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Correlation analysis between GNAS2 gene polymorphisms in children with arrhythmia

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Abstract. – OBJECTIVE: The objective of the present study was to analyze the correlation between GNAS2 gene polymorphisms and children with arrhythmia.

PATIENTS AND METHODS: We followed 89 children with arrhythmia treated in our hospital from April 2013 to April 2015, comprising the observation group, while 92 healthy subjects were taken as the control group. We analyzed polymorphisms of the GNAS2 gene from both groups by restriction fragment length polymorphism (RFLP).

RESULTS: There were no significant differences ($p>0.05$) in GNAS2 genotype or allelic distribution between the observation group and control group. However, there were significant differences in the gene frequency of homozygotes (TT and GG) and alleles between the control group and samples from children with arrhythmia ($p<0.05$) ($X^2=16.57$, $p<0.05$; $X^2=17.48$, $p<0.05$). Further study of samples from both groups indicate that gene frequency of homozygous (TT and GG) or heterozygous (TG) genes and alleles also had differences, indicating that homozygotes (TT and GG) are in relatively high proportion among patients with severe cases of arrhythmia.

CONCLUSIONS: Morbidity in patients with arrhythmia is related to GNAS2 gene polymorphisms. Differences in genotype and allele frequency are related to the severity of illness.

Key Words:
GNAS2 gene, Polymorphism, Children arrhythmia, RT-PCR, Correlation analysis.

Introduction

Arrhythmia refers to an irregular heart beat. This may be the result of abnormal pacemaker cell activity[1] in the sinoatrial node, or conduction disorders[2]. Clinical studies have shown that severe arrhythmias are often the cause of heart failure, Aspen syndrome, cardiogenic shock and even sudden death[3]. The study by Alakus et al[4] showed that immunity in newborn children was weak, making these children susceptible to diseases, such as arrhythmia. Arrhythmia in newborn children has become one of the most important factors affecting their lives. Otterbach et al[5] observed that roughly 1.3% of children in China presently are afflicted with arrhythmias of varying degree, and the number is increasing annually. At present, the pathogenesis of child arrhythmia remains unclear, and there are no therapeutic drugs or accurate detection methods to treat children with arrhythmias[6].

Yamawake et al[7] showed that the GNAS2 (human GS protein alpha subunit) gene on chromosome 12q13.2-13.3, is 1926 Kb in length and consists of 13 exons and 12 introns. Also, Mao et al[8] showed that a T393C mutation within the fifth exon of GNAS2 led to changes of GS protein function. They further demonstrated that the C-containing genotype was more frequent in the normal population than the TT genotype and correlated with changes in systolic blood pressure and left ventricular ejection fraction, which indicated that the function of the GS protein changed after the GNAS2 gene underwent T to C substitution. The result of the substitution was an enhancement of adenylate cyclase (AC) activity, an important factor[9] in maintaining a stable heart rate. Adenine nucleotides play an important role in the release of thyroid hormone, which affects the sympathetic nerves that regulate the heart. In the present study, we evaluated GNAS2 gene polymorphisms in patients between the control group (without arrhythmia) and the observation group (with arrhythmia) to provide a basis for the diagnosis and treatment of children with arrhythmia.

Patients and Methods

Patients

We followed 89 cases of children with arrhythmia who were treated in our hospital from April 2013 to April 2015 (the observation group). In the

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Table I. Primer sequences for GNAS2 exon 4 (cleavage site).

Primer name	Product length
Forward	CTCCTAACTGACATGGTGCA
Reverse	TAAGGCCACAAGTCGGGT

observation group, there were 45 males and 44 females and their average age was 5.6±3.2 years old. At the same time, we followed 92 healthy volunteers as the control group. There were 48 males and 44 females, and their average age was 6.2±3.6 years old in the control group. The patients were chosen in accordance with the relevant standards of pediatric cardiomyopathy and detection (ECG), and they had no other related diseases.

Sample Collection

In this work, we collected 6 ml of venous blood from patients with cardiomyopathy and healthy controls, and the blood was centrifuged for 10 min at 3000 rpm. Next, we collected the supernatant (serum) and stored it at -80°C. Cells were cryopreserved at -80°C for further use.

