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General Article

GENERAL ARTICLE

Relationship between glucose homeostasis and obesity in early life—a study of Italian children and adolescents

Zhanna Balkhiyarova[1,2,3], Rosa Luciano[4,5], Marika Kaakinen[1,6], Anna Ulrich[1,6], Aleksey Shmeliov[1], Marzia Bianchi[4], Laura Chioma[7], Bruno Dallapiccola[8], Inga Prokopenko[1,2,9,*] and Melania Manco[4,*,†]

[1]Section of Statistical Multi-Omics, Department of Clinical and Experimental Medicine, University of Surrey, Guildford GU2 7XH, UK, [2]Institute of Biochemistry and Genetics, Ufa Federal Research Centre Russian Academy of Sciences, Ufa 450008, Russian Federation, [3]Department of Endocrinology, Bashkir State Medical University, Ufa 450054, Russian Federation, [4]Research Area for Multifactorial Disease, Bambino Gesù Children's Hospital, IRCCS, Rome 00146, Italy, [5]Department of Laboratory Medicine, Bambino Gesù Children's Hospital, IRCCS, Rome 00146, Italy, [6]Section of Genetics and Genomics, Department of Metabolism, Digestion and Reproduction, Imperial College London, London SW7 2AZ, UK, [7]Unit of Endocrinology, Bambino Gesù Children's Hospital, IRCCS, Rome 00146, Italy, [8]Genetics and Rare Diseases Research Division, Bambino Gesù Children's Hospital, IRCCS, Rome 00146, Italy and [9]UMR 8199—EGID, Institut Pasteur de Lille, CNRS, University of Lille, Lille 59000, France

*To whom correspondence should be addressed. Tel: +39 668592649; Email: melania.manco@opbg.net (Melania Manco); Tel: +44 1483 684900; Fax: +44 1483 689790; Email: i.prokopenko@surrey.ac.uk (Inga Prokopenko)

Abstract

Epidemic obesity is the most important risk factor for prediabetes and type 2 diabetes (T2D) in youth as it is in adults. Obesity shares pathophysiological mechanisms with T2D and is likely to share part of the genetic background. We aimed to test if weighted genetic risk scores (GRSs) for T2D, fasting glucose (FG) and fasting insulin (FI) predict glycaemic traits and if there is a causal relationship between obesity and impaired glucose metabolism in children and adolescents. Genotyping of 42 SNPs established by genome-wide association studies for T2D, FG and FI was performed in 1660 Italian youths aged between 2 and 19 years. We defined GRS for T2D, FG and FI and tested their effects on glycaemic traits, including FG, FI, indices of insulin resistance/beta cell function and body mass index (BMI). We evaluated causal relationships between obesity and FG/FI using one-sample Mendelian randomization analyses in both directions. GRS-FG was associated with FG (beta = 0.075 mmol/l, SE = 0.011, $P = 1.58 \times 10^{-11}$) and beta cell function (beta = −0.041, SE = 0.0090 $P = 5.13 \times 10^{-6}$). GRS-T2D also demonstrated an association with beta cell function (beta = −0.020, SE = 0.021 $P = 0.030$). We detected a causal effect of increased BMI on levels of FI in Italian youths (beta = 0.31 ln (pmol/l), 95%CI [0.078, 0.54], $P = 0.0085$), while there was no effect of FG/FI levels on BMI. Our results demonstrate that the glycaemic and T2D risk genetic variants contribute to higher FG and FI levels and decreased beta cell function in children and adolescents. The causal effects of adiposity on increased insulin resistance are detectable from childhood age.

†Melania Manco, http://orcid.org/0000-0002-6581-975X
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Introduction

Childhood obesity is growing around the globe. In some developed countries, the disease incidence plateaued; nonetheless, the rate of severe obesity had increased worldwide (1). In Italy, the prevalence of overweight and 3rd grade obese children has decreased from 35.2% in 2008 to 30.6% in 2016, while the rate of severely obese has reached 2.1% (2). This caused the onset of health conditions previously considered exclusively adult diseases, such as prediabetes (impaired fasting glucose, IFG, and glucose tolerance, IGT) and type 2 diabetes (T2D), at an earlier age.

Obesity, especially in children, is a major risk factor of T2D and urges its study for diabetes prevention (3). Strong evidence suggests that pathogenic mechanisms are shared between obesity, prediabetes and T2D as well as causal effect of body adiposity on hyperinsulinaemia and T2D in adults (4). The rates of their occurrence differ among ethnicities, consistent with differences in genetic susceptibility to T2D, currently less characterized in youths than in adults. In Italy, 3% of adolescents with moderate and severe obesity present IFG and 5% IGT, while T2D is diagnosed in less than 0.5% of adolescents (5). An increased amount of adipose tissue leads to more severe insulin resistance (IR) via different mechanisms (i.e. lipotoxicity, release of some adipokines etc.), thus promoting the development of T2D (6). The early-life adiposity levels correlate with adulthood measures and might therefore represent a longitudinal causal risk factor for adult metabolic health deterioration, which requires further insight from studies dissecting health of young individuals (7).

To date, over 400 genetic variants have been implicated in the development of T2D and more than 500 genomic loci were uniquely associated with body mass index (BMI) (8–11). In general, these loci have small effect sizes, explaining approximately 6% of trait variance and disease susceptibility (12) when combined. Studies in children (13) and young adults (14–20) have demonstrated that genetic risk scores (GRSs) show stronger predictive ability in younger individuals than in older ones (21–25). Several obesity genes harbour loci that are associated with T2D but affect T2D susceptibility largely through their effect on BMI via increased IR (e.g. *FTO* and *MC4R* gene variants) (26). However, their effects on glucose homeostasis might be overestimated in the context of obesity (27).

Dissection of the genetic effects on the quantitative endophenotypes of T2D, including fasting glucose (FG), and insulin (FI), indices of beta cell function (HOMA-B) and IR (HOMA-IR) in individuals without diabetes, helps uncovering the disease pathophysiology (28). The Meta-Analyses of Glucose and Insulin-related traits Consortium (MAGIC) identified sixteen loci associated with FG/HOMA-B, and two loci associated with FI/HOMA-IR (29). Additional evidence was provided for 24 FG loci (12).

In this paper, we calculated GRS to combine the effects of multiple genetic variants to increase the power of the study. The choice of loci was based on reported associations of genetic variants with FG, FI and T2D (30).

The selection of SNPs for this study was based on prioritization of the variants with the largest effect size of the phenotype of interest. These variants, including *TCF7L2, FTO, MTNR1B* and *G6PC2*, were most frequently identified in the earliest GWAS studies (Supplementary Material, Table S1) and confirmed by multiple replications (28,31). This approach allowed us to detect the effect in individuals of young age, when genetic effects are usually smaller compared with adulthood. We hypothesized that the GRSs for FG, FI and T2D are associated with glycaemic traits, such as IR and beta cell function measured by HOMA-IR/HOMA-B, and adiposity (BMI) as a quantitative measure of obesity in children and adolescents. The aim of our study was to analyse 42 loci with the largest effects on T2D and glycaemic traits in a cohort of children enrolled at the Bambino Gesù Children's Hospital in Rome, Italy, and to define the effects of these variants on glycaemic traits and indices of IR, beta cell function, and BMI as well as to evaluate the causal relationships between these traits using genetic variants as instruments in a one-sample Mendelian randomization (MR) framework.

Results

Our study included 1660 young European-descent individuals from Italy with a mean BMI of 20.72 kg/m^2 (range 10.42–44.95 kg/m^2) and a mean age of 9.09 years (range 2.02–18.93 years) (Table 1). Most (1638; 98.67%) of the participants had FG level below 5.6 mmol/l. Twenty (1.20%) participants had FG values between 5.6 and 6.1 mmol/l and two (0.12%) had values between 6.1 and 7.0 mmol/l. Most of them fell within the BMI range between −2 and 2 of gender- and age-specific standard deviation scores (SDS) of BMI units (Material and Methods) with the exception of 205 individuals (12.35%) who were obese (BMI values ≥2SD). These individuals with obesity were older and had higher FG, FI, HOMA-B and HOMA-IR values when compared with non-obese individuals.

