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Pharmacogenetic analysis of the UKMRC (Medical Research Council) MAGIC trial: association of polymorphisms with toxicity and survival in patients treated with perioperative epirubicin, cisplatin and 5-fluorouracil (ECF) chemotherapy

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Abstract

Purpose—Germline polymorphisms may affect chemotherapy efficacy and toxicity. We examined the effect of polymorphisms in drug metabolism and DNA repair genes on pathological response rates, survival, and toxicity for patients randomised to surgery alone or perioperative ECF chemotherapy in the MRC MAGIC trial.

Experimental design—DNA was extracted from non-tumor resection FFPE blocks. *ERCC1*, *ERCC2*, *XRCC1*, *DYPD,* and *OPRT* SNPs were evaluated using Sequenom, *GSTP1*, *GSTT1* deletion and *TYMS* (*TS*) 5' 2R/3R using multiplex PCR. Post PCR amplification *TS* 2R/3R and *GSTT1* samples underwent gel electrophoresis.

Results—Polymorphism data was available for 289/456 (63.4%) operated patients. No polymorphism was statistically significantly associated with pathological response to chemotherapy. Median overall survival (OS) for patients treated with surgery alone with any *TS* genotype was not different (1.76 years 2R/2R, 1.68 years 2R/3R, 2.09 years 3R/3R). Median OS for patients with a *TS* 2R/2R genotype treated with chemotherapy was not reached, whereas median OS for 2R/3R and 3R/3R patients were 1.44 and 1.60 years respectively (log rank p value 0.0053). The p value for the interaction between treatment arm and genotype (3R/3R and 3R/2R vs 2R/2R) was 0.029. No polymorphism was statistically significantly associated with chemotherapy toxicity.

Conclusions—In MAGIC, patients with a *TS* 2R/2R genotype appeared to derive a larger benefit from perioperative ECF chemotherapy than patients with 3R containing genotypes. Further exploration of this potential predictive biomarker in this patient population is warranted.

Keywords

Gastric cancer; gastroesophageal cancer; perioperative chemotherapy; polymorphism; thymidylate synthase

Introduction

Gastric and oesophageal cancers are the third and sixth most common causes of cancer death annually worldwide.(1) Neoadjuvant or perioperative chemotherapy is one standard treatment for patients with operable gastric or gastroesophageal adenocarcinoma prior to surgical resection.(2–5) This approach is associated with a modest (6-13%) absolute overall survival advantage in terms of overall survival compared to surgery alone but also with chemotherapy related toxicities such as nausea and vomiting, and neutropenia. Furthermore, following multimodality therapy half of resected patients develop incurable, metastatic cancer and therefore do not benefit from neoadjuvant chemotherapy.(2,4) Better selection of patients for preoperative chemotherapy might avoid needless toxicity, however currently gastroesophageal cancer patients who are treated with neoadjuvant chemotherapy are selected for treatment based on radiological staging alone as there are no currently validated predictive biomarkers for chemotherapy.

Germline polymorphisms in genes associated with chemotherapy and drug metabolism have been validated as predictors of survival and toxicity outcomes across several tumour types

including colorectal and breast cancer.(6–8) Although similar studies have examined the effects of polymorphisms in germline genes relating to chemotherapy metabolism in gastroesophageal cancer, most of these have been retrospective, and all lack an untreated control group.(9–11) The UK MRC MAGIC trial was an open label, multicentre, phase III randomised trial comparing six cycles of perioperative ECF (epirubicin, cisplatin and infused 5-fluorouracil) chemotherapy (3 cycles pre- and 3 cycles post- resection) plus surgery to surgery alone in patients with resectable gastroesophageal cancer.(2) Patients treated with perioperative chemotherapy had improved overall survival (OS) compared to patients treated with surgery alone [5 year OS 36% vs 23%, HR 0.75, (95% CI 0.60-0.93) p=0.009]. As a result, perioperative ECF chemotherapy became one standard treatment regimen for patients with resectable gastroesophageal adenocarcinoma. We hypothesised that selected germline polymorphisms would be associated with pathological response to chemotherapy, overall survival or chemotherapy related toxicity in the MAGIC trial, and herein present the results of this analysis.

