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Original Article

Association of β-fibrinogen gene polymorphism and plasma fibrinogen and allergic purpura nephritis

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Abstract: Objective: To investigate the association of β-fibrinogen gene polymorphism with plasma fibrinogen and allergic purpura nephritis. Methods: We designed a case-control study (334 case and 300 control) to genotype the β-fibrinogen gene -455G/A polymorphism. The genotype and allele frequencies between the case and control group were compared. And we also compared the Fg concentration between different genotype. Results: In the case group, there were 143 cases of GA type, accounting for 42.8%; there were 168 cases of GG type, accounting for 50.3%; there were 23 cases of AA type, accounting for 6.9%. While in the control group, GG type was the most common. There were 228 cases of GG type, accounting for 76%; there were 66 cases of GA type, accounting for 22%; there was 6 case of AA type, accounting for 2%. The frequency of Fgβ-455G/A genotype between the two groups showed statistical significance ($P<0.05$). The levels of plasma Fg in the two groups showed statistical significance ($P<0.01$). In HSPN group Fgβ GA and AA-type the concentration of Fg [(4.2 ± 0.5) g/L], compared with the GG genotype [(3.1 ± 0.4) g/L], was significantly increased with statistical significance ($P<0.01$). Conclusion: The Fgβ-455G/A polymorphism was associated with the risk for HSPN and Fg concentration.

Keywords: β-fibrinogen, gene polymorphism, fibrinogen, allergic purpura nephritis

Introduction

Henoch-Schonlein purpura (HSP) is an allergic disease which is primarily based on acute inflammation of capillaries [1, 2] with an increasing rate. It often complicates with kidney damage and becomes henoch-schonlein purpura nephritis (HSPN), which is the most common secondary glomerular disease in pediatrics. In severe cases, it leads to renal fibrosis and renal failure, which seriously affects children's health. Szer [3] reported that about 25% to 60% of pediatric patients had urine abnormalities in the disease course. Based on biopsy, there will be more than 90% patients with varying degrees of renal involvement. Pathogenesis of pediatric patients has not yet been fully elucidated. In recent years, high pour factors and genetic mechanisms have become a trend in HSPN study. Early detection of patients with clotting abnormalities and treatment are very important for alleviating kidney damage and preventing complications. Fibrinogen (Fg) protein is in plasma with body's largest relative molecular mass, which was an important coagulation factor of body for physiological hemostasis. The relationship between β-fibrinogen (Fgβ) -455G/A polymorphism and hypercoagulable thrombotic diseases and the function of Fg are concentrated by more and more people. In this study, by detecting Fgβ-455G/A polymorphism and serum Fg concentration in HSPN patients, we studied its relationship with HSPN in order to provide new ideas for early clinical prevention, diagnosis and treatment of HSPN. Thus it has a broad application prospect.

Materials and methods

General information

From 2009 to 2014, 334 cases (HSPN group) of children with HSPN admitting in our hospital were selected. There were 175 males and 159 females aging from 3.5 to 16 years with mean year (8.8 ± 5.3). All the patients were in line with the following diagnostic criteria: with exact skin purpura history; with abnormal urinalysis;

excluding thrombocytopenic purpura and systemic lupus erythematosus and other systemic diseases. HSPN meets the 2001 Branch of revised nephritis diagnosis and treatment standard which were revised by nephrology group branch of pediatrics attached to Chinese Medical Sciences.

Clinical types

Type 1 is isolated hematuria or isolated proteinuria; type 2 is hematuria and proteinuria; type 3 is acute glomerulonephritis type; type 4 is nephrotic syndrome type; type 5 is rapidly progressive glomerulonephritis type; type 6 is chronic nephritis. The control group was consisted of 300 normal subjects from outpatient. There are 151 males and 149 females aging from 4 to 16 years with mean age (8.9 ± 4.5).

Methods

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was used to detect genotype of Fgβ-455G/A.

Genomic DNA extraction

Samples were 3 ml [ethylene diamine tetraacetic acid (EDTA) anticoagulant] peripheral blood. After the separation of plasma, whole blood cells were performed DNA extraction and preserved at -20°C for polymerase chain reaction (PCR) detection.

Primer design and synthesis

Primers were design and synthesized by the Biological Engineering Co., Ltd. Shanghai Generay. Primer sequences were as follows: Forward: GAA CAT TTT ACC TTA TGT GAA TTA AGG; Reverse: GAA GCT CCA AGA AAC CAT CC.