We performed the association analyses with FG, FI, HOMA-B, HOMA-IR and BMI SDS for the 42 variants, assuming an additive genetic model implemented in linear regression and detected nominally significant associations ($P < 0.05$) at eight FG, eight T2D and two FI loci with the phenotypes tested (Material and Methods, Supplementary Material, Table S1). For FG, we observed the most significant associations with rs560887 near *G6PC2* (glucose-6-phosphatase 2) ($P = 7.35 \times 10^{-6}$), rs4607517 near *GCK* (glucokinase) ($P = 3.24 \times 10^{-5}$) and rs10830963 at *MTNR1B* (melatonin receptor 1B) ($P = 4.37 \times 10^{-5}$), of which the signals at *G6PC2* and *MTNRB1* were also associated with HOMA-B ($P = 1.13 \times 10^{-2}$ and $P = 1.32 \times 10^{-4}$, respectively). Additionally, signals within MAP Kinase Activating Death Domain (*MADD*) and Coiled-Coil-Helix-Coiled-Coil-Helix Domain Containing 9/transducin-like enhancer protein 4 (*CHCHD9/TLE4*) were nominally associated with FG. For HOMA-B, we further observed an association with rs10440833 at CDK5 regulatory subunit-associated protein 1-like 1 (*CDKAL1*, $P = 9.72 \times 10^{-3}$). For FI, the variant rs340874 at Prospero Homeobox 1 (*PROX1*) showed a nominal association ($P = 2.37 \times 10^{-2}$). Furthermore, variation at Peroxisome Proliferator Activated Receptor Gamma (*PPARG*) was associated with FI ($P = 4.49 \times 10^{-2}$), HOMA-B ($P = 2.15 \times 10^{-3}$) and HOMA-IR ($P = 4.01 \times 10^{-2}$). HOMA-IR was further associated with rs12970134 at *MC4R* (melanocortin 4 receptor) ($P = 3.25 \times 10^{-2}$). Variation at *MC4R* was also associated with age- and gender-standardized BMI ($P = 8.45 \times 10^{-3}$), as were rs9939609 at *FTO* (fat mass and obesity-associated) and rs11558471 at *SLC30A8* (Solute Carrier Family 30 Member 8), ($P = 1.81 \times 10^{-2}$ and $P = 2.91 \times 10^{-2}$, respectively).

We calculated unweighted and weighted GRS for FG (20 SNPs), FI (5 SNPs) and T2D (36 SNPs) (Material and Methods, Supplementary Material, Table S1). Both unweighted and weighted GRSs were further multiplied by the proportion of successfully genotyped SNPs per individual. We compared the distributions of the number of trait-increasing risk alleles for FG, FI and T2D over the distributions of related phenotypes. As the number of FG risk alleles increased, concentrations of FG increased, whereas values of HOMA-B decreased (Fig. 1). However, contrary

Table 1. Characteristics of the study sample

Phenotype	Males ($N = 889$) Mean ± SD	Females ($N = 771$) Mean ± SD	P-value for difference*
Age (years)	9.07 ± 3.74	9.12 ± 3.91	0.82
BMI (kg/m^2)	20.83 ± 5.59	20.59 ± 5.47	0.37
BMI SDS	0.47 ± 1.52	0.47 ± 1.41	0.97
FG (mmol/l)	4.58 ± 0.48	4.47 ± 0.50	2.27×10^{-5}
FI (pmol/l)	54.91 ± 45.23	62.51 ± 49.62	0.0012
HOMA-B	108.28 ± 55.93	123.87 ± 60.88	7.74×10^{-8}
HOMA-IR	0.99 ± 0.80	1.13 ± 0.88	0.0013

BMI, body mass index; FG, fasting glucose; FI, fasting insulin; SDS, standard deviation score; T2D, type 2 diabetes.
*Difference between the mean values in male and female participants calculated by t-test.

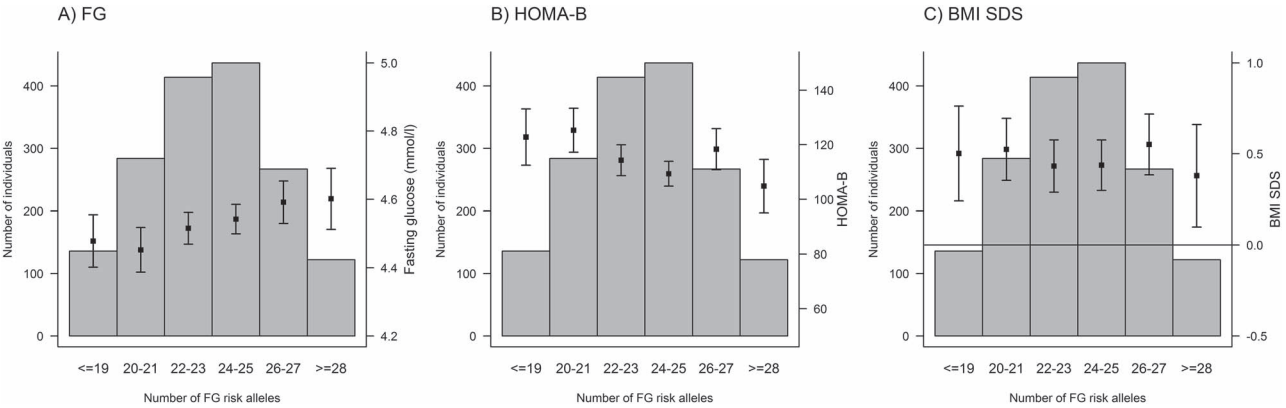

Figure 1. Relationships between the number of fasting glucose (FG) risk alleles and FG (A), HOMA-B (B) and age- and BMI SDS (C).

to our study hypothesis, the BMI SDS measurement remained relatively invariable compared with the number of FG risk alleles. For the number of FI risk alleles, no significant association was observed with FI, HOMA-IR or BMI SDS (Fig. 2). Similar results were obtained for the relationship between T2D risk alleles and FG, FI, HOMA-B, HOMA-IR and BMI SDS (Fig. 3).

When grouping the phenotype distributions according to percentiles, we observed that the individuals in the highest (>95%) percentile group of FG had a slightly increased number of FG risk alleles ($\text{mean}_{\text{group1}} = 22.86$, $\text{mean}_{\text{group2}} = 23.45$, $\text{mean}_{\text{group3}} = 23.84$; $P = 0.026$ in the linear regression of the number of FG risk alleles on the percentile group), whereas the opposite was true for HOMA-B ($\text{mean}_{\text{group1}} = 23.84$, $\text{mean}_{\text{group2}} = 23.46$, $\text{mean}_{\text{group3}} = 22.61$; $P = 0.0050$). No such effect was detected for the BMI SDS percentile groups ($\text{mean}_{\text{group1}} = 23.67$, $\text{mean}_{\text{group2}} = 23.43$, $\text{mean}_{\text{group3}} = 23.16$; $P = 0.24$) (Fig. 4).

A higher number of FG risk alleles was associated with elevated FG (beta = 0.075, 95%CI [0.053;0.097] mmol/l per unit increase in the weighted GRS) and lower HOMA-B values (beta = −0.041, 95%CI [−0.059;−0.024] ln(HOMA-B) units per unit increase in the weighted GRS) after adjustment for age, sex and BMI SDS (Material and Methods, Table 2). The weighted T2D GRS was associated with lower HOMA-B values (−0.020 [−0.038;−0.0019]) after adjusting for age, sex and BMI SDS (Table 2). The unweighted GRS provided mostly similar but weaker associations than the weighted GRS (Table 2), except for the unweighted FG GRS that showed a trend towards a negative association with FI levels (−0.024 [−0.050, 0.0029] ln(pmol/l), $P = 0.082$) and this was further strengthened (−0.028 [−0.055, −0.0016] ln(pmol/l), $P = 0.038$) when adding adjustment for family history of T1D and T2D. It is worth noticing that this additional adjustment had in general no noticeable effect on the other estimates (Material and Methods, Supplementary Material, Table S2). We did not observe any evidence for association between the FG and FI GRSs and BMI SDS or obesity status, including adjustments for sex, age, BMI SDS and family history of T1D and T2D (Table 3).

We evaluated the associations between FG/FI and BMI for causality in a bi-directional one-sample MR framework (Material and Methods). We identified a positive causal effect of BMI on FI ($P = 0.0085$) (Table 4). The IV estimator indicated a causal effect of 0.31 ln(pmol/l) higher FI (95%CI [0.078;0.54]) per unit increase in BMI SDS. We did not observe a causal effect in the other direction. Contrary to the observed epidemiological associations, we did not find evidence for a causal effect of BMI on the levels of FG and *vice versa*. However, this could be due to low power as the epidemiological effect estimates between BMI and FG in either direction are lower than those between BMI and FI (Table 4). Additionally, the low F-statistic of the FI IV (Table 4) suggests weak instrument bias and low power of the MR analysis testing the effect of FI levels on BMI.

