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Elevated Th17 and IL-23 in Hypertensive Patients with Acutly Increased Blood Pressure

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Abstract: Problem statement: Many severe cardiovascular complications such as stroke and heart attack can result from acutely increased blood pressure in patients with hypertension. The underlying mechanisms remain unclear despite of extensive study. To characterize the inflammatory mechanisms in hypertensive patients with acute blood pressure increase, we tested circulating Th17 levels, IL-23R expression and plasma levels of IL-17 and IL-23 in hypertensive patients with acute blood pressure increase. **Approach:** 50 patients (24 males and 26 females) and 20 healthy volunteers between the ages of 18 and 78 were enrolled in this study. 30 of them were hypertensive patients with acute blood pressure increase (group A). The other 20 patients were hypertensive patients with steady blood pressure (group B). 20 healthy volunteers that were sex- and age-matched to group B were recruited as normal controls (group C). IL-23R expression and Th17 ratio in $CD4^+$ T cells were examined by Flow Cytometry (FCM). The levels of IL-17 and IL-23 in plasma were measured using ELISA. **Results:** Increased Th17 and IL-$23R^+CD4^+$ T cells were detected in the patients of group A (1.4±0.6 and 8.6±3.3 respectively, $p<0.05$). No difference between group B and C was observed ($p>0.05$). The levels of IL-17 and IL-23 in group A were significantly higher than group B and C (11.9±5.1 v.s. 7.8±3.8 v.s. 6.0±1.1 for IL-17, 3017±950 v.s. 2143±927 v.s. 1916±935 for IL-23, $P<0.05$). No significant difference between group B and C was detected. Furthermore, there were a positive correlation between Th17/$CD4^+$T ratio and IL-17 level ($r = 0.514$, $p<0.05$) and a positive correlation between IL-17 and IL-23 level ($r = 0.837$, $p<0.05$) in all subjects. **Conclusion:** Our results suggested that IL-23 and Th17 cells may be involved in the pathogenesis of acute blood pressure increase in the patients with hypertension. Understanding its inflammatory characterization might be of fundamental importance for the prevention and treatment of acute blood pressure increase in hypertensive patients.

Key words: Th17, hypertension, IL-23R, IL-17, IL-23, pressure increase, significant difference, hypertensive patients, healthy volunteers

INTRODUCTION

As a chronic medical condition characterized by elevated blood pressure, hypertension is recognized as a major risk factor for a variety of life threatening diseases including stroke, myocardial infarction, heart failure and aortic aneurysm. And most of these severe complications occur in the hypertensive patients with a sudden increase of blood pressure. Despite of extensive study, the pathophysiology of the sudden/acute increase of blood pressure in hypertensive patients is not clearly characterized. The involvement of immune activation in hypertension has been well demonstrated by many research groups (Guzik *et al*., 2007; Schmid-Schonbein *et al*., 1991; Dorffel *et al*., 1999). Th17, a recently discovered subset of $CD4^+$ T cells, is believed to play a role in the pathogenesis of vascular dysfunction and hypertension (Madhur *et al*., 2009), In the current study, demonstrated an increased level of Th17 and IL-17 in hypertensive patients with acute increases of blood pressure, which is probably caused by the increased plasma IL-23 level and increased expression of IL-23R on $CD4^+$ T cells. The characterization of its pathophysiology might be beneficial for the prevention and therapy of the sudden/acute increase of blood pressure in hypertensive patients.

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MATERIALS AND METHODS

Subjects: 50 primary hypertensive patients including 24 males and 26 females, aged from 18-78 years, were recruited for this study. The patients were recruited from September 2010-February 2011. The patients were divided into two groups according to the symptoms of blood pressure: Group A, 30 patients with acute increase of blood pressure; Group B, 20 patients without acute increase of blood pressure. In addition to the 50 patients, we also included 20 healthy volunteers with normal blood pressure as control (group C).

Inclusion criteria for hypertensive patients with acute increase of blood pressure: (1) Onset of hypertension, SBP ≥ 160 mmHg and/or DBP ≥ 100mmHg, no previous history of hypertension found; (2) Blood pressure controlled in the normal course suddenly rise, SBP ≥ 160 mmHg and/or DBP ≥ 90mmHg; (3) Comply with the 2010 guidelines for hypertension prevention and treatment of hypertension in China definition and classification standards.

