Common Genetic Determinants of Glucose Homeostasis in Healthy Children

The European Youth Heart Study

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OBJECTIVE—The goal of this study was to investigate whether the effects of common genetic variants associated with fasting glucose in adults are detectable in healthy children.

RESEARCH DESIGN AND METHODS—Single nucleotide polymorphisms in *MTNR1B* (rs10830963), *G6PC2 (*rs560887), and *GCK* (rs4607517) were genotyped in 2,025 healthy European children aged 9–11 and 14–16 years. Associations with fasting glucose, insulin, homeostasis model assessment (HOMA)-insulin resistance (IR) and HOMA-B were investigated along with those observed for type 2 diabetes variants available in this study (*CDKN2A/B*, *IGF2BP2*, *CDKAL1*, *SLC30A8*, *HHEX-IDE*, and *Chr 11p12*).

RESULTS—Strongest associations were observed for *G6PC2* and *MTNR1B*, with mean fasting glucose levels (95% CI) being 0.084 (0.06–0.11) mmol/l, $P = 7.9 \times 10^{-11}$ and 0.069 (0.04–0.09) mmol/l, $P = 1.9 \times 10^{-7}$ higher per risk allele copy, respectively. A similar but weaker trend was observed for *GCK* (0.028 [−0.006 to 0.06] mmol/l, $P = 0.11$). All three variants were associated with lower β-cell function (HOMA-B $P = 9.38 \times 10^{-5}$, 0.004, and 0.04, respectively). *SLC30A8* (rs13266634) was the only type 2 diabetes variant associated with higher fasting glucose (0.033 mmol/l [0.01–0.06], $P = 0.01$). Calculating a genetic predisposition score adding the number of risk alleles of *G6PC2*, *MTNR1B*, *GCK*, and *SLC30A8* showed that glucose levels were successively higher in children carrying a greater number of risk alleles ($P = 7.1 \times 10^{-17}$), with mean levels of 5.34 versus 4.91 mmol/l comparing children with seven alleles (0.6% of all children) to those with none (0.5%). No associations were found for fasting insulin or HOMA-IR with any of the variants.

CONCLUSIONS—The effects of common polymorphisms influencing fasting glucose are apparent in healthy children, whereas the presence of multiple risk alleles amounts to a difference of >1 SD of fasting glucose. ***Diabetes*** **58:2939–2945, 2009**

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Diabetes is characterized by chronic hyperglycemia resulting from a defect in insulin secretion by the pancreatic β-cells and/or insulin action in peripheral tissues. Although the exact mechanisms underlying the development of type 2 diabetes remain unclear, it is known to be triggered by environmental and lifestyle factors in genetically susceptible individuals (1). Since 2006, an unprecedented number of novel genetic loci that are reproducibly associated with type 2 diabetes have been identified in genome-wide association (GWA) studies of adults (1,2). However, the contribution of known type 2 diabetes genes to differences in fasting glucose in healthy individuals is only small (3), and in contrast to the success in the discovery of these genes, much less is known about common genetic determinants of fasting glucose.

Common genetic variants in *G6PC2* (glucose-6-phosphatase catalytic 2) (4), *GCK* (glucokinase) (5), *GCKR* (glucokinase regulator) (6), and *MTNR1B* (melatonin receptor 1B) (7,8,9) have only recently been identified as the first loci regulating fasting glucose in GWA studies of healthy diabetes-free adults. *G6PC2*, *GCK*, and *GCKR* all encode proteins that are key regulators of the provision of fasting glucose. Rare mutations in GCK play a role for the development of maturity-onset diabetes of the young (MODY), a disorder characterized by mild stable fasting hyperglycemia (10). *G6PC2*, *GCK*, and *GCKR* thus represent likely candidates for altering an individual's physiologic glucostat set point in the absence of disease. In contrast, *MTNR1B* was the only fasting glucose loci shown to alter the risk of type 2 diabetes in addition to fasting glucose levels in healthy individuals (7,8,9). The reported associations are most likely due to increased expression of *MTNR1B* in pancreatic β-cells and melatonin-mediated impaired insulin secretion in risk allele carriers (8) and demonstrate that the identification of fasting glucose genes through GWA studies of healthy individuals can lead to the discovery of type 2 diabetes loci and provide important insight into the pathogenesis of type 2 diabetes.

