The co-occurrence of risk alleles in or near genes modulating insulin secretion predisposes obese youth to prediabetes

Cosimo Giannini\textsuperscript{1} MD, PhD, Chiara Dalla Man\textsuperscript{2} PhD, Leif Groop\textsuperscript{3} MD, PhD, Claudio Cobelli\textsuperscript{2} PhD, Hongyu Zhao\textsuperscript{4} PhD, Melissa M. Shaw\textsuperscript{1} BS, Elvira Duran\textsuperscript{1} BA, Bridget Pierpont\textsuperscript{1} MA, Allen E. Bale\textsuperscript{5} MD, Sonia Caprio\textsuperscript{1} MD, Nicola Santoro\textsuperscript{1} MD, PhD.

1) Department of Pediatrics, Yale University School of Medicine, New Haven, CT; 2) Department of Information Engineering, University of Padua, Padua, Italy; 3) Department of Clinical Sciences/Diabetes & Endocrinology and Lund University Diabetes Centre, Lund University, University Hospital, Malmö, Malmö, Sweden; 4) Department of Biostatistics, Yale School of Public Health, New Haven, CT; 5) Department of Genetics, Yale University School of Medicine, New Haven, CT.

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Address correspondence to:
Nicola Santoro, M.D. PhD
Yale University School of Medicine, Department of Pediatrics
330 Cedar Street, P.O. Box 208064, New Haven, CT 06520
Telephone number: (203) 737-6356
Fax Number: (203) 785-6421
E-mail: nicola.santoro@yale.edu

Sonia Caprio, M.D.
Yale University School of Medicine, Department of Pediatrics
330 Cedar Street, P.O. Box 208064, New Haven, CT 06520
Telephone number: (203) 785-5692
Fax Number: (203) 785-6421
E-mail: sonia.caprio@yale.edu
Abstract

**Background and aims:** Paralleling the rise of pediatric obesity, the prevalence of impaired glucose tolerance (IGT) and type 2 diabetes (T2D) is increasing among youths. In this study we asked whether the co-occurrence of risk alleles in or near 5 genes modulating insulin secretion (*TCF7L2* rs7903146, *IGF2BP2* rs4402960, *CDKAL1* rs7754840, *HHEX* rs1111875, and *HNF1A* rs1169288) is associated with a higher risk of IGT/ T2D in obese children and adolescents.

**Methods:** We studied 714 obese subjects (290 boys and 424 girls; mean age 13.6±3.1 years; mean z-score BMI 2.2±0.4), evaluated the insulin secretion by using the oral minimal model and, in a subgroup of 37 subjects, the hyperglycemic clamp. Also, 203 were followed-up for a mean of 2.1 years. **Results:** We observed that the increase of risk alleles was associated with a progressive worsening of insulin secretion (P<.001) mainly due to an impairment of the dynamic phase of insulin secretion (p=0.004). The higher was the number of the risk alleles the higher was the chance of progression from NGT to IGT/T2D (p=0.022), also for those who were IGT at baseline, a higher risk score was associated with a lower odds to revert to NGT (p=0.026).

**Conclusion:** Obese children and adolescents developing IGT/T2D have a higher genetic predisposition than those who do not show these diseases and this predisposition is mainly related to gene variants modulating the early phase of insulin secretion. Although these data are very interesting, they need to be replicated in other cohorts.

**Key words:** gene score, type 2 diabetes, youth, obesity
Introduction

Type 2 diabetes mellitus (T2D) has emerged as one of the greatest global health challenges of the twenty-first century, projected to affect roughly 1 out of 3 individuals born in the year 2000 during their lifetime (1). Recent population-based data from the SEARCH study (Search for Diabetes in Youth) indicate that T2D is diagnosed in about 3700 obese youths annually in US (2, 3). Also, using the most recent population-based estimates of diabetes incidence and prevalence and taking into account demographic changes over time, Imperatore et al reported that in the next 40 years the number of youth with T2D is projected to increase by 49% (4). Onset of type 2 diabetes in childhood or adolescence heralds many years of disease and an increased risk of the full range of both micro- and macrovascular complications that will occur when affected individuals are still relatively young. Therefore, it is imperative to identify early young subjects at increased risk of T2D when they are in the pre-diabetic state, particularly because the tempo of progression from impaired glucose tolerance to full blown diabetes seems to be faster in youth than in adults (5-8).

