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Expression quantitative trait loci in ABC transporters are associated with survival in 5-FU treated colorectal cancer patients

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Abstract

The chemotherapeutic efficacy in colorectal cancer (CRC) is limited due to the inter-individual variability in drug response and the development of tumour resistance. ATP-binding cassette (ABC) transporters are crucial in the development of resistance by the efflux of anticancer agents from cancer cells. In this study, we identified 14 single nucleotide polymorphisms (SNPs) in 11 *ABC* transporter genes acting as an expression of quantitative trait loci (eQTLs), i.e. whose variation influence the expression of many downstream genes. These SNPs were genotyped in a case–control study comprising 1098 cases and 1442 healthy controls and analysed in relation to CRC development risk and patient survival. Considering a strict correction for multiple tests, we did not observe any significant association between SNPs and CRC risk. The rs3819720 polymorphism in the *ABCB3/TAP2* gene was statistically significantly associated with shorter overall survival (OS) in the codominant, and dominant models [GA vs. GG, hazard ratio (HR) = 1.48; $P = 0.002$; AA vs. GG, HR = 1.70; $P = 0.004$ and GA + AA vs. GG, HR = 1.52; $P = 0.0006$]. Additionally, GA carriers of the same SNP displayed worse OS after receiving 5-FU based chemotherapy. The variant allele of rs3819720 polymorphism statistically significantly affected the expression of 36 downstream genes. Screening for eQTL polymorphisms in relevant genes such as *ABC* transporters that can regulate the expression of several other genes may help to identify the genetic background involved in the individual response to the treatment of CRC patients.

Introduction

Colorectal cancer (CRC) is one of the most common malignant diseases and a major cause of cancer-related deaths worldwide (1). Although the 5-year relative survival rate for CRC has improved in the last decade, the number of new CRC cases continues to grow every year globally (2).

Genetic variations play a key role in predisposition to CRC, initiation and in disease progression (3). Next generation sequencing (NGS) and genome-wide association studies (GWAS) have successfully identified new susceptibility loci for CRC predisposition; however, only a limited amount of data has clarified the functional mechanisms underlying these associations, which is essential to establish a link to cancer predisposition and progression (4–6).

Studying the association between polymorphisms and gene expression at genome-wide levels, known as expression quantitative trait loci (eQTL), could help in implementing the functional interpretation of genetic variants involved in CRC susceptibility (7). eQTL have been proposed as a method to find genes underlying the associations with disease risk (8), to improve the power of GWAS (9), or even to unravel gene/variant networks to discover new mechanisms/pathways related to disease or drug response (10).

ATP-binding cassette (ABC) transporters are responsible for the efflux of exogenous agents out of cells and they play a crucial role in the development of chemoresistance to anticancer agents (11). The coordinated expression of ABC transporters and their activity at the basolateral and apical side of transporting epithelia are significant determinants of drug disposition, drug–drug interactions and variability in drug response and toxicity (12).

We previously investigated the role of genetic variants in *ABC* transporter genes in CRC susceptibility (13–15). Here, we hypothesised that eQTLs variants (defined as polymorphisms whose variation could influence the expression of many downstream genes) could affect the drug metabolic pathways and consequently influence the treatment outcome (16). We, therefore, investigated whether single nucleotide polymorphisms (SNPs) in *ABC* transporters acting as eQTLs were associated with risk of developing CRC or in treatment response to the disease.

Materials and methods

Study population

The studied cohort comprised 1098 CRC patients and 1442 controls. The study group has been previously described elsewhere (17–24).

All subjects were informed and provided written consent to participate in the study and they approved the use of their biological samples for genetic analyses, according to the Helsinki declaration. The study design was approved by the Ethics Committee of the Institute of Experimental Medicine, Prague, Czech Republic.

Follow-up of the patients

All CRC cases were monitored with follow-up until December 31, 2016. For all subjects, clinical data at the time of diagnosis, including the location of the tumour, the International Union Against Cancer (UICC) tumour-node-metastasis (TNM) stage system, grade and adjuvant or first line 5-fluorouracil (5-FU)-based chemotherapy treatment were assembled, along with information about distant metastasis, relapse and date of death.

