Correlations of Calcium Voltage-Gated Channel Subunit Alpha1 A (CACNA1A) Gene Polymorphisms with Benign Paroxysmal Positional Vertigo

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Background: The aim of this study was to investigate the correlations of calcium voltage-gated channel subunit alpha1 A (CACNA1A) gene polymorphisms with benign paroxysmal positional vertigo (BPPV).

Material/Methods: A total of 120 BPPV patients and 60 healthy controls were enrolled according to the diagnostic criteria in the Guideline of Diagnosis and Treatment of Benign Paroxysmal Positional Vertigo (2017). Clinical and biochemical data were collected, the rs2074880 (T/G) polymorphisms in the CACNA1A gene were detected using TaqMan-MGB probe method, and the correlations of BPPV with predisposing factors were analyzed through logistic analysis.

Results: The BPPV group had higher levels of cholesterol and uric acid than in the control group (p<0.05). The cholesterol and uric acid levels were positively correlated with BPPV (p<0.05) [odds ratio (OR)=2.298 (1.252–4.350), 95% confidence interval (95% CI)=1.123 (0.987–1.987)]. The distribution frequency of TT genotype was higher than that of GG genotype (χ²=9.907, p=0.002, OR=0.279, 95% CI=0.123–0.633). In the BPPV group, cholesterol and uric acid levels of TT genotype were elevated compared with those in GG genotype (p<0.05).

Conclusions: The onset of BPPV is related to the increased levels of cholesterol and uric acid, as well as the dominant homozygous mutation of rs2074880 (T/G) in the CACNA1A gene.

MeSH Keywords: Meniere Disease • Polymorphism, Genetic • Polymorphism, Single Nucleotide

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Background
Benign paroxysmal positional vertigo (BPPV), commonly known as otolithiasis, is the most common self-limited peripheral vestibular disease seen in clinical practice. BPPV is often manifested as recurrent and transient vertigo associated with head and body positions, and accompanied with nystagmus and autonomic symptoms. Clinically, about 64.7% of patients have vertigo caused by peripheral vestibular disease, among which, BPPV patients account for 36.5% of those with peripheral vestibular disease. The prevalence of BPPV is high and it can seriously affect patient quality of life. Several studies have demonstrated a genetic contribution to the occurrence and pathogenesis of BPPV, as a number of BPPV patients have family histories of BPPV [1,2]. In addition, patients with BPPV were 5 times as likely to have relatives with BPPV compared to the dizzy control group [1]. Moreover, through whole-genome mapping with 400 microsatellite repeat markers and analyzing this trait using both autosomal dominant and recessive models of inheritance on a three-generation family in which multiple family members developed BPPV, Gizzi et al. showed that the BPPV gene in this family maps to a critical chromosomal 15 interval between markers GATA151F03N and GATA85D02 [3], further supporting the genetic predisposition in BPPV occurrence.

The CACNA1A gene, located at 19P13 and encoding Cav2.1 protein, is implicated in the formation of P/Q-type calcium channel as a subunit. The channel is located on the neuron membrane and distributed in the brain and neuromuscular junctions; it mediates the release of nerve-ending and synaptic transmitters and also participates in nervous system development [4,5]. Previous studies have shown that the calcium voltage-gated channel subunit alpha1 A (CACNA1A) gene can change calcium channel function through mutation, influencing the release of synapses and neurotransmitters, and having correlations with paroxysmal diseases of the nervous system [6,7]. Whether CACNA1A gene mutation is related to BPPV onset still remains unclear at present. Therefore, TaqMan-MGB probe method was used in this research to detect rs2074880 (T/G) polymorphisms in the CACNA1A gene, so as to investigate the relationship of CACNA1A gene polymorphisms with BPPV.

Material and Methods
Research objects
BPPV patients diagnosed in accordance with the diagnostic criteria of the Guideline of Diagnosis and Treatment of Benign Paroxysmal Positional Vertigo (2017) in the Navy General Hospital Department of Neurology from January 2016 to January 2018 were selected. Inclusion criteria were: 1) Patients with recurrent and transient vertigo or dizziness (≤ 1 min) after change of head position in relation to the gravity direction and 2) patients with vertigo and characteristic positional nystagmus during positional test. We excluded patients with other diseases, such as central and positional vertigo, Meniere’s disease, vestibular migraine, vestibular neuritis, vestibular paroxysmia, labyrinthitis, posterior-circulation ischemia, superior canal dehiscence syndrome, postural hypotension, or psychogenic vertigo. According to the above criteria, a total of 120 BPPV patients were enrolled in this study, including 53 males and 67 females, with an average age of (61.30±9.20) years old. We selected 60 healthy people in the physical examination center in the same time period as controls; there were 25 men and 35 women, with an average age of (61.32±9.54) years old. The participants in both groups signed the informed consent, and approval was obtained from the Ethics Committee of Navy General Hospital.

