

## Association of arginine vasopressin receptor 1a gene polymorphisms with hepatorenal syndrome

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### Abstract

**Objective:** To assess the association of arginine vasopressin receptor 1a gene single nucleotide polymorphisms with type I hepatorenal syndrome.

**Methods:** The case-control study was conducted at the Hangzhou City Xixi Hospital, Hangzhou, China, from January 2012 to June 2014, and comprised patients with type I hepatorenal syndrome and individuals with cirrhosis who acted as the control group. Arginine vasopressin receptor 1a gene rs113481894 locus single nucleotide polymorphisms were analysed by high-resolution melting methods. Statistical analysis was performed using SPSS 17.

**Results:** Of the 60 participants, 28(46.7%) were in the hepatorenal syndrome group and 32(53.3%) were controls. The mean age was 42.21±11.30years in the hepatorenal syndrome group and 43.69±12.60in the control group (p=0.64). Mean total bilirubin, albumin and prothrombin activity levels were 154.76±51.58, 49.30±24.67 and 33.42±3.69 in the hepatorenal syndrome group compared to 181.26±64.46, 41.78±17.52 and 32.98±4.81 among controls (p=0.09, p=0.18 and p=0.70). Statistically significant differences were found in the distributions of arginine vasopressin receptor 1a gene rs113481894 locus T allele between type I hepatorenal syndrome patients and the control group (odds ratio= 2.230; p= 0.040).

**Conclusion:** T allele located at arginine vasopressin receptor 1a receptor promoter rs113481894 locus may be associated with the pathogenesis of type I hepatorenal syndrome.

**Keywords:** Arginine vasopressin receptor, AVPR, Hepatorenal syndrome, HRS, Single nucleotide polymorphisms, SNPs, Liver cirrhosis. (JPMA 67: 577; 2017)

### Introduction

Type I hepatorenal syndrome (HRS) is a special type of acute kidney injury, which is one of the most serious complications of decompensated cirrhosis and acute liver failure,<sup>1</sup> and is an important sign of poor prognosis and independent prediction parameter for death.<sup>2</sup> During treatment, it was discovered that HRS prevalence in patients with advanced liver diseases is obviously different. Patients with the same liver function rating level would not all have HRS as complication, and it can be inferred that gene differences may constitute a risk factor for HRS.

Terlipressin has a selective effect on vasoconstriction, and thus is one of the recommended therapeutics in type I HRS.<sup>3</sup> Arginine vasopressin receptor 1a (AVPR1A) antagonist can block the vasoconstrictor effect.<sup>4</sup> It has been found that AVPR1A gene promoter -6951 locus' single nucleotide polymorphisms (SNPs) in primary hypertension patients are closely associated with vasoconstrictor effect.<sup>5</sup>

The current study was planned to assess the associations of genotype and allele frequencies by detecting AVPR1A

gene promoter SNPs-6951 locus in type I HRS patients, in order to identify molecular markers of HRS early detection.

### Subjects and Methods

The case-control study was conducted at the Hangzhou City Xixi Hospital, Hangzhou, China, from January 2012 to June 2014, and comprised patients with type I HRS and individuals with cirrhosis who acted as the control group. All patients met the HRS definition and diagnostic criteria suggested by the American Institute of Liver Diseases in its treatment recommendations for ascites due to cirrhosis in 2007:<sup>6</sup> (1) liver cirrhosis accompanied with ascites; (2) serum creatinine > 133 μmol/l (1.5 mg/dl); (3) serum creatinine with no improvement at least 2 days after application of albumin expansion and discontinuing diuretics usage, but not below 133μmol/l (the recommended albumin dose was 1 g/kg.d with a maximum of 100 g/d); (4) no clinical symptoms of shock; (5) no nephrotoxic drugs received recently; and (6) no renal parenchymal disease with urinary protein < 500 mg/d, i.e. no microscopic haematuria (red blood cells <50/HP) and/or abnormality during renal ultrasound examination. Rapid progression of renal dysfunction is one of the characteristics of type I HRS, including serum creatinine level twice as much as two weeks earlier (> 226μ mol/l).

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Patients in the control and type I HRS groups fasted in the morning after diagnosis, and 2ml peripheral blood were collected in ethylene diamine tetraacetic acid (EDTA) anticoagulant blood collection tubes. Blood genomic extraction kit (Guangzhou Bo Sheng) was then applied to extract deoxyribonucleic acid (DNA). Ultraviolet (UV)-visible spectrophotometer was used to assess DNA concentration, to make sure that concentrations of samples were consistent. Finally, DNA samples were stored at -20°C until use.

