CLINICAL STUDY

Association of a *MTNR1B* gene variant with fasting glucose and HOMA-B in children and adolescents with high BMI-SDS

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Abstract

Context: Genome-wide association studies have shown that the melatonin receptor 1B (*MTNR1B*) gene locus is strongly associated with fasting glucose and β -cell function. However, data are rather limited to the adult population and normal-weight children. So far, little is known whether similar associations are present in overweight and obese children and adolescents.

Objective: The aim is to investigate an *MTNR1B* polymorphism in a sample of 310 overweight and obese children and adolescents (mean body mass index standard deviation score (BMI-SDS): 2.74 (± 0.55), mean age: 14 (± 2) years), who participated in a short-term weight-loss program based on energy reduction, physical activity, and behavior therapy.

Methods: We investigated an association between genotype and fasting glucose, fasting insulin, homeostasis model assessment of insulin resistance (HOMA-IR) and of β -cell function (HOMA-B), and anthropometric parameters and their change during intervention.

Results: The minor G allele of polymorphism rs10830963 was significantly associated with increased fasting glucose (0.205 mmol/l, $P < 0.0001$) and decreased HOMA-B (-0.353 , $P < 0.0001$). Categorizing the sample into BMI-SDS groups, these significant associations were abolished in children with BMI-SDS below 2.5 but remained in those with higher BMI-SDS values with stronger β -estimates. The P value for the genotype $\times$ BMI-SDS category interaction was 0.012 for fasting glucose and 0.083 for HOMA-B. There was no significant association between genotype and anthropometric parameters and their change during intervention.

Conclusions: This is the first single study, replicating the association between the *MTNR1B* locus and diabetes-related traits in overweight and obese children and adolescents. The effect sizes in children and adolescents seem to be stronger than in adults and differed among BMI-SDS categories.

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Introduction

Type 2 diabetes is a complex disease in which blood glucose levels are elevated due to β -cell dysfunction and/or insulin resistance. The recent genome-wide association studies have identified new genetic loci associated with the risk of type 2 diabetes and glucose levels (1, 2).

A meta-analysis of 21 genome-wide association studies has reported that in the Framingham cohort about 10% of the inherited variation in fasting glucose levels is explained by 14 gene loci (1). Up to now, the glucose-6-phosphatase (*G6PC2*) gene and the melatonin receptor 1B (*MTNR1B*) gene are the loci

with the strongest association with fasting glucose (1, 3–7), whereas the initial genome-wide association studies reported the strongest association for the *MTNR1B* locus (5–7). Prokopenko *et al.* (6) published an increased fasting glucose level of 0.07 mmol/l per minor G allele in adults. This effect size was also observed in healthy normal-weight children (8). An analysis of 1309 European children has shown that the observations are consistent with that seen in adults, but the effect size estimate in children was smaller (1). In the normal-weight adolescents of the Northern Finland Birth Cohort 86 aged 16 years, an association between *MTNR1B* locus and fasting glucose has been replicated (5). In lean healthy adolescents from China, a

borderline significant association with fasting glucose was reported for the *MTNR1B* locus (9). Another *MTNR1B* polymorphism (rs8192552) with an amino acid exchange was associated with increased body mass index (BMI), waist circumference, and obesity risk in Danish and French adults (10).

Melatonin receptors are members of the family of G-protein-coupled receptors and the *MTNR1B* gene was predicted to encode one of the melatonin receptors, which was originally reported to be expressed in human retina and brain (11). It has recently been shown that the gene is also transcribed in human pancreatic islet cells (7, 12). Melatonin has an inhibitory effect on insulin secretion in clonal β -cells (7, 12–14), thereby explaining the association between the *MTNR1B* locus and fasting glucose as well as type 2 diabetes (15, 16).

Given the known link between melatonin and insulin as well as the recent findings between polymorphism rs10830963 and diabetes-related parameters in adults and the scarce knowledge of this association in overweight and obese children and adolescents, we investigated this polymorphism for the association with fasting glucose, fasting insulin, homeostasis model assessment of insulin resistance (HOMA-IR), and of β -cell function (HOMA-B) in a sample of 310 overweight and obese children and adolescents participating in a standardized weight-loss program. Analyses were extended to anthropometric parameters (weight, body mass index standard deviation score (BMI-SDS), waist circumference). Furthermore, using data from the well-standardized weight-loss program, we studied whether the risk allele G is associated with the change of anthropometric parameters during intervention.

Subjects and methods

Study sample

Overweight and obese children and adolescents participating in the 'Long-term effects of a lifestyle intervention in obesity and genetic influence in children (LOGIC)' study were recruited between January 2006 and June 2008. The children had no endocrine or syndromal disorder and participated in a standardized in-patient lifestyle intervention program for weight loss at the Rehabilitation Hospital Schönsicht in Berchtesgaden (Germany). Genotyping was performed in 310 children and adolescents (123 boys/187 girls) aged 8–19 years. All children were Caucasian and written informed consent for the genetic analysis was obtained for every participant. The study was approved by the ethics committee of the Faculty of Medicine of the Technische Universität München. The 4 to 6 week intervention program consisted of a moderate energy reduction of about 500 kcal/day, physical

activities such as swimming and walking (11 h/week), and behavior therapy. The duration of the lifestyle intervention was documented for every subject.

