European Review for Medical and Pharmacological Sciences 2017; 21: 3447-3451

Research on the relativity between gene polymorphism and children cardiac insufficiency

X.-H. HE, C.-L. LI, N. LING, Q.-W. WANG, Z.-Z. WANG, X.-J. AN

Department of Cardiology, Xuzhou Children's Hospital, Xuzhou, Jiangsu, Province, China

Abstract. – OBJECTIVE: We analyzed the relationship between Mink-S27 gene polymorphism and children with cardiac insufficiency.

PATIENTS AND METHODS: From April 2013 to April 2015, we enrolled 73 cases of children with cardiac insufficiency for this study, and all 73 were placed in the observation group. 76 normal cases were selected for the control group. Restriction fragment length polymorphism (RFLP) was used to make polymorphism analysis of the Mink-S27.

RESULTS: Our results showed no significant differences in Mink-S27 genotype and allele distribution in both observation and control groups ($p>0.05$). In lesion samples collected from children with cardiac insufficiency, we detected significant difference in AA, CC genotype frequency and allele frequency between the observation group and the control group ($p< 0.05$) ($X^2 = 15.43$, $p<0.05$; $X^2 = 16.27$, $p<0.05$). Further studies on samples obtained from both groups revealed certain differences of AA, CC, AC genotype frequency and allele frequency in the observation group. The proportion of homozygote (AA, CC) in children with severe cardiac insufficiency was relatively high.

CONCLUSIONS: GNAS2 gene polymorphism was associated with the prevalence of cardiac insufficiency in children. And also the patients' condition was correlated to the frequency of different genotypes and alleles.

Key Words

Mink-S27 gene, Gene polymorphism research, Children cardiac insufficiency, Relativity, RFLP.

Introduction

Children cardiac insufficiency, also known as cardiac failure, mainly refers to cardiac muscle's inability to pump blood. This condition can influence the organs, cell metabolism and normal bodily functions. The results of related studies realized by scholars like Xiaojin et al[1] showed that the reason for children cardiac insufficiency and its clinical manifestation were different from that in adults and the difference was most evident in infants and children. Soylu et al[2] showed that normal heart function depended on different factors, such as myocardial contractility, cardiac preload (capacity of ventricular end diastolic), cardiac afterload (tension after ventricle spray blood) and the harmony between heart rate and ventricular systole[3]. It was shown[4] that a single or multiple alterations in any of abovementioned factors could lead to heart failure to a certain degree. Another study[5] showed that most children cardiac insufficiency cases were caused by congenital obstructive valvular and excessive volume load. Currently, research on children cardiac insufficiency is mainly focused on children cardiac structure and dysfunction of related vessels, while studies reporting on molecular mechanism involved in children cardwiac insufficiency are rare[6].

Goiteinl et al[7] showed that genes related to atrial fibrillation were located in chromosome 10q22-24. Results from another research[8] demonstrated that atrial fibrillation genes were located in chromosome 11p15.5 and played a crucial role in ensuring normal function of heart. Xing et al[9] observed the effects of potassium transportation regulated by β subunit in channel protein that were coded by Mink in chromosome 21. And we know that potassium played a crucial regulatory role in myocardium repolarization. Ciofani et al[10] showed that 112A-G polymorphism changes existed in exon region had some relativity with atrial fibrillation, while Xuexian[11] demonstrated that the Mink gene expression level in patients with children cardiac insufficiency was different from that of normal people. In the same study[11], the degree of disease was somehow related to Mink expression. We designed this work to explore the possible link between Mink-S27 gene polymorphism and children cardiac insufficiency. We hoped to offer some theoretical and experimental foundation for better understanding of the molecular mechanism of children cardiac insufficiency.

Corresponding Author: Xinjiang An, MD; e-mail: anxinjian001@163.com

Patients and Methods

Patients

From March 2013 to July 2015, 73 children with cardiac insufficiency who were treated in our hospital were enrolled in this study. There were 36 males and 37 females, with average age of 5.6 ± 3.2 years who formed the observation group. Meanwhile, 76 healthy children were enrolled to form the control group. In the control group we had 38 males and 38 females (average age = 5.6 ± 3.2 years). While all members of observation group suffered from children cardiac insufficiency, children in the control group had no cardiac insufficiency or other illnesses. This study was approved by the Ethics Committee of Xuzhou Children's Hospital. Signed written informed consents were obtained from their guardians.