The majority of diabetes diagnosed before adulthood has until recently been attributed to type 1 diabetes, and with the majority of research focusing on adults only, limited data exist for children and adolescents with type 2 diabetes (11,12,13). Concomitant with the obesity epidemic, the prevalence and incidence of type 2 diabetes in youth have risen worldwide (11,12,14), and it is estimated that 45% of pediatric patients now diagnosed with diabetes in the U.S. have type 2 diabetes (12). Exposure to common genetic polymorphisms recently shown to contribute to

elevated glucose levels in adulthood starts at conception, yet it is unknown whether their effects can be detected in childhood. To understand whether common glucose-raising alleles exert an influence on glycemic control early in life and in the relative absence of insulin resistance, secondary to chronic obesity commonly present in older individuals (13), we studied associations of *G6PC2*, *GCK*, and *MTNR1B* as well as levels of fasting glucose, insulin, homeostasis model assessment (HOMA)-insulin resistance (HOMA-IR), and HOMA-B in a population-based study of healthy European children (n = 2,025) aged 9–11 and 14–16 years.

RESEARCH DESIGN AND METHODS

We studied children who participated in the European Youth Heart Study (EYHS), a mixed longitudinal population-based study of which the design and data collection have been described previously (15,16). DNA of children from Estonia (city and county of Tartu) and Denmark (city of Odense) was available for the study. Baseline measurements took place between 1997 and 1999 in Denmark and Estonia, and another survey of children aged 9 years was conducted in 2003 in Denmark only. Similar protocols for data collection were used in both countries. The study complied with international guidelines on biomedical research and ethical procedures of each participating country. Written informed consent was obtained from the child's parent or legal guardian after they were given a full written explanation of the goals of the study and its possible hazards, discomfort, and inconvenience. In addition, children had all procedures verbally explained to them, together with any possible discomfort they might encounter, and were given the option to withdraw at any time.

At each study location a defined population of children was identified, and from this population a two-stage cluster sample of boys and girls aged 9–11 and 14–16 years was randomly selected. The primary sampling units were schools, and secondary units were school registers. Age-groups 9–11 and 14–16 years were chosen to broadly represent children either side of puberty; in addition, detailed assessment of pubertal stage was performed as outlined below. In total, 2,194 children agreed to participate, with a similar proportion participating in each country (76% in Estonia and 75% in Denmark).

Each child had a blood sample taken and underwent a physical examination including anthropometric and blood pressure measurements using the same equipment in both countries. Blood samples were collected after an overnight fast and analyzed by a Clinical Pathology Accreditation (CPA) accredited laboratory in Bristol and Cambridge, England. Glucose concentrations were measured by standard methods using Olympus AU600 random-access analysers. Plasma-specific insulin was determined by two-site immunometric assays with either 125I or alkaline phosphatase labels. Between-laboratories correlations were 0.94–0.98 for 30 randomly selected samples analyzed in both Bristol and Cambridge. HOMA was used to estimate insulin resistance (HOMA-IR = [fasting glucose (mmol/l) × insulin (μU/ml)]/22.5) and β-cell function (HOMA-B = [insulin (μU/ml) × 20]/[glucose (mmol/l) − 3.5]) (17), both of which have been validated as surrogate markers in healthy children (18). These indexes based on fasting glucose and insulin concentrations reflect primarily hepatic insulin sensitivity/resistance (19), and HOMA-IR and fasting insulin have been suggested to predominantly measure hepatic rather than peripheral insulin resistance in prepubertal and early pubertal adolescents (20).

Weight was measured in light clothing to the nearest 0.1 kg using a calibrated beam balance scale. Height was measured without shoes to the nearest 0.5 cm using a transportable Harpenden stadiometer. Four skin-fold measurements (triceps, biceps, subscapula, and suprailiac) were taken on the left side of the body according to the criteria described by Lohman et al. (21). All measurements were taken twice and in rotation. If the difference between the two measurements differed by more than 2 mm, a third measurement was taken and the two closest were averaged. BMI was calculated as weight in kilograms divided by the square of height in meters. Pubertal status was assessed by trained personnel according to Tanner's five-level classification of biological maturity (22) using breast development in girls and pubic hair in boys. Children were later classified as being prepubertal (Tanner stage 1) or at early (Tanner stages 2–3) or later (Tanner stages 4–5) stages of puberty.

