Cancer cell research 10 (2016) 241-245

www.cancercellresearch.org Article

Study on the relationship between the rs6465976 polymorphism of elastin gene and the incidence of intracranial aneurysms

Qiang Liu, YuGong Feng*, XiaoQing Du

Department of neurosurgery, Affiliated Hospital of Qingdao University, Qingdao, 266027, China

Abstract: **Our aim is to study intracranial aneurysm (IA) patients with rs6465976 single nucleotide polymorphism in elastin of Qingdao, China population patients IA correlations. A case-control analysis method, randomly selected from March 2015 to February 2016 in the Affiliated Hospital of Qingdao University, patients were from the department of neurosurgery of no blood relationship of intracranial aneurysm patients 60 cases as the research object, also randomly selected 60 controls of healthy unrelated volunteers as control group. The genotypes ELN rs6465976 in 60 IA and 60 controls were examined by TaqMan allelic discrimination real-time polymerase chain reaction (PCR). In the end, the portion of the blood sample randomly selected for PCR amplification and DNA sequencing again to verify the correctness of the result of. In the case group rs6465976 site GG, AG and AA three genotypes frequencies were 30%, 43.3% and 26.7%, besides the control group were 36.7%, 46.7%, 16.6% and difference was not statistically significant (P > 0.05). The frequency distributions of allele G and A were 51.67% and 48.33%, respectively. While the control group was 60% and 40% respectively, and the difference was not statistically significant (P>0.05) Thus ELN rs6465976 polymorphism may not be the risk factors of intracranial aneurysm formation.**

Keywords: **Intracranial aneurysm; Genetic polymorphism; Elastin gene; Etiology**

Received 2 April2016, Revised 23 April 2016, Accepted 25 April 2016

* Corresponding Author: YuGong Feng, fengyugong@126.com

1. Introduction

With the progress of molecular genetic research, it is generally believed that the occurrence, development and rupture of intracranial aneurysm (IA) are closely related to the genetic polymorphisms under the influence of environmental factors. Elastin as constitute the main components of the artery wall (about 90%), the gene polymorphism has been confirmed and are related to the occurrence of Utah of the intracranial aneurysms. Intracranial aneurysm is a relatively common disease in the department of neurosurgery. It is mainly due to the abnormality of the intracranial vessels. It is a serious threat to people's life and health and its incidence in the adult population rate from 2% to 6%, including intracranial aneurysm rupture can cause serious subarachnoid hemorrhage and Patients died within a month accounted for 40-60% [1]. Survivors are left lifelong nerve function defect is neurosurgery to endanger the life of one of the diseases. IA is considered to be a disease caused by genetic and environmental factors. Old age, smoking, hypertension, family history and so on are high risk factors for IA [2]. IA at the molecular level the potential pathogenesis is very complex in recent years, with the continuous development of DNA technique and I are on the aneurysm susceptible gene of enthusiasm, found possible and aneurysm of genetics and pathogenesis related susceptible genes, including: tension in the elastin gene, based metal protease gene, endothelial gene and apolipoprotein E protein gene, type III collagen gene, vascular angiotensin converting enzyme gene, α-1 antitrypsin elastase gene [3].

Elastin (ELN) is constituted the main part of the extracellular matrix (accounting for about 90%), is a support and provide the vast most vascular physiology function material, ELN in which play an important role in the process of the arterial wall, due to the elastic protein, its physical and chemical properties the can bear of the arterial wall load and arterial systolic and diastolic tension. The destruction of the ELN will not cause the integrity of the arterial wall, and cause of the arterial wall systolic and diastolic function greatly reduced, aneurysm patients, aneurysm wall which were taken for routine histological examination can be found, in the aneurysmal wall elastic membrane large reductions [4].

ELN gene micro satellite sites D7s2421 located in intron region, ELN loci near cover length of 7cm linkage region of simple tandem repeats (simple tandem repeat (STR) sequence, the length of 88-102bP contained herein have four may lead to disease SNP, Japan and the United States has reported research found ELN changes and micro satellite SNP [5].

2. Material and methods

2.1. Subjects

60 cases of Affiliated Hospital of Qingdao University neurosurgery intracranial aneurysm patients were random selected as case group (2015 March 2015 December), which 26 cases were male, 34 female, male to female ratio was 1:1.3; the average age of (59±11) years old. All patients underwent brain CT, CTA or DSA and other imaging examinations confirmed that patients with intracranial aneurysms,

and underwent craniotomy aneurysm clipping or aneurysm interventional embolization surgery. At the same time, we selected 60 cases of volunteers as healthy control group, including 32 males and 28 females, and the ratio of male to female was 1.06:1, the average age of (63±9) years old. Have no influence on the experiment of other diseases (heart, brain, liver, kidney disease and infectious inflammation, etc.). Blood samples were taken before the diet for at least 6 hours, blood samples were taken from the morning fasting peripheral venous blood 5ml in the EDTA anticoagulant tube, -20℃ refrigerator frozen standby to avoid repeated freezing and thawing. This study was conducted with approval from the Ethics Committee of the Affiliated Hospital of Qingdao University.Written informed consent was obtained from participants.

