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# G39179T DNMT3B Gene Variants in Relation to Colorectal Cancer Risk in Kazakhstan Population

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## Abstract

**Objective:** Molecular-genetic study of the association between G39179T DNMT3B polymorphism and risk of the colorectal cancer in Kazakhstan population.

**Methods:** The method of site-specific PCR amplification with following restriction of amplified fragments was used for the genotyping of G39179T DNMT3B polymorphism.

**Results:** The DNMT3B 39179 GG genotype was associated with an increased risk of colorectal cancer development (OR=1.91, 95% CI=1.13-3.25, p=0.05). Separate analysis of population sub-groups showed that the GG genotype (vs GT+TT genotypes) was associated with a significantly increased risk of colorectal cancer among Russian (OR=2.10, 95% CI=1.07-4.10 p=0.03), individuals older than 60 years (OR=3.13, 95% CI=1.59-6.17 p=0.0008) and men (OR=3.96, 95% CI=1.52-10.31 p=0.004).

**Conclusion:** We suggested that the DNMT3B G39179T polymorphism participates in modulation of susceptibility to CRC. The obtained results showed the association of DNMT3B 39179 GG genotype with the development of colorectal cancer in Kazakhstan population.

**Keywords:** Methyltransferase; Single nucleotide polymorphism; Methylation; Colorectal cancer

## Introduction

Colorectal cancer (CRC) is one of the most common malignancies worldwide and the incidence is increasing in developed as well as in developing countries including Kazakhstan. According to the last epidemiological study colorectal cancer ranks (8.7 per 100 thousand) fourth in incidence of all types of cancer in Kazakhstan after breast (12.3), lung (11.4) and skin cancer (10.4) [1]. Mortality rate from this disease is also very high (70%). This is due to the fact that early-stage colorectal cancer (I and II stages) is difficult to detect. In addition most diagnosed cases of colorectal cancer are sporadic forms.

The cause of sporadic CRC includes variety of environmental and genetic factors that interact together to determine the risk of this cancer. One of the genetic factors is epigenetic alterations in the genome. The epigenetic hypermethylation is an alternative mechanism of tumor suppressor gene function loss, along with genetic mutations, and plays a crucial role in colorectal cancer initiation and progression [2,3]. Methylation occurs through enzymatic attachment of the methyl group to cytosine of DNA molecule. The group of enzymes that catalyze this process, called methyltransferases DNMT. There are 5 major DNMTs: DMNT1, DNMT2, DNMT3A, DNMT3B and DNMT3L. DNMT1 is the most common methyltransferase in adult cells [4]. DNMT1 primarily acts as a maintenance methyltransferase during each cell division and copies DNA methylation patterns to daughter strands during DNA replication. DNMT2, which is also

called TRDMT1 (transferRNA aspartic acid methyltransferase), participates in both DNA and RNA methylation [5]. DNMT3L has no catalytic activity, but DNMT3L methylation stimulates the de novo methylation by interacting with the catalytic domains of DNMT3A and DNMT3B [6]. The DNMT3A and DNMT3B proteins are required for genome-wide de novo methylation [7,8].

DNMT3B protein is encoded by DNMT3B gene which located on chromosome 20q11. 2.(23 exons, 22 introns,). Polymorphism of methyltransferase genes may underlie disorders of functional activity of the enzymes, which in its turn leads to disruption of the genomic DNA methylation and development of malignant processes. Thus, different allelic variants of the methyltransferase genes can contribute to predisposition to cancer.

21 polymorphisms have been found in the DNMT3B gene sequence, two of which are nonsynonymous (can induce substitution of one aminoacid for another). Several single nucleotid polymorphisms (SNP) can change the activity of the promoter [9].

A G39179T DNMT3B (-579G>T, rs1569686) single nucleotide polymorphism is localized in the DNMT3B gene promoter. The DNMT3B gene has two transcriptional start sites, which localized in exon 1A and 1B. The expression of gene is regulated by two promoters. One promoter was found in CpG-rich region, the other promoter was found in CpG-poor region. The DNMT3B G39179T polymorphism is located in the CpG-poor promoters: -579 bp from exon 1B transcription site [10]. Although it has been suggested that this polymorphism affects the function of the gene [11], it did not effect on the transcriptional activity of the DNMT promoter [9].

No study of G39179T DNMT3B gene polymorphism as risk factors for colorectal cancer development has been conducted in population of Kazakhstan. The studies to identify the polymorphism of candidate genes, which can contribute to the formation of the colorectal cancer risk, were initiated in Kazakhstan only in the last year [12,13]. The importance of such researches in Kazakhstan due not only to the novelty of the results, in particular information about the frequencies of alleles in a previously not studied Kazakhstan population, but also the possibility of practical application of these results. The study of polymorphisms of these genes can be used for development of molecular-genetic methods for early diagnosis, aimed at identification of patients with high risk of colorectal cancer.