Discussion

Our results confirm the ability of a GRS combining 20 independent genetic variants, associated with FG in previous GWAS (12,28,32,33), to predict values of fasting glucose and beta cell activity in children and adolescents already. Using a one-sample MR approach, we discovered that a causal effect of adiposity via BMI on FI levels is detectable as early as in childhood/teenage years.

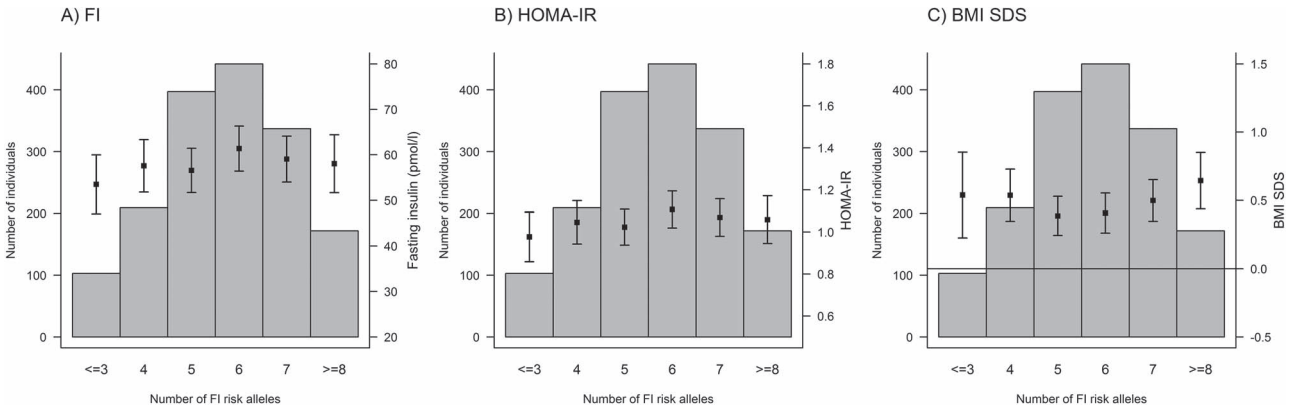

Figure 2. Relationships between the number of fasting insulin (FI) risk alleles and FI (A) HOMA-IR (B) and age- and BMI SDS (C).

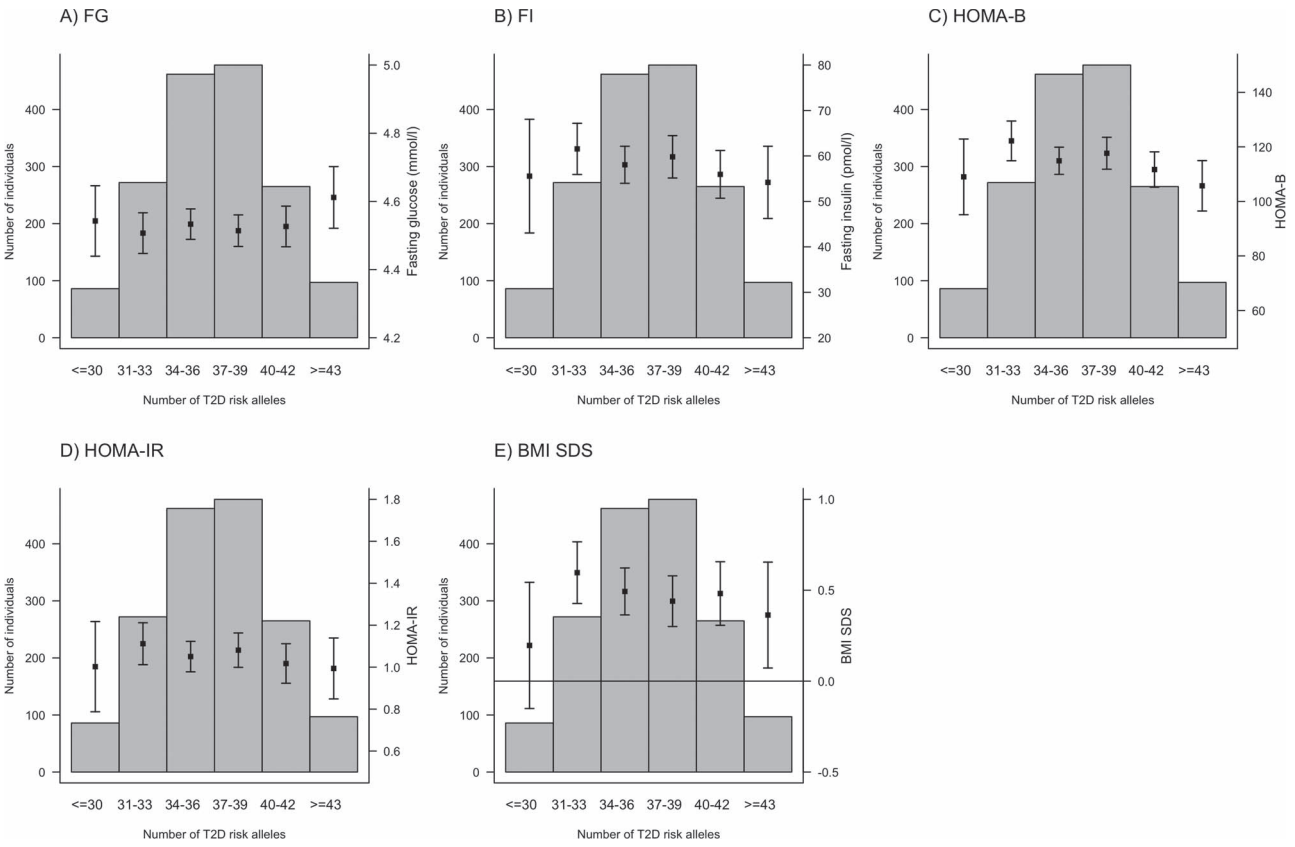

Figure 3. Relationships between the number of type 2 diabetes (T2D) risk alleles and FG (A), FI (B), HOMA-B (C), HOMA-IR (D), and BMI SDS (E).

Effects of adiposity on altered glucose metabolism

The present investigation expands on earlier findings in adults on *FTO/MC4R*-mediated adiposity's effect on increased fasting insulin levels (34,35) to its earlier age manifestation in children and adolescents. Despite much larger sample sizes in earlier adult studies, the causal effect of adiposity on FI in our study is comparable to that in adults (35). While we were able to observe a causal effect in the combined cohort, we were underpowered to perform sex-stratified analyses and to validate the larger causal effect of BMI on FI reported in men compared with women (4). This study did not find evidence for a causal effect of adiposity on FG, possibly due to lack of statistical power. The lack of causal effect of BMI on FG is in contrast to the findings of Dale *et al.* and Xu *et al.* who report a causal effect of BMI on glucose levels with markedly lower causal effect size than that between BMI and insulin (36,37). Even though our causal analyses from BMI to FI and FG used only two variants, namely those within *FTO* and *MC4R*, to date, these are the most strongly associated with BMI and have been successfully used as instrumental variables to estimate causal effects previously (4,38). The F-statistic of the BMI instrument (Table 4) demonstrates that these two variants make a strong enough IV in this study. Our findings

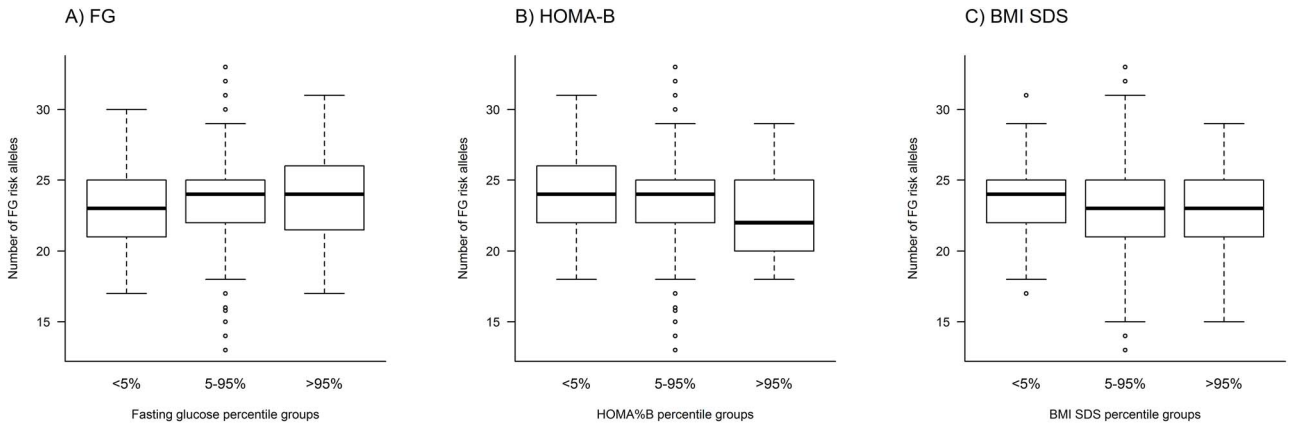

Figure 4. Relationship between the number of fasting glucose (FG) risk alleles and FG (A), HOMA-B (B), and BMI SDS (C). The results from the association analyses using linear regression of both GRS types, weighted and unweighted, confirmed the findings from the comparison of phenotypic distributions in relation to allele counts (Table 2).

support a crucial role of adiposity in the development of IR in young individuals. During puberty, IR is changing drastically, when insulin sensitivity undergoes a decline of around 25–50% during puberty and improves when puberty ends (39,40). Visceral and subcutaneous adipose tissue secrete free fatty acids and pro-inflammatory cytokines into blood that contribute to IR (41). Changes related to increased adiposity affect the complex interplay between pathophysiological processes already in early age, which might increase the risk of early onset T2D and comorbidities.

