# Evaluation of the prognostic usefulness of 420C/T, 750A/T, –857C/T polymorphisms in the *TNF-α* gene among Polish patients with Crohn's disease – original paper

Analiza przydatności prognostycznej polimorfizmów 420C/T, 750A/T oraz –857C/T w obrębie genu *TNF-α* w populacji polskiej u pacjentów z chorobą Leśniowskiego-Crohna

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**Key words:** Crohn's disease, polymorphisms, pyrosequencing, *TNF-α* gene.
**Słowa kluczowe:** choroba Leśniowskiego-Crohna, polimorfizmy, pirosekwencjonowanie, gen *TNF-α*.

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## Abstract

**Introduction:** Crohn's disease (CD), together with ulcerative colitis (UC), belongs to inflammatory bowel diseases (IBD). The aetiology of CD is still unknown but it is suspected that genetic, environmental and immunological factors play a major role in the background of CD. One of the genes investigated in the field of Crohn's pathogenesis is *TNF-α (tumor necrosis factor α)*.
**Aim:** To analyze three single nucleotide polymorphisms (SNP): two of them in the gene sequence and one in the promoter region (420C/T, 750A/T, –857C/T).
**Material and methods:** The investigated group consisted of 96 Polish patients with CD. Sequential analysis was performed using the pyrosequencing method.
**Results:** It was found that none of the three polymorphisms chosen for this analysis showed any differences among Polish patients with CD.
**Conclusions:** Polymorphisms 420C/T, 750A/T and –857C/T do not differentiate patients with CD and cannot be used for prognostics in the Polish population.

## Streszczenie

**Wprowadzenie:** Choroba Leśniowskiego-Crohna (ChLC) razem z wrzodziejącym zapaleniem jelita grubego (WZJG) należy do nieswoistych chorób zapalnych jelit (NChZJ). Do dziś etiologia schorzenia nie jest znana, ale podejrzewa się znaczący udział czynników środowiskowych, immunologicznych i genetycznych. Jednym z genów uważanych za kluczowy w podłożu ChLC jest gen *TNF-α* (*tumor necrosis factor α*).
**Cel:** Przeanalizowanie trzech polimorfizmów typu SNP (*single nucleotide polymorphisms*) w obrębie genu TNF-α: dwóch w regionie samego genu oraz jednego w obszarze paromotorowym (420C/T, 750A/T, –857C/T).
**Materiał i metody:** Badana grupa składała się z 96 pacjentów cierpiących na ChLC. Zastosowano metodę pirosekwencjonowania.
**Wyniki:** Wykazano, że w obrębie trzech badanych polimorfizmów nie występuje żadna zmienność genetyczna w populacji polskiej.
**Wnioski:** Polimorfizmy 420C/T, 750A/T i –857C/T nie różnicują pacjentów z ChLC i nie mogą być używane jako czynnik prognostyczny w tej chorobie w populacji polskiej.

## Introduction

Inflammatory bowel diseases (IBD) most commonly occur in two different forms: as ulcerative colitis (UC) and as Crohn's disease. In 10-15% of cases qualification to either of these disease categories is impossible and in this case indeterminate colitis (IC) is diagnosed.

Crohn's disease is becoming a more frequent cause of hospitalizations of Polish patients on hospital words. It is characterized by an inflammatory process in the wall of the GI tract, loose stools, ulcerations and in advanced stages of the disease also by fistulas and abscesses. Symptoms of the disease can occur in the whole length of the GI tract, although they occur most commonly in the terminal ileum and colon. Besides the symptoms from the gastrointestinal tract, extra-intestinal symptoms also occur, for example: iritis, primary sclerosing cholangitis, arthropathies, skin changes as well as stomatitis aphthosa. The cause of the disease to this day remains unknown, although more and more evidence points to the fact that environmental factors (smoking, diet, etc.) and genetic factors (studies of twins and patient family members) play a large role in the pathogenesis of Crohn's disease. These factors cause imbalances in the body's immune system initiating a cascade of immunological reactions which are seen as inflammatory changes in the wall of the gastrointestinal tract.

