



# Association between PDE4D polymorphism and ischemic stroke in young population

Xuejing Yue<sup>a</sup>, Liu Lixia<sup>a</sup>, Haiqing Yan<sup>b</sup>, Ping Zhang<sup>b,\*</sup>, Yongkun Gui<sup>b</sup>, Jinggui Song<sup>b,\*</sup>

<sup>a</sup>School of Basic Medical Sciences, Xinxiang Medical University, Xinxiang 453000, China

<sup>b</sup>The First Affiliated Hospital of Xinxiang Medical University, Weihui 453100, China

## ARTICLE INFO

### Article history:

Received 4 June 2018

Revised 9 April 2019

Accepted 9 April 2019

Available online 9 April 2019

### Keywords:

Phosphodiesterase 4D

Ischemic stroke

Gene polymorphism

## ABSTRACT

**Objective:** To explore the association between the polymorphisms of the phosphodiesterase (PDE) 4D gene (SNP83 and SNP87) and the risk of ischemic stroke (IS) in Chinese young population.

**Methods:** This study included 393 patients who were divided into IS group and non-IS group. Semiconductor high-throughput sequencing technology and multivariate logistic regression analysis were performed.

**Results:** In the case group, the frequency of CC genotype and C allele of the SNP83 gene was significantly higher than that in the control group. There was no significant difference in genotype frequency distribution of SNP87 between the two groups.

**Conclusion:** We found an association between SNP83 and the risk of IS in Chinese young population from northern Henan province. There was not a significant association between SNP87 and IS in Chinese young population.

© 2019 Production and hosting by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

## 1. Introduction

Stroke refers to acute or chronic cerebrovascular diseases caused by various causes. It is one of the most common diseases which may cause death or permanent disability in the world. In China, stroke is the first cause of disability and death (Eseyin et al., 2018; Wang et al., 2018; Xie et al., 2018). In recent years, the incidence of stroke has been increased at younger age (Bersano et al., 2013). Previous studies have demonstrated that the incidence rate of stroke among young population is approximately 97.7/100 000 in China, which is significantly higher than that in Western countries (Guo et al., 2016). 85% of strokes were confirmed to be ischemic stroke (IS) (Wang et al., 2017). It is important to properly implement the prevention, diagnosis and treatment of ischemic stroke in young adults (Arik et al., 2017;

Kevrekidis et al., 2018). It has been confirmed that IS was a result of multiple factors, such as chronic diseases, inflammation, and genetic variants (Banerjee et al., 2017). Genetic factors play a key predisposing role of IS in family and twin studies (Murray and Lopez, 2013).

The phosphodiesterase 4D (PDE4D) gene plays an important role in the degradation of cyclic adenosine monophosphate (cAMP). It is the only stroke-related gene acquired by the Genome-wide association study (GWAS) in recent years, which has been proven to be associated with the incidence of familial IS. There are six SNPs (SNP41, SNP45, SNP56, SNP83, SNP87 and SNP89) were reported to be associated with the risk of stroke. The SNP83 and SNP87 polymorphism have a focus for researches. However, the results are conflicting (Liu et al., 2013; Wu et al., 2017; Wei et al., 2017). We could not eliminate the feasibility that gene detection technology, sample size, genetic background and other factors contributed to these contradictory results.

In this study, high-throughput gene sequencing technology was adopted to analyze the association between the SNP83, SNP87 polymorphism and IS in young adults, aiming to investigate the genetic pathogenesis of stroke at the molecular level to prevent and provide theoretical basis for the prevention and treatment of stroke in young population.

\* Corresponding authors.

E-mail addresses: zhangpingsjnk@163.com (P. Zhang), songjg62@126.com (J. Song).

Peer review under responsibility of King Saud University.



Production and hosting by Elsevier

<https://doi.org/10.1016/j.sjbs.2019.04.007>

1319-562X/© 2019 Production and hosting by Elsevier B.V. on behalf of King Saud University.