Methods

Hematoxylin and eosin (H&E) stained tissue sections from resection specimens were reviewed by a histopathologist (AW). DNA was extracted from non-malignant tissue. Five sections (10 mm thick) were cut and deparaffinised using a standard protocol, and the area of interest was dissected using a sterile scalpel blade. Genomic DNA was extracted using the QIAmp DNA Micro Kit and QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. After dewaxing and rehydrating the slides, tissue was microdissected and placed into a 1.5-mL Eppendorf tube with buffer ATL and proteinase K for digestion (Qiagen). DNA was eluted in buffer ATE (Qiagen) with an elution volume of 60 mL. Quality control of the DNA was performed on the basis of 260:230 and 260:280 ratio values and visual inspection of the wavelength spectral pattern provided by the NanoDrop spectrophotometer (Thermo Scientific, Wilmington, DE). A 260:230 ratio of approximately 2.0, together with a 260:280 ratio of approximately 1.8 and the presence of a peak at 260 nm with a steep decrease toward 280 nm in the wavelength spectrum was considered sufficiently good quality DNA.

Slides from all cases were reviewed and pathological response in tumour graded using the Mandard tumour regression grading (TRG) system.(12)

Genotype Analysis

Ten polymorphisms were selected based on a review of the literature and expected interaction with epirubicin, cisplatin and 5 fluorouracil chemotherapy. These are listed in Table 1. For detailed description of genotype analysis methodology please see supplementary methods.

Statistical methods

OS was calculated from surgery to death from any cause or last date of follow up. Date of surgery was selected as the baseline for biomarker analysis to reduce potential bias as only patients with a surgical specimen were available for inclusion. Analyses were performed

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within treatment arms due to the differences in timing of surgery, to further reduce potential bias in the estimates of effects. Date of surgery could not be confirmed for nine patients in the chemotherapy plus surgery arm and these patients were excluded from the survival analyses. Differences in OS by polymorphism status were assessed using the Kaplan Meier method and compared using Cox regression. To mitigate multiplicity a p-value of <0.01 was considered significant when testing for associations of genotypes with survival and toxicity, and <0.05 when testing interactions Multiple imputation was performed to account for missing polymorphism data. OS results were adjusted for possible confounders of age, subtype, gender, site of primary, WHO, nodal status).

Proportions of patients with good pathological response (TRG 1-2) compared with poor pathological response (TRG 3-5) were compared for each genotype using the Fisher's exact test. Proportions of patients with toxicities according to genotype were compared using Pearson chi-squared test or Fisher's exact test where appropriate.

As TS 2R/2R genotype is the polymorphism of interest and is present in approximately 30% of patients, with median OS of 18 months in control (2R/3R + 3R/3R), power of 80%, 5% two sided significance level, to detect a HR of 0.5 would require 85 events. Alternatively as with the same assumptions as above with 70% power 67 events would be required. With respect to pathological response rate in TS genotyped patients, in order to detect a doubling in response rates from 15% to 30%, 206 patients would be required to achieve 70% power. This is based on based on a pathological response rate of 15% in the 3R group, which accounts for 70% of patients, and 30% in the 2R/2R group (which contains 30% of patients). Due to the trial design and retrospective nature of these analyses, all results can only be seem as hypothesis generating and suggestive of future work, with significance levels set to limit the possibility of a type II error.

All analyses were conducted using Stata version 14. The MAGIC protocol was approved by the relevant ethics committees, and patients gave written informed consent for participation in the trial. The translational MAGIC protocol (TransMAGIC) received separate national ethics approval (11/LO/0566).

Results

Polymorphism data was available for 289/456 (63.4%) patients who underwent surgery in the MAGIC trial. There was no difference in distribution of sex, performance status, site of tumour, age or treatment arm between patients with and without polymorphism data, however patients without polymorphism data were more likely to undergo a palliative resection in the view of the operating surgeon (Supplementary Table 1). This resulted in a borderline difference in survival between patients who had polymorphism data available and those who did not, which was more pronounced in the surgery only arm (Supplementary Figure 1).

Discordance in size based polymorphism assessment

We found that on duplicate runs that size based polymorphism assessment that discordance occurred at a rate of 32.7% and 4.2% for *GSTT1* and *TYMS (TS*) respectively.(13) Due to

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the high rate of discordance in *GSTT1* results for this polymorphism was not analysed further.

Genotype frequency

The frequency of each polymorphism genotype is described in Table 2. Genotype frequency was consistent with previously published data and all were in Hardy-Weinberg equilibrium with the exception of *DPYD* rs1801159.