As matched with United States National Centre for Biotechnology Information (NCBI), the locus was confirmed as rs113481894. In order to guarantee the study accuracy, two primer pairs were designed for this sequence, named as rs113481894 forward (F1) and rs113481894 reverse (R1), and rs113481894 forward 2 (F2) and rs113481894 reverse 2 (R2), with the following sequences: F1, GCACCGCAGTCTCAACC; R1, GTGGCTCATACTGTAATCCC; F2, ATCCTCCCACTTCAGCCTCC; R2, TTATGTGATAGTGTCTTAATGCGAGT. The product obtained with F1+R2 was relatively specific and stable as demonstrated in preliminary experiments.

The polymerase chain reaction (PCR) amplification reaction system included SsoFast Eva Green Supermix (10µL) forward primer at 10µmol/L (0.8µL), reverse primer

at 10 µmol/L (0.8µL), and genomic DNA (8.4µL). The cycling conditions were: 98.0°C for 2 hours (initial denaturation); 98.0°C for 2 minutes, 60.0°C for 10 minutes, 40 cycles; 95.0°C for 10 seconds. Melting curve was generated at 65°C to 95°C, with increment of 0.2°C for 5seconds.

High resolution melting curve analysis (HRM) was used for quantitative PCR (qPCR) product analysis, as well as cluster analysis.

SPSS 17 was used for data analysis. Allele and genotype frequencies were counted by the direct counting method. Measurement data was compared by t-test, and count data by chi-square test to confirm the Hardy-Weinberg equilibrium. TT genotype and C alleles were used as comparison. Unconditional logistic regression analysis was used to compute odds ratio (OR) and 95% confidence interval (CI) of distribution differences and frequencies of genotypes and alleles.

**Results**

Of the 60 participants, 28(46.7%) were in the HRS group and 32(53.3%) were controls. There were 22(78.6%) males and 6(21.4%) females in the HRS group compared to 28(87.5%) males and 4(12.5%) females in the control group (p=0.49), whereas the mean age was 42.21±11.30years and

**Table-1:** Baseline characteristics of patients in the two groups ( $\bar{x} \pm s$ ).

	HRSgroup	Control group	Tested value	P value
Cases	28	32		
Age (years)	42.21±11.30	43.69±12.60	t=-0.47	0.64
Gender (male/female)*	22/6	28/4	$\chi^2=0.84$	0.49
TB (µmol/L)	154.76±51.58	181.26±64.46	t=-1.74	0.09
PTA (%)	49.30±24.67	41.78±17.52	t=1.37	0.18
ALB (g/L)	33.42±3.69	32.98±4.81	t=0.39	0.70

HRS: Hepatorenal syndrome  
 TB: Total bilirubin  
 PTA: Prothrombin activity  
 ALB: Albumin.

**Table-2:** Distribution and risk of disease of genotypes and alleles for two groups.

Group	HRS group	Control group	$\chi^2$ value	P value	OR value(95%CI)*	P value*
Cases	28	32				
Genotype (cases)	CC	9	4.077	0.147	5.667(0.944~34.032)	0.058
	CT	13				
	TT	6				
	C	31				
	T	25				
			4.292	0.038	2.230(1.037~4.792)	0.040

Note: \* Unconditional logistic regression analysis method.  
 HRS: Hepatorenal syndrome.  
 OR: Odds ratio.  
 CI: Confidence interval.

43.69±12.60, respectively ( $p=0.64$ ). Mean total bilirubin (TB), albumin (ALB) and prothrombin activity (PTA) levels were 154.76±51.58, 49.30±24.67 and 33.42±3.69 in the HRS group compared to 181.26±64.46, 41.78±17.52 and 32.98±4.81 among controls ( $p=0.09, 0.18$  and  $0.70$ ) (Table-1).

Moreover, rs113481894 locus genotype frequency distributions all followed Hardy-Weinberg equilibrium ( $P_{\text{HRS group}}=0.98, P_{\text{control group}}=0.542$ ), indicating that gene frequencies of this locus had reached genetic equilibrium and it was group-representative.

The rs113481894 locus had three different genotypes, including CC, CT and TT, with no statistically significant difference among them ( $p=0.147$ ). Interestingly, distribution differences were found between the HRS and control groups for T allele frequencies, with statistical significance ( $p=0.038$ ). After adjustment for gender and age by unconditional logistic regression analysis, T allele significantly increased the risk of HRS (OR= 2.230; 95% CI: 1.037~4.792;  $p=0.040$ ) compared with the C allele (Table-2).