Genotyping. Single nucleotide polymorphisms (SNPs) most significantly associated with fasting glucose in recent GWA studies and located in *G6PC2* (rs560887), *GCK* (rs4607517), and *MTNR1B* (rs10830963) were the focus of this report and were genotyped at the Medical Research Centre Epidemiology Unit Research Laboratory using the TaqMan SNP Genotyping Assays (Applied Biosystems, Warrington, U.K.). In addition, SNPs that were identified in the initial round of type 2 diabetes GWA studies and had previously been successfully genotyped in EYHS were also included to compare effect sizes between type 2 diabetes and fasting glucose loci in children. These included SNPs located in or near *CDKN2A/B* (rs1063192 and rs10811661), *IGF2BP2* (rs11705729), *CDKAL1* (rs7756992, rs10946403, and rs10946398), *SLC30A8* (rs13266634), *HHEX-IDE* (rs5015480), and chromosomal region *11p12* (rs1828390).

The genotyping assays were undertaken on 10 ng of genomic DNA in a 5 μl 384-well TaqMan assay using a PTC-225 Thermal Cycler (MJ Research, Watertown, MA). The ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Warrington, U.K.) was used for end point detection and allele calling. The call rate was >98.9%, and concordance of 44 duplicate samples was 100%. All 12 SNPs were in Hardy-Weinberg equilibrium (all P values > 0.07).

Statistical analyses. Statistical analyses were performed using Stata 10.1. A total of 2,025 of 2,194 individuals had DNA available and had complete information on anthropometry, glucose, and insulin. Of all 2,025 participants, results from 21 children were excluded from analyses based on either nonfasting status (n = 17) or due to glucose levels ≥7 mmol/l (n = 4).

Linear regression models, assuming an additive effect, were used to test associations between each SNP and log-normalized outcomes (for insulin, HOMA-IR, and HOMA-B), adjusting for age, sex, country, and biochemistry laboratory. For all associations significant at 0.05 or less, differences by age-group, country, and sex were investigated.

To investigate the joint contribution of all SNPs associated with fasting glucose in children, a genetic predisposition score was calculated by adding the number of risk alleles based on SNPs associated with fasting glucose (*G6PC2*, *GCK*, *MTNR1B*, and *SLC30A8*). The score followed and approximated normal distribution and ranged from 0–7, the maximum observed number of risk alleles carried by children in the study. Sensitivity analyses were performed using alternative scores excluding *GCK* or the type 2 diabetes gene *SLC30A8*.

RESULTS

Overall mean ± SD fasting glucose was 5.1 ± 0.39 mmol/l (Table 1). Strongest overall associations were observed for variants in *G6PC2* and *MTNR1B* (Table 2), two of the genes recently identified as common genetic determinants of fasting glucose in healthy adults. Variant rs560887 in *G6PC2* showed the largest effect size, with fasting glucose being a mean 0.084 mmol/l (95% CI 0.06–0.11; $P = 7.9 \times 10^{-11}$) higher for each copy of the common C-allele (frequency 0.70 in HapMap CEU). For rs10830963 in *MTNR1B*, glucose levels were 0.069 mmol/l (0.04–0.09; $P = 1.9 \times 10^{-7}$) higher for each copy of the minor G-allele (frequency 0.30 in HapMap CEU). A similar but weaker trend was observed for rs4607517 in *GCK* (0.028 mmol/l [−0.006 to 0.06; P = 0.11]). Each of the three variants was also associated with significantly lower β-cell function ($P = 9.38 \times 10^{-5}$, 0.004, and 0.04, respectively).

Of the type 2 diabetes–susceptibility genes that were investigated, rs13266634 in *SLC30A8* was the only variant significantly associated with fasting glucose, with an effect size of 0.033 mmol/l (95% CI 0.01–0.06; P = 0.01) per copy of the common C-allele (frequency 0.75 in HapMap CEU); no significant association was observed with HOMA-B. The three SNPs in *CDKAL1* were associated with HOMA-B ($P \leq 0.03$) but not fasting glucose (Table 3). None of the observed associations were attenuated by adjustment for measures of adiposity estimated by either BMI or sum of the skin-fold thicknesses. No significant associations were present between any of the variants and fasting insulin or HOMA-IR (see supplemental Tables 1 and 2 in the online appendix, available at http://diabetes.diabetesjournals.org/cgi/content/full/db09-0374/DC1).

