The ADRB3 rs4994 polymorphism increases risk of childhood and adolescent overweight/obesity for East Asia’s population: an evidence-based meta-analysis

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ABSTRACT
Whether the Adrenoceptor Beta 3 (ADRB3) gene rs4994 polymorphism could affect the individual risk of childhood and adolescent overweight/obesity remains controversial. This meta-analysis was performed to estimate the prevalence of this polymorphism in overweight/obesity, and test the potential association by summarizing existing evidence. Comprehensive literature search in PubMed, Web of Science, Cochrane Library, Wanfang, and CNKI databases was performed to identify eligible data sets. Finally, 16 studies involving 5,147 overweight/obese cases and 7,350 non-obese controls were included for further synthetic analyses. Odds ratio (OR) and its corresponding 95% confidence intervals (CIs) were statistically calculated. Totally, 69.9% of the included subjects came from East Asia. In the meta-analysis for overall population, statistically significant associations with increased risk of childhood and adolescent overweight/obesity were identified in allele model (OR 1.23, 95% CI 1.10–1.38), heterozygote model (OR 1.39, 95% CI 1.16–1.68), and dominant model (OR 1.31, 95% CI 1.12–1.54). Further stratified analysis according to geographical regions revealed that the statistical significance could only be detected in the East Asia subgroup in allele model, homozygote model, heterozygote model, and dominant model. In summary, our meta-analysis indicated that the ADRB3 rs4994 polymorphism could significantly increase the risk of childhood and adolescent overweight/obesity, especially for the East Asia’s population.

Introduction
Emerging as a serious health problem worldwide, childhood and adolescent obesity, a complex and multifactorial metabolic disorder, could lead to not only improper physical and mental development [1–3], but also increased risk of medical complications like cardiovascular disease, dyslipidemia, asthma exacerbation, and metabolic syndrome [4]. The reciprocity between individual factors, including genetic variation like single nucleotide polymorphism (SNP), and lifestyle/environmental variables such as nutrition overbalance, lack of physical activity, and sedentary habit could interpret the variability in obesity predisposition between individuals in a given population.

Beta-adrenergic receptors, a subgroup of G-protein-coupled receptors, are involved in the regulation of energy expenditure [5]. As a member of this receptor family, the Adrenoceptor Beta 3 (ADRB3) gene, locating at 8p11.23 region of human genome, modulates catecholamine-induced stimulation of adenylate cyclase via the action of G proteins. This receptor expresses predominantly in adipocytes and functions in mediating lipolysis and thermogenesis [6]. Significantly decreased expression of the ADRB3 gene on both the mRNA and protein levels in adipose tissues of obese patients [7] and overweight individuals [8] was observed. Because of its demonstrated functions in lipid metabolism and observed gene expression dysregulation in obesity, ADRB3 could be reasonably expected to constitute a potential pharmacologic target for obesity treatment. The ADRB3 rs4994 polymorphism (Trp64Arg), a T to C switch leading to the replacement of tryptophan by arginine at position 64, has been related to lower resting metabolic rate [9], weaker response to obesity treatment [10], and increased...
capacity to gain weight [11] according to scattered evidence. These associations can be imputed to less efficient coupling with the G stimulating protein [12] and consequently impaired lipolytic activities [13] led by the rs4994 polymorphism. Given the significance of this ADRB3 polymorphism, it is necessary to quantitatively assess the strength of its relationship with overweight/obesity risk.

To date, many epidemiological assessments were completed to quantitatively determine the association between the ADRB3 rs4994 polymorphism and risk of childhood and adolescent overweight/obesity. However, the scattered reports remain inconclusive and did not reach a consensus [14–32]. These studies were plagued by multiple methodological shortcomings, including inadequate statistical power caused by a relatively small sample size, high potential risk of sampling bias, and inconsistent analysis strategies. The aim of this meta-analysis is to quantify more accurately the strength of the genotypic impact of the ADRB3 rs4994 polymorphism.

