Elevated serum levels of ghrelin and TNF-α in patients with cyanotic and acyanotic congenital heart disease

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Background: **The levels of ghrelin and tumor necrosis factor alpha (TNF-α) are considered biological markers of congenital heart diseases (CHD). The present meta-analysis was conducted to investigate the clinical significance of serum levels of ghrelin and TNF-α in children with (CHD).**

Methods: **Chinese and English scientific literature databases were searched to retrieve published studies relevant to ghrelin, TNF-α and CHD. Manual search was additionally employed to identify other relevant studies from cross-references. The retrieved studies were screened on the basis of our stringent inclusion and exclusion criteria to select high quality case-control studies for meta-analysis.**

Results: **We initially retrieved 108 published studies (20 in Chinese and 88 in English) from database searches. Finally, 6 case-control studies (5 in English and 1 in Chinese) were enrolled in our meta-analysis, and contained a total of 160 cyanotic congenital heart disease (CCHD) patients and 215 acyanotic congenital heart disease (ACHD) patients, along with 162 healthy controls. The results of meta-analysis showed that serum levels of ghrelin and TNF-α in CCHD or ACHD children were significantly higher than those in healthy controls.**

Conclusion: **Our meta-analysis results showed that serum levels of ghrelin and TNF-α are elevated in children with CHD, and could be used as effective biologic markers in early diagnosis of CHD.**

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Key words: **acyanotic;**
congenital heart disease;
cyanotic;
ghrelin;
meta-analysis;
TNF-α

Meta-analysis

Introduction

Congenital heart disease (CHD) occurs before birth, in the early days of gravidity, and is a malformation in one or more structures of the blood vessels or heart.[1] CHD is the major cause of congenital abnormalities across the world, and approximately 28% of all major congenital abnormalities constitute heart defects.[2] In infants and children, the incidence rates are 4-8 CHD patients per 1000 live births and about 25% of these patients present with other congenital defects, either as part of specific malformations or as part of genetic syndromes.[3] Modern surgical techniques and drugs have led to significant reduction in mortality among children, and the surviving adults with CHD are 2800 per 1 million people, with more than 1 million and 1.2 million adults with CHD in the USA and Europe, respectively.[4,5] CHD is functionally classified as acyanotic or cyanotic, both causing disruption in normal oxygenation of blood, but differ in whether the specific defect interferes with pulmonary blood flow.[6] The underlying causes of CHD can be further divided into genetic and non-genetic, and non-genetic causes include environmental factors (dioxins, polychlorinated biphenyls, and pesticides), maternal exposures (alcohol, thalidomide), and infectious agents.[7] Considering the high prevalence of CHD, it is urgent to identify effective biologic markers for diagnosis of CHD. Previous studies have suggested the clinical value of serum levels of ghrelin and tumor necrosis factor alpha (TNF-α) in CHD patients.[8,9]

Ghrelin is mainly generated in the stomach, and is a bioactive peptide of 28-amino-acids that is secreted into the blood circulation, as opposed to being secreted into the gastrointestinal tract like the digestive enzymes.[10] Ghrelin is the hunger hormone and stimulates growth

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hormone release that plays a pivotal role in regulating nutrient intake, weight, physical growth, energy intake and output.[11] TNF-*α* is a member of a large TNF ligand superfamily, and plays a homeostatic role under normal conditions in the central nervous system (CNS), but is highly elevated under pathological conditions, such as ischemia and brain injury, although its precise function in the brain remains to be discovered.[12,13] As an immune mediator, however, TNF-*α* is a pleiotropic pro-inflammatory cytokine that mediates a variety of immunological and metabolic effects.[1,14] TNF-*α* can activate endothelial cells to express adhesion molecules, which recruits inflammatory cells from blood stream to the vessel walls, and accelerate production of chemo-attractant cytokines, further stimulating the production of secondary pro-inflammatory cytokines, such as interleukin-6 (IL-6).[15,16] Thus, serum levels of ghrelin could be an important indicator in assessing growth, development and the nutritional status of CHD patients.[1] Accordingly, a few previous reports suggested that serum ghrelin and TNF-*α* levels are elevated in CHD patients, and elevated ghrelin levels represented malnutrition and growth retardation in these patients.[17,18] However, other studies failed to observe such a correlation.[1,19] To address this issue, we conducted the present meta-analysis to investigate the relationship between ghrelin, TNF-*α* and CHD.

