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Original Article

Correlation between IL-18 gene polymorphism and vascular dementia and effects on Th1/Th2 equilibrium

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Abstract: Vascular dementia (VD) is a common reason of dementia. Oxidative damage and inflammation are closely related to VD. Th1/Th2 imbalance occurs in the process of VD. It has been shown that IL-18 is related to the pathogenesis of VD. However, the relationship of IL-18 gene polymorphism with degree of VD and Th1/Th2 balance has not been fully elucidated. VD patients in our hospital were divided into a mild group, a middle group, and a severe group. Healthy volunteers in the corresponding period were selected as control. Peripheral IL-18, IL-2, IL-4, IL-6, and TNF-α expressions were tested by ELISA. The correlation of IL-18 with disease degree and Th1/Th2 balance was analyzed. IL-18 gene rs187238 SNP distribution was tested by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP). The correlation relationship between IL-18 polymorphism and VD was analyzed. IL-18, IL-2, and TNF-α increased, while IL-4 and IL-6 reduced in VD patients compared with control ($P < 0.05$). They changed more obviously following aggravation of disease level. IL-18 was positively correlated with VD degree, IL-2, and TNF-α, whereas negatively correlated IL-4 and IL-6. Rs187238 was positively correlated with VD degree and significantly increased the risk of VD (OR 1.79, 95% CI 1.02-2.31) ($P < 0.05$). IL-18 was upregulated in VD patients and participated in regulating Th1/Th2 balance. IL-18 rs187238 mutation was the risk factor of VD.

Keywords: Vascular dementia, IL-18, gene polymorphism, Th1/Th2

Introduction

Vascular dementia (VD) is a type of brain tissue damage caused by cerebrovascular disease, such as ischemic and hemorrhagic stroke, leading to chronic, acquired, progressive memory, cognitive, and behavioral disorders. It is a common cause of dementia [1, 2]. Recent research showed that the incidence of VD is only after Alzheimer's disease, which seriously impacts the quality of life, brings huge economic and mental stress, and generates heavy burden to the social and economic development [3]. VD prevention is a medical and social problem needs to be solved by geriatrics and related disciplines [4, 5]. Following progress of aging society, the incidence of VD caused by atherosclerosis, hypertension, or cerebrovascular disease keeps rising [6]. The occurrence of VD is suffered from multiple risk factors, and the specific mechanism has not yet fully elucidated. It was showed that oxidative stress injury and inflammation are closely related to VD [7, 8]. As a common type of senile dementia, VD is the most promising type to be controlled. However, there is still lack of efficient drug for the treatment of VD [9]. Thus, investigation of the mechanism of VD is helpful to find effective therapy.

Immune and inflammatory reactions are key factors of VD [10]. Cytokines participate in the regulation of VD as immunocompetent medium [11]. IL-18 a precursor peptide composed of 193 amino acids that locate on chromosome 11 q22.2-22.3. It becomes mature IL-18 through the effect of IL-1β invertase [12]. IL-18 could be secreted by several types of cells, such as mononuclear macrophages, thymus dependent lymphocytes (T cells), natural killer cells (NK cells), dendritic cells (DC), and fibroblasts. It plays an important role in inflammation and immune responses [13, 14]. It has been shown that IL-18 participates in VD by regulating glial cell activation and axon growth [15]. As the IL-18 polymorphism (rs187238) has been demonstrated to be strongly and specifically associated with a faster cognitive decline

Table 1. PCR primers of IL-18 SNPs

SNPs	Primer	5'-3'
rs187238	Forward	AGTAGAGTGCTGAACTCGATGG
	Reverse	CCAGTTCATACTTGCACCACTC

in patients with Alzheimer's disease during two year follow up [16], whether IL-18 gene polymorphism (rs187238) plays a role in the pathogenesis or development of VD has not been reported. T helper cells (Th) could be divided into two subgroups based on different cytokines section, including Th1 and Th2. It has been found that Th1 and Th2 regulate each other by secreting cytokines to maintain Th1 and Th2 balance [17]. However, the correlation relationship between IL18 and Th1/Th2 balance in VD has not been elucidated.