This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

## 2. Materials and methods

### 2.1. Study population

From June 2014 to January 2017, 193 young stroke patients admitted to the neurology department of the First Affiliated Hospital of Xinxiang Medical College were recruited in this study. Inclusion criteria (1) Those with initial episode of stroke. (2) Aged 18–45 years. (3) Sampling collection and necessary examinations were completed with 1 week after the onset of stroke. (4) Ischemic stroke was diagnosed according to the International Classification of Diseases, 10th revision (ICD10). Another matched 200 healthy subjects, aged 18–45 years, receiving physical examination in our hospital were assigned into the control group. All enrolled participants had no abnormality and with no history of cerebrovascular diseases according to medical history collection and physical examination. All study subjects were originally from the northern part of Henan province and lived there for >3 years. They were not married with other ethnicities for more than two consecutive generations. Their educational levels were above junior middle school. Exclusion criteria: those aged > 45 years or < 18 years; those with other types of cerebral diseases; severe systemic diseases; the time interval between onset of stroke and sampling collection > 1 week; refusal to participate in this study; inability to effectively cooperate with researches due to other reasons. In this single-center study, case-control study method was adopted to statistically compare the genetic types between two groups. Prior to this study, all subjects signed informed consents. The study procedures were approved by the Ethics Committee of Xinxiang Medical College.

### 2.2. Data collection

Baseline data of all participants were recorded and an electronic medical record was established. The registration data mainly included demographic data, current disease, medical history, family history, medication history, the results of physical examination, routine blood test and biochemical examinations, and imaging examination of the head and neck.

### 2.3. Detection of gene

A portion of 2 ml of venous blood was collected from the subjects and the cells were isolated by centrifuge at 4000 rpm. Sample DNA was extracted using the QIAGEN DNA extraction kit. After the sample DNA concentration was measured with a spectrophotometer, the PDE4D gene locus was subject to high-throughput sequencing using the Illumina Miseq high-throughput sequencing platform. The specific experimental conditions were performed strictly according to the manufacturer's instructions. GeneMapper 4.0 was used for sample genotype data analysis and raw data processing.

### 2.4. Statistical analysis

The statistical analyses were performed by SPSS version 19.0 software. Statistical significance was defined as two-sided  $P < 0.05$ . Mean  $\pm$  standard deviation or standard error were presented of the continuous variables. Counting data were represented by frequency or constituent ratio. Univariate and multivariate logistic regression analyses were used to present the odds ratio (OR). The chi-square test was performed to calculate the qualitative data comparison and the Hardy-Weinberg equilibrium test (HWE).

**Table 1**  
Baseline data.

	Control group (2 0 0)	Case group (1 9 3)	P
Age	31.1 $\pm$ 17.9	33.2 $\pm$ 12.8	<0.001
Gender ratio	2.64:1 (145:55)	3.2:1 (147:46)	<0.05
Smoking history	48% (96)	61.1% (1 1 8)	<0.05
Drinking history	52.5% (1 0 5)	54.9% (1 0 6)	<0.001
Diabetes history	9.5% (19)	14% (27)	<0.001
Hypertension history	16% (32)	38.9% (75)	<0.05
CVD family history	7.5% (15)	20.7% (40)	<0.05

**Table 2**  
SNP83 Genotype distribution frequency.

Genotype	Control group	Case group	$\chi^2$	P
CC	36 (18%)	66 (34.2%)	10.002	0.001
CT	97 (50.3%)	121 (60.5%)		
TT	43 (21.5%)	30 (15.5%)		

## 3. Results

### 3.1. Clinical characteristics

In the case group, there were 193 patients including 147 male and 46 female, aged 33.2 years on average. In the control group, 200 subjects were recruited with a male to female ratio of 2.64:1 and an average age of 31.1 years. There was no significant difference regarding all parameters between two groups (all  $P > 0.05$ ), as illustrated in [Table 1](#).

### 3.2. HWE test

In the case and control groups, the minimum allele frequency of SNP83 was 0.40 and 0.48, and the minimum allele frequency of SNP87 was 0.37 and 0.40. Chi-square test was consistent with HWE test, indicating that the distribution of data in this study can represent the gene distribution of the population.