Eight hundred and fifty-one of the CRC cases were monitored with follow-ups until December 31, 2016. Four hundred and eleven CRC cases received 5-FU-based chemotherapy as first line postoperative therapy. The therapy consisted of either a Mayo regimen, delivered as a bolus infusion of 5-FU (425 mg/m^2) and leucovorin (10 mg/m^2) for 5 days every four weeks repeated 6 times or a simplified De Gramont regimen which consisted of a 2 h intravenous (i.v.) infusion of leucovorin (200 mg/m^2), then a 5-FU i.v. bolus (400 mg/m^2) followed by a 46 h 5-FU continuous i.v. infusion (2400–3000 mg/m^2). Metastatic CRC (mCRC) patients were administrated with FOLFOX4 regimen: oxaliplatin (85 mg/m^2) and leucovorin (200 mg/m^2) infusions both given more than 120 min at the same time, followed by 5-FU (400 mg/m^2) bolus given more than 2–4 min, followed by 5-FU (600 mg/m^2) on the first day. Second day: leucovorin (200 mg/m^2) infusion both given more than 120 min at the same time, followed by 5-FU (400 mg/m^2) bolus given more than 2–4 min, followed by 5-FU (600 mg/m^2). The recommended dose schedule was given every 2 weeks. Four hundred and forty subjects did not receive any adjuvant chemotherapy after surgery. In this study, the outcome variables measured were 5-FU-based chemotherapy (yes/no), overall survival (OS) (time from diagnosis until death or censorship), and progression-free survival (PFS) (time of surgery or end of chemotherapy until the date of relapse, death or censorship).

Selection of eQTL polymorphisms

Seven subfamilies of *ABC* transporter genes encoding for 48 different proteins have been identified (25,26). For each *ABC* gene, the mutual information about the association between coding and non-coding SNPs and gene expression levels was obtained from the SCAN database (http://www.scandb.org/newinterface/about.html). The SCAN database provided for each SNP the number of differentially transcribed genes in lymphoblastoid cell lines (LCL) from individuals of Caucasian origin associated with the change of allele. Selected relevant eQTL SNPs were then filtered for a minor allele frequency (MAF) >5% in European population. Figure 1 depicts the workflow of the study. In addition, selected SNPs were further analysed for the possibility of functioning as 'master regulators of expression'. An arbitrary threshold of a minimum of 40 genes regulated by a single genetic variant was set up to define the 'master regulator of transcription' (16,27). Additionally, candidate eQTL SNPs were verified by Genotype-Tissue Expression project (GTEx; https://gtexportal.org/home/, version V6p). The GTEx project allows viewing and downloading computed eQTL results and aims to characterise variations in gene expression levels across individuals and diverse tissues of the human body, many of which are not easily accessible (28).

Gene set enrichment analysis

To identify a gene expression signature, we analysed all genes co-regulated by significant SNPs identified in our study. The list of regulated genes was tested using 'EnrichR' tool (29). The relevance of each gene set enrichment was estimated using an adjusted *P* value. Gene sets with $P < 0.05$ were considered as significant.

SNP genotyping

Genomic DNA was isolated from peripheral blood lymphocytes, using standard procedures. Genotyping of the selected 14 *ABC* SNPs was carried out by using the KASP^{ar} allele discrimination chemistry of LGC Genomics (Hoddesdon, Herts, UK: http://www.lgcgenomics.com/genotyping/kasp-genotyping-reagents/), as previously described (30). For quality control purposes, equal number of DNA samples from cases and controls were randomly placed on plates for simultaneous analysis, duplicate samples (5% of all samples) were repeated for each SNP, and no template controls were included in each plate. The genotypes with unclear results were

