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Genetic variants in DNA repair genes as potential predictive markers for oxaliplatin chemotherapy in colorectal cancer

Elisabeth J Kap, MSc.¹, Dr. Petra Seibold¹, Swantje Richter, MSc.¹, Dr. Dominique Scherer², Dr. Nina Habermann², Dr. Yesilda Balavarca², Dr. Lina Jansen³, Dr. Natalia Becker⁴, Dr. Katrin Pfützte^{5,6}, PD Dr. Odilia Popanda⁷, Dr. Michael Hoffmeister³, Prof. Dr. Med. Alexis Ulrich⁸, Dr. Axel Benner⁴, Prof. Dr. Cornelia M Ulrich^{2,9}, Prof. Dr. Barbara Burwinkel^{5,6}, Prof. Dr. Med. Hermann Brenner^{3,10}, and Prof. Dr. Jenny Chang-Claude¹

¹Division of Cancer Epidemiology, German Cancer Research Center (DKFZ), Heidelberg, Germany

²Division of Preventive Oncology, National Center for Tumor Diseases (NCT) and DKFZ, Heidelberg, Germany

³Division of Clinical Epidemiology and Aging Research, DKFZ, Heidelberg, Germany

⁴Division of Biostatistics, DKFZ, Heidelberg, Germany

⁵Division of Molecular Epidemiology, DKFZ, Heidelberg, Germany

⁶Division Molecular Biology of Breast Cancer, Department of Gynecology and Obstetrics, University of Heidelberg, Germany

⁷Division of Epigenomics and Cancer Risk Factors, DKFZ, Heidelberg, Germany

⁸Department of General, Visceral and Transplantation Surgery, University Hospital Heidelberg, University of Heidelberg, Germany

⁹Cancer Prevention Program, Fred Hutchinson Cancer Research Center, Seattle, Washington, United States of America

¹⁰German Cancer Consortium (DKTK), Heidelberg, Germany

Abstract

Oxaliplatin-based chemotherapy exerts its effects through generating DNA damage. Hence, genetic variants in DNA repair pathways could modulate treatment response. We used a prospective cohort of 623 CRC patients with stage II–IV disease treated with adjuvant/palliative chemotherapy to comprehensively investigate 1 727 genetic variants in the DNA repair pathways as potential predictive markers for oxaliplatin treatment. Single SNP associations with overall survival and recurrence free survival were assessed using a Cox regression model. Pathway analysis was performed using the gamma method. Patients carrying variant alleles of rs3783819

Correspondence to Prof. Dr. Jenny Chang-Claude, Division of Cancer Epidemiology, German Cancer Research Center (DKFZ), Im Neuenheimer Feld 581 D-69120 Heidelberg, Germany, Phone +49-6221-422373; Fax. +49-6221-422203; j.chang-claude@dkfz-heidelberg.de.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

(*MNAT1*) and rs1043953 (*XPC*) experienced a longer overall survival after treatment with oxaliplatin than patients who did not carry the variant allele while the opposite association was found in patients who were not treated with oxaliplatin (FDR adjusted p-values for heterogeneity 0.0047 and 0.0237, respectively). The nucleotide excision repair (NER) pathway was found to be most likely associated with overall survival in patients who received oxaliplatin (p-value 0.002). Our data show that genetic variants in the *NER* pathway are potentially predictive of treatment response to oxaliplatin.

Keywords

Colorectal cancer; DNA repair; nucleotide excision repair; *XPC*; *MNAT1*; oxaliplatin

INTRODUCTION

Colorectal cancer (CRC) is the fourth most common cancer in western countries, with around 65000 new diagnoses per year and a 5-year relative survival of 63% in Germany^{1,2}. The use of oxaliplatin in the treatment of stage III and IV CRC has been recommended in the German guideline for colorectal cancer treatment since 2004³. The current guideline also recommends the use of adjuvant chemotherapy in high risk stage II patients. Although the objective response rate of the addition of oxaliplatin to 5-fluorouracil (FOLFOX) is estimated to increase to ca. 50%, not all CRC patients benefit from oxaliplatin treatment and a substantial number of patients experience gastrointestinal, hematological and neurological adverse effects⁴⁻⁷. Currently, there are no markers used in clinical practice to identify patients who are likely to benefit from oxaliplatin treatment.

