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Polymorphisms of the *NR3C1* gene in Korean children with nephrotic syndrome

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= Abstract =

Purpose : Idiopathic nephrotic syndrome (NS) can be clinically classified as steroid-sensitive and steroid-resistant. The detailed mechanism of glucocorticoid action in NS is currently unknown.
Methods : In this study, we investigated 3 known single nucleotide polymorphisms (SNPs) (ER22/23EK, N363S, and BclI) of the glucocorticoid receptor gene (the *NR3C1* gene) in 190 children with NS using polymerase chain reaction-restriction fragment length polymorphism and analyzed the correlation between the genotypes and clinicopathologic features of the patients.
Results : Eighty patients (42.1%) were initial steroid nonresponders, of which 31 (16.3% of the total) developed end-stage renal disease during follow-up. Renal biopsy findings of 133 patients were available, of which 36 (31.9%) showed minimal changes in NS and 77 (68.1%) had focal segmental glomerulosclerosis. The distribution of the BclI genotypes was comparable between the patient and control groups, and the G allele frequencies in both the groups were almost the same. The ER22/23EK and N363S genotypes were homogenous as ER/ER and NN, respectively, in all the patients and in 100 control subjects. The BclI genotype showed no correlation with the NS onset age, initial steroid responsiveness, renal pathologic findings, or progression to end-stage renal disease.
Conclusion : These data suggested that the ER22/23EK, N363S, and BclI SNPs in the *NR3C1* gene do not affect the development of NS, initial steroid responsiveness, renal pathologic lesion, and progression to end-stage renal disease in Korean children with NS. **(Korean J Pediatr 2009;52:1260-1266)**

Key Words : Children, Nephrotic syndrome, Glucocorticoid receptor, *NR3C1*, Single nucleotide polymorphism

Introduction

Idiopathic nephrotic syndrome (NS) is one of the most common primary glomerular diseases in children[1]. NS can be clinically classified as steroid-sensitive (SSNS) and steroid-resistant nephrotic syndrome (SRNS) forms according to the responsiveness to oral glucocorticoid treatment, which is the first line of drug for childhood idiopathic NS[1]. However, the detailed mechanism of action of glucocorticoids in idiopathic NS is currently unknown, as is the pathogenesis of NS.

There have been intensive efforts to explain the difference in the response to glucocorticoid treatment in patients with idiopathic NS on the basis of genetic background by analyzing polymorphisms in various genes including angiotensin-converting enzyme (*ACE*)[2-6], cytokines or growth factors[7-18], apolipoprotein E (*APOE*)[19-21], paraoxonase 1 (*PON1*)[22], multidrug resistance protein 1 (*MDR1*, also called as *ABCB1*)[23-26], and glucocorticoid receptor (*NR3C1*) genes[27, 28]. However, the results of such studies are not consistent.

In this study, three single nucleotide polymorphisms (SNPs) of the *NR3C1* gene (ER22/23EK, N363S and BclI) were genotyped in a group of pediatric patients with NS to analyze the correlation between the genotypes and the clinico-pathological features of the patients.

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Materials and methods

1. Patients

One hundred ninety children (134 males and 56 females), who were diagnosed as idiopathic NS in the Department of Pediatrics, Seoul National University Children's Hospital, Seoul, Korea during the period from 1985 to 2006, were enrolled.

NS was defined as massive proteinuria of 40 mg/hour/m^2 or more with hypoalbuminemia of 2.5 g/dL or less and no known causes of nephrotic syndrome[1]. At initial presentation oral prednisolone 60 mg/m^2/day or equivalent dose of deflazacort was administered for 4 weeks followed by 40 mg/m^2/48hours for 4 weeks in all patients. However, treatment modalities for relapses were not uniform. Remission of nephrotic syndrome was defined as absence of proteinuria (4 mg/hour/m^2 or less or negative on dipstick test) for three consecutive days or longer, and steroid resistance was defined as the absence of remission within initial 8 weeks of oral steroid treatment.

The *NPHS2* gene (entire coding exons) and the *WT1* gene (exons 8 and 9) mutations were excluded in all patients with SRNS and patients with positive family history as well as *ACTN4* and *TRPC6* mutations in 3 patients with positive family history, which suggested autosomal dominant mode of inheritance[29, 30].

This study was approved by the Ethics Committee of Seoul National University Hospital, Seoul, Korea, and informed consent for the genetic analysis was obtained from all patients and/or their parents.