Materials and methods

Object of study

A total of 120 VD patients between Oct 2015 and Oct 2016 were enrolled from Zhejiang Chinese Medicine and Western Medicine Integrated Hospital. VD was diagnosed according to the criteria published by the American Psychiatric Association's Diagnostic and Statistical Manual-V (DSM-V) [18]. There were 65 males and 55 females with mean age at 57.5 ± 6.7 (41-72) years old. Exclusion criteria: severe organic disease, severe aphasia or extremity disability that cannot complete neuropsychology test, deprementia or psychosis, thyroid dysfunction, renal dysfunction, or hepatic failure that affect cognitive function, autoimmune disease, brain trauma, encephalitis, and epilepsy. Another 120 cases of healthy volunteers in the corresponding period were selected as control, including 68 males and 52 females with mean age at 56.1 ± 7.1 (42-71) years old. No statistical difference was observed on gender and age between two groups. All the subjects were in Han race. The study was approved by the Ethics Committee of Zhejiang Chinese Medicine and Western Medicine Integrated Hospital and obtained informed consent from the subjects.

Main reagents and instruments

IL-2, IL-4, IL-6, TNF-α, and IL-18 ELISA kits were purchased from R&D company (USA). AU680 fully automatic biochemical analyzer was bought from Beckmann Coulter (Germany). Labsystem Version 1.3.1 microplate reader was obtained from Bio-rad (USA).

General information and sample collection

VD patients were divided into mild, middle, and severe groups according to MMSE scoring. MMSE score 20-26 was defined as mild group including 35 cases, 10-19 was considered as middle group including 47 cases, and 0-9 was classified as severe group including 38 cases. Peripheral venous blood was collected in the fasting state.

ELISA

Serum IL-2, IL-4, IL-6, TNF-α, and IL-18 contents were tested by ELISA according to the manual. The standard substance was used to prepare the standard curve. A total of 50 μl sample was added to the well with three replicates. Then the plate was washed for five times and added with 50 μl conjugate reagent at 37°C for 30 minutes. Next, the plate was added with developer A and B at 50 μl and incubated at 37°C avoid of light for 10 min, respectively. At last, the plate was added with 50 μl stop buffer and read at 450 nm immediately. The standard linear regression equation was calculated based on the OD value of standard substance. The sample concentration was calculated according to the OD value.

PCR-RFLP

Total DNA was extracted using the blood DNA extraction kit and qualified on spectrophotometer. OD260/OD280 = 1.7-1.9 was considered as DNA. The primer sequence was designed by PrimerPremier 6.0 software and synthetized by Invitrogen (Shanghai, China) (**Table 1**). The PCR reaction system contained 10 pmol primers, 5 pmol dNTPs, 3 μl TaqDNA polymerase, and 1 μl DNA template. PCR product was digested by restriction endonuclease Nco I and Hinf I, respectively. The enzyme-digested product was tested by 3% agarose gel electrophoresis.

Statistical analysis

All statistical analyses were performed on SPSS 19.0 software. The measurement data are presented as mean ± standard deviation and compared by t test. Allele and genotype frequencies were compared by Chi-square test to determine whether genotype distribution was conformed to Hardy-Weinberg law. Correlation

Table 2. General information comparison

Index	Control	VD group		
		Mild	Middle	Severe
Cases	120	35	47	38
Age (year)	56.1 ± 7.1	55.5 ± 6.2	56.7 ± 7.4	57.2 ± 5.9
Male/female	68/52	18/17	25/22	22/16
Education (year)	7.2 ± 2.1	6.7 ± 3.8	6.8 ± 3.2	6.1 ± 2.5
Hypertension%	32 (26.7%)	22 (62.9%)*	40 (85.1%)*,#	35 (92.1%)*,#
Diabetes%	12 (10.0%)	11 (31.4%)*	18 (28.2%)*	22 (57.9%)*,#
MMSE	32.1 ± 2.1	24.0 ± 2.5*	17.2 ± 4.1*,#	7.1 ± 1.9*,#,&

*$P < 0.05$, compared with the control; #$P < 0.05$, compared with the mild group; &$P < 0.05$, compared with the middle group.