Genetic variants associated with adiposity

Our findings are in line with previous studies that linked genetic variants near *MC4R* and *FTO* genes with adiposity traits and T2D (42). *MC4R* rs12970134 is associated with increased risk of T2D and higher BMI in both European and trans-ethnic studies (8,26). The Nord-Trøndelag Health (HUNT) study assessing gene–environment interactions of *FTO* and *MC4R* on obesity in people with extreme phenotypes observed age- and gender-dependent associations of rs9939609 (*FTO*) and rs17782313 (*MC4R*) loci with BMI (43). Notably, the effect sizes of *FTO* tended to be the highest in the youngest age group for both genders; for *MC4R*, the highest effect on BMI was observed in the youngest age group, dipping in the middle age and increasing again after the age of sixty, while in men, it peaked at 40–60 years and became negligible later in life (43). The *FTO* locus is not only strongly associated with T2D (42) and higher BMI (44), but also with higher FI and HOMA-IR (29). These observations are in agreement with BMI playing a role in the *FTO* association with T2D via IR.

Effects on altered glucose metabolism in adult and paediatric populations

In our analysis, the GRS for FG comprising 20 DNA variants explained 2.76% of the variability in FG (beta = 0.075 mmol/l, $P = 1.58 \times 10^{-11}$) and 0.34% of the variability in β-cell function (beta = 0.042, $P = 5.13 \times 10^{-6}$). A number of studies (13–20,45) evaluated the performance of glucose homeostasis GRSs as a useful tool to estimate the effects of multiple risk alleles predicting prevalent or incident cases of T2D.

Similarly, in cross-sectional studies of normal-weight and overweight/obese children, T2D and FI GRSs comprising 62 (23) and 53 (9) SNPs were associated with different glycaemic traits, particularly with FG and estimates of beta cell function.

Genetic variants associated with fasting glucose and β-cell function

We confirmed previously established associations of some of the T2D susceptibility variants with glucose metabolism traits (Supplementary Material, Table S1). Specifically, among 20 loci previously implicated in FG variability, *G6PC2*, *GCK* and *MTNR1B* variants showed effects on FG in Italian children consistent with those found in European children in MAGIC (28,29) and in adults (46). According to our results and previous studies, two of these genes, *G6PC2* and *MTNR1B*, have been found to be associated with β-cell function in adults (47,48). Increased *MTNR1B* expression in individuals at risk of T2D suggests a direct inhibitory effect on beta cells (47). Our results demonstrate a significant association between *MADD* and *PPARG* loci and HOMA-B, consistent with previous reports (49,50). Our study provides evidence that *CDKAL1* variants confer risk of T2D through reduced insulin secretion, which is also in line with the findings of the genome-wide association study in European and Hong Kong populations (51).

Study limitations

We acknowledge the limitations of our study of which the limited sample size and the wide age range are the most evident. Other limitations include the cross-sectional design; the weights taken from older populations and effect sizes for risk variants can vary between different age groups and might not provide as good a predictive ability; the limited number of variants investigated; the proxy estimation of fasting IR and β-cell function; the lack of information on pubertal status and the enrolment of individuals with exclusively European descent. In an attempt to mitigate the limitation of wide age range, we used gender- and age-specific standardized obesity indices (BMI SDS). Future investigations must consider the changing physiology and hormonal levels during pubertal transition; they should enrol individuals of non-European descent to better reflect the evolving multi-ethnic nature of populations in Italy and worldwide; and the FG GRS should be validated in longitudinal studies. Additionally, the GRSs in our study are constructed based on a relatively small number of variants from pioneer GWAS studies. Therefore, as more variants are characterized, improved GRSs should be constructed.

Table 2. Association between the FG/FI/T2D genetic risk scores and unadjusted FG, FI, HOMA-B, HOMA-IR and age- and gender-standardized BMI

Model	FG (mmol/l)		ln(FI (pmol/l))		ln(HOMA-B)		ln(HOMA-IR)		BMI SDS	
	β (95% CI)	P-value	β (95% CI)	P-value	β (95% CI)	P-value	β (95% CI)	P-value	β (95% CI)	P-value
Weighted GRS, adjusted										
GRS_FG	**0.075 (0.053, 0.097)**	**1.58×10^{-11}**	−0.011 (−0.038, 0.016)	0.43	**−0.041 (−0.059, −0.024)**	**5.13×10^{-6}**	−0.0049 (−0.017, 0.0072)	0.43	0.034 (−0.033, 0.10)	0.32
GRS_FI	0.0023 (−0.020, 0.024)	0.84	0.020 (−0.0069, 0.046)	0.15	0.012 (−0.0061, 0.030)	0.20	0.0081 (−0.0040, 0.020)	0.19	0.013 (−0.055, 0.080)	0.71
GRS_T2D	0.0074 (−0.015, 0.029)	0.51	−0.025 (−0.052, 0.0016)	0.066	**−0.020 (−0.038, −0.0019)**	**0.030**	−0.0082 (−0.020, 0.0040)	0.19	−0.018 (−0.085, 0.050)	0.61
Weighted GRS, unadjusted										
GRS_FG	**0.082 (0.059, 0.11)**	**1.010×10^{-11}**	0.013 (−0.025, 0.050)	0.50	**−0.029 (−0.052, −0.0070)**	**0.010**	0.0052 (−0.011, 0.022)	0.54	0.051 (−0.020, 0.12)	0.16
GRS_FI	0.0016 (−0.022, 0.025)	0.90	0.023 (−0.014, 0.060)	0.23	0.014 (−0.0083, 0.037)	0.22	0.0095 (−0.0072, 0.026)	0.27	0.011 (−0.060, 0.082)	0.76
GRS_T2D	0.0064 (−0.018, 0.030)	0.60	−0.026 (−0.063, 0.011)	0.17	−0.020 (−0.042, 0.0027)	0.085	−0.0086 (−0.025, 0.0081)	0.31	−0.017 (−0.087, 0.054)	0.65
Unweighted GRS, adjusted										
GRS_FG	**0.045 (0.023, 0.067)**	**5.31×10^{-5}**	−0.024 (−0.050, 0.0029)	0.082	**−0.036 (−0.054, −0.018)**	**7.15×10^{-5}**	−0.0089 (−0.021, 0.0032)	0.15	−0.020 (−0.088, 0.047)	0.55
GRS_FI	0.0011 (−0.021, 0.023)	0.92	0.016 (−0.011, 0.042)	0.25	0.0097 (−0.0082, 0.028)	0.29	0.0065 (−0.0056, 0.019)	0.29	0.024 (−0.043, 0.092)	0.48
GRS_T2D	0.018 (−0.0044, 0.040)	0.12	−0.013 (−0.040, 0.013)	0.31	−0.017 (−0.035, 0.0010)	0.064	−0.0029 (−0.015, 0.0093)	0.65	−0.0021 (−0.070, 0.066)	0.95
Unweighted GRS, unadjusted										
GRS_FG	**0.048 (0.025, 0.072)**	**6.83×10^{-5}**	−0.017 (−0.054, 0.020)	0.37	**−0.033 (−0.056, −0.011)**	**0.0036**	−0.0064 (−0.023, 0.010)	0.45	−0.0090 (−0.080, 0.062)	0.80
GRS_FI	0.0022 (−0.022, 0.026)	0.86	0.021 (−0.016, 0.059)	0.26	0.013 (−0.0095, 0.035)	0.26	0.0089 (−0.0078, 0.027)	0.30	0.023 (−0.047, 0.094)	0.52
GRS_T2D	0.017 (−0.0073, 0.040)	0.17	−0.011 (−0.049, 0.026)	0.55	−0.015 (−0.037, 0.0077)	0.20	−0.0019 (−0.019, 0.015)	0.82	0.00036 (−0.070, 0.071)	0.99

BMI, body mass index; FG, fasting glucose; FI, fasting insulin; GRS, genetic risk score; SDS, standard deviation score; T2D, type 2 diabetes. Model adjusted for age, sex and BMI SDS. BMI SDS adjustment not done when BMI SDS is the outcome of interest. BMI SDS adjustment not done when BMI SDS is the outcome of interest. Bold values denote statistical significance at the $P < 1 \times 10^{-5}$.

