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Childhood Obesity Gene Risk: Intervention Outcome

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Genetic Susceptibility for Childhood BMI has no Impact on Weight Loss Following Lifestyle Intervention in Danish Children

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Objective: This study aimed to investigate the effect of a genetic risk score (GRS) comprising 15 single-nucleotide polymorphisms, previously shown to associate with childhood BMI, on the baseline cardiometabolic traits and the response to a lifestyle intervention in Danish children and adolescents.
Methods: Children and adolescents with overweight or obesity ($n = 920$) and a population-based control sample ($n = 698$) were recruited. Anthropometric and biochemical measures were obtained at baseline and in a subgroup of children and adolescents with overweight or obesity again after 6 to 24 months of lifestyle intervention ($n = 754$). The effects of the GRS were examined by multiple linear regressions using additive genetic models.
Results: At baseline, the GRS associated with BMI standard deviation score (SDS) both in children and adolescents with overweight or obesity ($\beta = 0.033$ [SE = 0.01]; $P = 0.001$) and in the population-based sample ($\beta = 0.065$ [SE = 0.02]; $P = 0.001$). No associations were observed for cardiometabolic traits. The GRS did not influence changes in BMI SDS or cardiometabolic traits following lifestyle intervention.
Conclusions: A GRS for childhood BMI was associated with BMI SDS but not with other cardiometabolic traits in Danish children and adolescents. The GRS did not influence treatment response following lifestyle intervention.

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Introduction

Obesity is a complex condition with multiple contributing factors, such as environmental and lifestyle factors, genetic susceptibility, and alterations in the gut microbiome (1-4), and children with obesity have a greater risk of developing metabolic and cardiovascular disorders (1,5). Obesity is highly heritable, and family, twin, and adoption studies have reported heritability estimates of 40% to 70% for BMI (6-9).

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Author contributions: MH, MF, JCH, and TH conceived the hypothesis regarding the study. JTK, TRHN, JCH, and TH planned and conducted the enrolment, clinical examination, and obesity treatment of study participants. MH, NG, HNK, OP, and TH planned and performed the genotyping, while EVRA, TMS, MH, and YM constructed the genetic risk scores. MH, YM, JTK, TRHN, JR, MØJ, and TH planned and performed statistical analyses, and MH, MF, and TH interpreted the data. MF wrote the initial draft, and all authors contributed with critical revision of the draft. MF and MH wrote the final draft, while TMS was responsible for the revision together with CTH, CFB, and MTL. All authors commented on and approved the final manuscript.

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The largest available genome-wide association meta-analysis comprising up to 47,541 children aged 2 to 10 years identified 15 genome-wide significant loci associated with childhood BMI (10). In a subset of 1,955 children, the authors showed that a genetic risk score (GRS) comprising these 15 loci explained 2.0% of the variance in childhood BMI (10).

It is not known whether children with a high genetic susceptibility for childhood obesity, as assessed by a GRS, will respond differently to lifestyle intervention. Treatment and prevention strategies may be improved by a deeper understanding of the underlying molecular mechanisms of obesity and by early identification of individuals at higher risk for developing obesity and related comorbidities. The heritable genetic influence on BMI increases during childhood, peaks around 20 years of age, and then weakens during adulthood (8,10-14), indicating that genetic variation has its strongest effect on BMI during early life. Nevertheless, children and adolescents are highly influenced by behavioral, social, parental, and environmental factors and may therefore be susceptible to lifestyle interventions (11). In a previous pediatric lifestyle intervention study ($n=168$), a GRS comprising nine BMI susceptibility loci was inversely associated with changes in BMI standard deviation score (SDS) and fat mass. However, the sample size was relatively small, and the GRS not only comprised childhood specific loci but also included loci found to be associated with BMI in adult populations (15).

The objectives for this present study were to investigate whether a GRS comprising 15 loci for childhood BMI associates with (1) BMI SDS and the overall cardiometabolic risk profile in Danish children and adolescents and (2) the response to lifestyle intervention in children and adolescents with overweight or obesity.