Oxaliplatin exerts its effects by binding to DNA and causing inter- and intra- strand crosslinks and bulky adducts. These DNA modifications will disrupt cellular replication, causing the cell to undergo apoptosis^{8,9}. Genetic variants in different biological pathways involved with DNA damage repair, drug transport, metabolism, and cell cycle regulation have been proposed as predictive markers^{10,11}. Genetic variants could reduce the functional activity of a DNA repair pathway for repairing DNA damage caused by oxaliplatin. This would lead to an increased number of DNA modifications and increased efficacy of the drug. Six different DNA repair pathways exist¹²⁻¹⁴ whereby mainly the nucleotide excision repair (NER) pathway repairs the DNA damage caused by oxaliplatin¹⁵. The nucleotide excision repair (NER) pathway is responsible for removing bulky DNA lesions and involves the incision of multiple nucleotides. Genetic variants in multiple DNA repair genes have been investigated in association with FOLFOX chemotherapy in epidemiological and in vitro studies, yielding conflicting results^{9,10,16-18}. However, these studies considered only a small proportion of genes involved in DNA repair. Therefore, we carried out a comprehensive investigation of the DNA repair pathway to identify potential predictive markers for oxaliplatin treatment in a prospective cohort of CRC patients treated with adjuvant/palliative chemotherapy.

PATIENTS AND METHODS

Study population

Our study population comprised CRC patients recruited for the DACHS (Darmkrebs: Chancen der Verhütung durch Screening) study^{19,20}, an ongoing population-based case-control study with additional long-term follow-up of colorectal cancer patients. Inclusion criteria for the DACHS study are: resident of the Rhein-Neckar-Odenwald region in Germany, ages 30 years or older at diagnosis, German speaking and the physical and mental ability to participate. For a total of 1 749 histological confirmed cases diagnosed between 2003 and 2007, both genotype and complete follow-up data until 31.12.2010 (for either 3 or 5 years) were available. The study was approved by the ethics committees of the Medical Faculty of the University of Heidelberg and the State Medical Boards of Baden-Wuerttemberg and Rhineland-Palatinate. All patients gave their written informed consent.

At baseline, trained interviewers collected socio-demographic, medical and lifestyle data via a standardized questionnaire. Furthermore, participants provided a blood sample (>99% of the analyzed patients) or a mouthwash at recruitment. At three and five years of follow-up, information on individual patient therapy was collected from the treating physician. Vital status, date and cause of death were collected through the local population registries.

Nearly all the patients received 5-FU or capecitabine and only a minority of the patients received in addition irinotecan, bevacizumab or cetuximab. Of those patients who received oxaliplatin almost 90% received oxaliplatin as first-line treatment. Less than 20% of all patients received second-line treatment. As shown in Figure 1, we excluded patients who did not receive adjuvant chemotherapy or received both adjuvant and neo-adjuvant chemotherapy, patients with unknown start date of chemotherapy or who died within 30 days of the start of chemotherapy. We defined patients receiving oxaliplatin as those who received at least four cycles. For this analysis, 623 CRC patients (Stage II–IV) were available of whom 201 received oxaliplatin.

Genotyping

DNA was extracted from EDTA blood and mouthwash samples using the Flexigene DNA kit (Qiagen GmbH, Hilden, Germany) and quantified using Quanti-iT PicoGreen dsDNA Reagent and Kit (Invitrogen life technologies). Tagging SNPs (tagSNPs) were selected to represent genetic variation across the DNA repair pathways. Genes were selected using the KEGG database and the DNA repair gene table by Wood et al.¹² (table available on http://sciencepark.mdanderson.org/labs/wood/dna_repair_genes.html). Haploview 4.2 was used for selection of tagSNPs, with a pairwise tagging approach based on the reference data from the HapMap project (CEU population, Phase II/Release 24) using $r^2 \geq 0.8$ as cutoff and excluding SNPs with a minor allele frequency below 0.05. Genotyping of the 688 SNPs was performed on a Illumina GoldenGate assay²¹. Nine SNPs that failed Illumina Golden Gate genotyping were genotyped using the iPLEX Assay for the MassArray system from SEQUENOM²².

SNPs with a success rate below 95% or poor quality of the cluster plot and samples with a call rate below 96% were excluded. Quality assurance also comprised 156 internal duplicate

samples (concordance >99.7%) and 74 external CEPH control samples (concordance >98%). SNPs with a MAF <0.01 or a deviation from Hardy-Weinberg equilibrium (HWE) in additionally genotyped controls ($p < 0.001$) were also excluded, after which 654 SNPs in the DNA repair pathways from the Illumina GoldenGate assay were available for analysis.

