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Gene polymorphism of vascular endothelial growth factor in children with Henoch-Schonlein purpura nephritis

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Abstract: **Objective** To study the relationship of -634G/C gene polymorphism of vascular endothelial growth factor (VEGF) with Henoch-Schonlein purpura nephritis (HSPN) in children. **Methods** One hundred ethnic Han children with HSP, including 50 children with concurrent nephritis (HSPN group) and 50 children without nephritis (HSP without nephritis group), were enrolled. Fifty age-, sex- and ethnics-matched healthy children were used as the control group. VEGF-634G/C genotypes were determined by PCR-RFLP. Plasma VEGF levels were measured using ELISA. **Results** CC genotype distribution (32%) and C allele frequency (56%) in the HSPN group were significantly higher than those in the control group (10% and 35% respectively) and the HSP without nephritis group (10% and 33% respectively) ($P<0.01$). The incidence of nephritis in HSP patients with CC genotype increased significantly when compared with those with GG genotype (76% vs 31%; $P<0.01$). Plasma VEGF levels in patients with CC genotype (180.5 ± 40.7 pg/mL) were significantly higher than those in patients with CG (145.2 ± 48.3 pg/mL) and GG (101.5 ± 26.5 pg/mL) genotypes ($P<0.05$). **Conclusions** VEGF-634G/C gene polymorphism may be associated with the development of HSPN. C allele may a susceptible gene of HSPN. [**Chin J Contemp Pediatr**, **2009**, **11** (**6**):**417 – 421**]

Key words: Henoch-Schonlein purpura nephritis; Gene polymorphism; Vascular endothelial growth factor; Child

儿童过敏性紫癜性肾炎血管内皮生长因子基因多态性的研究

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[**摘　要**] **目的** 探讨血管内皮生长因子(VEGF)-634G/C 基因多态性与汉族儿童紫癜性肾炎(HSPN)的关系。**方法** 应用聚合酶链反应 – 限制性内切酶片段长度多态性(PCR – RFLP)技术对 100 例过敏性紫癜(HSP)汉族儿童进行 VEGF -634G/C 基因型分析,其中包括合并紫癜性肾炎 50 例(HSPN 组),无合并肾炎者 50 例(单纯 HSP 组)。50 例年龄、性别匹配的健康汉族儿童作为对照组。并采用酶联免疫吸附试验(ELISA)检测各组血浆 VEGF 水平。**结果** HSPN 组 VEGF-634CC 基因型(32%)和 C 等位基因频率(56%)均高于对照组(分别为 10% 和 33%)及单纯 HSP 组(分别为 10% 和 35%,$P<0.01$)。HSP 患儿中,CC 基因型者肾炎的发生率比 GG 基因型者明显增加,差异有显著性意义(76% vs 31%,$P<0.01$)。CC 基因型者血浆 VEGF 水平(180.5 ± 40.7 pg/mL)较 CG(145.2 ± 48.3 pg/mL)及 GG(101.5 ± 26.5 pg/mL)基因型者显著上升,差异有显著性意义($P<0.05$)。**结论** VEGF-634G/C 基因多态性与儿童 HSPN 的发生有关。C 等位基因可能是儿童 HSPN 的易感基因。 [**中国当代儿科杂志,2009,11(6):417 – 421**]

[**关　键　词**] 过敏性紫癜性肾炎;基因多态性;血管内皮生长因子;儿童
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Topaloglu et al[1] reported that vascular endothelial growth factor (VEGF) may play a crucial role in the morphological and functional changes of the vascular bed and inflammatory reaction in Henoch-Schonlein

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purpura (HSP). Many polymorphisms of the VEGF gene have been identified. VEGF gene was first screened for polymorphisms by Watson et al[2]. Some studies have investigated the association of VEGF gene polymorphisms with diseases in which angiogenesis plays a major role in pathogenesis, such as diabetic retinopathy[3], renal cell carcinoma[4], acute renal allograft rejection[5], prostate cancer[6], and malignant melanoma[7]. The studies have shown that a few of the diseases are correlated with the variation in VEGF protein production[2,3,8,9].

The -634G/C polymorphism at the 5′-UTR of VEGF has been evaluated as a risk allele for the susceptibility of Henoch-Schonlein purpura nephritis (HSPN) through case-control studies in the Lugo region of Northwest Spain[10]. This study investigated whether the VEGF-634G/C polymorphism at the 5′- UTR is associated with HSPN in Chinese ethnic Han children.

