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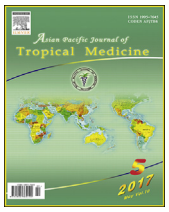

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Relationship between catecholamine level and gene polymorphism of β1 adrenergic receptor G1165C in children with EV71 infection in hand foot and mouth disease

Zhi-Xian Lei[1,#], Bang-Tao Li[1,#], Hong-Ai Li[2,3], Xiao-Ming Li[2], Man-Fang Xie[2], Ting Huang[4], Qi Wang[2], Ya-Zhou Wang[1], Qiu-Yu Lin[1], Li-Rong Zhou[1], Nai-Chao Feng[2], Xin Li[1], Dao-Mou Zhu[2], Yuan-Ping Hai[2], Lan Cui[2,5], Ya-Qin Zhang[2], Zhi-Wen Liu[2], Shou-Ye Wu[2], Yong-Zhao Chen[2], Ke-Qing Zhu[6], Xiao-Jie He[7✉], Wei Xiang[1✉]

[1]*Pediatric Intensive Care Unit, Maternal and Child Health Care Hospital of Hainan Province (Children's Hospital of Hainan Province), Haikou 570206, China*

[2]*Pediatrics, The Hainan Affiliated Hospital of University of South China, Haikou 570206, China*

[3]*Pediatric Nephrology, The Second Xiangya Hospital of Central South University, Changsha 410000, China*

[4]*Pediatrics, Maternal and Child Health Care Hospital of Hainan Province (Children's Hospital of Hainan Province), Haikou 570206, China*

[5]*Neonatal, Maternal and Child Health Care Hospital of Hainan Province (Children's Hospital of Hainan Province), Haikou 570206, China*

[6]*Labway Clinical Laboratory Shanghai Ltd, No. 74, Qing Chi Road, Changning District, Shanghai 200335, China*

[7]*Pediatrics, The Affiliated Hospital of Hainan Medical University, Haikou 570206, China*

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ABSTRACT

Objective: To investigate the relationship between the levels of plasma adrenaline and norepinephrine and gene polymorphism of β1 adrenergic receptor G1165C in children with enterovirus 71 (EV71) infection in hand foot and mouth disease (HFMD).

Methods: The polymerase chain reaction (PCR) was used to detect the expression of gene polymorphism of β1 adrenergic receptor G1165C *in vitro*. The levels of plasma adrenaline and norepinephrine were measured by enzyme-linked immunosorbent assay (ELISA).

Results: The plasma norepinephrine level of severe group was significantly higher than the mild group in children with EV71 infection in HFMD ($P < 0.05$); however, the levels of plasma adrenaline in two groups had no statistical differences ($P > 0.05$); There was no significant difference in the distribution of β1 adrenergic receptor G1165C genotype and allele between EV71 infection group and healthy control group ($P > 0.05$). Further analysis of EV71 infection group by dividing it into mild and severe groups showed that there was no significant difference in the distribution of genotype and allele between these two groups as well ($P > 0.05$). There was no significant difference in the levels of epinephrine and norepinephrine in different genotypes of EV71 infection group ($P > 0.05$), and in the levels of plasma epinephrine and norepinephrine in the mild and severe groups ($P > 0.05$).

Conclusions: As the disease gets worse, the plasma norepinephrine level has a rising trend in children with EV71 infection in HFMD, which is an important indicator to evaluate the progress of the disease. However, the gene polymorphism of β1 adrenergic receptor G1165C have no significant correlation, not only with the susceptibility and severity of EV71 infection in hand, foot and mouth disease, but also with the levels of catecholamine.

First author: Zhi-Xian Lei, Pediatric Intensive Care Unit, Maternal and Child Health Care Hospital of Hainan Province (Children's Hospital of Hainan Province), Haikou 570206, China.
Tel: +86 18689856355
E-mail: leix698@163.com
✉Corresponding authors: Wei Xiang, Pediatric Intensive Care Unit, Maternal and Child Health Care Hospital of Hainan Province (Children's Hospital of Hainan Province), Haikou 570206, Hainan, China.
Tel: +86 13907533259
Xiao-Jie He, Pediatric Nephrology, The Second Xiangya Hospital of Central South University, Changsha 410000, China.
Tel: +86 13786179396
E-mails: hexj7150@163.com, 13907533529@163.com
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[#] These authors contributed equally to this work.