Methods

Literature search

In order to retrieve eligible studies that investigated the relationship between serum levels of ghrelin and TNF-*α* with CHD, we searched PubMed, Springerlink, Wiley, EBSCO, Ovid, Web of Science, Wanfang database, China National Knowledge Infrastructure (CNKI) database, Weipu Journal database (last updated search in October, 2014) by applying a sensitive search strategy using combinations of the following keywords and relative words: ("ghrelin" or "GHRL protein" or "Ppghrelin" or "PpMTLRP" or "ghrelin-obestatin preprohormone" or "motilin-related peptide precursor" or "ghrelin precursor" or "obestatin" or "appetite-regulating hormone" or "motilin-related peptide") and ("tumor necrosis factor-alpha" or "tumor necrosis factor alpha" or "cachectin-tumor necrosis factor" or "TNFalpha" or "tumor necrosis factor" or "tumor necrosis factor ligand superfamily member 2" or "cachectin" or "TNF superfamily, member 2") and "congenital heart disease" or "congenital heart diseases" or "congenital heart defects". Further, manual search was conducted to identify other relevant articles from cross-references.

Selection and exclusion criteria

Published studies were enrolled if they met the following inclusion criteria: (1) case-control study of the correlations between either serum levels of ghrelin or TNF-*α* with CHD; (2) patients in the cases group were clinical diagnosed as CHD[20] and the controls were healthy people; (3) studies with complete data; (4) studies were published in Chinese or in English; (5) only the latest complete study was enrolled when the extracted studies were published by the same authors. Exclusion criteria were as follows: (1) the data of enrolled studies were incomplete; (2) significant differences between case-control studies in baseline characteristics; (3) duplicate articles; (4) the diagnostic criteria were uncertain.

Data extraction and quality assessment

To assure credibility, a standard data abstraction form was used and two investigators extracted data from the included studies independently. The following information was collected: first author, publication year, country, ethnicity of subjects, language, disease, age, gender, detection method and numbers of cases and controls. A third investigator resolved potential discrepancies or disagreements in data extraction, through reexamination of all items and discussions. The quality of enrolled studies was assessed by two or more investigators independently according to the critical appraisal skill program (CASP) criteria (http://www.casp-uk.net/). The CASP criteria are scored based on 11 aspects: Whether the study address a clearly focused issue or not (CASP01); Whether an appropriate method was used to answer their question or not (CASP02); Whether the cases were recruited in an acceptable way or not (CASP03); Whether the controls were selected in an acceptable way or not (CASP04); Whether the exposure was accurately measured to minimize bias (CASP05); What confounding factors have the authors accounted for or have the authors taken account of the potential confounding factors in the design and/or in their analysis (CASP06); What are the results of this study (CASP07); How precise are the results (CASP08); Do you believe the results (CASP09); Can the results be applied to the local population (CASP10); Do the results of this study fit with other available evidence (CASP11).

Statistical analysis

Statistical analysis was performed using STATA statistical software (Version 12.0, Stata Corporation, College Station, TX, USA). The differences in BMI, serum ghrelin and TNF-*α* were assessed using standard mean difference (SMD) with 95% confidence intervals (95% CI) by applying fixed effects model or random effects model and the *Z* test was utilized to evaluate

the significance of pooled SMDs.[21] The Cochran's Q-statistic (P<0.05 was considered significant) and I^2 test (0%, no heterogeneity; 100%, maximal heterogeneity) was conducted to evaluate the heterogeneity among studies.[22] Random effects model was applied in the case of significant heterogeneity (P<0.05 or I^2 test exhibited>50%), otherwise, a fixed effect model was applied.[23] In addition, the Contour-enhanced funnel plots and Egger's linear regression test were used to evaluate publication bias in enrolled studies to ensure the reliability of original results.[24,25] All tests were two-sided, and P values <0.05 were considered statistically significant.