Exclusion criteria: (1) acute coronary syndrome; (2) acute and chronic heart failure; (3) autoimmune diseases; (4) acute severe infection; (5) bronchial asthma; (6) tumor; (7) diabetes; (8) hyperlipidemia; (9) acute cerebral vascular accident; (10) liver and kidney dysfunction; (11) use of steroids or immunosuppressive drugs.

Specimen collection and cytokine detection: 5 ml venous blood was drawn from every patient after over 8 h fasting. Blood was collected in heparin anticoagulation tube. The blood samples of group A were collected within 24 h of acute blood pressure increase. Peripheral blood mononuclear cells were isolated for flow cytometry test. The plasma was separated from peripheral blood by centrifuging at 2000 rpm for 20 min. The levels of IL-17 and IL-23 in plasma were detected by corresponding ELISA kit (eBioscience, USA) as instructed by the manufacturer.

Flow cytometry detection: Peripheral Blood Mononuclear Cells (PBMCs) were isolated using Ficoll density according manufacturer's instruction. PBMCs were then resuspended with RPMI-1640 medium containing 10% fetal calf serum seeded in 24-well plate and treated with Phorbol 12-myristate 13 acetate (50 ng mL^{-1}), Ionomycin (750 μg L^{-1})and Monensin (500 μg L^{-1}) for 4 h. Cells were then collected and stained with Anti-Human CD4 PE-Cy5 and Anti-Human IL-23 p40/p70 PE. Cells were then fixed and permeabilized using Foxp3 Staining Buffer Set and stained with anti-human IL-17A FITC. Cells were analyzed by BD FACSCanto™ Flow Cytometer.

Statistical: All of data were analyzed by software SPSS17.0. The results were expressed as Mean ± SD, Comparison between multiple experimental groups was accomplished by one-way ANOVA with a post hoc test of LSD. $p<0.05$ was considered statistically significant. Correlation analysis was accomplished by using Pearson test.

RESULTS

The basic information and clinical data of subjects: The basic information (age, gender, body mass index) and clinical data [blood pressure, cholesterol (TC), High Density Lipoprotein (HDL) and Blood Sugar (BS)] of the three groups of subjects are listed in Table 1. No significant differences were detected except for Systolic Blood Pressure (SBP) and Diastolic Blood Pressure (DBP). The SBP and DBP of group A were higher than group B and group C ($p<0.01$). There was no significant difference between group B and group C in blood pressure ($p>0.05$).

Th17 level and IL-23R expression in hypertensive patients with acute increase of blood pressure: As indicated in Table 2 and Fig. 1-3, the ratio of Th17/$CD4^+$ of group A was significantly higher than group B and group C ($p<0.05$); There was no significant difference between group B and group C ($p>0.05$); The ratio of CD_4^+IL-$23R^+/CD_4^+$ of group A were significantly higher than group B and group C ($p<0.05$). There was no significant difference between group B and group C ($p>0.05$); the ratio of CD_4^+IL-17^+IL-$23R^+/CD_4^+$ of group A were significantly higher than group B and group C ($p<0.05$). There was no significant difference between group B and group C ($p>0.05$).

The levels of IL-17 and IL-23 in plasma: The plasma levels of IL-17 and IL-23 of group A were significantly higher than group B and group C ($p<0.05$). There was no significant difference between group B and group C ($p>0.05$) (Table 3).

The correlation between IL-17 and IL-23: There were significantly positive correlations between the level of IL-17 and IL-23 in three groups (Fig. 4-6). There was significantly positive correlation between the level of IL-17 and IL-23 in all subjects ($r = 0.837$, $p<0.05$; Fig. 7).

The correlation between Th17/CD_4^+T and IL-17: There were significantly positive correlations between the ratios of Th17/$CD4^+$T and the levels of IL-17 in all subjects ($r = 0.514$, $p<0.05$; Fig. 8).

