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The association between HERG gene expression and cardiac arrhythmia disease in children

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Abstract. We explored the possible link between the expression of *HERG* gene and cardiomyopathy in children. From April 2013 to April 2015, 73 children with cardiac arrhythmia who were treated were enrolled in the present study to serve as the observation group. At the same time, 76 normal individuals were also enrolled as the control group. HERG expression level in the observation group was compared with the control group. To determine the level of *HERG* gene expression we used fluorescent directional PCR, enzyme immunoassay and western blot analysis. The results showed that HERG mRNA level in the observation group was significantly higher than that of the control group. The level of HERG protein in the observation group was significantly higher as well. In the observation group, HERG expression gradually increased with time during the course of the disease. This result suggested that *HERG* gene expression was associated with the severity of cardiac arrhythmia in children. HERG expression may be the cause of deterioration in cardiomyopathy. The results have provided a theoretical and practical basis for the diagnosis and treatment of children cardiomyopathy. Thus, we established a correlation between HERG expression and cardiac arrhythmia in children.

Introduction

Any changes in physiological and biochemical status during childhood can lead to cardiac arrhythmia in children. These include heart-related myocardial cell excitability and automaticity and conductivity (1). Possible causes of cardiac arrhythmia in children are complex (2). This disease may be caused by congenital factors or be induced by certain acquired diseases, including myocarditis, rheumatic fever, toxins, drug side effects and cardiac surgery sequelae (3). Arrhythmia fatigue, nervousness and imperfect autonomic nervous function of children can induce myocarditis and congenital heart disease (4).

Poisoning caused by sympathomimetic amines and quinidine, acid-base balance disorders and electrolyte imbalance may lead to heart rate imbalances in children and may even deteriorate their condition (5). Due to the fact that heart rate imbalance in children is caused by complex factors, there is no specific therapeutic drug available (6). Results from a prior study revealed that the current treatment of heart rate imbalance is based on distinguishing heart rate imbalance symptoms using the frequency of premature beats and if the frequency of premature beats were reduced or disappeared after less activity, drug treatment would not be needed (7). Patients with long-standing symptoms of premature beat and diversity on electrocardiogram (ECG) can be treated by taking propafenone or propranolol β-blockers. However, there are studies showing that these drugs have no significant effects on heart rate imbalance symptoms of children (8).

The *HERG* gene product is known to be involved in cellular and molecular mechanisms controlling the metastasis and regulating the excitation of nerve and muscle cells (9). However, to the best of our knowledge, no study on the possible role of HERG in children's heart rate imbalance has been previously conducted. We explored the possible association between HERG expression and the heart rate imbalance of children and provide certain theoretical and experimental bases for rapid diagnosis and treatment of heart rate imbalances in children.

Materials and methods

General data. From April 2013 to April 2015, 73 cases of children with cardiac arrhythmia treated at the Xuzhou Children's Hospital (Jiangsu, China) were enrolled in this study to serve as the observation group. At the same time, 76 normal individuals were enrolled as the control group. The observation group comprised 38 males and 35 females with an average age of 6.2±3.8 years. The control group had 36 males and 40 females with an average age of 5.8±3.2 years. All participants were evaluated in accordance with the relevant standards and tests from ECG of children with cardiomyopathy and showed no signs of any related diseases. The study was approved by the Ethics Committe of Xuzhou Children's Hospital. Written informed consent was obtained from the parents of all the participants prior to the start of the study.

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Samples. We collected 5 ml venous blood from all the cases and centrifuged samples at 2,500 x g for 10 min at 4˚C to separate the serum, and the samples were stored at -80˚C. Freeze-stored solution was added to the remaining cells, and the cells were cryopreserved at -80˚C for follow-up experiments. HERG antibody used in the present study was purchased from Roche Diagnostics (Basel, Switzerland), RNA extraction kits were purchased from Axygen Biotechnology Co., Ltd. (Silicon Valley, CA, USA), the associated molecular reagents were purchased from Takara Biotechnology, Co., Ltd. (Dalian, China) and the fluorescence quantitative polymerase chain reaction (PCR) primers were designed by Primer 5 software and synthesized by Shanghai Sangon Biological Engineering Co. Ltd. (Shanghai, China).