Currently it is believed that Crohn's disease is a multifactorial and a polygenic disease. Genes *CARD15/NOD2*, *DLG5*, *OCTN1*, *OCTN2* as well as the *TNF-α* gene are suspected to be involved in the basis of Crohn's disease. The TNF-α (*tumor necrosis factor α*) gene is one of the key factors suspected to be involved in the pathogenesis of Crohn's disease [1-7]. This key pro-inflammatory cytokine takes part in many bodily functions including the metabolism of lipids, coagulation, insulin resistance, acute phase reactions as well as endothelial functions. Many studies have been done [8-11], as a result of which the *TNF-α* gene was localized on chromosome 6p21.3. It was found that interactions of TNF-α with TNF receptor play a key role not only in the immunological response but also in the process of programmed cell death, or apoptosis, proliferation of cells and cell differentiation [12-14].

Many polymorphisms of the SNP type are localized in the area of the *TNF-α* gene as well as in its promoter region. It was found that changes of the SNP type localized in the promoter region can influence the level of expression of the *TNF-α* gene as well as taking part in the basis of many diseases. For example, the nucleotides localized in positions –238A/G and –308A/G are believed to take part in the pathogenesis of asthma, psoriasis and rheumatoid arthritis [15-18].

This study was undertaken to investigate whether there are any significant SNP changes in the area of the *TNF-α* gene that can influence the future diagnostics of Crohn's disease and susceptibility to biological treatment.

## Material and methods

In the present work three polymorphisms were chosen for analysis: two are located in the vicinity of the *TNF-α* gene (420C/T and 750A/T), and one in the promoter region of the gene (–857C/T). These are polymorphisms not frequently analyzed in epidemiological studies, and when they were, they showed very little differences. Because it is sometimes observed that polymorphisms that occur frequently in one population do not show any changes in another, we decided to check whether there is any significant difference in the area of the *TNF-α* gene in Polish patients with CD. The objective of the work was to specify whether these particular polymorphisms show variability in a Polish population of patients with Crohn's disease.

Studies were conducted on the DNA isolated from systemic blood of 96 patients from the Department of Gastroenterology, Human Nutrition and Internal Diseases of Poznan University of Medical Scienes, Poland. Patients were qualified for the studies on the basis of clinical and radiological tests as well as histopathological results. Blood samples were collected for EDTA and then isolated by a standard GTC method [19]. For the analysis three polymorphisms of the SNP type were chosen: 420C/T, 750A/T, –857C/T. Sequential analyses were conducted for eachof the polymorphisms using a real-time sequencing method, called pyrosequencing. Specially designed starters were used: two for each of the standard polymerase chain reactions

**Table I.** List of starters used for analysis
***Tabela I.*** *Wykaz starterów użytych do pirosekwencjonowania*

| Starter | Labelling | Sequence |
|---|---|---|
| PiroTNF-α_-857_F | biotin | GGACCCCCCCTTAACGAA |
| PiroTNF-α_-857_seq | – | CTGGGGCCCTCTACA |
| PiroTNF-α_-857_R | – | ATCACCCCCGGGAATTCAC |
| PiroTNF-α_420_F | – | TAGGGGGGTATTTTCTAGGAAGTT |
| PiroTNF-α_420_seq | – | TCATCTTCTCGAACCC |
| PiroTNF-α_420_R | biotin | AGCGAGTCCTTCTCACATTGTC |
| PiroTNF-α_750_F | biotin | GGCCAAGCCCTGGTATGA |
| PiroTNF-α_750_seq | – | GACCCCTCCCAGATA |
| PiroTNF-α_750_R | – | ATAGTCGGGCCGATTGATC |

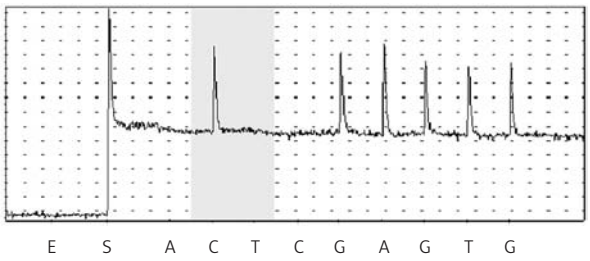


**Fig. 1.** Pyrosequencing of polymorphism *TNF-α* 420 C/T (Leu84Pro, rs4645843). Analysed sequence: C/TGAGTGAC AAGCCTGTAG CCCATGTT. Result: CC