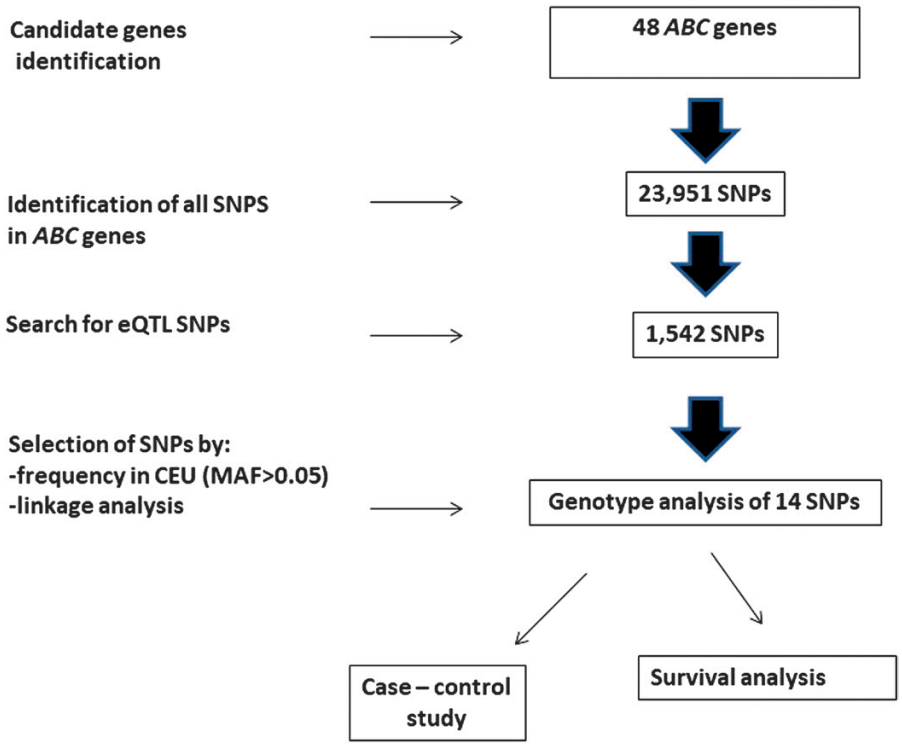

Figure 1. Workflow strategy for selection and analyses of expression quantitative trait loci in ABC transporter genes.

excluded from the study. Two genotyping assays failed to pass their validation. The genotype correlation between the duplicate samples was >95%. Genotype call rate between SNPs ranged between 94 and 99%.

Statistical analysis

Chi-square test (1 degree of freedom), with a Type I error threshold set at $\alpha = 0.05$, was used to verify whether the genotypes were in Hardy–Weinberg equilibrium in controls. The multivariate logistic regression (MLR) analysis was used to test the association between genotypes and risk of CRC. The covariates analysed in the multivariate model were: sex, age, smoking habit (non-smokers vs. smokers and ex-smokers), BMI, any positive familial history of CRC, education level (high, intermediate, and low), and living area (country, town neighbourhood, and town). The association between SNPs and CRC risk was calculated by estimating the odds ratios (ORs) and their 95% confidence intervals (CI), adjusted for both continuous and discrete covariates. For all SNPs with significant *P* values per genotype, the best model (dominant or recessive) was calculated. *P* values ≤0.05 were considered statistically significant. However, the Bonferroni-corrected significance threshold was 0.004 (as calculated for 12 SNPs and $\alpha = 0.05$).

OS in CRC patients was evaluated using the date of death or, for those alive, the date of the end of the study (December 31, 2016) as the end point of follow-up (6,22,24,31,32). PFS was defined as the time from surgery/end of therapy to the occurrence of distant metastasis, recurrence or death, whichever came first. The relative risk of death and recurrence was estimated as hazard ratio (HR) using Cox regression. The survival curves for overall and event-free survival were derived by the Kaplan–Meier method. Multivariate survival analyses were adjusted for age, sex, T, N, M and chemotherapy. Statistical analyses were performed using R (http://www.rproject.org, R version 2.14-2).

Results

SNP selection

Out of the 23,951 SNPs found in the 48 *ABC* transporter genes, 1542 were identified in the SCAN database for their possible influence in the expression of other genes according to the allele change (http://www.scandb.org/newinterface/about.html). These SNPs were then filtered for a MAF >5% in the European CEU population with the purpose to have sufficient power to evaluate genetic effects with the size of our study population. An additional filter based on linkage disequilibrium (LD) analysis was applied using an r^2 threshold of 0.80 among the selected SNPs.

In total, 26 eQTL in 15 *ABC* transporter genes complied with the above selection criteria. As an additional step, we applied the same selection criteria used in Pardini *et al.* (16,27), and we used SCAN database to identify as 'master regulators' those SNPs putatively acting as eQTLs and regulating more than 40 genes. According to this arbitrary selection, we identified six *ABC* SNPs as master regulators. To this list we decided to extend the study also to those SNPs working as eQTL but only regulating more than 25 genes. Finally, after this final selection, 14 SNPs indicating as eQTLs (six master regulators and eight eQTL SNPs regulating more than 25 genes) were included in the study and genotyped (supplementary Table 1, available at *Mutagenesis* Online). The genotyping assays for SNPs rs3758953 in *ABCC8* and rs12113924 in *ABCF2* failed to pass their validation as they showed only non-specific amplification, so were excluded from further analyses. The remaining 12 SNPs were genotyped successfully.