Research methods
Clinical and biochemical data
Data collected included name, sex, age, systolic blood pressure, diastolic blood pressure, hypertension, diabetes mellitus, and history of smoking and drinking. We drew 1 mL peripheral blood from the patients in the morning after fasting for at least 8 h to measure the cholesterol, low-density lipoprotein, uric acid, and fasting blood glucose.

Extraction of deoxyribonucleic acid (DNA)
Ws collected 1 mL elbow venous blood from each patient, and DNA was extracted using a kit for extracting genomic DNA from medium-dose whole blood (Biotek Corporation, Beijing) according to the specific steps in the kit instructions. The Nanodrop-2000 ultramicro-ultraviolet spectrophotometer (Thermo Fisher Scientific, USA) was used to determine the DNA purity and concentration. The purity and concentration of all the DNA samples met the experimental requirements.

Single-nucleotide polymorphism (SNP) genotyping
The rs2074880T/G SNP genotypes in the CACNA1A gene were detected and analyzed using TaqMan® SNP Genotyping Assays kits (Thermo Fisher Scientific, USA) following the steps detailed in the kit instructions.

Statistical methods
Statistical Product and Service Solutions (SPSS) 20.0 software was utilized for statistical analysis. The measurement data are expressed by (±s), the independent-samples t test was performed for comparison between 2 groups, and one-way analysis of variance was conducted for comparison among
multiple groups. The correlation of BPPV with its predisposing factors was analyzed by logistic analysis. Likelihood-ratio chi-square test was applied to assess whether the genotype distribution conformed to Hardy-Weinberg equilibrium. The R×C table chi-square test was performed to compare the genotype and allele frequencies in each group. \( p < 0.05 \) suggested that the difference was statistically significant.

## Results

### Comparisons of vascular risk factors

There were no differences in the vascular risk factors between the 2 groups \( (p>0.05) \) (Table 1).

### Comparisons of quantitative data

Levels of cholesterol and uric acid in the BPPV group were higher than those in the control group \( (p<0.05) \), and there were no differences in the remaining quantitative data between the 2 groups \( (p>0.05) \) (Table 2).

### Logistic regression analysis

According to the logistic regression analysis of predisposing factors in the BPPV group, the cholesterol and uric acid levels were positively correlated with BPPV \( (p<0.05) \) [odds ratio (OR)=2.298 (1.252–4.350), 95% confidence interval (95% CI)=1.123 (0.987–1.987)]. The differences in the correlations of the remaining predisposing factors with BPPV were not statistically significant \( (p>0.05) \) (Table 3).

### Genetic equilibrium test

Likelihood-ratio chi-square test was performed to assess the actual and theoretical frequencies of 3 genotypes between the BPPV group and control group. The distribution of all 3 genotypes was in line with Hardy-Weinberg equilibrium in both groups and were comparable \( (p>0.05) \) (Table 4).

### Comparisons of genotype distribution frequencies

The distribution frequencies of TT, TG and GG genotypes were 25.00%, 58.33%, and 16.67%, respectively, in the BPPV group, and 33.33%, 45.00%, and 21.67%, respectively, in the control group. The distribution frequency of TT genotype was lower than that of TG genotype \( (\chi^2=2.245, p=0.245, OR=0.579, 95\% CI=0.282–1.188) \), the distribution frequency of TG genotype was higher than that of GG genotype \( (\chi^2=9.907, p=0.002, OR=0.279, 95\% CI=0.123–0.633) \), and the distribution frequency of TG genotype was higher than that of GG genotype \( (\chi^2=1.544, p=0.214, OR=1.685, 95\% CI=0.737–3.855) \) (Table 5).

| Item                        | BPPV group | Control group | t       | p      |
|-----------------------------|------------|---------------|---------|--------|
| Age/years old               | 61.30±9.20 | 61.32±9.54    | −0.056  | 0.945  |
| Systolic blood pressure (mmHg) | 129.80±14.89 | 126.00±11.92 | 1.502  | 0.145  |
| Diastolic blood pressure (mmHg) | 73.57±8.78   | 75.54±7.43   | 0.369   | 0.702   |
| Cholesterol (mmol/L)        | 4.79±0.86   | 4.28±1.04    | 2.356  | 0.008   |
| Low-density lipoprotein (mmol/L) | 2.69±0.63    | 2.88±0.76    | −1.563 | 0.124   |
| Uric acid (mmol/L)          | 335.40±81.56 | 300.50±73.54 | 2.073  | 0.032   |
| Fasting blood glucose (mmol/L) | 4.93±1.00    | 5.00±0.57    | −1.269 | 0.215   |

Table 1. Comparisons of vascular risk factors between the 2 groups \( [n (%)] \).