### 3.3. Comparison of SNP83 polymorphism and allele frequency between the case and control groups

Comparison of SNP83 polymorphism of PDE4D gene between the case and control groups demonstrated that the distribution frequency of CC homozygote was 34.2% in the case group, significantly higher compared with 18% in the control group ( $P < 0.005$ ). The frequency of C allele in the case group was 59.3%, significantly higher than 48.3% in the control group ( $P < 0.001$ , OR: 1.564, 95% CI 1.180–2.075), as illustrated in [Table 2](#) and [3](#).

### 3.4. Comparison of SNP87 polymorphism and allele frequency between the case and control groups

After the SNP87 polymorphism of PDE4D gene was compared between two groups, the distribution frequency of CC homozygote was 49.2% in the case group, higher than 42.0% in the control group. No statistical significance was observed in terms of the distribution frequency of CC, CT and TT genotypes between two groups (all  $P > 0.05$ ). There was no significant difference in the distribution frequency of C and T alleles at the SNP87 polymorphism between two groups (both  $P > 0.05$ ), as demonstrated in [Tables 4](#) and [5](#).

**Table 3**  
SNP83 Allele frequency.

Genotype	Control group	Case group	$\chi^2$	P	OR	95%CI
C	193 (48.3%)	229 (59.3%)	9.693	0.002	1.564	1.180–2.075
T	207 (51.7%)	157 (40.7%)				

**Table 4**  
SNP87 Genotype distribution frequency.

Genotype	Control group	Case group	$\chi^2$	P
CC	84 (42%)	95 (49.2%)	2.913	0.233
CT	70 (35%)	53 (27.5%)		
TT	46 (23%)	45 (23.3%)		

#### 4. Discussion

PDE4D is distributed in vascular smooth muscle cells, endothelial cells and various types of inflammatory cells. It selectively degrades the second messenger cAMP and prevents the proliferation and migration of vascular smooth muscle induced by cAMP, ultimately affecting the occurrence and progression of stroke by inhibiting arteriosclerosis (Mika and Conti, 2016). In recent years, multiple studies focusing on the association between stroke and PDE4D gene polymorphisms have been conducted, whereas the results are inconsistent among these investigations. Several studies have proposed that stroke is correlated with the SNP83 and SNP87 polymorphisms of PDE4D gene, whereas others have identified no significant association between SNP87 gene polymorphism and the risk of stroke (Luo Man et al., 2014; Xu et al., 2010; Shao et al., 2015; Kumar et al., 2013). This phenomenon prompts that the correlation between genetic polymorphism and stroke is related to multiple factors, such as ethnicity, region and age, etc. The pathogenesis of ischemic stroke is complex and collectively affected by environmental and genetic factors. For young and middle-aged adults, the pathogenesis of ischemic stroke is more likely to be affected by genetic factors. At present, the studies analyzing the risk of stroke and genetic factors in young adults are still lacking. Gene sequencing technology is an approach to unravel the genetic code of humans. Since 1975 when British biochemist Frederick Sanger applied the first-generation gene sequencing of chain termination method to determine the genomic sequence of a type of phage, novel gene sequencing technologies have been increasingly discovered.

High-throughput sequencing enables simultaneous sequencing of hundreds of thousands to millions of DNAs, which significantly shortens the time and reduces the cost of gene sequencing (You et al., 2018). It allows for scanning and detection of mutation sites at the entire genome level, delivers accurate detection of gene polymorphism. High-throughput sequencing has been widely applied to identify the candidate genes of multiple diseases (Zhang et al., 2018).

