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Communication

Searching for association of the CAG repeat polymorphism in the mitochondrial DNA polymerase gamma gene (*POLG*) with colorectal cancer

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Mitochondrial DNA polymerase gamma (POLG) is the only DNA polymerase involved in maintaining the mitochondrial genome. Recent studies demonstrated an association of CAG repeat polymorphism in the second exon of *POLG* gene with the risk of cancer. We investigated the CAG repeat variability in the *POLG* gene in tumor and non-tumor tissues from colorectal cancer patients and in DNA samples isolated from blood obtained from age-matched healthy persons. Somatically occuring CAG-repeat alterations in cancer tissues have been observed in 10% of patients, but no association has been found between the CAG repeat variants in the *POLG* gene and colorectal cancer risk.

Key words: *POLG* gene; CAG repeat; colorectal cancer; somatic mutations.

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INTRODUCTION

Mitochondrial DNA was found to be highly mutated in colorectal cancer cells. One of the key molecules involved in the maintenance of the mitochondrial genome is polymerase gamma (POLG) which plays an essential role in mtDNA replication and repair. The polymerase gamma holoenzyme is a heterotrimer consisting of a single 140 kDa catalytic subunit (encoded by *POLG* at chromosomal locus 15q25) and a 55 kDa accessory subunit that forms a tight dimer (encoded by *POLG2* at chromosomal locus 17q24.1) (Stumpf & Copeland, 2011). The catalytic subunit belongs to the family of type A DNA polymerases. It comprises three domains: a N-terminal domain containing 3'–5' exonuclease (exo) activity, a spacer domain and a C-terminal domain containing polymerase (pol) activity. POLG also bears a 5'-deoxyribose phosphate lyase activity, but the location of its active site is unknown (Euro *et al.*, 2011). A feature unique to human POLG is the presence of a repeat of 13 glutamines, partly encoded by a run of 10 CAG codons near the NH2 terminus of the coding region. Deletion of the CAG repeat did not affect enzymatic properties, but modestly up-regulated expression (Spelbrink *et al.*, 2000). Polymorphism of CAG repeat is supposed to be associated with male infertility (Rovio *et al.*, 2001). Recent studies demonstrated an association of this polymorphism with the risk of cancer. Blomberg Jensen *et al.* (2008) and Nowak *et al.* (2005) found an association between the *POLG* gene polymorphism and testicular cancer. Azrak *et al.* (2012) suggested the possibility of an increased risk of breast cancer in women with minor CAG repeat variants of *POLG*, although differences in CAG repeat length observed between cases and controls were not statistically significant. In this study we investigated the association between CAG repeat variants of *POLG* gene and colorectal cancer in the Polish population.

MATERIALS AND METHODS

Sample collection and DNA extraction. Paired colorectal cancer and adjacent normal colorectal tissues were collected from 50 patients with colorectal adenocarcinoma undergoing surgery for treatment at the Department and Clinic of General Surgery, Collegium Medicum in Bydgoszcz. The control group comprised 100 DNA samples isolated from blood obtained from age-matched healthy persons from the Polish population. All samples were taken after obtaining written informed consent. The study was approved by the Bioethics Committee of the Ludwik Rydygier Collegium Medicum, Nicolaus Copernicus University in Bydgoszcz, Poland (approval no. KB 432/2008).

Molecular analysis. DNA was isolated from tumor and non-tumor tissues using *GeneMatrix Bio-Trace DNA Purification Kit* according to the manufacturer's protocol (Eurx, Gdańsk, Poland). To analyze the number of CAG repeats in colorectal cancer patients, the second exon of the *POLG* gene was amplified by PCR and sequenced directly using the *BigDye® Terminator v3.1 Cycle Sequencing Kit* (Applied Biosystems). PCR conditions were 94°C for 10 min; 35 cycles of 94°C for 1 min, 55°C–60°C for 30 sec and 72°C for 1 min and final extension 72°C for 10 min. The primers were used according to Van Goethem *et al.* (2001). Since extensive sequence analysis of the *POLG* CAG-repeat region in numerous populations of Eurasian ancestry (including Poles) was performed previously (Malyarchuk *et al.*, 2005), in this study the number of CAG repeats in the control group has been analyzed by capillary electrophoresis of PCR-amplified fragments. To determine the CAG repeat length in the control group, the second exon of the *POLG* gene was

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Abbreviations: POLG, mitochondrial DNA polymerase gamma

Table 1. Somatically occurring alterations in CAG track length in *POLG* gene identified in five colorectal cancer patients.

