

Preliminary Study Regarding the Association between Tumor Necrosis Factor Alpha Gene Polymorphisms and Childhood Idiopathic Nephrotic Syndrome in Romanian Pediatric Patients

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ABSTRACT

Background: Childhood idiopathic nephrotic syndrome (INS) is one of the most common glomerular diseases, characterized by heavy proteinuria, hypoalbuminemia, dyslipidemia and generalized edema. Although some progresses were made regarding the pathogenesis of this disease, there are a lot of questions still left unanswered. Some of them involve the implications of several cytokines, including tumor necrosis factor alpha (TNF-alpha), in the development and clinical course of INS.

Objective: Our objective was to analyze the role of two single nucleotide polymorphisms of TNF-alpha gene in the development of pediatric INS and their implication in the response to corticosteroid therapy.

Material and methods: Seventy patients with INS and 159 healthy controls were included in this study. They were analyzed for TNF-alpha gene polymorphisms by using polymerase chain reaction. The two SNPs (rs1799724/-857C/T and rs1800629/-308G/A) were genotyped by TaqMan Genotyping Assays, association tests were performed and p values <0.05 were considered significant.

Results: Minor alleles frequencies were 15.72% in INS patients versus 18.55% in controls for 857*T allele and 11.43% in INS versus 13.2% in controls for 308*A allele. Although the minor alleles were more frequent in controls than in patients, the difference was not statistically significant (p=0.46, OR=0.818 and p=0.59, OR=0.848).

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Analyzing the response to corticosteroid therapy, we found a low frequency of 857*T allele in steroid resistant patients (9.09%) compared to steroid sensitive patients (16.95%) and controls (18.55%). Regarding 308*A allele, the frequencies were 18.18% in the corticoreistant group and 10.17% in the corticosensitive one. None of them was statistically significant ($p>0.05$).

Conclusions: We conclude that neither -857C/T, nor-308G/A polymorphisms of TNF-alpha gene are associated with the susceptibility and response to steroid treatment of INS in our population. Given the small sample size used, future studies are necessary to clarify the results observed in the present study.

Keywords: nephrotic syndrome, TNF-alpha polymorphisms, steroid response

INTRODUCTION

Idiopathic nephrotic syndrome (INS) is the most frequent glomerular disease in childhood. The alterations of the selective glomerular permeability barrier – caused by damage to podocytes and foot process effacement – lead to intense proteinuria (1), which is the hallmark of this disease. In the USA and Europe, the annual incidence of pediatric INS has been estimated to be between 1 and 3 per 100,000 children below the age of 16 (2-4), varying with age, race and geography.

Children with NS have a decreased quality of life (5), are at risk of a wide range of complications associated with significant morbidity (infection, thromboembolism and dyslipidaemia) and experience mortality rates of up to 2.7% (6).

The pathogenesis of INS is yet unknown, but evidence such as efficacy of immunosuppressive treatment (especially prednisone), indicate that the immune system may play a crucial pathogenic role in non-genetic forms of this disease. However, despite extensive investigation, the mechanism by which immune dysregulation leads to disruption of the glomerular filtration barrier and consequently to proteinuria is poorly understood (7).

Cytokines play a critical role as mediators of inflammation and as progressive factors in INS, some of them being considered prime candidates for mediating INS progression (8-10). Among them, tumor necrosis factor alpha (TNF-alpha), a potent immunomediator and proinflammatory cytokine, has been implicated in the pathogenesis of a large number of diseases (11). Several studies reported the association of TNF-alpha gene variations with childhood INS (12-14).

First line treatment – corticotherapy – is effective in most of the patients, meaning they attain

remission following this treatment (steroid sensitive) (15). The ones who fail to respond to treatment are called steroid resistant. However, some of the patients who respond to therapy will undergo relapse at least once, with 50% of them being frequent relapsers or developing steroid dependence (15). The mechanisms underlying the difference in response to steroid therapy are not well understood, genetic factors may be involved (16).

The present study was conducted to investigate the possible association between TNF-alpha gene polymorphisms and INS in a group of Romanian children.