In the last decade genome-wide association studies (GWAS) have discovered a large number of T2D susceptibility loci (9-13). Among them, many are near or inside genes coding for proteins involved in insulin secretion (14, 15). Numerous studies mainly performed in adults showed that genetic risk scores obtained by combining the risk alleles (i.e. the alleles associated with the disease) of the SNPs associated with T2D are associated with an enhanced diabetes risk (16-23). However, the predictive power of any risk allele scores is yet insufficient to substitute or largely improve predictive power of known clinical risk factors (16, 24).
Although it is plausible to think that T2D and related metabolic traits have a substantially larger heritable component at younger than older ages, genetic studies in prediabetic children and adolescents are missing. To gain insights into the potential genetic underpinnings of IGT/T2D in obese children we analyzed a panel of 5 gene variants robustly associated with T2D susceptibility identified in adults by GWA studies (9-13). The rationale for selecting these gene variants is based on studies recently performed in human islets indicating that these genes are involved in the release of insulin granules from the beta cell (25). For instance, variants near the \textit{TCF7L2} were associated with reduced depolarization-evoked insulin exocytosis and susceptibility and variants near \textit{HHEX} gene were associated with granule docking (25). Although previous studies in the pediatric population have shown an association between some of these SNPs and fasting glucose and insulin levels (26, 27), so far there are no studies in youth assessing whether or not these risk alleles confer a high susceptibility to develop early onset impaired glucose tolerance. This study aims at filling this gap of knowledge; in particular herein, we sought 1) to examine whether in obese youth a genetic risk score for beta-cell dysfunction from 5 single nucleotide polymorphisms (SNPs) known to modulate insulin secretion, (\textit{TCF7L2} rs7903146, \textit{IGF2BP2} rs4402960, \textit{CDKAL1} rs7754840, \textit{HHEX} rs1111875, and \textit{HNF1A} rs1169288), might be associated with a higher risk to show prediabetes and T2D; 2) to determine the effects of these genetic variants on changes in insulin secretion and sensitivity; 3) to assess whether their co-occurrence might predict changes in glucose tolerance over time in obese youth.

\textbf{Research Design and Methods}
Subjects were recruited from a multiethnic cohort participating in the Yale Pathophysiology of Type 2 Diabetes Study, a long-term project aimed to study early alterations in glucose metabolism in obese children and adolescents. In order to be eligible, subjects needed to be obese and not to take medications that affect glucose metabolism (i.e., insulin and metformin, the only two medications approved to treat type 2 diabetes in pediatrics). The study was approved by the Human Investigations Committee of the Yale School of Medicine. Parental informed consent and child assent were obtained from all participants. From its original focus on determining the associations between early alterations in insulin sensitivity and secretion and the presence of impaired glucose tolerance in obese children and adolescents, the Yale Pathophysiology of Type 2 diabetes in Youth Study was broadened in scope to assess the genetic susceptibility and the identification of precursors of dysglycemia as they move through adolescence. The need for a follow-up study grew out of our clinical observational study indicating the rather unstable and vulnerable state of prediabetes in youngsters (28). Consequently, the study was expanded to include a second oral glucose tolerance test (OGTT) at approximately two years of follow-up. The rational for the two-year time interval was based on a previous study indicating that changes in categories of glucose tolerance in obese adolescents are likely to occur over a relatively short period (28).

As indicated in the supplementary table 1, we studied 714 obese children and adolescents (290 boys and 424 girls; mean age 13.6±3.1 years; mean z-score BMI 2.2±0.4) referred to the Yale Pediatric Obesity Clinic. To evaluate the effect of the gene variants on the risk of developing prediabetes, Two hundred and three subjects received a second OGTT after a follow-up of 2.1±1.2 years. During the follow-up time all participants received standard nutritional guidance and recommendations for physical activity. The same guidance was provided to all the subjects
attending to the obesity clinic. Unfortunately the follow-up rate was relatively low because, due to budgetary constraints, we were forced to limit the number of OGTT performed yearly. Additionally, it is likely that the low returned rates may be due to the fact that subjects are followed in the setting of clinic based pediatric weight management program. Further, most of these children come from economically disadvantaged families which may contribute to the high drop-outs rate. It should be noted that our drop-out rates is very consistent with that from other studies demonstrating high rates of attrition from pediatric weight management programs, ranging from 27% to 73% (29). To illustrate the number of children participating in the cross-sectional and longitudinal arms we created a flowchart shown in the supplementary figure 1.