1. Introduction

The mechanism of neurogenic pulmonary edema in children with severe hand, foot and mouth disease infected by enterovirus 71 (EV71) is very complex, in which catecholamines neurotransmitters (including adrenaline and norepinephrine) and other systemic hemodynamic changes involve in promoting and aggravating acute pulmonary edema [1–3]. The β1-adrenergic receptor (β1-AR) is an important tissue receptor that mediates catecholamine, among which the most significant β1-AR gene locus may be G1165C, replacement of guanine (G) with cytosine (C) results in a clinically significant amino acid substitution (Gly → Arg) [4]. In this paper, we studied the association of G1165C polymorphism with EV71 infection with hand, foot and mouth disease (HFMD) and plasma adrenaline and norepinephrine, and reported below.

2. Materials and methods

2.1. Study object

A total of 81 patients [57 males and 24 females, aged (2.2 ± 1.1) years old] with HFMD infected by EV71 in Maternal and Child Health Hospital of Hainan Province (Hainan Provincial Children's Hospital) from October 2015 to September 2016 were chosen with exclusion of those with primary heart, lung, kidney disease, hypertension and pheochromocytoma. Including 22 cases of mild HFMD and 59 cases of severe HFMD, the clinical diagnostic criteria were in line with the 'Hand Foot and Mouth Disease Diagnosis and Treatment Guide (2010 edition)', and the inclusion criteria was positive either in throat swab virus nucleic acid or serum EV71 IgM antibody of lab testing. Control group: 66 healthy subjects, including 31 boys and 35 girls and aged (2.9 ± 1.1) years old, were selected from the children's health clinics in the same period. The viral nucleic acid PCR and serum EV71 IgM were negative without hand, foot and mouth disease, central nervous system injury, pheochromocytoma, hypertension and heart, kidney disease and other medical history.

2.2. Method

2.2.1. PCR in vitro amplification and Sanger sequencing used to detect G1165C polymorphism

Blood samples were collected with K_2EDTA 5 mL anticoagulant tube and stored at ambient temperature of −70 °C. For DNA extraction, the genomic DNA of the collected samples was extracted with the OMEGA blood sample DNA extraction kit (D3471-02).

Based on the coding region of ADRB1 (NCBI Reference Sequence: NG_012187.1) published by NCBI, the amplification primers were designed: G1165C loci polymorphism: sense sequence 5′-CAGAAACATGCTGAAGTCCCG-3′, antisense sequence 5′-GATGGGCAGGAAGGACACC-3′. The primers above were synthesized by Shanghai LanWei Inspection Company.

The PCR total reaction system was 25 μL, of which 10 × LAmp Buffer: 2.5 μL; dNTP: 2 μL; ADRB1-F1/F2/F3/F4: 1 μL; ADRB1-R1/R2/R3/R4: 1 μL; LAmp Taq: 0.25 μL; 5 ×C Solution I: 5 μL; Template: 1 μL; ddH_2O: 12.25 μL.

The reaction conditions were pre-denaturation at 94 °C for 4 min; denaturation 30 s at 94 °C; annealing 4 s at 55 °C; extension at 94 °C for 4 min, a total of 35 cycles; terminal extension for 5 min. The PCR products were subjected to agarose gel electrophoresis and identified by gel electrophoresis to identify the genotype.

2.2.2. Determination of catecholamine in plasma by ELISA

Children with EV71 infection in HFMD in the acute phase (admission day) were collected 3 mL venous blood, set in the EDTA anticoagulant tube, and stored in the −20 °C freezer, and then adrenaline and norepinephrine level were measured by the use of ELISA. Human catecholamines (CA) enzyme immunoassay kit was provided by Wuhan Xinyi Di Biotechnology Limited Company.

2.3. Statistical analysis

In this study, SPSS 18.0 statistical software was used to establish and analyze the database, and the main test indicators were tested for normality. For the normal distribution, the data were expressed as mean ± standard deviation. For the data of the skewness distribution, median (quartile spacing) was used for description, and independent sample *t* test was used for comparison. Count data was expressed in frequency or percentage and χ^2 test was used for comparison. $P < 0.05$ was considered as a significant difference.

3. Result

3.1. Association of G1165C locus polymorphism with EV71 infection HFMD

As shown in Table 1, there was a G1165C polymorphism in 81 cases of EV71 infection in HFMD and 66 healthy children;

Table 1
Distribution of SNP at locus G1165C in infection group by EV71 and control group.

Group	*n*	Genotype frequency (%)		Allele frequency (%)		
		GG	GC	CC	G	C
Control	66	5 (7)	27 (41)	34 (52)	37 (28)	95 (72)
EV71 infection	81	9 (11)	34 (42)	38 (47)	52 (32)	110 (68)
Mild	22	4 (18)	12 (55)	6 (27)	20 (45)	24 (55)
Severe	59	5 (9)	25 (42)	29 (49)	35 (30)	83 (70)

Table 2
Comparison of the levels of epinephrine and norepinephrine in mild and severe group (pg/mL).

