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ARTICLE

Pediatrics

Causal relationships between adiposity and childhood asthma: bi-directional Mendelian Randomization analysis

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Abstract

Background/objectives Obesity and asthma are common chronic diseases and have been reported to be mutually causative. We investigated the causal direction of the relationship between adiposity and asthma using genetic markers as instrumental variables (IVs) in bi-directional Mendelian randomization (MR) analysis.

Subjects/methods We used data from the Taiwan Children Health Study with 24 body mass index (BMI)-single-nucleotide polymorphisms (SNPs, combined into a weighted allelic score) and 16 asthma-SNPs (combined into two weighted allelic scores, separately for asthma inflammatory and antioxidative genes) to yield genetic IVs for adiposity and asthma, respectively.

Results The weighted allele score for BMI was strongly associated with adiposity ($p = 2 \times 10^{-16}$) and active asthma ($p = 0.03$). The two-stage least square regression risk ratio (RR) for the effect of BMI on asthma was 1.04 (95% confidence interval: 1.00–1.07, $p = 0.03$). Although the weighted asthma genetic scores were significantly associated with asthma ($p = 8.4 \times 10^{-3}$), no association was seen for genetically instrumented asthma with BMI using MR. Central obesity was the most accurate predictor of asthma. Adiposity showed higher causal effects on asthma in boys and children with non-atopic asthma. Sensitivity analysis for MR revealed no directional genetic pleiotropy effects. The causal effect RRs of BMI on asthma were 1.04, 1.08, and 1.03 for inverse-variance weighted, MR–Egger regression (slope), and weighted median methods, respectively, all in accordance with the MR estimates.

Conclusions High adiposity may lead to asthma, whereas the effects of asthma on adiposity accumulation are likely to be small.

Introduction

Obesity and asthma are common chronic diseases in children that have been reported to be highly linked. Although most studies have supported that obesity antedates asthma and a meta-analysis of prospective studies [1] reported an increased incidence of asthma in obese children, the causal

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direction between the two diseases remains debatable [2]. Asthma is regarded as a barrier to children's physical activity that might lead to adiposity accumulation. A 10-year follow-up study revealed that children with asthma had a 51% increased risk of developing obesity in adolescence [3]. Moreover, possible obesogenic effects from long-term use of corticosteroid have been hypothesized as the reasons for the increased risk of obesity in individuals with asthma.

Mendelian randomization (MR) can provide a high evidence of causality mimicking randomized controlled trials. In MR analysis, genetic instrumental variables (IVs) act as proxies for an exposure, do not change with time or in response to disease, and are independent of confounders. If variants only affect the outcome through the selected exposure variables, then genetic IVs can be valid instruments. Using bi-directional MR, many previous literatures elucidate direction of causation between obesity and relevant factors [4, 5]. For example, by using 21 adult cohorts, Vimaleswaran et al. suggests that a higher body mass index (BMI) leads to lower Vitamin D level, but not vice versa [5]. In another reciprocal MR study, Timpson et al. discovered that the association between C-reactive protein level and BMI is likely to be driven by BMI, with CRP being a marker of higher BMI [4].

Through genetic IVs and MR analysis, Granell et al. have determined that a high BMI increased the risk of asthma in childhood [6]. However, they did not examine the possible causal pathway from asthma to adiposity accumulation. Moreover, the 32 BMI-related single-nucleotide polymorphisms (SNPs) used for genetic IVs comprised several shared SNPs for both obesity and asthma [7], making the pleiotropic effects of these genetic IVs a serious concern.

To determine the causal relationship between adiposity and asthma, we conducted a bi-directional MR analysis. The goals of this study were: (1) to investigate the causal direction between adiposity and asthma; (2) to examine the causal effect of different adiposity measures on asthma; and (3) to assess whether the strength of the effect varies with different sex or atopy status. To avoid the effect of selection bias for genetic variants with pleiotropic effects on the outcome, an MR sensitivity analysis was also performed.

Methods

The analysis herein involved children of the Taiwan Children Health Study, from the second cohort with an open-cohort design [8]. Details of cohort establishment were described in online Supplementary File. The repeated measures of adiposity indicators of the 10–11-year-old children were used for more reliable adiposity measures. Informed consent was obtained from all participating parents and children. The oral mucosa of each enrolled child was collected for genotyping. The present study was approved by the Institutional Review Board of National Taiwan University Hospital and followed the principles outlined in the Declaration of Helsinki.