Results

Baseline characteristics of included studies

This meta-analysis initially retrieved 108 studies through electronic database search and manual search. Subsequently, several studies were excluded, including duplicates (n=3), not human studies (n=10), and studies not related to our research topic (n=22). Of the remaining 73 studies, 12 studies were not case-control studies, 18 studies were not relevant to TNF-α or ghrelin, and 35 studies did not subgroup the study subjects as cyanotic congenital heart disease (CCHD), acyanotic congenital heart disease (ACHD) and control groups. Finally, 6 eligible studies, published between 2007 and 2014, were included in our meta-analysis, which containing a total of 537 subjects (160 CCHD patients, 215 ACHD patients and 162 healthy controls).[1,11,17,19,26,27] Among the 6 studies, study subjects in 2 trials were Asians, 1 trial was performed in Caucasians and 3 in Africans. On the basis of country, 1 study was from China, 1 from Iran, and 3 from Egypt. The baseline characteristics of the enrolled studies are shown in the Table.

Pooled outcome of meta-analysis

A total of 5 studies reported the BMI in CHD patients, and random effects model was applied due to heterogeneity (CCHD *vs.* control: I^2=87.7%, P<0.001; ACHD *vs.* control: I^2=90.6%, P<0.001). The results of meta-analysis showed that the BMI in both CCHD and ACHD patients was significantly lower than healthy controls (CCHD *vs.* control: SMD=-1.42, 95% CI: -2.23, -0.51, P=0.002; ACHD *vs.* control: SMD=-1.41, 95% CI: -2.37, -0.46, P=0.004), and the difference was statistically significant (Fig. 1A and B). However, no such statistical significance was found between CCHD and ACHD patients (SMD= -0.15, 95% CI: -0.40, 0.11, P=0.259) (Fig. 1C).