Methods

Study population

Data for this study were from The Danish Childhood Obesity Biobank (ClinicalTrials.gov identifier NCT00928473), comprising data from children and adolescents with normal weight, overweight, or obesity. Data material was collected between March 2007 and March 2015. A total of 1,069 children and adolescents (aged 6-18 years) with overweight or obesity were recruited through The Children's Obesity Clinic, Department of Pediatrics, Copenhagen University Hospital Holbæk in Denmark. Overweight was defined as a BMI above the 90th percentile (BMI SDS > 1.28) according to age and sex in a Danish reference population (16). Between September 2010 and March 2013, a population-based control sample of 719 Danish children and adolescents aged 6 to 18 years was recruited from schools across 11 municipalities in Denmark.

This study was conducted in accordance with the Helsinki Declaration of 1983. An informed written and oral consent was obtained from all participants or from their parents if the participant was younger than 18 years of age. This study was approved by the Ethics Committee of Region Zealand, Denmark (ID number SJ-104), and by the Danish Data Protection Agency.

Clinical examination

Anthropometric measurements. Height was measured using a stadiometer to the nearest 0.1 cm, and weight was measured to the nearest 0.1 kg on a Tanita BC418 scale (Tanita Corp., Tokyo, Japan) in the population-based control sample and on a Tanita digital medical scale, WB-110 MA in the children and adolescents with overweight or obesity. All measurements were conducted with the child or adolescent wearing light indoor clothes and without shoes. BMI was calculated as the weight in kilograms divided by the height in meters squared. BMI SDS was calculated using the LMS (Lambda for the skew, Mu for the median, and Sigma for the generalized coefficient of variation) methods (17) and expresses the standard deviation from the mean BMI in a representative Danish population (16).

Blood pressure (BP). A total of three BP measurements were made, and the mean of the last two measurements was reported. Hereafter, BP SDS was calculated according to an American reference population (18). BP was measured with the oscillometric device Omron 705IT (Omron Healthcare Europe, Hoofddorp, The Netherlands) on the right upper arm using an appropriate cuff size, a method that has been validated for use in children and adolescents (19).

Blood sampling. Venous blood samples were drawn from an antecubital vein after a 12-hour overnight fast. If required, a local anesthetic cream (lidocaine/prilocain mixture; Emla, AstraZeneca, Stockholm, Sweden) was applied 1 hour prior to venipuncture. The blood was analyzed immediately after venipuncture. A Cobas 6000 (Roche Diagnostics, Mannheim, Germany) was used to analyze serum concentrations of fasting plasma glucose, hemoglobin A_{1c} (HbA_{1c}), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), total cholesterol, and triglycerides.

When the interval between the date of the clinical examination and the date of the blood sample exceeded 30 days, biochemical measurements for the respective participant were excluded from our analyses.

Lifestyle intervention in children and adolescents with overweight or obesity

The lifestyle intervention consisted of an evidence-based treatment protocol, which was developed at the Department of Pediatrics at the Copenhagen University Hospital Holbæk in 2007 (20,21). The treatment protocol is based on the recommendations from the Expert Committee from 2007 (22). This protocol is family-centered and multidisciplinary and it comprises a range of behavioral lifestyle recommendations. The treatment is delivered by a team of pediatricians, dietitians, pediatric nurses, psychologists, and social workers. The recommended lifestyle changes are applicable for the entire family, and all family members are encouraged to attend the consultations. At the initial 1-hour consultation, a pediatrician obtains a detailed medical history and conducts a questionnaire-based interview aimed at identifying all the lifestyle changes necessary to reduce the patient's degree of overweight or obesity. These lifestyle changes are incorporated into an individualized treatment plan containing 15 to 20 items of advice regarding the implementation of the lifestyle changes into the daily lives of the families. Examples of lifestyle changes include dietary habits, means of transportation, physical activity, sedentary behaviors, sleep time, snacking, social activities, and sweets intake. At this consultation and at all subsequent consultations, the treatment plan is evaluated and adjusted to accommodate the specific challenges and needs of the families. Details of the treatment protocol have been reported in Holm et al. (20).

