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Original Article

Association of 5′-Untranslated Region Polymorphism of VEGF Gene with Henoch-Schönlein in North West of Iran

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Abstract

Background: Henoch-Schönlein purpura (HSP) is an lgA mediated small vessel systemic vasculitis disease in children. The etiology and pathogenesis of HSP disease remain unknown. However, environmental and genetic risk factors could play important roles in susceptibility to HSP disease. In this study we investigated the association of 5′-untranslated region polymorphism (*-634G/C*) of *VEGF* gene with HSP among Iranian Azeri Turkish population.
Methods: Thirty unrelated Iranian Azeri Turkish children with HSP and fifty healthy unrelated subjects without HSP and other inflammatory diseases were enrolled in this population. *-634G/C* polymorphism of VEGF gene was genotyped by polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) technique.
Results: The distribution of CC genotype in *VEGF -634G/C* polymorphism statistically showed a significant difference in HSP patients in compare to that of control group (*P*= 0.009).
Conclusions: The CC genotype of VEGF -634G/C polymorphism could be associated with susceptibility to HSP disease in Iranian Azeri Turkish ethnic group.

Key word: *-634G/C* polymorphism, vascular endothelial growth factor (*VEGF*), Henoch-Schönlein purpura (HSP), Iran

Introduction

Henoch-Schönlein purpura (HSP) is the most common small vessel systemic vasculitis disease in children (Dillon MJ. 2007). HSP generally occurs in children between 2 and 15 years (McCarthy JH, et al. 2010). It is more common in boys than in girls (Tizard EJ. 1999). The main symptom of HSP disease is a characteristic skin rash (Sohagia AB, et al.2010). Painful and swollen joints with limitation of movement are found in the majority of patients. Abdominal pain is present in patients when the vessels of the bowel become inflamed. Abdominal pain may be accompanied by gastrointestinal bleeding

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(hemorrhage) (Lawee, 2008). When the vessels of kidneys become inflamed, mild or severe hematuria and proteinuria may occur (Tizard, 1999).

The main causes of this disease remain unknown. Genetic and environmental risk factors could play important roles in susceptibility to HSP (YI ZW,et al. 2006). The polymorphisms of many genes are investigated in HSP disease, such as polymorphisms in Inter-Cellular Adhesion Molecule1 (Amoli et al., 2001), *PAX2* (Yi et al., 2006), *MEFV* (Gershoni-Baruch R. 2003), endothelial nitric oxide synthases (He X. 2013), rennin-angiotensin system (*RAS*) components (Nalbantoglu et al., 2013) and vascular endothelial growth factor (VEGF) gene (Rueda et al., 2006; Zeng et al. 2009). Some of these genes (*MEFV* in Israeli and Turkish children, *RAS* in Turkish population and *VEGF* in Spanish and Chinese children) significantly associated with development of HSP and/or increased susceptibility of nephritis in HSP patients.

The *VEGF* gene is chromosomally located at 6p21.3 and contains 8 exons (Vincenti et al., 1996; Neufeld et al., 1999). The disorders of the vasculature could cause many human diseases (Tammela et al., 2005). Recent study reported that the VEGF may play a main role in inflammatory reaction of the vascular bed in HSP disease (Topaloglu et al., 2011). Other studies have also shown that the *-634G/C* polymorphism of *VEGF* gene is associated with Henoch-Schönlein purpura nephritis (HSPN) in Spanish and Chinese ethnic groups (Rueda et al., 2006; Zeng et al., 2009). Therefore this study was planned to investigate the association of *-634G/C* polymorphism in *VEGF* gene with HSP disease among Iranian Azeri Turkish ethnic group.

Materials and methods

Patients

Thirty unrelated Iranian Azeri Turkish ethnic children with Henoch-Schönlein purpura constituted the study group. Fifty healthy unrelated people without HSP and other inflammatory diseases were enrolled as the control group. All patients were diagnosed by allergy and nephrologists and referred to the Molecular-Medical Genetic center of Tabriz.

Methods

Genomic DNA was isolated from peripheral blood using standard methods (Miller SA, et al. 1988). The *-634G/C* polymorphism of *VEGF* gene was genotyped by polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) technique.

PCR amplification was performed with an initial denaturation at 95°C for 5 min followed by 35 cycles of 95°C for 30sec, 60.3°C (annealing temperatures) for 45 sec and 72°C

for 35sec. Final extension was carried out at 72°C for 5 min, which amplified a fragment of 343bp. The amplicons were separated on 1.5% agarose gels electrophoresis.

The amplified fragments were digested with *BsmfI* restriction endonuclease at 65°C. Restriction fragment analyzed by 10% polyacrylamid gel electrophoresis and visualized with ethidium bromide. The GG genotype was cut into two fragments of 250bp and 93bp while the CC genotype displayed a single fragment of 343bp (Figures 1 and 2 respectively).