Considering the above, in this case-control study we analyzed G39179T DNMT3B gene polymorphism among CRC patients and controls to evaluate association between this genetic variant and susceptibility to CRC.

## Materials and Methods

### Sampling

Written informed consent was obtained from all participants before enrollment. The study was approved by the Ethics Committee of the Asfendiyarov Kazakh National Medical University, Almaty, Kazakhstan (Protocol No. 5 of 27 June 2012).

Blood samples were collected from 126 patients diagnosed with CRC at the Almaty Oncology Centre (Almaty, Kazakhstan). Diagnosis was performed according to clinical and histological examination. Control blood samples were collected from 116 healthy donors.

A standardized questionnaire was used to collect basic statistical information including information of socio-demographic status, diet, occupation, tobacco and alcohol habits, previous illness, illnesses of relatives, radiation exposure etc. The control group of healthy individuals was selected according to the ethnicity, age, gender, smoking and alcohol habits of CRC patient group. Controls were also biologically unrelated to the patients and had no known family history of malignancies.

### DNA isolation

Genomic DNA was isolated from peripheral blood leukocytes using Genomic DNA Purification Kit (Fermentas, Lithuania).The DNA was dissolved in distilled water and the quantity and quality of the dissolved DNA samples were evaluated by spectrophotometric analysis (Eppendorf BioPhotometer plus). Extracted DNA samples were stored at -20°C.

### Single nucleotide polymorphism genotyping

The PCR-RFLP assay was used for the genotyping of DNMT3B G39179T single nucleotide polymorphism [14]. 50ng of target genomic DNA was amplified in 20 µl PCR mixture, containing 10 µl 2 × PCR Master Mix (0.05 U/µL Taq DNA polymerase, reaction buffer, 4 mM $MgCl_2$, 0.4 mM of each dNTP (ThermoScientific, Lithuania)) and 5 pM of each primer. The following primers were used to amplify 343 bp PCR products: forward 5'-GGGGGCCTGGAGGTCTCATTAT-3' and reverse 5'-ACGGATGGGTTGGCAGGC TATA-3'. PCR cycling conditions consisted of an initial denaturation at 94°C for 10 min, followed by 32 cycles of 94°C for 50 s, 57°C for 50 s, 72°C for 50 s, and a final extension of 72°C for 10 min. PCR products were digested at

370C for 8-16 hours with 1-3U of PvuII (Fermentas) and analyzed on 2% agarose ("TopVision Agarose", Fermentas, Lithuania) gels using a 100 bp DNA ladder marker (Sigma-Aldrich, USA) for sizing of the amplified DNA fragments' length. Mutant variant T allele had an PvuII restriction site that resulted in two bands (241 and 102 bp), and wild-type G allele lacked the PvuII restriction site and therefore produced a single 343-bp band, GT heterozygote produced three bands.

### Statistical analysis

Differences in ethnicity, age, gender, smoking and alcohol habits between cancer patients and controls were evaluated by using the χ2 test. Association between the DNMT3B G39179T single nucleotide polymorphism and risk of CRC cancer was estimated by calculating the Odds ratios (ORs) and 95% confidence intervals (95%CIs). The level of statistical significance was set at 0.05. Statistical analysis was performed using "Case-Control Study Estimating Calculator" by TAPOTILI company (Laboratory of Molecular Diagnostics and Genomic Dactyloscopy of "GosNIIGenetika" State Scientific Centre of Russian Federation).

## Results

### Characteristic of the study population

A total of 126 CRC cases and 116 healthy controls were recruited in this study. The characteristics of the participants are summarized (Table 1).

| Characteristic | | CRC N (%) | Controls N (%) | tst | p value |
|---|---|---|---|---|---|
| Sample size | | 126 | 116 | | |
| **Ethnicity** | Kazakh | 43 (34) | 42 (36) | 0.273 | 0.601 |
| | Russian | 77 (61) | 70 (60) | 0.076 | 0.783 |
| | Other Asians | 6 (5) | 4 (4) | 0.486 | 0.486 |
| **Age (years)** | Median | 63,6 ± 9,90 | 64.5 ± 9,14 | 1.972 | 0.160 |
| | Range | 36-82 | 37-82 | | |
| **Gender** | Male | 47 (37) | 44 (38) | 0.080 | 0.780 |
| | Female | 79 (63) | 72 (62) | 0.062 | 0.803 |
| **Smoking status** | Ever | 35 (28) | 24 (21) | 1.075 | 0.300 |
| | Never | 66 (52) | 91 (78) | 2.389 | 0.122 |
| | did not respond | 25 (20) | 1 (1) | 0.975 | 0.323 |
| **Alcohol consumption** | Positive | 29 (23) | 31 (27) | 0.580 | 0.446 |
| | Negative | 72 (57) | 84 (72) | 1.455 | 0.228 |
| | did not respond | 25 (20) | 1 (1) | 0.975 | 0.323 |