Reagents

ACR1 antibodies used in this study were purchased from Roche (Basel, Switzerland), RNA extraction kit was from Axygen (Tewksbury, MA, USA), and the associated molecular reagents were purchased from Takara (Otsu, Shiga, Japan). Fluorescence quantitative PCR primers were synthesized by the Shanghai Biological Engineering Co. Ltd. (Shanghai, China).

ForK restriction enzymes were purchased from Roche (Basel, Switzerland), gelose was purchased from Shanghai Biological Engineering Co. Ltd; DNA extraction kit was purchased from Axygen. Primers were designed by Olige7.0 software, the sequences of the primers are shown in Table I.

PCR

We amplified the extracted DNA by PCR. The reaction mixture (total volume: 25 μl) was as follows: 16.9 μl ddH_2O, 2.5 μl 10×Buffer, 2 μl dNTP (250 μM), 0.8 μl or each forward and reverse primer (0.5 mmol/l), 1 μl DNA, 1 μl LA (DNA polymerase). The thermal profile was as follows: 95°C for 5 min, 95°C for 30 s, 62°C for 30 s, and 72°C for 30 s, for 30 cycles, 72°C for 5min and the heat insulation was set at 4°C. The obtained PCR products were identified by 1% agarose gel electrophoresis and observed by gel imaging.

Identification of Genotype

5 μl of the PCR products, as described above were mixed with 2 μl of ForK restriction endonucleases, 2 μl 10×buffer and 11 μl sterile purified water. The reaction mixture was placed in a water bath set to 37°C for 1 hour. Following enzyme digestion, 6 μl of the product was mixed with 1 μl of 6×buffer and underwent cataphoresis in 8% non-deformable polyacrylamide gel for 1 h (100 V, 40 mA) and, then, it was observed by a gel imaging system[10].

PCR Product Connection Transformation Sequencing

Following enzyme digestion and transformation, sequencing of the PCR products was carried out according to The Handbook of Molecular Cloning Technology. Four PCR products of the wild-type gene, heterozygous and homozygous mutants were randomly selected by the transformed gene and were sent to Shanghai Biological Engineering Co. Ltd to be sequenced and analyzed.

Classification of Arrhythmia in Children

We divided patients into the following three categories according to the related criteria in The Classification of Cardiac Arrhythmias: 1. Sinus arrhythmia: sinus rhythm tachycardia, sinus bradycardia, sinus irregularity, sinus arrest and sick sinus syndrome. 2. Ectopic beats and ectopic rhythm: premature beat (ventricular, atrial and nodal zone), supraventricular tachycardia, ventricular tachycardia, atrial fibrillation, atrial flutter and ventricular fibrillation. 3. Conduction disturbance: preexcitation syndrome, sinoatrial block, atrioventricular block, and bundle branch block.

Statistical Analysis

Data was analyzed by SPSS20.0 software (SPSS Inc., Chicago, IL, USA) and expressed as mean ± standard deviation. Allele frequency = (the number of homozygous cases ×2 + the number of heterozygous cases) ×2-1×n-1. Comparison between groups was done using independent Student's *t*-test. Percentage (%) was used to express the enumeration data and chi-square test was used for data analysis. The non-parametric total rank of independent samples of grade data was used to test. p-values < 0.05 were considered statistically significant.

Table II. Comparison of distribution of GNAS2 genotype and allele frequency between the control group and the observation group.

Group	N	Genotype frequency (%)			X^2	p
		GG	TT	GT		
Control group	92	46 (50.0)	34 (37.0)	12 (13.0)	16.31	0.000
Observation group	89	13 (14.6)	38 (42.7)	38 (42.7)		
Group	**N**	**Allele frequency (%)**			X^2	p
		T	G			
Control group	184	100 (54.3)	84 (45.7)		16.08	0.000
Observation group	178	121 (68.0)	57 (32.0)			

Results

PCR-restriction Fragment Length Polymorphism Electrophoresis and Sequencing

In this report, the PCR products underwent cataphoresis after treatment with the ForK1 restriction enzyme (the recognition site was AGCT). GNAS2-G/G genotypes could be cut by the Pst1 restriction enzyme, therefore after electrophoresis, two bands were produced: 125 bp and 220 bp; GNAS2-T/T genotype could not be cut by the Pst1 restriction enzyme; therefore, following electrophoresis, there was a single band of 345 bp; GNAS2-G/T genotype could be cut by the Pst1 restriction enzyme and generated 3 bands: 345 bp, 125 bp and 220 bp (Figure 1). The PCR results were consistent with the sequencing data.

