Mendelian randomization study of IGF-1, childhood obesity and obesity-related asthma

Tianyi Zhu[1#], Hao Liu[2#], Lan Ling[1], Yang Wang[3], Chao Jin[4], Jirong Qi[3*], Chen Zhou[1*]

[1] Department of Pediatrics, The First Affiliated Hospital of Nanjing Medical University, Nanjing, Jiangsu,210003, China
[2] Department of infection, Children's Hospital of Nanjing Medical University, Nanjing, Jiangsu,210003, China
[3] Department of Cardiothoracic Surgery, Children's Hospital of Nanjing Medical University, Nanjing, Jiangsu,210003, China
[4] Department of Outpatient, The First Affiliated Hospital of Nanjing Medical University, Nanjing, Jiangsu,210003, China

#These authors contributed equally to this work.

ARTICLE INFO

ABSTRACT

Insulin-like growth factor 1 (IGF-1) has been reported to potentially link with childhood obesity and obesity-related asthma, although a causal effect has not been illustrated. This study aimed to assess their association via multi-variable Mendelian randomization (MR) analysis with two-sample summary-level data on genetic variants as instrumental variables, thus estimating a causal effect. Genetic variants associated with serum IGF-1 at genome-wide significance (GWS) in the UK Biobank study involving 363,228 individuals of European descent were introduced as instrumental variables. Summary-level data on childhood obesity and obesity-related asthma were obtained from genome-wide association studies (GWAS). Here, MR-Egger, inverse-variance weighted (IVW), simple median, weighted median and penalized weighted median methods were used in the MR study. Results showed that there were strong causal associations of IGF-1 with childhood obesity (OR, 1.27; 95% CI 1.01-1.60; $P<0.05$) and obesity-related asthma (OR, 1.22; 95% CI 1.07-1.38; $P<0.005$). In conclusion, A causal association between high IGF-1 levels and high risks of childhood obesity and obesity-related asthma is estimated, which requires further validation in large-scale trials.

Introduction

Childhood obesity has become a global health issue due to rapid increases in the incidence and mean body mass index (BMI) of children and adolescents, which poses huge burdens on global public health and the economy (1). Obesity during childhood probably persists into adulthood, resulting in adverse health effects and high risks of heart diseases, metabolic syndromes, psychosocial comorbidities and cancers(2).

As a common chronic disease, asthma is estimated to affect 300 million children worldwide. Notably, the concern of obesity-related asthma has been on the rise with the sharply increased incidence of obesity(3). A thorough understanding of the pathogenesis contributes to formulating effective therapeutic strategies. Several factors are found to influence the pathogenesis of obesity-related asthma, including the inflammatory response, obesity-induced lung dysfunction, changes in the intake of macronutrients and micronutrients, and poor lifestyle (4). In addition, obesity causes immune system dysfunction, in which adipocytokines (e.g., leptin) have been considered the pathogenic basis for obesity-related asthma. In contrast to the pathogenesis, pathophysiology, and pulmonary dynamics of allergic asthma, obesity-related asthma is usually not linked with an allergic inflammatory response. Accumulating evidence has shown that conventional treatment of asthma is generally less effective in patients with obesity-related asthma, and therefore, novel effective therapeutic strategies are needed(5-7).

Insulin-like growth factor-1 (IGF-1) is a growth hormone (GH) that is structurally homologous to insulin. Circulating IGF-1 is mainly secreted by the liver and regulated by GH and relevant signaling pathways. Functionally, IGF-1 mediates the growth of bones and muscles, neuronal survival, lipolysis, hypoglycemic and lipid-lowering effects, and anti-inflammation(8, 9). Most likely due to residual confounding, the function of IGF-1 in childhood obesity and obesity-related asthma remains controversial(10-12). Clarifying the causal association of IGF-1 levels with childhood obesity and obesity-related asthma contributes to providing new avenues for the prevention and management of obesity. Well-designed studies are required to avoid biases produced in observational studies.