At present, most studies related to gene polymorphism are performed using the polymerase chain reaction-restriction fragment length polymorphism method (PCR-RFLP). The DNA fragments to

be detected are digested with restriction enzymes, and then the digested products are subject to electrophoresis. The diversity of gene sequences from different sources is compared by the diversity of the fragments. The limitations of this method are complicated procedures, time-consuming operation, relatively long cycle of experiment and limited sensitivity, which are not conducive to the identification of unknown gene mutations. In this study, high-throughput sequencing technology was first applied into gene detection and reported the association between the onset of ischemic stroke and with SNP83 and SNP87 polymorphisms of PDE4D gene in young stroke patients from the northern Henan province, China. By genotyping the SNP83 and SNP87 polymorphisms in 193 patients with ischemic stroke aged 18–45 years and 200 healthy controls, the genotype and allele frequencies of SNP83 and SNP87 polymorphisms were detected and statistically compared. The results demonstrated that the risk of stroke in young individuals carrying the SNP83C allele was higher than that in those carrying the T allele, whereas there was no significant association between the frequency of SNP87 gene and the incidence of stroke in young patients. These findings are consistent with previous investigations. Nevertheless, other studies have yielded different results. For example, a study conducted in 2014 reported that there was no association between SNP83 polymorphism of PDE4D gene, stroke patients and patients with 5 subtypes of stroke. Both two studies in 2009 and 2012 demonstrated that the SNP87C allele was a risk factor of stroke (Sun et al., 2009; Duo bin, 2012). The potential causes of the diverse findings are as follows: 1. The sample size is insufficient and small; 2. The frequency distribution of PDE4D genotypes and alleles varies among individuals from different countries, regions, races, ethnicities and age; 3. Inclusion criteria for ischemic stroke are different.

Taken together, the results of this study demonstrate that the SNP83 polymorphism of PDE4D gene is associated with the incidence of stroke in young population from the northern Henan province. However, The SNP87 polymorphism of PDE4D gene is not significantly associated with the onset of stroke in young stroke patients from the northern Henan province. Maybe the number of study subjects was relatively small, and multicenter studies with larger sample sizes are needed to confirm our findings.

#### 5. Conclusion

In conclusion, the SNP83 polymorphism of PDE4D gene was significantly associated with the risk of IS from the northern Henan province. This result may contribute to the young adult who carry the C allele to prevent the risk of IS in the future. And next, we will focus on how to reduce the incidence of the high-risk groups. Start with prevention in advance, depressing the incidence of IS.

**Table 5**  
SNP87 Allele frequency.

Genotype	Control group	Case group	$\chi^2$	P	OR	95% CI
C	238 (59.5%)	243 (63%)	0.535	0.464	1.114	0.834–1.487
T	156 (40.5%)	143 (37%)				

## Acknowledgement

The work was financially supported by Henan Key Laboratory Program of Neural Regeneration (Grant No.: HNSJXF-2018-007) and the National Natural Science Foundation of China (Grant No.:81471349).

