

## Original Article

# Visfatin expression and genetic polymorphism in patients with traumatic brain injury

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**Abstract:** Objective: To discover the expression of visfatin and its genetic mutation and clinical significance in patients with traumatic brain injury. Methods: 99 cases of traumatic brain injury patients admitted to Chongqing Emergency Medical Center from June 2013 to December 2014. There were 56 male and 43 female with an average age of 45.2 years. Through diagnosis, the 99 patient's conditions were all relatively in accordance with traumatic brain injury standard; meanwhile, the patients were divided into mild, moderate and severe traumatic brain injury according to GCS criteria when they admitted to clinics. 33 people were enrolled in each group. To highlight the results, 40 healthy people were enrolled in the control group with 25 males and 15 females. ELISA was used to detect serum visfatin level in 99 patients. Single base extension was used to detect visfatin gene promoter -1535C/T polymorphism. Results: By examination we found that visfatin gene -1535C > T locus had three genotypes: TT, TC and CC, respectively. By examination we obtained that genotype distribution and allele frequency showed no significance between the experimental group and the control group ( $P > 0.05$ ). Through analysis we can get that serum visfatin level in patients with traumatic brain injury was significantly higher than that in the control group. CC genotype are mostly patients with severe traumatic brain injury, and its serum visfatin level was higher than that with CT and TT genotype (all  $P < 0.05$ ). CT and TT genotype carriers were most mild and moderate traumatic brain injury patients. Conclusion: Expression of visfatin and its gene mutation in patients with traumatic brain injury were closely related to the severity of the disease.

**Keywords:** Visfatin, gene mutation, traumatic brain injury

## Introduction

The adipose tissue secretes several adipokines, including adiponectin, leptin, resistin and, of more recent discovery, nicotinamide phosphoribosyltransferase (NAMPT/Visfatin) [1]. It was suggested that these molecules could explain, at least in part, the link between obesity, insulin resistance, beta-cell dysfunction, endothelial dysfunction, cardiovascular disease (CVD), and inflammatory illness [1-6].

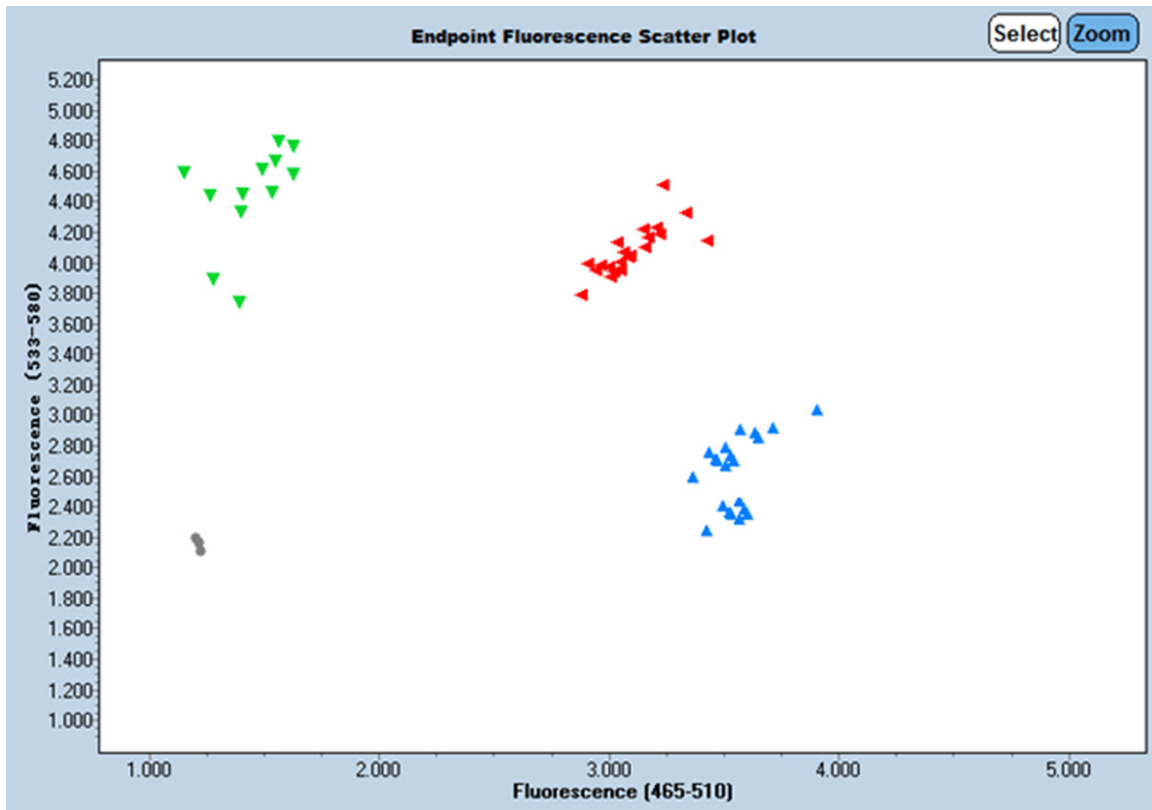
Visfatin is a newly identified pro-inflammatory adipokine and a genetic polymorphism -1535C > T located in the visfatin gene promoter has been suggested to be associated with the regulation of visfatin expression in some inflammatory illness. A lot of research reported that it had a close relationship with inflammation and tissue damage repair [7-9]. Also, It was found

