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Relationship between TIM-3 gene polymorphisms and steroid-resistant primary nephrotic syndrome in children

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Abstract: Nephrotic syndrome, also known as nephrosis, is a collection of symptoms in medicine and urology caused by damage to the basement membrane of the kidney glomeruli and the kidneys excrete a large amount of protein. This experiment was carried out to investigate the association of three single nucleotide polymorphisms (SNPs) of T-cell immunoglobulin and mucin-domain-containing-3 (Tim-3) with childhood primary nephrotic syndrome (PNS) steroid response in Han Chinese. For this purpose, a total of 218 children with steroid-resistant PNS and 189 children with steroid-responsive PNS were enrolled in this case-control study. Three single nucleotide polymorphisms (SNPs) of the TIM-3 gene promoter region (rs4704853, rs1051746, and rs10053538) were analyzed by polymerase chain reaction (PCR) and restriction enzyme digestion. Results showed that there were 124 males and 94 females in the steroid-resistant PNS group and 114 males and 75 females in the steroid-responsive PNS group. The mean ages of the two groups were 7.9 years and 7.7 years, respectively. The distribution of alleles of Rs1051746 and Rs10053538 were significantly different between the steroid-resistant PNS group and the steroid-responsive PNS group (P-value = 0.047 and 0.012, respectively). The distribution of their genotypes was also significantly different between the steroid-resistant PNS group and the steroid-responsive PNS group (P-value = 0.044 and 0.010, respectively). Haplotype G-C-G was less frequent among steroid-resistant PNS children than the steroid-responsive PNS children (P = 0.015). There was no significant difference between the three SNPs of TIM-3 and the clinical features of these PNS children (P>0.05). It concluded that this study provided evidence showing that the polymorphisms of Rs1051746 and Rs10053538 at the TIM-3 gene were related to childhood PNS steroid response. This result provided fundamental support for future studies on the role of TIM-3 in pathogenesis and therapy of childhood PNS.

Key words: Nephrotic syndrome; Mucin-domain-containing-3; Single nucleotide polymorphisms.

Introduction

Nephrotic syndrome is a glomerular disease characterized by proteinuria, hypoalbuminemia, hyperlipidemia, and edema. Primary nephrotic syndrome (PNS) is the most common nephrotic syndrome in childhood. The estimated incidence of PNS in children was about 2 to 7 cases per 100, 000 children (1). PNS treatment includes corticosteroids and alkylating agents. However, severe side effects of these treatments, drug resistance, and relapse are still challenges for PNS children.

So far, the pathogenesis of PNS is still unclear due to its complexity. Some studies suggested it may be due to primary T-cell disorder which leads to glomerular podocyte dysfunction (1). Some studies have shown that certain PNS susceptibility genetic features (2, 3). For example, F. Őktem and colleagues reported that the DD genotype of the angiotensin-converting enzyme gene was much higher in children with the nephrotic syndrome than the healthy control children (4). G Rainer and colleagues have shown that patients with mutations in Poducin don do not respond to standard steroid treatment (5). This evidence suggested the existence of PNS susceptibility genes.

T-cell immunoglobulin and mucin-domain-containing-3 (Tim-3) is a transmembrane protein containing immunoglobulin and mucin-like domain. TIM-3 is widely expressed on many immune cells, such as Th1 cells, dendritic cells, and macrophages (6-8). Tim-3 expressed specifically on Th1 cells inhibits autoimmune response by interacting with its ligand galectin-9 and suppressing the aggressive functions of Th1 cells (9, 10). What's more, decreased Tim-3 expression was observed in peripheral blood mononuclear cells from nephropathy patients (11). Association between polymorphisms of TIM-3 in promoter and coding regions and rheumatoid arthritis was also identified(12). However, whether the polymorphism of the Tim-3 gene is associated with childhood PNS steroid response is still unclear. The study of gene polymorphism can be done in several ways. They have used these methods in various researches. They have also linked these polymorphisms to various traits and diseases (13-17).

In this study, we investigated the relationship between polymorphisms of Tim-3 (Rs10053538 (A/C), Rs4704853 and Rs1051746) and steroid response in childhood PNS.

Materials and Methods

Clinical characteristics of the patients

A total of 218 steroid-resistant PNS children and 189

steroid-responsive PNS children were included in this study. These PNS children were diagnosed at Qilu Hospital, Shandong University from August 2010 to May 2014. The mean age of the steroid-resistant PNS group was 6.3 years old and that of the steroid-responsive PNS group was 6.7. There were 131 males and 87 females in the steroid-resistant PNS group and 114 males and 75 females in the steroid-responsive PNS group. No significant difference in age and gender was observed between these two groups. PNS was diagnosed according to the criteria of the Chinese Medical Association in 2001(18). Patients without urinary remission within 4 weeks of prednisone therapy (60 mg/m2/day) were considered as steroid-resistant. Peripheral blood of these children was collected for genomic DNA extraction. This study was approved by the Committee of Clinical Research in the hospital. All the included individuals were informed of the aim and process of this study and consent was signed by the children and their parents or legal guardians. Individuals with other chronic renal diseases or immune disorders were excluded from this study.