## Discussion

By assessing the AVPR1A gene locus rs113481894, we found that distribution differences of the three genotypes, i.e. CC, CT and TT, had no statistically significant difference between the HRS and control groups. These findings indicated that the above genotypes were not associated with HRS risk. Distribution frequencies of the dangerous allele T in rs113481894 were overtly higher in the HRS group compared with controls. After adjustment for gender and age, the risk of disease for T allele was 1.037~4.792 times that of C allele (OR= 2.230, 95% CI: 1.037~4.792,  $p=0.040$ ).

The associations of AVPR1A gene polymorphisms with behaviour personality, social cognition and emotional stress reaction have attracted attention from scientists,<sup>7-9</sup> but researches assessing HRS pathogenesis are scarce. Most studies suggested that HRS pathogenesis is associated with the vasoconstrictor system, including excessive vasopressin secretion.<sup>10,11</sup> Meanwhile, Oikawa et al.<sup>12</sup> demonstrated that AVPR1A antagonist has a potential treatment efficacy for primary hypertension. In addition, Hasan et al.<sup>5</sup> found that AVPR1A gene promoter -6951 SNPs are closely associated with vasoconstrictor effect. The current study further indicates that HRS pathogenesis may be associated with AVPR1A gene polymorphisms.

SNPs cause changes in encoding amino acid sequences and splicing of transcribed messenger ribonucleic acid (mRNA), therefore impacting gene expression and protein function. However, the exact impact of the biological

function of this locus in HRS remains unclear.

One of the limitations of the current study was its small sample size. Studies with larger sample sizes are recommended for the verification of our results.

## Conclusion

T allele located at AVPR1A receptor promoter rs113481894 locus may be associated with the pathogenesis of type-I HRS.

**Disclaimer:** None.

**Conflict of Interest:** None.

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## References

- Hartleb M, Gutkowski K. Kidneys in chronic liver diseases. *World J Gastroenterol* 2012; 18: 3035-49.
- Belcher JM, Garcia-Tsao G, Sanyal AJ, Bhogal H, Lim JK, Ansari N, et al. Association of AKI with mortality and complications in hospitalized patients with cirrhosis. *Hepatology* 2013; 57: 753-62.
- Moller S, Krag A, Bendtsen F. Kidney injury in cirrhosis: pathophysiological and therapeutic aspects of hepatorenal syndromes. *Liver Int* 2014; 34: 1153-63.
- Ryckwaert F, Virsolvy A, Fort A, Murat B, Richard S, Guillon G, et al. Terlipressin, a provasopressin drug exhibits direct vasoconstrictor properties: consequences on heart perfusion and performance. *Crit Care Med* 2009; 37: 876-81.
- Hasan KN, Shoji M, Sugimoto K, Tsutaya S, Matsuda E, Kudo R, et al. Association of novel promoter single nucleotide polymorphisms in vasopressin V1a receptor gene with essential hypertension in nonobese Japanese. *J Hum Hypertens* 2007; 21: 825-7.
- Salerno F, Gerbes A, Gines P, Wong F, Arroyo V. Diagnosis, prevention and treatment of hepatorenal syndrome in cirrhosis. *Gut* 2007; 56: 1310-8.
- Moons WG, Way BM, Taylor SE. Oxytocin and vasopressin receptor polymorphisms interact with circulating neuropeptides to predict human emotional reactions to stress. *Emotion* 2014; 14: 562-72.
- Vogel F, Wagner S, Baskaya O, Leuenberger B, Mobascher A, Dahmen N, et al. Variable number of tandem repeat polymorphisms of the arginine vasopressin receptor 1A gene and impulsive aggression in patients with borderline personality disorder. *Psychiatr Genet* 2012; 22: 105-6.
- Chang SC, Glymour MM, Rewak M, Cornelis MC, Walter S, Koenen KC, et al. Are genetic variations in OXTR, AVPR1A, and CD38 genes important to social integration? Results from two large US cohorts. *Psychoneuroendocrinology* 2014; 39: 257-68.
- Barbano B, Sardo L, Gigante A, Gasperini ML, Liberatori M, Giraldi GD, et al. Pathophysiology, diagnosis and clinical management of hepatorenal syndrome: from classic to new drugs. *Curr Vasc Pharmacol* 2014; 12: 125-35.
- Gines P, Schrier RW. Renal failure in cirrhosis. *N Engl J Med* 2009; 361: 1279-90.
- Oikawa R, Hosoda C, Nasa Y, Daicho T, Tanoue A, Tsujimoto G, et al. Decreased susceptibility to salt-induced hypertension in subtotaly nephrectomized mice lacking the vasopressin V1a receptor. *Cardiovasc Res* 2010; 87: 187-94.