Adiposity measures and asthma definition

Adiposity measures were recorded annually at schools during our research team visits, as described previously [9]. Central obesity indicator was determined by waist/hip ratio. BMI was converted into BMI *z*-scores, according to the WHO Growth Standards [10]. For a comprehensive comparison between different adiposity measures, each adiposity measure (except BMI) was transformed into *z*-scores using sex-specific and age-specific means and standard deviations from our cohort reference [9].

Asthma was defined in children by asking two questions from the parent-reported questionnaire [11]: "Has a doctor ever diagnosed your child as having asthma?" and "Did your child ever experience difficulty breathing, or did you observe any wheezing or whistling from his or her chest in the past 12 months?" If the answers were "Yes" to both questions, we classified the child as having active asthma. We classified the asthmatic children into atopic or non-atopic groups using fractional exhaled nitric oxide (Fe_{NO}) measurements, with a cut-off value of 20 ppb [12].

Genotyping

The 24 BMI-SNPs were identified by either Asian Genome-Wide Association Studies (GWAS) meta-analysis [13], Chinese GWAS [14], or Chinese children studies [15, 16]. The 16 asthma-SNPs were selected on the basis of GWAS [17], Chinese studies [18], and our previous findings [19, 20]. SNPs previously reported to be associated with both obesity and asthma were excluded to avoid the pleiotropic effects of genetic IVs. Tables E1 and E2 present the detailed references of candidate gene selection. The following criteria were used to identify the candidate SNPs: (1) SNPs exhibited a minor allele frequency of ≥5%; (2) the genotyping call rate was higher than 98% for all children; (3) SNPs having a linkage disequilibrium with candidate SNPs were not selected; and (4) the primers for SNPs were designed using the National Genotyping Center of Academia Sinica (http://lims.ngc.sinica.edu.tw/service/) platform [21]. The Supplementary File presents the detailed procedure.

Weighted genetic scores composed of BMI-SNPs and asthma-SNPs were used as IVs. The dosage of effect allele was multiplied by the regression coefficients of each gene on associated traits divided by the mean value of all

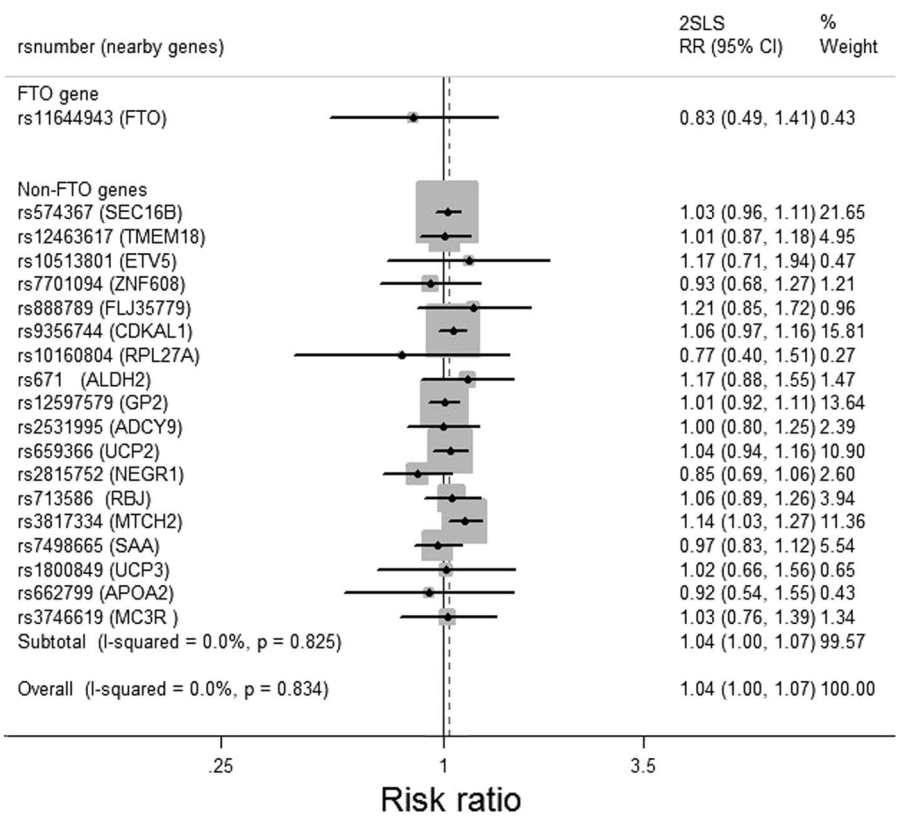

Fig. 1 Meta-analysis of individual causal effect of BMI-SNPs on active asthma at 10–11 years. rs11191580, rs651821, rs662799, rs764730, and rs9816226 were omitted from the forest plot because the corresponding standard error (SE) was >0.5