Table1 The clinical characteristics of cases and controls.

Characteristic	Cases (n=60)	Controls(n=60)	P value
average age(year)	59.5±11.3	63.7±9.6	>0.05
BMI(kg/m^2)	23.4±5.4	22.9±4.9	>0.05
FPG（mmol/L）	4.6±1.8	4.6±1.7	>0.05
Blood pressure(mmHg)	136.7±24.0	135.9±19.4	>0.05

2.2. Techniques

DNA was extracted from the whole-blood specimens using a Tiangen DNA extraction kit (Tiangen, shanghai, China). The concentration and quality of DNA were tested by a spectrophotometer,and the purifired DNA was kept at -20℃ until genotyping was conducted.Taqman allelic discrimination real-time PCR was used for genotyping the polymorphisms of rs6465976 in ELN in 7ul reaction mixture, containing probe and primers 1.20ul, PCR Master Mix 3.5ul, DNA 1.0ul and DNase-free water 1.3ul. The Taqman probes were designed by Applied Biosystems of Life Technologies. For rs6465976, the forward primer is 5'-TTTTTGTTCTTTTTCTTTTTCAC-3', and the reverse primer is 5'-CAAGTCTCGCTCTATCGCCAGGC-3'. PCR reaction conditions are: 95 degrees C activation 3min, 95 degrees Celsius denaturation 15s, 60 degrees Celsius annealing 1 min, a total of 45 cycles, the reaction performed by software (CFX -96, Bio-Rad) automatic genotyping.

2.3. Statical analysis

The Hardy-Weinberg equilibrium was evaluated to determine the distributions of gene frequencies in the case group and control group. Quantitative data are reported as means±standard deviation, and a comparison of the 2 group was obtained using the Student t test.The allelic and genotypic distributions of cases and controls were compared using χ^2 test. P＜0.05 was conducted statistically. All analyses were performed by statistical software package SPSS19.0.

3. Results

The clincial characteristics of the population enrolled and p-value for comparison are shown in Table 1.There was no statistically significant difference between the two groups (P>0.05). The genotypic distribution of the rs6465976 SNP followed Hardy-Weinberg equilibrium, as shown in Table 2.

The distributions of genotype and allele frequencies regarding SNP rs6465976 as well as statical comparisons are repored in Table 3 and Table 4. The results of DNA sequencing using PCR amplification products revealed 3 genotypes at the rs6465976 locus, including the AA, AG, and GG genotypes (Figure 1).

Table 2 The Hardy-Weinberg equilibrium.

	GG	GA	AA	P value
Cases(n=60)				
Actual number	18	26	16	P=0.3
Theory number	21.6	28.8	9.6	
Controls (n=60)				
Theory number	16.0	30.0	14.0	
Actual number	22	28	10	P=0.82

4. Discussion

Intracranial aneurysm (IA) is a common vascular disease in the department of neurosurgery. It is a kind of disease which is caused by the weakness of the arterial wall and the pressure of the local lumen. Most of the subarachnoid hemorrhage was caused by intracranial aneurysm rupture. With the advances in the technology of IA, the mortality of SAH and its 20%-30% is lower than before, but still a considerable proportion of patients have died due to the rupture of aneurysm for the first time. At present about aneurysm formation and rupture of different opinions, gender, age, hypertension, diabetes, external environment, geographical differences and aspirin anticoagulant medication use consider the risk factors of aneurysms [6]. Intense emotional fluctuations and fluctuations in blood pressure caused by a large number of physical activity lead to excessive pressure on the blood vessel wall, and ultimately lead to aneurysm rupture. And through the study of familial aneurysm, it is found that the volume of the aneurysm is larger than that in the

middle of the middle cerebral artery. So it is considered that the occurrence, location and rupture of the aneurysm are related to the genetic gene.

Table3 Comparison of the genotype frequencies of SNP rs6465976 in ELN gene between the cases and Controls.