Additional genotype data were available from the whole-genome Illumina CytoSNP v12.2.1 assay (> 299,000 SNPs) performed in collaboration with the Genetics and Epidemiology of Colorectal Cancer Consortium (GECCO)²³. Quality control for GECCO included exclusion based on call rate (< 98%), lack of HWE in controls and low MAF. Using the aforementioned criteria, 1 073 additional SNPs from 109 DNA repair genes as well as 52 additional genes were selected. Supplementary table 1 contains a full list of all 1 727 SNPs that were analyzed.

Imputation

In the DACHS study, missing genotype values for autosomal SNPs were imputed with IMPUTE2 using the 1,000 genome data as reference panel²⁴. In GECCO, missing genotype values for autosomal SNPs were imputed using MACH and the CEU population in HapMap II as reference panel²⁵. We also used IMPUTE2 and the 1 000 genome reference panel to impute additional SNPs from selected genes, which showed an association with overall survival in the main analysis to obtain more genetic information for the entire gene region²⁴.

Statistical analysis

The Chi-square goodness-of-fit test with one degree of freedom was used to check the genotype distribution for deviations from the HWE. The chi-square test was used to compare the frequency of the lifestyle factors between patients treated with oxaliplatin and patients not treated with oxaliplatin. All tests were two-sided with $\alpha = 0.05$. The associations between the genetic polymorphisms and overall survival (OS) were analyzed using Cox regression models, accounting for late entry. Survival time was defined as time from start of chemotherapy to date of death (by any cause) or date of last contact for the OS analysis or date of recurrence, death (by any cause) or last contact for the RFS analysis. Median follow-up was computed using the reverse Kaplan-Meier method²⁶. Age (in 10 year categories), sex and UICC stage were included into the model as relevant prognostic factors. Additional covariates were determined using backward selection of a set of variables including grade (1, 2 vs 3, 4), first-degree family history, smoking (never, former, current), BMI (18.5–25, 25–30, 30+), alcohol intake (0 and quartiles in subjects with alcohol intake >0g/day) and physical activity (median split). The variables grade, BMI and current alcohol intake, which were significantly associated with OS, were retained in the final model. Single SNP associations were assessed using the additive genetic model, whereby homozygous carriers of the common allele were taken as reference category. The proportional hazards assumption was tested according to Grambsch and Therneau²⁷.

Possible differential association of the SNP with OS in the patient groups with and without oxaliplatin treatment was assessed statistically using interaction terms between oxaliplatin treatment (yes, no) and the SNPs and evaluated by the log-likelihood ratio test. We adjusted for multiple testing by using the False Discovery Rate (FDR) correction according to

Benjamini and Hochberg, whereby an adjusted p-value of 0.05 was considered significant²⁸. We performed analyses separately for the CRC patients who received oxaliplatin and those who did not receive oxaliplatin treatment to quantify the SNP association with OS in the two groups. Kaplan-Meier curves were created to illustrate the different associations by treatment group. In addition, we performed subgroup analyses in the colon cancer patients with stage IV disease at diagnosis to allow for possible heterogeneity by UICC stage. For an exploratory analysis of the potential clinical use of multiple plausible predictive genetic markers, we assessed combined effects of all genes that showed a differential association by oxaliplatin treatment (nominal p-value <0.01). Since none of the genes yielded multiple independent associations we took one SNP per gene and constructed a polygenic score (PS) with the number of beneficial alleles per patient. We evaluated the predictive abilities of the associated SNPs by calculating the concordance probability estimate (CPE)²⁹ and evaluated the validity by calculating the model R^2 ³⁰. We reported the mean values and 95% CIs that were obtained from 1,000 bootstrap samples.

We employed the gamma method proposed by Biernacka et al.³¹ to evaluate the association of the different DNA repair pathways. Empirical p-values were obtained by performing 1000 permutations. The same variables included in the main analysis were included in this model. The statistical analysis was carried out using SAS 9.2 software (SAS Institute, NC, USA) and R version 2.15.2 (www.R-project.org).

RESULTS

The median age of all 623 patients was 67 (IQR 60–73) years and 38.8% of patients were female (Table 1). The median follow-up time was 58.5 months for the patients who received oxaliplatin and 60.0 months for the patients who did not receive oxaliplatin. Patients treated with oxaliplatin had a higher tumor stage (p-value <0.0001) and were younger (p-value = 0.0004) compared to patients who did not receive oxaliplatin.