regression coefficients. In additional analysis, 24 BMI-SNPs were divided into a fat mass and obesity (*FTO*)-associated gene and 23 non-*FTO* genes. Furthermore, 16 asthma-SNPs were divided into 8 SNPs each for inflammatory and antioxidative genes, according to the causative mechanisms of asthma.

Statistical analysis

To satisfy the IV assumption that the genotype should be (1) associated with the intermediate trait, (2) independent of the unmeasured confounders for the intermediate trait–outcome association, and (3) independent of the outcome conditioned on the intermediate trait (no pleiotropic effects on outcomes), we conducted a linear regression analysis for investigating the association between BMI-SNPs and z-BMI (Table E1), asthma-SNPs and z-BMI (Table E2), BMI-SNPs and confounders (Table E3), and asthma-SNPs and confounders (Table E4). For binary outcomes, such as active asthma, the logistic regression analysis was used to derive the risk ratio (RR) per allele change in both BMI-SNPs and asthma-SNPs (Tables E1 and E2). *F*-statistics were used to verify the association between genotypes and intermediate traits and the strength of these genetic IVs. The basic characteristics of each SNP, including the minor allele frequency and *F*-statistics, were evaluated with Plink v2.0.

For those missing data in variables such as adiposity measures, and confounders such as family history of atopy and in utero smoking, we performed multiple imputation method [22] by fully conditional-specific regression method, assuming the data were missing at random.

Generalized estimating equations (GEE) were implemented for the repeated measures of adiposity measures and asthma outcomes in children, accounting for the within-individual variance. Various potential confounders were incorporated into GEE models, including age, sex, parental educational levels, family income, a family history of atopy, and child's breastfeeding status and exposure to in utero maternal smoke, as described previously [11].

We performed an instrumental-variable regression analysis, the two-stage least squares (2SLS) method [23] to examine whether the BMI-genetic scores were associated with asthma through its associations with BMI and vice versa. 2SLS is a widely used MR method that can be used for binary exposure and outcomes. The first stage is the regression of intermediate traits on genetic scores, generating the predicted values of intermediate traits. The second stage is the regression of the outcome (active asthma or z-BMI) on the predicted intermediate traits. Meta-analysis and forest plot analysis were conducted to examine the existence of pleiotropy among each selected genetic variant and their relationships with the outcome (Figs. 1, 2). In addition, to maximize the robustness of the causal