Mendelian randomization (MR) is a novel genetic epidemiological approach that assesses the causal effect of exposure on outcomes with genetic variants as instrumental variables. Due to genetic variant inheritance, MR analysis estimates the exposure-outcome interaction by lowering the likelihood of potential confounding factors and reversing causal biases. Similar to a randomized controlled trial (RCT), MR analysis is frequently used to evaluate the causal association between modifiable factors and onset of an illness. In the present study, we performed a multivariable MR analysis to estimate the causal association of serum IGF-1 with childhood obesity and obesity-related asthma.

* Corresponding author. Email: qjr7@163.com; zhouchenvv0126@126.com

Materials and Methods

Study design

Multivariable MR analysis was performed to estimate the causal association of IGF-1 with childhood obesity and obesity-related asthma, in which two-sample summary-level data on genetic variants were considered as instrumental variables. Three important hypotheses require to be confirmed(13). First, the selected instrumental variables should be directly related to serum IGF-1 levels. Second, instrumental variables were independent of any potential confounders that may affect exposures and outcomes. Third, the selected instrumental variables affected childhood obesity and obesity-related asthma only by mediating serum IGF-1 levels. Being approved by the Ethics Committee, written informed consent was obtained prior to the study (Fig1).

Data source

The GWAS dataset provided reliable data for MR analysis. Data on genetic variants associated with serum IGF-1 levels were obtained from the UK Biobank involving 363,228 individuals of European descent, and genetic variants associated with circulating lipids were collected from a recently published GWAS analysis(14). A total of 430 independent SNPs at a genome-wide significant level ($P<5\times10^{-8}$) were included as genetic variants. The F-statistic of each SNP, ranging from 29-1478, was calculated as follows: $F=(\beta/\text{SE})^2$, where β represents the effect size, and SE represents the standard error of the estimator. Summary-level data on childhood obesity (ID: ieu-a-1096) were available from the GWAS dataset, and obesity-related asthma (ID: finn-b-ASTHMA_OBESITY) was available from the FinnGen biobank dataset(15). The former dataset included 5530 cases of childhood obesity with a minimum of 95% BMI before the age of 18Y and 8318 control cases with 2,442,739 genetic variants. The latter dataset included 4142 cases of obesity-related asthma and 135,449 control cases with 16,379,879 genetic variants.

Statistical analysis

MR–Egger, random-effects and fixed-effects IVW, simple median, weighted median and penalized weighted median methods were used in the MR study. Inverse-variance weighting was the most commonly applied MR analysis. With all valid instrumental variables and taking the inverse variance of an individual study as weights, IVW estimated the weighted mean of effect sizes, in which the intercept term was not considered in the regression model. MR–Egger was similar to IVW but added the intercept term. It is usually applied to evaluate horizontal pleiotropy, which is interpreted by the intercept. The weighted median represented the median of a weighted empirical density function, in which a causal estimator was obtained if at least half of the instruments were valid(16-18). A casual association with a significant difference was established if the p-value of IVW was less than 0.05; significant differences were not obtained in the direction of the estimates between the IVW, MR–Egger and weighted median methods; and the p-value of MR–Egger was greater than 0.05. Similar to that in a meta-analysis, sensitivity was analyzed by analyzing the estimation of remaining data with one SNP removed each time. The odds ratio (OR) and corresponding 95% confidence interval (CI) were cal-

culated. The two-sample multivariable MR analysis and IVW method were performed using the MR packages in R 3.4.2. A significant difference was set at $p<0.05$(19).

