)	0.23
	No	30 (41.1)	452 (48.3)	
	N/A	0 (0)	2 (0.2)	
Tamoxifen	Yes	58 (79.5)	736 (78.7)	0.94
	No	15 (20.5)	195 (20.9)	
	N/A	0 (0)	4 (0.4)	
Pyrs		447	5625	
Events		19	140	
Rate (per 1000 pyrs)		42.5 (27.1-66.6)	24.9 (21.1-29.4)	0.02

*Excludes two women with missing genotype; [#]two-sided *t* test (age), chi-squared test (size, grade, ER status, positive nodes, vascular invasion, surgery, radiotherapy, chemotherapy, tamoxifen), log rank test (events).

Table 2: Characteristics of 1,128* lung cancer cases according to rs45446698 genotype

		rs45446698 A:C + C:C n = 89	rs45446698 A:A n = 1039	P-value [#]
Mean age		62.8	64.8	
Age range		32 - 81	26 - 88	0.08
Gender	Male	41 (46.1)	435 (41.9)	0.44
	Female	48 (53.9)	604 (58.1)	
Smoking status	Never	7 (7.9)	72 (6.9)	0.74
	Ever	82 (92.1)	967 (93.1)	
Diagnosis	SCLC	28 (31.5)	245 (23.6)	0.15
	NSCLC (squamous)	30 (33.7)	383 (36.9)	
	NSCLC (adeno)	23 (25.8)	242 (23.3)	
	NCSLC (other)	8 (9.0)	169 (16.3)	
Stage	1	16 (18.0)	223 (21.5)	0.83
	2	10 (11.2)	110 (10.6)	
	3	32 (36.0)	335 (32.2)	
	4	31 (34.8)	371 (35.7)	
Surgery	Yes	14 (15.7)	179 (17.2)	0.72
	No	75 (84.3)	860 (82.8)	
Radiotherapy	Yes	24 (27.0)	265 (25.5)	0.76
	No	65 (73.0)	774 (74.5)	
Chemotherapy	Yes	67 (75.3)	745 (71.7)	0.47
	No	22 (24.7)	294 (28.3)	
Pyrs follow up		824	1151	
Events		59	644	
Rate (per 1000 pyrs)		715.8 (554.6-923.8)	559.7 (518.1-604.7)	0.09

* Excludes 14 women with missing genotype; [#]two-sided *t* test (age), chi-squared test (gender, smoking status, diagnosis, stage, surgery, radiotherapy, chemotherapy), log rank test (events).

Table 3: Characteristics of 347* CLL cases according to rs45446698 genotype

		rs45446698 A:C + C:C n = 23	rs45446698 A:A n = 324	P-value [#]
Median age		63.4	64.8	
Age range		42 - 83	46 - 84	0.44
Gender	Male	18 (78.3)	239 (73.8)	0.64
	Female	5 (21.7)	85 (26.3)	
Stage	A	2 (8.7)	88 (27.2)	0.15
	B	12 (52.2)	139 (42.9)	
	C	9 (39.1)	97 (29.9)	
13q deletion	Deletion	12 (52.2)	186 (57.4)	0.50
	No deletion	10 (43.5)	115 (35.5)	
	N/A	1 (4.4)	23 (7.10)	
<i>IGHV</i> Mutation status	Mutation	6 (26.1)	111 (34.3)	0.60
	No mutation	12 (52.2)	169 (52.2)	
	N/A	5 (21.8)	44 (13.6)	
Pyrs follow up		45	933	
Events		23	298	
Rate (per 1000 pyrs)		514.3 (341.8-773.9)	319.4 (285.1-357.8)	0.05

*Excludes 9 with missing genotype; [#]two-sided *t* test (age), chi-squared test (gender, stage, 13q deletion, mutation), log rank test (events).

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

Breast cancer (cytotoxic treatment) ¹	N treated (%)
Doxorubicin, cyclophosphamide	173 (17.2) ²
Cyclophosphamide , methotrexate, fluorouracil	18 (1.8)
Epirubicin, cisplatin, fluorouracil	13 (1.3) ³
Fluorouracil, epirubicin, cyclophosphamide	202 (20.0) ⁴
Methotrexate, mitoxantrone	93 (9.2) ⁵
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) ¹	
Tamoxifen	495 (49.1)
Tamoxifen, anastrozole	128 (12.7)
Tamoxifen, letrozole	171 (17.0)
Other/not known	75 (7.4) ⁶
No hormonal treatment	139 (13.8)
Total	1,008 (100)
Lung cancer	
Cisplatin/carboplatin, etoposide	206 (18.3) ⁷
Cisplatin/carboplatin, vinorelbine/vincristin	180 (16.0) ⁸
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)

Agents that are metabolised by a CYP3A enzyme are in bold. ¹for 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. ²includes 19 women also treated with a taxane, ³includes one woman also treated with a taxane, ⁴includes 20 women also treated with a taxane, ⁵includes 14 women also treated with a taxane, ⁶comprises 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. ⁷includes 4 individuals also treated with ifosfamide, and 1 who was also treated with both ifosfamide and vincristine, ⁸includes 2 individuals also treated with ifosfamide.