Genotyping

The anticipated length of the amplified products was 669 bp and 300 bp for β-Fg-455G/A and β-Fg-148C/T, respectively. Both amplified products included a HaeIII (New England Biolabs, Ipswich, MA, USA) restriction site, forming an RFLP (Bio-Rad, Hercules, CA, USA).

A total of 30 μL of the mixture was used for amplification and the PCR conditions for β-Fg-455G/A were: pre-denaturation at 95°C for 5 minutes; 35 cycles of denaturation at 95°C for 50 seconds, annealing at 58.2°C for 45 seconds and extension at 72°C for 60 seconds; and a final extension at 72°C for 7 minutes. Reaction was terminated by cooling to 4°C. Then, 6 μL of products were separated by 1.5% agarose gel electrophoresis (100 V) for 20 minutes and visualized with ethidium bromide staining.

Fg concentration detection

Enzyme-linked immunosorbent assay (ELISA) was used to detect serum Fg concentrations: according to specification requirements, remove 96 pieces and equilibrate them to room temperature. Except blank well, specimen or different concentrations of standard samples (100 μL/well) were added to the appropriate wells respectively, and plate wells were sealed with adhesive tape. They were incubated for 90 min at 37°C incubator. Plate was washed four times, adding biotinylated antibody solution (100 μL/hole) and incubated for 60 min at 37°C incubator. Plate was washed four times, adding enzyme conjugate working solution (100 μL/hole) and incubated at 37°C incubator for 30 min. Plate was washed 4 times and then measured absorbance (A) 450 value after color.

Statistical methods

SPSS 13.0 statistical software was used to processing the data. Date was expressed as Mean ± SD. T test was used for comparison between the groups. Classification data was expressed with frequency. χ^2 test was used for comparing differences between the two groups. $P<0.05$ was considered statistical significance.

Results

Relationship between the difference of Fgβ polymorphisms in different groups and their HSPN clinical manifestations:

By PCR-RFLP analysis, GG, GA, AA genotypes were obtained from the case and control group, as shown in **Figure 1**. Fgβ genotype distribution in HSPN group and control group showed different frequencies.

As shown in **Table 1**, there were 143 cases of GA type, accounting for 42.8%; There were 168 cases of GG type, accounting for 50.3%; There were 23 cases of AA type, accounting for 6.9%.

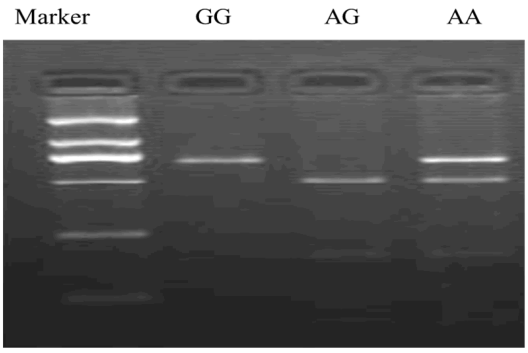

Figure 1. Genotyping results of PCR-RFLP in β-fibrinogen gene.

While in the control group, GG type was the most common. There were 228 cases of GG type, accounting for 76%; there were 66 cases of GA type, accounting for 22%; there was 6 case of AA type, accounting for 2%. χ^2 test indicated that the frequency of Fgβ-455G/A genotype between the two groups showed statistical significance (P<0.05) (**Table 1**).

Because the number of cases with AA genotype is too small in this group (2 group of 5 cases), the AA and GA genotypes were classified as the same group in analyzing the relationship between Fgβ-455G/A polymorphism and HSPN. That is carrying Fgβ-455 A allele. In HSPN group, the frequency of proteinuria and hematuria with Fgβ-455G/A GA and AA manifestations was significantly higher than that of GG genotype with statistical significance (P<0.05) **Table 2**.

Comparisons of plasma Fg levels

Because body weight, age and gender can affect the plasma Fg levels, we make sure that the body weight, age and sex composition between the two groups showed no significant difference when selecting subjects. The results showed that the levels of plasma Fg in the two groups showed statistical significance (P<0.01). In HSPN group Fgβ GA and AA-type the concentration of Fg [(4.2 ± 0.5) g/L], compared with the GG genotype [(3.1 ± 0.4) g/L], was significantly increased with statistical significance (P<0.01).

Discussion

The extent of involvement of kidney determines the long-term prognosis of HSPN. Its pathogenesis has not been fully elucidated. With the progress in molecular biology and immunology, nephritis is considered to be a kind of systemic vasculitis mediated by autoimmune reactions involved in the pathogenesis mainly through immunological mechanisms, mechanisms of inflammation and coagulation mechanisms [4, 5]. In recent years, the role of the relevant clotting mechanisms and genetic mechanism in HSPN has gradually been concerned [6-11].