Methods

Sample collection

We collected 8 ml of venous blood from cases in the observation and the control groups. After 10 minutes centrifugation, we collected the upper serum and kept it at -80°C. Cells were stored in liquid nitrogen after freeze-stored liquid handling. All molecular reagents used in this study were purchased from AXYGEN company (AXYGEN, Corning, NY, USA) and primers used were designed by Primer software, and synthesized by Shanghai biological engineering company (Shanghai, China) (Table I).

PCR reaction system

We extracted DNA samples using AXYGEN Genomic extraction kit, and amplified the Mink-S27 gene. PCR reaction system (50 µl) was set up as following: ddH2O 37.6 µl, 5 µl of 10X Buffer, 4 µl of dNTP (250 umol/l), 1.2 µl of F (0.5 mmol/l), 1.2 µl of R (0.5 mmol/l), 1 µl of DNA and 1 µl of LA. Reaction procedure was: 95°C for 5 min, 95°C for 30 s, 62°C for 30s, 72°C for 30 s set for 30 cycles and 72°C for 10 min followed by 4°C. Amplified Mink-S27 gene was identified using 1 % agarose gel and observed it with gel imaging instrument.

Identification of differences in genotype

To identify the link between the different gene mutations and children cardiac insufficiency, we added 2.5 µl of Pml1 restriction endonuclease along with 5 µl of 10X buffer and 1 µl of 37.5 distilled water on 5 µl PCR products. The reaction was incubated in water bath for 1 h at 37°C. Then, we added 1 µl of 6X buffer on 5 µl digestion product and conducted electrophoresis for 1 h in 1% non-deformation polyacrylamide gel (100 V, 40 mA) and observed the gel using the gel imaging system [12,13].

Conversion and sequencing of the product

To verify the possible link between gene mutations and cardiac insufficiency in children, we sequenced PCR products obtained after conversion. We connected the PCR products through digestion with T19 Simple carrier and then conducted transformation. After transformation we screened the colonies and selected PCR product including wild-type, heterozygous and homozygous mutation and samples were sequences and analyzed (Shanghai Public Health)[14].

Classification of children cardiac insufficiency

According to the Ninth Revision about Standards of children cardiac insufficiency, proposed by the American Heart Association (NYHA) in 1994, we divided cases into 4 classes: (i) Level I: physical activities were not restricted, normal physical activities did not lead to excessive fatigue, palpitations, and breathing difficulties and other symptoms. (ii) Level II (mild): physical activities were limited, no detectable symptoms after the rest, normal physical labor could lead to fatigue, palpitations, and breathing difficulties and other symptom. (iii) Level III (moderate): physical activities were greatly restricted, patient felt comfortable after a rest, mild physical labor could lead to heart disease symptoms, such as palpitations and dyspnea, (iv) Level IV (severe): Complete loss of physical ability, heart failure, angina and other symptoms after the break; slight physical labor could cause breathing difficulties and other symptoms.

Statistical Analysis

We used SPAA20.0 software for statistical analysis. Data were expressed as $\bar{x} \pm s$. Alleles and distribution of genotype frequency expressed by the relevant technical data. Allele frequency = (number of homozygote × 2 + number of heterozygote) × 2-1 × n-1. $p < 0.05$ was considered statistically significant.

Table I. Mink-S27 exon primer sequence.