Group	*n*	Epinephrine	Norepinephrine
Mild	22	71.46 (45.86–86.33)	148.40 (119.57–172.90)
Severe	59	87.24 (44.63–207.26)	166.60 (146.38–267.32)
u		−1.402	−2.378
P value		0.080	0.009

Table 3
Levels of epinephrine and norepinephrine in plasma between different genetypes (pg/mL).

Genetype	*n*	Epinephrine	Norepinephrine
GG	9	71.35 (33.29–290.21)	244.16 (149.25–407.94)
GC	34	87.16 (48.75–174.33)	164.59 (147.01–239.92)
CC	38	67.92 (43.12–118.50)	154.74 (125.11–211.98)
H		1.686	3.407
P value		0.430	0.182

Table 4
Levels of epinephrine and norepinephrine in plasma between different genetypes of mild group (n = 22) and severe group (n = 59) (pg/mL).

Genetype	Mild		Severe	
	Epinephrine	Norepinephrine	Epinephrine	Norepinephrine
GG	33.29 (29.98–62.06)	164.18 (113.11–233.49)	270.63 (148.57–328.00)	280.72 (196.54–651.11)
GC	83.03 (64.68–115.83)	152.08 (115.30–166.84)	92.58 (44.09–192.71)	190.38 (154.28–280.68)
CC	67.08 (42.24–89.16)	129.64 (118.40–174.45)	68.56 (41.60–141.14)	158.21 (130.77–248.95)
H	5.382	0.293	4.582	5.553
P value	0.068	0.864	0.101	0.062

the population distribution was in line with Hardy–Weinberg equilibrium in this study ($P > 0.05$). In EV71 infection in HFMD, the genotype frequencies of G1165C loci were GG11%, GC42%, CC47%, respectively, G allele frequency and C allele frequency were 32%, 68%, respectively; In healthy children, the frequencies of GG, GC and CC genotypes were 7%, 41%, 52%, respectively, G allele frequency and C allele frequency were 28% and 72%, respectively. Comparing EV71 infection group with the healthy control group, there was no significant difference in the distribution of genotype frequency by using χ^2 test analysis ($\chi^2 = 0.644$, $P > 0.05$); there was no significant difference in the distribution of allele frequency in these groups by using χ^2 test analysis ($\chi^2 = 0.570$, $P > 0.05$). Then it was further found that there was no significant difference in genotype frequencies between EV71 infection mild group and severe group ($\chi^2 = 3.654$, $P > 0.05$); there was no significant difference in allele frequency in two groups by using χ^2 test analysis ($\chi^2 = 3.565$, $P > 0.05$).

3.2. Relationship between polymorphism of G1165C locus and plasma catecholamine level

3.2.1. Detection of plasma catecholamines in children with EV71 infection in HFMD

A total of 81 cases of children with HFMD were selected and divided into mild group (n = 22) and severe group (n = 59) according to the clinical manifestations. Since the samples were skewed, plasma adrenaline and norepinephrine levels were expressed by median and quartile. As shown in Table 2, the plasma adrenaline in the mild group was 71.46 (45.86–86.33) pg/mL and in the severe group was 87.24 (44.63–207.26) pg/mL, as the condition worsened, plasma adrenaline levels increased, but Mann–Whitney U test indicated that the two groups of plasma adrenaline levels were no significant difference ($u = -1.402$, $P > 0.05$). The plasma norepinephrine level of mild group was 148.40 (119.57–172.90) pg/mL, and that of severe group was 166.60 (146.38–267.32) pg/mL, plasma norepinephrine levels increased gradually with the aggravation of the disease, the Mann–Whitney U test showed that there was a significant difference between the two groups of plasma norepinephrine levels ($u = -2.378$, $P = 0.009$).

3.2.2. Levels of epinephrine and norepinephrine in different genotypes of EV71 infection group

As shown in Table 3, according to the results of genotype detection, children with EV71 infection in HFMD was divided into 9 cases of GG type, 34 cases of type GC, 38 cases of type CC. The plasma epinephrine levels of three groups of were 71.35 (33.29–290.21) pg/mL, (48.75–174.33) pg/mL, 67.92 (43.12–118.50) pg/mL, respectively, there was no statistically significant difference between the groups ($H = 1.686$, $P > 0.05$). The plasma norepinephrine levels of three groups were 244.16 (149.25–407.94) pg/mL, (147.01–239.92) pg/mL, 154.74 (125.11–211.98) pg/mL, respectively, there was no significant difference between the groups ($H = 3.407$, $P > 0.05$).