Materials and methods

Literature and search strategy

A comprehensive literature search for relevant studies published on pre-reviewed journals in five databases [PubMed (https://www.ncbi.nlm.nih.gov/pubmed), Web of Science (https://www.webofknowledge.com/), Cochrane Library (https://www.cochranelibrary.com), China Academic Journals full-text database (CNKI, http://www.cnki.net), and Wanfang data (http://www.wanfangdata.com.cn)] from each database’s inception to 30 May 2019, in Chinese and English, was conducted. Terms for the ADRB3 gene and rs4994 polymorphism (‘Adrenoceptor Beta 3’, ‘ADRB3’, ‘Trp64Arg’, or ‘rs4994’), definitions for single nucleotide polymorphism (‘polymorphism’, ‘polymorphisms’, ‘SNP’, or ‘variant’), synonyms for obesity (‘obesity’, ‘obese’, ‘overweight’, ‘hyperadiposity’, ‘hyperadiposis’, or ‘fitness’), and keywords for ‘childhood and adolescent’ (‘children’, ‘adolescents’, ‘childhood’, or ‘adolescence’) were merged in the Boolean expression for database query.

Eligibility criteria

The eligibility of individual studies was evaluated by four investigators (YZ, JR, CX and WH) who independently used the predefined exclusion/inclusion criteria. Case–control studies providing original genotype data comparing the allele frequency difference between obese case and control samples were selected. All included studies had to meet the following criteria: (1) Language: studies published in Chinese or English; (2) Participants: children or adolescents; (3) Exposure of interest: genotypes of the ADRB3 rs4994 polymorphism; (4) Outcomes: obese or overweight. For publications with the same case-control population, only the largest or most complete study was included. Studies with the overall sample size less than 50 were excluded to avoid obvious sampling bias distorting the results.

Data extraction

A predefined data collection form was used for this review. The extracted data covered information regarding sample characteristics and features of study design. Author’s name, publication year, country, region, polymorphism detection method, definition method for obesity/overweight, and genotype data for case and control were recorded. The Newcastle–Ottawa Scale (NOS) quantification system (http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp, accessed on 1 June 2019) was used to comprehensively assess the quality of included studies in this meta-analysis. The summarized scores for all subscale point items were used to categorize study quality as either low (<4), medium (4–6), or high (>6). Deviations from Hardy–Weinberg equilibrium (HWE) in control populations were assessed with an online calculator (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl). P-values less than 0.05 indicate statistical significance for HWE deviation.

Statistical analysis

All statistical analyses were performed using STATA Statistical Software (Version 14.2; StataCorp LP, College Station, TX, USA). Initially, significance for heterogeneity between studies was evaluated using Chi-square based test (a $P < 0.10$ defines the statistical significance). If no significance was detected, a fixed effect model (the Mantel-Haenszel method) would be used [33]. Otherwise, random effects model (the DerSimonian–Laird method) would be applied [34]. Odds ratio (OR), 95% confidence intervals (CI) and the weight for each included study individually or in combination under fixed effect model and random effects model were calculated, estimating the single and pooled effect under homozygote model (CC versus TT), heterozygote model (TC versus TT), dominant model (CC +TC versus TT), recessive model (CC versus TT+TC), and allele model (C versus T). If $P$-value < 0.05, the null hypothesis that there is no genetic impact of the ADRB3 Trp64Arg polymorphism on the increased risk of childhood and adolescent overweight/obesity could be rejected. Subgroup analyses were performed according to the geographical
region (East Asia versus others), HWE status (consistent versus inconsistent and unassessable), sample size (no less than 200 versus less than 200), and sex (male versus female). Publication bias was not only visually measured based on the extent of asymmetry of Begg’s funnel plot, and also statistically evaluated by Egger’s regression test [35] and Begg’s rank test [36] (a P-value <0.05 defines the statistical significance). If significant publication bias was detected, the Duval and Tweedie’s rank-based ‘trim-and-fill’ method was applied to impute for potentially missing data sets and adjust the effect of publication bias [37]. Leave-one-out sensitivity analysis was performed to calculate the pooled estimates on each subset of the involved studies obtained by omitting exactly one individual study. All statistical variables were reported rounded to two decimal places.

This meta-analysis was deployed in strict accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) recommendations [38].

Results

Characteristics of included studies

Of the 239 individual publications initially identified, 16 unique studies [14–27,29,30] involving 5,147 overweight/obese cases and 7,350 non-obese controls were ultimately included for further synthetic analyses (Figure 1). Totally, 69.9% of the included subjects came from East Asia. One study for Romanian children was not included because its sample size is less than 50 [28]. Two studies from China were excluded due to duplicated data [31,32]. The characteristics of the

Figure 1. Systematic review flowchart for this meta-analysis.
The characteristics of included studies in this meta-analysis.

