



INSERTION/DELETION POLYMORPHISM OF ANGIOTENSIN CONVERTING ENZYME GENE AND KAWASAKI DISEASE IN SOUTH CHINA

Yan Pan¹ and Hongzhu Lu^{1,2*}

¹Department of Pediatrics, the First Affiliated Hospital of Yangtze University, Jingzhou, Hubei Province, China.

²Medical College of Yangtze University, Jingzhou, Hubei Province, China.

ABSTRACT

Background: Kawasaki disease (KD) is characterized by systemic vasculitis of unknown etiology. In this study, we investigated the relationship between KD and insertion/deletion polymorphism of ACE gene in south China. **Methods:** Sixty Kawasaki disease patients and 60 healthy children were enrolled. ACE genotype was genotyped through polymerase chain reaction (PCR). **Results:** Frequencies of ACE genotypes (DD, ID, II) were 16.7%, 60.0%, 23.3% in Kawasaki group, and 41.7%, 26.7%, 31.7% in control group respectively. Compared with the ACE I/I genotype, we found a significant association between the ACE I/D polymorphism and KD risk (DI vs II: OR = 3.05, 95%CI = 1.23-7.57). In Kawasaki group, both genotype and allelic frequencies were not statistically different between those with coronary dilatations and those without. **Conclusions:** This study suggests that I/D polymorphism in the ACE gene may be associated with susceptibility to KD in south China.

Key words: ACE; I/D polymorphism; kawasaki disease; Coronary artery lesions

INTRODUCTION

Kawasaki disease (KD) is an acute multi-system vasculitis that mainly affects children less than 5 years of age, especially in Asian countries. The clinical characteristics of KD include unremitting fever for more than 5 days, diffuse oral mucosal inflammation, bilateral non-purulent conjunctivitis, dysmorphic skin rashes, indurations over the hands and feet, and cervical lymphadenopathy. Coronary artery lesions (CAL), and coronary artery dilatation or aneurysms, are the most important complications, occurring in approximately 15-25% of affected patients. The etiology of the disease is unknown, but is generally believed to be an infectious agent[5].The annual incidence of KD in China is estimated to be 2.34-54.22/100,000 children, which is lower than in Japan and Korea[6].

Twins and siblings of affected children have a risk of KD that is 10-fold higher than that of the general population[7].This observation suggests that genetic factors may play key roles in the KD pathogenesis of this disease. Angiotensin-converting enzyme (ACE), a zinc-dependent peptidase, is an important component of the renin-angiotensin system (RAS) responsible for converting angiotensin (Ang) I to vasoconstrictor Ang II. It is located on human chromosome 17q23 and has been implicated in many physiologic processes such as blood pressure control, haematopoiesis, reproduction, renal development, renal function and the immune response[8].The ACE gene contains 26 exons and 25 introns.The presence of a common variant, the ACE I/D polymorphism in intron 16 of the ACE gene, may influence the serum and tissue ACE activity, accounting for half the variance of serum enzyme levels[9]. The I allele, which represents an insertion of 287-base pairs(bp), is associated with lower serum and tissue levels, and the deleted form of the variant (D allele) is associated with higher circulating and tissue ACE activity[10].

Previous studies have suggested that the ACE I/D polymorphism may increase the risk of heart diseases and ischemic stroke[11,12].However, few studies have investigated the association between ACE I/D polymorphism and risk of KD.In this study, we conducted a hospital-based case-control study to evaluate the association between ACE I/D polymorphism and the risk of KD in south China.