Genotype and pathological response to chemotherapy

The association between each polymorphism and pathological response to chemotherapy in chemotherapy treated patients is described in Table 3. No polymorphism was statistically significantly associated with pathological response to chemotherapy.

Genotype and overall survival

Median overall survival for patients treated with surgery alone who had *TS* 2R/2R genotype was 1.76 years, compared to 1.68 years for 2R/3R and 2.09 years for 3R/3R (Table 4, Figure 1). These differences were not statistically significant. In contrast, median overall survival for patients with a *TS* 2R/2R genotype treated with chemotherapy was not reached, whereas survival for 2R/3R and 3R/3R were 1.44 and 1.60 years respectively (log rank p value 0.0053). When all 3R genotypes were combined median overall survival was 1.44 years for chemotherapy treated patients vs not reached for 2R/2R genotype (HR 2.4, p=0.003). The effect of TS genotype on overall survival in chemotherapy treated patients remained statistically significant when adjusted for the potential confounders of age, subtype, gender, site of primary, WHO, nodal status) (Table 4). The p value for the interaction between treatment arm and TS genotype (3R/3R and 3R/2R vs 2R/2R) was 0.029 (with a HR of 0.46).

In order to assess the effect of a 4.2% discrepancy in *TS* genotype status assessment we performed 10000 simulations, randomly changing 4.2% of results. From these 10000 simulations, the 2.5 to 97.5 percentiles of the HR for the interaction between treatment arm and TS genotype (3R/3R and 3R/2R vs 2R/2R) was 0.37 – 0.60, compared to the estimate from the original data of 0.46.

Patients with the (AG) genotype of *DPYD* rs1801159 Ile543Val had numerically shorter survival compared to the AA genotype in the surgery alone arm of the trial, this difference was statistically significant (HR 1.75, p=0.008). There was no evidence of an interaction between treatment arm and DPYD status. Results were similar when multiple imputation was performed for missing data.

No other genotype was statistically significantly associated with overall survival (see Supplementary Tables 2 A-F).

Genotype and chemotherapy related toxicity

The presence of grade 3 or greater toxicity and association with polymorphism status are detailed in Supplementary Table 1. *DPYD2A* IVS14+1G>A GA variant was associated with

a non-statistically significant trend towards increased rates of ≥ Grade 3 diarrhoea (p=0.039), however only one patient was detected with this variant. No other polymorphism demonstrated a statistically significant relationship with chemotherapy related toxicity.

The mean number of cycles of chemotherapy received for most polymorphisms was five (Supplementary Table 3), with the exception of *ERCC1* rs3212986 (GT+TT) variant who had a mean of four cycles (Kruskal-Wallis equality-of-populations rank test p= 0.0425).

Discussion

Our study is the first to evaluate the association between germline polymorphisms and pathological response, overall survival, and chemotherapy related toxicity for patients with operable gastroesophageal cancer in a randomised trial with a control group. We found that patients who have a 2R/2R thymidylate synthase genotype who were treated with perioperative ECF chemotherapy had statistically superior overall survival compared to those who had a 2R/3R or 3R/3R genotype. This difference was not apparent in patients who were treated with surgery alone, and a significant interaction between *TS* genotype status and treatment arm was noted Additionally, in our study, patients with a *TS* 2R2R genotype had a non-statistically significant higher rate of good pathological response (TRG 1-2) at 24% compared to 3R allele containing patients. These findings are important as if validated pharmacogenomic genotyping could be used in future to select patients who are more likely to benefit from perioperative chemotherapy.

Thymidylate synthase (TS) acts to produce thymidylate which is an essential precursor for DNA synthesis. The activity of TS is blocked by 5-fluorodeoxyuridylate (5FdUMP), the active metabolite of 5-FU and it is via this mechanism that 5-FU exerts cytotoxicity. The human thymidylate synthase gene is polymorphic through the presence of either double (2R) or triplet (3R) 28 base tandem repeats which are sited upstream of the *TS* translational start site. [14] These repeats control the transcription and translation of the *TS* gene; individuals with 3R tandem repeats have higher levels of TS expression in tissue and consequently lower rates of response to fluoropyrimidine chemotherapy.[15] Our findings are in keeping with this biology. Several other series have reported comparable improvements in response rates overall survival similar results in gastric cancer patients with the favourable 2R genotype treated with fluoropyrimidine 5-FU based chemotherapy, however none of these were a randomised trial with an untreated control group.(14–16) However, opposing results have also been demonstrated.(17,18) Potential reasons for this include small, heterogeneous, ethnically diverse populations treated with variable chemotherapy regimens in both advanced and resectable disease settings, and the addition of other related polymorphisms such as the *TS* 3'UTR 6 base pair polymorphism to analyses.(19) We caveat our discussion with an awareness that length based polymorphism assessment resulted in a discordance rate of 4.2% for TS polymorphism status. However, as our findings for the 2R/2R genotype are quite striking, even when a stringent p.value is applied to correct for multiplicity, and were confirmed with repeated simulation testing to account for any discrepancy in *TS* genotype assessment we do not think that this is likely to have unduly affected these results.