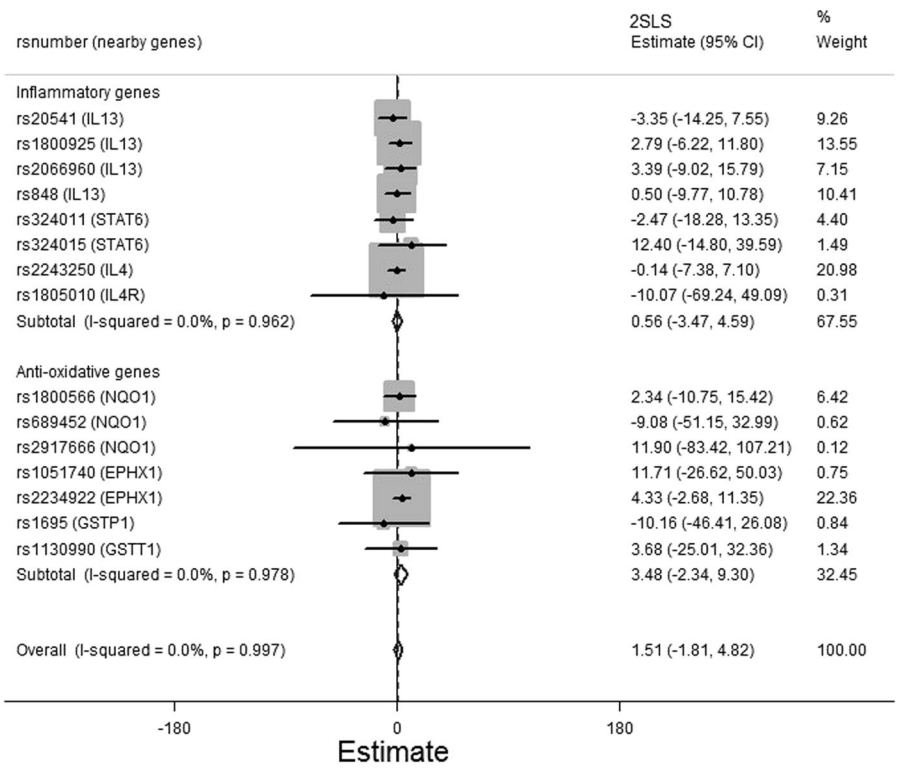

Fig. 2 Meta-analysis of individual causal effect of asthma-SNPs on z-BMI at 10–11 years. rs2239892 was omitted from the forest plot because the corresponding SE was >100

inference from the MR analysis and our multiple genetic variants, sensitivity analysis were performed [24]. Scatter plots were constructed to assess the heterogeneity of the genetic associations with the outcome (Fig. E1). Funnel plots were implemented to evaluate directional pleiotropy effects (Fig. E2). Table E5 (Supplementary File) presents the details of the sensitivity analysis. Statistical significance was inferred at $p < 0.05$. All model regression and sensitivity analysis were performed using R3.3.2. The forest plots were derived from Stata 11.0 (Stata Corporation, College Station, TX, USA).

Results

The study population comprised 2525 children at 10 years of age and 2613 children at 11 years of age (Table 1). The prevalence rates of active asthma were 5.5 and 4.8% among 10 and 11-year-old children, respectively. The percentage of non-atopic asthma among all asthma cases were 55.1% for children at 10 years and 67.9% for children at 11 years. The adiposity measures, such as BMI, total body fat, and fat-free mass, gradually increased at 10–11 years.

Genetic associations

Most of the 24 BMI-SNPs were associated with z-BMI at 10–11 years (Table E1), and little evidence was obtained on the associations of individual BMI-SNPs with active asthma and confounders (Tables E1 and E3). The weighted genetic scores of the 24 BMI-SNPs were robustly correlated with z-BMI (Table 2, $p = 2 \times 10^{-16}$). On the other hand, 16 asthma-related SNPs were not associated with z-BMI (Table E2) and confounders (Table E4). The weighted genetic scores for asthma were significantly correlated with active asthma (Table 2, $p = 8.4 \times 10^{-3}$).

Adiposity causes asthma but asthma does not lead to adiposity accumulation

Table 2 presents the coefficients of the bi-directional MR analysis. The weighted genetic scores of the 24 BMI-SNPs exhibited a positive association with z-BMI (per unit increase in regression coefficient: 2.70, 95% confidence interval [CI]: 2.21–3.18, $p = 2 \times 10^{-16}$). Even after the omission of the well-known obesity gene, *FTO*, the remaining 23 BMI-SNPs were significantly associated with z-BMI (per unit increase in regression coefficient: 2.64, 95% CI: 2.16–3.11, $p = 2 \times 10^{-16}$). Although the weighted asthma genetic scores were significantly associated with asthma ($p = 8.4 \times 10^{-3}$), no association was seen for genetically instrumented asthma with BMI using MR, and neither the asthma inflammatory nor the anti-oxidative genes were associated with z-BMI.

In the two-stage IV analysis for the direction and causality of the adiposity–asthma association, BMI was

associated with the risk of asthma (per unit increase in regression coefficient: 0.04, 95% CI: 0.003–0.07, $p = 0.03$). However, these analyses provided little evidence on the causal effect of asthma on z-BMI ($p = 0.55$). The causal effect estimates were similar after omitting the *FTO* locus from the genetic scores (Tables 2 and E6).

