The importance of *G2677T/A* and *C3435T* polymorphisms of the *MDR1* gene in the aetiology of colorectal cancer

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Abstract

Introduction: Colorectal cancer (CRC) is the most common cancer among patients, and its aetiology is still not precisely known. It is believed that 15–30% of colorectal cancers are genetically determined. P-glycoprotein (P-gp) encoded by the *MDR1* gene in normal conditions plays an important role in the action of colon epithelial cells. However, the *MDR1* polymorphism influences the P-gp expression and can weaken its effect against xenobiotics (procarcinogens) and increase the frequency of CRC.

Aim: To evaluate the correlation between the *MDR1 C3435T* and *G2677T/A* polymorphisms and the risk of colorectal cancer.

Material and methods: The study group with colorectal cancer included 47 women and 60 men while the control group consisted of 110 healthy patients. The diagnosis in patients suffering from CRC was confirmed by histopathological report. Genetic analysis was performed using PCR-RFLP method.

Results: We showed only a correlation between the frequency of CT and TT genotypes of *C3435T* polymorphism and the risk of colorectal cancer in younger age. There was no correlation between the *C3435T* and *G2677T/A* polymorphisms of the *MDR1* gene and other clinical parameters.

Conclusions: Our findings suggest that T allele carriers of *C3435T* polymorphism have an increased risk of CRC. However, further studies are needed on a much larger number of patients and genes associated with metabolism and transport of xenobiotics including procarcinogens.

Introduction

Colorectal cancer (CRC) is the most common newly diagnosed cancer and the third most common cause of cancer death among men and women. The risk of CRC is increased by several factors such as gene mutations, lifestyle factors, age, heredity, and polyps of the colon [1, 2]. The relationships between risk factors and colorectal cancer development are not exactly known. About 15–30% of all colon cancer cases are associated with genetic changes. The most common hereditary forms of CRC are familial adenomatous polyposis (FAP) and hereditary nonpolyposis colorectal cancer (HNPCC), although there are other syndromes associated with predisposition [3, 4]. The adenomatous polyposis coli (APC) gene encodes the APC protein that plays a substantial role in the regulation of the Wnt signalling

pathway participating in the activation of many genes (*MDR1*, c-Myc) [5, 6]. Hence, the genetic variability in genes associated with metabolism and transport of procarcinogens, DNA repair system, and activation of major signalling pathways may contribute to the appearance and progression of various cancers including CRC [7–10].

In addition, dietary factors that determine the environment of the intestinal epithelium are thought to play an important role in the process of oncogenesis because their toxic metabolites can cause genetic damage [11]. There are several mechanisms providing protection against toxic agents, such as P-glycoprotein (P-gp) belonging to the ATP binding cassette transporter superfamily, which functions as a transmembrane drug efflux pump, decreasing intracellular xenobiotic accumulation, and eliminates toxic agents from the cell. P-glycoprotein encoded by the *MDR1* gene, outside the protection of the body against exogenous compounds, plays a role in immune regulation of cell death [12–15]. In normal conditions, colon epithelial cells have a high concentration of P-glycoprotein, which plays an important role in their action. However, the *MDR1* gene polymorphism influencing the P-gp expression can weaken its effect against xenobiotics (procarcinogens) and increase the incidence of CRC [16, 17]. Furthermore, it is believed that the overexpression of the *MDR1* gene encoding the P-gp contributes significantly to the phenomenon of multidrug resistance (MDR) responsible for the failure of the pharmacotherapy of cancer. Multidrug resistance is one of the most important causes of reduced efficacy of cancer therapy. Among the best known and most significant polymorphisms of the *MDR1* gene are *C3435T* and *G2677T/A* [16, 18]. Many studies have shown a significant role of these polymorphisms in the pathogenesis of colorectal cancer [19–21].

Aim

The aim of our study was to determine the frequency of *C3435T* and *G2677T/A* polymorphisms of the *MDR1* gene in the group of colorectal cancer patients, in relation to healthy patients. In addition, we studied the impact of these polymorphisms on the CRC development and the correlation between the frequency of particular genotypes and the clinical factors.