Group	Numbe of cases	Genotype n(%)			χ^2	p
		GG	AG	AA		
Case group	60	18	26	16	1.859	0.395
		30.00%	43.33%	26.67%		
Control group	60	22	28	10		
		36.67%	46.67%	16.66%		

Through the gene bank inquiry, micro satellite loci D7s2421 that belongs to chromosome 7 is loci near the regional chain of short tandem repeats, covering a length of about 7cm, with a length of approx. 88-102bP, containing four single nucleotide polymorphisms, in gene bank position for 252184, play a role in the arterial wall is mainly dependent on the vascular wall in membrane (elastic membrane), a major component of film of the extracellular matrix, extracellular matrix is mainly composition of collagen and elastin, which elastin accounted for the vast majority of about 90%. Elastic protein in the three-dimensional space is in the folding of the sieve structure, and presents an orderly hierarchical arrangement. Under the influence of the external force, the length of the elastic protein can be changed greatly, the maximum elongation of 70%, which is an important part of the axial force of the artery. Collagen is embedded in the elastic protein structure in the shape of the structure, and the elasticity is far less than the elastic protein, which is about 1% of the expansion force. When the elastin is destroyed, the expansion of a sharp decline, decreased arterial elasticity, decreased ability to the regulation of smooth muscle cells, when sustained high level of blood pressure stimulation, vascular wall will appear morphological changes, cystic prominent and become the aneurysm. In addition, elastin also provides a micro fiber support framework for blood vessels. Its destruction will lead to a substantial decline in the integrity of the vessel wall. Therefore, the study of microsatellite loci D752421 will deepen our understanding of elastin, and better understand the pathogenesis of IA.

Table 4 Comparison of the allele frequencies of SNP rs6465976 in ELN gene between the cases and Controls.

Group	Numbe of cases	Allele n(%)		χ^2	p
		G	AA		
Case group	60	62	58	1.690	0.194
		51.67%	51.67%		
Control group	60	72	48		
		60.00%	40.00%		

Japan as the high incidence of arterial aneurysm, scholars of the aneurysms susceptibility genes were studied, through widely linkage analysis of gene and linkage disequilibrium method, et al. OndaH study the IA with 104 cases of twins, and aneurysm associated chromosomal 5q22-31, 7qll, ranging from 4q22 and related SNP, and between D55471 D552010, between D752415 D75657, microsatellite locus between D145258 D14574 research, that associated with IA gene locus located in chromosome 7 in the ELN gene in or near [7]. Familial aneurysm can help us to study the aneurysm susceptibility gene. M. Farnham James has studied 140 intracranial aneurysms that were found in each of the patients with aneurysm. The study found three microsatellite loci: D752415, D752472 and D752421. The results can be considered as IA and related (P=0.001) [8] D752421. And Japanese scholars Yamada s of the local 14 intracranial aneurysm family ELN locus, no genetic linkage association [9] and in the vicinity of the linkage region tandem repeats sequence of L, found that most Japanese intracranial aneurysm family and chromosome 7qll.

