

## NO ASSOCIATION BETWEEN THE Arg194Trp AND Arg399Gln POLYMORPHISMS OF THE XRCC1 GENE AND COLORECTAL CANCER RISK AND PROGRESSION IN A POLISH POPULATION

T. Sliwinski<sup>1</sup>, R. Krupa<sup>2</sup>, M. Wisniewska-Jarosinska<sup>3</sup>, J. Lech<sup>1</sup>, Z. Morawiec<sup>4</sup>, J. Chojnacki<sup>3</sup>, J. Blasiak<sup>1,\*</sup>

<sup>1</sup>Department of Molecular Genetics, University of Lodz, Banacha 12/16, 90-237 Lodz, Poland

<sup>2</sup>Laboratory of DNA Repair, Department of Molecular Genetics University of Lodz, Lodz, Poland

<sup>3</sup>Department of Gastroenterology and Internal Diseases, Medical University of Lodz, Lodz, Poland

<sup>4</sup>Department of Surgical Oncology, N. Copernicus Hospital, Lodz, Poland

**Background:** The risk of sporadic colorectal cancer can be associated with environmental and lifestyle factors that may be sources of physical and chemical carcinogens, modulated by products of many low penetrance genes. Polymorphisms of DNA repair genes may influence variation in individual DNA repair capacity, which is crucial for preventing genomic instability, which, in turn, may be associated with risk of cancer. XRCC1 is an essential protein for the base excision repair pathway which primarily deals with DNA base modifications, arisen spontaneously or as a consequence of the action of environmental factors. **Aim:** To perform a case-control study and test the association between two polymorphisms in the XRCC1 gene: Arg194Trp and Arg399Gln and colorectal cancer risk and progression. **Methods:** Genotypes were determined in DNA from peripheral blood lymphocytes of 100 colorectal cancer patients and 100 age, sex and ethnic-matched cancer-free controls by PCR RFLP. **Results:** We found that both polymorphisms of the XRCC1 gene were not associated with risk and progression of colorectal cancer in a Polish population. Moreover, there was not such association form the Arg194Trp/Arg399Gln haplotypes. **Conclusion:** The Arg194Trp and Arg399Gln polymorphisms of the XRCC1 gene may not be associated with colorectal cancer in Polish population.

**Key Words:** XRCC1, gene polymorphism, colorectal cancer.

The risk of sporadic colorectal cancer (CRC) can be associated with environmental and lifestyle factors that may be sources of physical and chemical carcinogens, modulated by products of many low penetrance genes [1]. Polymorphisms of DNA repair genes may influence variation in individual DNA repair capacity, which is crucial for preventing genomic instability, which, in turn, may be associated with risk of cancer [2]. Base excision repair (BER) is a DNA repair pathway dealing primarily with DNA base modifications, arisen spontaneously or as a consequence of the action of environmental factors. XRCC1 is a protein essential in BER. Two transitions C26304T and G28152A in the XRCC1 gene result in amino acid substitutions Arg194Trp and Arg399Gln, respectively, and are polymorphisms in many populations [3]. A case-control studies from Egypt and Asia showed that the Arg399Gln variant was associated with a significantly increased risk of CRC [4, 5], but results from Norway did not confirm those findings [6]. In the present work we tried to find an association between the Arg194Trp and Arg399Gln polymorphisms of the XRCC1 gene and CRC in a Polish population.

**Patients.** Blood samples were obtained from 100 patients (36 men and 64 women, median age 65, quartiles: 57, 75 years) with CRC treated from October 2000 to May 2007 at Department of Gastroenterology and Internal Diseases, Medical University of Lodz, Poland Internal Diseases and Department of Onco-

logical Surgery, N. Copernicus Hospital, Lodz, Poland. All patients had histologically confirmed advanced adenocarcinoma of the colon.

Control samples consisted of DNA extracted from blood of age- ( $\pm 5$  years) and sex-matched 100 cancer-free controls. Both patients and controls were of Central Poland origin. Samples were obtained under consideration of all ethical and legal requirements.

**Chemicals and reagents.** QIAamp DNA Blood Mini Kit for isolation of high-molecular-weight DNA was obtained from Qiagen (Chatsworth, CA, USA). *MspI* enzyme was purchased from NE Biolabs (USA). All reagents for PCR reaction were from Qiagen. Electrophoresis was conducted in TAE buffer.

**Genotyping.** Genomic DNA was prepared using the guanidine-isothiocyanate acid isolation method. PCR-RFLP was used to detect the genotypes of the Arg194Trp and Arg399Gln polymorphisms. PCR primers and conditions for amplification described previously by Abdel-Rahman *et al.* [4] were used to generate a 491-bp and a 615-bp products containing the polymorphic Arg194Trp and Arg399Gln sites, respectively. The restriction enzyme *MspI* was used to genotype these polymorphisms. More than 10% of the samples were repeated, and the results were 100% concordant.

**Data analysis.** Distribution of genotypes, haplotypes and alleles between groups were tested using chi-square tests. Potential linkage between genotype and cancer was assessed by the logistic regression. Analyses were performed using STATISTICA 6.0 package (Statsoft, Tulsa, OK, USA).

The 100 cases were divided into groups according to genotype, node status (N) and tumor size (T). The genotype and allele frequencies of the Arg194Trp and

Received: May 20, 2008.

\*Correspondence: Fax: 48042 635 4484

E-mail: januszb@biol.uni.lodz.pl

**Abbreviations used:** BER – base excision repair; CRC – colorectal cancer; XRCC1 – X-ray repair complementing defective repair in Chinese hamster cells 1.