Five studies reported the serum levels of ghrelin

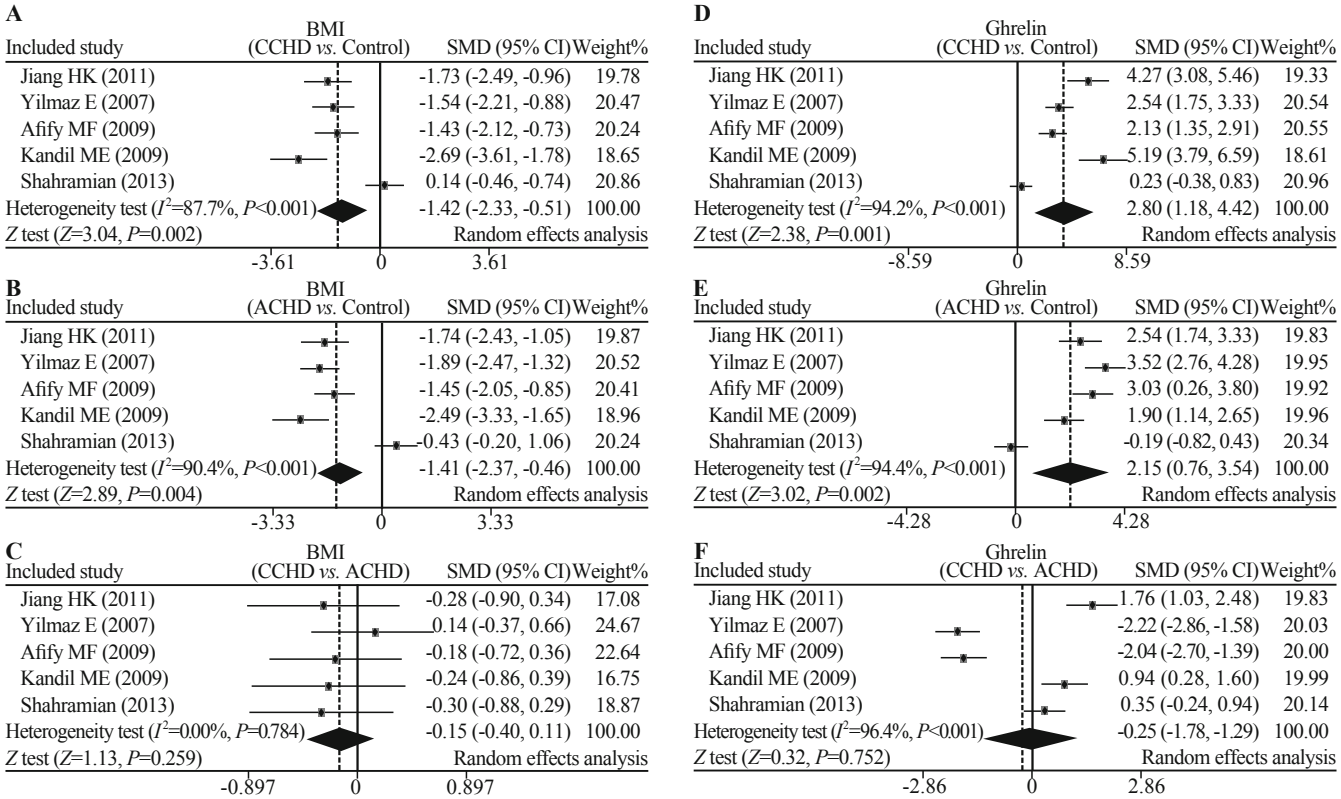

Fig. 1. Forest analyses for the comparisons of body mass index (BMI), serum levels of ghrelinbetween children with cyanotic congenital heart disease (CCHD), acyanotic congenital heart disease (ACHD) and healthy controls. **A**: CCHD *vs.* control, **B**: ACHD *vs.* control, **C**: CCHD *vs.* ACHD; **D**: CCHD *vs.* control; **E**: ACHD *vs.* control; **F**: CCHD *vs.* ACHD.

Table. Baseline characteristics of cyanotic congenital heart disease, acyanotic congenital heart disease and healthy controls in six included studies.

First author	Year	Ethnicity	Sample size			Gender (M/F)			Age (mon)		
			CCHD	ACHD	Control	CCHD	ACHD	Control	CCHD	ACHD	Control
Jiang HK[26]	2011	Asians	17	25	20	10/7	13/12	12/8	37.2±16.5	35.6±19.3	33.8±17.7
Yilmaz E[28]	2007	Caucasians	21	47	25	11/10	10/15	17/30	30.5±18.4	28.4±15.6	31.1±15.1
Afify MF[11]	2009	Africans	20	40	20	11/9	16/24	9/11	33.5±20.2	31.2±17.2	31.3±15.2
Kandil ME[17]	2009	Africans	18	22	18	22/18	-	11/7	13.23±8.38	-	15.67±7.81
Shahramian I[1]	2013	Asians	24	21	19	10/14	12/9	14/5	40.6±53.9	38.52±49.44	38.64±41.88
Nassef YE[19]	2014	Africans	60	60	60	26/34	35/25	30/30	12-48	-	-

CCHD: cyanotic congenital heart disease; ACHD: acyanotic congenital heart disease; M: male; F: female.

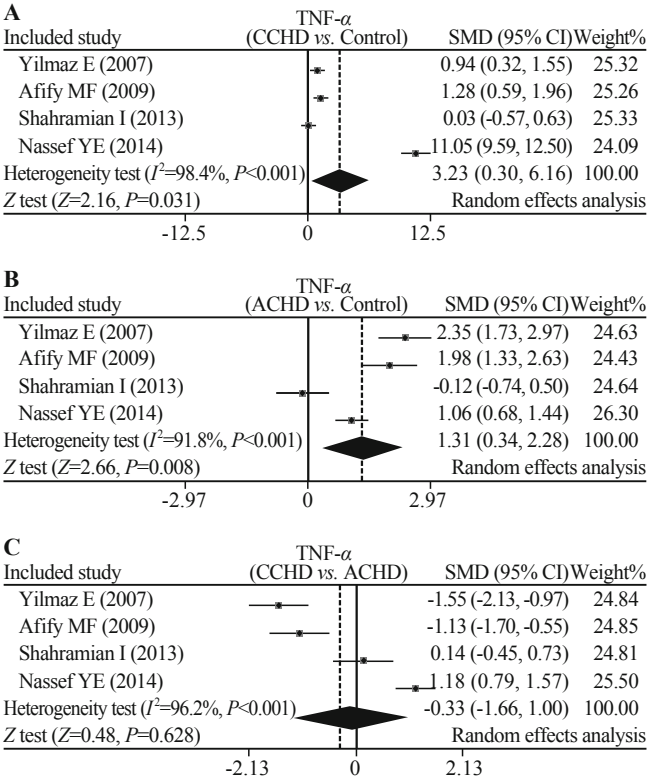

Fig. 2. Forest analyses for the comparisons of tumor necrosis factor alpha (TNF-α) level between children with cyanotic congenital heart disease (CCHD), children with acyanotic congenital heart disease (ACHD), and healthy controls. **A**: CCHD *vs*. control; **B**: ACHD *vs*. control; **C**: CCHD *vs*. ACHD. CI: confidence interval; SMD: standard mean difference.