| Author                  | Year | Ref ID | Country | Region      | Genotyping method | Obese/Overweight definition | Case  | Control | HWE | NOS |
|-------------------------|------|--------|---------|-------------|--------------------|----------------------------|-------|---------|-----|-----|
| Aradillas-Garcia        | 2017 | [29]   | Mexico  | Latin-America | TaqMan             | BMI                        | 348   | 698     | 0.23| 7   |
| Verdi                   | 2015 | [27]   | Turkey  | West Asia   | PCR-RFLP           | BMI                        | 130   | 121     | <0.01| 5   |
| Kuo                     | 2015 | [26]   | China   | East Asia   | TaqMan             | BMI                        | 1924  | 3901    | NA  | 5   |
| Oguri                   | 2013 | [24]   | Japan   | East Asia   | PCR-RFLP           | BMI                        | 73    | 59      | 0.83| 5   |
| Zhu                     | 2013 | [25]   | China   | East Asia   | PCR-RFLP           | BMI                        | 92    | 71      | 0.56| 6   |
| Csernus                 | 2013 | [23]   | Hungary | Europe      | PCR-RFLP           | BMI                        | 703   | 634     | NA  | 5   |
| Chou                    | 2012 | [22]   | China   | East Asia   | TaqMan             | BMI                        | 276   | 277     | 0.47| 6   |
| Peng                    | 2010 | [21]   | China   | East Asia   | PCR-RFLP           | BMI                        | 357   | 357     | 0.18| 7   |
| Wang                    | 2008 | [20]   | China   | East Asia   | PCR-RFLP           | BMI                        | 151   | 85      | 0.10| 5   |
| Zhang                   | 2008 | [30]   | China   | East Asia   | PCR-RFLP           | Obesity index              | 95    | 85      | NA  | 5   |
| Li                      | 2007 | [19]   | China   | East Asia   | PCR-RFLP           | WHO weight height chart    | 100   | 100     | 0.91| 6   |
| Erhardt                 | 2005 | [18]   | Hungary | Europe      | PCR-RFLP           | Body weight & body fat content | 295   | 147     | 0.54| 6   |
| Ochoa                   | 2004 | [17]   | Spain   | Europe      | PCR-RFLP           | BMI                        | 185   | 185     | 0.25| 5   |
| Mo                      | 2001 | [16]   | China   | East Asia   | PCR-RFLP           | NA                         | 90    | 87      | NA  | 6   |
| Endo                    | 2000 | [15]   | Japan   | East Asia   | PCR-RFLP           | Obesity index              | 90    | 463     | 0.80| 5   |
| Hinny                   | 1996 | [14]   | Germany | Europe      | PCR-RFLP           | BMI                        | 238   | 80      | 0.43| 5   |

HWE: Hardy–Weinberg equilibrium; NOS: Newcastle–Ottawa scale; NA: not available. BMI: body mass index, calculated according to the weight/height² formula. Obesity index: calculated according to the (real weight-standard weight)/standard weight * 100 formula.

The degree of publication bias was estimated based on the asymmetry of the funnel plots (data not shown). Begg’s rank correlation test for publication bias showed no statistical significance in all five genetic models (allele model: p = 0.36, homozygote model: p = 0.72, heterozygote model: p = 0.20, dominant model: p = 0.19, recessive model: p = 0.72). However, Egger’s regression test revealed there is slightly significant publication bias in the dominant model (p = 0.02), but not in the other models (allele model: p = 0.18, homozygote model: p = 0.57, heterozygote model: p = 0.10, recessive model: p = 0.71). The summary analysis incorporating four additional hypothetical studies using the trim-and-fill method continued to reveal a statistically significant association between the ADRB3 rs4994 polymorphism and risk of childhood and adolescent overweight/obesity (OR 1.20, 95% CI 1.03–1.40, Figure 3), suggesting the robustness of the pooled estimates from the dominant model.

Sensitivity analysis

The leave-one-out sensitivity analysis iteratively removed one data set at a time to determine whether the statistical significance of the pooled estimates was driven by any single study. No statistically significant changes could be observed (Figure 4), suggesting the stability of the results.
Table 2. Results of overall and subgroup analyses for the ADRB3 rs4994 polymorphism and risk of childhood and adolescent overweight/obesity.