Figure Legends:

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 colour 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.

Figure 2: Kaplan-Meier survival estimates based on (A) breast cancer specific mortality (1,008 breast cancer cases) (B) all cause mortality (1,128 lung cancer cases) and (C) progression (347 CLL cases), 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.

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 metabolised by a CYP3A enzyme.

Horizontal lines represent 95% CIs. Square boxes represent cancer specific fixed-effects estimates. Diamonds represent the combined, fixed-effects estimates of the HRs and 95% CIs in each stratum. The vertical line represents the null effect (HR = 1.0).

Figure 1: Regional association plot of the CYP3A locus at 7q22.1 (98,803,430-99,662,733)

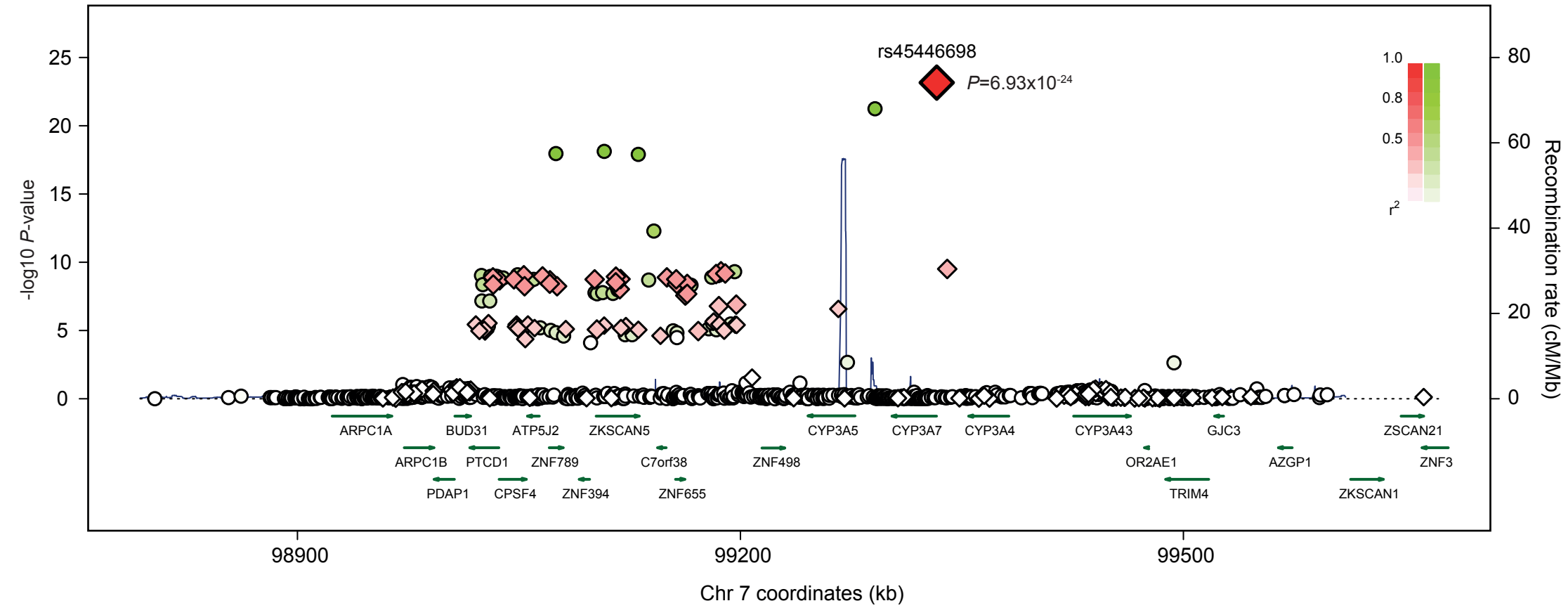
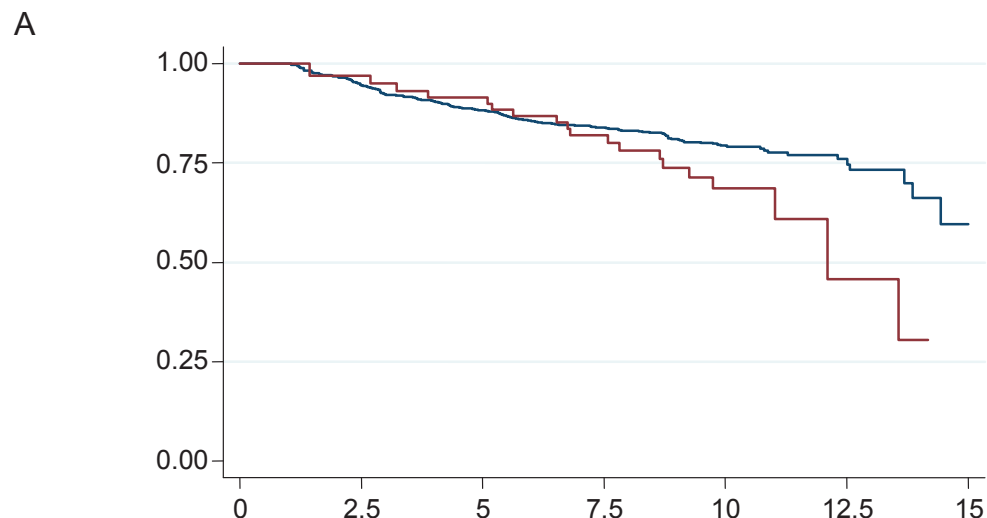
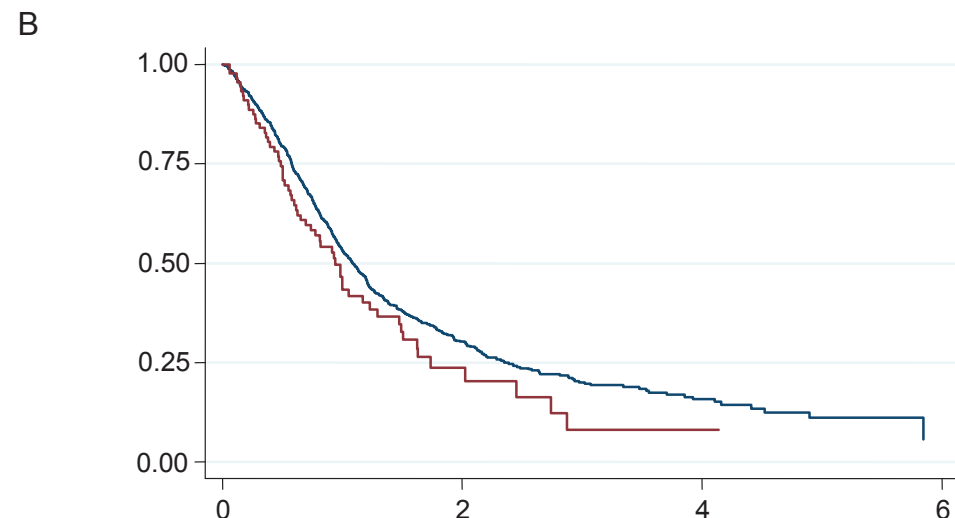