POLG-CAG variants	
Normal tissue	Tumor tissue
10/10	9/10
10/10	10/11
10/10	10/11
10/10	9/9
9/10	9/9

amplified in a 25 μl reaction containing 0.5 μM of both unlabelled (CCC TCC GAG GAT AGC ACT TGC GGC) and FAM-labelled primers (FAM-AGC GAC GGG CAG CGG CGG CGG CA). The PCR conditions were as follows: initial denaturation at 95°C for 2 min in 30 amplification cycles at 94°C for 30 sec, annealing at 60°C for 30 sec and 72°C for 30 sec, followed by a final extension step at 72°C for 10 min. Fluorescently labeled fragments generated by PCR were analyzed on a *3130xl Genetic Analyzer Automatic Sequencer* (Applied Biosystems).

Statistical analysis. The chi-squared test (adjusted by Yates correction where necessary) was used to compare genotype frequencies. Statistical calculations were performed using STATISTICA software (*STATISTICA v. 10* StatSoft Inc).

RESULTS

Pairwise comparison of the *POLG* sequence from tumor and non-tumor tissues revealed somatically occuring alterations in five tumor samples (Table 1). Somatic changes were excluded from the analysis. Only germline polymorphisms in *POLG* were used to determine an association with colorectal cancer risk. The most common 10/10 CAG repeat genotype was found in 39 cases (78%) and 75 controls (75%), while heterozygous 10/non-10 CAG repeats were observed in 11 cases (22%) and 24 controls (24%). We identified one individual in the control group with heterozygous non-10/non-10 CAG repeats (Table 2). No statistically significant difference was observed in the frequency of CAG repeats between cases and controls (p=0.9751).

Table 2. CAG repeat polymorphism in *POLG* gene in colorectal cancer patients and control group. Somatically occurring changes were excluded from the analysis.

POLG-CAG variants	*Frequency (%)*		p
	Case Group (n = 50)	Control group (n = 100)	
10/12	1 (2%)	7 (7%)	
10/11	8 (16%)	13 (13%)	
10/10	39 (78%)	75 (75%)	
10/9	2 (4%)	2 (2%)	0.9751
10/8	0	1 (1%)	
10/6	0	1 (1%)	
11/7	0	1 (1%)	

DISCUSSION

Mitochondrial disfunction is a key hallmark of cancer cells. Although mutations in the *POLG* gene have been shown to result in decreased OXPHOS, decreased mtDNA content and human mitochondrial diseases, their role in the pathogenesis of cancer is still unclear. Previous reports indicated the role of *POLG* in breast cancer (Singh *et al*., 2009) and testicular cancer risk (Blomberg Jensen *et al*., 2008; Nowak *et al*., 2005). This preliminary study is the first to investigate the role of CAG repeat polymorphism in the *POLG* gene in colorectal cancer. We found no association between CAG track length in *POLG* and colorectal cancer risk, but the principal limitation of this study is the small population sample which reduces the power to demonstrate significant association. However, it should be emphasized that the frequency of CAG-alleles observed in our study was consistent with the results reported by Malyarchuk *et al*. (2005) which included 102 Poles (p=0.7612), but significantly differed (p=0.0372) from the frequency observed in 55 Polish controls reported by Nowak *et al*. (2005). Moreover, the study reported by Nowak *et al*. (2005) of *POLG*-CAG repeat variants in cancer compared the frequency of these variants in tumor tissue to the frequency in blood samples from healthy individuals. Our study showed that somatically occuring CAG-repeat alterations in cancer tissue were observed in 10% of patients, probably due to microsatellite instability (MSI) caused by the loss of DNA mismatch repair activity, which is detected in about 15% of all colorectal cancers (Boland & Goel, 2010) and about 25% of testicular germ cell tumors (Velasco *et al*., 2004). Therefore, results of different studies may vary depending on the DNA source. Also, the inconsistency of the distribution of the genotypes raises the question of a possible chance finding in a small study. Thus, further, preferably population-based, studies are needed to draw any firm conclusions about the role of *POLG*-CAG repeat polymorphism in colorectal cancer.

Conflict of Interest

The authors have declared that no competing interests exist.

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