MATERIALS AND METHODS

Patients and controls

A total number of 229 unrelated Caucasian individuals of Romanian origin was included in the study. Idiopathic nephrotic syndrome patients (N=70 patients, 50 M/20 F, mean age nine years) were recruited from the Nephrology Department of "M. S. Curie" Emergency Hospital (Bucharest, Romania). All patients with INS were diagnosed according to the Kidney Disease Improving Global Outcomes (KDIGO) guideline criteria (17) and were categorized according to their response to corticotherapy: steroid sensitive – attainment of complete remission within initial four weeks of corticosteroid therapy (N=59, 42 M/17 F, mean age eight years) and steroid resistant – failure to achieve complete remission after eight weeks of corticosteroid therapy (N=11 patients, 8 M/3 F, mean age 9 years 11 months) (17).

We used a total number of 159 controls (103 M/56F, mean age 39 years), with no history of proteinuria and edemas.

The study was approved by the Ethic Committee of "Marie Curie" Hospital, Bucharest, Roma-

SNP	INS (N=70)	Control (N=159)	*P-value	OR, 95% CI
<i>TNF-alpha</i> – G308A rs1800629				
Genotypes				
GG	54 (77.15%)	122 (76.73%)		
GA+AA	16 (22.85%)	37 (23.27%)	p=0.94	0.977 (0.501-1.906)
Minor allele				
A	11.43%	13.2%	p=0.59	0.848 (0.459-1.566)
<i>TNF-alpha</i> – C857T rs1799724				
Genotypes				
CC	49 (70%)	106 (66.67%)		
CT+TT	21 (30%)	53 (33.34%)	p= 0.61	0.857 (0.467-1.575)
Minor allele				
T	15.72%	18.55%	p=0.46	0.818 (0.479-1.399)
*p-value < 0.05 is significant; OR: odds ratio; CI: confidence interval; INS: idiopathic nephrotic syndrome.				

TABLE 1. Genotypes and allele frequencies of *TNF-alpha* gene polymorphisms among INS and control groups

nia. The details were explained to all patients and controls and consent for genetic screening was obtained.

DNA extraction and genotyping

Genomic DNA was obtained from EDTA treated peripheral blood samples using QIAamp DNA Blood Mini Kit(Qiagen, Hilden, Germany) according to manufacturer protocol.

Two single nucleotide polymorphisms of *TNF-alpha* gene were selected for this study: rs1799724 (-857C/T) and rs1800629 (-308G/A). Single nucleotide polymorphisms (SNPs) genotyping was performed by real-time polymerase chain reaction (PCR) with TaqMan Allelic Discrimination AssaysC_11918223_10 and C_7514879_10, respectively (Real time PCR System, Applied Biosystems, Foster City, CA).

Statistics

The Hardy-Weinberg equilibrium (HWE) was tested using the Chi-square test. The association tests and HWE tests were performed with DeFinetti program (<http://ihg2.helmholtzmuenchen.de/cgi-bin/hw/hwa1.pl>) and p values ≤ 0.05 were considered statistically significant.

Alleles and genotypes frequencies of the studied SNPs were compared between INS patients and controls and between the steroid sensitive group and the steroid resistant group.

RESULTS

We have performed a case control association study of *TNF-alpha* gene single nucleotide polymorphisms in a group of Romanian idiopathic nephrotic syndrome patients versus ethnically matched controls.

The control and patients groups showed no departure from HWE for all studied SNPs.

The frequencies of *TNF-alpha* gene SNPs genotypes and alleles in controls and INS patients are presented in Table 1. For rs 1799724, the frequency for minor allele T was 15.72% in the INS group versus 18.55% in the control group. Although the minor allele T was more frequent in controls than in patients, the difference was not statistically significant (p=0.46, OR=0.818).

The analysis of the two groups (INS patients and controls) revealed similar distribution of the investigated polymorphisms.

When the INS patients were subdivided according to steroid responsiveness, no significant statistical difference was found in allele and genotypes frequencies between the steroid-sensitive and steroid-resistant subgroups when compared to each other, nor when compared to controls (Table 2).

