
Tumour Necrosis Factor-Alpha Gene Polymorphism (-308 G-A) in Turkish Pediatric Thrombosis Patients

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ABSTRACT

Tumour necrosis factor-alpha (TNF-a) plays an important role in clot formation by activating platelets, monocytes and endothelial cells and inducing procoagulant substances such as negatively charged phospholipids or tissue factor. There is a genetically controlled inter-individual variation of TNF-a production. Carrying TNF2 allele could have a slight protective effect against the occurrence of stroke in Sickle Cell Disease patients. We aimed to study this polymorphic site in Turkish children with the diagnosis of thrombosis.

Key Words: Tumour necrosis factor-alpha, Thrombosis.

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INTRODUCTION

Tumour necrosis factor-alpha (TNF-a) is an immunomodulatory cytokine, playing an important role in clot formation by activating platelets, monocytes and endothelial cells and inducing procoagulant substances such as negatively charged phospholipids or tissue factor. There exist inter-individual variation of TNF-a production, which is genetically controlled. A polymorphism due to a G to A transition at nucleotide -308 in the TNF-a gene promoter is a much stronger transcriptional activator than the common allele which is designated as TNF-2 allele^[1-3].

Although previous studies revealed no link between the genetic regulation of TNF production and venous thromboembolic disease, and antiphospholipid syndrome in adults; Carroll et al concluded that carrying TNF-2 allele could have a slight protective effect against the occurrence of stroke in Sickle Cell Disease patients^[2-4]. Our previous data on the genetic markers in pediatric cerebral infarcts revealed a discrepancy compared to adult studies^[5,6]. We aimed to study this polymorphic site in Turkish children with the diagnosis of thrombosis.

MATERIALS and METHODS

Seventy patients having thrombosis were included in the study. All patients were below the age of 18 (10 months to 18 years). All were clinically diagnosed, and the thrombotic event was verified with different techniques such as doppler ultrasonography, magnetic resonance imaging of the brain. Clinical distribution of the patients were given in Table 1.

Ninety-six controls were consecutively selected among healthy unrelated subjects from the same geographic area of Turkey without personal and family history of thrombosis and stroke^[7]. Blood for mutation analysis was obtained after written informed consent from the parents, and DNA was extracted by conventional methods.

TNF- α (nt-308) genotyping was performed by gene amplification and BspHI digestion (overnight at 37°C) according to previously described method^[2]. A 194 bp product was amplified using the primers 5'-AATGGAAATAGGTTTT-GAGGGT*CAT-3'; and 5'-TCTCGGTTTCTTCTC-CATCGC-3' in which T* was not present in the genomic sequence and was introduced to create a potential BspHI (Biolabs, USA) site. Cycling was at 94°C for 5 min followed by 35 cycles of 55°C for 20 s, 72°C for 20 s and 94°C for 20 s by a final extension step for 7 min at 72°C (Temp Cycler, England). PCR product was electrophoresed in a 1% agarose gel. PCR product was digested overnight at 37°C with BspHI and electrophoresed in a 2.5% agarose gel. BspHI digestion results in two fragments of 169 and 25 bp if an A is present at nt-

308.

RESULTS

The genotype distribution for the TNF-2 polymorphism is shown in Table 1. There wasn't any difference between the patients and controls in the frequency of the TNF-2 allele.

DISCUSSION

Patients with high TNF- α levels might be at increased risk of developing thrombotic complications due to the effect of this cytokine on the endothelium. However, this was not the case in adults with deep vein thrombosis and in patients with antiphospholipid syndrome in Systemic Lupus Erythematosus^[2,3]. Further, Carroll et al concluded that carrying TNF-2 allele in Sickle cell patients with stroke might have some slight protective effect of the gene against the occurrence of stroke^[4]. As there is a discrepancy between the published studies, we studied this polymorphism in our pediatric patients. Our data revealed no difference between the controls and thrombosis patients. However, it is interesting to note that none of the pediatric cerebral infarct patients carried the TNF- α -308 A (TNF-2) allele. Further, four of the seven Budd-Chiari patients carried the TNF-2 allele (55%).

Our data did not support the hypothesis that TNF-2 allele is associated with protection effect to cerebral infarcts in our pediatric cerebral infarct patients. But the possible role of TNF-2 allele in the pathogenesis of the Budd-Chiari syndrome

Table 1. TNF-2 allele distribution in Turkish pediatric thrombosis patients

	n	TNF-2	%	TNF-2 frequency
Control group	96	17(1)	17	0.093
Ped. Thrombosis patients	70	7	10	0.10
Cerebral infarct	50	0	0	
Budd-Chiari	7	4	55	
L-Asparaginase related	6	2		
Veno Occlusive Disease (BMT)	4	1		
Deep Vein Thrombosis	2	0		
DIC	1	0		

needs further investigation.

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