Dihydropyrimidine dehydrogenase (DPD) is the rate-limiting enzyme in 5-fluorouracil catabolism and variation in DPD levels and activity have profound effects of fluoropyrimidine metabolism and toxicity. The most well described of these is a *DPYD* 2* splice variant polymorphism which results in a non-functional enzyme and is associated with fluoropyrimidine related toxicity in many studies.(6,20–22) Although low patient deleterious allele frequency and lack of statistical significance due to correction for multiplicity means that we cannot be definitive in our conclusions, our results are consistent with these data. However, we think that these results are of secondary importance to the survival outcomes presented.

We asked two questions from our dataset, firstly, can genotyping be used to differentiate between those who derive a survival benefit from perioperative chemotherapy and those who do not, and secondly, if these genotypes were assessed preoperatively, would it possible to predict excessive toxicity prior to commencing chemotherapy? Regarding survival benefit, our findings relating the favourable effects of the *TS* 2R/2R genotype are shared with several other large studies. Therefore, is further validation with a clinical trial required? One small genotype directed clinical trial clinical trial evaluated FOLFOX chemotherapy in 25 patients with *TS* 2R containing genotypes (2R/2R and 2R/3R) and found that radiological response rates did not differ compared to historical control.(33) However, based on our results only the 2R/2R genotype would benefit from this approach; this was also suggested in subgroup analysis of that study. As patients with 3R containing genotypes did not appear to benefit from fluoropyrimidine based chemotherapy in MAGIC, we suggest that alternative treatment options should be evaluated for these patients. Omitting perioperative chemotherapy completely is unlikely to be acceptable as many patients (especially those with proximal tumours) require downstaging prior to surgical resection. Alternatively, patients with 3R containing genotypes could be treated with higher doses of fluoropyrimidines, although this could be result in increased toxicity. This approach in UGT1A1 genotyped patients has demonstrated that patients who are wildtype or heterozygous for the deleterious *28 allele can tolerate increased doses of irinotecan compared to UGT1A1 *28 homozygotes.(34,35) Finally, a non-fluoropyrimidine containing regimen could be considered; for patients with tumours of the gastroesophageal junction or oesophagus chemoradiotherapy with carboplatin and paclitaxel would seem a reasonable alternative.

With respect to avoiding toxicity, the relative rarity of alleles which predict for significant toxicity such as *DPYD 2A** is associated with significant screening costs even when toxicity is reduced by the use of pre-emptive dose reductions.(36) As such, neither the European Medicines Agency nor the US Food Drug Administration require testing for *DPYD* variants prior to treatment with fluoropyrimidines despite the availability of advice from expert groups such Clinical Pharmacogenetics Implementation Consortium and the Dutch Pharmacogenetics Working Group which provide clinical practice guidelines on genotype based drug dosing.(37,38) In the MAGIC trial, the most common grade 3 or greater chemotherapy associated toxicity was neutropenia which is likely to be due to epirubicin and which is not predicted by any of the polymorphisms which we examined. Therefore, routine testing for *DPYD 2A** polymorphisms is unlikely to significantly decrease toxicity in patients treated with MAGIC type chemotherapy.