Table 1 Characteristic of study participants in Taiwan Children Health Study

Characteristics	Children at 10 years ($n = 2525$)	Children at 11 years ($n = 2613$)
Age, years	10.6 ± 0.5	11.7 ± 0.5
Male sex	1259 (49.8)	1298 (49.7)
Breast feeding	880 (50.1)	952 (51.7)
Parental education		
High school or below	1178 (46.7)	1341 (51.3)
College or university	1103 (43.7)	1057 (40.5)
Post-graduate school	244 (9.7)	215 (8.2)
Family income		
<600,000	1101 (43.6)	1241 (47.5)
600,001–1,000,000	964 (38.2)	913 (34.9)
>1,000,001	460 (18.2)	459 (17.6)
Family history of atopy	600 (34.1)	627 (34.0)
In utero smoking	68 (3.7)	56 (2.1)
Adiposity measures		
BMI (kg/m^2)	19.3 (3.8)	20.4 (4.1)
Sum of skinfolds (mm)	37.3 (12.3)	33.7 (11.1)
Waist-to-hip ratio	0.82 (0.1)	0.78 (0.2)
Total body fat (kg)	7.9 (5.3)	9.6 (5.9)
Fat-free mass (kg)	33.5 (5.9)	36.4 (6.3)
Active asthma	136 (5.5)	109 (4.8)
Atopic asthma	46 (2.1)	32 (1.5)
Non-atopic asthma	75 (3.4)	74 (3.4)

All data are presented as mean ± SD or numbers (%)

The number of participants did not add up to the total number because of missing data

We further performed sensitivity analysis to examine the robustness of the causal inferences from MR analysis. The causal estimates from 2SLS of each genetic instrument were meta-analyzed to examine the small-study bias caused by pleiotropy (Figs. 1, 2). There was little evidence on the heterogeneity of the genetic instruments in both BMI-SNPs ($I^2 = 0$, $p = 0.83$) and asthma-SNPs ($I^2 = 0$, $p = 0.99$). The causal effect summary RR of the 24 BMI-SNPs on active asthma (Fig. 1) was identical to the weighted genetic score 2SLS RR (RR = 1.04, 95% CI: 1.00–1.07). By contrast, the causal effect summary RR of the 16 asthma-SNPs on z-BMI was non-significant (Fig. 2). The additional approaches of sensitivity analysis also provided evidence of a positive causal relationship between BMI and asthma (Table E6). The causal effect RRs of asthma for per one unit increase in z-BMI were 1.04, 1.08, and 1.03 for the inverse-variance weighted, MR–Egger regression (slope), and weighted median methods, respectively, all in accordance with the previous MR estimate of 1.04 (95% CI: 1.00–1.07, $p = 0.03$). Moreover, points in the scatter plot, which represented 24 BMI-SNPs, did not deviate much from the line under the null (Fig. E1), indicating the absence of any notable genetic pleiotropic effects. The symmetric funnel plot (Fig. E2) also confirmed the absence of any directional pleiotropy effects.

Central obesity is the most accurate predictor of childhood asthma

Table 3 presents the cross-sectional analysis of various adiposity measures with respect to asthma outcomes. Most of the associations between adiposity measures and active asthma were non-significant after adjustment for confounders. However, the estimated 2SLS RR for the causal effects of BMI on active asthma at 10–11 years was 1.04 (95% CI: 1.00–1.07; Table 4). Among the various adiposity measures, the waist/hip ratio, a central obesity indicator, was the strongest predictor (RR: 1.17, 95% CI: 0.99–1.40) of asthma in the IV analyses. The estimated RRs for the causal effects of the sum of skinfolds and

Table 2 Summary of coefficients used for bi-directional Mendelian randomization analysis

Instrumental variables (IV)	Genetic score with intermediate trait		Genetic score with outcomes		Two-stage IV analysis (asthma or z-BMI)	
	Coefficient (95% CI)	*p*-value	Coefficient (95% CI)	*p*-value	Coefficient (95% CI)	*p*-value
BMI genetic score (24 SNPs)	2.70 (2.21, 3.18)	2×10^{-16}	0.10 (0.01, 0.18)	0.03	0.04 (0.003, 0.07)	0.03
FTO gene (1 SNP)	2.00 (−2.81, 6.80)	0.42	−0.45 (−1.31, 0.40)	0.30	−0.18 (−0.71, 0.34)	0.49
Non-FTO genes (23 SNPs)	2.64 (2.16, 3.11)	2×10^{-16}	0.10 (0.01, 0.18)	0.03	0.04 (0.004, 0.07)	0.03
Asthma genetic score (16 SNPs)	0.09 (0.02, 0.16)	8.4×10^{-3}	0.14 (−0.30, 0.58)	0.55	1.50 (−3.46, 6.46)	0.55
Inflammatory genes (8 SNPs)	0.05 (0.00, 0.11)	0.05	−0.05 (−0.39, 0.30)	0.79	−0.89 (−7.62, 5.84)	0.80
Anti-oxidative genes (8 SNPs)	0.04 (−0.01, 0.08)	0.08	0.18 (−0.10, 0.45)	0.21	4.60 (−4.14, 13.33)	0.30