Using the genetic risk score, we examined the additive effect of an increasing number of risk alleles of glucose-associated variants on children's glucose levels (Fig. 1). Mean fasting glucose was 0.06 mmol/l (95% CI 0.04–0.07; $P_{trend} = 7.1 \times 10^{-17}$) greater for each additional risk allele

TABLE 1
Mean values of selected characteristics of the study population by country, sex, and age-group

	Denmark				Estonia			
	Girls		Boys		Girls		Boys	
	9–11 years	14–16 years	9–11 years	14–16 years	9–11 years	14–16 years	9–11 years	14–16 years
n	409	177	349	158	234	257	230	190
Age (years)	9.6	15.5	9.8	15.5	9.5	15.4	9.6	15.5
Prepubertal children/Tanner 1 (%)	96.7	0.0	100.0	1.9	91.5	0.0	92.6	1.1
Pubertal children/Tanner 2–3 (%)	3.3	6.0	0.0	5.7	8.6	8.6	7.4	23.2
Pubertal children/Tanner 4–5 (%)	0.0	94.0	0.0	92.4	0.0	91.4	0.0	75.8
Fasting glucose (mmol/l)	5.0	5.1	5.1	5.3	4.9	5.0	5.1	5.2
Fasting insulin (pmol/l)*	45.9	73.2	37.9	68.2	36.6	70.7	32.9	57.0
HOMA-IR*	1.7	2.8	1.4	2.6	1.3	2.6	1.2	2.2
HOMA-B*	101.7	144.7	79.2	128.7	90.0	164.0	68.1	109.1
Triglycerides (mmo/l)	0.83	1.04	0.75	0.92	0.77	0.85	0.69	0.67
HDL cholesterol (mmol/l)	1.49	1.40	1.61	1.33	1.44	1.45	1.51	1.32
LDL cholesterol (mmol/l)	2.68	2.45	2.55	2.28	2.71	2.56	2.52	2.29
Systolic blood pressure (mmHg)	101.5	108.8	103.8	118.5	100.0	107.3	101.3	114.2
Diastolic blood pressure (mmHg)	60.8	64.1	61.2	63.7	59.1	64.1	58.7	62.5
Height (cm)	139.5	165.9	139.9	174.8	137.3	164.9	137.6	174.1
Weight (kg)	34.0	57.5	34.3	63.6	31.4	55.4	31.9	61.7
BMI (kg/m^2)	17.4	20.9	17.4	20.8	16.6	20.3	16.8	20.3
Sum of skin-fold measurements (cm)	40.1	53.2	33.0	37.0	31.5	44.3	24.8	28.2

*Data are median values.

of *G6PC2*, *GCK*, *MTNR1B*, and *SLC30A8*. *GCK* was included in this score despite not reaching conventional levels of statistical significance in this study because previous evidence demonstrated its association with fasting glucose in healthy children (5). In addition, the fasting glucose effect sizes of *GCK* and *SLC30A8* were found to be of similar magnitude. The total difference in fasting glucose between individuals carrying zero (0.5% of all children) compared with seven (0.6%) glucose-rising risk alleles was 0.43 mmol/l.

Alternative scores using risk alleles of *G6PC2*, *MTNR1B*, and *GCK* or *G6PC2*, *MTNR1B*, and *SLC30A8* showed a similar trend. Similar results were obtained when investigating the additive effect of fasting glucose risk alleles on HOMA-B levels, with children having a greater number of risk alleles showing successively lower β-cell function ($P_{trend} = 3.0 \times 10^{-5}$; supplemental Fig. 2 in the online appendix).

We observed no difference in associations by age-group, sex, or country (all interaction *P* values > 0.05), except for rs10830963 in *MTNR1B* for which a significantly stronger effect on fasting glucose was observed in the older age-group (interaction *P* value = 0.004). Among the 9–11 year olds, fasting glucose levels were 0.037 mmol/l (95% CI 0.005–0.07) higher for each minor G-allele, whereas this effect was 0.11 mmol/l (0.07–0.15) for children aged 14–16 years. More detailed investigation showed similar effect modification of rs10830963 on fasting glucose by pubertal status based on Tanner stage, with greater effect sizes at later stages of maturity (interaction *P* value = 0.02), independent of age, sex, or country. In prepubertal children (Tanner stage 1) the per-allele difference in fasting glucose was 0.046 mmol/l (0.01–0.08) but 0.071 mmol/l (−0.04 to 0.18) at Tanner stages 2–3 and 0.11 mmol/l (0.07–0.16) at Tanner stages 4–5. Although the effect of rs10830963 on HOMA-B did not differ significantly by age-group (*P* = 0.41), its effect was found to be marginally stronger at later Tanner stages (interaction *P* value = 0.079), mirroring the interaction observed for fasting glucose.