RT-PCR

RNA extraction. For RNA extraction, we followed the standard protocol of Axygen kit (10), and the specific schemes were as follows: i) 0.6 ml of RNA Plus was added to 0.2 g of frozen cell sample (-80˚C) and the mix was transferred to a precooled mortar and pestle. The sample was then moved into a 1.5 ml sterilized EP tube. The mortar was washed with 0.3 ml of RNA Plus and the liquid was added to the EP tube; ii) chloroform (200 μl) was added and the tube was agitated for 15 sec and left at room temperature for 15 min; iii) the sample was centrifuged at 10,000 x g for 15 min at 4˚C; iv) supernatant was transferred into EP tube with RNase and then isovolumetric isopropanol was added. After mixing, tube was incubated at room temperature for 10 min; v) samples were centrifuged again (10,000 x g at 4˚C for 10 min); vi) the supernatant was discarded and 750 μl of 75% ethanol was added to the pellet and mixed gently. It was then centrifuged at 10,000 x g for 10 min at 4˚C; vii) supernatant was discarded and the residual ethanol was removed; and viii) appropriate amount of water was added to remove the RNase. The quality of the extracted RNA was evaluated according to a previous report (11).

Fluorescence quota PCR. Fluorescence quota PCR kit was purchased from Takara Biotechnology, Co., Ltd. Three-step method was used according to the instructions provided by the manufacturer's manual. Primer sequences are shown in Table I.

Enzyme-linked immunosorbent assay (ELISA). ELISA was used to verify the level of HERG protein in the serum. We followed a standard ELISA protocol (12):

i) Coating: 100 μl of diluted HERG primary antibody (5-10 μg/ml) was added into a 96-well plate and incubated at 4˚C overnight and solution was discarded the following morning. Wells were then washed with washing buffer solution provided in the kit (5 washes and 1 min/wash).

ii) Samples: 100 μl of sample was added to each well and plate was incubated at 37˚C for 1 h. The plate was then washed (5 times, 1 min/wash).

iii) Secondary antibody: 100 μl of second antibody was added and incubated at 37˚C for 30-80 min. The plate was then washed with washing buffer solution (5 times, 2 min/wash).

iv) Chromogenic substrate: 100 μl of the freshly-prepared chromogenic substrate solution was added followed by incubation at 37˚C for 30 min.

Table I. PCR primer sequences.

Gene	Primer sequence	Fragment length (bp)
HERG	F: 5'-TGACGTAGTCAAGTACGGTAG-3'	128
	R: 5'-TGCGTATTGCCGTAGCTGCGGC-3'	
GAPDH	F: 5'-GAAGGTGAAGGTCGGAGTC-3'	226
	R: 5'-GAAGATGGTGATGGGATTTC-3'	

PCR, polymerase chain reaction; F, forward; R, reverse.

v) Stop buffer: 20 μl of 0.2 M sulfuric acid was added to stop the reaction.

vi) Qualitative and quantitative tests: For qualitative observation, a plate was placed on a blank paper and color depth was compared. For the quantitative test, we used a microplate reader (Bio-Rad, Hercules, CA, USA) which was set at 450 nm.

Western blot analysis. We used the standard protocol for western blot analysis (all the antibodies were purchased from Roche Diagnostics).

Immunohistochemical detection of AVR1 in myocardial tissue. In the present study, we conducted the conventional antibody incubation and staining in cardiomyopathy tissue samples by streptomycin and enzymes (S-P). The immunohistochemical standard protocol was as follows (9): Membrane staining <10% or cardiomyocytes after staining presenting negative was considered as negative. Membrane staining or the membrane staining of >10% of myocardial cells was considered positive (+). When >10% of myocardial cells showed weak or moderate staining we considered it as (++). When >10% of myocardial cells were showing strong complete membrane staining, we considered it as (+++).