For 32 SNPs in 15 different genes, which showed effect heterogeneity by oxaliplatin treatment (unadjusted p-value for interaction <0.01), the associated hazard ratios (HR) for OS according to chemotherapy with oxaliplatin are presented in Table 2. Four interactions remained significant after correction for multiple comparisons and included 3 SNPs in *MNAT1* and one SNP in *XPC*. Compared to non-carriers, carrying each additional variant allele of rs3783819 (*MNAT1*) was associated with a decreased risk of dying in patients who received oxaliplatin (HR 0.51, 95% confidence interval (CI) 0.36–0.73) but not in patients who did not receive oxaliplatin (FDR p-value <0.005). The same direction and magnitude of association were found for rs973063 and rs4151330 (both *MNAT1*), which are in high linkage disequilibrium (LD) with rs3783819 and each other (Supplementary Figure S1a). The number of variant alleles in rs1043953 (*XPC*) was also associated with a decreased HR in patients who received oxaliplatin (HR 0.45 (95% CI 0.29–0.70)), but not in patients who were not treated with oxaliplatin (FDR p=0.02). An association in the opposite direction was found for another *XPC* SNP, rs3731108 (HR 1.40 (95% CI 1.01– 1.94), in low LD with rs1043953 $R^2=0.07$ (Table 2). Figure 2 shows the Kaplan-Meier survival curves according to genotype for rs3783819 (*MNAT1*) and rs1043953 (*XPC*), visualizing the differing associations in patients who received chemotherapy with oxaliplatin and without. The SNPs

within *MNAT1* and within *XPC* are in high LD (Supplementary Figures S1a and S1b). Therefore, independently associated SNPs were not identified in the joint analysis of marginally significant SNPs within each gene.

The subgroup analysis restricted to only colon cancer patients with stage IV disease yielded similar results to that in the total population (Table 3). We also tested for heterogeneity of association by tumor stage in colon cancer patients and found none (data not shown). In addition, we assessed the association of the top 4 SNPs in relation with recurrence free survival (RFS). Our results confirm the magnitude of association in the subgroup by treatment, however the interaction was not significant (p-value for interaction between 0.09–0.35). In patients who received oxaliplatin, the associated HRs for rs3783819, rs4151330, rs973063, and rs1043953 were 0.86 (0.63–1.19), 0.83 (0.60–1.14), 0.79(0.58–1.09) and HR 0.65 (0.43–0.98), respectively. The corresponding HRs in patients who did not receive oxaliplatin were 1.38 (1.09–1.74), 1.34 (1.07–1.69), 1.35(0.07–1.70) and HR 1.28 (0.98–1.66), respectively.

We constructed a polygenic score using 15 SNPs that showed heterogeneity by oxaliplatin treatment (unadjusted $p < 0.01$, see table 2). The multivariate model including the polygenic score and the interaction with oxaliplatin treatment had a high discriminative power, with a CPE of 0.76 (95% CI 0.74–0.79) and explained 55% (95% CI 54%–57%) of the variability in OS. Compared to the model containing only the adjustment variables (CPE: 0.72 95% CI 0.70–0.75), this was an increase in CPE of 0.04 and 13% increase in R^2 . Nearly half of this increase in validity and discriminant ability could be achieved by taking rs3783819 and rs1043953 (CPE: 0.75 95% CI (0.72–0.77), $R^2=48\%$). This model improvement was solely due to the inclusion of the interaction term. The pathway analysis using the gamma method yielded only the NER pathway to be significantly associated with OS in CRC patients treated with oxaliplatin (empirical p-value 0.002) (Table 4).

To identify potential functional candidates in *MNAT1* and *XPC*, we imputed the gene regions and additional 50 kb around the gene using the 1 000 genome reference data. In total, 4 571 sites were imputed. Using the information metric provided by the IMPUTE2 software we excluded 4 003 SNPs that were imputed with low certainty (info metric < 0.3)³². Furthermore, non-bi-allelic SNPs, SNPs with a minor allele frequency (MAF) < 0.05 and SNPs that deviated from HWE were excluded leaving 220 SNPs available for analysis. Six SNPs from *MNAT1* showed an interaction with oxaliplatin treatment (p-value < 0.001), with SNPs rs7142844 and rs17256107 showing the strongest association with decreased OS in CRC patients who received oxaliplatin (HR 0.48, 95% CI 0.34–0.68) (Supplementary Table S2). Of twenty SNPs from *XPC* showing heterogeneity by oxaliplatin treatment (p-value < 0.001), nineteen were in high LD with each other and similarly associated with reduced OS in the patients treated with oxaliplatin as the genotyped SNP rs1043953. One SNP, rs6442429, was associated with increased OS (HR 1.60 (1.14–2.25)) and is in high LD ($r^2=0.72$) with the genotyped SNP rs3731108 (Supplementary Figures S2a and S2b).