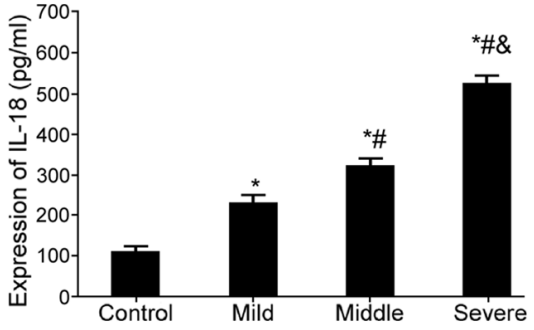

Figure 1. IL-18 expression in the serum of VD patients. *P < 0.05, compared with the control; #P < 0.05, compared with the mild group; &P < 0.05, compared with the middle group.

analysis was performed using Spearman rank correlation. OR and 95% CI were calculated. P < 0.05 was depicted as statistical significance.

Results

General information analysis

General information and MMSE score in VD patients and healthy control were analyzed. No statistical difference was found on age, gender, and education between two groups. MMSE score significantly decreased, while the ratio of hypertension and diabetes obviously elevated in VD patients compared with control (P < 0.05) (**Table 2**).

IL-18 expression in the serum of VD patients

ELISA was applied to test IL-18 expression in the serum of VD patients. Serum IL-18 significantly increased in VD patients compared with control (P < 0.05). Its level kept rising following the aggravation of disease (**Figure 1**).

IL-2, TNF-α, IL-4, and IL-6 expression in the serum of VD patients

ELISA was adopted to test Th1 cytokines IL-2 and TNF-α levels in the serum of subjects. Serum IL-2 and TNF-α contents significantly elevated in VD patients compared with control, and further increased following disease aggravation (P < 0.05). In contrast, Th2 cytokines IL-4 and IL-6 levels markedly reduced in VD patients compared with healthy control and kept declining following aggravation (P < 0.05) (**Figure 2**).

The correlation between IL-18 and VD degree

The relationship between IL-18 and VD degree was further analyzed. IL-18 was negatively correlated with MMSE score (P < 0.05), positively correlated with hypertension (P < 0.05), and showed no relationship with age, gender, or diabetes (**Table 3**).

The relationship between IL-18 and Th1/Th2 balance in VD patients

The relationship between IL-18 and Th1/Th2 balance in VD patients was analyzed. IL-18 was positively correlated with IL-2 and TNF-α, whereas negatively correlated IL-4 and IL-6 (P < 0.05) (**Table 4**).

Correlation analysis of IL-18 SNP and VD pathogenesis

IL-18 gene rs187238 genotype was detected by PCR-RFLP. SNP genotyping success rate reached 99.2%. Allele frequency conformed to Hardy-Weinberg law. Rs187238 mutation homozygote and heterozygote were CC and GC, respectively. Rs187238 frequency constituent ratio exhibited statistical difference (P < 0.05) (**Table 5**). IL-18 rs187238 variant allele (GC+CC; GC and CC) was related to VD pathogenesis. It obviously increased the risk of VD (1.79, 95% CI 1.02-2.31) (P < 0.05).