Genotyping

Genotyping was performed on 1,788 individuals using the Illumina Infinium HumanCoreExome BeadChip (Illumina, San Diego, California). Genotypes were called using the Genotyping module (version 1.9.4) of GenomeStudio Software (version 2011.1; Illumina). We excluded individuals identified as duplicates, ethnic outliers, or with extreme inbreeding coefficients, mislabeled gender, or a call-rate < 95%, leaving 1,618 individuals ($n_{\text{overweight/obesity}} = 920$, $n_{\text{population-based sample}} = 698$) who passed all quality control criteria. Additional genotypes were imputed into the 1000 Genomes phase 1 panel using IMPUTE2. The imputation quality was high (INFO score > 0.95) for all imputed variants included in the current study and GRS construction, except for one (rs13387838, INFO score = 0.77). All variants were in Hardy-Weinberg equilibrium ($P > 0.05$).

GRS construction

Genotypes were coded according to the number of BMI-increasing alleles based on 15 independent loci previously shown to be associated with childhood BMI at genome-wide significance ($P < 5 \times 10^{-8}$) (10). We constructed a weighted GRS (wGRS) by summing the number of BMI-increasing alleles weighted by the effect size of the variant estimated in the genome-wide association discovery study (10) and an unweighted GRS (uGRS) by summing the number of BMI-increasing alleles. Seven of the fifteen BMI single-nucleotide polymorphisms (SNPs) identified in the genome-wide association discovery study (rs7550711, rs543874, rs13130484, rs987237, rs7132908, rs12429545, rs1421085) were directly genotyped, while the remaining genotypes (rs3829849, rs4854349, rs6567160, rs8092503, rs11676272, rs12041852, rs13253111, rs13387838) were retrieved from imputed data, and the derived dosage information for the SNPs was included into the GRS calculation (Supporting Information Table S1).

Statistical analyses

We included all children and adolescents with eligible data at baseline in our analysis. In analyses focusing on the lifestyle intervention outcome, only individuals having completed 6 to 24 months of lifestyle intervention were included. Furthermore, we performed all statistical analyses with and without inclusion of individuals with diseases, conditions, or medication intake that may have an effect on weight and/or appetite regulation ($n = 72$ individuals). Prior to statistical analyses, non-normally distributed traits at baseline (fasting blood glucose, LDL-C, and triglycerides) and following intervention (fasting blood glucose, HDL-C, LDL-C, and triglycerides) were logarithmically transformed to comply with the assumption of normal distribution.

All statistical analyses were performed using the statistical software program R (version 3.3.0; The R Foundation for Statistical Computing, Boston, Massachusetts). At baseline, differences in anthropometric and biochemical measures between study samples were examined using unpaired *t* tests, while the difference in sex ratios between study samples was evaluated using Pearson χ^2 test. The effects of the wGRS and uGRS on cardiometabolic traits were estimated using multiple linear regressions while adjusting for the first three genome-wide principal components, age, age^2, and sex, as appropriate. When analyzing the lifestyle intervention response outcome, linear multiple regressions were used to calculate the association between the wGRS and uGRS with end-point values while adjusting for the first three genome-wide principal components, treatment duration, and baseline value of the examined trait, as well as age, age^2, and sex, as appropriate. Furthermore, end-point values of biochemical measures were analyzed with and without adjustment for baseline BMI SDS. While non-normally distributed traits were logarithmically transformed to achieve a normal distribution, and respective *P* values are given from analyses of logarithmically transformed traits, all effect sizes (β) and standard errors (SE) are given from analyses of nonstandardized traits.

When estimating statistical power of the present analyses, we took into account that we are using imputed markers for many of the SNPs in the GRS. Our power calculation was based on a simulation in which, for a randomly sampled population of 10,000 individuals, the per-allele effects on a random normal phenotype (mean = 0 [SD 1]) correspond to the weights specified in the wGRS, but the imputed dosages correlate to the degree implied by the imputation quality INFO score from our real imputed data. Statistical power is calculated by repeatedly subsampling to a specific sample size and testing whether a $P < 0.05$ phenotype correlation exists for a GRS constructed from the dosages (Supporting Information Figure S1).

For our primary hypothesis, BMI SDS, $P < 0.05$ was considered statistically significant. For secondary hypotheses, we performed 16 tests (8 cardiometabolic traits tested in the two different groups of children), and a *P* of 0.05/16 = 0.0031 was considered statistically significant.