Arg399Gln polymorphisms for the patients did not differ from those for the controls (Table), and genotypes in controls were in Hardy-Weinberg equilibrium ( $P = 0.05$ ). Logistic regression analysis indicated that neither genotype nor allele variants of the polymorphisms were risk factors for CRC in the population under study (see Table). There was not any association between Arg194Trp/Arg399Gln haplotypes and CRC occurrence (data not shown). Additionally, we did not find any correlation between the polymorphisms and clinical factors N and T (data not shown).

**Table.** The genotype and allele frequency and odds ratio (OR) of the Arg194Trp and Arg399Gln polymorphism of the *XRCC1* gene in colorectal cancer

Genotype or Allele	Frequency		OR (95% CI)
	Patients (n = 100)	Controls (n = 100)	
Arg194Arg	0.92	0.91	1.14 (0.42–3.08)
Arg194Trp	0.05	0.08	0.61 (0.19–1.92)
Trp194Trp	0.03	0.01	3.06 (0.31–29.95)
194Arg	0.94	0.95	0.90 (0.38–2.18)
194Trp	0.06	0.05	1.11 (0.46–2.67)
Arg399Arg	0.47	0.39	1.39 (0.78–2.43)
Arg399Gln	0.37	0.45	0.72 (0.41–1.26)
Gln399Gln	0.16	0.16	1.00 (0.47–2.13)
399Arg	0.66	0.62	1.19 (0.79–1.79)
399Gln	0.34	0.38	0.84 (0.56–1.26)
Arg194Arg/Arg399Arg	0.46	0.36	1.51 (0.86–2.67)
Arg194Arg/Arg399Gln	0.33	0.40	0.74 (0.41–1.32)
Arg194Arg/Gln399Gln	0.13	0.25	0.85 (0.38–1.89)
Arg194Trp/Arg399Arg	0.01	0.02	0.49 (0.04–5.55)
Arg194Trp/Arg399Gln	0.03	0.05	0.59 (0.14–2.53)
Arg194Trp/Gln399Gln	0.01	0.01	1.00 (0.06–16.21)
Trp194Trp/Arg399Arg	0.00	0.01	–
Trp194Trp/Arg399Gln	0.01	0.00	–
Trp194Trp/Gln399Gln	0.02	0.00	–

Notes: "CI" – confidence interval, "–" – not estimated.

Our data did not provide evidence to support the reports of Abdel-Rahman et al. [4] and Hong et al. [5] that the 399Gln variant of the Arg399Gln polymorphism could be associated with increased risk of CRC, although our data are in agreement with those reported for a Caucasian population from Norway [6]. This discrepancy is likely due to different allele frequency. Distribution data indicate that the frequency of the 399Gln allele in our controls was 0.38, much higher than those reported by

Abdel-Rahman et al. (0.14), and Hong et al. (0.20). The frequency of the 399Gln allele in our study (0.34) was comparable with that reported for Caucasians (0.37) and higher than that reported for Egyptian and Asian (0.14 and 0.25, respectively). These differences suggest a possible ethnic variability in the allelic distribution of *XRCC1*. We also find that variant Arg194Trp of the *XRCC1* gene is not associated with risk of CRC in a Polish population, what is consistent with data from other populations [4–6]. In conclusion — the Arg194Trp and Arg399Gln polymorphism of the *XRCC1* gene may not be associated with colorectal cancer in Polish population.

## ACKNOWLEDGEMENTS

This work was supported by the grant 505/378 from University of Lodz, Poland and the "Spoleczny Komitet Walki z Rakiem" Lodz Foundation.

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## ОТСУТСТВИЕ АССОЦИАЦИИ МЕЖДУ ПОЛИМОРФИЗМАМИ Arg194Trp И Arg399Gln ГЕНА *XRCC1* И РИСКОМ И ПРОГРЕССИЕЙ РАКА ТОЛСТОЙ КИШКИ В ПОЛЬСКОЙ ПОПУЛЯЦИИ

Риск развития спорадического рака прямой кишки может быть обусловлен факторами окружающей среды, а также образом жизни больных. Эти факторы могут быть источником физических и химических канцерогенов, которые модулируются многими низко-пенетрантными генами. Полиморфизм генов, отвечающих за репарацию ДНК, может влиять на индивидуальную эффективность репарации, которая играет решающую роль в предотвращении геномной нестабильности и может быть ассоциирована с повышенным риском развития рака. Белок *XRCC1* выполняет важную функцию в процессе эксцизионной репарации нуклеотидных оснований, которая распознает модификации нуклеотидных оснований ДНК, появляющихся спонтанно либо в результате действия внешних факторов. **Цель:** провести исследование методом случай-контроль и проверить ассоциацию между двумя полиморфизмами гена *XRCC1* — Arg194Trp и Arg399Gln и риском развития и прогрессии рака толстой кишки. **Методы:** для определения генотипа методом PCR RFLP использовали ДНК, выделенную из лимфоцитов периферической крови больных раком толстой кишки (n = 100) и здоровых доноров (n = 100), без отбора по возрасту, полу или этнической группе. **Результаты:** показано, что полиморфизмы гена *XRCC1* не ассоциированы с риском и прогрессией рака толстой кишки в польской популяции. Более того не выявлена ассоциация с формами Arg194Trp/Arg399Gln. **Выводы:** полиморфизм Arg194Trp и Arg399Gln в гене *XRCC1* не ассоциирован с развитием рака прямой кишки в польской популяции.

**Ключевые слова:** *XRCC1*, полиморфизм гена, рак толстой кишки.