DISCUSSION

We found evidence that genetic variants in *MNAT1* and *XPC* could be used as predictive markers for oxaliplatin treatment in CRC patients. In comparison with non-carriers, carrying the variant allele of rs3783819 (*MNAT1*) or rs1043953 (*XPC*) was associated with a significantly improved OS in CRC patients after treatment with oxaliplatin but not in patients not treated with oxaliplatin (FDR adjusted p-values for heterogeneity 0.0047 and 0.0237). This suggests that patients with altered DNA repair capacity experience greater benefit from treatment by oxaliplatin, which is consistent with its anti-tumor effect.

The MNAT1 protein is part of the CDK activating kinase (CAK) complex³³. Together with XPB, XPD and GTF2H family, CAK forms the transcription factor II H complex (TFIIH). TFIIH opens the DNA helix for incision during NER³⁴. TFIIH is also involved in transcription, cell cycle regulation and it activates estrogen receptor α ^{35–37}. There are no previous studies on *MNAT1* variants and CRC prognosis. However, Wu et al. (2009) found that *MNAT1* rs12888332 was associated with a higher risk of recurrence of head and neck cancer³⁸. Genetic variants in *MNAT1* have otherwise been investigated in relation to cancer risk, with reports of different SNPs being associated with nasopharyngeal cancer and prostate cancer^{39,40}, but not with CRC, cervical cancer and pancreatic cancer^{41–43} and inconsistent findings for lung cancer risk^{44,45}.

The three significantly predictive SNPs, rs3783819, rs973063 and rs4151330, and the six additional imputed SNPs, are located in the introns of *MNAT1*. RNA seq data from the Cancer Genome Atlas (TCGA) project was downloaded from the TCGA data portal (<https://tcga-data.nci.nih.gov/tcga/>) and used to investigate differences in gene expression between normal and tumor colon tissue. Matched tumor and normal tissue data were available for 14 pairs showing a significantly higher expression of *MNAT1* in tumor tissue. However, no significant association between *MNAT1* expression and OS in patients treated with chemotherapy was found. Based on the Genotype-Tissue expression (GTEx) project portal rs3783819 was not associated with differences in gene expression in colon tissue or in whole blood⁴⁶. We used the UCSC genome browser to investigate if any of the significantly associated SNPs are located in known regulatory regions and used Targetscan (6.2) to predict miRNA targets in the gene region⁴⁷. The SNPs rs2020892 and rs973063 are in a region with a high level of H3K4me1 marks (indicating an enhancer region) whereas the independently associated SNPs, rs72722276 and rs4151330, are located at a transcription factor binding site (Supplementary Figure S3a).

When analyzing all nine *MNAT1* SNPs simultaneously, rs2020892, rs1952401 and rs4151330 remained significantly associated with OS in CRC patients treated with oxaliplatin. Rs2020892 and rs1952401 are in high LD with each other, but not with rs4151330 (Supplementary Figures S2a). Therefore, there might be two independent variants in *MNAT1* associated OS after oxaliplatin treatment. Although genetic variants in *MNAT1* have not been studied extensively, the role of MNAT1 in the NER pathway and our results suggest that these genetic variants have predictive potential for oxaliplatin treatment.

XPC has been investigated previously since gene mutations can lead to the autosomal disorder Xeroderma Pigmentosum⁴⁸, a disease characterized by increased sensitivity to sunlight and therefore a higher risk of developing skin cancer. *XPC* can recognize DNA damage that can be repaired by NER and recruits the suitable proteins to the damaged site, including TFIIH⁴⁹. Two studies investigated rs2228001, another missense variant in *XPC*, and CRC survival after treatment with oxaliplatin and found no significant associations in 343 and 432 CRC patients from all pathological stages^{50,51}. We also found no association with rs2228001, which is in high LD with rs2607734.