The interaction between chemotherapy and genotype is complex, and coloured by many other clinical variables such as age, ethnicity, gender, hepatorenal function, and the interaction between individual components of each chemotherapy regimen. This has profound implications for the accuracy of toxicity or outcome prediction using genotyping. One potential flaw relating specifically to this work is that not all MAGIC trial participants were included in the current study as not all provided tissue for analysis, therefore we caution that the analysis could be underpowered to detect small effect sizes. On one hand, if a patient did not undergo surgery due to failure to respond to treatment then no tissue was available for analysis. Alternatively, patients with excessive toxicity due to chemotherapy may also have stopped chemotherapy prior to surgery. These potential biases may be reflected in the borderline improved overall survival demonstrated for patients with polymorphism data available. Thus although germline genotype will not be altered by treatment, any true assessment of the predictive power of genotype would preferentially be performed in pre-treatment samples for these reasons. A second issue relates to the technical challenges associated with length based polymorphism assessment; moving forward advances in high throughput next generation sequencing technologies should ensure improved accuracy and speed of results with decreased DNA requirements.

In summary, ours is the first study to examine the effect of germline polymorphisms on pathological response and survival outcomes for patients treated with perioperative chemotherapy for operable oesophagogastric cancer, with a randomised control group. We found that patients with a *TS* 2R/2R genotype (representing 34% of the population) had excellent survival when treated with perioperative ECF chemotherapy. In contrast, patients with a 3R containing genotype did not appear to derive a similar benefit from standard dose fluoropyrimidine based chemotherapy when compared to patients treated with surgery alone. It is salutary to note that despite recent progress in our understanding of the molecular biology underpinning gastroesophageal cancer that only one targeted drug, trastuzumab, is licenced in this disease, and that almost all patients will receive platinum and fluoropyrimidine based chemotherapy as a component of their treatment.(39,40) Therefore use of available data relating to patient selection for standard of care chemotherapy to design a prospective would appear to be a sound choice.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Statement of translational relevance

Neoadjuvant or perioperative cisplatin and 5-fluorouracil based chemotherapy plus surgery is one standard of care for patients with resectable gastroesophageal cancer. However, chemotherapy benefits only a small proportion of patients, and validated biomarkers predictive of response or toxicity have been elusive. We analysed the effect of multiple germline polymorphisms putatively associated with response or toxicity to chemotherapy in patients treated with chemotherapy plus surgery or surgery alone in the UK Medical Research Council MAGIC Trial. One polymorphism in thymidylate synthase (*TS*), a 2R/2R tandem repeat, was significantly associated with overall survival in patients treated with chemotherapy, but not in patients treated with surgery alone. These findings suggest that neoadjuvant chemotherapy for patients with operable gastroesophageal cancer could be personalized based on germline polymorphism status.

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Statement of significance

We demonstrate in a randomised trial with a chemotherapy control arm that the presence of the thymidylate synthase 2R/2R genotype is associated with significantly improved overall survival for patients with operable gastroesophageal cancer treated with fluoropyrimidine based chemotherapy plus surgery. Further validation of this potential predictive biomarker for chemotherapy efficacy may be appropriate.