Figure 2: Kaplan-Meier survival estimates based on (A) breast cancer specific mortality (1,008 breast cancer cases) (B) all cause mortality (1,128 lung cancer cases) and (C) progression (347 CLL cases), according to rs45446698 genotype



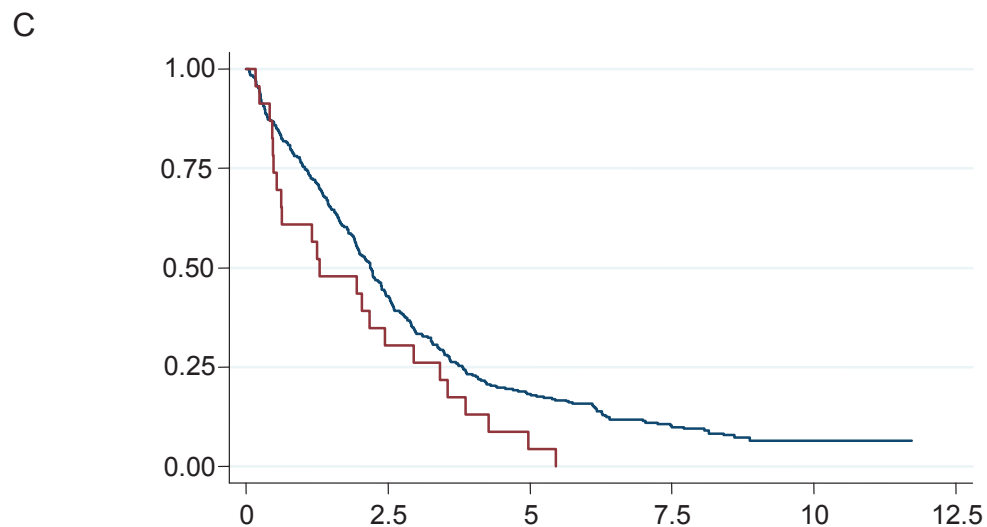
Number at risk

		0	2.5	5	7.5	10	12.5	15
Reference	—	9	577	738	544	316	56	8
Variant	—	0	47	60	44	24	3	0



Number at risk

		0	2	4	6
Reference	—	1039	143	26	0
Variant	—	89	7	1	0



Number at risk

		0	2.5	5	7.5	10	12.5
Reference	—	324	139	59	25	3	0
Variant	—	23	7	1	0	0	0

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 metabolised by a CYP3A enzyme.