2. Genotyping

Genotypes of 3 SNPs in the *NR3C1* gene (ER22/23EK, N363S and BclI) were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) methods (Table 1). Genomic DNA was extracted and purified from peripheral blood by using a QIA Amp DNA Blood Mini Kit (Qiagen, Hilden, Germany). The PCR products were purified with a QIA Quick PCR Purification Kit (Qiagen, Hilden, Germany), and, then were digested with corresponding restriction enzymes. The digested PCR products were visualized in ethidium bromide-stained 2.5% agarose gel using a UV camera. Genotypes of 100 healthy blood donors, as a normal control group, were also determined.

3. Statistical analyses

The Hardy-Weinberg equilibrium (HWE) assumption was assessed for case and control groups by comparing the observed numbers of each genotypes with those expected under HWE for the estimated allele frequency. The distribution of the genotypes between two groups was compared using the Mann-Whitney U-test. Fisher's exact test was used to estimate odds ratios and 95% confidence intervals to gauge the relationship between the genotype and the risk of nephrotic syndrome.

Table 2. Characteristic of the Patients with Idiopathic Nephrotic Syndrome (n=190)

Parameter	Number of patients (%)
Age (mean±SD)	4.95±3.26 years
Male gender	134 (70.5)
Family history of nephrotic syndrome	11 (5.8)
Initial steroid-resistance	80 (42.1)
End-stage renal disease	31 (16.3)
Renal biopsy findings (n=113)	
Minimal change nephrotic syndrome	36 (31.9)
Focal segmental glomerulosclerosis	77 (68.1)

Abbreviations : SD, standard deviations

Table 1. The Primer Sets and Restriction Enzymes (RE) used for the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP)

Variations (rs number)	RE	Primers used for PCR
ER22/23EK (rs6189/rs6190)	*Mnl*I	F: 5'-ATCCCAGGTCATTTCCCATC-3' R: 5'-CGGATCAGGAAGATAATGTGAC-3'
N363S (rs56149945)	*Tsp*509I	F: 5'-TCATGGTGTGAGTACCTCTGGAG-3' R: 5'-CTGTTCGACCAGGGAAGTTCA-3'
BclI*	*Bcl*I	F: 5'-GCAGTGAACAGTGTACCAGACC-3' R: 5'-AAATTGAAGCTTAACAATTTTGGC-3'

*No rs number is assigned yet.

Table 3. Comparison of *Bcl*I in *NR3C1* Genotype Distribution in the Patients and Control Subjects

Genotype	Patients (n=190)	Control (n=100)	OR (95 % CI)	*P* value
CC	105 (55.3%)	59 (59.0%)	1 (reference)	
CG	73 (38.4%)	32 (32.0%)	1.282 (0.759-2.164)	0.353
GG	12 (6.3%)	9 (9.0%)	0.749 (0.298-1.882)	0.539
G allele frequency	25.5%	25.0%	1.028 (0.693-1.526)	0.890

Abbreviations : OR, odds ratio

Results

1. The clinical and pathological findings of the patients

The clinico-pathological profiles of the patients were summarized in Table 2. Among total 190 patients, males were 134 and females were 56. The mean age at the onset of idiopathic NS was 4.95±3.26 years (range, 1-16 years). Family history of NS was positive in 11 patients (5.8%); SRNS in one or more siblings of 4 patients, SRNS in one of the parents of 2 patients, SSNS in one sibling of 4 patients, and SSNS in father of 1 patient. Eighty (42.1%) patients were resistant to initial steroid treatment, and 31 (16.3% of total) of them progressed to end-stage renal disease (ESRD). Renal histologic examination was available in 113 patients; focal segmental glomerulosclerosis (FSGS) in 77 (68.1%) patients and minimal change nephrotic syndrome (MCNS) in 36 (31.9%) patients.

2. Comparison of the genotype distribution in the patients and the control subjects

The distribution of BclI genotypes was comparable between in the patients group (CC 55.3%, CG 38.4%, and GG 6.3%) and in the control group (CC 59.0%, CG 32.0%, and GG 9.0%), and the G allele frequencies in both groups were almost same (25.5% vs 25.0%) (Table 3).

The genotypes of ER22/23EK and N363S were homogenous as ER/ER and NN, respectively, in all of the patients and 100 control subjects, and, thus, analysis of these two SNPs was not performed further.

3. Correlation of BclI genotype to clinical and pathological features of the patients

The onset age of NS was not affected by any of the BclI genotypes (Table 4).

The genotype distribution and allele frequencies showed no significant difference between the initial steroid responders and initial steroid nonresponders (Table 5).