Data processing. SPSS 20.2 statistical software (Chicago, IL, USA) was used for the statistical analysis. Measureable data were presented as mean ± SD and categorical data were analyzed using the Chi-square test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

HERG transcription level. Results obtained from the fluorescence quantitative PCR showed that the level of HERG mRNA was considerably higher in the observation group compared to the control group (Fig. 1). The difference was statistically significant ($P<0.05$). These results showed that *HERG* gene was actively transcribed in children with cardiomyopathy.

HERG protein expression measured by ELISA. Results obtained from ELISA showed that the level of HERG protein expression was markedly stronger in the observation group compared to the control group (Fig. 2). The difference was statistically significant ($P<0.05$).

These results were in accordance with fluorescence quantitative PCR results showing higher levels of HERG expression in children suffering from cardiomyopathy.

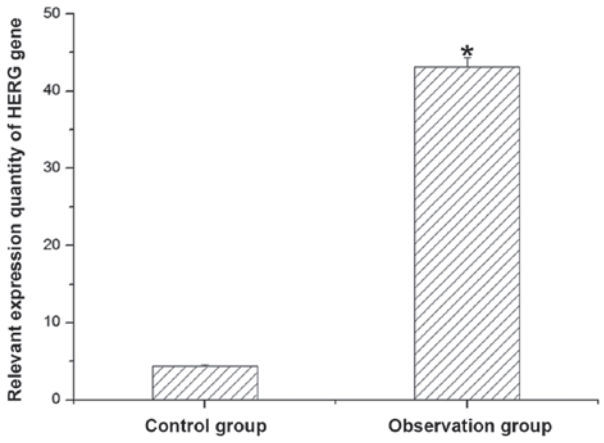

Figure 1. HERG mRNA level. *P<0.05.

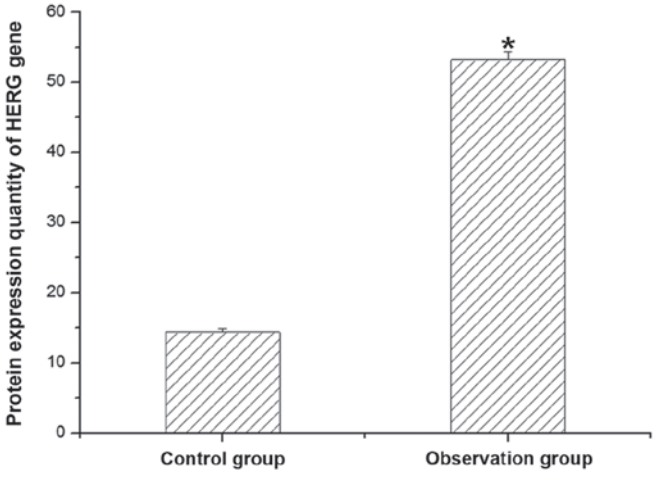

Figure 2. HERG protein expression measured by enzyme-linked immunosorbent assay (ELISA). *P<0.05.

HERG protein expression measured by western blot analysis. To verify and confirm the results obtained from ELISA, a western blot analysis was conducted on samples. The results obtained from the western blot analysis showed that the level of HERG protein expression was markedly stronger in the observation group compared to the control group (Fig. 3). The difference was statistically significant (P<0.05). This was in accordance with the ELISA results and confirmed that

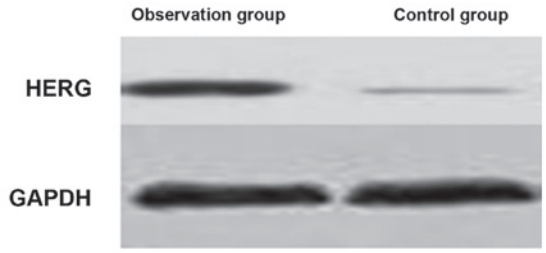

Figure 3. HERG protein expression levels measured by western blot analysis.