| Item                        | BPPV group | Control group | t       | p      |
|-----------------------------|------------|---------------|---------|--------|
| Age/years old               | 61.30±9.20 | 61.32±9.54    | −0.056  | 0.945  |
| Systolic blood pressure (mmHg) | 129.80±14.89 | 126.00±11.92 | 1.502  | 0.145  |
| Diastolic blood pressure (mmHg) | 73.57±8.78   | 75.54±7.43   | 0.369   | 0.702   |
| Cholesterol (mmol/L)        | 4.79±0.86   | 4.28±1.04    | 2.356  | 0.008   |
| Low-density lipoprotein (mmol/L) | 2.69±0.63    | 2.88±0.76    | −1.563 | 0.124   |
| Uric acid (mmol/L)          | 335.40±81.56 | 300.50±73.54 | 2.073  | 0.032   |
| Fasting blood glucose (mmol/L) | 4.93±1.00    | 5.00±0.57    | −1.269 | 0.215   |

Table 2. Comparisons of quantitative data between the 2 groups.
Comparisons of allele distribution frequencies

The distribution frequencies of T and G alleles were 54.17% and 45.83%, respectively, in the BPPV group, and 55.83% and 44.17%, respectively, in the control group. By comparing T allele with G allele, we found $\chi^2 = 0.090, p = 0.765, OR = 0.935, and 95\% CI = 0.602–1.453$ (Table 6).

Table 3. Logistic regression analysis on predisposing factors in BPPV group.

| Item                  | p   | OR    | 95% CI       |
|-----------------------|-----|-------|--------------|
| Sex                   | 1.049 | 0.865 | 0.564–1.875  |
| Age                   | 0.876 | 0.701 | 0.032–0.358  |
| Smoking               | 0.461 | 0.654 | 0.401–0.914  |
| Drinking              | 0.472 | 1.410 | 0.517–2.743  |
| Systolic blood pressure | 0.369 | 1.101 | 0.841–1.320  |
| Diastolic blood pressure | 0.271 | 0.940 | 0.871–1.210  |
| Cholesterol           | 0.007 | 2.298 | 1.252–4.350  |
| Low density lipoprotein | 0.493 | 1.349 | 0.528–2.735  |
| Uric acid             | 0.042 | 1.123 | 0.987–1.987  |
| Fasting blood glucose | 0.352 | 0.748 | 0.398–1.298  |

Table 4. Genetic equilibrium law of rs2074880 genotypes in CACNA1A gene.

| Group       | n | TT                  | TG                    | GG                  | $\chi^2$ | p   |
|-------------|---|---------------------|-----------------------|---------------------|---------|-----|
| BPPV group  | 120 | 30 (25.00) | 70 (59.58) | 20 (16.42) | 3.67  | 0.16 |
| Control group | 60 | 20 (33.33) | 27 (45.00) | 13 (21.67) | 0.46  | 0.79 |

Table 5. Comparisons of genotype distribution frequencies of rs2074880 in CACNA1A gene [n (%)].

| Genotype | BPPV group | Control group | $\chi^2$ | p   | OR | 95% CI | $\chi^2$ | p   | OR | 95% CI |
|----------|------------|---------------|---------|-----|----|--------|---------|-----|----|--------|
| TT       | 30 (25.00) | 20 (33.33)    |         |     |    |        |         |     |    |        |
| TG       | 70 (58.33) | 27 (45.00)    | 2.245   | 0.134 | 0.579 | 0.282–1.188 | 2.144  | 0.027 | 1.233–3.331 |
| GG       | 20 (16.67) | 13 (21.67)    | 9.907   | 0.002 | 0.279 | 0.123–0.633 | 1.544  | 0.021 | 1.233–3.331 |

$\chi^2$, p, OR, and 95% CI stand for the $\chi^2$, p, OR and 95% CI, respectively, for comparison between TT genotype and TG and GG genotypes. $\chi^2$, p, OR, and 95% CI stand for the $\chi^2$, p, OR and 95% CI, respectively, for comparison between TG genotype and GG genotype.

Table 6. Comparisons of allele distribution frequencies of rs2074880 in CACNA1A gene [n (%)].

| Allele | BPPV group | Control group | $\chi^2$ | p   | OR | 95% CI |
|--------|------------|---------------|---------|-----|----|--------|
| T      | 130 (54.17) | 67 (55.83)    |         |     |    |        |
| G      | 110 (45.83) | 53 (44.17)    | 0.090   | 0.765 | 0.935 | 0.602–1.453 |

Comparisons of allele distribution frequencies

T allele with G allele, we found $\chi^2 = 0.090, p = 0.765, OR = 0.935$, and 95% CI = 0.602–1.453 (Table 6).
Correlation analysis of rs2074880 genotypes in CACNA1A gene with quantitative data in the BPPV group

The 3 rs2074880 genotypes in the CACNA1A gene were associated with cholesterol and uric acid levels in the BPPV group (p<0.05). Pairwise analyses were further conducted for the cholesterol and uric acid levels of the 3 genotypes, and the results showed that the cholesterol and uric acid levels of the TT genotype were elevated compared with those in the GG genotype (p<0.05). The correlations of the remaining quantitative data with the 3 rs2074880 genotypes in CACNA1A gene were not significant (p>0.05) (Table 7).