Rs1043953, which showed a significant interaction with oxaliplatin treatment, is located approximately 5,000 base pairs from *XPC* in the 3' UTR region of *TMEM43* and is in LD with multiple genetic variants in *XPC*. *TMEM43* is a transmembrane protein of which the function is not yet clear. Mutations in this gene have not been associated with cancer. Rs1043953 is in high LD with rs2228000, a missense SNP located in *XPC*. Only one of the associated imputed SNPs, rs67353494, which is in high LD with the genotyped SNPs rs1043953 and rs2228000, is also a missense variant but located upstream of *XPC*. The other variants are all located in the 3' UTR or intron regions of *XPC* or *TMEM43*. The TCGA data showed no significant difference between *XPC* expression in normal and colon tissue. A higher *XPC* expression was non-significantly associated with a lower survival in patients treated with oxaliplatin, while the opposite association was found in those who did not receive oxaliplatin. Using the GTEx portal we found that rs1043953 was associated with reduced *XPC* expression in whole blood (p-value 0.004), but not in colon tissue. Rs2470458 is located at a predicted miRNA target (miRNA 3124-3p). Rs1043953 and six imputed SNPs (rs13063, rs8516, rs2733531, rs2470458, rs67353494 and rs10468) are in a highly transcribed region with multiple regulatory marks, such as transcription factor binding sites, DNase I hypersensitivity clusters and histone modifications (Supplementary Figures S3b). Confirmation of this finding and functional studies would be necessary to clearly identify the implicated SNPs.

Previous investigations mainly focused on *XRCC1*, *ERCC1* and *ERCC2*. We did not observe significant interactions between SNPs in these genes and oxaliplatin treatment. A meta-analysis from 2011 on rs11615 (*ERCC1*) and rs13181 (*ERCC2*) showed consistent associations with reduced OS in patients treated with oxaliplatin⁵². However, most of these studies have investigated the SNP association solely in patients receiving oxaliplatin and were therefore not able to formally test for interaction. Thus, these studies did not rule out the possibility that the same associations existed in patients who did not receive oxaliplatin. Furthermore, sample sizes in most previous studies were relatively small.

Our results are based on a large population-based prospective patient cohort that received standard care. Using the detailed patient and treatment information, we were able to account for differences in patient characteristics as possible confounding factors in addition to adjustment for standard prognostic factors. The assessment of the DNA repair genes was comprehensive and the 1 000 genome data was used to impute additional SNPs in the associated genes. To identify predictive factors, we formally tested for possible differential effects by oxaliplatin treatment. We also evaluated possible differences by stage of disease and tested for heterogeneity (stage 2–3 vs. stage 4), but found no evidence for differential

association. We also demonstrated by using pathway analysis that - as hypothesized - the NER pathway was associated with OS in patients receiving oxaliplatin. Finally, we showed that the models including the two most significant predictive genetic variants already had a high discriminative power, but this could be improved by including more genetic variants.

Although our sample size was relatively large, we were unable to investigate rare variants and could not adjust for or stratify by all known prognostic markers.

In conclusion, our data show that variants in *XPC* and *MNAT1* have potential as predictive markers for oxaliplatin treatment. Replication in large independent patient cohorts is necessary to confirm these findings. Although we showed that potential predictive markers are most likely to be involved in the NER pathway, discriminative power was improved when accounting for multiple associated variants in several DNA repair pathways. Therefore, a clinically relevant genetic predictive profile for oxaliplatin may require consideration of genetic variation in further pathways.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Inclusion of cases CRC patients from the DACHS study.

A) Rs3783819 **B) Rs1043953** **Figure 2.**

Kaplan – Meier curves for rs3783819 (*MNAT1*) and rs1043953 (*XPC*).

Table 1

Descriptive characteristic of 623 CRC patients which received adjuvant/palliative chemotherapy.

Characteristic	All patients on chemotherapy (CT) (N=623)	Patients with oxaliplatin-based CT (N=201)	Patients with non-oxaliplatin-based CT (N=422)
Sex			
Female	242 (38.8)	78 (38.8)	164 (38.9)
Male	381 (61.2)	123 (61.2)	258 (61.1)
Age (years)			
<60	147 (23.6)	65 (32.3)	82 (19.4)
60 to <70	241 (38.7)	81 (40.3)	160 (37.9)
70 to <80	183 (29.4)	44 (21.9)	139 (32.9)
80	52 (8.4)	11 (5.5)	41 (9.7)
Median ¹	67 (60–73)	68 (61–75)	64 (57–70)
UICC stage			
Stage II	99 (15.9)	14 (7.0)	85 (20.1)
Stage III	360 (57.8)	116 (57.7)	244 (57.8)
Stage IV	164 (26.3)	71 (35.3)	93 (22.1)
CRC site			
colon	386 (62.0)	149 (74.1)	237 (56.2)
rectum/rectosigmoid	237 (38.0)	52 (25.9)	185 (43.8)
Recurrence	279 (44.8)	99 (49.3)	180 (42.7)
BMI[^]			
Normal weight	238 (38.2)	85 (42.3)	153 (36.3)
Overweight	262 (42.1)	82 (40.8)	180 (42.7)
Obese	123 (19.7)	34 (16.9)	89 (21.1)
Grade			
G1+G2	396 (63.6)	278 (65.9)	118 (58.7)
G3+G4	227 (36.4)	144 (34.1)	83 (41.3)
Current alcohol intake²			
0 g/day	170 (27.3)	49 (24.4)	121 (28.7)
0.1–6.1 g/day	107 (17.2)	34 (16.9)	73 (17.3)
6.1–15.6 g/day	118 (18.9)	39 (19.4)	79 (18.7)
15.6–32.6 g/day	131 (21.0)	46 (22.9)	85 (20.1)
>32.6 g/day	97 (15.6)	33 (16.4)	64 (15.2)