Table 3. Effects of FG/FI/T2D genetic risk scores on obesity

Model	Obese (N = 205) versus non-obese (N = 1350) (control group 1) a				Obese (N = 205) versus non-overweight (N = 639) (control group 2) b			
	Unadjusted		Adjusted c		Unadjusted		Adjusted c	
	OR (95% CI) c	P-value	OR (95% CI) c	P-value	OR (95% CI) c	P-value	OR (95% CI) c	P-value
Weighted GRS								
GRS_FG	1.00 (0.86–1.15)	0.96	0.97 (0.83–1.13)	0.74	1.00 (0.86–1.18)	0.96	1.00 (0.84–1.19)	0.99
GRS_FI	0.98 (0.85–1.13)	0.78	0.98 (0.84–1.13)	0.74	0.94 (0.81–1.10)	0.48	0.93 (0.79–1.10)	0.41
GRS_T2D	0.93 (0.80–1.07)	0.31	0.91 (0.78–1.06)	0.22	0.99 (0.85–1.15)	0.89	0.99 (0.84–1.17)	0.91
Unweighted GRS								
GRS_FG	0.95 (0.82–1.10)	0.48	0.93 (0.80–1.09)	0.36	0.94 (0.30–1.03)	0.45	0.93 (0.79–1.11)	0.44
GRS_FI	0.99 (0.86–1.15)	0.93	0.99 (0.85–1.15)	0.86	0.96 (0.82–1.11)	0.60	0.95 (0.80–1.12)	0.52
GRS_T2D	0.97 (0.84–1.12)	0.68	0.96 (0.82–1.12)	0.58	1.02 (0.88–1.20)	0.77	1.02 (0.86–1.20)	0.83

FG, fasting glucose; FI, fasting insulin; GRS, genetic risk score; SDS, standard deviation score; T2D, type 2 diabetes.
[a]Obese individuals: BMI SDS > =2; non-obese individuals: −2 < BMI SDS < 2.
[b]Obese individuals: BMI SDS > =2; non-overweight individuals: −1 < BMI SDS < 1.
[c]Adjusted for age, sex and family history of T1D and T2D.

Table 4. Mendelian randomization analysis

Causal relationship tested (exposure on outcome)	Epidemiological association		Causal effect, instrumental variable (IV) approach		
	β (95%CI)	P-value	F-stat (IV)	β_{IV}(95%CI)	P-value
FG on BMI	0.68 (0.54, 0.82)	6.70×10^{-21}	46.98	0.61 (−0.22, 1.43)	0.15
FI on BMI	0.96 (0.89, 1.043)	1.75×10^{-109}	0.79	−2.55 (−11.15, 6.053)	0.56
BMI on FG	0.077 (0.061, 0.093)	6.70×10^{-21}	15.72	0.049 (−0.12, 0.21)	0.56
BMI on FI	0.27 (0.25, 0.29)	1.75×10^{-109}	15.72	**0.31 (0.078, 0.54)**	**0.0085**

Mendelian randomization analyses using the Two-Stage-Least Squares method. FG, fasting glucose (mmol/l); BMI, body mass index (kg/m^2); FI, fasting insulin (ln(pmol/l)). For the instrumental variable analyses, estimates are reported in units of the outcome as described in the previous sentence per amount of increase in the exposure attributable to one unit increase in the genetic instrument of the exposure. For the statistically significant causal relationship of BMI on FI, one unit increase in BMI GRS corresponds to 0.14 (95%CI [0.070;0.21]) standard deviation score increase in BMI.

Conclusion

We report that T2D risk genetic variants contribute to higher FG levels and beta cell function in Italian children and adolescents. We provide novel evidence for a causal effect of childhood adiposity on higher FI levels validating previously published results in adults. Further larger studies in children are mandatory to expand present knowledge of the genetic overlap between childhood/adolescent age obesity and risk of T2D.

Materials and Methods

Study sample

The study sample comprised individuals referred to the Bambino Gesù Children's Hospital in Rome, Italy, between July 2012 and July 2013 by general practitioners from the Metropolitan Area of Rome (Italy) to participate in 'The Bambino Gesù Study: Profiling the genetic risk of complex diseases in the Italian population'. The primary aim of the study was to dissect the genetic architecture of glucose homeostasis in the Italian children and adolescents. The study was approved by the Ethics Committee of the Bambino Gesù Hospital, and written informed consent was obtained from the child's parents or legal guardians in accordance with the Helsinki declaration (52).

In total, 1806 participants were enrolled in the study. We excluded participants below 2 or above 19 years of age (N = 10), non-Europeans (N = 135) and one individual with an FG value ≥7 mmol/l. The final study sample included 1660 (889 male) participants of European descent aged between 2 and 19 years (Supplementary Material, Fig. S1). None of the participants were following a weight loss diet or an intensive exercise program, and, at the time of enrolment, all study participants were healthy. Information on family history of diabetes in the first-degree relatives was obtained by a short questionnaire completed by both parents (53).

Anthropometric measurements and biochemical assays

Weight and height were measured using standard procedures (54). All participants were asked to refrain from intensive physical activity in the 3 days prior to the study. Fasting glucose was measured by glucose oxidase technique (Cobas Integra, Roche) and insulin by a chemiluminescent immunoassay method (ADVIA Centaur analyzer; Bayer Diagnostics).

Phenotypes

Body mass index (BMI) (kg/m^2). Gender- and age-specific standard deviation scores (SDS) of BMI were calculated (Supplementary Material, Fig. S2) with the Growth Analyser RCT tool (version 3.0, https://www.growthanalyser.org/; Dutch Growth Research Foundation, Rotterdam, the Netherlands). Within the Growth Analyser, BMI data of 2- to 20-year-olds from Italy were used as the reference (54). We also dichotomized the BMI-SDS scores into obese (SDS ≥ 2) and non-obese using two different definitions for the control groups: (1) non-obese (−2 < BMI SDS < 2) and (2) non-overweight (−1 < BMI SDS < 1). The distribution of the BMI SDS scores according to age of the participants is shown in Figure 5.

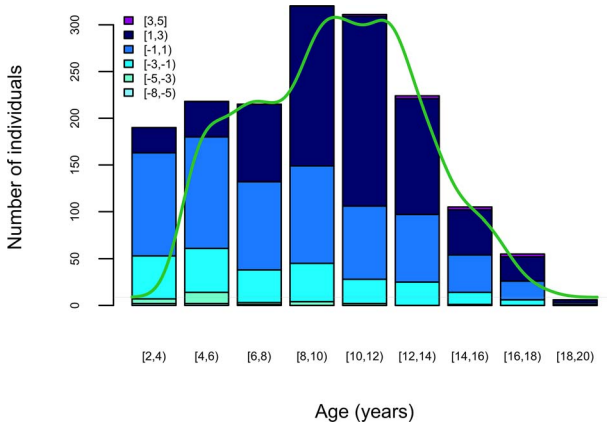

Figure 5. BMI SDS values of the Bambino Gesù study sample within their age distribution. Colours represent BMI SDS groups as displayed in the figure insert. The bars represent subject counts within each BMI SDS group by the age group (x-axis). The green line shows the age density of the study sample.

Fasting insulin (FI) and glucose (FG). None of the included children had diabetes according to WHO criteria (55). We used different units for FG and FI (mmol/l and pmol/l, respectively) for the calculation of indices. Indices of insulin sensitivity and beta cell function, namely HOMA-IR and HOMA-B, were calculated using the HOMA calculator provided by the University of Oxford (https://www.dtu.ox.ac.uk/homacalculator/). To reduce skewness, FI, HOMA-IR and HOMA-B were natural logarithm transformed.

DNA extraction

DNA was extracted from 300 ul of whole blood using the QIAsymphony DSP DNA kit. The extraction was performed on the automated extractor QIAsymphony SP workstation Qiagen, Hilden, Germany) according to manufacturer procedure. DNA was eluted in 200 ul deionized water.