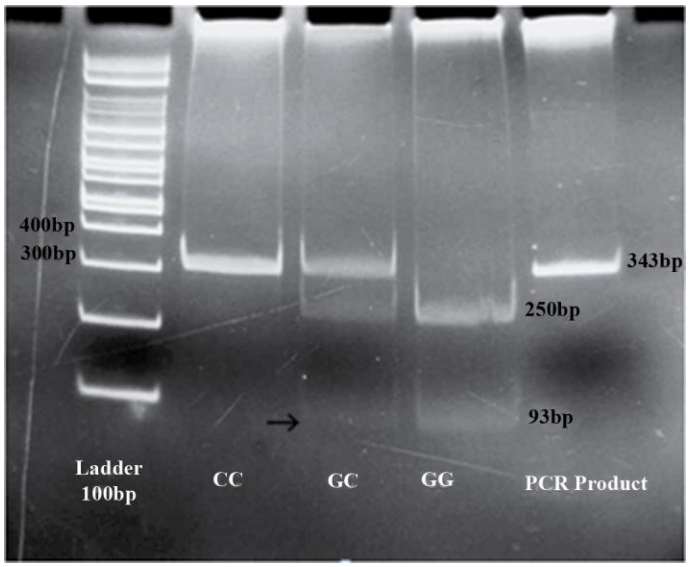

Figure 1. The Vascular endothelial growth factor genotypes in henoch-schönlein

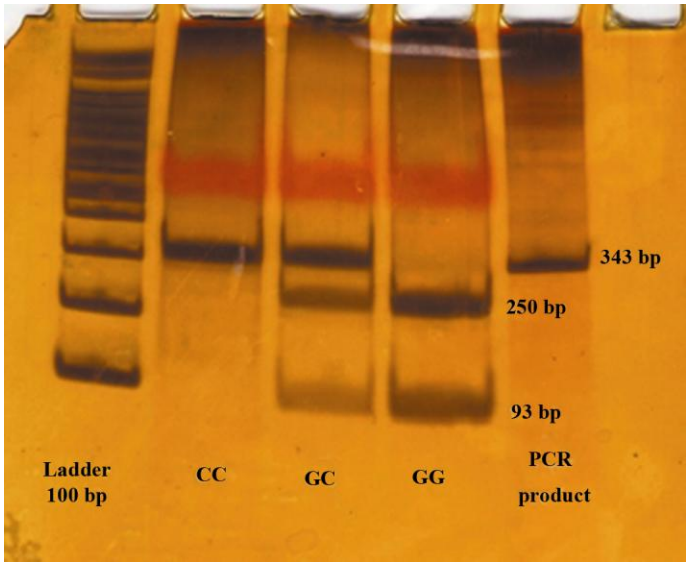

Figure 2. The genotypes of vascular endothelial growth factor in henoch-schönlein

Statistical analysis

The differences in VEGF allele and genotype frequencies between the HSP patients and the control group were analyzed for statistical significance at the 95% confidence interval (CI) using Chi-Square Test. *P*-values <0.05 were considered as statistically significant difference.

Results

The allele and genotype frequencies of VEGF gene were calculated for the HSP patients and a control group (Table 1). The CC genotype distribution in the HSP patients was significantly higher as compare to that of control group (*P*= 0.009). A significant increase in the frequency of GC genotype was observed in the control group as compared to HSP patients (*P*= 0.001). Whereas, there were no significant difference in the *VEGF*-634 allelic distribution in HSP patients and the control group.

Table1. Genotype and allele frequencies of the *VEGF -634G/C* polymorphism in HSP patients and control group

Genotypes And Alleles	HSP N=30 (%)	Control N=50 (%)	Odd ratio (95% CI)	*P*-value
CC	8 (26.67)	6 (12)	2.667	0.009*
GC	17 (56.67)	39 (78)	0.369	0.001*
GG	5 (16.67)	5 (10)	1.800	0.165
C	33 (55)	51 (51)	1.174	0.571
G	27 (45)	49 (49)	0.852	0.571

*Statistically significant (*P<0.05*)
†CI= confidence intervals

Discussion

Although HSP is a self-limited disorder in children, it could sometimes lead to gastrointestinal involvement or renal insufficiency in adults (Rai et al., 1999). Identification of the susceptibility genes in HSP could be implicated in treatment and diagnostic process of this disease (Zeng et al., 2009).

In previous functional studies, the *-634C* allele and the CC genotype were associated with increased *VEGF* expression (Yang et al., 2010). Therefore the CC genotype of *VEGF* -634G/C polymorphism could increase the plasma VEGF levels in our patients and could play an important role in susceptibility to the development of HSP disease.

Based on the results of this study it could be suggested that *VEGF* -634G/C polymorphism can play a key role in the pathogenesis of Henoch-Schonlein purpura in this ethnic group.

Recent studies have evaluated the association between -634G/C polymorphism in the *VEGF* gene and HSP disease. According to Reuda et al. (2006) the -634G/C polymorphism was not associated with HSP in related population. However, the C allele of -634G/C

polymorphism was associated with the development of nephritis in patients with HSP. It has also been reported that the C allele and CC genotype of *VEGF-634G/C* polymorphism were associated with development of HSPN (Zeng et al., 2009). The inconsistency in results between our study and reports in Chinese and Spanish populations might be due to ethnic differences. To confirm the observed association, we suggest further study of this polymorphism among HSP patients in other ethnic groups.

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