DISCUSSION

This is the first study to show that common genetic variants in *G6PC2*, *MTNR1B*, and *GCK*, all of which are associated with fasting glucose in diabetes-free adults, significantly influence levels of fasting glucose and/or β-cell function in healthy children.

Importantly, the joint effects of these common variants were additive and substantial. Higher levels of glucose were observed in children carrying more risk alleles, with a difference of >1 SD of fasting glucose comparing the small proportion of children in the extreme groups with the lowest, opposed to the highest, genetic susceptibility.

Effect sizes of *MTNR1B* and *G6PC2* variants in children of this study were close to those recently reported in adults, with differences in fasting glucose of 0.08 and 0.06 mmol/l per risk allele, respectively (7,4). Weedon et al. (5) previously showed that rs1799884 in *GCK* is associated with fasting glucose in adults and also children of the Avon Longitudinal Study of Parents and Children (ALSPAC) cohort. Compared with this study and a more recent meta-analysis of adults reporting an effect size of 0.06 mmol/l (7), we observed a somewhat weaker and nonsignificant effect for rs4607517, a SNP in perfect linkage disequilibrium with rs1799884 in European-descent HapMap participants ($r^2 = 1$), suggesting that low power is likely to underlie the lack of statistical significance in our study.

In the absence of any direct measure of β-cell function, we use HOMA-B. We report significant associations for *MTNR1B*, *GCK*, and *G6PC2* with HOMA-B in healthy children, adding to the evidence from initial GWA studies (7) and showing for the first time that early effects on β-cell function can be demonstrated for these loci. *GCK* and *G6PC2* are expressed in the pancreas and code for

TABLE 2
Mean fasting glucose by genotype and per-allele difference (95% CI)

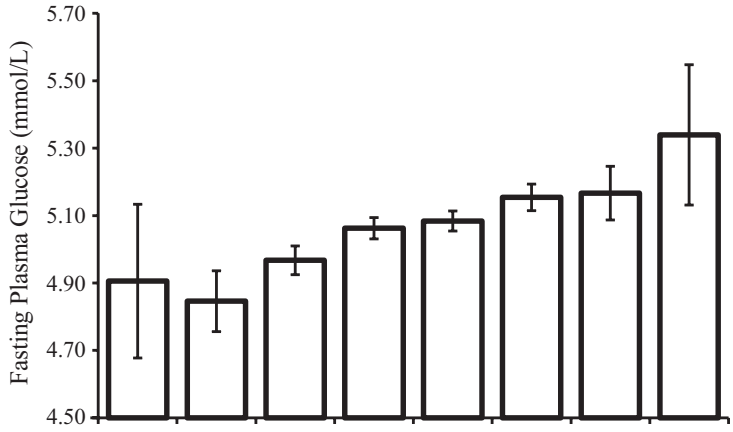
Gene	SNP	Non-risk allele (1)*	Risk allele (2)*†	Risk allele frequency (HapMap)	Risk allele frequency (EYHS)	Mean Glucose 11	12	22	β-coefficient (95% CI)	P‡	P§
G6PC2	rs560887	T	C	0.70	0.70	4.95	5.04	5.12	0.084 (0.06–0.11)	7.98×10^{-11}	6.73×10^{-11}
MTNR1B	rs10830963	C	G	0.30	0.29	5.03	5.10	5.13	0.069 (0.04–0.09)	1.90×10^{-7}	1.93×10^{-7}
GCK	rs4607517	G	A	0.20	0.14	5.06	5.10	5.10	0.028 (−0.006 to 0.06)	0.11	0.11
SLC30A8	rs13266634	T	C	0.75	0.67	5.05	5.04	5.10	0.033 (0.01–0.06)	0.01	0.01
CDKAL1	rs7756992	A	G	0.25	0.30	5.05	5.09	5.07	0.024 (−0.002 to 0.05)	0.07	0.07
	rs10946403	A	G	0.15	0.18	5.05	5.11	5.03	0.029 (−0.002 to 0.06)	0.07	0.06
	rs10946398	A	C	0.31	0.34	5.06	5.08	5.09	0.019 (−0.01 to 0.04)	0.14	0.14
CDKN2A/B	rs1063192	G	A	0.60	0.54	5.05	5.08	5.06	0.003 (−0.02 to 0.03)	0.81	0.82
	rs10811661	C	T	0.79	0.84	5.06	5.07	5.07	0.002 (−0.03 to 0.03)	0.92	0.93
IGF2BP2	rs11705729	T	A	0.71	0.55	5.09	5.05	5.09	8.11×10^{-5} (−0.02 to 0.02)	1.00	0.99
Chr 11p12	rs1828390	T	C	0.12	0.10	5.07	5.05	5.03	−0.033 (−0.07 to 0.01)	0.09	0.09
HHEX-IDE	rs5015480	T	C	0.57	0.61	5.10	5.06	5.06	−0.014 (−0.04 to 0.01)	0.26	0.26