**Assessment of Insulin sensitivity and secretion: The Oral Glucose Minimal Model.**

Subjects were studied at the Yale Clinical Center Investigation (YCCI) at 8 am, after a 10 hour overnight fast (25). A 3-h oral glucose tolerance test (OGTT) (1.75 g/Kg body weight, up to 75 g) was performed in all children and adolescents, as previously reported (29, 30). Two baseline samples were obtained at –15 and 0 min for measurements of plasma glucose, insulin and C-peptide. Thereafter, flavored glucose (Orangedex; Custom Laboratories, Baltimore, MD, USA) was given orally, and blood samples were obtained at 10, 20 and 30 minutes and every 30 min thereafter for 180 min for the measurements of plasma glucose, insulin and C-peptide. Glucose tolerance status was defined according to American Diabetes Association guidelines (31).

The Insulin Sensitivity ($S_I$) was estimated from plasma glucose and insulin concentrations measured during the 3-h OGTT using the Oral Glucose Minimal Model (30- 32). This index has been validated against the euglycemic clamp, showing a correlation of 0.81 ($P = 0.001$) (32). Beta-cell responsivity indices were estimated from plasma glucose and C-peptide concentrations measured during the OGTT by using the oral C-peptide minimal model (32-36).
The model assumes that insulin secretion is made up of two components. The dynamic component is likely to represent secretion of promptly releasable insulin and is proportional to the rate of increase of glucose concentration through a parameter, \( \Phi_d \left(10^{-9}\right) \), which defines the dynamic responsivity index. The static component derives from provision of new insulin to the releasable pool and is characterized by a static index, \( \Phi_s \left(10^{-9}\text{ min}^{-1}\right) \), and by a delay time constant, \( T \) (min). From \( \Phi_d \) and \( \Phi_s \) one can also calculate a single, overall \( \beta \)-cell responsivity index, \( \Phi_{\text{total}} \left(10^{-9}\text{ min}^{-1}\right) \). Finally a basal \( \beta \)-cell responsivity index, \( \Phi_b \left(10^{-9}\text{ min}^{-1}\right) \) can also be calculated from basal C-peptide and glucose concentrations. In order to determine whether \( \beta \)-cell function is appropriate for the degree of insulin resistance, \( \Phi_{\text{total}} \) can be expressed in relation to insulin sensitivity through the disposition index \( DI=\Phi_{\text{total}} S_I \) (37).

**Hyperglycemic clamp**

To quantify insulin secretion, plasma glucose concentration was raised to 11 mmol/liter by infusion of 20% dextrose at variables rates and kept at that value for 120 min (1). Samples were drawn at 2, 4, 6, 8, 10, and every 20 min thereafter for glucose, insulin, and C-peptide concentrations. Incremental first-phase concentration of insulin and C-peptide was calculated as the mean of 2, 4, 6, 8, and 10 min values minus the mean of -20, -10 and 0 fasting levels. Mean second-phase concentration of insulin and C-peptide was calculated as the mean value of 60 and 120 min as previously described (5, 37, and 38). Only 37 subjects volunteered to have this test as it was offered to them as an option.

**Genotyping**

We genotyped 5 SNPs in 5 genes that in recent cross-sectional GWAS have shown consistent association with T2D and found to modulate insulin secretion: \( TCF7L2 \) rs7903146, \( CDKAL1 \), and \( ... \)
rs7754840, *IGF2BP2* rs4402960, *HHEX* rs1111875, and *HNF1A* rs1169288 (14). Genomic DNA was extracted from peripheral blood leukocytes. Genotyping was performed with the use of a matrix assisted based laser desorption-ionization time of flight mass spectrometry on the MassARRAY platform (Sequenom) (16). We obtained an average genotyping success rate of more than 95% and an average genotyping accuracy of more than 98% by re-genotyping 11% of the samples using the Sequenom platform. The allele frequencies were consistent with those shown in similar ethnic groups in the Allele Frequency Database (ALFRED, http://alfred.med.yale.edu) as well as in HAPMAP (http://hapmap.ncbi.nlm.nih.gov/) (supplementary table 2). Within each ethnic group there was no evidence against the null hypothesis that the genotype distribution was in Hardy Weinberg equilibrium for all of the variants (all p>0.003) (supplementary table 2).

**Biochemical analyses**

Plasma glucose was determined using the YSI 2700 Analyzer. Plasma insulin was measured by the Linco RIA, which has less than 1% cross-reactivity with C-peptide and proinsulin. Plasma C-peptide was assayed with an assay made by Diagnostic Product (Los Angeles, CA).