GNAS2 Genotype and Allele Frequency of the Observation and Control Groups

We carried out statistical tests on the G allele, T frequency, GG genotype, and TT frequency between the observation group and the control group. Our findings show that there were significant differences between the control group and the observation group (X^2=16.31, p<0.05; X^2=16.08, p<0.05). The verification results of the X^2-test, the genotype frequency and allele frequency between the control group and the observation group show that it was in accordance with Hardy-Weinberg equilibrium (Table II).

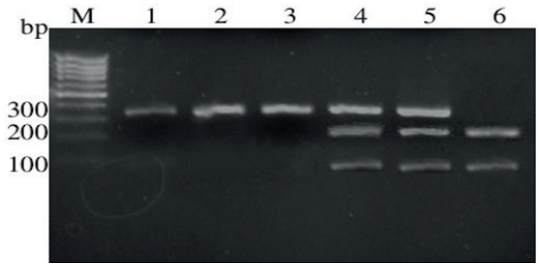

Figure 1. Electrophoresis of PCR-RFLP of GNAS2 gene. 1-3: TT type, 4-5: G/T type, 6: G/G type.

GNAS2 Genotype and Allele Frequency of Patients in the Observation Group

We categorized patients in the observation group (with arrhythmias) by standard practice, into three groups: sinus arrhythmia, ectopic beats and ectopic rhythm, and patients with abnormal conduction. We carried out statistical tests on the genotypes (GG, TT) and allele frequency (G, T) in the above three groups. The results showed that there were significant differences (X^2=16.82, p<0.05; X^2=16.36, p<0.05) in genotype and allele frequencies among patients with different degrees of illness in the observation group. The verification results of the X^2 test, the genotype frequency and allele frequency between the control group and the observation group showed that it was in accordance with Hardy-Weinberg equilibrium (Table III).

Discussion

Arrhythmias are a form of severe heart disease that can seriously affect the healthy development of children[12]. The work by Fecteau et al[13] shows that there are about 78 million children who suffer from different forms of fever-induced arrhythmias. Death caused by arrhythmia account for up to 32.7% of overall deaths in children per month[14]. Halperin et al[15] observed that G-protein coupled receptors can

Table III. Comparison of distribution of GNAS2 genotype and allele frequency of patients in the observation group.

Group	N	Genotype frequency (%)			X^2	*p*	Hardy-Weinberg equilibrium
		GG	TT	TG			
Sinus arrhythmia	32	14	12	6	16.82	$p<0.05$	0.142
Ectopic beats and ectopic rhythm	28	11	12	5			
Abnormal conduction	29	12	11	6			
Group	**N**	**Allele frequency (%)**					
		T	G		X^2	*p*	Hardy-Weinberg equilibrium
Sinus arrhythmia	64	46 (72.0)	18 (28.0)		16.36		
Ectopic beats and ectopic rhythm	56	38 (67.9)	18 (32.1)				
Abnormal conduction	58	41 (70.6)	17 (29.4)				

provide important auxiliary effects on both transport and conduction of many signaling molecules in the body. Jons et al[16] showed that G-proteins can be divided into four categories: GS, GI, Gq and G12. Further studies indicate that the proteins in these categories are composed of 3 subunits (α, β and γ), and their differences in function are primarily determined by the α-subunit[17]. Sileikiene et al[18] concluded that adrenaline released by sympathetic nerves mainly acts on the β-adrenergic receptor (β-AR) expressed on cardiac myocyte plasma membranes. Activated β-AR can, then, activate AC after coupling with the α-subunit on GS protein, to increase the contents of cAMP inside cells. A rise in cAMP, which is a second messenger, can activate the AC-cAMP-PKA signaling pathway, which causes internal flow of slow Ca^{2+}. It can also promote the release of Ca^{2+} from the cytoplasm of cells in the network. Ca^{2+} plays an important role in maintaining normal activity of cardiac muscle cells and cardiac pacing[19].