in CHD patients, and significant heterogeneity was detected (CCHD *vs*. Control: I^2=94.2%, P<0.001; ACHD *vs*. control: I^2=94.4%, P<0.001; CCHD *vs*. ACHD: I^2=96.4%, P<0.001), therefore random effects model was applied. The result of meta-analysis demonstrated that serum levels of ghrelin in both CCHD and ACHD patients were markedly higher than healthy controls (CCHD *vs*. control: SMD=2.80, 95% CI: 1.18, 4.42, P=0.001; ACHD *vs*. control: SMD=2.15, 95% CI=0.76-3.54, P=0.002), and the difference was statistically significant (Fig. 1D and E). However, the serum levels of ghrelin in comparison between CCHD and ACHD patients were not statistically significant (SMD=-0.25, 95% CI: -1.78, 1.29, P=0.752) (Fig. 1F).

Four studies reported the levels of TNF-α in CHD patients, and significant heterogeneity was detected (CCHD *vs*. control: I^2=98.4%, P<0.001; ACHD *vs*. control: I^2=91.8%, P<0.001; CCHD *vs*. ACHD: I^2=96.2%, P<0.001). The results of meta-analysis revealed that the levels of TNF-α in both CCHD and ACHD patients were significantly higher than those in the healthy controls (CCHD *vs*. control: SMD=3.23, 95% CI=0.30, 6.16, P=0.031; ACHD *vs*. control: SMD=1.31, 95% CI=0.34, 2.28, P=0.008), suggesting statistically significant differences (Fig. 2A and B). Nevertheless, there was no statistical significance in the TNF-α levels between CCHD and ACHD patients (SMD=-0.33, 95% CI=-1.66, 1.00, P=0.628) (Fig. 2C).

Sensitivity analysis and publication bias

The sensitivity analysis showed that all enrolled studies had no evident influence on the pooled SMD on relation between BMI, the serum levels of ghrelin and TNF-α in CHD patients. The contour-enhanced funnel plots showed most of enrolled studies in the area of P>5%. Additionally, the Egger linear regression analysis further confirmed the publication bias between ghrelin serum levels of CCHD, ACHD patients and controls, and TNF-α levels of CCHD patients and controls (all P<0.05) (Figs. 3 and 4).

Discussion

We conducted a systematically meta-analysis to investigate the clinical relevance of serum levels of ghrelin and TNF-α in CHD patients. The outcomes of our research revealed that serum levels of ghrelin and TNF-α were significantly increased in the CHD patients, compared with the healthy subjects, implying that elevated serum concentrations of ghrelin and TNF-α may play crucial roles in the development and progression of CHD. Whereas, previous studies revealed that no changes of ghrelin and TNF-α were found in CCHD and ACHD patients by which the