Table 3 Associations between adiposity measures and asthma outcomes in children at 10–11 years, Taiwan Children Health Study

Adiposity measures (*z*-score)	Active asthma		Non-atopic asthma		Atopic asthma	
	Adjusted RR (95% CI)[a]	*p*-value	Adjusted RR (95% CI)[a]	*p*-value	Adjusted RR (95% CI)[a]	*p*-value
BMI	1.11 (1.01, 1.23)	0.04	1.16 (1.02, 1.32)	0.02	1.04 (0.88, 1.23)	0.66
Males	1.20 (1.06, 1.35)	5×10^{-3}	1.26 (1.07, 1.49)	0.01	1.08 (0.88, 1.32)	0.47
Females	0.95 (0.80, 1.14)	0.60	1.01 (0.81, 1.25)	0.96	0.89 (0.63, 1.27)	0.52
p for interaction[b]		0.29		0.16		0.93
Sum of skinfolds	1.12 (0.99, 1.28)	0.07	1.19 (1.02, 1.39)	0.03	1.12 (0.90, 1.39)	0.33
Males	1.28 (1.09, 1.49)	3×10^{-3}	1.47 (1.21, 1.80)	1×10^{-4}	1.08 (0.83, 1.40)	0.56
Females	0.90 (0.73, 1.12)	0.36	0.87 (0.67, 1.13)	0.28	1.14 (0.76, 1.71)	0.53
p for interaction[b]		0.52		0.42		0.39
Waist/hip ratio	1.08 (0.98, 1.20)	0.10	1.12 (1.01, 1.24)	0.04	1.03 (0.86, 1.24)	0.75
Males	1.08 (0.97, 1.20)	0.15	1.12 (1.00, 1.26)	0.04	0.96 (0.72, 1.28)	0.79
Females	1.10 (0.84, 1.43)	0.49	1.13 (0.83, 1.54)	0.44	1.27 (0.82, 1.98)	0.29
p for interaction[b]		0.13		0.05		0.48
Total body fat	1.11 (0.98, 1.25)	0.11	1.12 (0.96, 1.31)	0.13	1.18 (0.96, 1.45)	0.12
Males	1.20 (1.03, 1.40)	0.02	1.28 (1.05, 1.55)	0.01	1.18 (0.92, 1.51)	0.20
Females	0.95 (0.76, 1.18)	0.62	0.92 (0.70, 1.20)	0.52	1.14 (0.76, 1.70)	0.52
p for interaction[b]		0.44		0.54		0.20
Fat-free mass	1.07 (0.94, 1.23)	0.29	1.05 (0.89, 1.25)	0.54	1.16 (0.92, 1.46)	0.21
Males	1.17 (0.98, 1.38)	0.08	1.17 (0.94, 1.45)	0.16	1.17 (0.88, 1.54)	0.28
Females	0.93 (0.74, 1.16)	0.52	0.91 (0.70, 1.19)	0.49	1.13 (0.73, 1.74)	0.59
p for interaction[b]		0.71		0.96		0.30

[a]Models were adjusted for age, sex, parental education, family income, family history of atopy, breastfeeding, and in utero smoking

[b]Test for null hypothesis that RR in males is the same as the RR in females

total body fat were 1.06 (95% CI: 1.00–1.12) and 1.05 (95% CI: 1.00–1.10), respectively.

Adiposity shows higher causal effects on asthma in boys and children with non-atopic asthma

The adjusted associations between z-BMI and active asthma were stronger in boys than in girls (1.20 [95% CI: 1.06–1.35] vs. 0.95 [95% CI: 0.80–1.14]; Table 3). Consistent with previous study findings [6], the associations were stronger for non-atopic asthma than for atopic asthma. The estimated 2SLS RRs were significantly higher in boys than in girls for all adiposity measures (Table 4). Furthermore, subgroup analysis revealed that the causal effect RRs were higher for non-atopic asthma than for atopic asthma across all adiposity measures.

Discussion

In accordance with previous findings [6], the present study suggests that higher adiposity leads to asthma. However, the causal effects of asthma on adiposity accumulation in children was less likely. Moreover, among various adiposity measures, central obesity is the strongest predictor of asthma in the present study. The causal effects of adiposity on asthma were higher in boys than in girls with significant sex interactions and were more significant in non-atopic asthma than in atopic asthma. Sensitivity analysis for MR estimates also strengthened the robustness of our findings with similar causal effect estimates.