Jeffrey et al[20] reported that, at present, the genes encoding sodium voltage-gated channel alpha subunit 5 (SCN5A), potassium voltage-gated channel subfamily E regulatory subunit 1 (KCNE1), and sarcoplasmic reticulum calcium channels (RyR2) are all related to heart rate imbalance[21]. However, the interrelation between GNAS2 gene polymorphisms and arrhythmia in children remains poorly understood.

Conclusions

In the present study, we examined polymorphisms of the GNAS2 gene between patients in the observation and control groups, but there were no significant differences ($p>0.05$) in the GNAS2 allelic distribution between the observation group (children patients of arrhythmia) and control group.

However, there were significant differences in GNAS2 gene frequency (i.e. TT, GG and alleles between the control group and patients with arrhythmia ($p<0.05$) ($X^2=16.57$, $p<0.05$; $X^2=17.48$, $p<0.05$). The further study of samples from the control group and observation group indicates that TT, GG and TG gene frequencies and allelic frequencies also had differences, indicating that homozygosity (TT, GG) is in relatively high proportion among pediatric patients with severe arrhythmia.

Conflict of interest

The authors declare no conflicts of interest.

References

1) Nakamura H. [Recent progress in molecular etiology of arrhythmia and anti-arrhythmia drug therapy]. Masui 2004; 53 Suppl: S81-S90.
2) Hardt SE, Geng YJ, Montagne O, Asai K, Hong C, Yang GP, Bishop SP, Kim SJ, Vatner DE, Seidman CE, Seidman JG, Homcy CJ, Vatner SF. Accelerated cardiomyopathy in mice with overexpression of cardiac G(s)alpha and a missense mutation in the alpha-myosin heavy chain. Circulation 2002; 105: 614-620.
3) Raybaud F, Camous JP, Tibi T, Baudouy M, Morand P. [Severe arrhythmia in the elderly: a prospective hospital study]. Arch Mal Coeur Vaiss 1995; 88: 27-33.
4) Alakus H, Monig SP, Warnecke-Eberz U, Alakus G, Winde G, Drebber U, Schmitz KJ, Schmid KW, Riemann K, Siffert W, Bollschweiler E, Holscher AH, Metzger R. Association of the GNAS1 T393C

polymorphism with tumor stage and survival in gastric cancer. World J Gastroenterol 2009; 15: 6061-6067.

5) Otterbach F, Callies R, Frey UH, Schmitz KJ, Wreczycki C, Kimmig R, Siffert W, Schmid KW. The T393C polymorphism in the gene GNAS1 of G protein is associated with survival of patients with invasive breast carcinoma. Breast Cancer Res Treat 2007; 105: 311-317.

6) Arora R, Ng J, Ulphani J, Mylonas I, Subacius H, Shade G, Gordon D, Morris A, He X, Lu Y, Belin R, Goldberger JJ, Kadish AH. Unique autonomic profile of the pulmonary veins and posterior left atrium. J Am Coll Cardiol 2007; 49: 1340-1348.

7) Yamawake N, Nishizaki M, Hayashi T, Niki S, Maeda S, Tanaka Y, Fujii H, Ashikaga T, Sakurada H, Hiraoka M. Autonomic and pharmacological responses of idiopathic ventricular tachycardia arising from the left ventricular outflow tract. J Cardiovasc Electrophysiol 2007; 18: 1161-1166.

8) Mao YM, Liu ZQ, Chen BL, Guo D, Han CT, Yang LJ, Wang SY, Fan L, Zhou HH. Effect of 393T>C polymorphism of GNAS1 gene on dobutamine response in Chinese healthy subjects. J Clin Pharmacol 2009; 49: 929-936.

9) Regard JB, Cherman N, Palmer D, Kuznetsov SA, Celi FS, Guettier JM, Chen M, Bhattacharyya N, Wess J, Coughlin SR, Weinstein LS, Collins MT, Robey PG, Yang Y. Wnt/beta-catenin signaling is differentially regulated by Galpha proteins and contributes to fibrous dysplasia. Proc Natl Acad Sci U S A 2011; 108: 20101-20106.