*Alleles are annotated according to the positive strand (NCBI35). †Allele associated with higher glucose and/or risk of diabetes in previously published GWA studies. ‡Adjusted for country, age, sex, and biochemistry laboratory. §Additionally adjusted for BMI.

TABLE 3
Median HOMA-B by genotype and per-allele difference (95% CI) in HOMA-B

Gene	SNP	Non-risk allele (1)*	Risk allele (2)*†	Risk allele frequency (HapMap)	Risk allele frequency (EYHS)	Median HOMA-B¶ 11	12	22	β-coefficient (95% CI)	P‡	P§
G6PC2	rs560887	T	C	0.70	0.70	109.19	107.35	98.25	−0.09 (−0.13 to 0.04)	9.38×10^{-5}	3.53×10^{-4}
MTNR1B	rs10830963	C	G	0.30	0.29	108.10	98.80	103.33	−0.07 (−0.12 to −0.02)	0.004	0.003
GCK	rs4607517	G	A	0.20	0.14	104.92	101.47	80.49	−0.03 (−0.10 to 0.04)	0.04	0.01
SLC30A8	rs13266634	T	C	0.75	0.67	103.97	105.11	101.67	−0.04 (−0.08 to 0.01)	0.39	0.72
CDKAL1	rs7756992	A	G	0.25	0.30	105.92	103.33	96.87	−0.07 (−0.12 to −0.02)	0.002	0.002
	rs10946403	A	G	0.15	0.18	104.45	100.54	110.22	−0.03 (−0.09 to 0.02)	0.03	0.03
	rs10946398	A	C	0.31	0.34	105.16	102.19	103.33	−0.07 (−0.11 to −0.02)	0.02	0.03
CDKN2A/B	rs1063192	G	A	0.60	0.54	105.31	101.54	106.43	−0.004 (−0.05 to 0.04)	0.28	0.16
	rs10811661	C	T	0.79	0.84	89.31	104.52	104.16	0.02 (−0.04 to 0.08)	0.80	0.96
IGF2BP2	rs11705729	T	A	0.71	0.55	105.51	101.74	105.08	0.005 (−0.04 to 0.05)	0.43	0.51
Chr 11p12	rs1828390	T	C	0.12	0.10	103.33	110.78	82.09	0.05 (−0.02 to 0.13)	0.68	0.70
HHEX-IDE	rs5015480	T	C	0.57	0.61	106.78	100.56	106.12	0.02 (−0.02 to −0.07)	0.24	0.22

*Alleles are annotated according to the positive strand (NCBI35). †Allele associated with higher glucose and/or risk of diabetes in previously published GWA studies. ‡Adjusted for country, age, sex, and biochemistry laboratory. §Additionally adjusted for BMI. ¶Median untransformed HOMA-B levels; adjusted regression analyses are based on log-normalized HOMA-B (β, *P* values).



Number of risk alleles	0	1	2	3	4	5	6	7
Number of individuals in each category	10	64	286	518	588	335	82	12
Percentage of individuals in each category (%)	0.5	3.4	15.1	27.3	31.0	17.7	4.3	0.6

FIG. 1. Mean (95% CI) fasting plasma glucose level (mmol/l) by number of glucose risk alleles (*MTNR1B*, *G6PC2*, *SLC30A8*, and *GCK*).

proteins that play an important role for the regulation of glucose levels. *MTNR1B* is a less obvious candidate, and expression of *MTNR1B* in pancreatic β-cells and the role of *MTNR1B* risk alleles for impaired insulin secretion has only recently been identified (8).