**Statistical analyses**

Distribution of continuous variables was examined for skewness and variables were logarithmically transformed when appropriate. A Chi square test was used to assess the Hardy Weinberg equilibrium for each of the studied SNPs and to compare proportions. The genetic score was calculated by a risk allele counting. In each ethnic group the association between the genetic score and the main outcome ($\Phi_{\text{total}}$) and secondary (2-hour glucose, DI, SI) outcomes
was assessed by a general linear model using an additive model and age, gender and z-score BMI were used as covariate. Age, gender and BMI expressed as z-score were chosen as covariates because insulin secretion is influenced by changes occurring with age; also those physiological modifications of insulin sensitivity and, as consequence of insulin secretion, are different between males and females, and are exacerbated by the obesity degree. This approach has been used previously in other pediatric association studies (27). No other covariates have been investigated. To assess whether there was an interaction between the gene score and the ethnicity in modulating Φ_total or DI, a model including the ethnicity and gene score separately and an interaction term (gene score X ethnicity) including as covariates age, sex and z-score BMI was run. The odds of showing IGT according to the number of risk alleles at baseline was evaluated by a logistic regression analysis and age, gender and z-score BMI were used as covariates. To evaluate whether the addition of genetic data to BMI degree was associated with a higher risk of showing alteration of glucose tolerance, we divided the population into quartiles of BMI (1st quartile≤28.7 kg/m^2; 2nd quartile between 28.7 and 33.0 kg/m^2, 3rd quartile between 33.0 and 38.5 kg/m^2, 4th quartile ≥ 38.5 kg/m^2) as well as by gene score groups (1st group gene score ≤2; 2nd group gene score between 3-5; and 3rd group gene score ≥ 6). Thereafter, we tested the differences of IGT prevalence across the three genetic score groups in each BMI quartile, using a Chi square test. Also to explore the additive effect between BMI and the number of risk alleles on the development of IGT a logistic regression model was run, and the odds for subjects carrying more than 6 risk alleles of showing IGT across the BMI groups was calculated.

In the longitudinal analysis, the three ethnic groups were merged and the risk at follow-up of NGT subjects to progress to IGT and the risk for IGT subjects to stay IGT or progress to T2D was calculated by using a logistic regression model by including as covariates age, gender,
ethnicity, baseline z-score BMI, delta z-score BMI and follow-up time. Statistical analyses were performed with SPSS (19.0 for Windows, SPSS Inc., Chicago, IL). All data were expressed as mean± standard deviations (SD) or median and inter-quartile range.

Results
The study population consisted of three ethnic groups, 316 Caucasians, 188 African Americans, 210 Hispanics and included 530 (74.4%) subjects with normal glucose tolerance (NGT) and 184 (25.6%) with impaired glucose tolerance (IGT). Family history of T2D was collected in all subjects at recruitment. All participants underwent a 3 hours OGTT, whereas a subgroup of 37 obese youths (16 Caucasians, 10 African Americans and 11 Hispanics, 17 boys and 20 girls, mean age 13.6±2.9 years, BMI 35.3±6.9 kg/m², z-score BMI 2.2±0.4) agreed to undergo a hyperglycemic clamp. To evaluate potential changes in glucose tolerance, in a sub-group of 203 (36%) subjects (115 NGT/ 88 IGT), who came back for a follow-up visit, a second OGTT was performed after a mean 2.1±1.2 years. A flowchart showing the study participants is shown in supplementary figure 1 Out of 115 obese youth who were NGT at baseline, 103 (90.5%) remained NGT while 12 (10.5%) progressed to IGT. Of 88 subjects who were IGT at baseline, 53 (60%) converted to NGT, 31 (35%) remained IGT and 4 (4.5%) progressed to T2D at follow-up.

Effect of a genetic score on the risk of IGT at baseline

The prevalence of IGT was significantly different across the genetic risk score groups in all the ethnicities: the higher was the genetic score the higher was the prevalence of IGT (figure 1). In particular, the odds to show prediabetes per additional risk allele was OR=1.454 (95%CI 1.207-1.751) in Caucasians, OR=1.541 (95%CI 1.210-1.962) in African Americans and OR=1.458 (95%CI 1.182-1.799) in Hispanics. To analyze whether genetic risk factors would further
increase the risk imposed by a high BMI we stratified the entire cohort by quartiles of BMI and within each quartile by increasing genetic risk score and then examined the prevalence of IGT across the genetic score group for each BMI quartile as reported in figure 2. In each BMI quartile group, we found a significant increase in IGT prevalence, therefore independently of BMI quartile group the presence of 6 or more risk alleles resulted in a significant increase of having IGT and most importantly the risk of showing IGT according to the number of risk alleles increased across the BMI quartiles. In particular, carriers of more than 6 risk alleles in each of BMI quartiles compared to the first quartile had an odds ratio to show IGT of 6.99 in the second BMI quartile, (95% CI, 1.58 to 30.91; P=0.10), of 4.60 in the third BMI quartile (95% CI, 1.26 to 16.77; P=0.02), and 5.63 in the fourth BMI quartile (95% CI, 1.65 to 19.17; P=0.006).

