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Vesicoureteric reflux and tumor necrosis factor-α gene polymorphism

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Abstract *Aim:* To determine the role of tumor necrosis factor-α (TNF-α) gene polymorphism in promoting renal scarring among patients with vesicoureteric reflux (VUR). This genetic variant involves a guanosine to adenine transition at position -308, and this single-base polymorphism is associated with increased transcription of the TNF-α gene. Recent studies suggest that the TNF-α gene may be associated with predisposition to renal scarring.
Patients and methods: A total of 195 (51.8% females) patients with VUR demonstrated by voiding cystourethrogram were recruited, 126 of them with reflux nephropathy diagnosed by dimercaptosuccinic scan. The control group included 266 healthy individuals. Genotyping was performed by polymerase chain reaction and digestion with a restriction enzyme.
Results: Allele frequencies of -308G and -308A were 83.8% and 16.2%, respectively in patients with VUR and 88.9% and 11.1%, respectively in controls ($P < 0.05$). No differences were found in genotype distribution related to presence/absence of renal scars. There was no relationship between TNF-α genotype and grade of VUR or the presence of proteinuria.
Conclusions: Our data suggest that the TNF-α AA genotype is not associated with reflux nephropathy. The TNF-α-308A allele could be related to a higher susceptibility to VUR.

Introduction

Reflux nephropathy (RN) is defined by the presence of renal scars in children with VUR. RN is an important cause of hypertension and chronic renal

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failure (CRF) in children [1]. Current knowledge indicates that evolution of VUR is not equal in all patients, suggesting the influence of different factors, including genetics [2]. Recent studies have shown that proinflammatory cytokines, including tumor necrosis factor-α (TNF-α), initiate the parenchymal damage leading to renal scarring [3]. TNF-α is produced by monocytes/macrophages, and renal mesangial and epithelial cells, and it stimulates the synthesis of other cytokines and growth factors [4].

A single-base polymorphism at position -308 in the promoter region of the TNF-α gene, involving a transition of guanine to adenine, has been described [5]. The -308A genotype (TNF2) is associated with increased transcription in vitro of TNF-α [6] that may be related to predisposition to inflammatory diseases. Carriage of TNF2 has been studied in patients with IgA nephropathy [7], end-stage renal failure [8,9], nephrotic syndrome [10] and RN [11], with controversial results. The aim of our study was to determine the influence of the TNF-α-308 polymorphism in promoting renal scarring among patients with VUR.

Patients and methods

Patients and controls

This study involved 195 patients at a pediatric nephrology center who were classified into three groups according to the clinical and radiological data: VUR ($n = 69$), RN ($n = 99$) and CRF due to RN ($n = 27$). Each patient was evaluated for the presence of hypertension, febrile UTI and family history of VUR. Renal function was evaluated by creatinine clearance, estimated by the method of Schwartz et al. [12]. VUR was determined by VCUG and classified as grade I—V according to the International Reflux Study [13]. A technetium-99m DMSA renal scan (99mTc-DMSA) was performed in all patients at least 6 months after the last episode of UTI, and RN was defined as a hypogenic area. CRF was defined as creatinine clearance below 70 ml/min/1.73 m^2. Patients with neurogenic bladder, urethral valves or any associated urological malformations were excluded. Informed consent was required from patients or their guardians prior to inclusion in this study. The control group consisted of 266 healthy blood donors.

Determination of genotypes

Genomic DNA was extracted from peripheral blood leukocytes using a salting out procedure. DNA concentrations were determined by spectrophotometry. For determination of -328G/A polymorphism of the TNF-α gene, the primers used were 5′-GCAATAGGTTTTGAGGGCCAT-3′ and 5′GGGACAC ACAAGCATCAAG-3′. Briefly, the polymerase chain reaction consisted of a denaturing cycle at 94 °C for 2 min, 30 cycles at 94 °C for 30 s, 58 °C for 40 s and 72 °C for 45 s, and a final extension at 72 °C for 5 min. Products were digested with *Nco*I and visualized on a 3% agarose gel. The -308G generated two fragments of 122 bp and 25 bp while -308A gave a single 147-bp fragment.

Statistics

All data were expressed as means with standard deviations. Allele frequency was defined as the number of occurrences of the test allele in the population divided by the total number of alleles. The distribution of the genotypes among the different groups was compared using the Chi-square test or Fisher's exact test, as appropriate. Differences were considered statistically significant if $P < 0.05$.

Results

A total of 195 patients (51.8% females) were recruited. The mean age was 10.2 years (range 3 months to 22 years). One hundred and twenty-six patients developed renal scarring, and 27 of them had CRF due to RN. One hundred and ninety-two patients had VUR (90 grade I—III, 102 grade IV—V) and we found three patients with RN and no evidence of VUR. Forty-seven per cent of patients with VUR presented bilateral forms. Twenty-three children had familiar VUR (11.7%). Proteinuria was present in 22 patients in the RN and CRF groups. Twenty-nine children had hypertension (four in VUR, 18 in RN and seven in CRF groups), predominantly males. Seventy-seven patients required surgical treatment of VUR.