Table 4. Influence of BclI in *NR3C1* Genotypes on the Onset Age of Nephrotic Syndrome

Genotype	No. of Pts	Age at the onset (years)*
CC	105	4.92±3.09
CG	73	5.00±3.41
GG	12	4.91±3.98

*These data were expressed with mean±standard deviations
P value was >0.05 for all comparisons
Abbreviation : Pts, patients

There was no significant difference in any genotype or allotype distribution between patients with MCNS and patients with FSGS (Table 6). The genotype distribution and allele frequencies showed no significant difference between the patients with ESRD and the patients without ESRD (Table 7).

Discussion

In this study, three known SNPs of the *NR3C1* gene (ER22/23EK, N363S and BclI) were genotyped in a group of pediatric patients with idiopathic NS to analyze the correlation between the genotypes and the clinico-pathologic features of the patients.

Glucocorticoid, the first line of drug for childhood idiopathic NS, exerts its effects by its binding to the glucocorticoid receptor (GR), a ligand-dependent transcription factor, which belongs to the superfamily of nuclear receptors[31]. It is well known that the response to glucocorticoid treatment is variable in patients with glomerular diseases including childhood idiopathic NS, asthma or other common diseases, and the association of *NR3C1* polymorphisms and response to glucocorticoid treatment have been analyzed in several studies[27, 28, 32-38]. In vitro studies have demonstrated that T cells from patients with glucocorticoid-resistant asthma showed a reversible cytokine-induced reduction in GR binding affinity and an irreversible reduction in GR number[39]. This finding suggests that *NR3C1* gene polymorphisms affecting its affinity to glu-

Table 5. Comparison of BclI in *NR3C1* Genotypes in the Initial Steroid Responders and Initial Steroid Nonresponders

Genotype	Responders (n=110)	Nonresponders (n=80)	OR (95 % CI)	*P* value
CC	62 (56.4%)	43 (53.8%)	1 (reference)	
CG	40 (36.4%)	33 (41.3%)	1.190 (0.651-2.174)	0.573
GG	8 (7.3%)	4 (5.0%)	0.721 (0.204-2.546)	0.611
G allele frequency	25.5%	25.6%	1.009 (0.633-1.609)	0.970

Abbreviations : OR, odds ratio; CI, Confidence interval

Table 6. Comparison of BclI in *NR3C*1 Genotypes in the Patients with Minimal Change Nephrotic Syndrome and the Patients with Focal Segmental Glomerulosclerosis

Genotype	MCNS (n=36)	FSGS (n=77)	OR (95 % CI)	*P* value
CC	18 (50.0%)	43 (55.8%)	1 (reference)	
CG	12 (33.3%)	30 (39.0%)	1.047 (0.440-2.489)	0.918
GG	6 (16.7%)	4 (5.2%)	0.279 (0.070-1.109)	0.070
G allele frequency	33.3%	24.7%	0.655 (0.355-1.208)	0.174

Abbreviations : MCNS, minimal change nephrotic syndrome; FSGS, focal segmental glomerulosclerosis; OR, odds ratio; CI, Confidence interval

Table 7. Influence of BclI in *NR3C1* Genotypes to Renal Prognosis

Genotype	ESRD (-) (n=159)	ESRD (+) (n=31)	OR (95 % CI)	*P* value
CC	86 (54.1%)	19 (61.3%)	1 (reference)	
CG	64 (40.3%)	9 (29.0%)	0.637 (0.270-1.499)	0.301
GG	9 (5.7%)	3 (9.7%)	1.509 (0.373-6.106)	0.564
G allele frequency	25.8%	24.2%	0.919 (0.488-1.730)	0.792

Abbreviations : ESRD, end-stage renal disease; OR, odds ratio; CI, Confidence interval

cocorticoids can play an important role in the response to glucocorticoids treatment. In vitro studies by Russcher et al.[40] have demonstrated that two polymorphisms in *NR3C1* gene (ER22/23EK and N363S polymorphisms) directly affected glucocorticoid-regulated gene expression, which was confirmed in clinical studies demonstrating that patients with the ER22/23EK allele are relatively more resistant to the effects of glucocorticoids with respect to the sensitivity of the adrenal feedback mechanism than non-carriers, resulting in a better metabolic health profile[41]. However, the exact influence of these polymorphisms in *NR3C1* gene remains to be controversial. Tissing et al.[42] demonstrated that these polymorphisms including ER22/23EK, N363S, BclI are not related to glucocorticoid resistance in childhood acute lymphoblastic leukemia. This finding is compatible with our study. Similarly, several studies of other diseases have shown inconsistent results with each other[32-38].

