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Association of eNOS gene intron 4 a/b VNTR polymorphisms in children with nephrotic syndrome

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ABSTRACT

To investigate the association of endothelial nitric oxide synthase gene intron 4 (eNOS4) polymorphisms with nephrotic syndrome, the eNOS4 genotypes were assessed in 161 children with nephrotic syndrome in comparison with 78 healthy subjects. We classified the children with nephritic syndrome into 2 groups: as steroid-sensitive nephrotic syndrome (SSNS) ($n = 125$) and steroid-resistant nephrotic syndrome (SRNS) ($n = 36$). The eNOS4 polymorphisms were analyzed by polymerase chain reaction. The frequencies of eNOS4 *aa, ab* and *bb* genotypes were 3%, 31%, and 66% in all the nephrotic syndrome groups, and 1%, 23%, and 76% in the control group ($x^2 = 2.87$, $p > 0.05$). In addition, the frequencies of eNOS4 *aa, ab* and *bb* genotypes were 2%, 33%, and 65% in SSNS group, and 5%, 28%, and 67% in the SRNS group ($x^2 = 1.13$, $p = 0.567$). The present study is the first to investigate eNOS4 gene polymorphisms in children with SSNS and SRNS. Our data show that the eNOS4 gene polymorphisms were not associated with the development, frequent relapse and response to steroid in nephritic syndrome.

1. Introduction

Nitric oxide (NO) is synthesized from L-arginine by the enzyme nitric oxide synthase (NOS). There are three known NOS in humans: endothelial (eNOS), neuronal (nNOS), and inducible (iNOS) (Lamas et al., 1992). All three NOS isoforms can be expressed in the kidney: nNOS is principally expressed in the macula densa; iNOS has been localized in several tubule segments, glomeruli and interlobular arteries, and eNOS is expressed in the endothelium of glomerular capillaries, afferent and efferent arterioles and intracranial arteries. eNOS is encoded by a gene located on chromosome 7q35-3b, comprising 2b exons that span 21 kb (Marsden et al., 1993). This gene has a common larger allele and a smaller allele; the larger has five tandem 27-bp repeats (allele *b*), while the smaller has only four repeats (allele *a*) (Wang et al., 1996).

Heavy glomerular proteinuria, otherwise known as nephrotic syndrome (NS), is marked by edema formation and avid sodium retention. Given the pivotal role of NO in regulation of renal sodium handling, vascular resistance and sympathetic activity, we considered that sodium retention and hypertension in NS may be associated with impaired NO system (Trachtman et al., 1996). In the kidney, as well as other solid organs physiologic concentrations of NO function as a tonic vasodilator, working essentially instantaneously. Several studies have shown that a decrease of NO production may be important in the progression of renal diseases (Bachmann et al., 1995; Baylis et al., 1992; Tolins et al., 1990). The first in vivo evidence of its role in the pathogenesis of glomerulonephritis was reported by Weinberg et al. (1994). Baylis et al. showed that chronic NO blockade causes proteinuria and glomerular sclerosis as well as systemic hypertension in rats (Baylis et al., 1992). In another study Trachtman et al. showed an increased level of urinary nitrite in children with NS (Trachtman et al., 1996).

There is a strong association between the "*a*" allele of the eNOS4 and decreased plasma NO levels (Tsukada et al., 1998). eNOS4 a/b variable number of tandem repeat (VNTR) polymorphisms is considered the deterioration factor for progressive renal disease and it may be implicated in the pathogenesis of human glomerular diseases. Furusu et al found a decreased expression of eNOS in patients with IgA nephropathy and lupus nephritis suggesting diminished physiological effect of eNOS in damaged glomeruli (Bellini et al., 2007; Furusu et al., 1988).

Abbreviations: eNOS, endothelial nitric oxide synthase; eNOS4, endothelial nitric oxide synthase gene intron 4; ESRD, end stage renal disease; iNOS, inducible nitric oxide synthase; NO, nitric oxide; NOS, nitric oxide synthase; nNOS, neuronal nitric oxide synthase; NS, nephrotic syndrome; SSNS, steroid-sensitive nephrotic syndrome; SRNS, steroid-resistant nephrotic syndrome; VNTR, variable number of tandem repeats.

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Recent studies have also reported that increased NO production may contribute to hyperfiltration and microalbuminuria that characterize early diabetic nephropathy (Prabhakar et al., 2004; Shin Shin et al., 2004). In an experimental study it was demonstrated that intrarenal production of nNOS increased in the rats with chronic diabetes, and this overproduction of nNOS in the kidneys suppresses the tubuloglomerular feedback system, and this suppression might be a critical mechanism for the development of diabetic renal hyperfiltration (Subrata et al., 2009; Yabuki et al., 2006; Yanming et al., 2011).

The pivotal role of NO in regulation of renal and systemic hemodynamics and renal sodium handling which are markedly deranged in NS is known. Studies have been published related to eNOS4 polymorphisms in several renal diseases (Alasehirli et al., 2009; Nagase et al., 2003; Shimizu et al., 2002), however, not many studies have been conducted to investigate this polymorphisms in patients with NS and especially in SRNS. We hypothesized that eNOS4 polymorphisms may influence the development and/or progression of NS. The aim of this study is to investigate whether the eNOS4 a/b VNTR polymorphisms are associated with susceptibility to NS and its clinical features.