Methods

Subjects

One hundred children with HSP, including 50 patients with concurrent nephritis (HSPN group, mean age 7.7 ± 2.3 years) and 50 patients without nephritis (HSP without nephritis group, mean age 8.0 ± 2.0 years), were enrolled. All of the patients were unrelated Chinese ethnic Han individuals. The patients were recruited from Guangzhou Children's Hospital between January 2005 and December 2006. They were required to have a follow-up of at least 6 months. Diagnosis of HSP was based on The American College of Rheumatology 1990 Criteria for the Classification of Henoch-Schonlein Purpura. HSPN was diagnosed when haematuria and/or proteinuria occurred in HSP. Haematuria was defined by the presence of five or more red blood cells per high power field, and macrohaematuria was defined when blood was visible to the naked eye in the urine. Proteinuria was evaluated by 24 hours quantitative measurements of urinary protein.

As ethnically matched controls for the allele frequency of the specific polymorphism, 50 healthy unrelated Chinese children (mean age 7.5 ± 2.1 years) were included. The control population was matched for age, sex, and ethnics.

Analysis of the VEGF-634 G/C polymorphism

Genomic DNA was extracted from whole blood of each individual using whole blood genome DNA extract kit following the manufacturer's protocol. Concentration and purity of DNA were determined by ultraviolet spectrophotometry. Genotyping of each polymorphism was carried out by PCR-RFLP analysis. For the -634G/C polymorphism the following primers amplified a fragment of 401 bp: 5′-CGCTCGGTGCTGGAATTTG-3′ (forward) and 5′-GTATGTCTGTCTGTCCGTCA-3′ (reverse). The -634G allele results in the gain of BsmFI site. The -634G allele was cut into two fragments of 300 bp and 101 bp while the -634C allele remained uncut (401 bp). PCR products and restriction fragments were analyzed by 2.0% agarose gels electrophoresis, stained with ethidium bromide, and photographed.

Measurement of plasma VEGF concentration

Venous blood samples were taken from all of subjects before breakfast. Blood samples were centrifuged after full clotting at room temperature and plasma were stored at −80°C. Plasma VEGF levels were measured using enzyme linked immunosorbent assay (ELISA).

Statistical analysis

Statistical analyses were done with SPSS 11.5. Differences in the distribution of VEGF genotypes and allele frequencies between the study and control groups were analyzed by the χ^2-test or the Fisher's exact test. The statistical significances for deviations from the Hardy-Weinberg equilibrium were tested using the Pearson test. Plasma VEGF levels were compared by the unpaired Student's t test. Data were expressed as mean values ± standard deviation ($\bar{x} \pm s$). A P value of less than 0.05 was believed to be statistically significant.

Results

Genotype and allele frequencies of the VEGF-634G/C polymorphism

The Hardy-Weinberg equilibrium was observed for VEGF-634 allele and genotypes in all subjects. Allele and genotype frequencies for VEGF -634G/C are shown in Table 1. There were no significant differences in the VEGF -634 genotype ($\chi^2 = 0.179$, $P > 0.05$) and allele frequencies ($\chi^2 = 0.089$, $P > 0.05$) between the HSP without nephritis and the control groups. There were significant differences in the VEGF-634 genotype frequency between the HSPN and the control groups ($\chi^2 = 9.116$, $P < 0.05$). Significant differences were also found in the VEGF-634 genotype frequency between the HSPN and the HSP without nephritis groups ($\chi^2 = 10.283$, $P < 0.01$). The VEGF-634 CC genotype frequency in the HSPN group increased significantly as compared with the control group ($\chi^2 = 7.294$, $P < 0.01$). A significant increase in the frequency of C allele was observed in the HSPN group as compared with the control group ($\chi^2 = 8.892$, $P < 0.01$). The HSPN group demonstrated increased

VEGF-634 CC genotype frequency ($\chi^2 = 7.294$, $P < 0.01$) and increased frequency of C allele compared with the HSP without nephritis group ($\chi^2 = 10.710$, $P < 0.01$). VEGF-634 genotypes detected by PCR-RFLP are shown in Figure 1.

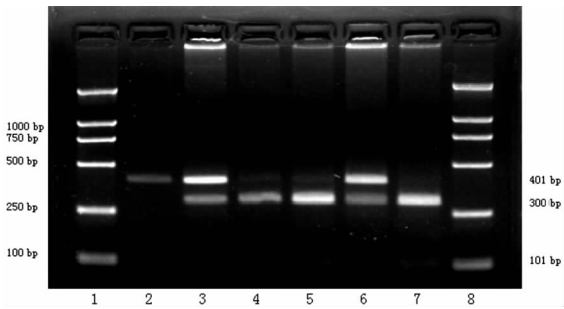

Figure 1 VEGF-634 genotypes detected by PCR-RFLP 1, 8: DNA marker; 2: CC genotype, one fragment of 401 bp; 4, 5, 7: GG genotype, two fragments of 300 bp and 101 bp; 3, 6: CG genotype, three fragments of 300 bp, 101 bp and 401 bp.