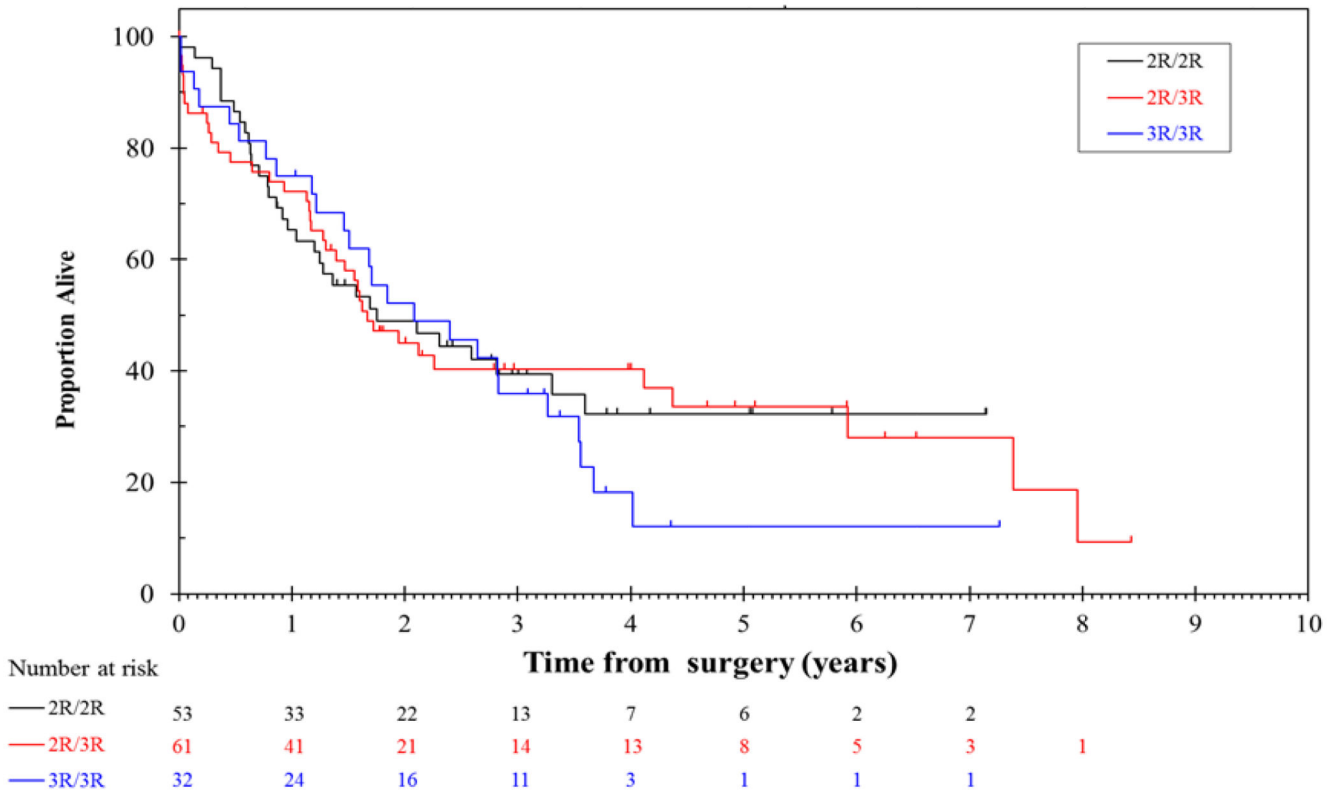

Figure 1. Overall survival from surgery by TS genotype (surgery alone arm)

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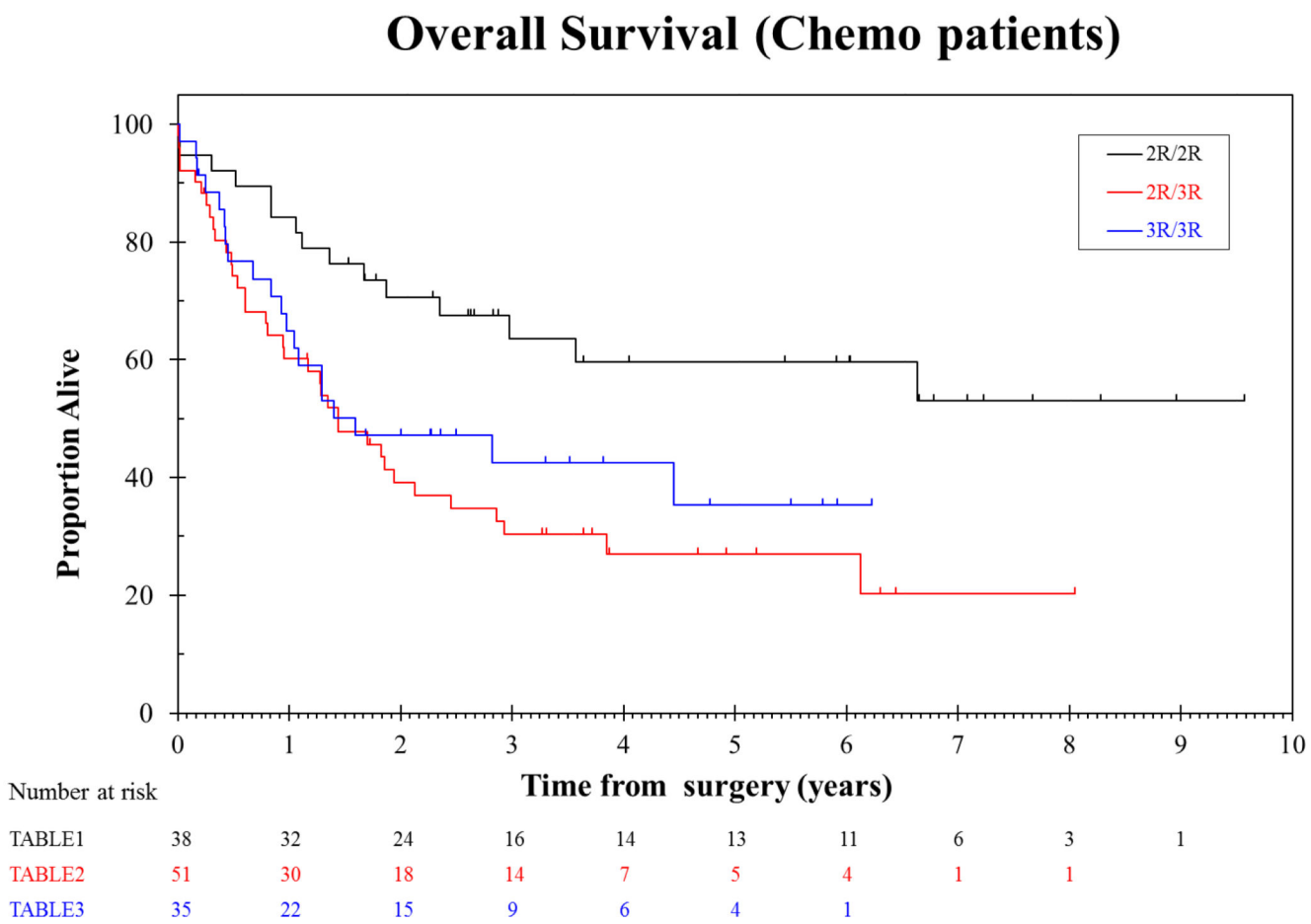

