

## Determination of iNOS-2087A>G Polymorphism in Acute Pancreatitis Patients

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**ABSTRACT:** Purpose: To determine whether single nucleotide polymorphism (SNP) of inducible nitric oxide synthase (iNOS) is involved in susceptibility for acute pancreatitis. Material and Methods: Genomic DNA was extracted from blood samples collected from cases of acute pancreatitis (n=110) and normal population controls frequency matched for age and sex (n=232). iNOS – 2087A>G polymorphism was genotyped using TaqMan allelic discrimination assays. The association of the genetic polymorphism with clinical and pathological data of the patients was evaluated. Results: We have found no significant statistical association between this polymorphism and an increased risk of developing acute pancreatitis. Conclusion: In Romanian population, the risk of developing acute pancreatitis is not increased by the presence of iNOS-2087A>G polymorphism.

**KEYWORDS:** acute pancreatitis, polymorphism, genotype, iNOS

### Introduction

Acute pancreatitis is a localized pathological condition of the pancreatic gland that involves a systemic inflammatory response [1,2]. This is the consequence of an imbalance between pro-inflammatory mediators and anti-inflammatory mechanisms produced through an excess of pro-inflammatory mediators [3,4]. It was demonstrated by several studies that the main players of the pro-inflammatory mediators group are the cytokines [2,3,5,6]. High levels of cytokines are responsible for the activation of reactive oxygen species pathway involved in oxidative stress mechanisms [1,7,8]. In this way a large amount of nitric oxide, a highly reactive free radical, is produced by nitric oxide synthase (NOS) starting from the amino acid L-arginine [7]. It was shown that under certain conditions, nitric oxide has protective and deleterious actions in cardiovascular, neuronal, digestive, and immunological systems [1, 9-11].

Several studies revealed that nitric oxide levels are also increased in the early stages of acute pancreatitis, being associated with a high risk of sepsis and shock (12-14). High levels of nitric oxide are the result of an increased activity of one of the nitric oxide synthase (NOS) isozymes = inducible - NOS (iNOS) [8,14, 15]. Inducible nitric oxide synthase is encoded by the *iNOS* gene and has an increased activity during inflammation, suggesting an important involvement of this enzyme in disorders like acute pancreatitis [1,8,14,15]. Enzyme expression and nitric oxide production might be influenced and modified by several *iNOS* gene polymorphisms [16]. Higher nitric oxide

production was thus associated with an increased enzyme expression determined by single-nucleotide polymorphisms (SNPs) located in the promoter region of the *iNOS* gene (*iNOS* – 954G>C or -2087A>G) [17, 18].

The role of iNOS polymorphisms have been studied in disorders like diabetes, asthma, atopic diseases, achalasia, malaria, acute pancreatitis or malignant tumors where nitric oxide production has been implicated in the pathogenic mechanisms [1, 18-23].

### Subjects and Methods

#### Subjects

The study population comprised 110 patients with acute pancreatitis (AP) and 232 controls with no evidence of pancreatic pathology, either inflammatory or tumoral. Cases and controls were aged >18 years, were of Romanian origin and consented to provide biological samples for genetic analysis. AP was diagnosed based on both clinical symptoms and imaging signs. Biological samples (peripheral whole blood) from both groups were obtained from patients who were admitted at the Emergency County Hospital of Craiova, Romania between January 2013 and July 2014. Controls were selected from individuals who attended the same hospital and had no history of acute or chronic inflammatory diseases, infectious, cancer or autoimmune disorders. The study design was approved by the Ethics Committee of University of Medicine and Pharmacy of Craiova, Romania. All participants were properly informed and signed a written consent and approval form for genetic analysis in accord with the Helsinki

declaration. Demographic data, age, gender, body mass index, diabetes, clinical information (family/personal history of cancer and long-term - at least six consecutive months - drug use) were also collected for each patient.

### SNP genotyping

All participants were genotyped for *iNOS* -2087A>G (rs2297518). The genotyping was performed in a 5- $\mu$ L reaction volume using TaqMan probes fluorescently labeled with FAM or VIC and following the protocol recommended by the supplier (Applied Biosystems, Foster City, CA, USA).

Real Time PCR cycling conditions (Real Time ViiA7 - Applied Biosystem) for the denatured reactions were 95°C for 10 minutes, followed by 45 cycles of 92°C for 15 seconds and 60°C for 90 seconds annealing temperature.

Interpretation of samples was done using ViiA™ 7 Software v1.0 with the Allelic Discrimination option.

### Statistical analysis

The Hardy-Weinberg equilibrium was tested to compare the observed and expected genotype frequencies among cases and controls. To estimate the association between *iNOS* polymorphism and AP, we calculated odds ratios (ORs) and 95% confidence intervals (95%

CI) using logistic regression analysis. Genotypes were assessed using indicator variables with the common homozygote as reference. A two-sided P value < 0.05 was considered to be statistically significant.

### Results

All 342 samples harvested from AP patients and healthy controls were genotyped. Genotyping was performed in 110 AP patients and 232 controls.

The average age of our subjects with acute pancreatitis was 59.54, while for the control group it was 60.65. Based on disease severity, out of the total 110 cases of acute pancreatitis 23% (25 cases) had a mild form of the disorder, while 77% (85 cases) were severe cases of pancreatitis.

The polymorphism we studied was in Hardy-Weinberg equilibrium for both acute pancreatitis and healthy control groups.

The genotype frequency for *iNOS* -2087A>G polymorphism is shown in table 1. As shown, with a p value of 0.631 and an OR value of 0.902 (95%CI: 0.590 – 1.378) we have no significant statistical association between the presence of this polymorphism and the increased risk for patients to develop acute pancreatitis.

**Table 1: Acute pancreatitis risk associated with *iNOS* – 2087A>G genotype**

<i>iNOS</i> – 2087A>G	Acute pancreatitis	Control	OR(95%CI)	p
<b>GG</b>	76(69.09%)	158 (68.10%)	Reference	
<b>AG</b>	31 (28.18%)	63 (27.16%)	1.023 (0.614 – 1.703)	0.930
<b>AA</b>	3 (2.73%)	11 (4.74%)	0.567 (0.154 – 2.092)	0.373
<b>A allele Carriers</b>	37 (16.82%)	85 (18.32%)	0.902 (0.590 – 1.378)	0.631

### Discussion

*iNOS* is induced in response to inflammation. *iNOS* is an enzyme that can generate large quantities of nitric oxide in response to cytokines and endotoxins, being involved in the pathway of reactive oxygen species [1, 8]. *iNOS* gene comprises 27 exons and is found on chromosome 17q11.2 [24, 25]. Several studies associated polymorphisms of this with the risk of developing various diseases [16,20,21,23, 24,26]. In a study conducted by Ozhan et al it was shown that there is an association between

*iNOS* -2087A>G polymorphism and susceptibility to acute pancreatitis [1].

In the present study, no statistical association between *iNOS* -2087A>G polymorphism presence /absence and risk of developing acute pancreatitis has been found. Furthermore, a stratified analysis based on disease severity (mild and severe acute pancreatitis) has been done. Likewise the stratified analysis performed showed no statistically significant results that could be associated with the risk of developing acute pancreatitis (data not shown).

## Conclusion

According to these results, *iNOS* – 2087A>G polymorphism was not associated with an increased risk of Romanian population for acute pancreatitis. Further extensive studies are needed on larger groups in order to clarify the role of *inducible nitric oxide synthase (iNOS)* polymorphisms in acute pancreatic inflammation.

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