Polymerase chain reaction and genotyping

QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) was used to extract DNA from blood samples (19). The manufacture's instruction was followed. The Real-time polymerase chain reaction (PCR) was performed to amplify DNA. Primer sequences were: Rs10053538 (forward primer 5`- GCCTTGACCAAGTTCATGCT-3`, reverse primer 5`- ACCACCCCGGATAATTTTGT-3`), Rs4704853 (forward primer 5`- CTTTTGCTTTTAAGGTGTC-3`, reverse primer 5`- TTCAAACTTCCAACTCTTC-3`), and Rs1051746 (forward primer 5`- AGAAGAAGGATGAGAGTGAGGCTTATGCTGGGAGTT-3`, reverse primer 5`-ACTCAAATCAGTCCCTTCATC-3`). Genotyping was performed using restriction enzyme digestion. Rs1051746 segments were digested by Taq I and resulted in 132bp and 37bp segments (genotype of GG), 169bp segments (genotype of TT), and 169bp + 132bp + 37bp segments (genotype of TT). Rs4704853 segments were digested by BsoB I enzyme which generated 141bp and 132bp segments (genotype of CC), 273bp segments (genotype of TT), and 141bp, 132bp, and 273bp segments (genotype of CT). Rs10053538 segments were digested by Bsi I enzyme which generated 66bp and 338bp segments (genotype of GG), 404bp segments (genotype of TT), and 66pb, 338bp plus 404bp segments (genotype of GT). 2% agarose gel was used to separate these segments, and ethidium bromide was used for visualization.

Statistical analysis

SPSS software 17.0 (SPSS Inc., Chicago, IL, USA) was used to evaluate the difference between genotypes and alleles frequencies between the steroid-resistant PNS group and steroid-responsive PNS group (χ^2 test). Odds ratios and 95% confidence interval (CI) were calculated. Arlequin software was used to analyze the Hardy-Weinberg equilibrium. Linkage Disequilibrium and haplotypes were analyzed and created by the expectation-maximization algorithm. All the data were shown as mean ± standard deviation (SD) or frequencies (n) and percentages. Two-tail P-values less than 0.05 was considered as significant.

Results

Clinical characteristics

This study included 218 children with steroid-resistant PNS and 189 steroid-responsive PNS children. The mean age of the steroid-resistant PNS group was 7.9 ± 2.5 years, and that of the steroid-responsive PNS group was 7.7 ± 2.6 years. There was no difference in age was observed in these two groups. Average body mass index (BMI) was 15.8 ± 3.1 kg/m^2 in steroid-resistant PNS group and 16.1 ± 3.0 kg/m^2 in the steroid-responsive PNS group (P > 0.05).

Distribution of alleles of TIM-3 gene

As shown in Table 1, in polymorphism Rs1051746 and Rs10053538, G allele was significantly more frequent in steroid-responsive PNS group (n = 372 (98.4%), 366 (96.8%)) than in steroid-resistant PNS group (n = 419 (96.1%), 366 (92.9%)), while the T allele was more frequent in steroid-resistant PNS group (n = 17 (3.9%), 31 (7.1%)) than in the steroid-responsive PNS group (n = 6 (1.6%), 12 (3.2%)) with P-values of 0.047 and 0.012, respectively. However, no significant difference of alleles of Rs4704853's distribution was observed between the steroid-resistant PNS group and steroid-responsive PNS group (P-value = 0.304).

Distribution of genotypes and haplotypes of TIM-3

Genotypes of Rs1051746 contained GG and GT; genotypes of Rs4704853 included CC and CT; and the genotypes of Rs1005358 contained GG, GT, and TT. As shown in Table 2, there was a higher ratio of GT carriers of Rs1051746 and Rs10053538 (including TT carriers) in the steroid-resistant PNS group than the steroid-responsive PNS group (P-values of 0.044, and 0.010, respectively). No significant difference of Rs4704853 genotypes was observed between the steroid-resistant PNS group and the steroid-responsive PNS group (P = 0.300).

There was relatively weak linkage disequilibrium between Rs4704853 and Rs10053538 (r^2 = 0.351). Ha-

Table 1. Distribution of alleles at TIM-3 in the steroid-responsive PNS children and steroid-resistant PNS children.

Polymorphism	Allele	Steroid-resistant PNS, n (%)	Steroid-responsive PNS, n (%)	*OR*(95%*CI*)	*P*
Rs1051746	G	419 (96.1)	372 (98.4)	1.000	0.047
	T	17 (3.9)	6 (1.6)	2.516 (0.982, 6.447)	
Rs4704853	C	426 (97.7)	373 (98.7)	1.000	0.304
	T	10 (2.3)	5 (1.3)	1.751 (0.593, 5.169)	
Rs10053538	G	405 (92.9)	366 (96.8)	1.000	0.012
	T	31 (7.1)	12 (3.2)	2.335 (1.181, 4.614)	

Table 2. Distribution of genotypes at TIM-3 in the steroid-responsive PNS children and steroid-resistant PNS children.