Material and methods

Patients

In the present study 107 patients with diagnosed colorectal cancer were evaluated. The patients were diagnosed and treated between 2010 and 2012 in the Department of General and Gastroenterological Surgery of SPSK1 Hospital in Szczecin. The study group included 47 women and 60 men. The age range of patients suffering from CRC was 47–83 years. In every case, the diagnosis of CRC was confirmed by histopathological report. The clinical data from the patients was collected, including age, gender, tumour localisation, staging, grading, and clinical symptoms (anaemia, weight loss, bowel obstruction). The control group consisted of 110 healthy patients of similar age. The Bioethical Committee of the Pomeranian Medical University approved the study. All patients were informed about the aim of the study and gave written consent to perform genetic testing.

Among the 53 patients in the study group, the most common histological type of colorectal cancer was adenocarcinoma (the degree of differentiation – G2) constituting 78% (41 cases), adenocarcinoma G1 – 10% (5 cases), adenocarcinoma G3 – 6% (3 cases), carcinoma mucinosum – 4% (2 cases), and carcinoma gelatinosum – 2% (1 case). The 54 remaining patients did not agree to make available data with histopathological examination.

Genetic analysis

Genetic testing was performed at the Laboratory of Experimental Pharmacogenetics, Department of Clinical Pharmacy and Biopharmacy, Poznan University of Medical Sciences. The *C3435T* and *G2677T/A* polymorphisms of the *MDR1* gene were determined using polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) methods. The primers (TibMolBiol, Poland) used in the PCR reaction, length of amplified products, and the conditions of the PCR reaction were applied as previously described [22, 23].

The results of PCR-RFLP were analysed on agarose gels by visualisation in UV light using a documentation system (KS 4000/Image PC, Syngen Biotech Molecular Biology Instruments).

Statistical analysis

The statistical significance of the difference between the control and study groups was assessed by SPSS 17.0 software using one-way ANOVA test (SPSS Inc.). Values of $p < 0.05$ were considered to represent a statistically significant difference.

Results

No differences were found in the *MDR1 C3435T* polymorphism frequencies between colorectal patients and the control group ($p = 0.94$) (Table I). Among the study group, the most common genotype was heterozygous CT (49.5%) then homozygous TT genotype (34.6%) while homozygous CC genotype occured in 17 (15.9%) cases. The frequency of the individual alleles also showed no statistically significant differences between the study

Table I. The frequency of genotypes and alleles of the *MDR1 C3435T* and *G2677T/A* polymorphisms in the study group with colorectal cancer and in the control group

Variable	Study group (n = 107)		Control group (n = 110)		Value of p
	Observed value n (%)	Expected value %	Observed value n (%)	Expected value %	
C3435T Genotype:					
CC	17 (15.9)	16.5	17 (15.5)	17.1	0.94
CT	53 (49.5)	48.3	57 (51.8)	48.5	
TT	37 (34.6)	35.2	36 (32.7)	34.4	
Allele:					
C	87 (40.7)	–	91 (41.4)	–	0.88
T	127 (59.3)	–	129 (58.6)	–	
G2677T/A Genotype:					
GG	39 (36.4)	(38.6)	34 (30.9)	27.3	0.02
GT	54 (50.5)	(45.9)	47 (42.7)	50.0	
TT	12 (11.2)	(13.6)	29 (26.4)	22.8	
TA	1 (0.9)	(0.7)	0 (0)	0	
GA	1 (0.9)	1.2	0 (0)	0	
AA	0 (0)	0	0 (0)	0	
Allele:					
G	133 (62.2)	–	115 (52.3)	–	0.03
T	79 (36.9)	–	105 (47.7)	–	
A	2 (0.9)	–	0 (0)	–	

*$p < 0.05$.

group and controls (p = 0.88). In the control group, the genotype distribution was very similar (CT – 51.8%, TT – 32.7%, CC – 15.5%). Also, the risk of developing colorectal cancer has been studied in three models: recessive, dominant, and additive. None of these models showed a statistically significant increase in risk of colorectal cancer (TT homozygotes OR = 1.03; CT heterozygotes OR = 0.93) (Table II).

No statistically significant impact of *C3435T* polymorphism on the appearance of CRC was found. The increase of risk of colorectal cancer was only observed for CT and TT genotypes of *C3435T* polymorphism in younger age. There were no significant differences between the clinical factors of colorectal cancer patients and genotypes frequencies (Table III).