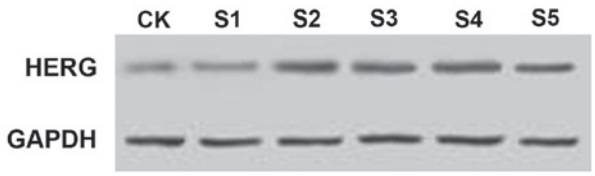

Figure 4. HERG protein level in the serum at different times in the course of the disease in the observation group. CK, the control group; S1-S5, the observation group in 3, 16, 28, 36, 45 and 52 months.

relatively higher amounts of HERG protein were present in in children suffering from cardiomyopathy.

To examine the association between the expression level of HERG and the condition of children with cardiomyopathy, we measured HERG expression in the serum of the ill children in the observation group at different times of the disease (Fig. 4). The results showed that in the observation group, HERG expression gradually increased during the course of the disease. After a certain time in the course of the disease the HERG expression decreased indicating that HERG can suppress the deterioration of children's codition to some extent.

HERG in myocardial tissue tested by immunohistochemistry. The immunohistochemistry results showed that the positive staining was mainly concentrated in myocardial cell membrane in sick children from the observation group.

Cardiac arrhythmia of children (Fig. 5). This suggested that the HERG level in children with cardiomyopathy was higher than the normal control group.

Discussion

As a common pediatric clinical disease with very complex causes, cardiac arrhythmia of children can be caused by

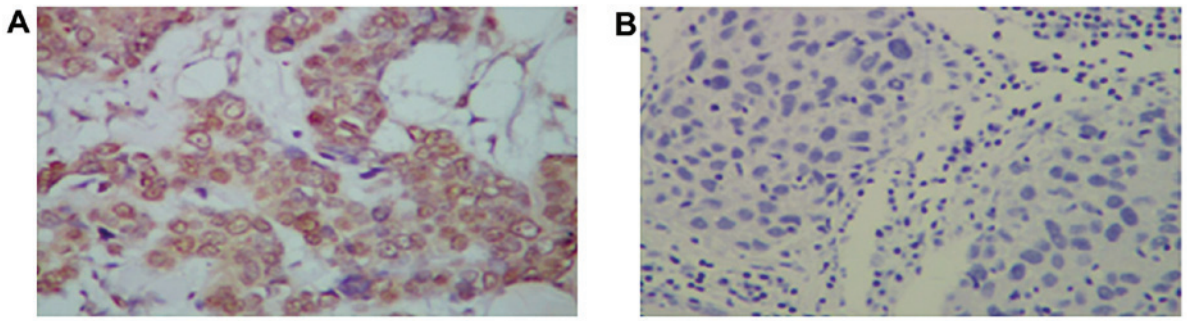

Figure 5. HERG protein in myocardial tissue tested by immunohistochemistry. (A) The observation group; (B) the control group.

pediatric heart disease and non-cardiac diseases, such as hypoxia, and electrolyte imbalance to some extent. Therefore, the proper diagnosis and treatment for arrhythmia of children have become an important issue. Brembilla-Perrot *et al* (13) showed that the current diagnosis for arrhythmia in children is mainly achieved through ECG. There are some complications associated with ECG diagnostics, therefore the diagnosis of cardiac arrhythmias in children of different regions in China is not easy (14). There is still no specific medication for treating arrhythmia disease of children. According to Bian *et al* (15), HERG protein expression is linked to regulation of neural cells in human body (16) as well as the expression of potassium channel membrane proteins.

Results from a study by Phartiyal *et al* (17) showed higher levels of HERG in human gastric cancer tissues. Significantly lower levels of HERG have been reported in necrotic muscle cells (18,19). This result indicates that HERG may be involved in the improvement of muscle cell excitability (20).

In the present study, from the measured HERG expression it was demonstrated that HERG expression in children suffering from cardiac arrhythmia increased significantly.

In the course of the disease, HERG expression increased gradually, suggesting that there was a correlation between HERG expression and arrhythmia disease in children. It also suggested that the expression of HERG may cause the arrhythmia or help its deterioration. Our results provided a theoretical and experimental basis for cardiac arrhythmia disease research in children.

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