3.2.3. Genotypes and plasma levels of epinephrine and norepinephrine in children with EV71 infection in HFMD

It could be seen from Table 4 that there were no significant differences in the levels of epinephrine and norepinephrine in different genotypes of the mild and severe groups ($P > 0.05$).

4. Discussion

At present, the research of the relationship between G1165C locus polymorphism and disease of β1 adrenergic receptor gene is mainly focused on cardiovascular disease [5–8]. The research of gene polymorphism of hand-foot-mouth disease associated with EV71 is mainly CXCL-10, TLR7, MBL2, TNF IL-10, IFN-γ, TNF-α, IL-17F, IL-10, IL-10, IFN-18, OAS2-751 + C/A, OAS, IL-2, IL-10-1082, SELS, *etc*. [9–12], but the relationship between the G1165C polymorphism of the β1 adrenergic receptor gene and the EV71 infection was rarely. There were no significant differences in frequency and allele frequency of G1165C genotype in EV71 infected patients with hand, foot and mouth disease and healthy control group. There was no significant difference in EV71 infection group and severe group. Possible causes are: (1) There are other susceptibility genes (such as those described above) that may be associated with EV71 infection in hand, foot and mouth disease, or other SNPs on the β-AR gene interact with 1165 SNPs in the coding region of β1-AR gene Or chain imbalance. (2) EV71 hand, foot and mouth disease susceptibility and disease progression, may be not only infected by genetic factors, but also by virulence [13], host reaction [14], environmental and other aspects while of a single gene mutation plays a smaller role.

Catecholamines play an important role in hemodynamic changes in severe hand, foot and mouth disease [15,16]. The epinephrine is mainly secreted by adrenaline medulla, and the norepinephrine is mainly secreted by the sympathetic nerve. They can damage the blood vessels, strengthen the myocardial contraction, and then cause the blood pressure increase and the heart rate accelerating. Compared with other enteroviruses, the infection of hand, foot and mouth disease caused by the EV71 is more prone to central nervous system injury owing to the EV71 is more neurotoxicity. When involving the hypothalamus, brain stem and spinal cord of the neck part,

increased intracranial pressure would cause sympathetic nervous system over-excitement, resulting in a large number of catecholamines. It is then commonly seen for adrenaline, norepinephrine increase and strong contraction of peripheral blood vessels and sharply blood pressure increase caused by direct impact of blood flow vascular endothelial cells damage, then resulting in pulmonary edema, pulmonary hemorrhage, *etc*. [15–17]. The research found that the level of adrenaline and norepinephrine in plasma was significantly increased with the EV71 infection hand, foot and mouth disease getting worse, but after test, only norepinephrine has a statistical difference. Combined with domestic and foreign literature, there was a significantly increase in the level of adrenaline and/or norepinephrine in plasma of the children in severe hand, foot and mouth disease, and it has a certain reference value to assess the progress of the disease [18,19]. Many scholars found that EV71 infection in severe hand, foot and mouth disease in children with the level of adrenaline and norepinephrine significantly increase in the plasma [20,21], may be accompanied by a significant increase in blood pressure and heart rate. Some scholars have found that the level of catecholamine is mainly increased by the norepinephrine in the children with severe hand, foot and mouth disease, and it is more significant in the more severe type, while adrenaline, though elevated, it was not statistically significant after the test [22,23]. When the blood catecholamine levels were significantly increased, 'two high' and 'two fast' appear clinically. 'two high' is high blood pressure and high blood sugar while 'two fast' is fast heart rate, breathing fast. This is also a sign of the progress of hand, foot and mouth disease as severe.

However, there was no significant difference between plasma adrenaline and norepinephrine at different G1165C loci groups, and there was no difference between different genotype groups in EV71 infection with hand, foot and mouth disease and severe group. There may be other genetic polymorphisms that affect catecholamine metabolizing enzymes, such as catechol-O-methyltransferase (COMT), which can cause G and A replacement mutations that can lead to a decrease in activity of the enzyme 3–4 times, COMT enzyme activity phenotype changes affect the metabolism of children with catecholamines [24]. It was found by Mr. Cao that the catecholamines (such as norepinephrine) was associated with COMT gene polymorphism, and the level of plasma norepinephrine in wild type group was significantly lower than that in genotype group with mutation site [25]. Therefore, high levels of plasma norepinephrine can further accelerate the process of EV71 infection in hand, foot and mouth disease and it also is an early warning index of severe hand, foot and mouth disease, but there was no significant correlation with the β1 adrenergic receptor gene G1165C polymorphism.

Conflict of interest statement

We declare that we have no conflict of interest.

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