Results

Influences of serum IGF-1 levels on childhood obesity

Using the IVW method, a positive correlation was identified between genetic variants associated with serum IGF-1 and childhood obesity (OR, 1.275; 95% CI 1.01-1.60; P=0.039). A similar causal association was identified by the IVW fixed effects meta-analysis (OR, 1.275; 95% CI 1.025-1.588; P=0.029), which was supported by the MR–Egger regression (OR: 1.049, 95% CI 0.557-1.977; P=0.605), although a significant difference was not detectable considering the possible effect of horizontal pleiotropy (Fig2A). However, evidence to support a causal association between IGF-1 levels and risk of childhood obesity was sufficient according to the MR analysis. To validate the robustness of our findings, the included SNPs of serum
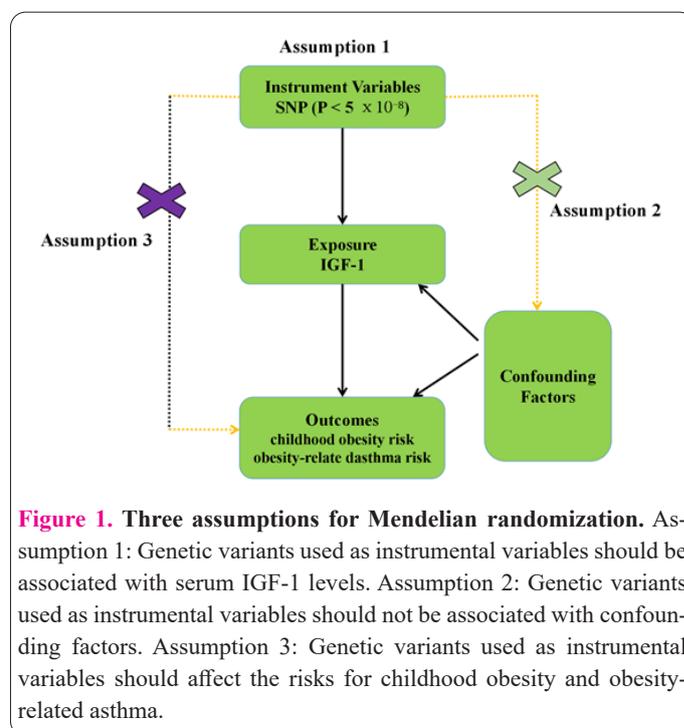

Figure 1. Three assumptions for Mendelian randomization. Assumption 1: Genetic variants used as instrumental variables should be associated with serum IGF-1 levels. Assumption 2: Genetic variants used as instrumental variables should not be associated with confounding factors. Assumption 3: Genetic variants used as instrumental variables should affect the risks for childhood obesity and obesity-related asthma.
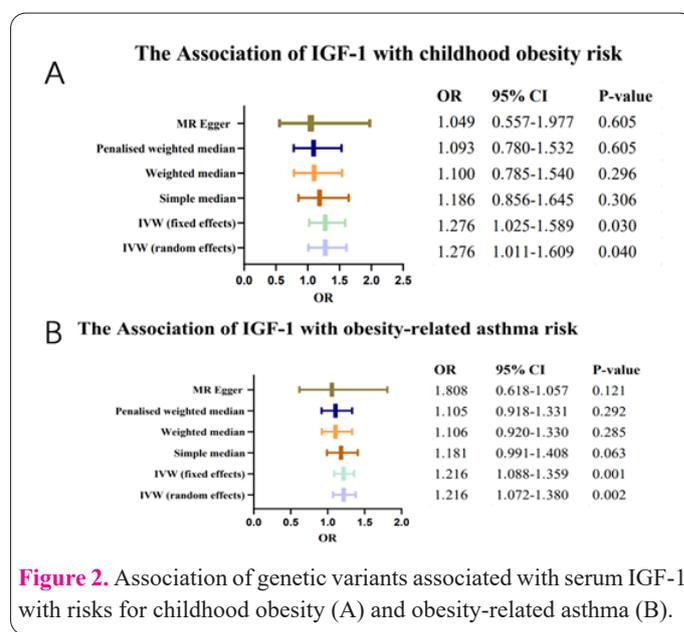

Figure 2. Association of genetic variants associated with serum IGF-1 with risks for childhood obesity (A) and obesity-related asthma (B).

IGF-1 were further subjected to MR–Egger. Neither horizontal pleiotropy nor potential biases were determined by MR–Egger and funnel plots, respectively (Fig3 A, C and Fig4A). Taken together, we have validated a causal association of IGF-1 with childhood obesity.