Table 1 Genotype and allele frequencies of the VEGF-634G/C polymorphism [n(%)]

Group	n	Genotype frequency			Allele frequency	
		CC	CG	GG	C	G
Control	50	5(10)	25(50)	20(40)	35(35)	65(65)
HSP without nephritis	50	5(10)	23(46)	22(44)	33(33)	67(67)
HSPN	50	16(32)[a]	24(48)	10(20)	56(56)[a]	44(44)

a: $P < 0.01$, vs the control and the HSP without nephritis groups.

Association of VEGF genotypes with the development of nephritis

The incidence of nephritis in HSP patients with VEGF-634 CC genotype was significantly higher than that in patients with VEGF- 634 GG genotype ($\chi^2 = 10.247$, $P < 0.01$). The CC genotype of the - 634G/C polymorphism was associated with an increased risk for the development of nephritis in HSP patients ($P < 0.01$; $OR = 7.040$; 95% $CI = 2.013$-24.618). See Table 2.

Table 2 Association of VEGF genotypes with the development of nephritis

Genotype	n	Incidence (%)
GG	10	31
CG	24	51
CC	16	76[a]

a: $P < 0.01$, vs the GG genotype group.

Plasma VEGF levels in patients with different genotypes

As shown in Table 3, plasma VEGF levels in patients with CC genotype were significantly higher than those in patients with other genotypes ($P < 0.05$).

Table 3 Plasma VEGF levels ($\bar{x} \pm s$)

Genotype	n	VEGF (pg/mL)
GG	52	101.5 ± 26.5
CG	72	145.2 ± 48.3
CC	26	180.5 ± 40.7[a]

a: $P < 0.05$, vs the GG and CG genotype groups.

Discussion

HSP is the most common vasculitis syndrome in children. It is an immunoglobulin A (IgA)-mediated immune-complex vasculitis that affects predominantly the skin, joints, gastrointestinal tract and kidneys. It is generally a benign, self-limited disorder that follows an intercurrent illness, usually of the upper respiratory tract. The spectrum of the clinical expression of HSP may vary from only a minimal petechial rash to severe gastrointestinal, renal, neurologic, pulmonary, and joint diseases. In the long-term follow-up, systemic involvements or serious sequelae are not frequent. Its prognosis is mainly predicted by the severity of renal involvement. The proportion of patients reported to have renal involvement varies between 20% and 80%. The majority of children with HSPN present only with haematuria and/or low-grade proteinuria, or both, and have a good chance to recover. However, patients with massive proteinuria at onset frequently have a progressive course [11,12]. In some reports, the proportion of children with HSPN who progress to renal failure or end-stage renal disease varies from 12% to 19% [13-16]. Substantial research has found that genetic factors might play an important role in the pathogenesis of HSPN. In order to improve the prognosis of HSP, it is very important to find the susceptibility gene to HSPN for early diagnosis and treatment of HSPN.

VEGF is an endothelial cell-specific mitogen that promotes angiogenesis and is a potent mediator of vascular permeability[17]. The gene encoding VEGF is located on chromosome 6p21.3 and comprises a 14 kb coding region with 8 exons and 7 introns[18]. The biological activities of VEGF are mediated through two high-affinity receptor tyrosine kinases, fms-like tyrosine kinase-1 (Flt-1) (VEGF receptor-1) and fetal liver kinase-1 (flk1) (VEGF receptor-2)[19], and their expression is mainly restricted to endothelial cells[20]. A possible role of VEGF in the activation and recruitment of monocytes is mediated through the Flt-1 receptor [21,22]. Functional studies have shown that the VEGF - 634C allele is associated with increased transcriptional levels of VEGF, both *in vitro* and *in vivo*[23]. This allele is also related to a higher activity at ribo-

some site B (IRESB)[24-26], suggesting that it also influences VEGF translation. The GG genotype is embedded in haplotypes with lower VEGF expression, whereas the CC genotype is present in most of the haplotypes with the highest VEGF transcription levels[24,27]. Presently, the researchers have been focusing on the association between VEGF gene polymorphism and HSPN.