Also, the genetic score significantly correlated with the family history of T2D (P<0.001), thus with the increasing of risk allele number we found a significant increase in the prevalence of a positive family history (supplementary table 3).

**Risk score and insulin secretion**

We found a significant association between the number of the risk alleles and the main outcome: the beta-cell glucose responsiveness expressed as $\Phi_{\text{total}}$ (figure 3). In fact, as shown in figure 3, in the three ethnic groups, we observed a significant and positive association between the genetic score and measures of insulin secretion ($\Phi_{\text{total}}$) and similarly, the genetic score was associated with the DI values (supplementary table 4). Also, the association between the gene score and Log $\Phi_{\text{total}}$ remained statistically significant also after multiple comparisons adjustment (significant value p =0.013). There was no interaction between ethnicity and gene score in modulating the $\Phi_{\text{total}}$ (p=0.49) or DI (p=0.12). Of note, no association was documented with the $S_I$ (supplementary table 4) suggesting that the effect of the risk score on insulin secretion was
independent of insulin sensitivity. The association between the genetic score and beta-cell function indexes was further evaluated by using hyperglycemic clamp derived measures of insulin secretion. As shown in the supplementary figure 2, we documented a significant association between the gene score and the first phase insulin secretion \( (p=0.004) \); no association was found between the gene score and the second phase insulin secretion \( (p=0.75) \).

**Follow-up cohort**

We also determined whether the co-occurrence of risk alleles might increase the risk of progression from NGT to IGT or for IGT subjects to remain IGT or progressing to diabetes. The odds ratio of progressing to IGT in those youths who were NGT at baseline was \( 1.89 \) (95% C.I. 1.099-3.265; \( P=0.022 \)) per each risk allele, while the odds ratio of staying IGT or progressing to T2D at follow up in those youth who were IGT at baseline was \( 1.44 \) (95% C.I. 1.046-1.983; \( P=0.026 \)) per risk allele. In those subjects who were IGT at baseline, to further illustrate the dynamic changes in glucose tolerance status by risk allele we calculated the prevalence of subjects that converted from IGT to NGT and that stayed IGT or converted to T2D for each risk allele. As shown in figure 4, with increasing of risk alleles the prevalence of subjects converting from IGT to NGT decreased significantly, in contrast with the increase of risk alleles the prevalence of subjects remaining IGT or progressing to T2D increased significantly.

**Discussion**

The current study provides, for the first time, insights about the importance of the association of common genetic variants with prediabetes in obese youth. Herein, we show that in obese youth
the combined effect of five common variants in or near genes involved in the pathway of insulin release is associated with a reduced insulin secretion, a higher odds of showing IGT and a higher risk over time to develop IGT and T2D. In fact, the co-occurrence of the risk alleles is associated with odds of showing IGT between 1.454 and 1.551 independent of age, gender, and BMI; also subjects carrying multiple risk alleles have almost 90% increased risk of developing IGT. Thus, these data clearly show that obese children and adolescents carrying gene variants affecting the early phase of insulin secretion are more prone to develop IGT; this data is also consistent with our previous finding that in obese youth an impaired early phase insulin secretion is a required condition to develop IGT (5).

Previously we showed that the degree of obesity represents one of the most important risk factor for T2D in youth (39). In this study we further demonstrate that the risk alleles have an additive effect on the risk of IGT related to adiposity. In fact, in each BMI quartile category the presence of 6 or more risk allele induces a significant increase risk of showing IGT compared to those with the same degree of obesity.

We also show that carrying multiple risk alleles provide subjects who were IGT at baseline with a higher probability to stay IGT or progress to T2D at follow-up. In fact, the odds ratio of staying/progressing to- IGT/T2D at follow up was 1.44, independent of age, follow up time, gender, BMI and changes in BMI.