Influences of serum IGF-1 levels on obesity-related asthma

We similarly explored the influences of serum IGF-1 levels on obesity-related asthma. The IVW data revealed a positive correlation between genetic variants associated with serum IGF-1 levels and obesity-related asthma (OR, 1.216; 95% CI 1.072-1.380; P=0.002). The causal association between them was also validated by the IVW fixed effects meta-analysis (OR, 1.216; 95% CI 1.088-1.360; P=0.001), which, however, was not detected by MR–Egger regression (Fig2 B). Neither horizontal pleiotropy of SNPs of serum IGF-1 nor potential biases were determined by MR–Egger and funnel plots (Fig3 B, D and Fig4B), respectively, suggesting that a causal association of IGF-1 with obesity-related asthma was proven with strong robustness.

Discussion

IGF-1 is gradually up-regulated with aging and peaks after puberty. However, the correlation between IGF-1 and childhood obesity remains controversial. Aino et al. reported that the percentage of body fat in children is positively correlated with serum IGF-1, which is linked with diet, physical activities and sedentary behavior(20). In a large-scale longitudinal intervention study, serum IGF-1 is comparable between normal-weight children and obese children, which is still similar in obese children after weight loss(21). After the interventions of moderate-intensity aerobic exercises and daily dietary for 4 weeks, body fat and waist circumference in 9 Chinese obese teenagers with 18-19Y of age and 30 Chinese obese girls with 14-16Y. Their glucose and lipid metabolism are also greatly ameliorated. Moreover, serum IGFBP-3 levels decrease with increased IGF-1/IGFBP-3 levels, although the total IGF-1 levels remain similar(22). The heterogeneity of IGF-1 levels is mainly attributed to the influence of obesity-related factors on the IGF regulatory system like the distribution of adipose tissues, regulation of glucose metabolism, hyperinsulinemia and free fatty acids. In addition, it can be even more influenced by the phenotypic traits of subjects, such as obesity, age, sex, and race. Differences in the normal range of IGF-1 levels also contribute to the controversial conclusion. As a result, multiple factors and their combination altogether confuse the role of IGF-1 levels in obese people. Here, we performed the first MR analysis on the causal association between genetically predicted IGF-1 levels and childhood obesity. Our data revealed that high serum IGF-1 levels predicted a high risk of childhood obesity. We screened SNPs with GWS and independent inheritance as instrumental variables to estimate the causal exposure-outcome association, thus providing valuable epidemiological evidence for the intervention of childhood obesity. Our findings should be further validated in clinical populations to acquire sufficient clinical evidences.

Many diseases are correlated with obesity, especially asthma. Currently, childhood obesity has been identified to significantly influence lung function. It is well known
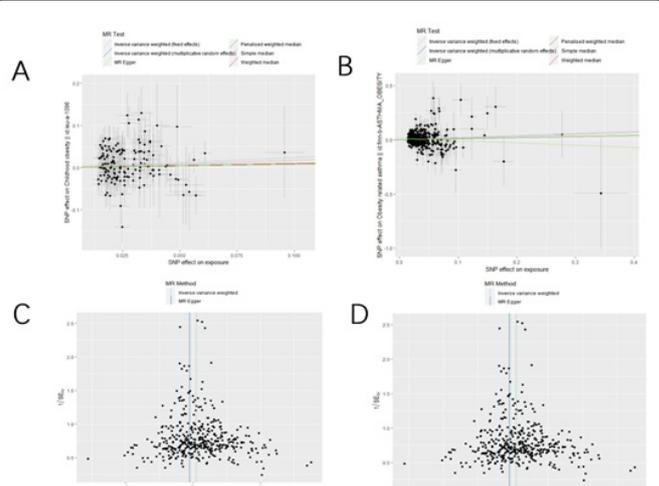