Reuda et al[10] analyzed VEGF- 634G/C gene polymorphism of 57 HSP cases and 226 healthy controls. They found that there were no significant differences in the allele and genotype frequencies for the VEGF - 634G/C polymorphism between HSP patients and controls. However, they found that the C allele of VEGF- 634G/C gene polymorphism was associated with the development of HSPN and that the C allele may be a susceptible gene of HSPN. The results of this study were consistent with those of Reuda et al. In this study, the HSPN group demonstrated increased VEGF- 634 CC genotype and C allele frequencies compared with the control and the HSP without renal involvement groups. Further, this study showed that the incidence of nephritis in HSP patients with VEGF- 634 CC genotype was significantly higher than that in patients with VEGF- 634 GG genotype.

This study found that plasma VEGF levels in patients with CC genotype of the - 634G/C polymorphism were significantly higher than those in patients with CG or GG genotypes and that there were no significant differences in plasma VEGF levels in patients with CG and GG genotypes. It suggested that plasma VEGF levels were associated with the - 634G/C polymorphism. Awata et al[3] tested VEGF levels and VEGF - 634G/C polymorphism in 64 healthy controls, and reported that plasma VEGF levels in patients with CC genotype were higher than those with CG and GG genotypes and there were no significant differences in plasma VEGF levels between patients with CG and GG genotypes. But Watson et al[2] found that plasma VEGF levels in patients with the GG genotype were the highest and those were lowest in patients with the CC genotype. The differences of results may be related to means of test, the size of sample, sampling error and sources of VEGF.

In summary, this study showed that the VEGF - 634 CC genotype and - 634 C allele frequencies increased in children with HSPN compared with HSP children without nephritis and healthy controls and that the CC genotype of the - 634G/C polymorphism was associated with an increased risk for the development of nephritis in HSP patients. Furthermore, plasma VEGF levels were significantly higher in HSP patients with the CC genotype of the - 634G/C polymorphism than those in patients with CG and GG genotypes. This suggests that VEGF- 634G/C gene polymorphism may be associated with the development of HSPN and that C allele may be a susceptible gene of HSPN.

[References]

[1] Topaloglu R, Sungur A, Baskin E, Besbas N, Saatci U, Bakkaloglu A. Vascular endothelial growth factor in Henoch-Schonlein purpura[J]. J Rheumatol, 2001, 28(10):2269-2273.

[2] Watson CJ, Webb NJ, Bottomley MJ, Brenchley PE. Identification of polymorphisms within the vascular endothelial growth factor (VEGF) gene: correlation with variation in VEGF protein production[J]. Cytokine, 2000, 12(8):1232-1235.

[3] Awata T, Inoue K, Kurihara S, Ohkubo T, Watanabe M, Inukai K, et al. A common polymorphism in the 5′-untranslated region of the VEGF gene is associated with diabetic retinopathy in type 2 diabetes[J]. Diabetes, 2002,51(5):1635-1639.

[4] Abe A, Sato K, Habuchi T, Wang L, Li Z, Tsuchiya N, et al. Single nucleotide polymorphisms in the 3′untranslated region of vascular endothelial growth factor gene in Japanese population with or without renal cell carcinoma[J]. Tohoku J Exp Med, 2002, 198(3):181-190.

[5] Shahbazi M, Fryer AA, Pravica V, Brogan IJ, Ramsay HM, Hutchinson IV, et al. Vascular endothelial growth factor gene polymorphisms are associated with acute renal allograft rejection[J]. J Am Soc Nephrol, 2002,13(1):260-264.

[6] McCarron SL, Edwards S, Evans PR, Gibbs R, Dearnaley DP, Dowe A, et al. Influence of cytokine gene polymorphisms on the development of prostate cancer[J]. Cancer Res, 2002,62(12): 3369-3372.

[7] Howell WM, Bateman AC, Turner SJ, Collins A, Theaker JM. Influence of vascular endothelial growth factor single nucleotide polymorphisms on tumour development in cutaneous malignant melanoma[J]. Genes Immun, 2002, 3(4):229-232.

[8] Renner W, Kotschan S, Hoffmann C, Obermayer-Pietsch B, Pilger E. A common 936 C/T mutation in the gene for vascular endothelial growth factor is associated with vascular endothelial growth factor plasma levels[J]. J Vasc Res, 2000, 37(6):443-448.

[9] Brogan IJ, Khan N, Isaac K, Hutchinson JA, Pravica V, Hutchinson IV. Novel polymorphisms in the promoter and 5′ UTR regions of the human vascular endothelial growth factor gene[J]. Hum Immunol, 1999, 60(12):1245-1249.

[10] Rueda B, Perez-Armengol C, Lopez-Lopez S, Garcia-Porrua C, Martín J, Gonzalez-Gay MA. Association between functional haplotypes of vascular endothelial growth factor and renal complications in Henoch-Schönlein purpura[J]. J Rheumatol, 2006, 33 (1):69-73.

