

RESEARCH ARTICLE

Correlation of 5-HTR6 gene polymorphism with vestibular migraine

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Abstract

Objective: To investigate the correlation of 5-hydroxy tryptamine receptor 6 (5-HTR6) gene polymorphism with vestibular migraine (VM).

Methods: A total of 92 VM patients were enrolled as the observation group, and 100 healthy people receiving physical examinations as the control group. Their general clinical information was collected, and the level of 5-HT in plasma and the vestibular function test indexes were detected. Moreover, the polymorphism of 5-HTR6 rs770963777 was detected with the TaqMan-MGB probe.

Results: The observation group had a lower level of 5-HT than the control group ($P < .05$), and the abnormality rates of the vestibular function tests, including the caloric test, head-shaking test, and vestibular autorotation test, were obviously higher than those in the control group ($P < .01$). The comparisons showed that the distribution frequencies of the genotypes and alleles were different between the two groups ($P < .05$). According to the analysis of the genetic mode, there were differences in recessive and additive modes between the two groups ($P < .05$), but the dominant mode was not different between the two groups ($P > .05$).

Conclusion: The level of 5-HT and the vestibular function test indexes can serve as the effective indicators for observing VM, and the polymorphism of 5-HTR6 rs770963777 site is correlated with VM onset.

KEYWORDS

5-hydroxy tryptamine, 5-hydroxy tryptamine receptor 6, single nucleotide polymorphism, vestibular function test, vestibular migraine

1 | INTRODUCTION

Vestibular migraine (VM) is a common central vestibular disease and mainly manifests the cross-attack of vestibular dysfunction and migraine, with the more prominent symptom of vertigo. The epidemiological survey displays that the incidence rate of VM is 4%–5.7% among people, while that in migraine patients is 10.3%.^{1,2} The pathogenesis of VM has not been clarified yet, and the studies of some scholars have suggested that its onset is characterized by

familial aggregation and related to genetic inheritance.^{3–5} 5-hydroxy tryptamine (5-HT) is a kind of important humoral mediator and neurotransmitter, and studies have revealed that 5-HT is closely related to the occurrence and development of migraine.^{6–8} In addition, the studies on many subtypes of 5-HT have found that 5-HT receptor 6 (5-HTR6) bears a close relationship with the occurrence of pain.^{9–11} Therefore, in this project, the VM patients were enrolled from our department to detect the polymorphism of 5-HTR6 rs770963777 site with the TaqMan-MGB probe and explore the correlation of

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5-HTR6 gene polymorphism with VM, hoping to provide a theoretical support for the genetic polymorphism of VM.

2 | MATERIALS AND METHODS

2.1 | Study subjects

The VM patients who were treated from January 2016 to January 2018 in Neurology Department of the Sixth Medical Center of Chinese PLA General Hospital were selected, and they met the following criteria: (a) patients who met the diagnostic criteria for VM in 2013 ICHD and (b) those with favorable compliance and complete information. Exclusion criteria were as follows: (a) patients with dizziness or headache due to other causes, (b) patients who were complicated with mental diseases or other cognitive dysfunctions and could not cooperate, or (c) those who suffered from dysfunctions of heart, kidney, liver or other major organs. This study included 92 cases of VM as the observation group based on the above criteria. They consisted of 44 males and 48 females, with the mean age of 44.22 ± 9.30 years old. Meanwhile, 100 healthy people receiving physical examinations in the same period were selected from the Medical Center of our hospital as the control group. They consisted of 48 males and 52 females, with the mean age of 41.71 ± 8.66 years old. All study subjects were unrelated Chinese Han individuals and signed the informed consent. The project was approved by the ethics committee (committee's reference number: SYXK 2015-0189).