Results

Baseline characteristics

During the study period, 1,618 children and adolescents (935 girls) were included. Children and adolescents with overweight or obesity were younger (median age: 11.63 years; interquartile range: 9.59-13.87) compared with the population-based control sample (median age: 12.50 years; interquartile range: 10.09-15.10; $P < 0.001$) (Table 1).

Association between GRS and anthropometry at baseline

Our results did not differ on inclusion of individuals ($n = 72$) with diseases, conditions, or medication intake with a potential effect on weight and/or appetite regulation, so results obtained in the full data set are provided. The wGRS was associated with a 0.033-higher BMI SDS (SE: 0.01; $P = 0.001$) per risk allele in children and adolescents with overweight or obesity and with a 0.065-higher BMI SDS in the population-based control study sample (SE: 0.02; $P = 0.001$) (Table 2). Similarly, a positive association in both study samples was observed when analyzing the uGRS (Supporting Information Table S2). No associations with systolic or diastolic BP were observed for the wGRS or the uGRS (Table 2 and Supporting Information Table S2, respectively).

Furthermore, we performed additional sensitivity analyses to investigate whether there was an age effect on the association between the wGRS and BMI SDS in children with overweight or obesity. For this, we stratified the study population into three age groups (6-10, >10-14, and >14-18 years) and observed a significant positive association of the wGRS, with BMI SDS only in the two oldest age groups (>10-14 and >14-18 years) (Figure 1). However, there was no interaction between the wGRS and age on BMI SDS in children with overweight or obesity when performing a formal test for interaction ($P > 0.05$, data not shown).

TABLE 1 Study population characteristics

	Children and adolescents with overweight or obesity		Population-based control sample		Differences between groups
	n	**Median (IQR)**	***n***	**Median (IQR)**	***P***
Sex (girls/boys)	514/406	–	421/277	–	NS
Age (y)	920	11.63 (9.59-13.87)	698	12.50 (10.09-15.10)	*
BMI SDS	920	2.87 (2.47-3.29)	698	0.33 (–0.38-1.01)	*
Systolic BP SDS	855	1.54 (0.71-2.55)	664	1.56 (0.77-2.58)	NS
Diastolic BP SDS	855	0.54 (0.04-1.12)	664	0.41 (–0.02-0.85)	*
Biochemical measures obtained after fasting					
Glucose (mmol/L)	703	5.10 (4.90-5.40)	685	4.97 (4.75-5.19)	*
HbA_{1c} (mmol/mol)	730	34.00 (33.00-37.00)	655	35.00 (33.00-36.00)	NS
LDL-C (mmol/L)	723	2.50 (2.10-3.00)	655	2.10 (1.80-2.50)	*
HDL-C (mmol/L)	723	1.20 (1.00-1.40)	655	1.50 (1.30-1.70)	*
Total cholesterol (mmol/L)	723	4.20 (3.70-4.80)	655	3.900 (3.60-4.40)	*
Triglycerides (mmol/L)	723	0.90 (0.70-1.30)	655	0.60 (0.50-0.80)	*

*$P < 0.001$.
BP, blood pressure; HbA_{1c}, hemoglobin A_{1c}; HDL-C, high-density lipoprotein cholesterol; IQR, interquartile range; LDL-C, low-density lipoprotein cholesterol; NS, not significant; SDS, standard deviation score.

TABLE 2 Association between weighted GRS and metabolic traits

	Children and adolescents with overweight or obesity			Population-based control sample		
	n	**β (SE)**	***P***	***n***	**β (SE)**	***P***
BMI SDS	920	0.033 (0.01)	**0.001**	698	0.065 (0.02)	**0.001**
Systolic BP SDS	855	–0.003 (0.02)	0.89	664	0.04 (0.03)	0.13
Diastolic BP SDS	855	0.01 (0.01)	0.45	664	0.001 (0.01)	0.95
Biochemical measures obtained after fasting						
Glucose (mmol/L)	702	0.002 (0.01)	0.60[a,b]	683	–0.01 (0.02)	0.71[a,b]
HbA_{1c} (mmol/mol)	730	0.01 (0.07)	0.91[a]	655	–0.05 (0.07)	0.50[a]
LDL-C (mmol/L)	723	–0.002 (0.01)	0.90[a,b]	655	–0.01 (0.01)	0.19[a,b]
HDL-C (mmol/L)	723	0.003 (0.01)	0.51[a]	655	0.0002 (0.01)	0.98[a]
Total cholesterol (mmol/L)	723	–0.001 (0.02)	0.93[a]	655	–0.02 (0.01)	0.23[a]
Triglycerides (mmol/L)	723	–0.001 (0.01)	0.91[a,b]	655	–0.004 (0.01)	0.21[a,b]