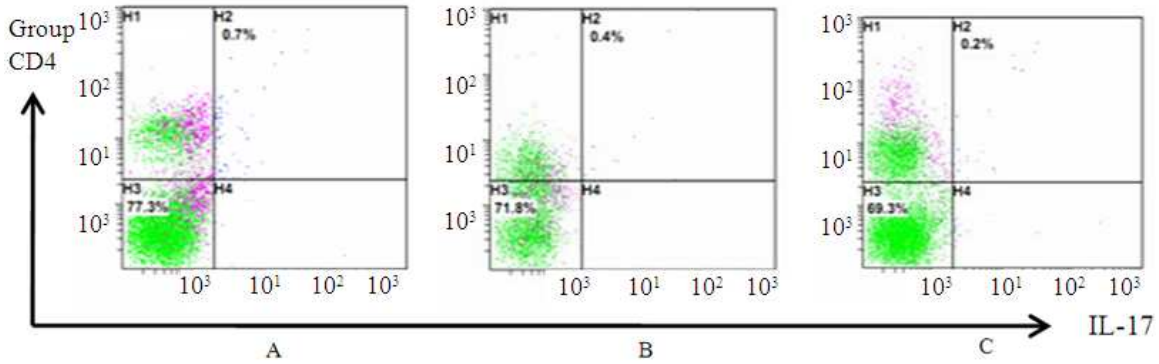

Fig. 1: Th17 level PBMCs isolated from patients and controls were stimulated with PMA, ionomycin, and Monensin for 4 h, followed by staining with anti-human CD4, anti-human IL-23R and anti-human IL-17 Ab. Cells were then analyzed by BD FACSCanto™ Flow Cytometer. The percentage of Th17 in $CD4^+$ T cells was shown in Fig. 1

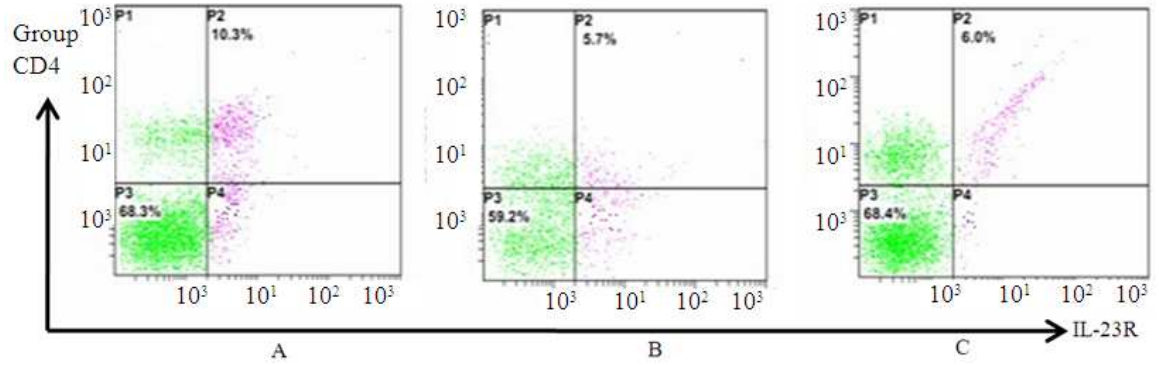

Fig. 2: IL-23R expression PBMCs isolated from patients and controls were stimulated with PMA, ionomycin, and Monensin for 4h, followed by staining with anti-human CD4, anti-human IL-23R and anti-human IL-17 Ab. Cells were then analyzed by BD FACSCanto™ Flow Cytometer. The percentage of $CD4^+$ $IL\text{-}23R^+$ T cells in $CD4^+$ T cells was shown