MATERIAL AND METHODS

Subjects:

Our study included KD patients from the Department of Pediatrics at the First Affiliated Hospital of Yangtze University from January 2016 to July 2017.The 60 patients suffering KD included in our study group included 32 boys and 28 girls aged 0.6 to 3.2 years, with a mean age of 1.6 years, all of whom satisfied the appropriate diagnostic criteria for KD.All patients received treatment with intravenous gamma globulin(1 g/kg/day for 2 day) and were treated with oral aspirin, 30 mg/kg per day during the acute febrile phase and 5 mg/kg per day as a single daily dose during the convalescent phase.Two-dimensional echocardiography

was used to detect the presence of any coronary artery lesions. Coronary arterial lesion was defined as following: 1) inner diameter that is >3 mm in children <5 yrs old and >4 mm in children ≥5 yrs old; 2) internal diameter of a segment ≥1.5 times that of an adjacent segment; or 3) lumen with irregular surface. The control group included 60 healthy subjects randomly selected during health check-ups at our hospital (33 boys and 27 girls aged 0.4-3.0 years, with a mean age of 1.8 years). The study was approved by the Ethics Review Board of the First Affiliated Hospital of Yangtze University. Informed consent was obtained from the parents of all the subjects who were studied.

Genotype analysis:

We collected blood samples from KD patients and controls in disposable blood collection tubes (containing anticoagulant EDTA-K2). Samples were immediately transferred to cryopreservation tubes and stored at -70°C. Genomic DNA was extracted from blood samples using a DNA blood mini kit (QIAGEN, Valencia, CA). DNA fragments were amplified via polymerase chain reaction (PCR), which was carried out in a total volume of 10 L containing 50 ng of genomic DNA, 200 mM dNTPs, 0.3 mM/mL of each primers (5'-CTGGAGACCACTCCCATCCTTTCT); (5'-GATGTGGCCATCACATTCGTGAT) in PCR buffer with 0.5 units Taq DNA polymerase (Takara, Shiga, Japan). After the initial denaturation step (10 min at 95° C), 35 cycles were repeated for 30 sec at 94° C, 30 sec at 52° C, 90 sec at 72° C, and 5 min at 72° C. DNA fragments were then separated by electrophoresis on 2.5% agarose gel.

Statistical analysis:

All of the allele frequencies were in line with the Hardy-Weinberg equilibrium. The direct counting method was used to calculate the allele and genotype frequencies. The chi-square test was used to examine the differences in genotype and allele frequencies between the two groups. The odds ratio (OR) with its 95% confidence intervals (95% CI) were estimated for associations between risk alleles and genotypes with KD. *p* value less than 0.05 was considered significant. SPSS version 16.0 (SPSS Inc., Chicago, IL, USA) was used for data analysis.

RESULTS

The mean age of Kawasaki group (32 boys and 28 girls) was 20.4±8.4 months, and that of the control group (33 boys and 27 girls) was 22.5±7.2 months. Among 60 Kawasaki disease patients, coronary dilatation was observed in 15 patients (8 boys and 7 girls). The frequency of the ACE genotype was as follows: in controls: I/I, 31.7% (n=19); I/D, 26.7% (n=16); D/D, 41.7% (n=25); in KD patients: I/I, 23.3% (n=14); I/D, 60.0% (n=36); D/D, 16.7% (n=10) (Table 1). The odds ratio for developing Kawasaki disease in individuals with ACE I/D genotype was 3.05 (95% CI, 1.23-7.57) compared with the ACE I/I genotype. Comparing allelic (I, D) frequencies, the ACE genotypes did not significantly differ between control

and KD patients(Table 2).

In Kawasaki group, both genotype (DD, ID, II) and allelic frequencies were not statistically different between those with coronary dilatations and those without(Table 3).

Table 1. Prevalence of angiotensin converting enzyme genotype in Kawasaki disease patients and control group.

No. (%)	ACE genotype			Control
	DD	ID*□	II	
Control	25(41.7)	16(26.7)	19(31.7)	60(100.0)
Kawasaki	10(16.7)	36(60.0)	14(23.3)	60(100.0)

* $p < 0.05$, odds ratio, 3.05; 95% CI, 1.23-7.57.

Table 2. Deletion/insertion allelic prevalence of angiotensin converting enzyme gene in Kawasaki disease patients and control group.