Importantly, adjustment for BMI or skin-fold thickness did not alter the observed associations, and our findings thus demonstrate that the effects of genetic variants contributing to elevated levels of fasting glucose are present from an early age and can help to identify children at different levels of β-cell function, independent of their level of adiposity. We provide epidemiological evidence for common genetic variants contributing to variation in the human homeostatic set point for blood glucose levels from an early age, which may or may not translate into clinically significant differences and longer term complications related to chronic hyperglycemia.

In contrast, *SLC30A8* was the only type 2 diabetes locus investigated that was found to be associated with variations in fasting glucose in children, with an effect that did not follow a clear linear trend/additive model and did not influence levels of HOMA-B. *SLC30A8* encodes a zinc transporter involved in insulin secretion by the β-cell (2) and has previously been shown to be associated with fasting glucose levels in healthy adults (3) but not children. Studies of adults have likewise shown an effect of *SLC30A8* (3,23) and also *HHEX* (23,24) and *CDKAL1* (24) on β-cell function assessed by HOMA-B (3) as well as oral glucose tolerance test or hyperinsulinemic-euglycemic clamp (23,24) measures. In children, *CDKAL1* was the only locus significantly associated with HOMA-B, and future studies including a larger number of children may be able to detect significant effects for loci of smaller effect sizes.

We did not observe any associations with fasting insulin or HOMA-IR, consistent with previous studies in adults that showed little or no association between glucose or type 2 diabetes–susceptibility genes with insulin levels or insulin resistance (3,7,23,24), pointing toward β-cell function rather than insulin action underlying the higher glucose levels and risk of type 2 diabetes caused by variants identified in recent GWA studies (8,25). It is important to consider the clinical and public health implications of such interindividual differences in fasting glucose among normoglycemic individuals. Elevated adult glucose concentration per se is a strong predictor of future type 2 diabetes (26), and Morrison et al. (27) have shown that the presence of impaired fasting glucose in girls of Caucasian and African descent aged 9–10 years is associated with an increased risk of type 2 diabetes 1 decade later. Higher levels of fasting glucose have been linked to an increased risk of coronary heart disease in healthy individuals and those with type 2 diabetes (28,29), but the strength of this association in the nondiabetic glucose range is a matter of debate (30). Whether early differences in fasting glucose levels due to genetic susceptibility translate into an increased risk of type 2 diabetes and related metabolic and cardiovascular disorders and whether or how these are modified by environmental risk factors remain to be investigated.

Children included in this study were randomly selected by age to broadly represent groups before and after entering puberty. Although we found no evidence for differences in the observed associations by age, sex, or country, one exception is noteworthy. The *MTNR1B* variant, recently associated with fasting glucose in adults (7) and located in a gene coding for one of the two known human melatonin receptors (31), displayed a significantly stronger effect on fasting glucose—and to a somewhat lesser degree on HOMA-B—in older or pubertal children compared with younger or prepubertal children. The *MTNR1B* gene is expressed in β-cells in both human and rodent islets, and the translated receptor is thought to mediate the inhibitory effect of melatonin on insulin secretion (8,32). During puberty, children go through a transient state of relative insulin resistance (12,14,33). Although our cross-sectional epidemiological findings do not allow strong causal or temporal inference, they may point toward the effect of *MTNR1B* on β-cell function being more pronounced in the context of insulin resistance and increased secretory demand. The recent observation that the increased expression of *MTNR1B* in G-allele carriers of rs10830963 was greater in older individuals than those <45 years of age (8) lends some support to this hypothesis.

We conclude that effects of genetic variants contributing to differences in fasting glucose and β-cell function are apparent at early ages. Although the strength of each

individual association appears to be of limited clinical significance, joint additive effects are substantial, amounting to a difference in 1 SD in fasting glucose in healthy children. Ongoing efforts in the identification of novel fasting glucose genes will help develop more comprehensive genetic risk scores that can identify a larger proportion of children with elevated fasting glucose levels and decreased β-cell function and provide useful tools for future studies of the longer-term consequences of chronic elevations in fasting glucose, as well as opportunities for targeted prevention of diabetes.

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