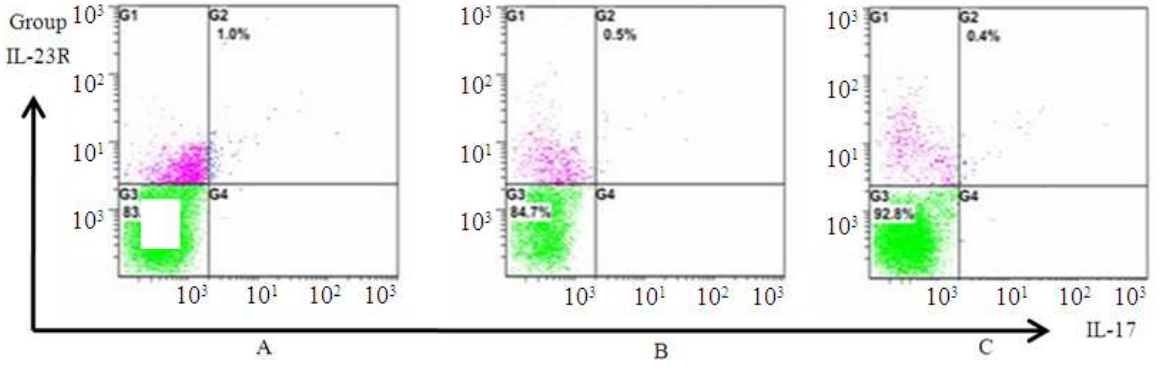

Fig. 3: Co-expression of IL17 and IL-23R PBMCs isolated from patients and controls were stimulated with PMA, ionomycin, and Monensin for 4h, followed by staining with anti-human CD4, anti-human IL-23R and anti-human IL-17 Ab. Cells were then analyzed by BD FACSCanto™ Flow Cytometer. The co-expression of $IL\text{-}23R^+$ and IL-23R in $CD4^+$ T cells was shown

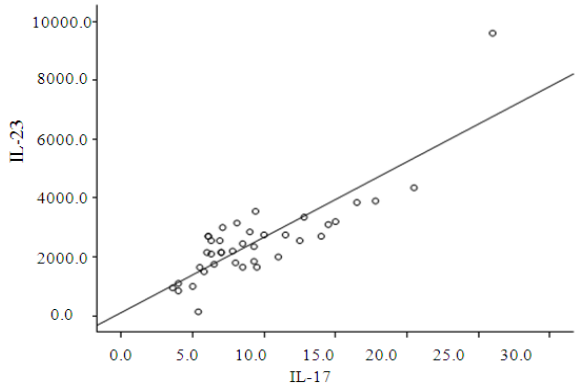

Fig. 4: The scatter graph between IL-17 and IL-23 of group A. There was a significant positive correlation between the level of IL-17 and IL-23 in group A (r = 0.832, p<0.05)

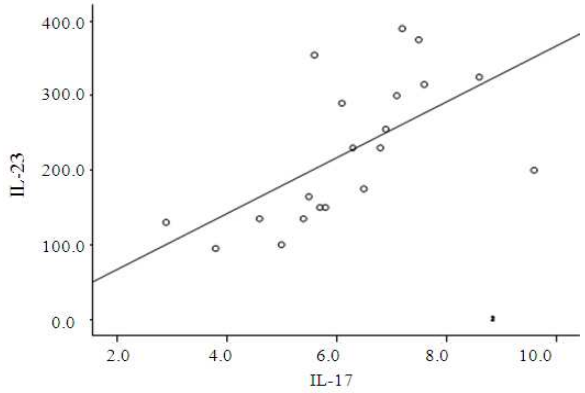

Fig. 5: The scatter graph between IL-17 and IL-23 of group B. There was a significant positive correlation between the level of IL-17 and IL-23 in group B (r = 0.613, p<0.05)

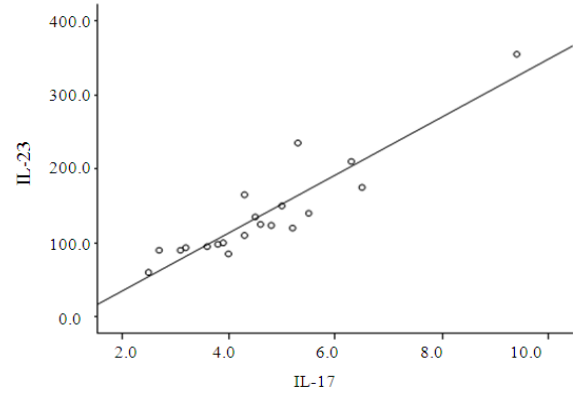

Fig. 6: The scatter graph between IL-17 and IL-23 of group C. There was a significant positive correlation between the level of IL-17 and IL-23 in group C (r = 0.838, P<0.05)