SNP genotyping

For the analysis, we selected 42 DNA variants reported in previous publications (Supplementary Material, Table S1) (12,28,33,56). The SNP genotyping was performed using Agena MassArray® System (Agena Bioscience, San Diego, USA). SNPs were assayed and typed using iPLEX® chemistry on a matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometer. All biochemical reactions were performed as recommended by the manufacturer. The iPLEX single base extension was spotted on 384-SpectroChips and analysed in the MassARRAY Analyzer. MassARRAY Typer 4.0 software was used for evaluating and managing the genotype results.

Quality control of genetic data

For the quality control (QC) purposes, missing rate per individuals and missing rate per SNP were calculated. In addition, for QC of SNP genotyping, positive and negative template control samples were included in each assay plate. Any assay found as positive in the negative template control was removed from the study. We kept SNPs with missingness ≤ 0.02, Hardy–Weinberg equilibrium test P-value $< 1 \times 10^{-6}$, SNP genotyping call rate $\geq 95\%$ and minor allele frequency (MAF) $> 1\%$.

Statistical analyses

All statistical analyses were performed using the software package R version 3.5.1 (57).

Genetic risk scores (GRSs). We calculated unweighted and weighted GRS for FG (20 SNPs), FI (5 SNPs) and T2D (36 SNPs) (see Supplementary Material, Table S1 for the list of SNPs used). Effect sizes of genetic variants on each specific phenotype were obtained from large-scale consortia with mean age ranging from 31.0 to 73.4 (12,28,33,56). For the unweighted GRS, the numbers of effect alleles (0, 1, 2) for each SNP were added up, while for the calculation of the weighted GRS, each effect allele count for each SNP was multiplied by the reported effect size of the effect allele (beta for all, i.e. log(OR) for T2D). If the reported SNP was not available in our data, we used an SNP in linkage disequilibrium and further weighted the SNP by the r^2 value (58). Both unweighted and weighted GRSs were further multiplied by the proportion of successfully genotyped SNPs per individual. The distributions of the GRSs for each respective set of established loci by phenotype were investigated against the distributions of the phenotypes of interest, e.g. FG, FI and T2D. For the association analyses described below, the GRSs were standardized to have a mean value of 0 and standard deviation of 1 to allow comparison of effect estimates across different GRSs and the outcome variables.

Association analysis. We performed the association analysis with FG, FI, HOMA-B, HOMA-IR and BMI SDS for the 42 genotyped SNPs assuming an additive genetic model using linear regression. Unweighted and weighted GRSs were also tested for association with FG, FI, HOMA-B, HOMA-IR and BMI SDS via linear regression. Logistic regression was used for the association analysis of GRS and obese versus non-obese individuals. For the linear regression, we report the effects as regression coefficients with their 95% confidence intervals (CIs), whereas for logistic regression, we provide estimates of odds ratios (OR) with their 95% CIs. We report unadjusted associations as well as analyses adjusted for (1) age, sex and BMI SDS (BMI adjustment not done when BMI or obesity is the outcome), and (2) age, sex, BMI SDS and family history of T1D and T2D. We applied a Bonferroni correction to adjust for multiple testing. The P-value thresholds for statistical significance after Bonferroni correction were $P = 0.0025$, 0.0014 and 0.008 for 5, 20 and 36 tests for FI, FG and T2D SNPs, respectively.

Mendelian randomization. We evaluated the casual relationship between FG/FI and BMI in a one-sample MR framework (Fig. 6) using Two-Stage-Least Squares (2SLS) as implemented in the *ivreg(v.0.5–0)* R package. In 2SLS, the first regression model regresses the exposure on the genetic instruments providing fitted exposure values independent of the confounders. The second stage of 2SLS regresses the outcome on the fitted values of the exposure. The genetic instrument for FG was the same as the GRS for FG described previously, whereas the instrument for FI comprised of four variants after excluding the *FTO* variant from the FI GRS. The genetic instrument for BMI was constructed from the *FTO* and *MC4R* variants, and we used the effect sizes as reported (8). Since Locke *et al.* reported different variants for these two loci, we further weighted the effect sizes by the r^2 values between the reported lead variants and the variants used

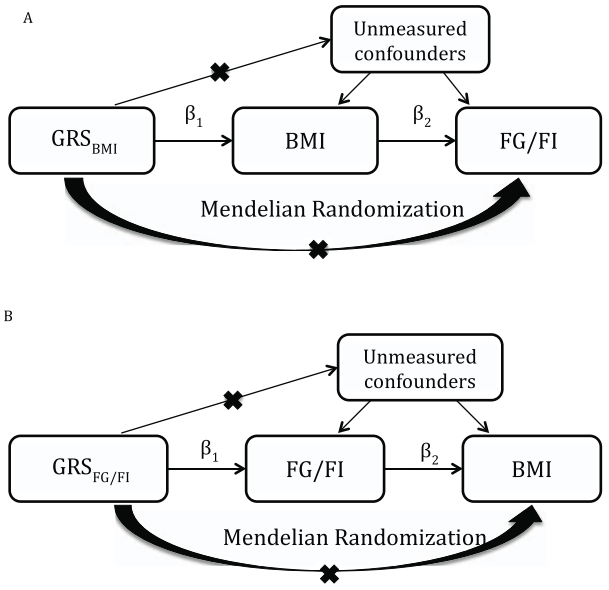

Figure 6. Mendelian randomization analysis to explore causality between BMI and FG/FI. (**A**) IV estimator is calculated as the beta coefficient from the association of GRS_{BMI} with FG or FI divided by the beta coefficient from the association of GRS_{BMI} with BMI (IV estimator = 0.31 pmol/l/BMI unit). The IV estimator is equivalent to what is seen when FI is regressed on BMI. These results are supportive of a causal, non-confounded relationship. (**B**) The relationship of FG or FI with BMI.

in the present study. We report *F*-statistics from the regression model of the exposure on the corresponding IV as a measure of instrument strength. Causal effects estimated in MR are only valid if the following core assumptions hold true: (1) the genetic instrument has a true effect on the exposure and that (2) it only affects the outcome through its effect on the exposure as well as (3) it is independent of any measured and unmeasured confounding factors of the exposure–outcome relationship.

Supplementary Data

Supplementary Material is available at *HMG* online.

Data availability

The datasets generated during and/or analysed during the current study are not publicly available for reasons related to privacy and participant consent but are available from the corresponding author on reasonable request.

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Conflict of Interest statement. The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Contribution statement

Z.B., M.K., A.U., M.M. and I.P. wrote the manuscript and researched the data. M.K., A.U. and A.S. performed the statistical analyses. M.M., I.P. and B.D. designed the study and reviewed and edited the manuscript. R.L., M.B. and L.C. contributed to data/sample collection and genotyping and reviewed and edited the manuscript. B.D. obtained funding for the study. All authors approved the final version of the manuscript.

I.P. and M.M. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Duality of interest

The authors declare that there is no duality of interest associated with this manuscript.

References

1. Shashaj, B., Bedogni, G., Graziani, M.P., Tozzi, A.E., DiCorpo, M.L., Morano, D., Tacconi, L., Veronelli, P., Contoli, B. and Manco, M. (2014) Origin of cardiovascular risk in overweight preschool children: a cohort study of cardiometabolic risk factors at the onset of obesity. *JAMA Pediatr.*, **168**, 917–924.
2. Lauria, L., Spinelli, A., Buoncristiano, M. and Nardone, P. (2019) Decline of childhood overweight and obesity in Italy from 2008 to 2016: results from 5 rounds of the population-based surveillance system. *BMC Public Health*, **19**, 618.
3. Chen, L., Magliano, D.J. and Zimmet, P.Z. (2011) The worldwide epidemiology of type 2 diabetes mellitus–present and future perspectives. *Nat. Rev. Endocrinol.*, **8**, 228–236.
4. Fall, T., Hägg, S., Ploner, A., Mägi, R., Fischer, K., Draisma, H.H., Sarin, A.P., Benyamin, B., Ladenvall, C., Åkerlund, M. *et al.* (2015) Age- and sex-specific causal effects of adiposity on cardiovascular risk factors. *Diabetes*, **64**, 1841–1852.
5. Di Bonito, P., Pacifico, L., Chiesa, C., Valerio, G., Del Giudice, E.M., Maffeis, C., Morandi, A., Invitti, C., Licenziati, M. and Loche, S. (2017) Impaired fasting glucose and impaired glucose tolerance in children and adolescents with overweight/obesity. *J. Endocrinol. Investig.*, **40**, 409–416.
6. Day, C. and Bailey, C.J. (2011) Obesity in the pathogenesis of type 2 diabetes. *Br. J. Diabetes Vasc. Dis.*, **11**, 55–61.
7. Cousminer, D.L. and Freathy, R.M. (2020) Genetics of early growth traits. *Hum. Mol. Genet.*, **29**, R66–R72.

