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Mutation of the cyclooxygenase 2 gene promoter and anastomotic leakage in colorectal cancer patients: retrospective cohort study

Oskar Grahn[1,*], Klas Holmgren[1], Mun-Gwan Hong[2], Malin Sund[1,3] and Martin Rutegård[1,4]

[1]Department of Surgical and Perioperative Sciences, Surgery, Umeå University, Umeå, Sweden
[2]Department of Biochemistry and Biophysics, National Bioinformatics Infrastructure Sweden, Science for Life Laboratory, Stockholm University, Solna, Sweden
[3]Department of Surgery, University of Helsinki and Helsinki University Hospital, Helsinki, Finland
[4]Wallenberg Centre for Molecular Medicine, Umeå University, Umeå, Sweden

Correspondence to: Oskar Grahn, Department of Surgical and Perioperative Sciences, Surgery, Umeå University, Umeå SE-901 85, Sweden (e-mail: oskar.grahn@umu.se)

Introduction

Anastomotic leakage after surgery for colorectal cancer is a serious complication, causing an increased morbidity rate and mortality rate[1,2].

There is a debate and conflicting evidence on whether non-steroidal anti-inflammatory drugs (NSAIDs) increase the risk of leak[3,4]. NSAIDs act by inhibiting cyclooxygenase (COX) enzymes, which can be subdivided into isoenzymes COX-1 and COX-2. In a seminal study by Reisinger *et al.*[5], knocking out the COX-2 gene resulted in an increase of colonic anastomotic leaks in mice. In a complementary cohort of colorectal cancer patients[5], an increased frequency of anastomotic leaks was demonstrated among those homozygous for the COX-2 gene promoter mutation −765G > C (also known as rs20417). This finding could potentially be translated into clinical use following external validation.

Biological effects might not only be present among those homozygous for the minor allele of −765C/C. For example, the heterozygous state of −765G/C has been associated with a decreased postoperative inflammatory response[6].

The present study aimed to evaluate the prevalence of the polymorphism 765G > C in a Swedish cohort of colorectal cancer patients, and its association with postoperative peritoneal infection.

Methods

This is a retrospective matched case-control study on colorectal cancer patients treated with resection with a curative intent and a primary anastomosis between 1 January 2010 and 31 December 2015, at the Uppsala or Umeå University Hospitals. The regional ethical review board at Umeå University and the national ethical review board provided ethical approval (dnr 2015/425-31, dnr 2019-05594 and dnr 2021-02568). The included patients consented at the time they agreed to give samples to the involved biobank. Those with disseminated disease before surgery were excluded. Demographic and clinical data including complications within 30 days were extracted from the Swedish ColoRectal Cancer Registry (SCRCR)[7]. The study compared patients with peritoneal infection (anastomotic leak and/or intra-abdominal abscess within 30 days) with controls who had a complication-free postoperative course. Case and control status were verified by chart review. Cases and controls were matched 1:1 according to: operating hospital (Uppsala or Umeå), tumour location (colon or rectum), pathological tumour stage (pTNM I, II or III), age (±5 years at the index operation) and sex (male/female).

Preoperative whole-blood samples were requested from the Uppsala-Umeå Cancer Consortium biobank (U-CAN)[8]. These samples were sent to the National Genomics Infrastructure Science for Life Laboratory, Uppsala, Sweden, for genotyping using the Illumina Infinium assay and GenomeStudio 2.0.3.

Frequencies of the allele compositions of the COX-2 gene promoter −765G > C were tabulated. The association between allelic combinations and peritoneal infection was evaluated with logistic regression for additive effects. Quality control and statistical analysis were performed with Plink v.1.9[9].

A protein quantitative trait loci analysis (pQTL) was also performed, including 266 proteins and 547 294 variants (see Supplementary Methods).

Results

Forty-seven patients with peritoneal infection were matched with 47 controls with a complication-free postoperative course (*Fig. S1*). A higher ASA score, higher BMI and neoadjuvant therapy were more frequent among patients with peritoneal infection (*Table 1*).

Only one patient homozygous for the minor allele (that is C/C) was identified in both the case and control group. Ten individuals in the case group and 14 individuals in the control group were heterozygous for the minor allele (that is G/C), whereas 36 individuals in the case group and 32 individuals in the control group were homozygous for the major allele (that is G/G). There were more individuals with heterozygosity in the control group than in the case group (*Table 2*). The logistic regression model

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Table 1 Clinical data for 94 colorectal cancer patients