Results shown for weighted GRS. Effect sizes and standard errors (SE) calculated using untransformed variables. *P* values calculated using either untransformed or log transformed variables. Analyses adjusted for first three genome-wide principal components.
[a]Analyses adjusted for age, age^2, and sex.
[b]*P* value calculated using logarithmically transformed variables.
P values in bold indicate $P < 0.05$.
BP, blood pressure; GRS, genetic risk score; HbA_{1c}, hemoglobin A_{1c}; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SDS, standard deviation score.

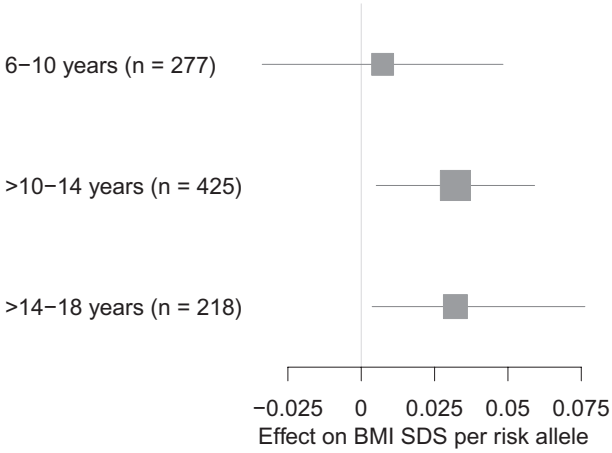

Figure 1 Association of the weighted genetic risk score on baseline BMI SDS in children with overweight or obesity (n_{total} = 920) when stratified into three age groups. BMI SDS, body mass index standard deviation score.

Association between GRS and biochemical measures obtained after fasting at baseline

No associations were identified between the wGRS and fasting plasma glucose, HbA_{1c}, LDL-C, HDL-C, total cholesterol, or triglycerides in the children and adolescents with overweight and obesity or in the population-based control sample (all $P > 0.20$) (Table 2). Similar results were found for the uGRS (Supporting Information Table S2).

Association between GRS and trait-specific principal components at baseline

Considering that most of the BMI-related cardiometabolic traits are correlated, we performed additional analyses by producing trait-specific principal components instead of studying the genetic associations with individual cardiometabolic traits at baseline (Supporting information Figure S2). The trait-specific principal component showing negative trait correlations with total cholesterol, LDL-C, and HDL-C as well as BMI SDS was negatively associated with the wGRS in children and adolescents with overweight or obesity ($P = 0.034$ [unadjusted P value]; Supporting Information Figure S2). In contrast, the trait-specific principal component showing negative trait correlations with HbA_{1c}, triglycerides, total cholesterol, and LDL-C and a positive trait correlation with BMI SDS was positively associated with the wGRS in the population-based control sample ($P = 0.018$ [unadjusted P value]; Supporting Information Figure S2). Both associations were not statistically significant after applying Bonferroni correction for multiple testing and hence presented null findings.

Association between GRS and response to lifestyle intervention

The influence of the GRS on lifestyle intervention outcome was investigated in a subgroup of 754 children and adolescents with overweight or obesity. The wGRS did not influence changes in BMI SDS ($\beta = -0.007$ [SE: 0.01]; $P = 0.34$) (Table 3). The same result was found after stratifying the subgroup into age groups (6-10, >10-14, and >14-18 years; data not shown).