that the visfatin gene-mutation-induced various genotypes were greatly related with the conditions of patients with traumatic brain injury [10]. And the main reasons of traumatic brain injury are the inflammatory reactions and brain ischemia secondary to brain damage [11, 12]. Therefore, the slight differences in serum visfatin levels between patients with traumatic brain injury and normal controls are closely related with the development of the disease. In order to explore the expression and clinical significance of visfatin and its gene mutation in patients with traumatic brain injury, we detected the serum visfatin levels and analyzed the mutant genotypes in patients in the present study.

## Material and methods

### General information

A total of 99 cases were included in the monitoring group; the 99 patients with traumatic



**Figure 1.** The genotyping results of -1535C > T polymorphism by real time-PCR. Green: CC genotype; Red: CT genotype; Blue: TT genotype.

**Table 1.** The basic characteristics of patients and controls

Groups	n	Age (Year)	Sex (M/F)	Smoking (n, %)	Drinking (n, %)	BMI (Kg/m <sup>2</sup> )
Control group	40	48.3 ± 8.5	28/12	22 (55.0)	18 (45.0)	25.5 ± 5.4
Traumatic brain injury	99	49.1 ± 9.3	61/38	50 (50.5)	44 (44.4)	25.8 ± 4.9
P values		0.423	0.103	0.887	0.901	0.762

**Table 2.** Visfatin level between case and control group

Groups	N	visfatin level (µg/L)
Control group	40	16.98 ± 2.35
Mild traumatic brain injury	33	22.32 ± 2.45
Moderate traumatic brain injury	33	29.15 ± 2.53
Severe traumatic brain injury	33	37.32 ± 2.68

brain injury were mainly collected from Chongqing Emergency Medical Center between June 2013 and December 2014, including 56 cases of male and 43 cases of female, with an average age of 45.2 years. Among them, 65 cases of brain injury were caused by car accidents, 26 cases of traumatic brain injury were caused by falling from a height, and the rest 8 cases were caused by heavy machinery. Pa-

tients were admitted within 12 hours and no wound infection was found in 99 patients; CT scan showed that the patients had primary or secondary brain injury, and by asking the patient families we found that these patients had no psychiatric history. Through diagnosis, these patients' conditions were basically compliant with the criteria of traumatic brain injury. In order to highlight the results, 40 healthy individuals were taken as controls, including 25 males and 15 females.