## References

- Arik, F., Arik, B.E., Ozer, O., Kalan, U., Uyaniker, N., Cetinkaya, A., 2017. Fatal intoxication with colchicine. *Acta Medica Mediterranea* 33 (4), 592–596.
- Banerjee, K., Thiagarajan, N., Thiagarajan, P., 2017. Formulation optimization, rheological characterization and suitability studies of polyglucoside-based azadirachta indica a. Juss emollient cream as a dermal base for sun protection application. *Indian J. Pharm. Sci.* 79 (6), 914–922.
- Bersano, A., Borellini, L., Motto, C., et al., 2013. Molecular basis of young ischemic stroke. *Curr. Med. Chem.*
- Duo bin, 2012. Polymorphism of phosphodiesterase 4D gene in ischemic stroke patients in Xinjiang Uygur and Han nationalities. Medical University, Xinjiang.
- Eseyin, O.A., Sattar, M.A., Rathore, H.A., Aigbe, F., Afzal, S., Ahmad, A., Lazhari, M., Akthar, S., 2018. Gc-ms and hplc profiles of phenolic fractions of the leaf of telfairia occidentalis. *Pakistan J. Pharm. Sci.* 31 (1), 45–50.
- Guo, Y., Yue, X.J., Li, H.H., et al., 2016. Overweight and obesity in young adulthood and the risk of stroke: a meta-analysis. *J. Stroke Cerebrovasc. Dis.* 25 (12), 2995–3004.
- Kevrekidis, D.P., Minarikova, D., Markos, A., Malovecka, I., Minarik, P., 2018. Community pharmacy customer segmentation based on factors influencing their selection of pharmacy and over-the-counter medicines. *Saudi Pharm. J.* 26 (1), 33–43.
- Kumar, A., Sagar, R., Kumar, P., et al., 2013. Identification of genetic contribution to ischemic stroke by screening of single nucleotide polymorphisms in stroke patients by using a case control study design. *Bmc Neurol.* 13 (1), 136.
- Liu, X., Zhu, R., Li, L., et al., 2013. Genetic polymorphism in PDE4D gene and risk of ischemic stroke in Chinese population: a meta-analysis. *Plos One* 8 (6), e66374.
- Luo Man, Xu., Xiaowei, Li Jiaoxing, et al., 2014. The relationship between phosphodiesterase 4D gene polymorphism and ischemic stroke in Guangdong Han population. *Chinese J. Senile Cardio Cerebral Vascul. Dis.* 16 (5), 503–506.
- Mika, D., Conti, M., 2016. PDE4D phosphorylation: a coincidence detector integrating multiple signaling pathways. *Cell. Signal.* 28 (7), 719–724.
- Murray, C.J., Lopez, A.D., 2013. Measuring the global burden of disease. *N Engl J Med* 369, 448–457.
- Shao, M., Yi, X., Chi, L., et al., 2015. Ischemic stroke risk in a southeastern Chinese population: Insights from 5-lipoxygenase activating protein and phosphodiesterase 4D single-nucleotide polymorphisms. *J. Formos. Med. Assoc.* 114 (5), 422–429.
- Sun, Y., Huang, Y., Chen, X., et al., 2009. Association between the PDE4D gene and ischaemic stroke in the Chinese Han population. *Clin. Sci.* 117 (7), 265–272.
- Wang, L., Ge, S., Liu, Z., Zhou, Y., Yang, X., Yang, W., Li, D., Peng, W., 2018. Properties of antibacterial bioboard from bamboo macromolecule by hot press. *Saudi J. Biolog. Sci.* 25 (3), 465–468.
- Wang, W., Jiang, B., Sun, H., et al., 2017. Prevalence, incidence, and mortality of stroke in china clinical perspective: results from a nationwide population-based survey of 480 687 adults. *Circulation* 135 (8), 759.
- Wei, L.K., Au, A., Menon, S., et al., 2017. Polymorphisms of MTHFR, eNOS, ACE, AGT, ApoE, PON1, PDE4D and Ischemic stroke: meta-analysis. *J. Stroke Cerebrovasc. Dis. Official J. National Stroke Assoc.*
- Wu, W.L., Feng, X.W., Qiu, C.F., et al., 2017. A meta-analysis of PDE-gene polymorphism and cerebral infarction risk. *Exp. Therapeutic Med.* 13 (6), 2905–2911.
- Xie, Y., Ge, S., Jiang, S., Liu, Z., Chen, L., Wang, L., Chen, J., Qin, L., Peng, W., 2018. Study on biomolecules in extractives of camellia oleifera fruit shell by gc-ms. *Saudi J. Biolog. Sci.* 25 (2), 234–236.
- Xu, X., Li, X., Li, J., et al., 2010. Meta-analysis of association between variation in the PDE4D gene and ischemic cerebral infarction risk in Asian populations. *Neurogenetics* 11 (3), 327–333.
- You, Q., Yang, X., Peng, Z., et al., 2018. Development and applications of a high throughput genotyping tool for polyploid crops: single nucleotide polymorphism (SNP) array. *Front. Plant Sci.*, 9
- Zhang, R.Q., Lai, F.N., Wang, J.J., et al., 2018. Analysis of the SNP loci around transcription start sites related to goat fecundity trait base on whole genome resequencing. *Gene* 643, 1–6.