| Comparison       | Allele model | Homozygote model | Heterozygote model | Dominant model | Recessive model |
|------------------|--------------|------------------|--------------------|---------------|----------------|
|                  | OR (95% CI)  | P     | OR (95% CI)  | P     | OR (95% CI)  | P     | OR (95% CI)  | P     | OR (95% CI)  | P     |
| Overall          | 1.23(1.10,1.38) | <0.01 | 1.36(0.90,2.06) | 0.15 | 1.39(1.16,1.68) | <0.01 | 1.31(1.12,1.54) | <0.01 | 1.26(0.83,1.90) | 0.28 |
| Region           |              |       |              |       |              |       |              |       |              |       |
| East Asia        | 1.47(1.25,1.71) | <0.01 | 1.97(1.09,3.56) | 0.02 | 1.60(1.34,1.90) | <0.01 | 1.50(1.22,1.84) | <0.01 | 1.68(0.94,3.00) | 0.08 |
| Others           | 1.04(0.89,1.22) | 0.62 | 0.92(0.50,1.69) | 0.79 | 1.01(0.82,1.26) | 0.90 | 1.01(0.82,1.24) | 0.96 | 0.92(0.50,1.68) | 0.79 |
| HWE Consistent   | 1.25(1.11,1.41) | <0.01 | 1.45(0.95,2.21) | 0.09 | 1.28(1.11,1.47) | <0.01 | 1.34(1.11,1.62) | <0.01 | 1.34(0.88,2.04) | 0.18 |
| Others*          | 1.17(0.90,2.52) | 0.25 | 0.33(0.03,3.23) | 0.34 | 2.16(1.32,3.53) | <0.01 | 1.11(0.99,1.25) | 0.08 | 0.30(0.03,2.97) | 0.31 |
| Sample Size      |              |       |              |       |              |       |              |       |              |       |
| <200             | 1.82(1.16,2.84) | <0.01 | 1.27(0.30,5.32) | 0.74 | 2.30(1.54,3.45) | <0.01 | 2.05(1.39,3.02) | <0.01 | 1.02(0.25,4.17) | 0.98 |
| ≥200             | 1.20(1.07,1.35) | <0.01 | 1.37(0.89,2.11) | 0.16 | 1.24(1.07,1.48) | <0.01 | 1.15(1.05,1.26) | <0.01 | 1.28(0.83,1.97) | 0.26 |
| Sex              |              |       |              |       |              |       |              |       |              |       |
| Male             | 1.31(1.03,1.67) | 0.03 | 1.45(0.46,4.54) | 0.53 | 1.38(1.05,1.83) | 0.02 | 1.44(1.12,1.85) | <0.01 | 1.35(0.43,4.21) | 0.61 |
| Female           | 1.74(1.29,2.35) | <0.01 | 2.47(0.54,11.18) | 0.24 | 1.90(1.36,2.66) | <0.01 | 2.01(1.48,2.73) | <0.01 | 1.98(0.44,8.97) | 0.37 |

HWE: Hardy–Weinberg equilibrium; OR: odds ratio; 95% CI: 95% confidence interval; NA: not available. * Studies inconsistent with HWE and unassessable were classified into subgroup ‘Others’.
Discussion

There is still uncertainty regarding the genetic impact of the ADRB3 rs4994 polymorphism on the risk of childhood and adolescent overweight/obesity despite previous case–control studies [14–32]. Our systematic literature review and meta-analysis identified statistical significance under allele model, heterozygote model, and dominant model, which suggest that the C allele of this polymorphism could be a risk factor. Subgroup analysis by sex showed that statistical significance for allele model, heterozygote model, and dominant model could be detected in both male and female subgroups. However, given the limited number of included studies and sample size, these significances would not conclude whether or not gender could influence the impact of this polymorphism. Further stratified analyses according to geographical region revealed significantly higher risk of overweight/obesity in the East Asia subgroup under allele model, homozygote model, heterozygote model, and dominant model, suggesting the possibility that there may be some interaction effects with other genetic variables with ethnicity or regional specificity. The variances of the lifestyle should also be taken into account.