Discussion

Immune responses and inflammation are key factors of VD occurrence and development [19,

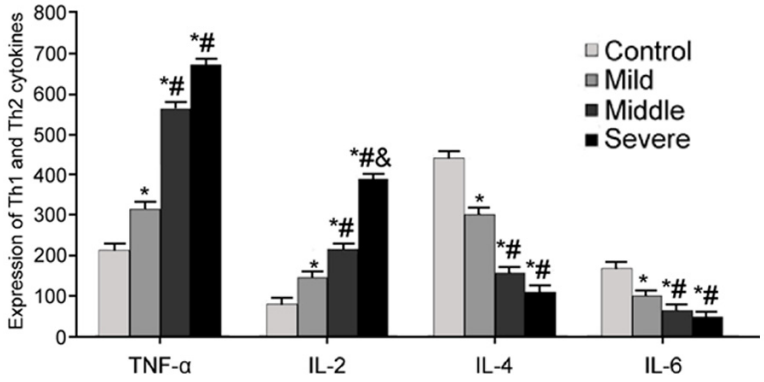

Figure 2. IL-2, TNF-α, IL-4, and IL-6 expression in the serum of VD patients. *P < 0.05, compared with the control; #P < 0.05, compared with the mild group; &P < 0.05, compared with the middle group.

Table 3. Correlation analysis of IL-18 and clinical parameter in VD patients

	Age	Gender	Education	Hypertension	Diabetes	MMSE score
r value	0.198	0.147	0.341	0.782	0.356	-0.817
P value	0.752	0.889	0.671	0.036	0.751	0.012

Table 4. Relationship between IL-18 and Th1/Th2 balance in VD patients

	IL-2	TNF-α	IL-4	IL-6
r value	0.42	0.631	-0.941	-0.623
P value	0.026	0.031	0.028	0.033

Table 5. IL-18 SNP gene frequency analysis in VD patients

Group	rs187238		
	GG	GC	CC
Control	58.8	37.7	3.5
VD group	41.6	49.7	8.7
χ^2		6.862	
P value		0.032	

20]. Th1 and Th2 cells regulate each other to maintain balance by regulating the secretion of cytokines, thus play a key role in maintaining normal immune function [21]. Th1 cells mainly secreted cytokines IL-2 and TNF-α, while Th2 cells majorly secrete IL-4 and IL-6 [22]. Th1/Th2 homeostasis plays a crucial role in cellular immunity and humoral immunity through self-regulation and regulating cytokines secretion [23]. T cell subgroup Th1 mediated immune reaction is involved in the pathogenic process, while Th2 cells mediate a protective role by secreting cytokines [24].

In the central nervous system disease, macrophages and microglia play critical roles in various pathological changes. When the brain cortex and midbrain are stimulated by inflammation, glial cells promote Th1 cells to release inflammatory cytokine TNF-α and IL-2, whereas microglia can be activated to phagocytes in acute inflammation and further promote Th1 cells to secrete inflammatory cytokines, neurotoxic molecules, and activation signals, thus aggravating nerve inflammation [25]. IL-4 and IL-6 secreted by Th2 cells mainly play a protective role by activating macrophages in immune response [26]. IL-18 is an inflammatory factor with a wide range of immune regulatory effects to participate in tumor, inflammation, immune and other pathophysiologic processes [27]. This study found that serum IL-18 increased in VD patients and kept rising following aggravation, which was consistent with previous report [15].

A further study analyzed the impact of IL-18 expression on Th1/Th2 balance in VD patients. The results suggest that T1 cytokines IL-2 and TNF-α upregulated, while Th2 cytokines IL-4 and IL-6 reduced in VD patients, which was in accordance with previous report [25, 26]. Moreover, this study verified that Th1/Th2 cytokines changes more significantly following disease aggravation. IL-18 was positively correlated with disease condition, IL-2, and TNF-α, while negatively correlated with IL-4 and IL-6. IL-18 SNP was found in different populations. IL-18 SNP was not only related to gram-negative bacteria and fungal infections, but also associated with cardiovascular and cerebrovascular diseases [27]. However, the role of IL-18 gene polymorphism in VD has not been clarified. These results indicate that IL-18 was correlated with the occurrence and development of VD. Further in-depth investigation is

needed to explore the mechanism of IL-18 in regulating VD.

Conclusion

IL-18 was increased in VD patients and involved in regulating Th1/Th2 balance. IL-18 rs187238 mutation was the risk factor of VD. This study may provide a new theoretical basis for selection of VD as a therapeutic target.

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Disclosure of conflict of interest

None.

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