[11] Habib R, Niaudet R, Levy M. Schoenlein-Henoch purpura nephritis and IgA nephropathy[M].//Tisher CC, Brenner BM. Renal Pathology with Clinical and Functional Correlations. 2nd ed. Philadelphia: Lippincott, 1994, 427-523.

[12] Haycock GB. The nephritis of Henoch-Schoenlein purpura[M]. //Cameron JS. Oxford Textbook of Nephrology. 2nd ed. Oxford: Oxford University Press, 1998, 585-612.

[13] Niaudet P, Habib R. Methylprednisolone pulse therapy in the treatment of severe forms of Schoenlein-Henoch purpura nephritis [J]. Pediatr Nephrol, 1998, 12(3):238-243.

[14] Watanabe T, Takahashi S, Nakajo S, Hamasaki M. Pathological

improvement of IgA nephropathy and Henoch-Schoenlein purpura nephritis with urokinase therapy[J]. Acta Paediatr Japonica, 1996, 38(6):622-628.
[15] Coppo R, Mazzucco G, Cagnoli L, Lupo A, Schena FP. Long-term prognosis of Henoch-Schonlein nephritis in adults and children. Italian Group of Renal Immunopathology Collaborative Study on Henoch-Schonlein purpura[J]. Nephrol Dial Transplant, 1997, 12(1):2277-2283.
[16] Counahan R, Winterborn MH, White RH, Heaton JM, Meadow SR, Bluett NH, et al. Prognosis of Henoch-Schonlein nephritis in children[J]. Br Med J, 1977, 2(6078):11-14.
[17] Ferrara N, Houck K, Jakeman L, Leung DW. Molecular and biological properties of the vascular endothelial growth factor family of proteins[J]. Endocr Rev, 1992, 13(1):18-32.
[18] Vincenti V, Cassano C, Rocchi M, Persico G. Assignment of the vascular endothelial growth factor gene to human chromosome 6p21.3[J]. Circulation, 1996, 93(8):1493-1495.
[19] Ferrara N, Davis-Smyth T. The biology of vascular endothelial growth factor[J]. Endocr Rev, 1997, 18(1):4-25.
[20] Rahimi N, Dayanir V, Lashkari K. Receptor chimeras indicate that the vascular endothelial growth factor receptor-1 (VEGFR-1) modulates mitogenic activity of VEGFR-2 in endothelial cells[J]. J Biol Chem, 2000, 275(22):16986-16992.
[21] Barleon B, Sozzani S, Zhou D, Weich HA, Mantovani A, Marmé D. Migration of human monocytes in response to vascular endothelial growth factor (VEGF) is mediated via the VEGF receptor flt-1[J]. Blood, 1996, 87(8):3336-3343.
[22] Clauss M, Weich H, Breier G, Knies U, Rockl W, Waltenberger J, et al. The vascular endothelial growth factor receptor Flt-1 mediates biological activities: implications for a functional role of placenta growth factor in monocyte activation and chemotaxis[J]. J Biol Chem, 1996, 271(30):17629-17634.
[23] Awata T, Kurihara S, Takata N, Neda T, Iizuka H, Ohkubo T, et al. Functional VEGF C-634G polymorphism is associated with development of diabetic macular edema and correlated with macular retinal thickness in type 2 diabetes[J]. Biochem Biophys Res Commun, 2005, 333(3): 679-685.
[24] Lambrechts D, Storkebaum E, Morimoto M, Del-Favero J, Desmet F, Marklund SL, et al. VEGF is a modifier of amyotrophic lateral sclerosis in mice and humans and protects motoneurons against ischemic death[J]. Nat Genet, 2003, 34(4): 383-394.
[25] Bornes S, Boulard M, Hieblot C, Zanibellato C, Iacovoni JS, Prats H, et al. Control of the vascular endothelial growth factor internal ribosome entry site (IRES) activity and translation initiation by alternatively spliced coding sequences[J]. J Biol Chem, 2004, 279(18):18717-18726.
[26] Huez I, Bornes S, Bresson D, Creancier L, Prats H. New vascular endothelial growth factor isoform generated by internal ribosome entry site-driven CUG translation initiation[J]. Mol Endocrinol, 2001, 15(12):2197-2210.
[27] Lambrechts D, Devriendt K, Driscoll DA, Goldmuntz E, Gewillig M, Vlietinck R, et al. Low expression VEGF haplotype increases the risk for tetralogy of Fallot: a family based association study[J]. J Med Genet, 2005, 42(6):519-522.

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