Primer name		PCR product length
F	GTGACGCCCTTTCTGACCA	494bp
R	CCCCTCACCCCTTACAACAACG	

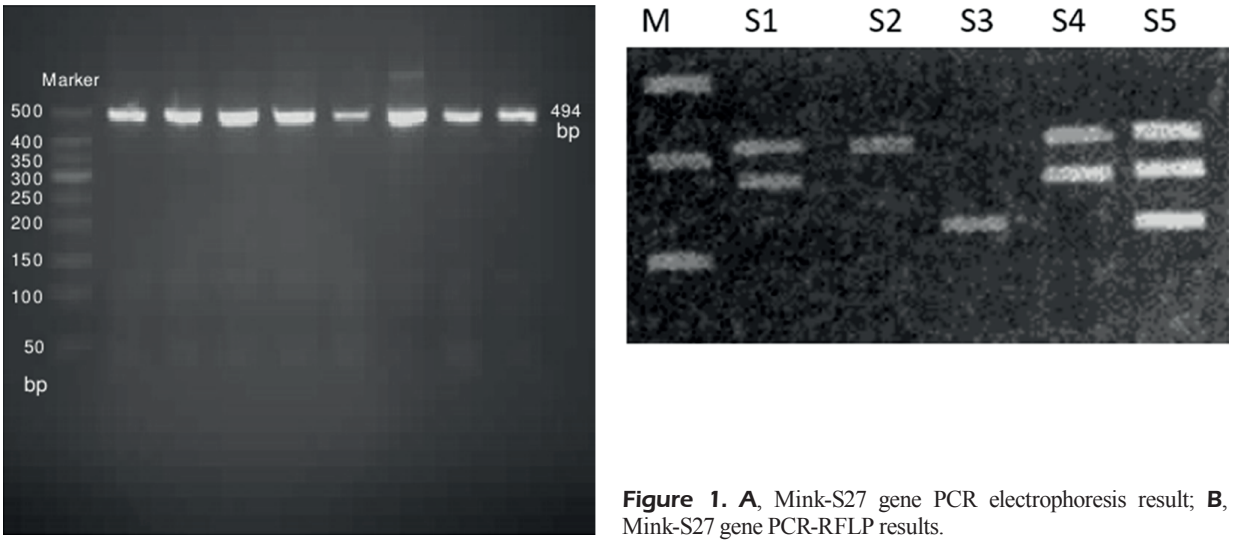

Figure 1. A, Mink-S27 gene PCR electrophoresis result; **B**, Mink-S27 gene PCR-RFLP results.

Results

Mink-S27 gene PCR-RFLP electrophoresis and sequencing results

Because GNAS2-G / G genotype could be incised by Pml1 restriction enzyme, the electrophoresis results showed 2 bands, one at 185bp and the other at 309bp. Mink-S27 genotype could not be incised by Pml1 restriction enzyme, so PCR product contained only one band at 494bp. After digestion with Pml1 restriction enzyme, Mink-S27 genotype showed three bands: 494bp, 185bp and 309bp (Figure 1). PCR digestion results were consistent with the sequencing results.

Frequencies comparison of genotype and alleles

We conducted statistical tests to compare frequency of Alleles A, C as well as genotype AA, CC in both groups. Results showed that significant differences existed between the two groups ($X^2 = 16.31$, $p < 0.05$; $X^2 = 16.08$, $p < 0.05$).

Through X^2-test and frequencies comparison between the control group and the observation group, as well as genotype and allele, we found that our results complied with Hardy-Weinberg's law of equilibrium (Table II).

Genotype frequency and allele frequency of different patients in observation group

Patients in the observation group were divided into three types: sinus arrhythmia, ectopic beats patients, ectopic patients and patients with abnormal conduction. We conducted statistical tests on genes (AA, CC) frequency and allele (A, C) frequency of above three types. The result showed that, significant differences existed between the genotype and allele frequency in the patients in the observation group, who had different degrees of the disease ($X^2 = 15.43$, $p <$

Table II. Comparison of genotype and alleles frequencies.

Group	N	Genotype frequency (%)			X^2	*P*
		AA	CC	AC		
Control group	76	38 (50.0)	23 (30.3)	15 (19.7)	15.43	0.000
Observation group	73	21 (28.8)	19 (26)	33 (45.2)		
Group	**N**	**Allele frequency (%)**			**X^2**	***P***
		A	**C**			
Control group	152	105 (69)	47 (31)		16.27	0.000
Observation group	146	113 (77.4)	33 (22.6)			

Table III. Comparison of frequency distribution of alleles.