**Table 1:** Demographic characteristics of CRC patients and controls.

The ethnic heterogeneity of both groups were identical: 34% Kazakh, 61% Russian, 5% other Asians in CRC group and 36% Kazakh, 60% Russian, 4% other Asians in controls. The median age of the CRC patients (at the time of diagnosis) was 63.6 years (range: 36-82 years) and of the controls-64.5 years (range: 37-82 years).The male/female

ratios were analogous for the CRC patients (37%/63%) and the controls (38%/62%). 58 (46%) of CRC patients were colon cancer cases, 68 (54%)-were rectal cancer cases. The I, II, III and IV stages were diagnosed in 3%, 37%, 51%, and 9% CRC cases, respectively.

### Investigation the association of G39179T DNMT3B polymorphism with risk of the colorectal cancer in a Kazakhstan population

The genotypes distributions in controls and cases were in agreement with the Hardy-Weinberg equilibrium ($\chi2$ =1.18 p=0.28 and $\chi2$ =9.42 p=0.002). The distribution of the genotypes for the DNMT3B G39179T polymorphism in CRC patients and control subjects and the CRC risk related to the DNMT3B G39179T genotypes is summarized (Table 2).

| Gene polymorphism | Genotype | Genotypes distribution | | OR (95% CI) | p value |
|---|---|---|---|---|---|
| | | Case (126) | Controls (116) | | |
| DNMT3B G39179T | GG | 57 | 35 | 1.91 (1.13-3.25) | 0.05 |
| | GT | 43 | 52 | 0.64 (0.38-1.07) | |
| | TT | 26 | 29 | 0.78 (0.43-1.42) | |
| DNMT3B G39179T dominant model | GG+GT | 100 | 87 | 1.28 (0.70-2.34) | 0.42 |
| | TT | 26 | 29 | 0.78 (0.43-1.42) | |
| DNMT3B G39179T recessive model | GG | 57 | 35 | 1.91 (1.13-3.25) | 0.02 |
| | GT+TT | 69 | 81 | 0.52 (0.31-0.89) | |

**Table 2:** The DNMT3B G39179T genotypes association with CRC.

As shown in Table 2, the DNMT3B 39179 GG genotype was associated with a significantly increased risk of colorectal cancer (OR=1.91, 95% CI=1.13-3.25, p=0.05). Furthermore, a significantly increased risk of colorectal cancer was found for GG genotype versus the combined variant of GT+TT genotypes (OR=1.91, 95% CI=1.13-3.25, p=0.02) in recessive model.

We further calculated the association of the DNMT3B G39179T polymorphism with colorectal cancer risk in the population sub-groups divided into ethnicity, age, gender, smoking and alcohol status. Although these sub-groups were small in most cases, we received some statistically significant results (Table 3).

| Population sub-groups | Genotype | Cases/Controls | OR (95% CI) | p-value |
|---|---|---|---|---|
| Ethnicity | | | | |
| Kazakh | GG | 17/11 | 1.84 (0.73 – 4.62) | 0.19 |
| | GT+TT | 26/31 | 0.54 (0.22 – 1.36) | |
| Russian | GG | 39/23 | 2.10 (1.07 – 4.10) | 0.03 |
| | GT+TT | 38/47 | 0.48 (0.24 – 0.93) | |
| Age | | | | |
| ≤ 60 | GG | 14/14 | 0.83 (0.34 – 2.02) | 0.68 |
| | GT+TT | 35/29 | 1.21 (0.50 – 2.94) | |
| >60 | GG | 43/21 | 3.13 (1.59 – 6.17) | 0.0008 |
| | GT+TT | 34/52 | 0.32 (0.16 – 0.63) | |
| Sex | | | | |
| Male | GG | 22/8 | 3.96 (1.52 – 10.31) | 0.004 |
| | GT+TT | 25/36 | 0.25 (0.10 – 0.66) | |
| Female | GG | 35/27 | 1.33 (0.69 – 2.54) | 0.4 |
| | GT+TT | 44/45 | 0.75 (0.39 – 1.45) | |
| Smoking status | | | | |
| Never | GG | 30/28 | 1.88 (0.97 – 3.62) | 0.06 |
| | GT+TT | 36/63 | 0.53 (0.28 – 1.03) | |
| Ever | GG | 16/6 | 2.53 (0.81 – 7.89) | 0.11 |
| | GT+TT | 19/18 | 0.40 (0.13 – 1.24) | |
| Alcohol consumption | | | | |
| Positive | GG | 12/6 | 2.94 (0.92 – 9.36) | 0.06 |
| | GT+TT | 17/25 | 0.34 (0.11 – 1.08) | |
| Negative | GG | 34/28 | 1.79 (0.94 – 3.42) | 0.08 |
| | GT+TT | 38/56 | 0.56 (0.29 – 1.07) | |