2. Material and methods

A total of 161 Turkish children with NS (108 boys and 53 girls, ages between 1 and 15 years) and 78 ethnically matched healthy subjects (39 men and 39 women, ages between 18 and 55 years) were enrolled into this study. Informed written consent was obtained from all subjects participating to the study and local ethics committee approval was granted. The control group consisted of unrelated healthy adult volunteers without renal and cardiac diseases. We classified children with NS into 2 groups: as SSNS ($n = 125$) and SRNS ($n = 36$). In SSNS group, 3 children have focal segmental glomerulosclerosis, 5 children have mesangiopathic glomerulonephritis, and other 113 children have minimal change glomerulonephritis. However in SRNS group, 2 children have minimal change glomerulonephritis, 3 children have membranoproliferative glomerulonephritis and other 31 children have focal segmental glomerulosclerosis.

Venous blood samples from all subjects were obtained for DNA extraction and collected in EDTA tubes. Genomic DNA from leukocytes was purified according to the method of Miller et al. (1988). The eNOS gene intron 4, 27 bp VNTR polymorphisms were detected by polymerase chain reaction according to the method described by Wang et al. (1997). Polymerase chain reaction products of NO gene locus were examined by gel electrophoresis (2% NuSieve agarose–agarose) at 150 V for 30 min and visualized at room temperature under UV after ethidium bromide staining (Fig. 1). Clinical manifestations and laboratory parameters were analyzed in each patient and correlated with the genotypes. We compared frequencies of eNOS4 VNTR polymorphisms among all the patients and the healthy controls.

The data was analyzed by SPSS package program (version 16.0). Allelic frequencies were calculated by a gene-counting method. The genotype and allele frequencies in patients with NS were compared to those in the control subjects; and also the genotype and allele frequencies in patients with SSNS were compared to those in the SRNS patients. A chi-square test was used to test expected type frequencies, assuming Hardy–Weinberg equilibrium. Results were considered statistically significant if the p values were less than 0.05.

3. Results

Among the 161 NS patients studied, 108 (67%) were boys and 53 (33%) were girls. Mean age at diagnosis was 5.25 ± 3.48 years (range between 1 and 15). Clinical and laboratory features of all NS, SSNS and SRNS patients are shown in Table 1.

The distribution of *aa, ab* and *bb* genotypes was 3%, 32% and 65% in NS patients compared with 1%, 23% and 76% in the controls (Table 2). The frequencies of eNOS4 genotypes are not different in the NS group when compared with control group ($x^2 = 2.87$, $p = 0.238$). The *a* and *b* allele frequencies were 19% and 81% in the NS group, 13% and 87% in the control group. The allele frequencies in NS and control were compared with the chi-square test and no significant difference was found ($x^2 = 3.33$, $p = 0.189$).

The frequencies of eNOS4 *aa, ab* and *bb* genotypes were 2%, 33%, and 65% in SSNS group, and 5%, 28%, and 67% in the SRNS group respectively (Table 3). The frequencies of eNOS4 genotypes are not different in the SSNS group as compared with the SRNS group ($x^2 = 1.13$, $p = 0.567$). The *a* and *b* allele frequencies were 19% and 81% in the SSNS group, 13% and 87% in the SRNS group. The allele frequencies in SSNS and SRNS group were compared with the chi-square test and no significant difference was found ($x^2 = 0.015$, $p = 0.511$). Compared with SSNS patients, age, serum creatinine, systolic blood pressure and serum albumin were higher, but serum complement 3 and erythrocyte sedimentation rate were lower in SRNS patients (Table 1).

The frequencies of eNOS4 *aa, ab* and *bb* genotypes were 3%, 32%, and 65% in SSNS with < 4 relapses group, and 0%, 36%, and 64% in the SSNS with ≥4 relapses group respectively. The frequencies of eNOS4 genotypes are not different in the SSNS with <4 relapse group than in the SSNS with ≥4 relapse group ($x^2 = 0.75$, $p = 0.687$). The *a* and *b* allele frequencies were 19% and 81% in the SSNS with <4 relapses group compared with 18% and 82% in the SSNS with ≥4 relapses group. The allele frequencies in SSNS with <4 relapse group and SSNS with ≥4 relapse group were compared with the chi-square test and no significant difference was found ($x^2 = 0.013$, $p = 0.550$).

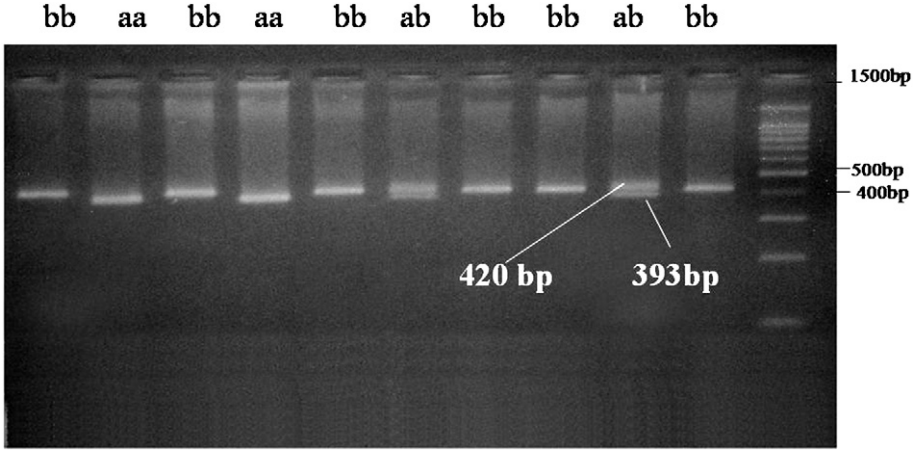

Fig. 1. Polymorphism in intron 4 of the eNOS gene. The PCR products on 2% NuSieve agarose–agarose gel and visualized by ethidium bromide staining (90 min and 150 V).