DISCUSSION

In the present paper we studied the association between two *TNF-alpha* gene polymorphisms

SNP	SSNS (N=59)	SRNS (N=11)	*P-value	OR, 95% CI
<i>TNF-alpha</i> – G308A rs1800629				
Genotypes				
GG	47 (79.66%)	7 (63.64%)		
GA+AA	12 (20.34%)	4 (36.36%)	p=0.24	2.238 (0.562-8.917)
Minor allele				
A	10.17%	18.18%	p=0.29	1.963 (0.570-6.763)
<i>TNF-alpha</i> – C857T rs1799724				
Genotypes				
CC	40 (67.8%)	9 (81.82%)		
CT+TT	19 (32.2%)	2 (18.18%)	p= 0.35	0.46 (0.092-2.38)
Minor allele				
T	16.95%	9.09%	p=0.53	0.49 (0.106-2.265)
*P-value < 0.05 is significant; OR: odds ratio; SSNS: steroid sensitive nephrotic syndrome; SRNS: steroid resistant nephrotic syndrome.				

TABLE 2. Genotypes and allele frequencies of *TNF-alpha* gene polymorphisms among SSNS and SRNS groups

(G308A and C857T) and the susceptibility for developing INS in Romanian population. We investigated as well the implication of those polymorphisms in the response to corticosteroid therapy.

Tumor necrosis factor alpha (*TNF-alpha*) is a potent immunomodulator and pro-inflammatory Th1 cytokine (19). Polymorphism at position -308 of the *TNF-alpha* gene promoter (G to A base transition) has been linked to increased TNF transcription (20, 21), earlier studies showing an increase of *TNF-alpha* synthesis and gene expression in patients with INS (22, 23).

Several studies have investigated the association between cytokines gene polymorphisms and INS in worldwide populations, but not in Romanian population (12-14, 18). The results of our study showed no significant difference between the patient group and controls regarding the *TNF-alpha* gene SNPs genotypes or alleles distribution. This is in agreement with Kim Sung-Do *et al.*, who genotyped the G to A exchange at position -308 of the *TNF-alpha* gene, and found no difference in allele frequency between the two groups (18). In contrast, Madani *et al.* concluded in their study that AA genotype and A allele were significantly higher in the INS group than controls (12). The same results were obtained by Jafar T *et al.* in a study conducted in 2011 (14).

Given the fact that corticosteroids are the first line agents used to treat pediatric INS, but their long term use is marked by a lot of serious ad-

verse effects, it is very important to see if we can find some early markers that would allow us to determine the effectiveness of these agents in remission induction.

We could not find any association between the studied polymorphisms and the response to corticosteroid therapy. In 2014, Madani *et al.* investigated the role of cytokine genes polymorphisms (*TNF-alpha*-G308A, IL6-G174C and IL4-C590T) and the response to corticosteroid therapy in children with INS in Egypt. When comparing steroid-sensitive patients with steroid-resistant ones, the results showed that AA genotype is higher in the first group (though not reaching statistical significant difference), but A allele distribution is significantly higher in the steroid-sensitive group compared to the steroid-resistant one (12). More than that, in the study of Jafar T *et al.*, when the steroid-resistant group was compared with steroid-sensitive group, significant association was found at both the genotypic level, and allelic level, in the steroid-resistant group (14). In 2008, Tripathy G *et al.* investigated the association between some cytokine gene polymorphisms (IL-4, IL-6, *TNF-alpha*) and the response to corticosteroid therapy, in 150 children with INS. They observed that *TNF-alpha* showed a strong association at both the genotypic and allelic levels in steroid-sensitive and steroid-resistant groups, demonstrating that it may be considered one of the genetic risk

factors affecting the steroid response among the North Indian INS patients (13).

Regarding -857C/T TNF alpha gene polymorphism, to our knowledge, this is the first paper addressing this issue. Our data did not show any significant difference between INS population and controls in terms of genotype and allele frequencies; also, we found no role of this polymorphism in predicting response to steroid treatment in Romanian pediatric INS patients.

DNA data should be interpreted carefully, as different populations present different genetic background, making results difficult to apply in practice outside the studied population. Given the small sample size used, future studies with a

larger scale are necessary to clarify the results observed in the present study and to determine whether *TNF-alpha* polymorphisms play a role in INS development and course of treatment.

CONCLUSION

Our results show that, in our population, neither -857C/T, nor -308G/A polymorphisms of TNF-alpha gene are associated with the susceptibility and the response to steroid treatment of INS. □

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