8. Locke, A.E., Kahali, B., Berndt, S.I., Justice, A.E., Pers, T.H., Day, F.R., Powell, C., Vedantam, S., Buchkovich, M.L., Yang, J. *et al.* (2015) Genetic studies of body mass index yield new insights for obesity biology. *Nature*, **518**, 197–206.
9. Mahajan, A., Taliun, D., Thurner, M., Robertson, N.R., Torres, J.M., Rayner, N.W., Payne, A.J., Steinthorsdottir, V., Scott, R.A., Grarup, N. *et al.* (2018) Fine-mapping type 2 diabetes loci to single-variant resolution using high-density imputation and islet-specific epigenome maps. *Nat. Genet.*, **50**, 1505–1513.
10. Ingelsson, E. and McCarthy, M.I. (2018) Human genetics of obesity and type 2 diabetes mellitus. *Circ. Genom. Precis. Med.*, **11**, e002090.
11. Barroso, I. and McCarthy, M.I. (2019) The genetic basis of metabolic disease. *Cell*, **177**, 146–161.
12. Morris, A.P., Voight, B.F., Teslovich, T.M., Ferreira, T., Segre, A.V., Steinthorsdottir, V., Strawbridge, R.J., Khan, H., Grallert, H. and Mahajan, A. (2012) Large-scale association analysis provides insights into the genetic architecture and pathophysiology of type 2 diabetes. *Nat. Genet.*, **44**, 981.
13. Graae, A.-S., Hollensted, M., Kloppenborg, J.T., Mahendran, Y., Schnurr, T.M., Appel, E.V.R., Rask, J., Nielsen, T.R., Johansen, M.Ø. and Linneberg, A. (2018) An adult-based insulin resistance genetic risk score associates with insulin resistance, metabolic traits and altered fat distribution in Danish children and adolescents who are overweight or obese. *Diabetologia*, **61**, 1769–1779.
14. Stančáková, A., Kuulasmaa, T., Kuusisto, J., Mohlke, K.L., Collins, F.S., Boehnke, M. and Laakso, M. (2017) Genetic risk scores in the prediction of plasma glucose, impaired insulin secretion, insulin resistance and incident type 2 diabetes in the METSIM study. *Diabetologia*, **60**, 1722–1730.
15. Lotta, L.A., Gulati, P., Day, F.R., Payne, F., Ongen, H., Van De Bunt, M., Gaulton, K.J., Eicher, J.D., Sharp, S.J. and Rolfe, E.D.L. (2017) Integrative genomic analysis implicates limited peripheral adipose storage capacity in the pathogenesis of human insulin resistance. *Nat. Genet.*, **49**, 17.
16. Vassy, J.L., Hivert, M.-F., Porneala, B., Dauriz, M., Florez, J.C., Dupuis, J., Siscovick, D.S., Fornage, M., Rasmussen-Torvik, L.J. and Bouchard, C. (2014) Polygenic type 2 diabetes prediction at the limit of common variant detection. *Diabetes*, **63**, 2172–2182.
17. Vaxillaire, M., Yengo, L., Lobbens, S., Rocheleau, G., Eury, E., Lantieri, O., Marre, M., Balkau, B., Bonnefond, A. and Froguel, P. (2014) Type 2 diabetes-related genetic risk scores associated with variations in fasting plasma glucose and development of impaired glucose homeostasis in the prospective DESIR study. *Diabetologia*, **57**, 1601–1610.
18. Jensen, A.C., Barker, A., Kumari, M., Brunner, E.J., Kivimäki, M., Hingorani, A.D., Wareham, N.J., Tabák, A.G., Witte, D.R. and Langenberg, C. (2011) Associations of common genetic variants with age-related changes in fasting and postload glucose: evidence from 18 years of follow-up of the Whitehall II cohort. *Diabetes*, **60**, 1617–1623.
19. Andersson, E.A., Allin, K.H., Sandholt, C.H., Borglykke, A., Lau, C.J., Ribel-Madsen, R., Sparsø, T., Justesen, J.M., Harder, M.N. and Jørgensen, M.E. (2013) Genetic risk score of 46 type 2 diabetes risk variants associates with changes in plasma glucose and estimates of pancreatic β-cell function over 5 years of follow-up. *Diabetes*, **62**, 3610–3617.
20. Go, M.J., Lee, Y., Park, S., Kwak, S.H., Kim, B.-J. and Lee, J. (2016) Genetic-risk assessment of GWAS-derived susceptibility loci for type 2 diabetes in a 10 year follow-up of a population-based cohort study. *J. Hum. Genet.*, **61**, 1009–1012.
21. Lyssenko, V., Jonsson, A., Almgren, P., Pulizzi, N., Isomaa, B., Tuomi, T., Berglund, G., Altshuler, D., Nilsson, P. and Groop, L. (2008) Clinical risk factors, DNA variants, and the development of type 2 diabetes. *N. Engl. J. Med.*, **359**, 2220–2232.
22. de Miguel-Yanes, J.M., Shrader, P., Pencina, M.J., Fox, C.S., Manning, A.K., Grant, R.W., Dupuis, J., Florez, J.C., D'Agostino, R.B. and Cupples, L.A. (2011) Genetic risk reclassification for type 2 diabetes by age below or above 50 years using 40 type 2 diabetes risk single nucleotide polymorphisms. *Diabetes Care*, **34**, 121–125.
23. Vassy, J., Durant, N., Kabagambe, E., Carnethon, M.R., Rasmussen-Torvik, L.J., Fornage, M., Lewis, C., Siscovick, D. and Meigs, J. (2012) A genotype risk score predicts type 2 diabetes from young adulthood: the CARDIA study. *Diabetologia*, **55**, 2604–2612.
24. Vassy, J.L., DasMahapatra, P., Meigs, J.B., Schork, N.J., Magnussen, C.G., Chen, W., Raitakari, O.T., Pencina, M.J., Jamal, S.M. and Berenson, G.S. (2012) Genotype prediction of adult type 2 diabetes from adolescence in a multiracial population. *Pediatrics*, **130**, e1235–e1242.
25. Walford, G.A., Porneala, B.C., Dauriz, M., Vassy, J.L., Cheng, S., Rhee, E.P., Wang, T.J., Meigs, J.B., Gerszten, R.E. and Florez, J.C. (2014) Metabolite traits and genetic risk provide complementary information for the prediction of future type 2 diabetes. *Diabetes Care*, **37**, 2508–2514.
26. Mahajan, A., Go, M.J., Zhang, W., Below, J.E., Gaulton, K.J., Ferreira, T., Horikoshi, M., Johnson, A.D., Ng, M.C., Prokopenko, I. *et al.* (2014) Genome-wide trans-ancestry meta-analysis provides insight into the genetic architecture of type 2 diabetes susceptibility. *Nat. Genet.*, **46**, 234–244.
27. Florez, J. (2008) Newly identified loci highlight beta cell dysfunction as a key cause of type 2 diabetes: where are the insulin resistance genes? *Diabetologia*, **51**, 1100.
28. Dupuis, J., Langenberg, C., Prokopenko, I., Saxena, R., Soranzo, N., Jackson, A.U., Wheeler, E., Glazer, N.L., Bouatia-Naji, N., Gloyn, A.L. *et al.* (2010) New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk. *Nat. Genet.*, **42**, 105–116.
29. Scott, R.A., Lagou, V., Welch, R.P., Wheeler, E., Montasser, M.E., Luan, J., Magi, R., Strawbridge, R.J., Rehnberg, E., Gustafsson, S. *et al.* (2012) Large-scale association analyses identify new loci influencing glycemic traits and provide insight into the underlying biological pathways. *Nat. Genet.*, **44**, 991–1005.
30. Grarup, N., Sandholt, C.H., Hansen, T. and Pedersen, O. (2014) Genetic susceptibility to type 2 diabetes and obesity: from genome-wide association studies to rare variants and beyond. *Diabetologia*, **57**, 1528–1541.
31. Voight, B.F., Scott, L.J., Steinthorsdottir, V., Morris, A.P., Dina, C., Welch, R.P., Zeggini, E., Huth, C., Aulchenko, Y.S., Thorleifsson, G. *et al.* (2010) Twelve type 2 diabetes susceptibility loci identified through large-scale association analysis. *Nat. Genet.*, **42**, 579–589.
32. Scott, R.A., Chu, A.Y., Grarup, N., Manning, A.K., Hivert, M.-F., Shungin, D., Tönjes, A., Yesupriya, A., Barnes, D. and Bouatia-Naji, N. (2012) No interactions between previously associated 2-hour glucose gene variants and physical activity or BMI on 2-hour glucose levels. *Diabetes*, **61**, 1291–1296.
33. Voight, B.F., Scott, L.J., Steinthorsdottir, V., Morris, A.P., Dina, C., Welch, R.P., Zeggini, E., Huth, C., Aulchenko, Y.S. and Thorleifsson, G. (2010) Twelve type 2 diabetes susceptibility loci identified through large-scale association analysis. *Nat. Genet.*, **42**, 579.
34. Freathy, R.M., Timpson, N.J., Lawlor, D.A., Pouta, A., Ben-Shlomo, Y., Ruokonen, A., Ebrahim, S., Shields, B., Zeggini,