Recently, there have been studies to analyze the association of *NR3C1* polymorphisms and response to glucocorticoid treatment in NS. Zalewski et al.[27] studied a three-point haplotype (BclI C/G, rs33389 C/T and rs33388 A/T) within intron B of *NR3C1* in 118 children with SSNS and showed that the GTA haplotype was associated with a higher glucocorticoid sensitivity and was found to be more prevalent in early than late prednisone responders. However, Ye et al.[28] found no significant association between *NR3C1* haplotypes and steroid resistance in Chinese children with sporadic NS. In our study, BclI polymorphism in *NR3C1* was not associated with development of NS, response to steroid therapy, renal pathology, or renal outcome in Korean children with idiopathic NS. While among these studies, the distribution of intro B polymorphisms in children in Poland is compatible with that in adults in UK[27, 37], this is not similar to that observed in our study. This finding may be speculated that this disagreement observed in the studies is attributable to the difference of ethnics. Though the study by Ye et al.[28], showed to similar conclusion to our study, the polymorphisms which they found are different with ours;

therefore large scale clinical studies are necessary to prove the ethical difference in the distribution of *NR3C1* polymorphisms and establish the role of *NR3C1* polymorphisms.

In our study, the genotypes of ER22/23EK and N363S were homogenous as ER/ER and NN in all of the patients and control subjects, and BclI genotype distribution in the patient group revealed no difference from that in control group. Furthermore, BclI genotype showed no significant correlation with the onset age of nephrotic syndrome, initial steroid responsiveness, renal pathologic findings, or progression to end-stage renal disease. Because of ER/ER and NN in all of the patients and control subjects, it is impossible that we analyze the association of the *NR3C1* three-marker haplotype and steroid response in patients with NS. In the future, using haplotype analysis with other target polymorphism, we can clarify the role of the haplotypes in steroid response.

These data suggested that the ER22/23EK, N363S and BclI SNPs in the *NR3C1* gene do not affect the development and the clinical course of NS in Korean children. This is the first study to demonstrate the lack of association of *NR3C1* gene polymorphism with NS and response to glucocorticoid treatment in Korean children.

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한 글 요 약

한국 신증후군 환아에서 *NR3C1* 유전자 다형성 분석

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목 적: 특발성 신증후군은 소아의 가장 흔한 일차성 사구체 질환 중의 하나이다. 신증후군은 초기 경구 스테로이드 치료에 대한 반응에 따라서 임상적으로 스테로이드 반응성 신증후군과 스테로이드 저항성 신증후군으로 분류될 수 있다. 그러나 현재까지 신증후군에서 스테로이드의 정확한 작용 기전은 알려져 있지 않다. 신증후군 환자를 대상으로 여러 가지 유전자 다형성을 분석함으로써 스테로이드 치료에 대한 반응의 차이를 설명하려는 여러 시도들이 있어왔다.

방 법: 본 연구에서는 190명의 신증후군 환자를 대상으로 *NR3C1* 유전자 다형성(ER22/23EK, N363S, BclI)을 확인하여 유전형과 임상-병리 양상의 연관성에 대해서 분석하였다.

결 과: 신증후군 환자의 평균 연령은 4.95세였고 남아가 134명이었다. 11명의 환자는 신증후군의 가족력이 있었다. 그러나 이 환자들을 대상으로 *NPHS2, WT1, ACTN4, TRPC6* 유전자 분석을 시행한 결과 이상 소견은 발견되지 않았다. 80명의 환자(42.1%)는 초기 스테로이드 저항성이었고 그 중 31명의 환자는 말기 신질환으로 진행하였다. 신장 조직 검사는 113명의 환자를 대상으로 시행되었고 그 중 36명의 환자(31.9%)는 미세변화 신증후군이었고 77명의 환자(68.1%)는 초점성 분절성 사구체 경화증이었다. BclI 유전형을 비교하였을 때 G allele 빈도는 환자군과 대조군에서 차이가 없었다. ER22/23EK과 N363S 유전형은 각각 ER/ER과 NN으로 환자군과 대조군에서 동일한 양상을 보였다. BclI 유전형은 신증후군의 발병 나이, 초기 스테로이드 반응 여부, 신장의 병리학적 소견, 말기 신질환으로의 진행여부와 연관성을 보이지 않았다.

결 론: 한국 신증후군 환아를 대상으로 한 이 연구 결과는 *NR3C1* 유전자의 ER22/23EK, N363S 및 BclI 유전자 다형성이 신증후군의 발병, 초기 스테로이드 치료에 대한 반응, 신장의 조직학적 소견 및 신 기능의 저하에 영향을 미치지 않음을 보여준다.

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