Figure 2.

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Table 1

Germline polymorphisms analysed

Gene	Polymorphism	rsID
OPRT	G638C (Gly213Ala)	1801019
DPYD	IVS14+1G>A	DPYD2A
DPYD	A1627G	1801159
ERCC1	C118T	11615
ERCC1	C8092A	3212986
ERCC2	Lys751Gln	13181
XRCC1	A399G	25487
GSTP1	I105V	1695
TS 2R/3R 5'UTR	2R/3R repeat	
GSTT1 deletion		

Table 2

Frequency of each polymorphism

		Chemo+Surgery	Surgery alone	Total
TS 2R/3R 5'UTR	2R/2R	38 (31%)	53 (36%)	91 (34%)
	2R/3R	51 (41%)	61 (42%)	112 (41%)
	3R/3R	35 (28%)	32 (22%)	67 (25%)
	Total	**124**	**146**	**270**
GSTP1 rs1695	A	74 (56%)	73 (47%)	147 (51%)
	AG	53 (40%)	67 (43%)	120 (42%)
	G	6 (5%)	16 (10%)	22 (8%)
	Total	**133**	**156**	**289**
OPRT rs1801019	C	5 (4%)	3 (2%)	8 (3%)
	G	92 (69%)	92 (59%)	184 (64%)
	CG	36 (27%)	60 (39%)	96 (33%)
	Total	**133**	**155**	**288**
DPYD2A IVS14+1G>A	G	131 (99%)	152 (100%)	283 (>99%)
	GA	1 (1%)	0 (0%)	1 (<1%)
	Total	**132**	**152**	**284**
DPYD rs1801159	A	96 (73%)	100 (65%)	196 (68%)
	AG	27 (20%)	48 (31%)	75 (26%)
	G	9 (7%)	7 (5%)	16 (6%)
	Total	**132**	**155**	**287**
ERCC1 rs11615	C	19 (14%)	29 (19%)	48 (17%)
	CT	70 (53%)	68 (44%)	138 (48%)
	T	44 (33%)	57 (37%)	101 (35%)
	Total	**133**	**154**	**287**
ERCC1 rs3212986	G	61 (46%)	84 (55%)	145 (51%)
	GT	64 (48%)	60 (39%)	124 (43%)
	T	7 (5%)	10 (6%)	17 (6%)
	Total	**132**	**154**	**286**
ERCC2 rs13181	G	23 (17%)	20 (13%)	43 (15%)
	GT	62 (47%)	63 (41%)	125 (44%)
	T	48 (36%)	71 (46%)	119 (41%)
	Total	**133**	**154**	**287**
XRCC1 rs25487	A	19 (14%)	18 (12%)	37 (13%)

		Chemo+Surgery	Surgery alone	Total
	AG	56 (42%)	67 (44%)	123 (43%)
	G	57 (43%)	69 (45%)	126 (44%)
	Total	**132**	**154**	**286**