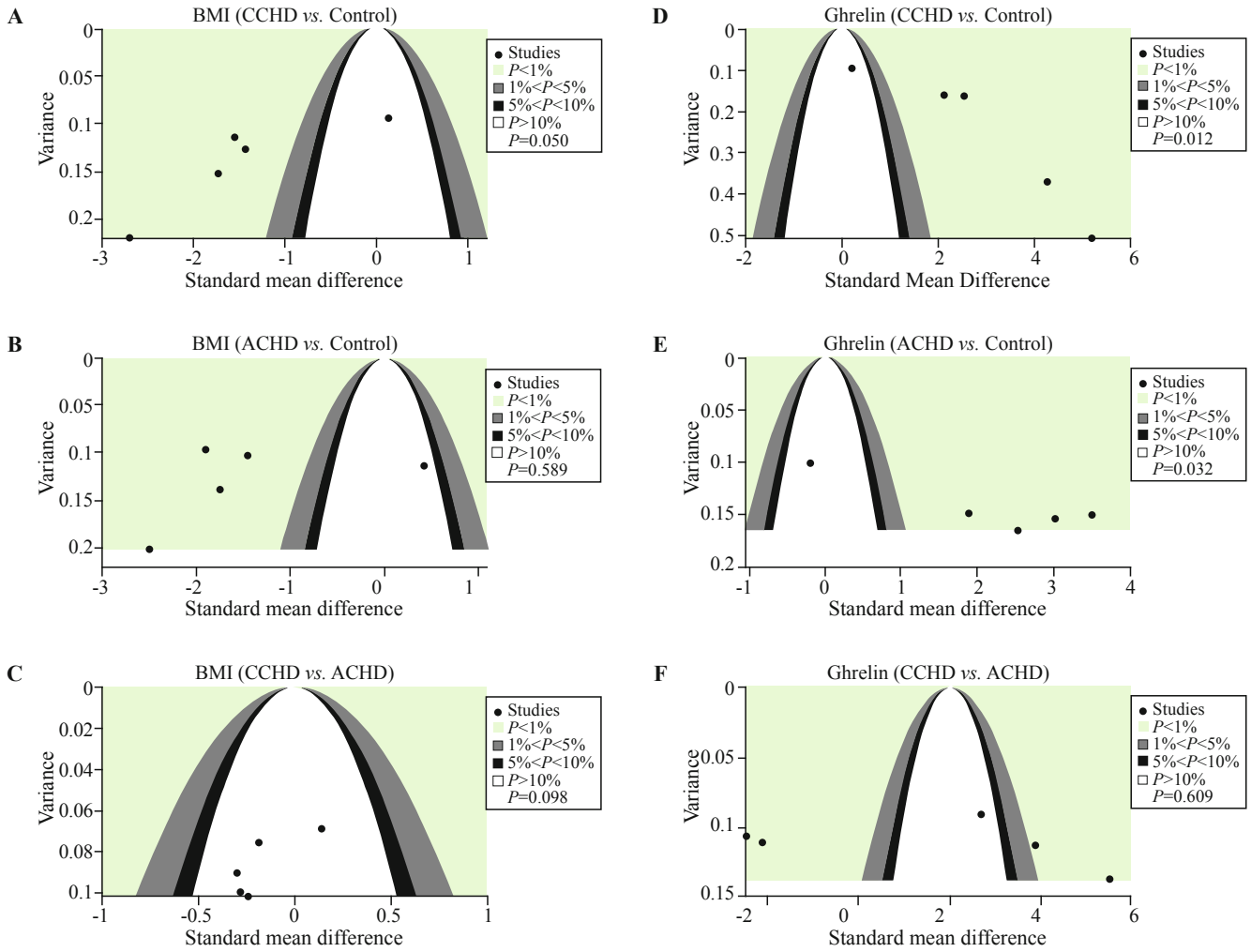

Fig. 3. Funnel plot of publication biases on the comparisons of body mass index and serum levels of ghrelin between children with cyanotic congenital heart disease, children with acyanotic congenital heart disease, and healthy controls. **A**: CCHD *vs.* control; **B**: ACHD *vs.* control; **C**: CCHD *vs.* ACHD; **D**: CCHD *vs.* control; **E**: ACHD *vs.* control; **F**: CCHD *vs.* ACHD. BMI: body mass index; CCHD: cyanotic congenital heart disease; ACHD: acyanotic congenital heart disease.

most dominant causes of growth retardation in CHD were increased metabolism and inadequate caloric intake.[1,19] But the present meta-analysis indicated a converse relationship of ghrelin and TNF-α with CHD. CHD is the most common congenital abnormality among children, which leads to growth retardation and malnutrition.[2,19] Ghrelin and TNF-α serum levels regulate nutrient intake, physical development and growth, weight and energy intake and output in CHD patients.[11,17] Additionally, ghrelin enhances feeding and weight gain and regulates energy homeostasis, affecting physical development and growth.[29] With the functions of increasing stomach acid secretion and stimulating gastric motility, ghrelin serum level is elevated by fasting and reduced by feeding and hyperglycaemia, that is to say, ghrelin serum level is low in obese people and high in lean people.[30] CHD patients exhibit significant malnutrition, and evidence suggests that serum level of ghrelin is a marker for the nutritional status, and is prominently elevated in conditions of malnutrition.[11] In this respect, increased ghrelin levels in CHD patients represent malnutrition and growth retardation, suggesting that elevated serum concentration of ghrelin is significantly associated with the development of CHD. TNF-α, a pro-inflammatory cytokine, located on chromosome 6, can induce catabolism and has multiple immunologic and metabolic actions.[31,32] It is synthesized as a 26 kDa transmembrane monomer, which induces smooth muscle proliferation and causes the increasing adherence of leucocytes to endothelial cells by impelling the expression of cell adhesion molecules.[28] The increase of TNF-α may release multifarious endothelial adhesion molecules, activate leukocytes and excrete platelet-activating factor and then lead to the development of CHD.[33,34] Evidence shows that circulating TNF-α