For *G2677T/A* polymorphism, statistically significant differences between colorectal cancer patients and the control group were observed (p = 0.02) (Table I). The most common genotype in the study group and the control group was heterozygous GT (50.5% vs. 42.7%).

Table II. The odds ratio (OR) and 95% confidence intervals (95% CI) for the developing colorectal cancer

Variable	OR (95% CI)	Value of p
C3435T polymorphism:		
Recessive model TT vs. CT + CC	1.09 (0.62–1.90)	0.77
Dominant model TT + CT vs. CC	0.97 (0.47–2.01)	0.93
Additive model CT vs. CC TT vs. CC	0.93 (0.43–2.01) 1.03 (0.46–2.32)	0.85 0.95
G2677T/A polymorphism:		
Recessive model TT vs. GT + GG	0.36 (0.17–0.75)	0.007
Dominant model TT + GT vs. GG	0.76 (0.43–1.33)	0.34
Additive model GT vs. GG TT vs. GG	1.00 (0.55–1.83) 0.36 (0.16–0.82)	1.00 0.01

Table III. The selected clinical parameters in patients with colorectal cancer compared to different polymorphic variants of the *MDR1* gene

Parameter	*C3435T* polymorphism				*G2677T/A* polymorphism			
	CC	CT	TT	Value of *p*	GG	GT	TT	Value of *p*
Gender:								
Male	10	31	19	0.77	21	31	8	0.73
Female	7	22	18		18	23	4	
Age [years]:								
Mean	73	68	79	0.013	73	69	73	0.40
Range	57–82	54–81	68–-83		57–82	54–83	65–80	
pT								
Tis	0	2	0		0	2	0	
T1	0	0	0		0	0	0	
T2	1	2	2		2	3	0	
T3	7	22	5	0.64	12	18	3	0.92
T4	2	7	3		4	6	2	
pN								
N0	5	16	7		8	18	1	
N1	2	10	2	0.80	5	6	3	0.50
N2	2	7	1		4	5	1	
M								
M0	8	23	9	0.14	15	22	3	0.20
M1	1	9	0		1	6	2	
Location:								
Colon ascedens	2	6	3		3	7	1	
Colon transverses	1	6	2		2	5	2	
Colon descedens	0	3	1		2	2	0	
Colon sigmoideum	1	15	3	0.10	6	12	1	0.86
Rectum	6	3	0		5	3	1	
Histological type:								
Adenocarcinoma	9	32	9	0.48	17	27	5	0.81
Others	1	1	1		1	2	0	
Degree of differentiation:								
G1	0	4	1		2	3	0	
G2	8	26	8	0.72	15	22	4	0.56
G3	1	2	0		0	2	1	
Symptoms of anaemia:								
No	5	8	2	0.07	7	6	2	0.52
Yes	5	25	8		11	23	3	
Body weight loss:								
No	5	14	6	0.79	11	12	1	0.28
Yes	5	19	4		7	17	4	
Obstruction:								
No	7	18	7	0.72	9	20	3	0.33
Yes	3	15	3		9	9	2	
Radical surgery:								
No	2	6	1	0.82	3	4	1	0.89
Yes	8	27	9		15	25	4	

TNM classification of malignant tumours (T – size or direct extent of the primary tumour (Tis: carcinoma in situ; T0: no signs of tumour; T1, T2, T3, T4: size and/or extension of the primary tumour), N – degree of spread to regional lymph nodes (N0: tumour cells absent from regional lymph nodes; N1: regional lymph node metastasis present; N2: tumour spread to an extent between N1 and N3), M – presence of distant metastasis (M0: no distant metastasis, M1: metastasis to distant organs)).

A protective role of TT genotype of *G2677T/A* polymorphism against the development of CRC was observed (OR = 0.36, 95% CI = 0.17–0.75) (Table II). No dependence between colorectal cancer clinical factors and *G2677T/A* polymorphism was found (Table III).

Discussion

In the present study we showed no effect of *C3435T* polymorphism of the *MDR1* gene on the occurrence of colorectal cancer. We only observed an increase of risk of CRC for CT and TT genotypes of *C3435T* polymorphism in younger age. This suggests that the T allele carriers have an evaluated predisposition to the occurrence of colorectal cancer. Similar results were obtained by Petrova *et al.* [24]. The authors investigated the frequencies of *MDR1 C3435T* polymorphism in patients with colorectal cancer and a control group in a Bulgarian population. They did not show the relationship between the studied polymorphism and the occurrence of colorectal cancer. The obtained values of the risk were not statistically significant and amounted to OR = 0.81 (95% CI: 0.43–1.52) for CT heterozygotes and OR = 1.33 (95% CI : 0.77–2.30) for TT homozygotes in comparison to CC genotype.