Consistent with previous studies (40, 41), herein we show that the studied variants influence the beta-cell function possibly by affecting the early, dynamic phase of insulin secretion; indeed the higher is the number of the risk alleles, the lower is the beta cell response to the glucose either administered orally or intravenously. Our observations suggest that obese youths who develop
IGT or T2D tend to carry a high number of risk alleles especially affecting processes modulating the rapid granule recruitment and exocytosis (41).

Although the natural history of prediabetes is not yet well known in youth, we report the rather dynamic nature of these conditions in obese children. For the first time, we show that those children that converted back to normal glucose tolerance had a low number of risk alleles whereas those that either stayed IGT or progressed to T2D were enriched with a high number of risk alleles. Although these findings need to be extended to larger cohorts followed over longer time, the data suggest strongly the importance of genetic factors implicated in the development of early onset T2D in youth.

Although ethnicity seems not to influence the association between the studied gene variants and glucose dysregulation (the association between the gene score and the studied outcomes are actually evident in all the ethnicities), African American obese youths with a high risk score tended to have a higher risk than Caucasians and Hispanics of having IGT. Also, this would help explaining why, African American youth, in spite of a more favorable adipose tissue disposition, characterized by a low visceral fat and an almost absent intra-hepatic fat accumulation (42), still show a higher risk of developing IGT/T2D than Caucasian and Hispanic obese kids.

We acknowledge that this study has a number of limitations. One limitation is that we elected to analyze only 5 SNPs in or near genes encoding for protein mainly involved in insulin secretion. While there are more than 60 SNPs shown to be associated with type 2 diabetes, whose effects are not taken into account in our analysis, it is rather remarkable to see such a high predictive value with only five SNPs. The sample size of the cohort is rather small and the study lacks replication in another independent cohort of obese children. The latter is a critical point, since replication helps ensuring that a genotype-phenotype association observed in an association
study represents a credible association and is not a chance finding or an artifact due to uncontrolled biases. Also the small sample size did not allow to run any gene-gene interaction or haplotype analyses. Further, we do acknowledge that the high attrition observed in our study is a strong limitation and that this data should be interpreted as preliminary needing further future evaluation in longitudinally designed studies that dedicate significant effort and resources aimed at avoiding high attrition rates among these participants. Also, the high attrition rates did not allow to analyze the data according to the ethnicity, this analysis would have provided more information about the gene-race interaction in the modulation of insulin secretion. On the other hand, to our best knowledge this is the only existing cohort comprehensive of children and adolescents with and without IGT and T2D carefully phenotyped with regard to insulin secretion and sensitivity and followed-up over time. Another limitation is that we did not evaluate the effect of other covariates besides age, gender and BMI in our models. Also strengths of the study are i) the use of state of art measures of insulin secretion, ii) the study of a young population in which the effects of aging on insulin secretion and sensitivity are not evident yet, iii) the multiethnic background of the cohort.

In conclusion, obese children and adolescents developing IGT or T2D have a higher genetic predisposition than those who do not show these diseases and this susceptibility is mainly related to gene variants modulating the early, dynamic phase of insulin secretion.
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Authors Contributions NS, CG and HZ analyzed the data. CDM, CC, BP, ED, MS, AEB and LG researched the data. CG, SC and NS reviewed the data, wrote and edited the MS. SC and NS are the guarantor of this work and as such had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the analyses.
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Figures Legend.

Figure 1. The figure displays the prevalence of IGT according to the number of risk alleles in the three ethnic groups.

Figure 2. The prevalence of IGT subjects in the entire cohort divided into quartile of BMI (1st quartile ≤ 28.7 kg/m²; 2nd quartile between 28.7 and 33.0 kg/m², 3rd quartile between 33.0 and 38.5 kg/m², 4th quartile ≥ 38.5 kg/m²) as well as by gene score groups (1st group gene score ≤ 2; second group: gene score between 3-5; and third group; gene score ≥ 6). The p-values refer to the prevalence of IGT according to the number of risk alleles in each quartile group.

Figure 3. Association between the genetic risk score and Log Φ total in the three ethnic groups (P-values adjusted for age, gender, z-score BMI, and glucose tolerance).

Figure 4. Changes of glucose tolerance status at follow up of subjects IGT at baseline. The white part of the bars describes those who moved from IGT to NGT, while the black portion of the bars shows those who moved from NGT to IGT/T2D.
Figure 1. The figure displays the prevalence of IGT according to the number of risk alleles in the three ethnic groups.