The workflow strategy for the selection and the analysis of eQTL SNPs used in the study is depicted in Figure 1.

Identification of candidate SNPs through eQTL analysis

In the SCAN database, for the rs6958591, rs2188966, rs2608288, rs4148105, rs4147825 and rs480562 polymorphisms the presence

of a specific allele was shown to significantly affect the expression of 124, 72, 57, 49, 44 and 41 genes, respectively. Therefore, we defined these SNPs as master regulators of eQTLs. The list of differentially expressed genes associated with the selected SNPs according to the SCAN database is presented in supplementary Tables 1 and 2, available at *Mutagenesis* Online.

According to GTEx data, the SNPs rs2254884, rs4147825, rs9895649, rs11185610, rs2188966, rs6958591, rs2608288, rs4148105 and rs11685669 were not associated to significant eQTLs in any tissues. Regarding rs4147864 in the *ABCB4* gene, this SNP appeared to have a significant impact as eQTLs in the small intestine terminal ileum. Significant *cis*-eQTL effect was observed for rs3819720 in *ABCB3/TAP2* and *HLA-DQB1* in thyroid, muscle, skin, artery, oesophagus, heart, whole blood, adipose tissue, breast and small intestine tissues. Strong eQTL effect was also observed for rs3819720 and *HLA-DQB2* in colon, pancreas, lung and artery tissues. Similar eQTL effects were observed for rs3819720 and *HLA-DMA, HLA-DOB, PSMB9, SKIV2L, HLA-DQA1, HLA-DQA2* and *C4A* genes. Finally, the rs480562 in *ABCB11* gene, the SNP functioned as an eQTL in altering *G6PC2* expression in lung, skin exposed and unexposed to sun, nerve, adipose and stomach tissues. Similarly, the same SNP had an eQTL effect on *NOSTRIN* gene expression in the testis.

These *in silico* analyses highlighted the most significant genes that were involved in major histocompatibility complex (MHC) (HLA-DQB1, HLA-DQB, etc.) in intestine tissues. It is well known that tumour antigens represented by the MHC on the cell surface are essential for eliciting a tumour immune response (33).

Case–control study

The characteristics of the study participants are given in supplementary Table 3, available at *Mutagenesis* Online. Among the 1098 CRC cases, 708 patients were diagnosed with colon cancer, and 369 with rectal cancer, 21 cases lacked information about the tumour located but were included in survival analysis. Compared to the control group, CRC cases were more likely to be older and had a slightly higher BMI and were more likely to have a positive family history of CRC and lower formal education. The control group had a significantly higher number of male individuals and current smokers and non-smokers (supplementary Table 3, available at *Mutagenesis* Online).

For all SNPs, the distribution of genotypes in controls was in agreement with Hardy–Weinberg equilibrium. SNPs significantly associated with CRC risk (before the multiple testing correction) are reported in supplementary Table 4, available at *Mutagenesis* Online.

Briefly, carriers of the GA genotype in *ABCB3/TAP2* rs3819720 were associated with an increased colon cancer risk compared to GG carriers (GA vs. GG; OR = 1.30; 95% CI = 1.03–1.65; $P = 0.03$). Similar result was observed in the dominant model when patients presented the variant A allele (GA + AA vs. GG; OR = 1.26; 95% CI = 1.00–1.57; $P = 0.05$). By considering multiple testing adjustment (for instance considering the minimal Bonferroni correction with 12 SNPs and $\alpha = 0.05$; $P = 0.004$) these associations were not considered as statistically significant.

Survival study

In total, 1098 CRC cases were included in the survival analyses. In the univariate assessment, several covariates were associated with decreased survival, including established prognostic factors such as male sex, higher age, smoking habit and tumour stage (Table 1).

Five SNPs were associated with either OS or PFS according the nominal *P* value (supplementary Table 5, available at *Mutagenesis* Online); however, by considering the minimal multiple testing adjustment ($P = 0.004$) the majority of these associations were not interpreted as statistically significant.