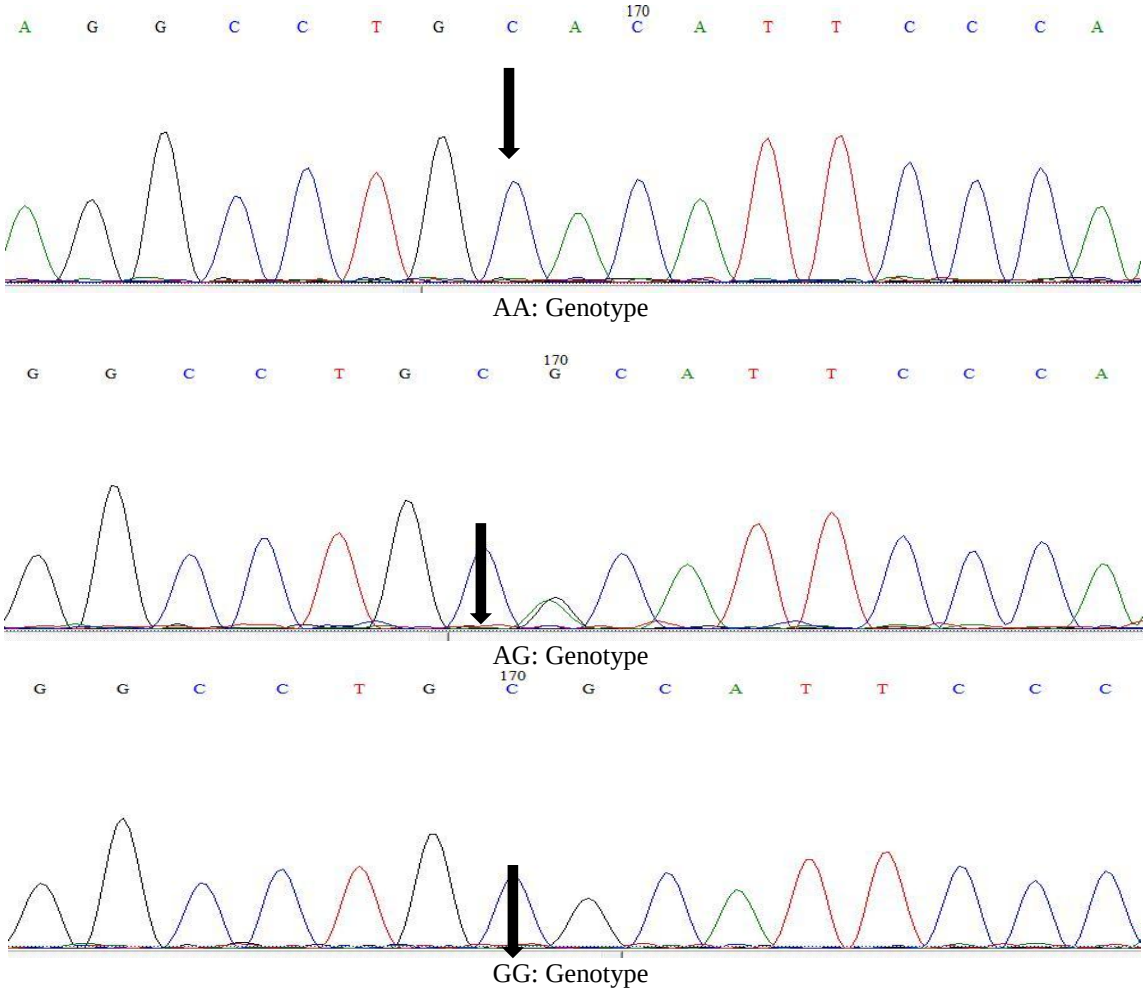

Figure1. DNA direct sequencing results of the ELN rs6465976 SNP.

In Austria and Germany, Hofer a collected a total of 30 intracranial aneurysms pedigree, 175 cases of sporadic patients with ELN and selected 235 controls found on the Japanese scholars OndaH of two elastic protein gene single nucleotide polymorphism were studied and found that elastin SNP and intracranial aneurysm between independent and said the study showed the Europeans and the Japanese population in gene genetic heterogeneity [10]. The first application fluorescence quantitative PCR method of populations in the coastal city of Qingdao area micro satellite sites D7s2421 single nucleotide polymorphism of loci: rs6465976G/A (located in intron region) of genetic polymorphism analysis, results showed that: microsatellite loci D7s2421 of SNP rs6465976G/A in intracranial ruptured artery aneurysm group GG genotype frequency and non intracranial aneurysm group no correlation, suggesting that micro satellite D7s2421 SNP rs6465976G/A sites may and ruptured intracranial aneurysm without correlation.

In conclusion, the SNP rs6465976 in ELN gene may not be associated with intracranial aneurysm development. Thus, additional studies are necessary to determine the funrmine the functions of SNPs in ELN pathogenesis.

References

[1] Famham JM, Camp NJ, Neuhausen SL. Confirmation of chromosome 7qll locus for predisposition to intracranial aneurysm. Hum Genet, 114 2014 250-255.

[2] Ruigrok YM, Rinkel GJ, Van't Slot R. Evidence in favor of the contribution of genes involved in the maintenance of the extracellular matrix of the arterial wall to the development of intracranial aneurysms. Human Molecular Genetics, 15(22) 2012 3361-3368.

[3] Krischek B, Moue I. The genetics of intracranial aneurysms. Hum Genet, 51 2013 587-594.

[4] Ynte MR, Gabriel JER, Cisca W. Genetics of intracranial anenrvcmcfTl-lancet. Neurnl, 4(a) 2005 179-182.

[5] Onda H, Kasuya H, Yoneyama T. Genomewide-linkage and haplotype-association studies map intracranial aneurysm to chromosome 7q11. Human Genetics, 69(4) 2011 804-819.

[6] Ruigrok YM, Rinkel GJ, Van' t Slot R. Evidence in favor of the contribution of genes involved in the maintenance of the extracellular

matrix of the arterial wall to the development of intracranial aneurysms. Human Molecular Genetics, 15(22) 2009 3361-3368.
[7] Onda H, Kasuya H, Yoneyama T. Genomewide-linkage and haplotype-association studies map intracranial aneurysm to chromosome 7q11. Human Genetics, 69(4) 2011 804-819.
[8] James M. Farnham, Nicola J. Camp, Susan L. Neuhausen. Confirmation of chromosome 7q11 locus for predisposition to intracranial aneurysm. Hum Genet, 114(3) 2014 250-255.
[9] Yamada S, UtsunomiyaM, moue K. Absence of linkage of familial intracranial aneurysms to 7q11 in highly aggregated Japanese families. Stroke, 34(4) 2013 892-900.
[10] Hofer A, Hermans M, Kubassek N. Elastin Polymorphism Haplotype and Intracranial Aneurysms Are Not Associated in Central Europe. Stroke, 34(5) 2013 1207-1211.