

# NOD2 Arg702Trp Polymorphism in Romanian Patients with Gastric Cancer

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**ABSTRACT: Purpose.** Our study aimed to assess a possible correlation between NOD2 Arg702Trp (rs2066844) polymorphism and gastric cancer risk in a Romanian population. **Material/Methods.** A total of 322 subjects (72 patients with gastric adenocarcinoma and 250 healthy controls) were included. Genomic DNA was extracted from blood leukocytes and NOD2 Arg702Trp polymorphism was genotyped by Real-Time PCR using specific TaqMan probes. **Results.** No statistically significant difference was observed between gastric cancer patients and controls when we compared one genotype with other genotype (the CC genotype serves as reference) (OR 0.45, 95% CI: 0.10 - 2.05) or when we compared allele frequencies (the C allele serves as reference) (OR 0.46, 95% CI: 0.11 - 2.04). We examined separately the association of this polymorphism with tumor site and histologic type and no correlation was found. **Conclusion.** NOD2 Arg702Trp polymorphism is not associated with gastric cancer risk and further investigations are needed to elucidate the contribution of NOD2 gene in gastric carcinogenesis.

**KEYWORDS:** gastric cancer, gene, NOD2 polymorphism, genotype

## Introduction

Gastric cancer remains a major health problem worldwide and despite the advances in surgery and chemotherapy it is still the second leading cause of cancer death across the world [1]. Currently, it is accepted a multifactorial model in gastric carcinogenesis where environmental factors, diet and genetic susceptibility interact in producing the host disease.

NOD2 gene, also called CARD15, has been mapped on 16q21 and encodes nucleotide-binding oligomerization domain-containing protein 2. NOD2 protein is involved in immune system response produced by macrophages or monocytes and defence processes against invading pathogens. Upon triggered by various substances released by viruses or bacteria, NOD2 activates a protein known as nuclear factor-kappa-B (NF-kB), which regulates inflammatory reactions and immune responses [2]. Also, NOD2 protein has been proven to have a key role in autophagy, a conserved process that removes cellular parts that are no longer functional or needed. NOD2 gene has been linked to several diseases such as Blau syndrome or Crohn disease [3,4,5]. The role of NOD2 gene in cancer pathogenesis is controversial, some studies found a positive

correlation whereas others were not able to reproduce it [6].

Our study aimed to assess a possible correlation between NOD2 Arg702Trp (rs2066844) polymorphism and gastric cancer risk in a Romanian population.

## Material And Methods

### Patients

In this research, we included 72 patients diagnosed with gastric adenocarcinoma and 250 controls. All gastric cancer cases were diagnosed by endoscopy and histopathologically confirmed at Clinical Hospital from Craiova, Romania and at the Research Centre in Gastroenterology and Hepatology, University of Medicine and Pharmacy of Craiova, Romania. According to Lauren classification gastric cancer cases were classified into two subgroups: intestinal and diffuse type. The control group consisted of 250 unrelated and healthy volunteers without gastroduodenal lesions or cancer. Written informed consent was obtained from all participants and the Ethics Committee of the University of Medicine and Pharmacy of Craiova approved our study.

### Taq Man Genotyping assay

Blood samples were collected in EDTA tubes from both groups. Genomic DNA was extracted from blood leukocytes using Wizard Genomic

DNA Purification Kit (Promega, Madison, WI), following the manufacturer protocol. NOD2 Arg702Trp polymorphism was genotyped by Real-Time PCR using specific TaqMan probes for each allele (rs2066844, assay C\_11717468\_20, Applied Biosystems Foster City, CA). RealTime PCR was performed on a ViiA™ 7 Real Time PCR System (Life Technologies, Carlsbad, USA) and components of reaction were: sample DN, Universal Master Mix (Applied Biosystems, Foster City, CA), TaqMan SNP Genotyping Assay 40x (Applied Biosystems, Foster City, CA) and DNase-free, sterile-filtered water.

**Statistical analysis**

Genotyping frequencies in control group were tested for compliance with Hardy-Weinberg equilibrium (HWE) using the  $\chi^2$  test. Odds ratios (OR) with 95 % confidence intervals (CIs) were used to determine the association between NOD2 genotypes and gastric cancer. A P-value less than 0.05 was considered statistically significant. All data were analyzed by SPSS software version 17.

**Results**

We genotyped 322 subjects (72 patients with gastric adenocarcinoma and 250 healthy

controls). The mean age of the gastric cancer patients was 67 years. The distribution of cases and controls according to age, sex, and ethnicity are comparable. All gastric adenocarcinoma were Helicobacter pylori positive. The tumor site was cardia in 24 cases and non-cardia in 48 cases (Table 1). Histologically, 43 (59.72%) were intestinal-type and 29 (40.28%) were diffuse-type.