No. (%)	Allele		Total
	D	I*	
Control	66(55.0)	54(45.0)	120(100.0)
Kawasaki	56(46.7)	64(53.3)	120(100.0)

* $p > 0.05$.

Table 3. Angiotensin converting enzyme genotype of Kawasaki patients with and without coronary dilatation.

Genotype	CAL (%)	Without (%)	Allele	CAL (%)	Without (%)
	(n=45)	(n=15)		(n=90)	(n=30)
DD	7(15.6)	2(13.3)	D	42	12
ID	28(62.2)	8(53.4)	I	48	18
II	10(22.2)	5(33.3)			

* $p > 0.05$.

DISCUSSION

KD is an immune-mediated disease. Possible causes proposed for the disease have included infection, autoimmune diseases, and genetic susceptibility. However, the exact causative agent remains unknown. In Kawasaki disease, the associated endothelial cell damage subsequently lowers the ACE level[13]. The ACE gene is localized on chromosome 17q23 and is characterized by a major insertion/deletion polymorphism consisting of the presence or absence of a 287-base pair Alu repeat sequence within intron 16[14]. The ACE I/D polymorphism accounts for 47% of the variation in ACE plasma activity[15]. This polymorphism has been

studied in connection with several diseases, including Alzheimer's disease, cerebral infarction and hypertension[16-18].

Wu et al. first reported that the ACE I/D polymorphism influences susceptibility to KD[19]. Our study was conducted to verify the relationship of this polymorphism with KD in south China. In our study, the ID genotype was more prevalent in the Kawasaki group than in the control group. However, genotypes (DD, ID, II) and allelic frequencies were not statistically different between KD patients with coronary dilatations and those without. Therefore, the ACE gene I/D polymorphism may be associated with the occurrence of KD but not with the development of coronary lesions. These results are different from those of Takeuchi et al.[20] that the II genotype of ACE gene is more prevalent in KD and those with coronary aneurysms. The discrepancies may be due to racial differences, heterogeneity of the population, sampling bias, or possibly by environmental factors.

The present study has some limitations. First, the study was conducted in one hospital, and this population may not be representative of the entire population of south China. Second, KD is a disease induced by multiple genes and environmental factors, and other genetic and environmental factors should be examined in future studies. Third, the number of cases and controls in our study was relatively small.

In conclusion, we found that subjects carrying the ACE gene I/D polymorphism was associated with an increased risk of KD in south China. Further large-scale studies are required to determine whether ACE polymorphisms play a role in the development of KD.

Acknowledgements: This study was supported by 2016 Jingzhou science and technology project(20161PE1-6).

REFERENCES

1. Nakamura Y1, Yashiro M, Uehara R, Oki I, Kayaba K, Yanagawa H. Increasing incidence of Kawasaki disease in Japan: nationwide survey. *Pediatr Int.* 2008;50(3):287-90.
2. Park YW, Han JW, Park IS, Kim CH, Cha SH, Ma JS, Lee JS, Kwon TC, Lee SB, Kim CH, Lee HJ, Yun YS. Kawasaki disease in Korea, 2003-2005. *Pediatr Infect Dis J.* 2007;26(9):821-3.
3. Kuo HC, Liang CD, Wang CL, Yu HR, Hwang KP, Yang KD. Serum albumin level predicts initial intravenous immunoglobulin treatment failure in Kawasaki disease. *Acta Paediatr.* 2010;99(10):1578-83.
4. Newburger JW, Takahashi M, Gerber MA, Gewitz MH, Tani LY, Burns JC, Shulman ST, Bolger AF, Ferrieri P, Baltimore RS, Wilson WR, Baddour LM, Levison ME, Pallasch TJ, Falace DA, Taubert KA; Committee on Rheumatic Fever, Endocarditis, and Kawasaki Disease, Council on Cardiovascular Disease in the Young, American Heart Association. Diagnosis, treatment, and long-term management of Kawasaki disease: a statement for health professionals from the Committee on Rheumatic Fever, Endocarditis, and Kawasaki