2.2 | Study methods

2.2.1 | Collection of general clinical information

The name, age, sex, symptoms, and signs of the study subjects were collected. After 4 mL venous blood was taken from the elbows of the study subjects, the level of 5-HT was determined using the high-performance liquid chromatography system (Gilson), the 5-HT standard sample (Sigma), and the high-performance liquid chromatography ultraviolet light method. In brief, for detection of 5-HT, the equipment was connected to the UV diode array detector. We actualized the chromatographic separation of 5-HT with C18 Column (4.6×250 mm, $5 \mu\text{m}$) under isocratic elution. The mobile phase is 0.05 mol/L KH_2PO_4 (apparent pH = 5)/acetonitrile (90:10, V/V), and the wavelength of the UV detector was set at 280 nm. The stock solution containing 100 $\mu\text{g}/\text{mL}$ serotonin hydrochloride was diluted subsequently into 6 calibrators with concentrations of

1000, 500, 250, 125, 62.5, and 31.25 ng/mL, respectively. Each solution is prepared in 3 mL volume, and all solutions were preserved at 4°C. Additionally, all subjects received the vestibular function tests, including the caloric test (less than 0.025 Hz), head-shaking test (1-2 Hz), and vestibular autorotation test (2-6 Hz) to assess the horizontal semicircular canal functions at different frequency. The above operations were conducted by the attending physicians from Neurology Department.

2.2.2 | Extraction of the deoxyribonucleic acid (DNA)

After 1 mL venous blood was taken from the elbows of the study subjects, DNA was extracted using the medium-amount whole blood genomic DNA extraction kit (BioTeke Corporation) according to the instructions in the kit. Moreover, the TaqMan® single nucleotide polymorphism (SNP) genotyping assay kit (Thermo Fisher Scientific) was employed to detect and analyze the genotypes of the samples (Table 1).

2.3 | Statistical methods

SPSS 20.0 software was used for statistical analysis. The measurement data were expressed as ($\bar{x} \pm s$), and the independent-samples *t* test was employed for the comparisons of measurement data between two groups. Chi-square (χ^2) test was adopted to compare the count data between two groups. The likelihood-ratio χ^2 test was performed to analyze whether the genotype distribution met the Hardy-Weinberg equilibrium law. $R \times C \chi^2$ test was applied for the comparison of the frequency of genotypes and alleles in each group. $P < .05$ suggested that the difference was statistically significant.

3 | RESULTS

3.1 | Comparisons of the general information and 5-HT level between the two groups

There were no differences in sex and age between the two groups ($P > .05$), and the observation group had a lower level of 5-HT than the control group ($P < .05$) (Table 2).

3.2 | Comparison of the vestibular function test between the two groups

The abnormality rates of the caloric test, head-shaking test, and vestibular autorotation test were evidently higher in the observation group than those in the control group ($P < .01$) (Table 3).

3.3 | Genetic equilibrium test

The likelihood-ratio χ^2 test was conducted for the actual and theoretical frequencies of three genotypes in the observation group and the

TABLE 1 TaqMan®-MGB probe information of 5-HTR6 rs770963777 site

SNP reference	rs770963777
Assay ID	C_363289632_10
SNP type	Intron
Context sequence	CATGGCATCCCTAGTCCTCAGTCCT[C/T] GGAGCATCTCGTCCTTTTCCACAT

TABLE 2 Comparisons of the general information and 5-HT level between the two groups

Group	n	Sex (male/female)	Age (years old)	5-HT (ng/mL)
Observation group	92	44 (47.83)/48 (52.17)	44.22 ± 9.30	133.88 ± 75.30
Control group	100	48 (48.00)/52 (52.00)	41.71 ± 8.66	250.61 ± 91.74
χ^2/t		0.001	1.642	3.831
P		.981	.525	.037

TABLE 3 Comparison of the vestibular function test between the two groups

Group	n	Caloric test (n)		Head-shaking test (n)		Vestibular autorotation test (n)	
		Normal	Abnormal	Normal	Abnormal	Normal	Abnormal
Observation group	92	58 (63.04)	34 (36.96)	35 (38.04)	57 (61.96)	69 (75.00)	23 (25.00)
Control group	100	95 (95.00)	5 (5.00)	98 (98.00)	2 (2.00)	98 (98.00)	2 (2.00)
χ^2		30.231		80.920		22.381	
P		.000		.000		.000	

control group. The frequency distributions of 5-HTR6 rs770963777 genotypes in both groups were consistent with Hardy-Weinberg equilibrium law ($P > .05$) and comparable (Table 4).