Few studies have reported the development of obesity in children with asthma [2, 3]. Several proposed pathophysiologies of this hypothesis include reduced physical activity levels among individuals with asthma and potential adverse effects from inhaled corticosteroid medications [25]. Although Chen et al. observed that children with asthma are at an increased risk of obesity [3], they did not provide adequate physical activity level measurements. Similarly, Hasler et al. reported that asthma is associated with increased subsequent weight gain without considering the temporal changes between asthma and physical activity levels [2]. In contrast to previous pediatric studies [26], children with asthma were not inactive compared with those without asthma. In clinical practice, exercise is recommended to all individuals with asthma. Low physical activity, which might be a consequence of adiposity, has been proved to be a risk factor for new-onset asthma in

Table 4 Instrumental variable estimates of adiposity measures on asthma outcomes in children at 10–11 years, Taiwan Children Health Study

Adiposity measures (*z*-score)	Active asthma		Non-atopic asthma		Atopic asthma	
	2SLS RR (95% CI)	*p*-value	2SLS RR (95% CI)	*p*-value	2SLS RR (95% CI)	*p*-value
BMI	1.04 (1.00, 1.07)	0.03	1.03 (1.00, 1.06)	0.04	1.02 (1.00, 1.04)	0.11
Males	1.06 (1.01, 1.12)	0.02	1.07 (1.02, 1.11)	0.01	1.01 (0.98, 1.04)	0.58
Females	1.02 (0.97, 1.06)	0.50	1.00 (0.96, 1.04)	0.96	1.02 (1.00, 1.05)	0.09
p for interaction[a]		0.03		0.08		0.58
Sum of skinfolds	1.06 (1.00, 1.12)	0.04	1.05 (1.001, 1.1)	0.04	1.03 (0.99, 1.06)	0.11
Males	1.09 (0.97, 1.22)	0.16	1.05 (0.96, 1.15)	0.27	1.03 (0.96, 1.11)	0.40
Females	1.04 (0.98, 1.10)	0.19	1.04 (0.99, 1.09)	0.11	1.02 (0.99, 1.05)	0.18
p for interaction[a]		3×10^{-3}		3×10^{-4}		0.94
Waist/hip ratio	1.17 (0.99, 1.40)	0.07	1.15 (0.98, 1.35)	0.09	1.08 (0.97, 1.21)	0.16
Males	1.16 (0.92, 1.47)	0.22	1.11 (0.9, 1.37)	0.32	1.07 (0.91, 1.26)	0.43
Females	1.17 (0.90, 1.52)	0.25	1.2 (0.91, 1.58)	0.20	1.09 (0.94, 1.27)	0.25
p for interaction[a]		0.63		0.45		0.46
Total body fat	1.05 (1.00, 1.10)	0.03	1.04 (1.00, 1.08)	0.05	1.02 (0.99, 1.05)	0.11
Males	1.07 (0.98, 1.17)	0.16	1.04 (0.97, 1.12)	0.26	1.03 (0.97, 1.09)	0.4
Females	1.03 (0.98, 1.08)	0.19	1.04 (0.99, 1.08)	0.10	1.02 (0.99, 1.04)	0.18
p for interaction[a]		0.84		0.03		0.66
Fat-free mass	1.08 (1.004, 1.15)	0.04	1.06 (1.00, 1.13)	0.05	1.04 (0.99, 1.08)	0.11
Males	1.08 (0.97, 1.20)	0.16	1.05 (0.96, 1.15)	0.27	1.03 (0.96, 1.11)	0.40
Females	1.06 (0.97, 1.15)	0.20	1.07 (0.98, 1.16)	0.12	1.03 (0.98, 1.08)	0.19
p for interaction[a]		0.07		0.13		0.66

[a]Test for null hypothesis that RR in males is the same as the RR in females

meta-analysis [27]. However, our previous longitudinal study [11] did not support the direct correlation between asthma and low physical activity. We indicated that both low physical activity levels and obesity are preceding factors for asthma. Moreover, no evidence suggests that asthma medication treatment may contribute to the development of obesity [25]. The evidence supporting the causal pathway from asthma to adiposity accumulation was relatively weak and was in line with our findings in this study.