Variables	Peritoneal infection	
	Complication-free ($n = 47$)	Infected ($n = 47$)
Age (years), median (i.q.r.)	70 (63–76)	69 (62–77)
Sex		
Male	26 (55.3)	26 (55.3)
Female	21 (44.7)	21 (44.7)
ASA fitness grade		
ASA I–II	40 (85.1)	34 (72.3)
ASA III	7 (14.9)	12 (25.5)
Missing	0 (0.0)	1 (2.1)
BMI (kg/m^2)		
<25	22 (46.8)	17 (36.2)
25–30	18 (38.3)	19 (40.4)
>30	7 (14.9)	11 (23.4)
Tumour location		
Colon	25 (53.2)	25 (53.2)
Rectum	22 (46.8)	22 (46.8)
Neoadjuvant treatment		
No	34 (72.3)	27 (57.4)
Radiotherapy	7 (14.9)	11 (23.4)
Chemoradiotherapy	6 (12.8)	9 (19.1)
Diverting stoma		
No	29 (61.7)	27 (57.4)
Yes	18 (38.3)	20 (42.6)
Intraoperative blood loss (ml), median (i.q.r.)	100 (50–300)	200 (100–500)
Surgical approach		
Open	38 (80.9)	35 (74.5)
Laparoscopic	7 (14.9)	10 (21.3)
Conversion to open	2 (4.3)	2 (4.3)
Year of surgery	2013 (2012–2014)	2013 (2012–2015)
pTNM		
I	11 (23.4)	11 (23.4)
II	20 (42.6)	20 (42.6)
III	16 (34.0)	16 (34.0)

Cases with peritoneal infection and complication-free controls. Values are *n* (%) unless otherwise stated. i.q.r., interquartile range; pTNM, pathological tumour stage.

for additive effects testing −765G > C and case/control status demonstrated an odds ratio of 0.71 ($P = 0.413$).

The pQTL did not demonstrate any significant findings (see *Supplementary material*).

Discussion

In this retrospective matched cohort study, the previous findings of Reisinger *et al.*[5] could not be reproduced, in which homozygosity for the polymorphism of the COX-2 gene promoter −765G > C minor allele was associated with peritoneal infection after colorectal cancer resection with an anastomosis. The calculated point estimate demonstrated a non-significant reverse association in the present study.

In the study by Reisinger *et al.*[5], tissue samples from 148 consecutive colorectal cancer patients were collected at a single centre, and all were genotyped for PTGS2-765G > C. Seven patients were homozygous for the polymorphism, and three of them experienced anastomotic leakage, compared with 16 of 141. This corresponds to a leakage frequency of 43% *versus* 11% and a corresponding odds ratio of 5.86, in stark contrast to the present study.

Possible explanations for these differing results could be that the present study was underpowered, resulting in false negative findings. A notable analytic difference is that all categories of −765G > C were included, while the former study only compared

Table 2 Frequency table of the single-nucleotide polymorphism of the COX-2 gene promoter −765G > C for 94 patients with colorectal cancer undergoing surgical resection, all with a primary anastomosis

	Number of patients with specific allelic combinations		
Peritoneal infection	G/G	G/C	C/C
Infected	36	10	1
Complication-free	32	14	1

Patients with peritoneal infection *versus* complication-free controls.

C/C *versus* the rest (that is G/C and G/G grouped together). In the present study, heterozygotes were included as a separate group in the analyses as previous studies have demonstrated alterations in biological status associated with the minor allele of C even in the heterozygote state[6,10].

Another difference between the studies is the outcome definition, as the composite of peritoneal infection (that is anastomotic leakage and/or abscess) was used here, in contrast to anastomotic leakage exclusively. In addition, medical charts of all cases and controls were reviewed to avoid misclassification of case-control status, while classification of anastomotic leakage in the study by Reisinger *et al.*[5] is unclear. Another aspect to consider is adjustment for confounding, which to some degree was performed through matching in this case-control study; this was not adjusted in the former study. Notably, there were also differences in allele frequencies, as the study by Reisinger *et al.* had 4.7% homozygosity, in comparison to 2.1% in the present report. It was unknown what frequency to expect of the −765G > C variant in this Swedish cohort. The present findings could be valuable for future research, including power calculations.

Regarding the expressional effect of −765G > C, reports are conflicting. An initial hypothesis was that this minor allele would reduce promoter activity and affect COX-2 gene expression levels and function[6], while subsequent studies reported conflicting evidence[11–13], that is that G/C and C/C can lead to increased production of prostaglandins, displayed in both *in vivo* and *in vitro* studies. Moreover, COX-2 expression has been suggested to be important for anastomotic healing[5]. If the minor allele of −765G/C does not result in a decreased, but rather an increased COX-2 function, this could be in line with these findings of numerically less peritoneal infections in those with a higher frequency of the minor allele.

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Disclosure

The authors declare no conflict of interest.

Supplementary material

Supplementary material is available at *BJS Open* online.

Data availability

The data for this paper will be stored at the Department of Surgical and Perioperative Sciences at Umeå University hospital

for 10 years. This data can only be made available with relevant ethical permits due to the sensitive information contained.

Author contributions

Oskar Grahn (Data curation, Formal analysis, Methodology, Project administration, Software, Validation, Visualization, Writing—original draft, Writing—review & editing), Mun-Gwan Hong (Formal analysis, Investigation, Methodology, Software, Writing—review & editing), Klas Holmgren (Data curation, Formal analysis, Writing—review & editing), Malin Sund (Formal analysis, Methodology, Supervision, Writing—review & editing) and Martin Rutegård (Conceptualization, Formal analysis, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing—review & editing)

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