Group	N	Genotype frequency (%)			X^2	*P*	H-W balance
		AA	CC	AC			
Cardiac insufficiency type I	17	5	6	6	15.43	P<0.05	0.142
Cardiac insufficiency type II	18	4	9	5			
Cardiac insufficiency type III	20	10	8	2			
Cardiac insufficiency type IV	18	9	7	2			
Group	**N**	**Allele frequency (%)**			X^2	*P*	H-W balance
		A	C				
Cardiac insufficiency type I	34	16 (47)	18 (53)		16.27	P<0.05	0.164
Cardiac insufficiency type II	36	13 (36)	23 (64)				
Cardiac insufficiency type III	40	22 (55)	8 (45)				
Cardiac insufficiency type IV	36	20 (55.6)	16 (44.4)				

0.05; $X^2 = 16.27$, $p < 0.05$). Through X^2-test and comparison of frequencies between the control group and the observation group, as well as genotype and allele, we found that it also complied with Hardy-Weinberg's law of equilibrium (Table III).

Discussion

As a harmful disease to healthy growth of children, in recent years, children cardiac insufficiency's incidences in China are growing. It was shown that the number of children suffering from this condition were about 320 thousand cases in 2014, which accounted for 1.2% of the total number of newborns[15]. Therefore, diagnosis and treatment of this condition has become the focus of attention in clinical research.

Results obtained from a prior study[16] showed that the cause of disease, among other factors, was mainly myocardial systolic and diastolic dysfunction, cardiac overload. Another related work[17] showed that the cause of the disease was very complex and could be resulted from infection, imbalance of water and electrolyte, cardiac arrhythmia and many other factors, such as emotional volatility and excessive exercise. Despite much progress in recent years, the molecular mechanism of heart failure in children remains unclear. Lai et al[16] showed that Mink-21 gene mainly codes KCNQ1 protein. However, Radicke et al[18] reported that KCNQ1 mainly transported and metabolized potassium. In our study, potassium and calcium played an important role in maintaining the normal heart function.

According to our data, β subunit and KCNQ1 constitute the slow delayed rectifier potassium channel together (Iks current complete ion channels). There are two potassium channel in atrial tissue, Ikr and Iks. They are both composed of four subunit encoded by the genes: KCNQ1 encodes α subunit of Iks, KCNH2 (HERG gene) and mink gene related peptide -1 (MiRP-1) constitute the α subunit together in Ikr channel, and KCNE1 gene (Mink) and KCNE2 gene encodes β subunit in two channels respectively. Mink gene was expressed at the mRNA level and protein level in atrial tissue. In regards to distribution of Mink-S27 genotype and allele, we found that there was no significant difference between the control and the observation groups ($p<0.05$). However further research showed that frequency of AA, CC genotypes and allele of patients with children cardiac insufficiency were significantly different from that in the control group ($p<0.05$). Moreover, further investigations on patients in the observation group showed that the frequency of AA, CC genotypes and alleles were different among patients. In severe patients the homozygous (AA, CC) ratio was relatively high.

