



## Vesicoureteric reflux and tumor necrosis factor- $\alpha$ gene polymorphism

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### KEYWORDS

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**Abstract** *Aim:* To determine the role of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) 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- $\alpha$  gene. Recent studies suggest that the TNF- $\alpha$  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- $\alpha$  genotype and grade of VUR or the presence of proteinuria.

*Conclusions:* Our data suggest that the TNF- $\alpha$  AA genotype is not associated with reflux nephropathy. The TNF- $\alpha$ -308A allele could be related to a higher susceptibility to VUR.

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### 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- $\alpha$  (TNF- $\alpha$ ), initiate the parenchymal damage leading to renal scarring [3]. TNF- $\alpha$  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- $\alpha$  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- $\alpha$  [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- $\alpha$ -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<sup>2</sup>. 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- $\alpha$  gene, the primers used were 5'-GCAATAGGTTTTGAGGGCCAT-3' and 5'GGGACACACAAGCATCAAG-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 NcoI 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- $\alpha$ -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

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