254x190mm (300 x 300 DPI)
Figure 2. The prevalence of IGT subjects in the entire cohort divided into quartile of BMI (1st quartile ≤ 28.7 kg/m²; 2nd quartile between 28.7 and 33.0 kg/m², 3rd quartile between 33.0 and 38.5 kg/m², 4th quartile ≥ 38.5 kg/m²) as well as by gene score groups (1st group gene score ≤ 2; second group: gene score between 3 to 5; and third group; gene score ≥ 6). The p-values refer to the prevalence of IGT according to the number of risk alleles in each quartile group.

254x190mm (96 x 96 DPI)
Figure 3. Association between the genetic risk score and Log $\Phi$total in the three ethnic groups (P-values adjusted for age, gender, z-score BMI, and glucose tolerance).

254x190mm (300 x 300 DPI)
Figure 4. Changes of glucose tolerance status at follow up of subjects IGT at baseline. The white part of the bars describes those who moved from IGT to NGT, while the black portion of the bars shows those who moved from NGT to IGT/T2D.
Supplementary Table 1. Main clinical characteristics of the pediatric cohort evaluating β-cell function assessed from OGTT by the Oral C-peptide Minimal Model

|                        | Total Cohort | NGT Obese Youth | IGT Obese Youth |
|------------------------|--------------|-----------------|-----------------|
| Number †               | 714          | 530             | 184             |
| Gender (M/F)           | 290/424      | 224/306         | 66/118          |
| Race (C/AA/H)          | 316/188/210  | 240/144/146     | 76/44/64        |
| GT (NGT/IGT)           | 530/184      | 530/0           | 0/184           |
| Age                    | 13.6±3.1     | 13.7±3.1        | 13.1±2.9        |
| BMI (kg/m²)            | 33.4±7.4     | 33.2±7.3        | 34.2±7.4        |
| BMI-Z                  | 2.2±0.4      | 2.2±0.4         | 2.2±0.4         |
| Fasting Glucose (mg/dl)| 92±8         | 91±7            | 97±10           |
| 2-h Glucose (mg/dl)    | 122±25       | 111±16          | 155±14          |
| Fasting Insulin (µU/ml)| 33.9±21.3    | 31.7±20.4       | 40.3±22.5       |
| Phi total *            | 84.8 (60.6-112.8) | 89.3 (64.8-125.6) | 72.5 (55.9-97.0) |
| SI *                   | 19.4 (10.6-32.6) | 22.8 (14.1-36.6) | 10.7 (6.1-20.1) |
| DI total *             | 2512 (1401-4274) | 3121 (1909-5543) | 1294 (727-2217) |

Data are means±SD or *median (interquartile range).
†Number of participants. M: Male; F: Female; C: Caucasians; AA: African Americans; H: Hispanics; NGT: Normal Glucose Tolerance; IGT: Impaired Glucose Tolerance; BMI: Body Mass Index; BMI-Z: Body Mass Index Zeta Score; SI: Insulin Sensitivity; DI: Disposition Index
### Supplementary Table 2. Minor allele frequencies (MAF) genotype distribution (101, 102 and 202) and Hardy Weinberg Equilibrium for each of the studied SNPs

| Gene          | rs       | MAF  | 101 | 102 | 202 | HWE p-value |
|---------------|----------|------|-----|-----|-----|-------------|
| TCF7L2 (C/T)  | rs7903146| 0.31 | 150 | 135 | 31  | 0.94        |
| HHEX (C/T)    | rs1111875| 0.37 | 126 | 144 | 46  | 0.64        |
| IGF2BP2 (G/T) | rs4402960| 0.32 | 143 | 143 | 30  | 0.50        |
| CDKAL1 (G/C)  | rs7754840| 0.41 | 113 | 147 | 56  | 0.49        |
| HNF1A (A/C)   | rs1169288| 0.35 | 138 | 138 | 40  | 0.54        |

#### Caucasians

| Gene          | rs       | MAF  | 101 | 102 | 202 | HWE p-value |
|---------------|----------|------|-----|-----|-----|-------------|
| TCF7L2 (C/T)  | rs7903146| 0.29 | 93  | 78  | 17  | 0.92        |
| HHEX (C/T)    | rs1111875| 0.28 | 104 | 64  | 20  | 0.04        |
| IGF2BP2 (G/T) | rs4402960| 0.51 | 42  | 99  | 47  | 0.45        |
| CDKAL1 (G/C)  | rs7754840| 0.52 | 45  | 91  | 52  | 0.67        |
| HNF1A (A/C)   | rs1169288| 0.14 | 139 | 45  | 4   | 0.87        |