Kurzawski *et al.* also obtained similar results [25] analysing the frequency of *C3435T* polymorphism of the *MDR1* gene in the Polish population. The authors did not show significant differences in the presence of polymorphisms between the study groups and the patients with colorectal cancer. However, they showed a statistically significant increased risk of colorectal cancer in carriers with 3435TT genotype younger than 50 years (OR = 2.74, 95% CI: 1.02–7.53). The authors explain the reduced amount of P-gp in the cell membrane of the intestine, which leads to decreased removal of substances from the body that affect carcinogenesis.

According to Osswald *et al.* [26], the obtained results showed statistically significant decreased risk of colorectal cancer in carriers with TT and CT genotypes. Moreover, they noted an increased risk of developing colorectal cancer in patients with chronic smoking (OR = 3.9, 95% CI: 1.4–10.6) compared to the same polymorphism in the *MDR1* gene. Another study demonstrated the influence of environmental factors on the correlation with *C3435T* polymorphism and the occurrence of colorectal cancer [20]. The authors analysed the effect of eating meat, smoking, administration of non-steroidal anti-inflammatory drugs (NSAIDs), and hormone replacement therapy. They showed a slightly increased incidence of colorectal cancer among homozygous CC patients consuming an increased amount of red meat (OR = 1.08, 95% CI: 1.00–1.16). Also, the CC genotype correlated with the intake of NSAIDs characterised by a more than two-fold increase in the risk of colorectal cancer (OR = 2.34, 95% CI: 1.22–4.48). Other variants had no effect on the incidence of CRC in correlation with NSAIDs. Furthermore, the authors have not proved the impact of smoking on the risk of colorectal cancer in relation to the *MDR1 C3435T* polymorphism. Additionally, Andersen *et al.* demonstrated statistically significantly reduced risk of colorectal cancer among homozygous TT patients (OR = 0.69, 95% CI: 0.48–1.00) [20]. The lack of a direct effect of the *MDR1* gene *C3435T* polymorphism on the risk of colorectal cancer has also been shown in a meta-analysis conducted by Wang *et al.* [27]. Similar results on the *C3435T* polymorphism and the occurrence of CRC were obtained in the meta-analysis by Sheng *et al.* [28].

By analysing polymorphism *G2677T/A* we showed a statistically significant difference between patients with colorectal cancer and the control group ($p = 0.02$). In the individual comparative model we demonstrated a significant reduction in the risk of colorectal cancer in the additive model for the homozygotes TT (OR = 0.36, 95% CI: 0.16–0.82, $p = 0.01$) and recessive model (OR = 0.36, 95% CI: 0.17–0.75, $p = 0.007$). Osswald *et al.* [26] obtained similar results in a study of the Russian population. The obtained results showed a statistically significant reduction in the risk of CRC in the case of heterozygotes GT compared to the homozygous GG (OR = 0.65, 95% CI: 0.45–0.96) and confirmed a protective effect of *G2677T/A* polymorphism of the *MDR1* gene for the development of colorectal cancer. Furthermore, other studies among the populations of Bulgaria [24], Japan [29], and Italy [30] showed no significant association between *G2677T/A* polymorphism and the development of colorectal cancer.

The results of the presented studies do not provide a clear answer about the relationship between the investigated polymorphisms and the occurrence of colorectal cancer. This may be because of the need for performance analysis of the multi-gene polymorphisms in combination with existing environmental factors that may lead to malignant tumours.

Conclusions

Our results showed that the *C3435T* polymorphism of the *MDR1* gene has no direct impact on the development of colorectal cancer. However, the relationship was found between the frequency of CT and TT genotypes and the risk of colon cancer in younger age. On the other hand, the TT genotype carrier of *G2677T/A* polymorphism has a protective effect on the incidence of CRC. However, further studies are needed on a much larger number of patients and genes associated with the metabolism and transport of xenobiotics (procarcinogens).

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Conflict of interest

The authors declare no conflict of interest.

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