***Ryc. 1.*** *Pirosekwencjonowanie TNF-α 420 C/T (Leu84Pro, rs4645843). Analizowana sekwencja: C/TGAGTGAC AAGCCTGTAG CCCATGTT. Wynik: CC*

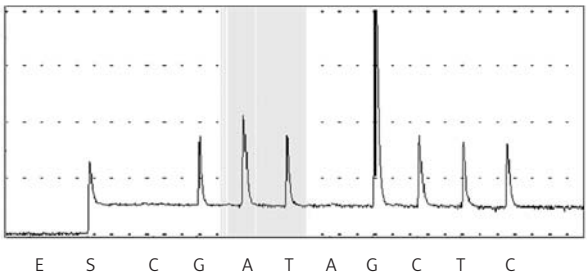


**Fig. 2.** Pyrosequencing of polymorphism *TNF-α* 750A/T (Asn194Ile, rs11574936). Analysed sequence: GA/TTGGGCT CATACCAGGG CTTGGCCTC. Result: AA

***Ryc. 2.*** *Pirosekwencjonowanie TNF-α 750A/T (Asn194Ile, rs11574936). Analizowana sekwencja: GA/TTGGGCT CATACCAGGG CTTGGCCTC. Wynik: AA*

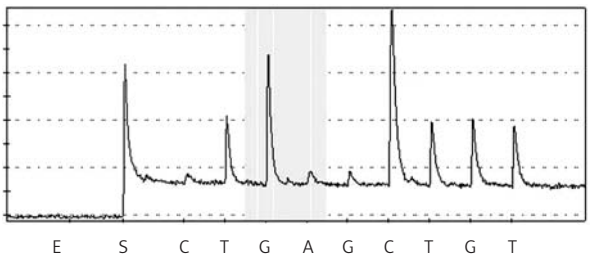


**Fig. 3.** Pyrosequencing of polymorphism *TNF-α* in the promoter region –857C/T. Analysed sequence: TGA/GCCCTG TCTTCGTTAA GGGGGGGTCC (sequenced reverse strand of DNA). Result: GG

***Ryc. 3.*** *Pirosekwencjonowanie TNF-α region promotorowy –857C/T. Analizowana sekwencja: TGA/GCCCTG TCTTCGTTAA GGGGGGGTCC. Wynik: GG*

(PCR) and one sequential starter (labelled with biotin) (Table I).

The project was accepted by the ethics committee of the University School of Medical Sciences in Poznan.

## Results

While in the NCBI database 420C/T, 750A/T and –857C/T polymorphisms in the *TNF-α* gene region show very little polymorphism, in the population of Polish patients with Crohn's disease no differences were observed in these genome locations. Polymorphism 420C/T in all the cases analyzed showed CC variant (Figure 1), in 750A/T variant AA was the only one present (Figure 2), and for polymorphism –857C/T only variant CC was shown (Figure 3).

## Discussion

The *TNF-α* gene is thought to be one of the key factors responsible for Crohn's disease conditioning. In many other research projects investigators have shown the major role of different polymorphisms in the area of the *TNF-α* gene that can play a major role in the pathogenesis of the disease. Because sometimes it can be observed that among different populations the frequency of specific alleles or genotypes varies, we decided to check whether such differences can be observed in the Polish population in a group of Crohn's patients. In the NCBI database polymorphisms 420C/T, 750A/T and –857C/T show slight diversity (among others studied were African, Caucasian and Hispanic populations). Conducted studies showed that in the Polish population, these polymorphisms were not observed in any of the 96 patients. It did not escape our attention that the group of patients was quite small, but due to financial restrictions and no significant results in the first part of the studies we decided to finish with the group of 96 individuals.

According to this study we expect that there are other genetic factors in the Polish population which play a key role in Crohn's disease conditioning.

## Conclusions

Although the *TNF-α* gene is expected to play one of the major roles in the background of Crohn's disease, no differentiation was observed in the Polish population. Because of this, it cannot be used for prognostics in the Polish population.

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