#### Specimen collection and processing

3 mL antecubital vein blood were collected in the fasting state for all subjects at the time points of 0, 1, 3, 8, 16d and within 12 hours after admission (including the control group),

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**Table 3.** Visfatin levels at different time point ( $\mu\text{g/L}$ )

Groups	0 d	3 d	8 d	16 d
Mild traumatic brain injury	19.32 $\pm$ 2.15	23.22 $\pm$ 2.55	18.32 $\pm$ 2.25	17.32 $\pm$ 2.24
Moderate traumatic brain injury	22.34 $\pm$ 2.24	27.72 $\pm$ 2.46	24.35 $\pm$ 2.24	20.15 $\pm$ 2.53
Severe traumatic brain injury	31.01 $\pm$ 2.12	39.23 $\pm$ 2.45	35.34 $\pm$ 2.15	26.32 $\pm$ 2.48

and injected into glass test tubes without anti-coagulant treatment; after standing at room temperature for 30 min, samples were centrifuged at 3000 r/min for 15min and the serum was separated, and stored at  $-70^{\circ}\text{C}$  for detection.

### SNP selection

There were three SNPs in visfatin gene to be reported previously. Of which, -1535C/T, a promoter SNP, was reported to be associated with glucose and lipid metabolism in a Chinese population. Therefore, we selected -1535C/T as a candidate SNP in the present study.

### Genotyping

We genotyped the -1535C/T locus according to the protocol reported in previous study. Genomic DNA was extracted by a standard phenol-chloroform extraction procedure. SNP genotyping was performed using the rapid and reliable allele-specific real-time PCR method. The single nucleotide variations were discriminated by the 3' end of the allele-specific primer. Amplification was monitored by SYBR-Green I fluorescence (Invitrogen, Carlsbad, CA, USA). All reactions contained 30 ng human genomic DNA. When the allele, detected by the respective primer, is present, the amplification curve rises before cycle 30. When it is not present, the amplification curve is shifted by about +5 cycles. For a heterozygous template DNA, both amplification curves rise at the same cycle number before cycle 30. Allele-specific real-time PCR reactions were performed using ABI Prism 7300 instrument (Applied Biosystems, Foster City, CA, USA). The genotyping results were shown as **Figure 1**.

### Serum visfatin levels detection

ELISA was used to determine these 99 patients' serum visfatin levels according to the protocol of the ELISA kit.

### Statistical methods

In this study, SPSS13.0 software was used for statistical analysis. All measurement data were

represented as average  $\pm$  standard deviation; when  $P < 0.05$ , the difference was statistically significant.

## Results

### *The basic characteristics of the patients and the control group*

As shown in **Table 1**, we did not find significant differences between the patients and the control subjects in age, sex, and other indexes (All  $P > 0.05$ ).

### *Serum visfatin levels in patients with traumatic brain injury*

The detection showed that serum visfatin levels in patients with three different degrees of traumatic brain injury in experimental group were significantly higher than those in the individuals in control group ( $P < 0.01$ ); and we also found that patients with higher extent of brain injury had higher serum visfatin level ( $P < 0.01$ ); serum visfatin level in patients with severe traumatic brain injury was significantly higher than that in patients with moderate traumatic brain injury ( $P < 0.01$ ); serum visfatin level in patients with moderate traumatic brain injury was significantly higher than that in mild traumatic brain injury patients ( $P < 0.01$ , **Table 2**).

### *Dynamic changes of serum visfatin level in patients with traumatic brain injury*

The higher the degree of brain damage, the higher the serum visfatin levels in patients ( $P < 0.01$ ); serum visfatin level in patients with severe traumatic brain injury was significantly higher than that in patients with moderate traumatic brain injury ( $P < 0.01$ ); serum visfatin level in patients with moderate traumatic brain injury was significantly higher than that in mild traumatic brain patients ( $P < 0.01$ ). In mild traumatic brain injury group, we found that the serum visfatin level of patients continuously increased in the beginning 1-3 days after admission, and peaked on the third day, fol-

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**Table 4.** Distribution of genotype and allele in each group [n (%)]

Groups	n	CC	CT	TT
Control group	40	2 (5.0)	28 (70.0)	10 (25.0)
Mild traumatic brain injury	33	3 (9.1)	16 (48.5)	14 (42.4)
Moderate traumatic brain injury	33	5 (15.2)	11 (33.3)	17 (51.5)
Severe traumatic brain injury	33	21 (63.6) <sup>a,b,c</sup>	6 (18.2) <sup>a,b,c</sup>	6 (18.2) <sup>a,b,c</sup>

Note: a, Compared to control group,  $P < 0.01$ ; b, Compared to mild traumatic brain injury group,  $P < 0.01$ ; c, Compared to moderate traumatic brain injury,  $P < 0.01$ .