**Table 3:** Association analysis based on ethnicity, age, gender, smoking and alcohol status.

We found that the GG genotype versus GT+TT combined genotypes was associated with a significantly increased risk of colorectal cancer in Russian (OR=2.10, 95% CI=1.07-4.10 p=0.03), individuals older than 60 years (OR=3.13, 95% CI=1.59-6.17 p=0.0008) and men (OR=3.96, 95% CI=1.52-10.31 p=0.004). Analysis of tobacco and alcohol consumers did not show significant association of GG genotype with CRC (OR=2.53, 95%CI=0.81 - 7.89, p=0.11 and OR=2.94, CI 95%= 0.92-9.36, p=0.06, respectively).

### Discussion

DNMT3B as de novo methyltransferase plays an important role in normal development but also in the development of cancer. It has been reported that mRNA of DNMT3B is over expressed in human colorectal cancer compared with matched normal colonic mucosa [15]. Using human oral cancer cell lines it has been revealed that over expression of DNMT3b were significantly associated with a higher incidence of lymph node metastasis, a higher recurrence rate after treatment and shorter survival of cancer patients. The inhibition of DNMT3b using a silencing vector resulted in slower tumor growth [16]. It was showed that DNMT3B may play an important role in widespread promoter CpG island methylation during the colorectal carcinogenic process [17].

Polymorphisms in this gene may affect expression and functions of the protein encoded by the gene and therefore, may be associated with the development of cancer.

In the present molecular epidemiological study, we investigated the association of G39179T promoter polymorphism in DNMT3B gene with the risk of colorectal cancer in Kazakhstan population.

The association between cancer and DNMT3B G39179T polymorphism has been studied for different types of cancer. The association of DNMT3B G39179T polymorphism with the risk of gastric cancer has been demonstrated by Hu et al. [18]. Their results showed that individuals with at least one G allele were at significantly decreased risk of gastric cancer compared with those having a TT genotype. The meta-analysis performed by Coppedè F et al. [19] suggested that the DNMT3B 39179 T allele may contribute to the risk of developing Myasthenia gravis in thymoma patients, particularly in homozygous TT subjects. Other studies have not shown association of DNMT3B G39179T polymorphism with head and neck squamous cell carcinoma [20], esophagus [21], cervical [22] and ovarian [23] cancer.

Concerning colorectal cancer a case-control study of Iranian population [14] showed that TT genotype was significantly associated with an increased risk of colorectal cancer. Compared to DNMT3B TT genotype, the GT and GG genotypes had lower risk of sporadic colorectal cancer development.

Bao Q et al. [24] studied the DNMT3B G39179T polymorphism in 544 colorectal cancer patients (including 280 cases of colon cancer and 264 cases of rectal cancer) and 533 control subjects. The results showed that the G allele could significantly decrease the risk of CRC compared to the TT genotype, also the G allele could decrease the risk of colon and rectal cancer, separately.

Another case-control study showed that the combination of GG and GT genotypes was associated with significantly decreased risk of colorectal cancer development [25]. In the study of the correlation between DNMT3B G39179T polymorphism and the susceptibilities to colorectal adenomatous polyps and adenocarcinoma, the G allelotype (G/T+G/G genotypes) had lower risk for colorectal cancer development, but there was no significant difference between the colorectal adenomatous polyps patients and control individuals [26]. In the stratification analysis, G/T+G/G genotypes showed significant association with colorectal cancer in subgroup of age<55 and men. Meanwhile, G/T+ G/G genotypes had a lower risk of both colorectal adenomatous polyps and adenocarcinoma development between non-drinkers.

In this study a total of 126 CRC cases and 116 healthy controls were recruited. To reach the significance of the association between investigated polymorphism and the risk of colorectal cancer we have selected the group of healthy people maximally matched to cohort of CRC patients on many parameters (ethnicity, age, gender, smoking and alcohol habits).