Table 1 shows the TNF-α-308G/A allele and genotype frequencies in patients and controls. We found a slightly higher presence of the A allele (TNF2) in patients with VUR compared to controls (16.2% vs 11.1%, $P < 0.05$), but failed to demonstrate an association between either the AA genotype or A allele and susceptibility to renal scarring. There were no significant differences in genotype distribution between patients with VUR, RN and CRF (Table 2). Other known risk factors for renal scarring in patients with VUR (such as familial forms of VUR, hypertension, proteinuria, grade of

Table 1 Genotype and allele frequency of TNF-α gene polymorphism in patients with VUR and controls

TNF-α gene	VUR ($n = 195$)	Controls ($n = 266$)
Genotype		
GG	138 (70.7)	214 (80.5)
AG	51 (26.1)	45 (16.9)
AA	6 (3.2)	7 (2.6)
Allele		
G (TNF1)	(83.8)	(88.9)
A (TNF2)	(16.2)*	(11.1)*

Values are number of patients, with percentage in brackets.
*$P < 0.05$.

VUR or unilateral/bilateral forms) were not associated with TNF-α polymorphism.

Discussion

The evolution of patients with VUR is variable, as we observe in daily practice. It is well known that RN hypertension and even CRF can occur in these patients. Reflux nephropathy is the final result of an inflammatory response partly regulated by cytokines such as TNF-α, one of the first immunological mediators to appear after an infection is established [4]. TNF-α stimulates the synthesis of other proinflammatory growth factors and cytokines (interleukin-1β, transforming growth factor-β, etc.) leading to interstitial fibrosis and parenchymal damage. A single-base polymorphism of the TNF-α gene at position -308 of the promoter region was associated with changes in production and transcription of this cytokine [7]. The A allele (TNF2) is related to higher levels of TNF-α [14], thus inflammatory nephropathy could be more frequent in patients with the A allele because of the increased production of this cytokine.

The -308A allele has been associated with a higher predisposition to several inflammatory diseases, including alopecia areata [15] and systemic lupus erythematosus [16]. Kidney diseases have also been studied: Shu et al. [7] reported an influence of TNF-α polymorphism in susceptibility to IgA nephropathy, but Syrjanen et al. [17] failed to demonstrate this. Kim et al. [10] did not find any difference in genotype distribution between patients with nephrotic syndrome and controls. Recently, Solari et al. [11] found a higher proportion of the TNF-α AA genotype in patients with RN compared to healthy controls, suggesting that this genotype could be related to higher susceptibility to renal scarring. We failed to demonstrate a relationship between this polymorphism and renal scarring in patients with VUR, in contrast to the previously published data [11]. The distribution of the polymorphism differs between the samples of the two research groups; this could be partly explained by the fact that we included all grades of RN, not only the moderate/severe forms. When we compared genotype distribution between patients with RN and those with end-stage renal failure we did not find a higher presence of the A allele. These data support the previous hypothesis that TNF-α polymorphism is not associated with disease progression [7]. We have studied other factors known to be related to renal scarring in patients with VUR such as proteinuria, hypertension, high-grade VUR, bilateral forms and familial history of VUR, and we did not find any correlation with genotype/allele distribution. Our healthy control group showed a -308A allele frequency of 11%. This is similar to the distribution reported by other authors [7,10], indicating that this genetic variant is not influenced by racial factors.

Table 2 Distribution of TNF-α gene polymorphism

TNF-α gene	CRF due to RN ($n = 27$)	RN ($n = 99$)	VUR ($n = 69$)
Genotype			
GG	19 (70.3)	75 (75.8)	44 (63.8)
AG	8 (29.7)	23 (23.2)	20 (29)
AA	0	1 (1)	5 (7.2)
Allele			
G (TNF1)	(85.2)	(87.3)	(78.2)
A (TNF2)	(14.8)	(12.7)	(21.8)

Values are number of patients, with percentage in brackets.

It is well known that renal prognosis in a patient with VUR may be influenced not only by genetic factors: the number of UTI (specially pyelonephritis), age at first UTI, severity of VUR and adequacy of follow up are some factors that could be even more important than genetic variations. A renal scar detected by DMSA could be not only the result of an UTI, since congenital malformations (hypoplasia or dysplasia) may be involved [18,19]. Thus, the term 'reflux nephropathy' probably covers at least two types of diseases: acquired renal scarring secondary to UTI and VUR that affect mainly females, and congenital renal scarring in males with prenatal VUR and dysplastic kidneys [20]. All of these point to the need for studies, as homogeneous as possible, to detect the real impact of each factor, including genetics, in the natural history of VUR and RN.

Our data show that the -308A (TNF2) allele is more frequent in patients with VUR than controls, supporting the idea that the polymorphism could be related to disease susceptibility. The A allele is

related to higher activity of this cytokine, and recent studies have shown increased levels of TNF-α in fetal dysplastic kidneys [21]. This allele could thus be related to the development of ureteral malpositioning leading to VUR.

Conclusions

In conclusion, our data show a significant association between the -308A allele (TNF2) of the TNF-α gene and the presence of VUR, suggesting that this genetic variant could be related to a higher susceptibility of carriers to have reflux than those with the other form of the allele. We did not find that this polymorphism is associated with renal scarring or disease activity. More studies are needed to determine the role of this genetic variant in VUR and the development of renal scarring.

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