- Disease, Council on Cardiovascular Disease in the Young, American Heart Association. *Pediatrics*. 2004;114(6):1708-33.
5. Wang CL, Wu YT, Liu CA, Kuo HC, Yang KD. Kawasaki disease: infection, immunity and genetics. *Pediatr Infect Dis J*. 2005;24(11):998-1004.
 6. Pan JY. Epidemiological features of Kawasaki disease in China. *Int J Pediatr*. 2013;40:466-469.
 7. Fujita Y, Nakamura Y, Sakata K, Hara N, Kobayashi M, Nagai M, Yanagawa H, Kawasaki T. Kawasaki disease in families. *Pediatrics*. 1989;84(4):666-9.
 8. Guilherme L, Kalil J. Rheumatic heart disease: molecules involved in valve tissue inflammation leading to the autoimmune process and anti-s. pyogenes vaccine. *Front Immunol*. 2013;30:4:352.
 9. Zhang YF, Cheng Q, Tang NL, Chu TT, Tomlinson B, Liu F, Kwok TC. Gender difference of serum angiotensin-converting enzyme (ACE) activity in DD genotype of ACE insertion/deletion polymorphism in elderly Chinese. *J Renin Angiotensin Aldosterone Syst*. 2014;15(4):547-52.
 10. Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F. An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. *J Clin Invest*. 1990;86(4):1343-6.
 11. You FJ, Shen DM. Association between angiotensin-converting enzyme insertion/deletion polymorphisms and the risk of heart disease: an updated meta-analysis. *Genet Mol Res*. 2016;15:15017194.
 12. Tian Y, Ge Z, Xing Y, Sun Y, Ying J. Correlation of angiotensin I-converting enzyme gene insertion/deletion polymorphism with rheumatic heart disease: a meta-analysis. *Biosci Rep*. 2016;36. pii: e00412.
 13. Caldwell PR, Seegal BC, Hsu KC, Das M, Soffer RL. Angiotensin converting enzyme: vascular endothelial localization. *Science*. 1976; 191:1050-1051.
 14. Manning MW, Cassis LA, Daugherty A. Differential effects of doxycycline, a broad-spectrum matrix metalloproteinase inhibitor, on angiotensin II-induced atherosclerosis and abdominal aortic aneurysms. *Arterioscler Thromb Vasc Biol*. 2003; 23: 483-488.
 15. Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F. An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. *J Clin Invest*. 1990;86:1343-1346.
 16. Belbin O, Brown K, Shi H, Medway C, Abraham R, Passmore P, Mann D, Smith AD, Holmes C, McGuinness B, Craig D, Warden D, Heun R, Kölsch H, Love S, Kalsheker N, Williams J, Owen MJ, Carrasquillo M, Younkin S, Morgan K, Kehoe PG. A multi-center study of ACE and the risk of late-onset Alzheimer's disease. *J Alzheimers Dis*. 2011;24:587-597.
 17. Tao HM, Shao B, Chen GZ. Meta-analysis of the ACE gene polymorphism in cerebral infarction. *Can J Neurol Sci*. 2009;36:20-25.
 18. Li Y. Angiotensin-converting enzyme gene insertion/deletion polymorphism and essential hypertension in the Chinese population: a meta-analysis including 21,058 participants. *Intern Med J*. 2012;42:439-444.

19. Wu SF, Chang JS, Peng CT. Polymorphism of angiotensin-1 converting enzyme gene and Kawasaki disease. *Pediatr Cardiol.* 2004;25:529-533.
20. Takeuchi K, Yamamoto K, Kataoka S, Kakihara T, Tanaka A, Sato S, Uchiyama M. High incidence of angiotensin 1 converting enzyme genotype II in Kawasaki disease patients with coronary aneurysm. *Eur J Pediatr.* 1997; 156: 266-268.