Conclusions

We observed a correlation not only between GNAS2 gene polymorphism and the prevalence of cardiac insufficiency in children but also between the patients' condition and the frequency of different genotypes and alleles.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Xiaojin L, Liao Z, Mingsong A. Analysis of ventricular wall motion and m echocardiography evaluation on relevance of wall motion and coronary angiography in all direction. Chinese Journal of Ultrasound in Medicine 2007; 4: 262-265.
2) Soylu MO, Altun I, Basaran O, Uzun Y, Dogan V, Ergun G, Akin F, Biteker M. Impact of QRS morphology on heart rate turbulence and heart rate variability after cardiac resynchronization therapy in patients with heart failure. Eur Rev Med Pharmacol Sci 2016; 20: 317-322.
3) Liu J, Murata K, Fujino T, Ueda K, Kimura K, Wada Y, Oyama R, Tanaka N, Matsuzaki M. Effect of dobutamine on regional diastolic left ventricular asynchrony in patients with left ventricular hypertrophy. Circ J 2003; 67: 119-124.
4) Mor-Avi V, Sugeng L, Weinert L, MacEneaney P, Caiani EG, Koch R, Salgo IS, Lang RM. Fast measurement of left ventricular mass with real-time three-dimensional echocardiography: Comparison with magnetic resonance imaging. Circulation 2004; 110: 1814-1818.
5) McCarthy RR, Boehmer JP, Hruban RH, Hutchins GM, Kasper EK, Hare JM, Baughman KL. Long-term outcome of fulminant myocarditis as compared with acute (nonfulminant) myocarditis. N Engl J Med 2000; 342: 690-695.
6) Yang XO, Pappu BP, Nurieva R, Akimzhanov A, Kang HS, Chung Y, Ma L, Shah B, Panopoulos AD, Schluns KS, Watowich SS, Tian Q, Jetten AM, Dong C. T helper 17 lineage differentiation is programmed by orphan nuclear receptors ROR alpha and ROR gamma. Immunity 2008; 28: 29-39.
7) Goitein O, Matetzky S, Beinart R, Di Segni E, Hod H, Bentancur A, Konen E. Acute myocarditis: noninvasive evaluation with cardiac MRI and transthoracic echocardiography. AJR Am J Roentgenol 2009; 192: 254-258.
8) O'Shea JJ, Plenge R. JAK and STAT signaling molecules in immunoregulation and immune-mediated disease. Immunity 2012; 36: 542-550.
9) Chang X, Liu F, Wang X, Lin A, Zhao H, Su B. The kinases MEKK2 and MEKK3 regulate transforming growth factor-beta-mediated helper T cell differentiation. Immunity 2011; 34: 201-212.
10) Ciofani M, Madar A, Galan C, Sellars M, Mace K, Pauli F, Agarwal A, Huang W, Parkurst CN, Muratet M, Newberry KM, Meadows S, Greenfield A, Yang Y, Jain P, Kirigin FK, Birchmeier C, Wagner EF, Murphy KM, Myers RM, Bonneau R, Littman DR. A validated regulatory network for Th17 cell specification. Cell 2012; 151: 289-303.
11) Yang XO, Nurieva R, Martinez GJ, Kang HS, Chung Y, Pappu BP, Shah B, Chang SH, Schluns KS, Watowich SS, Feng XH, Jetten AM, Dong C. Molecular antagonism and plasticity of regulatory and inflammatory T cell programs. Immunity 2008;29:44-56.
12) Zhiyu Z, Dongfeng G. Study on single nucleotide polymorphisms of KCNQ1, KCNE1 and KCNE4 genes in patients with atrial fibrillation. Chinese Journal of Cardiovascular Diseases 2005; 33: 987-991.
13) Qing L. Analysis the cause of 62 cases of atrial fibrillation and clinical study anti coagulation therapy. Digest World Latest Med Inf 2015; 80: 67-70.
14) Xiao J, Deng SB, She Q, Li J, Kao GY, Wang JS, Ma Y. Allopurinol ameliorates cardiac function in non-hyperuricaemic patients with chronic heart failure. Eur Rev Med Pharmacol Sci 2016; 20: 756-761.
15) Chung Y, Chang SH, Martinez GJ, Yang XO, Nurieva R, Kang HS, Ma L, Watowich SS, Jetten AM, Tian Q, Dong C. Critical regulation of early Th17 cell differentiation by interleukin-1 signaling. Immunity 2009; 30: 576-587.
16) Lai LP, Su MJ, Yeh HM, Lin JL, Chiang FT, Hwang JJ, Hsu KL, Tseng CD, Lien WP, Tseng YZ, Huang SK. Association of the human minK gene 38G allele with atrial fibrillation: Evidence of possible genetic control on the pathogenesis of atrial fibrillation. Am Heart J 2002; 144: 485-490.
17) Bond CT, Herson PS, Strassmaier T, Hammond R, Stackman R, Maylie J, Adelman JP. Small conductance Ca2+-activated K+ channel knock-out mice reveal the identity of calcium-dependent afterhyperpolarization currents. J Neurosci 2004; 24: 5301-5306.
18) Radicke S, Cotella D, Graf EM, Banse U, Jost N, Varro A, Tseng GN, Ravens U, Wettwer E. Functional modulation of the transient outward current Ito by KCNE beta-subunits and regional distribution in human non-failing and failing hearts. Cardiovasc Res 2006; 71: 695-703.