#### African Americans

| Gene          | rs       | MAF  | 101 | 102 | 202 | HWE p-value |
|---------------|----------|------|-----|-----|-----|-------------|
| TCF7L2 (C/T)  | rs7903146| 0.27 | 113 | 79  | 18  | 0.43        |
| HHEX (C/T)    | rs1111875| 0.33 | 89  | 102 | 19  | 0.18        |
| IGF2BP2 (G/T) | rs4402960| 0.32 | 98  | 91  | 21  | 0.98        |
| CDKAL1 (G/C)  | rs7754840| 0.43 | 77  | 86  | 47  | 0.02        |
| HNF1A (A/C)   | rs1169288| 0.37 | 80  | 104 | 26  | 0.37        |

#### Hispanics

| Gene          | rs       | MAF  | 101 | 102 | 202 | HWE p-value |
|---------------|----------|------|-----|-----|-----|-------------|
| TCF7L2 (C/T)  | rs7903146| 0.27 | 113 | 79  | 18  | 0.43        |
| HHEX (C/T)    | rs1111875| 0.33 | 89  | 102 | 19  | 0.18        |
| IGF2BP2 (G/T) | rs4402960| 0.32 | 98  | 91  | 21  | 0.98        |
| CDKAL1 (G/C)  | rs7754840| 0.43 | 77  | 86  | 47  | 0.02        |
| HNF1A (A/C)   | rs1169288| 0.37 | 80  | 104 | 26  | 0.37        |

### Supplementary Table 3. Prevalence of Family History (FH) of type 2 diabetes according to the genetic risk score

| Number of Risk Alleles | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|------------------------|---|---|---|---|---|---|---|---|---|
| Negative FH (%)        | 92| 83| 76| 77| 72| 71| 41| 67| 50|
| Positive FH (%)        | 8 | 17| 24| 23| 28| 29| 59| 33| 50|
Supplementary Table 4. Linear regression analysis evaluating the association between 2h-glucose, Insulin Sensitivity (SI) or Disposition Index (DI) total and the gene risk score adjusted for Age, Gender, BMI-Z and Glucose Tolerance, divided by ethnicity

| Dependent Variables | Independent variables | Ethnic Group | Caucasians | African Americans | Hispanics |
|---------------------|-----------------------|--------------|------------|-------------------|-----------|
|                     |                       |              | Beta       | P                 | Beta      | P         | Beta      | P         |
| Log 2h glucose      | Age                   |              | -.139      | .013              | -.068     | .346      | .022      | .755      |
|                     | Gender                |              | .094       | .088              | .080      | .258      | .090      | .195      |
|                     | BMI-Z                 |              | .174       | .002              | .102      | .159      | .040      | .571      |
|                     | Gene score            |              | .221       | .000              | .327      | .000      | .307      | .000      |
| Log SI              | Age                   |              | -.116      | .022              | -.111     | .079      | -.236     | .001      |
|                     | Gender                |              | -.059      | .240              | -.159     | .011      | -.136     | .044      |
|                     | BMI-Z                 |              | -.460      | .000              | -.374     | .000      | -.219     | .001      |
|                     | GT                    |              | -.360      | .000              | -.469     | .000      | -.364     | .000      |
|                     | Gene score            |              | .041       | .423              | .101      | .113      | .024      | .722      |
| Log DI Total        | Age                   |              | -.136      | .009              | -.106     | .088      | -.171     | .009      |
|                     | Gender                |              | -.049      | .333              | -.053     | .387      | -.134     | .037      |
|                     | BMI-Z                 |              | -.378      | .000              | -.306     | .000      | -.186     | .005      |
|                     | GT                    |              | -.415      | .000              | -.489     | .000      | -.387     | .000      |
|                     | Gene score            |              | -.120      | .025              | -.157     | .014      | -.254     | .000      |

BMI-Z: Body Mass Index Zeta Score; GT: Glucose Tolerance.
Obese
n=714

NGT
n=530 (74.4%)

Follow-up
n=115 (21.6%)

NGT-NGT
n=103 (89.5%)

NGT-IGT
n=12 (10.5%)

No follow-up
n=415 (78.4%)

IGT
n=184 (25.6%)

Follow-up
n=88 (47.8%)

IGT-NGT
n=53 (60%)

IGT-IGT
n=31 (35%)

IGT-T2D
n=4 (4%)

No follow-up
n=96 (52.2%)
Supplemental Figure 2

Association between the allele score and Clamp derived measures of beta cell secretion (first and second phase for c-peptide, respectively)