Considering all CRC patients, rs3819720 in the *ABCB3/TAP2* gene was statistically significantly associated with OS in the codominant, and dominant models at least after the minimal Bonferroni correction for multiple test (GA vs. GG; HR = 1.48; 95% CI = 1.15–1.90; $P = 0.002$; AA vs. GG; HR = 1.70; 95% CI = 1.18–2.43; $P = 0.004$; and GA + AA vs. GG; HR = 1.52; 95% CI = 1.20–1.93; $P = 0.0006$; log-rank test $P = 0.05$; Figure 2). After the stratification for tumour localisation, the associations remained significant for all the sites only at the nominal *P* value (colon: GA vs. GG; HR = 1.38; 95% CI = 1.01–1.88; $P = 0.04$; and GA + AA vs. GG; HR = 1.40; 95% CI = 1.04–1.89; $P = 0.03$; rectum: GA + AA vs. GG; HR = 1.57; 95% CI = 1.03–2.40; $P = 0.04$).

Survival and therapy

To examine the association of analysed SNPs with therapy outcome we further stratified patients into three separate groups according to the treatment received: CRC patients receiving (i) no treatment; (ii) receiving 5-fluorouracil (5-FU) regimen without or (iii) in combination with oxaliplatin. From the univariate model analysis several genotypes were significantly associated with OS or PFS in patients stratified according to the therapy regimen but only not considering correction for multiple tests (Table 2).

Considering only nominal *P* value, in those patients receiving no treatment, no SNPs were significantly associated with survival outcomes. In patients undergoing 5-FU-based chemotherapy without oxaliplatin, heterozygous GA carriers of rs3819720 in the *ABCB3/TAP2* gene displayed worse OS after receiving 5-FU based chemotherapy (GA vs. GG; HR = 1.59; 95% CI = 1.05–2.41; $P = 0.03$ and GA + AA vs. GG; HR = 1.60; 95% CI = 1.07–2.38; $P = 0.02$; log-rank test $P = 0.008$; Figure 3). By considering the Bonferroni adjustment for multiple testing, these associations were not statistically significant, not even when considering the minimal calculation for multiple test ($P = 0.004$).

Enrichment analyses of the co-regulated genes

Gene set enrichment analysis using gene ontology (GO) revealed significant representation of several biological processes involving the co-regulated genes by significant SNPs. The most significant processes for rs3819720 were observed: ”Interferon-gamma-mediated signalling pathway (GO:0060333; adjP = 2.9e-13); ‘antigen processing and presentation of exogenous peptide antigen via MHC class II’ (GO:0019886; adjP = 1.1e-12; supplementary Table 2, available at *Mutagenesis* Online).

Discussion

Inherited variation in genes encoding membrane-bound ABC transporters are emerging as useful approaches to identify patients at risk for aberrant pharmacokinetic or pharmacodynamic effects (12). Predicting response and limiting drug-induced toxicity are two important challenges faced by clinicians in the treatment of CRC. The introduction of genetic testing to individualise treatment regimens will hopefully enable a better response prediction and reducing the toxicity induced by drugs (34,35). One strategy to improve the efficacy and reduce toxicity is the identification of subgroups of CRC patients best suited to 5-FU–based chemotherapy allowing.

Table 1. Clinical and anamnestic characteristics significantly affecting OS and PFS of the CRC patients with complete follow-up (Cox regression)