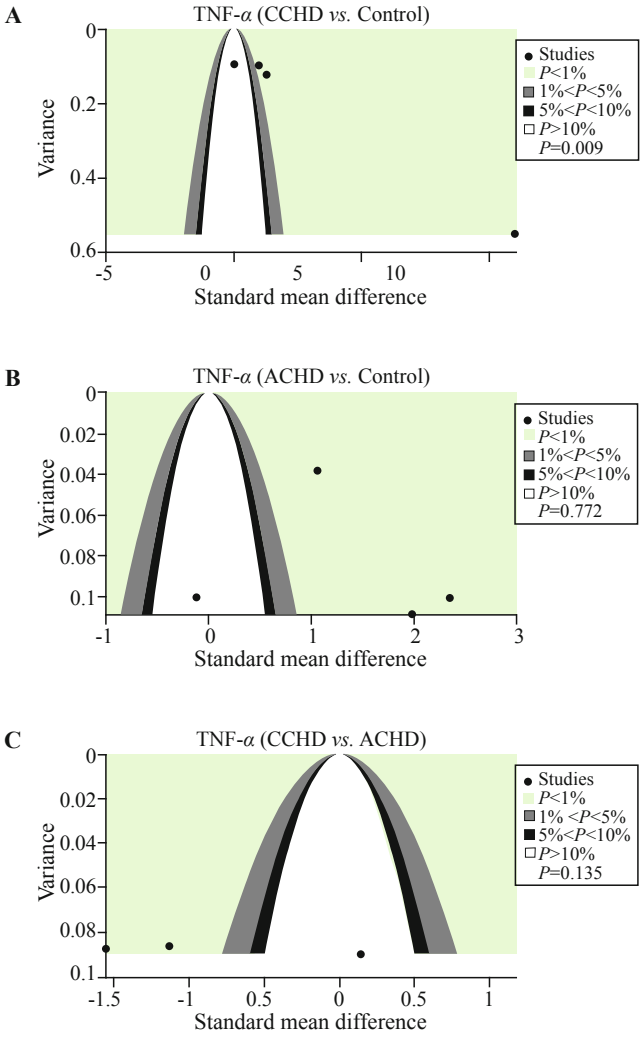

Fig. 4. Funnel plot of publication biases on the comparisons of tumor necrosis factor alpha level between children with cyanotic congenital heart disease, children with acyanotic congenital heart disease and healthy controls. TNF-α: tumor necrosis factor alpha; CCHD: cyanotic congenital heart disease; ACHD: acyanotic congenital heart disease; **A**: CCHD *vs.* control; **B**: ACHD *vs.* control; **C**: CCHD *vs.* ACHD.

can lead to cardiac cachexia, which correlates with weight loss, reduced muscle strength and function, and consequently compromise normal immune functions.[11,35]

We acknowledge limitations in the current meta-analysis. First, the sample sizes in all enrolled studies were relatively small, which may reduce the reliability of our conclusions. Second, the statistical power may be inadequate because of the small number of included studies, which may limit the conclusions in this meta-analysis. Third, selection bias in current meta-analysis is also a potential limitation since the six studies enrolled for analyses involved 5 English articles and 1 article in Chinese. Therefore, further studies are needed to confirm our findings.

In conclusion, we propose that elevated serum ghrelin and TNF-α levels closely correlate with poor development in CHD patients, based on the data from our systematic review using meta-analysis approach, and ghrelin and TNF-α may be effective biological markers for early diagnosis of CHD patients.

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