**Table 1. Subjects characteristics**

	<b>Gastric adenocarcinoma (n=72)</b>
Male/Female	42/30
Age (years), mean±SD	67,3±9,1
Location	
- cardia	24
- noncardia	48
Histological type	
- intestinal	43
- diffuse	29

**Table 2. Risk of gastric cancer by genotype**

Polymorphism	Gastric cancer (n=72)	Control (n=250)	OR (95%CI)	p value
NOD2 rs2066844C/T Arg702Trp				
CC	70 (97.22%)	235 (94.00%)	Reference	-
CT	2 (2.78%)	15 (6.00%)	0.45 (0.10 - 2.05)	0.25
TT	0 (0%)	0 (0%)	/	/
C:T	98.61% : 1.39%	97.00% : 3.00%	0.46 (0.11 -2.04)	0.26

**Table 3. Risk of gastric cancer according to tumor site and histological type**

	NOD2 rs2066844C/T Arg702Trp			OR (95%CI); p
	CC	CT	TT	
<b>Tumor site</b>				
- Cardia (24)	23 (95.83%)	1 (4.17%)	0 (0%)	0.68 (0.09-5.39); 0.70
- Noncardia (48)	47 (97.92%)	1 (2.08%)	0 (0%)	0.33 (0.04-2.59); 0.22
<b>Histological type</b>				
- Intestinal (43)	42 (97.67%)	1 (2.33%)	0 (0%)	0.37 (0.05-2.90); 0.28
- Diffuse (29)	28 (96.55%)	1 (3.45%)	0 (0%)	0.56 (0.071-4.39); 0.55

NOD2 rs2066844 (C/T) in the control group showed no deviation from expected genotype frequencies under the Hardy-Weinberg equilibrium ( $p>0.05$ ,  $X^2 = 0.24$ ). The CC and

CT genotype frequencies for both groups are shown in Table 2 and no homozygous for TT genotype was found (Table 2). No statistically significant difference was found between gastric

cancer patients and healthy subjects when we compared one genotype with other genotype (the CC genotype serves as reference) (OR 0.45, 95% CI: 0.10 - 2.05) or when we compared allele frequencies (the C allele serves as reference) (OR 0.46, 95% CI: 0.11 - 2.04). Association of this polymorphism with tumor site and histological type were also examined separately and no important difference was noticed between gastric cancer site (noncardia and cardia) or gastric cancer histology (intestinal and non-intestinal) and controls in the stratified analysis (Table 3).

## Discussion

We evaluated a possible correlation between NOD2 Arg702Trp (rs2066844 C/T) polymorphism and gastric cancer susceptibility in a Romanian population, and no association was observed. Similar results were reported in several studies which assessed NOD2 mutations and gastric cancer susceptibility. The polymorphism Arg702Trp was not associated with histological types of gastric cancer, but 3020insC variant represents a risk factor for developing gastric cancer in Portugal, mainly for intestinal type [7]. Also, no association was found between NOD2 polymorphisms and gastric cancer susceptibility in a German study that included 171 gastric cancer patients and 153 healthy controls [8]. In contrast, an Italian study which included 170 gastric cancer patients and 156 healthy controls reported that Arg702Trp and 1007fs polymorphisms were highly correlated with gastric cancer. Environmental carcinogens and NOD-induced proinflammatory cytokines could be possible reasons by which the studied polymorphisms may increase the risk for gastric cancer [9].

A meta-analysis evaluated several NOD2 polymorphisms such as rs2066842 C/T, rs2066844 C/T, rs2066845 C/G, rs2066847, L1007fsinsC and risk of cancer. The NOD2 rs2066844 C/T polymorphism was associated with increased risk of cancer for individuals bearing TT or CT genotype compared to individuals with CC genotype. The subgroup of analysis revealed that TT+CT genotype was associated with high risk of colorectal cancer, but there was no important association with the development of gastric cancer. This meta-analysis concluded that several NOD2 polymorphisms might be correlated with an increased risk of developing gastrointestinal cancers [10].

Different bacterial and viral impacts in the aetiology of cancer, as well as diagnostic and genotyping methods, differences in sample size, clinical and histological characteristics, ethnicity and chance have been suggested as possible explanations for controversial published findings in various populations [11]. NOD2 variants (Arg702Trp, Gly908Arg and 3020insC) could be risk factors in developing colorectal cancer, and a much higher frequency of all the mutations was found in the gastric cancer group compared to the healthy group in a Greek study [12]. Furthermore, NOD2 polymorphisms have been evaluated in relation with other pathological entities such as inflammatory bowel diseases (IBD) [13,14,15] and NOD2 variants are found associated with increased risk for developing IBD [16, 17].

## Conclusion

NOD2 Arg702Trp polymorphism is not correlated with gastric cancer risk in Romanian population and further investigations are needed to elucidate the contribution of NOD2 gene in gastric carcinogenesis.

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