3.4 | Comparison of the genotype distribution frequency

The distribution frequencies of genotype CC, CT, and TT in the observation group were 63.04%, 31.52%, and 5.44%, respectively, and those in the control group were 45.00%, 48.00%, and 7.00%, respectively, displaying difference in genotype distribution frequency between the two groups ($P < .05$) (Table 5).

3.5 | Comparison of the allele distribution frequency

The distribution frequencies of C and T alleles in the observation group were 78.80% and 21.20%, respectively, and those in the control group were 69.00% and 31.00%, respectively. The comparison revealed differences in the allele distribution frequency between the two groups ($P > .05$) (Table 6).

3.6 | Analysis of 5-HTR6 rs770963777 genetic mode

According to the genetic mode analysis, there were differences in dominant and additive modes between the two groups ($P < .05$),

but the recessive mode was not different between the two groups ($P > .05$), suggesting that the dominant and additive modes are suitable for describing the 5-HTR6 rs770963777 genetic mode (Table 7).

4 | DISCUSSION

VM is a kind of recurrent functional headache, and its pathogenesis remains unclear now, but one of the recognized theories is the hypothesis of the trigeminal vascular pathway. Once the trigeminal ganglion and its fibers are stimulated, such neurotransmitters as substrate P and calcitonin gene-related peptides are released. They can act on the vascular wall, thus causing the neurogenic inflammatory response and central sensitization of the endocranium. 5-HT in the central nervous system can bind to its different receptors to affect the release of substrate P and calcitonin gene-related peptides and mediate the contraction and relaxation of the meningeal vessels, thereby exerting different pain modulation effects.¹²⁻¹⁴ The abnormalities of the content and functions of 5-HT play important roles in the occurrence and development of VM. Studies have manifested that the discharge of 5-hydroxyindoleacetic acid, a metabolite of 5-HT, is increased when migraine patients experience headache. According to the study of Drummond et al, reducing the synthesis of 5-HT in the nerve center via the consumption of tryptophan can lead to the symptoms of headache, photophobia, and

TABLE 4 Genetic equilibrium test of 5-HTR6 rs770963777 genotype

Group	n	CC		CT		TT		χ^2	P
		Actual frequency	Theoretical frequency	Actual frequency	Theoretical frequency	Actual frequency	Theoretical frequency		
Observation group	92	58	57.13	29	30.73	5	4.13	0.29	.86
Control group	100	45	47.61	48	42.78	7	9.61	1.49	.48

Group	n	Genotype [n (%)]			χ^2	P
		CC	CT	TT		
Observation group	92	58 (63.04)	29 (31.52)	5 (5.44)	6.340	.042
Control group	100	45 (45.00)	48 (48.00)	7 (7.00)		

TABLE 5 Comparisons of the genotype distribution of 5-HTR6 rs770963777 between the two groups

Group	n	Allele [n (%)]		χ^2	P
		C	T		
Observation group	92	145 (78.80)	39 (21.20)	4.752	.029
Control group	100	138 (69.00)	62 (31.00)		

TABLE 6 Comparisons of the distributions of 5-HTR6 rs770963777 C/T alleles between the two groups [n (%)]

Item	Observation group	Control group	χ^2	P	
					Recessive mode
Recessive mode	CC vs. CT + TT	58 (63.04)/34 (36.96)	45 (45.00)/55 (55.00)	6.273	.012
Dominant mode	CC + CT vs. TT	87 (94.56)/5 (5.44)	93 (93.00)/7 (7.00)	0.200	.654
Additive mode	CC vs. CT vs. TT	58 (63.04)/29 (29.41)/7 (6.86)	45 (45.00)/48 (48.00)/7 (7.00)	6.340	.042