In our study the GG genotype was associated with a significantly increased risk of colorectal cancer (OR=1.91, 95% CI=1.13-3.25, p=0.05). Furthermore, a significant increased risk of colorectal cancer was found for GG genotype versus combined variant GT+TT genotype (OR=1.91, 95% CI=1.13-3.25, p=0.02). Thus, our results seem to contradict the results of other studies that showed the association of the 39179 TT genotype with the risk of colorectal cancer. Additional study in a larger cohort of patients is required to verify our results.

It should be noted, that several studies also demonstrated correlation between 39179 G allele and cancer, but not colorectal. Zheng et al. [7] suggested that the G allele served as a risk factor for acute myeloid leukemia [1]. The GG genotype appeared to be 5.76

times more susceptible to acute myeloid leukemia compared to the TT genotype. Wang et al. [27] found that individuals with the GG+TG genotypes were significantly associated with poor prognosis in gastric cancer compared to those carrying the TT genotype. The DNMT3B 39179 GG and TG genotypes were found to be non-significantly associated with an overall increased risk of gallbladder carcinoma in North Indian Population [28].

Analysis of sub-groups in our study demonstrated that compared to the GT+TT genotypes, the GG genotype was significantly more associated with the risk of colorectal cancer in Russian (OR=2.10, 95% CI=1.07-4.10, p=0.03), individuals older than 60 years (OR=3.13, 95% CI=1.59-6.17, p=0.0008) and men (OR=3.96, 95% CI=1.52-10.31 p=0.004). Analysis among tobacco and alcohol consumers sub-groups revealed non-significant association of GG genotype with CRC (OR=2.53, 95% CI= 0.81-7.89, p=0.11 and OR=2.94, 95% CI=0.92-9.36, p=0.06, respectively), perhaps because of a small number of these sub-groups and relatively large percentage of people who did not report whether they smoked or drank.

We found no published data regarding the analysis of association between the DNMT3B G39179T polymorphism and risk of colorectal cancer in the subgroup of the Kazakhstan population. However, in relation to other types of polymorphism it has been shown the strong association of GSTM1 null genotype (OR=2.01, 95% CI=1.45-2.79, p=0.0001 for ethnically mixed population) with colorectal cancer, which is higher in Kazakhs (OR=2.36, 95% CI = 1.35-4.10, p=0.006) than in Russians (OR=1.84, 95% CI=1.17-2.89, p=0.003), and also significantly correlates with smoking (OR=3.37, 95% CI=1.78-6.38, p=0.0007) [13].

In another study for the MLH1 G-93A (rs 1800734) polymorphism separate analysis of ethnic groups revealed that -93G/G genotype strongly associated with increased CRC risk in Kazakhs (OR=2.67, 95 %CI=1.35-5.30, $\chi2$=10.61, p=0.005) but not in Russians (OR=1.02, 95%CI=0.63-1.64, $\chi2$=0.01, p=1) [12]. In addition in this study the CRC risk for TP53 Arg72Pro was studied. In the Russian population the risk was high for the Pro/Pro (OR=4.69, 95 %CI=2.53-8.66, p<0.0001) and Pro/Arg (OR=1.55, 95 %CI=0.96-2.53, p<0.0001) genotypes. In the Kazakh population, however, the CRC risk is associated only with the Pro/Pro genotype (OR=3.40, 95 %CI=1.63-7.06, p=0.003) which also demonstrated a strong association with smoking (OR=5.51, 95% CI=2.15-14.08, p<0.0001).

Interestingly that a meta-analysis of 24 case-control studies indicated that DNMT3B G39179T polymorphism is associated with risk of different types of cancer in Asians (OR=0.68, 95% CI=0.53-0.87 for GT vs. TT) but not in Europeans OR=0.82, 95% CI=0.63-1.07 for GT vs. TT) [29].

In conclusion we hypothesized that the mutant T allele can suppress the functions of the methyltransferase enzyme which leads to disruption in the traditional process of tumor suppressor gene methylation in cancer cells. Thus, the T allele can reduce the risk of colorectal cancer development. Other studies are needed to evaluate the role of DNMT3B G39179T variants in the DNMT3B protein functions in patients with colorectal cancer.

Our study suggests that the DNMT3B G39179T polymorphism participates in modulation of susceptibility to CRC. The obtained results showed the association of DNMT3B 39179GG genotype with the development of colorectal cancer in a Kazakhstan population, especially in older Russian men. The study of polymorphisms of this

gene can be used for development of molecular-genetic methods for identification of patients with high risk of colorectal cancer.

## Acknowledgement

## Conflict of interest

The authors declare no conflict of interest.

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