The results of the study showed that: statistically significant differences had been found in Fgβ-455G/A genotype frequencies between the two groups. In HSPN group, the frequencies of proteinuria and hematuria in patients with GG genotype, and the difference were statistically significant. There was a statistically significant difference in plasma Fg level between the two groups. In HSPN group, Fg concentration of FgβGA and AA genotypes was significantly increased compared with AA genotype and GG genotype, and the difference was statistically significant. Studies have shown that intravascular coagulation was particularly prominent in this disease [12, 13]. Fg is the highest coagulation protein in all coagulation factors; up-regulation of Fg can slow down blood flow velocity and easy the adhesion of erythrocytes and platelets on the damaged surface of endothelial cells. Fg could bind to the Fg receptor on platelet surface and promote platelet aggregation by oxidative stress to increase platelet cohesiveness, which is conducive for thrombosis and hypercoagulable state [14]. Balance disorders of coagulation and fibrinolysis system is an important factor in the disease progression of various kidney diseases; in the development of kidney disease, coagulation hyperthyroidism appeared in the peripheral blood and renal tissue, expressing as thrombosis and thrombotic microangiopathy, thus leading to progressive glomerular sclerosis and clinical chronic renal failure [15, 16]. Therefore, prevention and early detection of nephritis are very important. Fg is an acute-phase protein significantly increased in the acute phase, which is a sensitive indicator reflecting the body's inflammatory response. The mechanism may be that: Fg, as an acute-reactive protein, is significantly increased in acute extensive acute necrotizing vasculitis. Since a large number of urinary albumin loss, plasma hypoalbuminemia stimulates the enhancement of liver Fg synthesis capacity,

Table 1. The genotypes and allele distribution between control group and HSPN group

Group	Number	Genotype (n, %)			Allele (n, %)	
		GG	GA	AA	G	A
Control group	300	228 (76.0)	66 (22.0)	6 (2.0)	522 (87.0)	78 (13.0)
HSPN group	334	168 (50.3)	143 (42.8)	23 (6.9)	479 (71.7)	189 (28.3)

Table 2. The frequencies of complications between different genotypes in HSPN group

Genotypes	n	Proteinuria (n, %)	Microscopic hematuria (n, %)	Visible haematuria (n, %)
GG	168	24 (14.3)	54 (32.1)	6 (3.6)
GA	143	125 (87.4)	67 (46.9)	58 (40.7)
AA	23	6 (26.1)	2 (8.7)	6 (26.1)

which is another important factor for Fg increase. In the 334 patients of HSPN group, levels of plasma Fg were increased in 76% of patients, while only 38% of the control group with increased Fg level; the difference was statistically significant. Therefore, Fg can provide reliable experimental basis for clinical treatment. Regularly detecting Fg to understand the body's clotting mechanism, has a certain clinical significance for early detection and intervention in the occurrence and development of purpura nephritis.

In acute reaction phase in the body, under the stimulation of inflammatory factors, mRNA synthesis of Fg α, β, γ chains was increased in liver cells; wherein, mRNA transcription of β chain determined the mRNA transcription of α and γ chains, playing the rate-limiting role in the synthesis of entire Fg molecule and having an important impact on the level of Fg. Brown and Fuller [17] found that liver cells had 3 proteins, Complex I, II, III which can combine with DNA fragments on both sides of β-455 sites (-468~-439 bP). The combination between Complex III and -455 A was loose, while the -455 G had a solid combination with Complex III, having a "repressor" effect and thereby reducing the transcriptional activity of β chain. Because of the vulnerable repression of -455 G, Fg levels of Fgβ-455 A allele carriers were significantly higher than those of GG genotype carriers, consistent with the previous research results [17].

Genotype A in Fgβ-455 locus was closely related with the increase in Fg levels. In HSPN group, frequencies of proteinuria and hematuria in Fgβ-455G/A GA and AA genotypes were significantly higher than those in GG genotype, and the difference was statistically significant.

From the results of this study we can speculate that GA or AA genotype related to the elevated levels of Fg which may be a major factor for individual differences in susceptibility to HSPN. Once HSPN occurred in individuals with GA or AA genotypes, the clinical manifestations were heavier. Early detection of patients with clotting abnormalities and treatment is very important for relieving from renal impairment and preventing from complications.

Disclosure of conflict of interest

None.

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