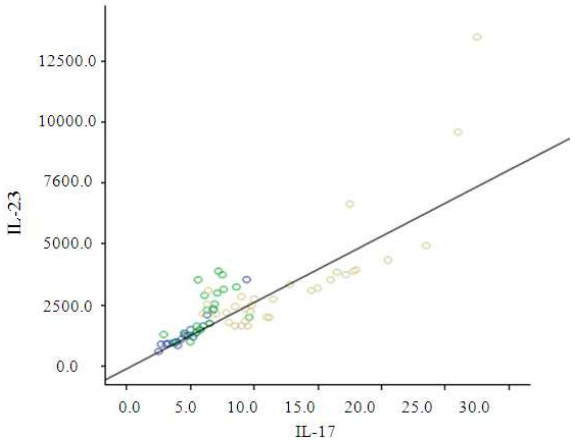

Fig.7: The scatter graph between IL-17 and IL-23 of all subjects. There was significantly positive correlation between the level of IL-17 and IL-23 in all subjects (r = 0.837p<0.05)

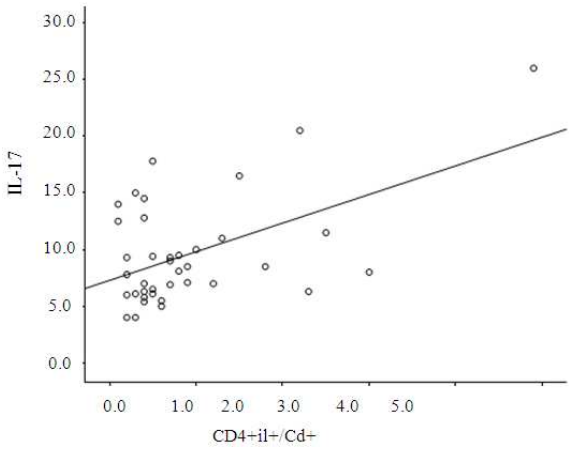

Fig.8: Th17/CD4$^+$ T ratio was positively correlated and IL-17 level. There were significantly positive correlations between the percentage of Th17 and the level of IL-17 in all subjects(r = 0.514, p<0.05)

Table 1: The clinical data of subjects

	Group A	Group B	Group C
Age	53. 8± 14.1	55.0± 13.6	49.8. ±16.1
Number (male/femal	30(13/17)	20(11/9)	20(12/8)
SBP(mmHg)	172.5±17.6$^{\#}$	130.4±3.5	120.8±4.2
DBP(mmHg)	100.6± 8.5$^{\#,}$	75.3±6.8	65.3±5.4
BMI(kg/m^2)	24.1± 3.9	23.8±3.	23.2±3.5
TC (mmol/L)	3.95± 0.34	3.56±0.27	3.32±0.30
TG(mmol/L)	1.56±0.37	1.43±0.41	1.23±0.21
LDL-C(mmol/L)	2.57±0.45	2.16±0.40	2.07±0.34
HDL-C (mmol/L)	2.55 ±0. 28	2.75 ±0. 46	3.04 ±0. 36
FBS(mmol/L)	4.67±0.49	4.52±0.34	4.34±0.28

Table 2: The data of Flow cytometry (%)

Group	D_4^+IL-17$^+$/CD_4^+	D_4^+IL-23R$^+$/CD_4^+	D_4^+IL-17$^+$IL-23R$^+$/CD_4^+
Group A	1. 4±0.6$^{\#,*}$	6±3. 3$^{\#,*}$	1. 4±0.7$^{\#,*}$
Group B	0. 5±0.3	4.2±1.4	0.7±0.4
Group C	0. 3±0.2	3.2±0.4	0.4±0.3

# P<0.05when compared with group B; * P<0.05 when compared with group C

Table 3: The levels of IL-17 and IL-23 (pg/mL)

Group	IL-17	IL-23
Group A	11. 9±5.1$^{\#,*}$	3017±950$^{\#,*}$
Group B	7.8±3.8	2143±927
Group C	6. 0±1.1	1916±935