		OS		PFS	
Characteristics	N[a]	HR (95% CI)	*P*	HR (95% CI)	*P*
Sex					
Females	427	Ref		Ref	
Males	656	1.54 (1.23–1.92)	**0.0001**	1.35 (1.09–1.68)	**0.006**
Age (years)					
≤55	293	Ref		Ref	
56–62	248	1.43 (1.05–1.95)	**0.02**	1.41 (1.06–1.87)	**0.02**
63–70	294	1.39 (1.04–1.88)	**0.03**	1.19 (0.90–1.58)	0.22
>70	248	2.02 (1.50–2.72)	**<0.0001**	1.04 (0.76–1.42)	0.80
Smoking habit[b]					
No	533	Ref		Ref	
Yes	493	1.26 (1.02–1.56)	**0.03**	1.14 (0.93–1.41)	0.19
T					
1	50	Ref		Ref	
2	166	2.64 (0.94–7.40)	0.06	2.18 (0.85–5.55)	0.10
3	535	5.84 (2.17–15.71)	**0.0005**	5.58 (2.30–13.53)	**0.0001**
4	136	9.21 (3.36–25.26)	**<0.0001**	6.96 (2.80–17.27)	**<0.0001**
N					
0	498	Ref		Ref	
1	260	2.17 (1.69–2.79)	**<0.0001**	1.87 (1.46–2.41)	**<0.0001**
2	68	3.40 (2.35–4.91)	**<0.0001**	3.43 (2.45–4.81)	**<0.0001**
M					
0	725	Ref		Ref	
1	177	4.80 (3.83–6.02)	**<0.0001**	4.56 (3.68–5.65)	**<0.0001**
5-FU–based chemotherapy					
Yes	411	Ref		Ref	
No	440	1.42 (1.13–1.790)	**0.003**	0.85 (0.68–1.06)	0.14
Stage					
I	149	Ref		Ref	
II	293	2.14 (1.32–3.48)	**0.002**	2.47 (1.51–4.05)	**0.0003**
III	244	3.75 (2.33–6.03)	**<0.0001**	3.87 (2.38–6.31)	**<0.0001**
IV	177	11.87 (7.44–18.95)	**<0.0001**	11.86 (7.42–18.98)	**<0.0001**

Significant results are given in bold.

[a]Numbers may not add up to 100% of available subjects because of missing information.

[b]Ex-smokers included in non-smokers.

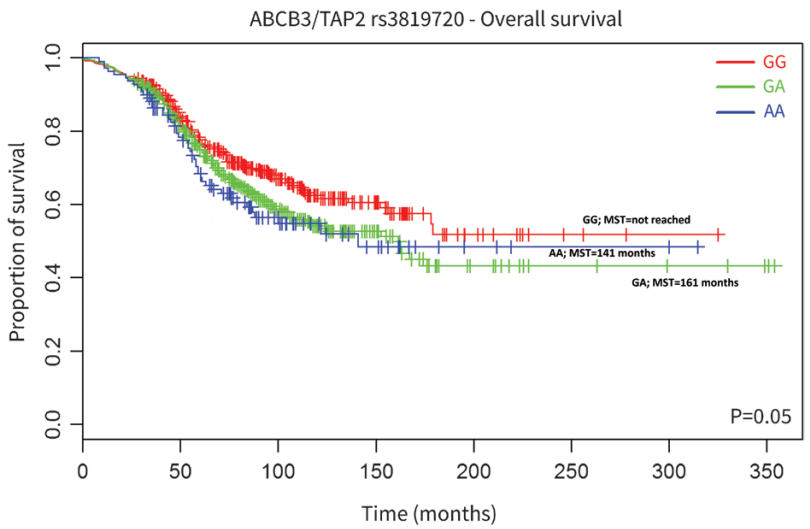

Figure 2. Kaplan–Meier OS curves in CRC patients stratified for rs3819720 in *ABCB3/TAP2* gene.

In the present study, we showed that out of 12 *ABC* gene polymorphisms, selected on the basis of being regulators of a high number of genes downstream, no significant association with the risk of CRC was found for any of the studied polymorphisms. However, *ABCB3/TAP2* rs3819720 was associated with OS in both rectal and colon cancer patients after the treatment with 5-FU. The

Table 2. SNPs associated with OS and PFS in all CRC patients stratified by chemotherapy regimen (Cox regression for adjusted estimates)