¹ Presented as median (Interquartile Range)² At time of diagnosis

* Presented as median (IQR)

Associations between polymorphisms in DNA repair genes and overall survival in 623 colorectal cancer patients who received adjuvant chemotherapy according to chemotherapy with oxaliplatin ($p < 0.01$).

Table 2

SNP	gene	Pathway	Patients with oxaliplatin-based CT			Patients with non-oxaliplatin-based CT		
			Cases/Deaths	Hazard Ratio	p	Cases/Deaths	Hazard Ratio	p
rs3783819*	MINAT1	NER	192/89	0.51 (0.36–0.73)	0.0002	401/158	1.36 (1.08–1.71)	0.0080
rs973063	MINAT1	NER	201/96	0.52 (0.37–0.72)	0.0001	418/167	1.29 (1.03–1.62)	0.0248
rs4151330	MINAT1	NER	201/96	0.53 (0.38–0.75)	0.0002	418/167	1.26 (1.00–1.57)	0.0489
rs2020892	MINAT1	NER	192/89	1.53 (1.11–2.11)	0.0100	401/158	0.75 (0.59–0.97)	0.0283
rs12889598	MINAT1	NER	201/96	0.68 (0.47–0.98)	0.0396	418/167	1.23 (0.94–1.60)	0.1266
rs4151351	MINAT1	NER	192/89	0.72 (0.49–1.04)	0.0773	401/158	1.27 (0.97–1.66)	0.0892
rs1043953*	XPC	NER	201/96	0.45 (0.29–0.70)	0.0004	418/167	1.34 (1.04–1.74)	0.0253
rs2228000	XPC	NER	201/96	0.46 (0.29–0.71)	0.0005	418/167	1.22 (0.95–1.56)	0.1270
rs3731143	XPC	NER	201/96	0.43 (0.20–0.90)	0.0246	418/167	1.66 (1.08–2.55)	0.0209
rs3731108	XPC	NER	201/96	1.40 (1.01–1.94)	0.0407	418/167	0.77 (0.59–1.00)	0.0503
rs4781555*	ERCC4	NER	192/89	1.45 (1.01–2.09)	0.0454	401/158	0.72 (0.53–0.99)	0.0396
rs12214686*	CDKN1A	BER	201/96	0.72 (0.47–1.12)	0.1455	418/167	1.77 (1.30–2.42)	0.0003
rs1043180*	NEIL2	BER	201/96	0.34 (0.17–0.69)	0.0028	418/167	1.06 (0.80–1.41)	0.6714
rs1059262*	UNG	BER	201/96	1.62 (1.07–2.44)	0.0216	418/167	0.79 (0.58–1.08)	0.1410
rs3852507*	MGMT	Direct repair	201/96	0.64 (0.46–0.90)	0.0110	418/167	1.16 (0.92–1.46)	0.2031
rs574831	MGMT	Direct repair	192/89	0.67 (0.47–0.94)	0.0212	401/158	1.20 (0.94–1.52)	0.1403
rs4750759	MGMT	Direct repair	192/89	1.41 (1.00–1.98)	0.0518	401/158	0.80 (0.63–1.02)	0.0723
rs553371	MGMT	Direct repair	192/89	1.41 (1.00–1.98)	0.0518	401/158	0.81 (0.64–1.03)	0.0809
rs7116503*	ALKBH3	Direct repair	192/89	0.45 (0.24–0.84)	0.0121	401/158	1.30 (0.94–1.81)	0.1161
rs12477063	BARD1	DSBR	192/89	0.56 (0.39–0.80)	0.0015	401/158	1.05 (0.84–1.31)	0.6778
rs6757091	BARD1	DSBR	201/96	0.57 (0.40–0.81)	0.0016	418/167	1.00 (0.80–1.24)	0.9923
rs3768708*	BARD1	DSBR	192/89	0.56 (0.36–0.87)	0.0096	401/158	1.03 (0.79–1.34)	0.8246
rs10234749*	XRCC2	DSBR	192/89	1.55 (1.08–2.24)	0.0183	401/158	0.82 (0.62–1.09)	0.1748
rs11960003*	XRCC4	DSBR	192/89	0.20 (0.06–0.69)	0.0109	401/158	1.18 (0.75–1.86)	0.4718