Discussion

The pathogenesis of BPPV, which is a clinically common peripheral vertigo, is still unclear. The currently popular theory of otolith involvement argues that the pathogenesis of BPPV is that calcium carbonate particles fall off from the otolithic membrane of the utricle due to various reasons, enter into the semicircular canal and stimulate the hair cells of semicircular canal, thereby triggering nausea, vomiting, and other discomforts [8–10]. In the present study, we found that levels of cholesterol and uric acid in the BPPV group were elevated compared with those in the control group (p<0.05). Further logistic regression analysis also revealed that cholesterol and uric acid levels were positively correlated with BPPV (p<0.05). These results are consistent with the findings of other scholars [11–13], indicating the close association of cholesterol and uric acid levels with the development or pathogenesis of BPPV.

Enormous strides have been made in the analysis on gene loci with the rapid development of gene detection technology in recent years, and research on BPPV has revealed that the occurrence of BPPV is correlated with spinocerebellar ataxia type 6, familial hemiplegic migraine, episodic ataxia type 2, epilepsy, and other neurological diseases [17–20]. However, its correlation with BPPV has not been clarified yet. In the present study, CACNA1A gene polymorphism site rs2074880 (T/G) was selected, and the genotype and allele frequencies in the BPPV group and control group were analyzed by means of SNPscan multiplex SNP genotyping technology. The results indicated that the distribution frequency of TT genotype was higher than that of GG genotype ($\chi^2=9.907$, p=0.002, OR=0.279, 95% CI=0.123–0.633), illustrating that the occurrence of BPPV is correlated with the dominant homozygous mutation of rs2074880 (T/G) in the CACNA1A gene, and the risk of BPPV is increased due to TT mutation of rs2074880 in the CACNA1A gene, suggesting CACNA1A is involved in the development and pathogenesis of BPPV, possibly through regulating calcium channels. However, the exact molecular mechanism by which CACNA1A is involved in the occurrence of BPPV remains unclear and requires further investigation.

Based on the finding that BPPV onset was associated with rs2074880 (T/G) in the CACNA1A gene, the correlation of rs2074880 genotypes in CACNA1A gene with quantitative data in BPPV group was further analyzed. We discovered that in the BPPV group, the 3 rs2074880 genotypes in the CACNA1A gene were associated with cholesterol and uric acid levels (p<0.05), and the cholesterol and uric acid levels of TT genotype were elevated compared with those in GG genotype (p<0.05). Our results suggest that the TT genotype mutation of rs2074880 in the CACNA1A gene is correlated with increased levels of cholesterol and uric acid.

Table 7. Correlation analysis of rs2074880 genotypes in CACNA1A gene with quantitative data in BPPV group.

| Item                          | TT             | TG             | GG             | $\chi^2$ | p          |
|-------------------------------|----------------|----------------|----------------|----------|------------|
| n (Male/Female)               | 20/2423        | 13/20          | 20/23          | 0.430    | 0.806      |
| Age (years old)               | 61.33±9.40     | 62±7.83        | 60.54±8.72     | −0.256   | 0.276      |
| Systolic blood pressure (mmHg)| 130.12±14.78   | 129.56±13.65   | 127.78±15.90   | 1.436    | 0.134      |
| Diastolic blood pressure (mmHg)| 73.67±7.89    | 74.59±8.97     | 72.48±7.98     | 0.376    | 0.698      |
| Cholesterol (mmol/L)          | 4.78±0.83      | 4.74±0.92      | 4.80±0.78      | 3.142    | 0.006      |
| Low-density lipoprotein (mmol/L)| 2.70±0.64     | 2.67±0.71      | 2.68±0.74      | 1.453    | 0.136      |
| Uric acid (mmol/L)            | 335.12±81.34   | 334.45±79.92   | 337.69±80.56   | 2.189    | 0.047      |
| Fasting blood glucose (mmol/L)| 4.92±1.01      | 5.01±0.95      | 4.93±0.99      | 1.272    | 0.235      |
Conclusions

The onset of BPPV is related to the increased levels of cholesterol and uric acid as well as the dominant homozygous mutation of rs2074880 (T/G) in the CACNA1A gene. However, due to the limited number of patients enrolled in this study, a large-cohort clinical study is required to confirm these findings in the future.

Conflict of interest

None.

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