10) Sakaguchi T, Shimizu W, Itoh H, Noda T, Miyamoto Y, Nagaoka I, Oka Y, Ashihara T, Ito M, Tsuji K, Ohno S, Makiyama T, Kamakura S, Horie M. Age- and genotype-specific triggers for life-threatening arrhythmia in the genotyped long QT syndrome. J Cardiovasc Electrophysiol 2008; 19: 794-799.

11) Siddappa R, Mulder W, Steeghs I, van de Klundert C, Fernandes H, Liu J, Arends R, van Blitterswijk C, de Boer J. CAMP/PKA signaling inhibits osteogenic differentiation and bone formation in rodent models. Tissue Eng Part A 2009; 15: 2135-2143.

12) Aksoy H, Okutucu S, Sayin BY, Ercan EA, Kaya EB, Ozdemir O, Inanici F, Aytemir K, Oto A. Assessment of cardiac arrhythmias in patients with ankylosing spondylitis by signal-averaged P wave duration and P wave dispersion. Eur Rev Med Pharmacol Sci 2016; 20: 1123-1129.

13) Fecteau RE, Lutterbaugh J, Markowitz SD, Willis J, Guda K. GNAS mutations identify a set of right-sided, RAS mutant, villous colon cancers. PLoS One 2014; 9: e87966.

14) Dasmahapatra HK, Jamieson MP, Brewster GM, Doig B, Pollock JC. Permanent cardiac pacemaker in infants and children. Thorac Cardiovasc Surg 1986; 34: 230-235.

15) Estes NA 3rd, Halperin JL, Calkins H, Ezekowitz MD, Gitman P, Go AS, McNamara RL, Messer JV, Ritchie JL, Romeo SJ, Waldo AL, Wyse DG, Bonow RO, DeLong E, Goff DC Jr, Grady K, Green LA, Hiniker A, Linderbaum JA, Masoudi FA, Piña IL, Pressler S, Radford MJ, Rumsfeld JS; American College of Cardiology; American Heart Association Task Force on Performance Measures; Physician Consortium for Performance Improvement. ACC/AHA/Physician Consortium 2008 clinical performance measures for adults with nonvalvular atrial fibrillation or atrial flutter: a report of the American College of Cardiology/American Heart Association Task Force on performance measures and the physician consortium for performance improvement (Writing committee to develop clinical performance measures for atrial fibrillation) developed in collaboration with the Heart Rhythm Society. J Am Coll Cardiol 2008; 51: 865-884.

16) Jons C, Raatikainen P, Gang UJ, Huikuri HV, Joergensen RM, Johannesen A, Dixen U, Messier M, McNitt S, Thomsen PE. Autonomic dysfunction and new-onset atrial fibrillation in patients with left ventricular systolic dysfunction after acute myocardial infarction: a CARISMA substudy. J Cardiovasc Electrophysiol 2010: 21: 983-990.

17) Rosenbaum DM, Rasmussen SG, Kobilka BK. The structure and function of G-protein-coupled receptors. Nature 2009; 459: 356-363.

18) Sileikiene R, Baksiene D, Sileikis V, Kazakavicius T, Vaskelyte J, Kevalas R. Changes in electrophysiologic properties of the conductive system of the heart in children with atrioventricular nodal reentrant tachycardia after 2-8 years following radiofrequency catheter ablation of the slow pathway. Medicina (Kaunas) 2009; 45: 632-638.

19) Pass RH, Gates GG, Gellis LA, Nappo L, Ceresnak SR. Reducing patient radiation exposure during paediatric SVT ablations: Use of CARTO(R) 3 in concert with “ALARA” principles profoundly lowers total dose. Cardiol Young 2015; 25: 963-968.

20) Kim JJ, Macicek SL, Decker JA, Kertesz NJ, Friedman RA, Cannon BC. Magnetic versus manual catheter navigation for ablation of free wall accessory pathways in children. Circ Arrhythm Electrophysiol 2012; 5: 804-808.

21) Schwagten B, Witsenburg M, De Groot NM, Jordaens L, Szili-Torok T. Effect of magnetic navigation system on procedure times and radiation risk in children undergoing catheter ablation. Am J Cardiol 2010; 106: 69-72.