		OS					PFS			
Gene dbSNP ID	Genotype	N[a]	Events	Expected	HR[b] (95% CI) [b]	P	Events	Expected	HR[b] (95% CI) [b]	P
					5-FU–based therapy					
ABCA4	CC	347	99	103.6	Ref		135	135.2	Ref	
rs4147864	CT	53	20	15.7	1.67 (1.01–2.77)	**0.04**	20	20.3	0.99 (0.60–1.66)	0.98
	TT	1	1	0.4	6.43 (0.84–49.2)	0.07	1	0.5	4.32 (0.58–32.4)	0.15
	CT + TT	54	21	16.1	1.73 (1.06–2.84)	**0.03**	21	20.8	1.04 (0.63–1.71)	0.88
	CC + CT	400	119	119.3	Ref		155	155.5	Ref	
	TT	1	1	0.4	5.85 (0.77–44.6)	0.09	1	0.5	4.32 (0.58–32.4)	0.15
ABCB3/TAP2	GG	176	39	56.1	Ref		65	71.0	Ref	
rs3819720	GA	176	67	53.5	1.59 (1.05–2.41)	**0.03**	75	68.8	1.24 (0.87–1.78)	0.23
	AA	47	16	12.5	1.63 (0.88–3.00)	0.12	17	18.2	1.08 (0.62–1.85)	0.79
	GA + AA	223	83	66	1.60 (1.07–2.38)	**0.02**	92	87	1.20 (0.86–1.69)	0.28
	GG + GA	352	106	109.6	Ref		140	139.8	Ref	
	AA	47	16	12.5	1.26 (0.72–2.20)	0.41	17	18.2	0.97 (0.58–1.61)	0.89
ABCG1	TT	129	42	36.4	Ref		53	49.1	Ref	
rs4148105	TC	192	53	59.7	0.71 (0.46–1.10)	0.13	80	73.7	1.02 (0.70–1.49)	0.91
	CC	81	26	24.9	0.86 (0.51–1.45)	0.56	25	35.3	0.59 (0.36–0.98)	**0.04**
	TC + CC	273	79	84.6	0.75 (0.50–1.13)	0.17	133	122.8	0.887 (0.61–1.25)	0.45
	TT + TC	321	95	96.1	Ref		133	122.8	Ref	
	CC	81	26	24.9	1.06 (0.67–1.68)	0.80	25	35.3	0.58 (0.37–0.92)	**0.02**
					5-FU + Oxaliplatin–based therapy					
ABCA1	AA	45	12	14.2	Ref		28	23.7	Ref	
rs2254884	AC	28	10	9.0	1.32 (0.44–3.97)	0.61	17	19.2	0.78 (0.40–1.55)	0.48
	CC	5	2	0.9	6.10 (1.11–33.4)	**0.04**	1	3.1	0.36 (0.04–2.80)	0.33
	AC + CC	33	12	9.9	1.70 (0.60–4.78)	0.32	18	22.3	0.73 (0.38–1.43)	0.37
	AA + AC	73	22	23.2	Ref		45	42.9	Ref	
	CC	5	2	0.9	5.57 (1.06–29.3)	**0.04**	1	3.1	0.41 (0.05–3.07)	0.38

Significant results are given in bold.

[a]Numbers may not add up to 100% of available subjects because of missing information.

[b]Adjusted for sex, age, TNM and chemotherapy.

heterozygous and homozygous carriers of the variant allele were at more than 1.5-fold increased risk of death than the non-carriers. Based on cell-line functional studies from SCAN database, rs3819720 SNP has been identified as a *cis* transcription regulator for 36 genes, mainly involved in major histocompatibility complex (MHC) as additionally verified by gene enrichment analysis. In addition, this intronic variant was classified according the Regulome (http://www.regulomedb.org/snp) as variant which might likely affect the binding and might be linked to expression of a gene target. *ABCB3* is known to be involved in antigen presenting by transporting peptides necessary to assemble MHC class I molecules from the cytoplasm into the endoplasmic reticulum (36). The reduced expression of *ABCB3* is associated with HLA class I deficient human tumour cell lines (37) and it has been suggested that it could be related to the aggressive features of CRC tumours (38). We hypothesise that rs3819720 genetic variant may be implicated in the functionality of *ABCB3/TAP2* and the regulation of the expression of a vast number of genes may be one of the reasons for the observed associations with the survival of CRC patients treated with 5-FU. eQTL studies connect variation at DNA level with the quantitative variation in mRNA transcripts, leading to the identification of regulatory regions. To the best of our knowledge, no other studies previously examined these selected SNPs in relation to CRC susceptibility and clinical outcomes after diagnosis.