TABLE 7 Analysis of 5-HTR6 gene rs770963777 genetic mode in both two groups [n (%)]

nausea.¹⁵ Besides, it has also been found in the studies on VM patients that the decrease in the synthesis of 5-HT can cause balance disorder, while applying 5-HT receptor agonists to VM patients can prevent VM.¹⁶ All studies above have demonstrated that there is a correlation between 5-HT and the onset of VM, and the decrease in the level of 5-HT can induce VM. Hence, the level of 5-HT can objectively reflect the severity of VM to a certain degree, providing a basis for the clinical diagnosis and treatment. In this study, consistently, the VM patients were enrolled strictly in accordance with the diagnostic criteria for VM in 2013 ICHD and requirements of favorable compliance and complete information. The result revealed that 5-HT was significantly increased in VM patients compared to that in control group. Of note, partially different from our result, in a recent study by Kowalska et al,¹⁷ migraine patients were selected according to the ICHD-3 criteria. MA patients (migraine with aura) and MO patients (migraine without aura) were further divided. The result indicated that significantly higher 5-HT plasma concentration was found in MA patients (migraine with aura) as compared to MO patients (migraine without aura) or control group, but there was no significant difference between MO patients and control group. We propose that the aim of our study is the correlation of 5-HTR6 gene polymorphism with vestibular migraine whereas the factor of aura (MA or MO) in migraine patients was not further involved.

In the vestibular function tests, the caloric test can reflect the vestibular peripheral low-frequency function, while the head-shaking test and vestibular autorotation test can simultaneously reflect the vestibular peripheral and central high-frequency functions. The present study compared the vestibular function test between the

observation group and the control group and showed that the observation group had markedly higher abnormality rates in the caloric test, head-shaking test, and vestibular autorotation test than the control group, indicating that VM can affect the vestibular peripheral and central functions. The central and peripheral vestibular regions participate in the pathogenic process of VM together, and when the integration of the vestibular and pain signaling pathways is abnormal, the migraine signal at the specific cortex will be integrated by the abnormal region and induces vestibular symptoms.¹⁸⁻²⁰

The current studies have suggested that VM, a multigenic disease, is associated with the functional mutations of multiple ion channels and receptors in the vestibular and pain transmission pathways. 5-HTR6 is one of the 5-HT receptors, and its gene, located on chromosome 1p36-p35, can encode the proteins of the G protein-coupled receptor family and is expressed in tissues, such as the frontal cortex, caudate nucleus, and amygdala.²¹ In this study, the polymorphism of 5-HTR6 rs770963777 in VM patients was detected so as to elucidate its correlation with VM. The results of this study were subjected to the Hardy-Weinberg satisfaction test, and those in the two groups met the Hardy-Weinberg equilibrium law, namely there were no differences in the observation value and expectancy value of the genotype frequency between the two groups, suggesting that the frequency distribution of the alleles in the two groups can represent the distributions among their respective groups. The frequencies of 5-HTR6 rs770963777(C/T) genotypes and alleles were different between the two groups, indicating that the polymorphism of 5-HTR6 rs770963777(C/T) is correlated with the onset of VM. The mutation of 5-HTR6 gene can cause the abnormal integration of

the vestibular and pain transmission pathways, thereby stimulating the inflammatory responses to induce VM. Furthermore, the genetic mode of 5-HTR6 rs770963777(C/T) was analyzed, and it was discovered that there were differences in recessive and additive modes between the two groups, indicating that these two modes are suitable for describing the genetic mode of 5-HTR6 rs770963777(C/T).

5 | CONCLUSION

In conclusion, the polymorphism of 5-HTR6 rs770963777(C/T) is correlated with the risk of VM.

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