The wGRS showed a nominal positive association with changes in systolic BP SDS ($\beta = 0.039$ [SE: 0.02]; $P = 0.039$) (Table 3), yet this association became insignificant upon adjustment for baseline BMI SDS ($P = 0.06$). In addition, the wGRS associated nominally with changes in HbA_{1c} concentrations ($\beta = -0.104$ mmol/mol [SE: 0.05]; $P = 0.027$) (Table 3). None of these associations remained statistically significant upon correction for multiple testing (data not shown). Similar associations were found for the uGRS (Supporting Information Table S3).

SNP-specific associations with baseline BMI SDS and changes in BMI SDS following lifestyle intervention

In addition to examining the cumulative effect of the 15 SNPs composing the GRS, SNP-specific associations with baseline BMI SDS and changes in BMI SDS following intervention were also examined. In children and adolescents with overweight or obesity, overall, 12 SNPs showed the expected direction of effect (i.e., BMI SDS increasing), while *SEC16B* rs543874 and *FTO* rs1421085 were nominally associated with baseline BMI SDS ($\beta = 0.07$ [SE: 0.03], $P = 0.04$, and $\beta = 0.06$ [SE: 0.03], $P = 0.05$, respectively). Only one SNP, *LMX1B* rs3829849, was significantly associated with decreased change in BMI SDS following intervention (i.e., a lower degree of weight loss) (Supporting Information Table S1). Similarly, 12 out of the 15 SNPs showed the expected direction of effect in the population-based control group, while only *TMEM18* rs4854349 significantly associated with baseline BMI SDS ($\beta = 0.19$ [SE: 0.08]; $P = 0.01$) (Supporting Information Table S1).

Discussion

The present study demonstrates that a GRS comprising 15 known childhood BMI susceptibility loci was associated (as expected) with BMI SDS but not with the overall cardiometabolic risk profile in Danish children and adolescents. We further showed that the intervention response of children and adolescents with overweight or obesity was independent of their genetic susceptibility to childhood obesity across all examined age groups.

While we found that the wGRS displayed the highest effect estimate on BMI SDS (0.065 increase in BMI SDS per weighted risk allele) in the population-based control sample compared with the sample of children and adolescents with overweight or obesity (0.033 increase in BMI SDS per weighted risk allele), these effect sizes were not significantly different from each other. A similar allelic effect of the GRS on BMI SDS at adiposity peak ($\beta = 0.039$; 95% CI: 0.006-0.073; $P = 0.023$) was reported in a population-based sample of children ($n = 3{,}975$; mean age: 6 years; range 5.7-7.8) (23). Our observed allelic effect of the wGRS was, however, slightly smaller compared with the 0.073 increase in BMI SDS that was reported in the discovery study ($n = 1{,}955$; age range: 2-10 years) (10).

We observed no association between the wGRS and BMI SDS in children and adolescents aged 6 to 10 years when stratifying our population into three age groups. This discrepancy may be due to our

TABLE 3 Effect of weighted GRS on intervention response in children with overweight or obesity

		Children and adolescents following intervention	Intervention response	Per allelic effect of GRS	
	n	**Median (IQR)**	**Median Δ (IQR)**	***β* (SE)**	***P***
Duration of intervention (y)	754	1.25 (1.00 to 1.59)	NA	NA	NA
BMI SDS	754	2.63 (2.09 to 3.10)	−0.20 (−0.50 to 0.01)	−0.01 (0.01)	0.34
Systolic BP SDS	600	1.70 (0.90 to 2.68)	0.24 (−0.39 to 0.99)	0.04 (0.02)	**0.039**
Diastolic BP SDS	600	0.52 (0.06 to 1.03)	0.04 (−0.46 to 0.57)	−0.01 (0.01)	0.60
Biochemical measures obtained after fasting					
Glucose (mmol/L)	403	5.10 (4.90 to 5.40)	0.10 (−0.35 to 0.40)	−0.01 (0.01)	0.89[a,b]
HbA_{1c} (mmol/mol)	427	34.00 (32.00 to 37.00)	0.00 (−2.00 to 1.00)	−0.10 (0.05)	**0.027**[a]
LDL-C (mmol/L)	424	2.30 (1.900 to 2.700)	−0.10 (−0.40 to 0.20)	0.01 (0.01)	0.58[a,b]
HDL-C (mmol/L)	426	1.20 (1.10 to 1.50)	0.10 (−0.10 to 0.20)	0.0003 (0.005)	0.95[a]
Total cholesterol (mmol/L)	425	4.10 (3.70 to 4.50)	−0.10 (−0.40 to 0.30)	0.007 (0.01)	0.70[a,b]
Triglycerides (mmol/L)	425	0.90 (0.60 to 1.20)	0.00 (−0.30 to 0.20)	−0.003 (0.01)	0.79[a,b]