Polymorphism	Genotype	Steroid-resistant PNS, n (%)	Steroid-responsive PNS, n (%)	*OR* (95%*CI*)	*P*
Rs1051746	GG	201 (92.2)	183 (96.8)	1.000	0.044
	GT	17 (7.9)	6 (3.2)	2.580 (0.996, 6.683)	
Rs4704853	CC	208 (95.4)	184 (97.4)	1.000	0.300
	CT	10 (4.6)	5 (2.6)	1.769 (0.594, 5.271)	
Rs10053538	GG	187 (85.8)	177 (93.7)	1.000	0.010
	GT or TT	31 (14.2)	12 (6.3)	2.445 (1.217, 4.911)	

Table 3. Distribution of haplotypes of TIM-3 in the steroid-responsive PNS children and steroid-resistant PNS children.

Haplotype	Steroid-resistant PNS, n (%)	Steroid-responsive PNS, n (%)	*OR* (95%*CI*)	*P*
G-C-G	82.1	90.5	0.483 (0.266, 0.877)	0.015
G-C-T	8.7	5.8	1.545 (0.716, 3.336)	0. 265
T-C-G	3.2	1.6	2.057 (0.524, 8.059)	0.291

plotypes of Rs1051746, Rs4704853 and Rs10053538 were summarized in Table 3. G-C-G haplotype was less frequent in steroid-resistant PNS group (82.1%) than the steroid-responsive PNS group (90.5%, P-value = 0.015). However, no significant difference between G-C-T and T-C-G haplotypes' distribution was observed (P-value = 0.265, and 0.291, respectively).

Relationship between clinical features of steroid-resistant PNS children and TIM-3 genotypes

The relationship between clinical features (age, gender, and BMI) of steroid-resistant PNS children and TIM-3 genotypes were analyzed. As shown in Table 4, the mean age of steroid-resistant PNS children with different genotypes of Rs1051746, Rs4704853, and Rs10053538 was similar; no significant difference was observed (P-value > 0.05). Similar results were observed in gender and BMI.

Discussion

Focal segmental glomerulosclerosis and minimal change nephrotic syndrome are two of the major cause of childhood PNS (1, 20). Genetic disorders also contribute to childhood PNS (1, 2, 4). Corticosteroids are usually the first choice for PNS treatment (21). Patients who are responsive to steroid therapy usually have a good prognosis (22, 23). However, a part of PNS children cannot be tolerant of the side effects of steroid therapy, and many PNS children are not even responsive to steroid therapy (20, 23). Therefore, understanding the mechanism of childhood PNS pathogenesis and finding new therapeutic strategies are still highly needed.

In this study, we screened the TIM-3 gene's polymorphisms, Rs1051746, Rs4704853, and Rs10053538 in steroid-resistant PNS children and steroid-responsive PNS children. We found that the ratio of allele T and its carriers of Rs1051746 and Rs10053538 were significantly higher among steroid-resistant PNS children than steroid-responsive PNS children. What's more, haplotype G-C-G were relatively infrequent in steroid-resistant PNS children compared to the steroid-responsive PNS children. This evidence suggested that polymorphism of the TIM-3 gene might be a susceptibility gene of childhood steroid-resistant PNS.

Angiotensin-converting enzyme (ACE) gene insertion/deletion polymorphism (DD) was reported to be more frequent in focal segmental glomerulosclerosis patients and related to lower responsiveness to corticosteroid therapy (2). Wasilewski and colleagues reported that the polymorphisms of MDR-gene were significantly related to the time to respond to initial prednisone therapy of steroid-responsive nephrotic syndrome in children (3). Considering the strong evidence provided by our study showing that the polymorphism of the TIM-3 gene was more frequent in the steroid-resistant PNS children, it is highly likely that the polymorphism of the TIM-3 gene might also relate to the responsiveness of childhood steroid-resistant PNS to steroid therapy. More studies with larger sample sizes are needed to develop these polymorphism features into clinical biomarkers.

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Table 4. Relationship between clinical features of the steroid-resistant PNS children and TIM-3 genotypes.

Parameters	Rs1051746			Rs4704853			Rs10053538		
	GG	GT	*P*	CC	CT	*P*	GG	GT or TT	*P*
Age, years	7.937	7.600	0.599	7.892	8.300	0.620	7.879	8.104	0.648
Gender, n (%)									
Male, n (%)	113 (91.1)	11 (8.9)	0.497	118 (95.2)	6 (4.8)	0.838	106 (85.5)	18 (14.5)	0.886
Female, n (%)	88 (93.6)	6 (6.4)		90 (95.7)	4 (4.3)		81 (86.2)	13 (13.8)	
BMI, kg/m^2	15.775	15.952	0.820	15.818	15.175	0.518	15.751	15.795	0.940

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