Figure 3. Scatter plots of genetic variants associated with serum IGF-1 with risks for childhood obesity (A, C) and obesity-related asthma (B, D).
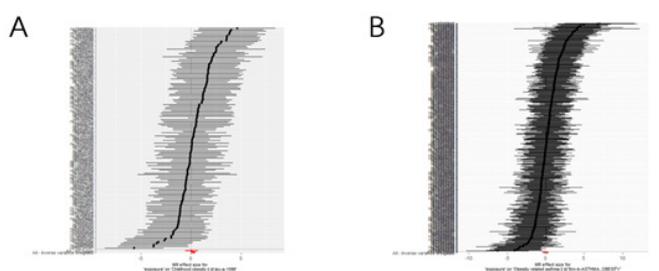

Figure 4. Forest plots of the causal effect of serum IGF-1 on childhood obesity (A) and obesity-related asthma (B).

that lung function is an extremely important diagnostic criterion in asthma(23). Obese children tend to have worse disease control of asthma and quality of life than those of normal-weight children(3, 7, 23, 24). In our research, higher serum IGF-1 levels may predict a higher risk of childhood obesity. To date, the correlation between IGF-1 and obesity-related asthma has been less studied, especially in population-based epidemiological studies. In this study, a causal association of IGF-1 with obesity-related asthma was proven with strong robustness. Consistent with our findings, a case-control study illustrated a positive correlation between serum IGF-1 and susceptibility to asthma(25). Bronchial biopsy showed the upregulated IGF-1 in asthma patients, which is closely linked with subepithelial fibrosis, suggesting the role of IGF-1 in airway inflammation and remodeling(26). A prospective study demonstrated that body weight gain is a risk factor for airway hyperresponsiveness (AHR), which is a typical manifestation of asthma(27). IGF-1 contributes to aggravating airway inflammation by inhibiting the phagocytosis of apoptotic cells and stimulating the release of inflammatory components by apoptotic cells(28). As a special type of asthma, highly specific therapeutic strategies for obesity-related asthma are needed. Immune factors are of great significance in obesity-related asthma. Interventions targeting immune factors (e.g., gut microbiota, immune modulators) are expected to effectively control obesity-related asthma(29). Our data validated that genetically predicted IGF-1 was a risk factor for obesity-related asthma, serving as a vital biomarker for its prevention and treatment. Thus, we speculated that IGF-1 is involved in the pathogenesis of obesity-related asthma. Basic mechanistic research

and clinical observational research are required to further prove our findings.

Several strengths of the present study were highlighted. Firstly, it was an MR study that minimized residual confounding and biases produced by reverse causality, thus strengthening the causal association of serum IGF-1 levels with childhood obesity and obesity-related asthma. Second, genetic testing of IGF-1 was effective, which has been previously applied to ensure robust findings. In addition, using genetic variants associated with serum IGF-1 as instrumental variables (F-statistic>10) greatly lowered the possibility of biased causal estimates from the observed associations. Third, more than 400 SNPs of IGF-1 were screened, which reduced the likelihood of biases produced by invalid SNPs. However, some limitations should be considered. Genetic data were extracted from an online dataset involving individuals of European descent. Although principal components of the top 40 genotypes were adjusted to prevent deviations in population structure, whether our findings could be applied to other populations remains unclear. In addition, we did not comprehensively analyze sequencing data on childhood obesity, such as methylation modifications and other epigenetic regulations, and the interaction between genes and environmental exposures may also influence the association of serum IGF-1 levels with childhood obesity and obesity-related asthma.

In conclusion, a causal association between high IGF-1 levels and high risks of childhood obesity and obesity-related asthma is estimated. We speculated that obesity is a vital risk factor for childhood asthma due to its effect on IGF-1. In-depth studies are needed in the future to validate our findings.

Competing interests
The authors report no conflict of interest.

Authors contributions
Jirong Qi and Chen Zhou conceived and designed the project. Tianyi Zhu, and Hao Liu wrote and revised the paper. Lan Ling generated the data. Yang Wang analyzed the data. Chao Jin supervised this research. Tianyi Zhu and Hao Liu contribute equally in this work. All authors gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Funding
Not applicable.

Acknowledgements
Not applicable.

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