Median outcome for all traits following 6 to 24 months of intervention. Per allelic effects shown for weighted GRS. Effect sizes and standard errors (SE) calculated using untransformed variables. Analyses adjusted for first three genome-wide principal components. All analyses adjusted for baseline value and intervention duration. BP, blood pressure; GRS, genetic risk score; HbA_{1c}, hemoglobin A_{1c}; HDL-C, high-density lipoprotein cholesterol; IQR, interquartile range; LDL-C, low-density lipoprotein cholesterol; NA, not applicable; SDS: standard deviation score.
[a]Analyses adjusted for age, age^2, and sex.
[b]*P* value calculated using logarithmically transformed variables.
P values in bold indicate $P<0.05$.

smaller sample size in this age group ($n=277$) and the reduced statistical power when investigating the age-group-specific associations, as demonstrated in Supporting Information Figure S1. Nevertheless, it is noteworthy that the present study demonstrated a significant positive association between the wGRS and BMI SDS in children and adolescents aged >10 to 18 years, suggesting that in a Danish population, the included 15 loci also exerted a significant cumulative effect on BMI SDS in children older than 10 years. The two previous studies reported larger effect estimates for the BMI GRS in children compared with adults (10,23), suggesting that these loci displayed age-dependent effects. Age-dependent effects have previously been demonstrated for the alpha-ketoglutarate dependent dioxygenase (*FTO*) (11,13) and the melanocortin 4 receptor (*MC4R*) loci (24), with genetic effects initially strengthening in the age range of 3 to 7 years (25) and decreasing during adulthood, potentially because of an increasing influence of environmental factors and gene-environment interactions throughout a life course (8,13). Both the *FTO* and *MC4R* loci were included in the GRS, and other included loci may also exert age-dependent effects. When examining the SNP-specific effects of the 15 included SNPs on baseline BMI SDS, nominally significant associations were identified for only three SNPs (*SEC16B* rs543874, *FTO* rs1421085, and *TMEM18* rs4854349). However, a positive direction of effect was observed for the majority, namely 12 out of 15, SNPs in both children and adolescents with overweight and obesity as well as the population-based sample. Furthermore, only one SNP (*LMX1B* rs3829849) was associated with decreased change in BMI SDS following intervention. Nevertheless, given our limited statistical power to detect SNP-specific associations, these results should be interpreted cautiously.

In the present study, the GRS showed no significant association with BMI-related cardiometabolic traits, suggesting that the 15 included loci have no cumulative effect on these traits, at least not an effect that is strong enough to be detected in our study. Similarly, previous studies examining the combined effect of SNPs in or near the *FTO* or *MC4R* loci on anthropometric or metabolic measures in children have observed no significant associations (26,27). Several other studies have indicated that both childhood and adulthood BMI susceptibility loci are also associated with eating behavior, particularly appetite regulation (3,23,28), food intake, and energy expenditure (29), which is mediated through the central nervous system. Eating behavior could complicate the associations but was not investigated in the present study. In sensitivity analysis, we tested the association between GRS and trait-specific principal components instead of studying the genetic association with individual cardiometabolic traits, but the results presented null findings. While it could be interesting to study the drivers behind these trait-specific principal component associations in more detail, this would warrant a larger sample size. Therefore, the results of these additional trait-specific principal component analyses can only be speculative and should be interpreted with caution.