**Table 5.** Visfatin levels among each genotype in the case group

Genotype	N	Visfatin level ( $\mu\text{g/L}$ )
CC	29	$38.12 \pm 2.87^{\text{a,b}}$
CT	33	$28.44 \pm 2.67$
TT	37	$21.02 \pm 2.10$

Note: a, Compared to CT genotype,  $P < 0.01$ ; b, Compared to TT genotype,  $P < 0.01$ .

lowed by continuous decline in 3-8 days; and on the eighth day, it had nearly dropped to the normal level; In moderate traumatic brain injury group, we found that serum levels in patients continuously increased in the beginning 1-3 days after admission, and peaked on the third day, followed by continuous decline in 3-16 days; and on the 16<sup>th</sup> day, it had nearly dropped to the normal level; in severe traumatic brain injury group, we found that the serum visfatin level in patients continuously increased in the beginning 1-3 days after admission and peaked on the third day, followed by continuous decline in 3-16 days; and on the 16<sup>th</sup> day, it was still relatively high (Table 3).

### *The distribution of genotype and allele of -1535C > T polymorphism between patients and control subjects*

As shown in Table 4, there were significant differences in the distribution of genotype and allele between the patients and the control subjects ( $P < 0.05$ ).

### *Relationship between visfatin gene -1535C > T locus mutation and serum visfatin level*

Through testing we found that visfatin gene -1535C > T loci of patients had three genotypes; patients with CC genotype mostly belonged to the patients with severe traumatic brain injury, and the serum visfatin levels were higher than those in patients with CT and TT genotypes (all  $P < 0.01$ ); while the patients with

CT and TT genotypes mostly belonged to the patients with mild and moderate traumatic brain injury (Table 5).

## Discussion

In the present study, we found Visfatin expression and genetic polymorphism was associated with traumatic brain injury.

In fact, primary trauma is not necessarily life-threatening, but most of the patient's condition is still very serious, mainly due to the various secondary brain diseases after traumatic brain injury, especially the brain inflammation. After traumatic brain injury, complement, thrombin and fibrin degradation products can induce a series of inflammatory reactions [13] to sustainably increase the generation and the concentration of most inflammatory factors, thus leading to the increased permeability of brain cell membrane and thereby increasing the incidence of cerebral edema.

Visfatin is a cytokine discovered recently, which is mainly secreted by adipocytes. Research confirmed that it was closely related with inflammation and re-repair after tissue damage. Especially visfatin -1535C > T locus gene, located on chromosome 7q22.17-q31.33, with a length of 37.14 kb, including 11 exons and 10 introns. Visfatin gene -1535 polymorphism includes three genotypes (CC, CT, and TT). Visfatin -1535C > T locus gene mutation may alter the binding activity of NF1, AP1, AP2, NF- $\kappa$ B and STAT transcription factors to regulate visfatin expression, thus affecting its biological effects [14-16]. Studies in different populations, including a meta-analysis, suggested that high levels of circulating visfatin are positively associated with cardiovascular disease and inflammation [14, 16]. The pathophysiological basis for the association between visfatin levels and traumatic brain injury is still unclear.

This study mainly analyzed the traumatic brain patients in the Chongqing Emergency Medical Center. We analyzed the difference in visfatin levels between these two groups, and found that the visfatin level in patients was higher than that in normal individuals. Also, we found genetic polymorphism was associated with visfatin levels.

There were several limitations in our study. Firstly, the sample size is relative small, which may reduce the power in the present study. Secondly, we only analyzed on SNP in the present study, the relation between other SNPs and traumatic brain injury is still unclear. Finally, in the present study, we did not consider the impacts of other risk factors, such as obesity, diabetes and other cardiovascular disease, which may result in selected bias in the present study.

Although there were several limitations in our study, our results indicated that both the higher visfatin levels and CC genotype were associated with traumatic brain injury in Chinese population.

### Disclosure of conflict of interest

None.

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