Most studies on adiposity and asthma have supported the causal direction from adiposity to asthma. Several meta-analyses of prospective children studies have revealed that obesity precedes asthma [1, 28], we also found that central obesity is the strongest predictor of asthma. The possible mechanisms include the mechanical compression of abdominal obesity on the thoracic cavity and the influence of proinflammatory mediators on airway remodeling. Consistent with previous findings [6], we demonstrated that the effects of fat-free mass on asthma were slightly higher than those of total body fat. The possible hypothesis could be that elevated intramyocellular lipids within lean tissue may exert proinflammatory effects [29]. Scott et al. also revealed that neutrophilic airway inflammation was positively associated with lean mass but not fat mass [29]. Additional intervention studies are warranted to reduce central obesity and explore the differential distribution of fat mass and fat-free mass.

In childhood studies on the predictability of obesity on asthma outcomes, a significant debate on sex differences exists due to inconsistent findings [30]. Our finding that adiposity contributes to the increased risk of asthma only in boys was consistent with our previous meta-analysis of six prospective cohorts [1]. In our previous meta-analysis, obese boys had a significant 2.5-fold risk of asthma than obese girls in a dose-dependent manner. Males have disproportionately smaller airway diameters (dysanapsis) [31], and previous studies have revealed that the effects of obesity on pulmonary function are higher in males than in females [32]. By contrast, in Granell's MR study [6], female-specific obesity effects on asthma were inconsistent across different ages and various asthma outcomes. In countries such as Taiwan, where the prevalence of overweightness/obesity is 1.4-fold higher in males than in females [33], parents should focus on reducing childhood obesity in boys, rather than in girls, to prevent asthma.

Most pediatric studies have suggested that obesity is more strongly associated with non-atopic asthma than with atopic asthma [12], which is confirmed by the present findings. The obesity–asthma phenotype, in which obesity promotes the development of Th1-mediated non-atopic

asthma, is unique. A review article indicated that non-atopic asthma has been late-onset and was associated with neutrophilic inflammation, rather than eosinophilic inflammation [34]. Adiponectin, which was inversely associated with obesity, has been shown to inhibit vascular smooth muscle proliferation. Decreased adiponectin levels could further lead to non-allergic airway inflammation. Moreover, truncal obesity, which reduces the compliance of respiratory muscles, may be the non-atopic pathway from obesity to asthma.

The major strengths of our study were population-based children cohort that involved multiple adiposity measures. Unlike GWAS studies in which most of the genotyping were imputed, our study provided the DNA samples for the exact genotyping of each SNP to obtain our multiple genetic IVs. Furthermore, we adopted several new analytical techniques, such as the sensitivity analysis for MR inferences, to overcome difficulties caused by directional pleiotropy. Our findings confirmed the causal direction from adiposity to asthma and are robust in the three distinct statistical analyses (2SLS, inverse-variance weighted, and MR–Egger regression [slope] methods).

Our study was limited by a relatively small sample size for MR analysis; therefore, we used repeated measurements at 10–11 years, which improved the validity and accuracy of the measurements. Furthermore, we used Fe_{NO} levels, rather than serum IgE or the skin prick test, to categorize the children into atopic and non-atopic asthma groups. Fe_{NO}, a surrogate marker of airway inflammation, has been used to categorize atopy status without obvious bias [9, 12]. In addition, our study was limited by the relatively weak instrument of asthma while detecting the effect of asthma on adiposity. Using multiple SNPs to index asthma, we were able to explain more variance in asthma and can address the problem of weak instrument. However, due to the complicated variety of asthma genes reported in GWAS, we were unable to include all asthmatic genes in our genetic IVs. Our candidate asthmatic genes were based on the Chinese susceptible asthma gene, which could still be satisfactory genetic IVs for MR analysis.

Regarding the long-term debate on the causal direction between adiposity and asthma, the present study suggested that obesity is a causal risk factor for asthma. Importantly, this does not fully exclude that asthmatic children may experience adiposity accumulation, and a "vicious cycle" being initiated. Together with the increased incidence of asthma in obese individuals, our study highlighted the importance of future study to target interventions to reduce obesity and their expected asthma reduction effects.

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Author contributions Y-CC contributed the cohort data collection, interpretation of data, and writing. H-YF and Y-TH assisted in the critical part of the statistical analysis, data interpretation, and revised it critically for important intellectual content. S-YH and T-HL contributed to analysis and interpretation of data, critically revising this manuscript for intellectual content. YL reviewed the study design, acquisition of data, interpretation of data, supervised the study, and revised the manuscript critically for important intellectual content. All authors approved the final manuscript as submitted and published, and agreed to be accountable for all aspects of the work.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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