E., Weedon, M.N. *et al.* (2008) Common variation in the FTO gene alters diabetes-related metabolic traits to the extent expected given its effect on BMI. *Diabetes*, **57**, 1419–1426.
35. Fall, T., Hagg, S., Magi, R., Ploner, A., Fischer, K., Horikoshi, M., Sarin, A.P., Thorleifsson, G., Ladenvall, C., Kals, M. *et al.* (2013) The role of adiposity in cardiometabolic traits: a Mendelian randomization analysis. *PLoS Med.*, **10**, e1001474.
36. Xu, L., Borges, M.C., Hemani, G. and Lawlor, D.A. (2017) The role of glycaemic and lipid risk factors in mediating the effect of BMI on coronary heart disease: a two-step, two-sample Mendelian randomisation study. *Diabetologia*, **60**, 2210–2220.
37. Dale, C.E., Fatemifar, G., Palmer, T.M., White, J., Prieto-Merino, D., Zabaneh, D., Engmann, J.E.L., Shah, T., Wong, A., Warren, H.R. *et al.* (2017) Causal associations of adiposity and body fat distribution with coronary heart disease, stroke subtypes, and type 2 diabetes mellitus: a Mendelian randomization analysis. *Circulation*, **135**, 2373–2388.
38. Hägg, S., Fall, T., Ploner, A., Mägi, R., Fischer, K., Draisma, H.H., Kals, M., de Vries, P.S., Dehghan, A., Willems, S.M. *et al.* (2015) Adiposity as a cause of cardiovascular disease: a Mendelian randomization study. *Int. J. Epidemiol.*, **44**, 578–586.
39. Whincup, P.H., Gilg, J.A., Papacosta, O., Seymour, C., Miller, G.J., Alberti, K.G. and Cook, D.G. (2002) Early evidence of ethnic differences in cardiovascular risk: cross sectional comparison of British south Asian and white children. *BMJ (Clinical Research ed.)*, **324**, 635.
40. Tagi, V.M., Giannini, C. and Chiarelli, F. (2019) Insulin resistance in children. *Front. Endocrinol.*, **10**.
41. Preis, S.R., Massaro, J.M., Robins, S.J., Hoffmann, U., Vasan, R.S., Irlbeck, T., Meigs, J.B., Sutherland, P., D'Agostino, R.B., Sr., O'Donnell, C.J. *et al.* (2010) Abdominal subcutaneous and visceral adipose tissue and insulin resistance in the Framingham heart study. *Obesity (Silver Spring)*, **18**, 2191–2198.
42. Scott, R.A., Scott, L.J., Magi, R., Marullo, L., Gaulton, K.J., Kaakinen, M., Pervjakova, N., Pers, T.H., Johnson, A.D., Eicher, J.D. *et al.* (2017) An expanded genome-wide association study of type 2 diabetes in Europeans. *Diabetes*, **66**, 2888–2902.
43. Bjørnland, T., Langaas, M., Grill, V. and Mostad, I.L. (2017) Assessing gene-environment interaction effects of FTO, MC4R and lifestyle factors on obesity using an extreme phenotype sampling design: results from the HUNT study. *PLoS One*, **12**.
44. Speliotes, E.K., Willer, C.J., Berndt, S.I., Monda, K.L., Thorleifsson, G., Jackson, A.U., Lango Allen, H., Lindgren, C.M., Luan, J.a., Mägi, R. *et al.* (2010) Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. *Nat. Genet.*, **42**, 937–948.
45. Morandi, A., Bonnefond, A., Lobbens, S., Yengo, L., Miraglia del Giudice, E., Grandone, A., Lévy-Marchal, C., Weill, J., Maffeis, C. and Froguel, P. (2016) Associations between type 2 diabetes-related genetic scores and metabolic traits, in obese and normal-weight youths. *J. Clin. Endocrinol. Metabol.*, **101**, 4244–4250.
46. Prokopenko, I., Langenberg, C., Florez, J.C., Saxena, R., Soranzo, N., Thorleifsson, G., Loos, R.J., Manning, A.K., Jackson, A.U., Aulchenko, Y. *et al.* (2009) Variants in MTNR1B influence fasting glucose levels. *Nat. Genet.*, **41**, 77–81.
47. Lyssenko, V., Nagorny, C.L., Erdos, M.R., Wierup, N., Jonsson, A., Spégel, P., Bugliani, M., Saxena, R., Fex, M., Pulizzi, N. *et al.* (2009) Common variant in MTNR1B associated with increased risk of type 2 diabetes and impaired early insulin secretion. *Nat. Genet.*, **41**, 82–88.
48. THart, L.M., Simonis-Bik, A.M., Nijpels, G., van Haeften, T.W., Schäfer, S.A., Houwing-Duistermaat, J.J., Boomsma, D.I., Groenewoud, M.J., Reiling, E., van Hove, E.C. *et al.* (2010) Combined risk allele score of eight type 2 diabetes genes is associated with reduced first-phase glucose-stimulated insulin secretion during hyperglycemic clamps. *Diabetes*, **59**, 287–292.
49. Gupta, D., Kono, T. and Evans-Molina, C. (2010) The role of peroxisome proliferator-activated receptor γ in pancreatic β cell function and survival: therapeutic implications for the treatment of type 2 diabetes mellitus. *Diabetes Obes. Metab.*, **12**, 1036–1047.
50. Li, L.-c., Wang, Y., Carr, R., Haddad, C.S., Li, Z., Qian, L., Oberholzer, J., Maker, A.V., Wang, Q. and Prabhakar, B.S. (2014) IG20/MADD plays a critical role in glucose-induced insulin secretion. *Diabetes*, **63**, 1612–1623.
51. Steinthorsdottir, V., Thorleifsson, G., Reynisdottir, I., Benediktsson, R., Jonsdottir, T., Walters, G.B., Styrkarsdottir, U., Gretarsdottir, S., Emilsson, V., Ghosh, S. *et al.* (2007) A variant in CDKAL1 influences insulin response and risk of type 2 diabetes. *Nat. Genet.*, **39**, 770–775.
52. (2013) World medical association declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA*, **310**, 2191–2194.
53. Shashaj, B., Luciano, R., Contoli, B., Morino, G.S., Spreghini, M.R., Rustico, C., Sforza, R.W., Dallapiccola, B. and Manco, M. (2016) Reference ranges of HOMA-IR in normal-weight and obese young Caucasians. *Acta Diabetol.*, **53**, 251–260.
54. Cacciari, E., Milani, S., Balsamo, A., Spada, E., Bona, G., Cavallo, L., Cerutti, F., Gargantini, L., Greggio, N., Tonini, G. *et al.* (2006) Italian cross-sectional growth charts for height, weight and BMI (2 to 20 yr). *J. Endocrinol. Investig.*, **29**, 581–593.
55. WHO/IDF (2006) *Definition and Diagnosis of Diabetes Mellitus and Intermediate Hyperglycaemia : Report of a WHO/IDF Consultation*. World Health Organization, Geneva.
56. Scott, R.A., Lagou, V., Welch, R.P., Wheeler, E., Montasser, M.E., Mägi, R., Strawbridge, R.J., Rehnberg, E., Gustafsson, S. and Kanoni, S. (2012) Large-scale association analyses identify new loci influencing glycemic traits and provide insight into the underlying biological pathways. *Nat. Genet.*, **44**, 991.
57. R Core Team. (2021) *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.
58. Belsky, D.W., Moffitt, T.E., Sugden, K., Williams, B., Houts, R., McCarthy, J. and Caspi, A. (2013) Development and evaluation of a genetic risk score for obesity. *Biodemography Soc. Biol.*, **59**, 85–100.