# P<0.05 when compared with group B; * P<0.05 when compared with group C

DISCUSSION

Studies have shown that immune system plays a critical role in the development and progression of hypertension. Increased level of circulating cytokines and their ligands was detected in patients with primary hypertension (Eriksson *et al*., 2001; Virdis and Schiffrin, 2003). Higher numbers of activated mononuclear cells and lymphocytes were found in spontaneously hypertensive rat especially in adult rats (Schmid-Schonbein *et al*., 1991; Dorffel *et al*., 1999). Consistenly, the activation of inflammatory cells is also found in the peripheral blood of hypertensive patients (Dorffel *et al*., 1999). Inflammation and immune response, mediated by T lymphocyte can directly lead to vascular remodeling and increase of blood pressure (Schiffrin, 2010). Guziks have proven Th1 plays an important role in the pathogenesis of Ang II/DOCA-salt-induced hypertension and Th1/Th2 imbalance leads to hypertension (Guzik *et al*., 2007). Th17 is a recently discovered subgroup of helper T cell characterized by the secretion of IL-17. It is believed that Th17 may play a role in the pathogenesis of hypertension. However, its underlying mechanism is still unknown. In addition, it is unclear whether Th17 plays a role in the pathophysiology of acute blood increase within hypertensive patients, a common trigger for various severe cardiovascular complications. In this study, we found that the number of Th17 and plasma level of IL-17 in hypertensive patients with acutely increased blood pressure are significantly higher than that of both hypertensive patients without acutely increased blood pressure and healthy controls. Correlation analysis showed that Th17 cell percentage in peripheral blood cells is positively correlated with plasma IL-17 level. We failed to detect a statistical difference of Th17 and IL-17 between hypertensive patients without acutely increased blood pressure and healthy controls, despite of a slightly higher level in hypertension group. This is probably because the blood pressure in hypertensive patients without acute increase blood pressure was controlled to a similar level as healthy controls. Therefore we believe that Th17/IL-17 is one of the inflammatory mediators in the development and progression of essential hypertension.

In our study, acutely increased blood pressure group contains some incipient essential hypertension patients and some patients with previously controlled blood pressure but with a sudden increase of blood pressure. What they have in common is the sudden increase of blood pressure in a short time. Various factors can cause the sudden increase of blood pressure, such as weather changes, agitation, nervousness, fatigue and poor sleep. There are also some patients without any incentives. Based on the previous findings (Chen *et al*., 2004), we speculated that lymphocytes and monocytes are activated by AngII or other stimuli. The activation of those inflammatory cells results in an increase of Thl7 cells which in turn cause vascular inflammation and endothelial dysfunction by the secretion of IL-17, IL-6andTNF-α. The vascular inflammation and endothelial dysfunction finally lead to the increase of blood pressure. The elevation of blood pressure may also lead to endothelial damage which in turn induces inflammation and exacerbates hypertension. This may also explain why some essential hypertension patients who have controlled their blood pressure by taking antihypertensive drugs can have acutely increased blood pressure. We observed these patients and found that the same changes mentioned above. If we could break one of the above mentioned steps, the blood pressure might return to the stable stage again.

IL-23 is a new member of IL-12 family and mainly secreted by mononuclear macrophages and dendritic cells. It consists of a unique p19 subunit and a p40 subunit shared with the IL-12. IL-23 R is a new erythropoietin receptor family member and only expressed in the activated or memory cells.

Signaling through IL-23R, IL-23 activates STAT3 signal pathway and promotes the proliferation and migration of memory CD4 $^+$T cell. It is an important factor in Th17 cell survival and proliferation (Aggarwal *et al*., 2003). In this study, IL-23R expression and the level of IL-23 in group A were higher than group B and group Candthere was no significant difference between group B and group C. It has been shown that IL-23R and IL-23 are involved in the pathogenesis of hypertension.

Increased blood pressure activated monocyte-macrophage which secreted more IL-23. IL-23 in turn enhanced the cytokine secretion and antigen presentation of monocyte-macrophage and dendritic cell (Bastos *et al*., 2004). IL-23 plays an important role in the differentiation and proliferation of Thl7 cell

through its binding to the IL-23R on T-cell. In this study, we found a positve correlation between IL-23 and IL-17 expression, indicating IL-23 might participate in the increase of blood pressure through promoting the differentiation of Thl7. Therefore, inflammation axis of IL-23/Th17/IL-17 is closely associated with the change of blood pressure and may be involved in the reoccurrence of hypertension.

CONCLUSION

In the current study, We demonstrated that IL-23 and Th17 cells may be involved in the pathogenesis of acute blood pressure increase in the patients with hypertension. Understanding its inflammatory characterization might be of fundamental importance for the prevention and treatment of acute blood pressure increase in hypertensive patients.

ACKNOWLEDGEMENT

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J.W. researched data, wrote manuscript. S.H. and J.C. contributed to the sample collection. J.C. and C.C. designed the experiments. J.Z. and X.R. edited manuscript.

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