No effect of GRS on changes in BMI SDS was found across all examined age groups. While the GRS was initially inversely associated with changes in systolic BP and concentrations of HbA_{1c}, these associations were insignificant when corrected for multiple testing. Our findings indicate that children and adolescents with a high genetic susceptibility based on the 15 selected SNPs are likely to respond to the same degree and to achieve a similar decrease in degree of obesity following lifestyle intervention compared with individuals with a lower genetic susceptibility. Several studies have examined the effects of single adulthood or childhood BMI and/or obesity susceptibility SNPs or loci on obesity intervention outcome. However, these studies have generally been performed in smaller study populations, and the durations of the applied interventions have been limited (26,27,30-35).

Moleres et al. (15) constructed a GRS comprising nine obesity susceptibility SNPs (in or near *MC4R, FTO, PPARG, TMEM18, IL6,* and *ADPOQ*) previously identified in adult populations and examined the association between this GRS and the response to a lifestyle intervention

in Spanish adolescents ($n = 168$; age: 12-16 years) with overweight or obesity. At baseline, the GRS was positively associated with BMI SDS and fat mass, and following 3 months of obesity treatment, the GRS was inversely associated with changes in both BMI SDS and fat mass (15). These findings suggest that a GRS comprising adult obesity and/or BMI susceptibility loci and not necessarily childhood BMI-specific loci may also influence baseline BMI SDS in adolescents, and that a high genetic susceptibility, as based on the nine included SNPs, may influence treatment outcome. In the Spanish study, however, the adolescents were treated according to a more intensive program based on food intake questionnaires, individualized balanced diet, physical activity, and weekly group sessions (15). In comparison, our study consisted of an individualized treatment plan containing 15 to 20 items of advice regarding the implementation of the lifestyle changes and individual consultations every 2 months (20). Furthermore, our study comprised a larger study population of both children and adolescents, and the duration of the lifestyle intervention was longer, ranging from 6 to 24 months. Discrepancy between the reported effects of the applied GRSs may thus be due to differences in selected SNPs as well as differences in study and intervention methodology.

As more genetic variants associated specifically with childhood BMI or childhood obesity are identified, we will likely gain a better understanding of how the effect of these gene variants differ compared with gene variants associated exclusively with adulthood obesity. In time, this can expand our understanding of biological pathways influencing obesity development differently during childhood and adulthood. Future studies examining cumulative effects of childhood BMI susceptibility loci are thus likely to include a larger number of genetic loci, and such studies may divide susceptibility SNPs into pathway-specific GRSs as a means to examine metabolic effects from a pathway perspective.

A strength of the present study was that we constructed a GRS comprising 15 SNPs known to be associated with childhood BMI (10). Furthermore, the present study included a relatively large study population compared with similar previous studies in children, especially in our analyses of intervention outcome. Importantly, the children and adolescents with overweight and obesity underwent treatment using The Children's Obesity Clinic protocol, which has, in several nonrandomized studies, shown improvement in metabolic risk variables and psychosocial well-being, including degree of obesity in both patients and their parents (20,36), blood pressure (37), plasma lipid fractions (38), body composition (39), liver and muscle fat content (40), and quality of life (41,42).

A limitation of our study is that the selected 15 childhood BMI susceptibility SNPs were identified in a sample of children aged 2 to 10 years (10), whereas our population included children and adolescents between 6 and 18 years of age. Although the cumulative effect of these SNPs may not be directly comparable between the study samples, we demonstrated that the GRS was significantly associated with BMI SDS in children and adolescents above 10 years of age. Furthermore, as the lifestyle intervention program was highly individualized, measures of compliance were not registered, and statistical analyses were therefore performed using an intention-to-treat approach. Finally, as we have used data from a clinical database, information on family history of diabetes, coronary vascular disease, and related diseases were not systematically obtained, and statistical analyses could therefore not be adjusted for these potential confounders. Likewise, as the children and adolescents were not assessed according to pubertal development, statistical analyses were not adjusted accordingly.

Conclusion

A GRS for childhood BMI was associated with increased BMI SDS in Danish children and adolescents but was not associated with other BMI-related cardiometabolic measures at baseline. The GRS was not associated with the outcome of 6 to 24 months of structured lifestyle intervention, as assessed by changes in BMI SDS, systolic or diastolic BP, and related biochemical measures. Our study indicates that children and adolescents with overweight or obesity are likely to obtain a similar decrease in degree of obesity regardless of their common variant genetic susceptibility for childhood obesity.O

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