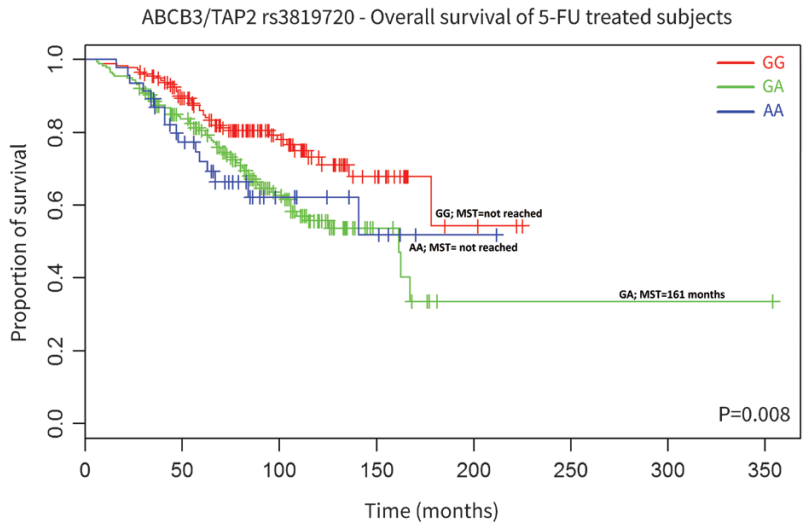

Figure 3. Kaplan–Meier OS curves in CRC patients undergoing 5-FU-based chemotherapy stratified for rs3819720 in *ABCB3/TAP2* gene.

Tumour-specific antigen expression plays an important role in the durability of the antitumor immune response by enhancing the development of antitumor-specific T cells. Recent studies have suggested that tumours with larger numbers of novel antigens tend to be more immunogenic, and thus constitute better targets for immunotherapy. For example, microsatellite-instable (MSI) colon cancers, which have mutations in the mismatch repair genes, generate from 10 to 50 times more neoantigens than microsatellite-stable (MSS) colon cancers (39). This increased antigen expression in colon cancer patients with MSI (vs. MSS) is associated with significantly increased T-cell infiltration and better prognoses (40).

Additionally, some evidence indicate that certain tumours can have reduced expression of MHC I receptors, inhibiting the presentation of tumour-derived antigens (41). Reductions in MHC I expression have been associated with poor prognosis in patients with CRC as well (42). Decreased expression of MHC I receptors and of transporters associated with antigen processing (TAPs), Types 1 and 2, have also been associated with progression of breast cancer (43). Although the loss of MHC I receptors can partially explain the loss of immunogenicity of certain tumours, it cannot fully account for tumour resistance. This may be due to the actions of other oncolytic mechanisms that are independent of MHC I expression, such as oncolysis mediated by NK cells (41).

As stated before, *ABCB3/TAP2* is involved in the development of multidrug resistance and in the aetiology of immunological diseases (44). 5-FU resistance can result from various causes including alteration of drug influx/efflux, enhancement of drug inactivation and mutation of the drug target(s) (45). Kasajima *et al.* (46) showed that the expression of TAP1 and TAP2 protein is tightly linked to the density of tumour-infiltrating lymphocytes (TIL), which are positive prognostic factors in CRC. Lately, it was observed that breast cancer patients with reduced *TAP2* expression showed increased benefit from neoadjuvant chemotherapy (47). This is in agreement with the observation that an upregulation of expression of genes connected with multiple drug resistance is associated with breast cancer patients' resistance to neoadjuvant chemotherapy (44). In addition, with a bacterial model, Lerebours *et al.* (48) demonstrated that the alteration of *TAP2* expression could change the metal accumulation level and survival ratio in *Escherichia coli* cells, indicating the importance of TAP2 in metal detoxification.

Variable gene expression levels among individuals can be analysed like other quantitative phenotypes (9). However, it must be taken into consideration that the observed associations have been detected on lymphoblastoid cell lines, which have demonstrated to be very useful for the interpretation of human disease associations, but at the same time present several limitations due to potential artefacts associated with their immortalisation, subsequent passages and growth conditions before their harvest (49). Until recently, the lack of information available about regulators of expression in association with cancer risk was mainly due to the difficulties in eQTL studies on cell lines other than the lymphoblastoid ones (16). However, with the advantage of next generation sequencing and improvements in bioinformatic approaches these limitations can be overcome. Therefore, the role of eQTL loci needs to be elucidated and elaborated in future studies.

It should be also considered that the majority of the associations reported in the present work were statistically significant only at the nominal *P* value or under a Bonferroni correction for multiple tests that was considering only the minimal number of comparisons. This is a limitation but since it was an exploratory study referring to the potential of eQTLs in specific genes we were considering anyway these results a good starting point for further investigations on larger cohorts and with higher statistical power.

In summary, screening for eQTL polymorphisms in relevant genes that can regulate the expression of many other genes may aid to identify the genetic background involved in the individual response to treatment of CRC patients.

Supplementary data

Supplementary Tables 1–5 are available at *Mutagenesis* Online.

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