SNP	gene	Pathway	Patients with oxaliplatin-based CT			Patients with non-oxaliplatin-based CT			p	fdr_p
			Cases/Deaths	Hazard Ratio	p	Cases/Deaths	Hazard Ratio	p		
rs6734662*	XRCC5	DSBR	201/96	0.46 (0.27–0.79)	0.0046	418/167	1.16 (0.85–1.57)	0.3566	0.497	0.497
rs17036952*	MSH6	MMR	192/89	0.58 (0.28–1.21)	0.1438	401/158	1.77 (1.09–2.85)	0.0201	0.402	0.402
rs12318289*	RECQL	MMR	201/96	0.70 (0.51–0.96)	0.0282	418/167	1.18 (0.95–1.47)	0.1304	0.402	0.402
rs7324651	RFC3	MMR	192/89	0.63 (0.46–0.87)	0.0056	401/158	1.23 (0.96–1.57)	0.0993	0.374	0.374
rs9597711	RFC3	MMR	192/89	0.37 (0.17–0.82)	0.0135	401/158	1.10 (0.77–1.56)	0.5946	0.420	0.420
rs9540133*	RFC3	MMR	192/89	1.65 (1.15–2.35)	0.0065	401/158	0.79 (0.60–1.03)	0.0799	0.497	0.497
rs7323331	RFC3	MMR	192/89	1.47 (1.01–2.13)	0.0421	401/158	0.85 (0.65–1.12)	0.2530	0.497	0.497
rs1359388	RFC3	MMR	192/89	1.52 (1.06–2.18)	0.0233	401/158	0.80 (0.62–1.03)	0.0853	0.497	0.497

* SNPs that were used to create polygenic score

Presented as hazard ratio (95% Confidence Interval)

^ Analysis were adjusted for Age (in 10 year categories), sex, UICC stage, BMI (18.5–25, 25–30, 30+), alcohol intake (0 and quartiles in subjects with alcohol intake >0g/day)

Table 3

Significant association of polymorphisms in DNA repair genes with overall survival in stage IV colon cancer patients who received adjuvant chemotherapy according to chemotherapy with oxaliplatin.

SNP	gene	Pathway	Patients with oxaliplatin-based CT			Patients with non-oxaliplatin-based CT		
			Cases/Deaths	Hazard Ratio	p	Cases/Deaths	Hazard Ratio	p
rs3783819	MNAT1	NER	45/40	0.46 (0.25–0.86)	0.0143	54/45	2.05 (1.13–3.71)	0.0182
rs4151330	MNAT1	NER	48/43	0.50 (0.28–0.89)	0.0193	56/46	1.59 (0.98–2.59)	0.0609
rs973063	MNAT1	NER	48/43	0.51 (0.28–0.92)	0.0262	56/46	1.78 (1.00–3.20)	0.0518
rs1043953	XPC	NER	48/43	0.24 (0.09–0.63)	0.0037	56/46	2.91 (1.44–5.90)	0.0029

* presented as hazard ratio (95% Confidence Interval)

^ Analysis were adjusted for Age (in 10 year categories), sex, UICC stage, BMI (18.5–25, 25–30, 30+), alcohol intake (0 and quartiles in subjects with alcohol intake >0g/day)

Table 4

Empirical p-values obtained after 1000 permutations using the gamma method for all patients who received adjuvant/palliative chemotherapy and by oxaliplatin treatment

	All patients on CT	Patients with oxaliplatin-based CT	Patients with non-oxaliplatin-based CT
BER	0.69700	0.17600	0.91300
Direct repair	0.39700	0.38600	0.22300
DSBR	0.01300	0.10000	0.01700
Fanconi	0.89300	0.33600	0.75600
MMR	0.64600	0.21700	0.87500
NER	0.08900	0.00200	0.29400
Related	0.79400	0.90900	0.55800

[^] Analysis were adjusted for Age (in 10 year categories), sex, UICC stage, BMI (18.5–25, 25–30, 30+), alcohol intake (0 and quartiles in subjects with alcohol intake >0g/day)