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Table 3
Genotype and pathological response to chemotherapy

Genotype	TRG 1-2	TRG 3-5	p	OR for TRG 3-5	95%CI
TS					
2R/2R	9 (24.3)	28 (75.7)	(0.536)	1.0	
2R/3R	8 (16.7)	40 (83.3)	0.384	1.61	0.55-4.68
3R/3R	5 (14.7)	29 (85.3)	0.313	1.86	0.56-6.25
2R/2R	9 (24.3)	28 (75.7)		1.0	
2R/3R + 3R/3R	13 (15.9)	69 (84.2)	0.274	1.71	0.66-4.44
GSTP1 rs1695					
A	11 (15.5)	60 (84.5)	(0.812)	1.0	
AG	10 (20.0)	40 (80.0)	0.520	0.73	0.29-1.89
G	1 (16.7)	5 (83.3)	0.939	0.92	0.10-8.62
OPRT					
C	2 (40.0)	3 (60.0)	(0.308)	1.0	
G	16 (18.2)	72 (81.8)	0.249	3.0	0.46-19.5
GC	4 (11.8)	30 (88.2)	0.128	5.0	0.63-39.7
DPYD rs1801159					
A	14 (15.4)	77 (84.6)	(0.413)	1.0	
AG	5 (18.5)	22 (81.5)	0.698	0.80	0.26-2.46
G	3 (33.3)	6 (66.7)	0.186	0.36	0.08-1.62
ERCC1 rs11615					
C	3 (16.7)	15 (83.3)	(0.211)	1.0	
CT	15 (22.7)	51 (77.3)	0.580	0.68	0.17-2.67
T	4 (9.3)	39 (90.7)	0.417	1.95	0.39-9.77
ERCC1 rs3212986					
G	8 (13.6)	51 (86.4)	(0.496)	1.0	
GT	12 (19.7)	49 (80.3)	0.371	0.64	0.24-1.70
T	2 (28.6)	5 (71.4)	0.308	0.39	0.06-2.38
ERCC2 rs13181					
G	3 (14.3)	18 (85.7)	(0.879)	1.0	
GT	11 (19.0)	47 (81.0)	0.631	0.71	0.18-2.85
T	8 (16.7)	40 (83.3)	0.804	0.83	0.20-3.51
XRCC1 rs25487					
A	2 (11.1)	16 (88.9)	(0.752)	1.0	
AG	10 (18.9)	43 (81.1)	0.453	0.54	0.11-2.72

Genotype	TRG 1-2	TRG 3-5	p	OR for TRG 3-5	95%CI
G	10 (17.9)	46 (82.1)	0.503	0.58	0.11-2.91

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Table 4
Association between TS genotype and overall survival

(2nd HR & p-value are adjusted for: age, subtype, gender, site of primary, WHO, nodal status)

	Chemotherapy			**Surgery alone**			**Overall**		
	2R/2R	**2R/3R**	**3R/3R**	**2R/2R**	**2R/3R**	**3R/3R**	**2R/2R**	**2R/3R**	**3R/3R**
Patients	38 (31%)	51 (41%)	35 (28%)	51 (36%)	59 (41%)	32 (23%)	89 (34%)	110 (41%)	67 (25%)
Events	15	36	20	31	38	25	46	74	45
Median survival	Not reached	1.44	1.60	1.76	1.68	2.09	3.31	1.62	1.84
Log-rank p-value	0.0053			0.7212			0.0326		
Hazard ratio	1 (REF)	2.66 3.06	2.10 2.64	1 (REF)	1.03 1.07	1.23 1.45	1 (REF)	1.59 1.73	1.53 1.79
HR p-value		0.002 0.001	0.032 0.009		0.896 0.778	0.448 0.190		0.013 0.005	0.043 0.008
Combined analysis									
	2R/2R	2R/3R + 3R/3R		2R/2R	2R/3R + 3R/3R		2R/2R	2R/3R + 3R/3R	
Patients	38 (31%)	86		51 (36%)	91		89 (34%)	177	
Events	15	56		31	63		46	119	
Median survival	Not reached	1.44		1.76	1.84		3.31	1/71	
Log-rank p-value	0.002			0.6519			0.009		
Hazard ratio	1 (REF)	2.43 2.89		1 (REF)	1.10 1.15		1 (REF)	1.57 1.75	
HR p-value		0.003 0.001			0.652 0.531			0.010 0.002	