Meta-analyses pooling scattered epidemiological studies are generally prone to between-study heterogeneity and bias, which could partly impact the difficulty in drawing conclusions based on pooled estimates [39]. In the heterozygote model and dominant model, substantial heterogeneity with regard to the overall population was detected. The conclusion of individual study with small sample size may be undermined by methodological weaknesses such as failure to control potential confounding factors, which could lead to significant between-study heterogeneity in meta-analysis. Stratified analyses according to sample size

| Study ID         | OR (95% CI) | % Weight |
|------------------|-------------|----------|
| Others           | 0.86 (0.43, 1.72) | 4.05     |
| Ochoa (2004)     | 0.80 (0.45, 1.44) | 5.32     |
| Erhardt (2005)   | 1.28 (0.66, 2.46) | 4.47     |
| Verdi (2015)     | 1.56 (0.76, 3.20) | 3.82     |
| Aradillas-Garcia (2017) | 0.97 (0.74, 1.28) | 12.72   |
| Subtotal (I-squared = 0.00%, p = 0.598) | 1.00 (0.81, 1.24) | 30.38 |

**East Asia**

| Study ID         | OR (95% CI) | % Weight |
|------------------|-------------|----------|
| Endo (2000)      | 1.74 (1.10, 2.74) | 7.64     |
| Mo (2001)        | 1.87 (1.03, 3.40) | 5.12     |
| Li (2007)        | 1.88 (0.99, 3.59) | 4.56     |
| Wang (2008)      | 1.56 (0.91, 2.67) | 5.96     |
| Peng (2010)      | 1.27 (0.91, 1.77) | 10.77    |
| Chou (2012)      | 1.50 (1.03, 2.18) | 9.48     |
| Oguri (2013)     | 1.96 (0.90, 4.27) | 3.36     |
| Zhu (2013)       | 2.37 (1.21, 4.65) | 4.26     |
| Kuo (2015)       | 1.08 (0.95, 1.22) | 18.57    |
| Subtotal (I-squared = 53.0%, p = 0.030) | 1.50 (1.22, 1.84) | 69.62 |
| **Overall** (I-squared = 42.6%, p = 0.046) | 1.31 (1.12, 1.54) | 100.00 |

**Note:** Weights are from random effects analysis.
remarkably reduced the significance level of heterogeneity in this study. However, the conclusions based on the pooled estimates in both subgroups remained stable. This supported the robustness of our meta-analysis. In addition, the results of the leave-one-out sensitivity analysis ensured that no single data set could dominate the statistical
The significance of the pooled estimates, indicating the validity of our conclusions. Although statistical significance for publication bias was detected in the dominant model, the trim-and-fill method adjusting for asymmetry of funnel plot confirmed that a positive association between this polymorphism and overweight/obesity risk is unlikely to be due to publication bias, suggesting the authenticity of our results. In brief, these above aspects could be regarded as the manifestation of concrete reliability for this meta-analysis.

This meta-analysis has several merits. Our study endorsed the recommendations by the PRISMA guidelines [38]. The broad-scope search of multiple literature databases, stringent study selection and data extraction, standardized statistical analysis processes, and rigorous interpretation of final results substantially reinforced our confidence in the validity of this study. However, a clear understanding of the drawbacks inherent in our approach should be reached to disclose the limitations of the results. First of all, the primary limitation of this meta-analysis is the differing definitions of overweight/obesity across ethnicities and countries. Although the heterogeneity is not unexpected given methodological nature of the included studies, this complicating factor does impact the ability to precisely calculate the pooled estimates. Furthermore, most of the included studies were conducted in East Asia, which significantly impacted the ethnic diversity and could lead to sampling bias. Moreover, this quantitative synthesis only assessed the genetic impact of the rs4994 polymorphism. It is still unable to exclude the likelihood that other functional polymorphisms of the ADRB3 gene also contribute to the risk of overweight/obesity. In addition, besides the individual contribution of each polymorphism, whether the combined polymorphisms, so-called haplotypes, could exert a synergistic effect has yet to be answered. Last but not least, confounding factors including over- or underdiagnosis, comorbid diseases, error in the genotyping assay, environmental exposure, socioeconomic status, and behaviour and lifestyle habits of the family should also be taken into account in future studies. Thus, the results of this meta-analysis should be interpreted with caution.

In conclusion, the results of this meta-analysis evaluating the genetic impact of the ADRB3 rs4994 polymorphism on the risk of overweight/obesity among children and adolescents are reassuring. There is a statistically significant association between the rs4994 polymorphism and overweight/obesity risk. However, this meta-analysis shows that the C allele of this polymorphism in the ADRB3 gene is a risk factor for overweight/obesity only in children and adolescents from East Asia.

Authors’ contributions
XY and XX conceived and designed this study. XY led the investigations and recruited the team. JR, YZ, WH and CX performed the systematic literature review and analyses. XY and XX drafted the manuscript. All authors read and approved the proof of the manuscript before submission.

Disclosure statement
No potential conflict of interest was reported by the authors.

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Ethical statement
This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent
For this type of study, formal consent is not required.

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