Assessment of demographic and clinical characteristics

Standardized questionnaires were used to obtain demographic variables. All clinical examinations were conducted by trained medical staff. Body weight was measured in underwear to the nearest 0.1 kg (Tanita BC-420 P MA Profi, Tanita Europe B V, Hoofddorp, The Netherlands) and height to the nearest 0.5 cm by a rigid stadiometer. BMI-SDS was calculated using an established equation (17, 18). Waist circumference (cm) was measured on the midway between the lowest rib margin and the superior border of the iliacal crest using a nonstretch measuring tape to the nearest 0.5 cm. After an overnight fast, blood was taken and glucose and insulin levels were measured after admission. Plasma glucose was measured by the hexokinase method (COBAS INTEGRA 800, Roche Diagnostics GmbH) and insulin by an ELISA (Mercodia, Uppsala, Sweden). HOMA-IR as a measure of insulin sensitivity and HOMA-B as an index of β -cell function were calculated according to Matthews *et al.* (19). These indexes have been validated in healthy children (20).

Genotyping

The top signal (rs10830963) within the *MTNR1B* gene reported by Prokopenko *et al.* (6) was selected for genotyping. The *MTNR1B* gene is localized at chromosome 11 and the minor G allele of the intronic polymorphism rs10830963 has a frequency of 30% (HapMap, dbSNP b126). Samples were genotyped with the MassARRAY system using the iPLEX Gold Chemistry (Sequenom, San Diego, CA, USA). The samples were analyzed in a matrix-assisted laser desorption ionization time of flight mass spectrometer (MALDI TOF MS, Bruker Daltonik, Leipzig, Germany). χ^2 test was used to test for deviation from the Hardy–Weinberg equilibrium (HWE). The single nucleotide polymorphism (SNP) rs10830963 fulfilled HWE ($P > 0.05$), and the genotyping success rate was 99%. The minor allele frequency in our sample was 28%, which is comparable to the literature (6). Owing to the gender test with glycogenin 2 (GYG2) gene and the double-genotyping of samples, high genotyping quality was reached.

Statistical analysis

Means and s.d. or medians and inter quartile ranges (IQR) were used for descriptive statistics at study entry, and after 4 and 6 weeks of intervention. Fasting insulin, HOMA-IR, and HOMA-B were approximately log-normal distributed and therefore log-transformed

(natural log) for the analyses. For HOMA-B, negative values were excluded. Associations between the polymorphism and fasting glucose, fasting insulin, HOMA-IR, and HOMA-B at study entry were analyzed by linear regression.

In a secondary analysis, the genotype–glucose and the genotype–HOMA-B association were studied for different BMI-SDS groups (A) according to sample size ($x < 2.5$; $2.5 \leq x < 3.0$; $x \geq 3.0$) and (B) dichotomized according to the mean ($x < 2.74$; $x \geq 2.74$). SNP–parameter interaction analyses were performed by including additionally the respective parameter and an interaction term, $\text{SNP} \times \text{parameter}$, in the regression equation. The interaction terms $\text{SNP} \times \text{BMI-SDS}$ (continuous trait), $\text{SNP} \times \text{BMI-SDS}$ category, and $\text{SNP} \times \text{gender}$ were calculated for fasting glucose and HOMA-B.

To study the association between genotype and anthropometric parameters and their changes during intervention, linear regression was performed for weight, BMI-SDS, and waist circumference.

All linear analyses were in the first model, adjusted for sex and age as well as in a second model for BMI-SDS. In all linear models, an additive genetic effect was assumed. In a sensitivity analysis, values below 1% and above 99% percentile and for HOMA-B values below 10% and 90% percentile were excluded.

Statistical analyses were performed using SAS Version 9.1 (SAS Institute, Cary, NC, USA).

Results

Description of study sample

Main characteristics of the study sample are shown in Table 1. A proportion of 60% of the participants were girls. Means (s.d.) or medians (IQR) of fasting glucose, fasting insulin, HOMA-IR, and HOMA-B as well as of anthropometric parameters according to genotype are shown in Table 2. BMI-SDS itself was significantly correlated with fasting glucose, fasting insulin, HOMA-IR, and HOMA-B (Pearson correlation coefficient: 0.15, 0.43, 0.42, or 0.18 respectively).

Diabetes-related traits

Polymorphism rs10830963 was significantly associated with fasting glucose levels and HOMA-B (Table 2). There was an increase in fasting glucose per minor G allele by 0.205 mmol/l ($P = 1.17 \times 10^{-7}$). Additional adjustment for BMI-SDS did not change the association (0.201 mmol/l, $P = 1.41 \times 10^{-7}$). Moreover, the minor G allele showed a significant association with HOMA-B independent of BMI-SDS (not BMI-SDS adjusted: -0.353 , $P = 1.58 \times 10^{-6}$; BMI-SDS adjusted: -0.353 , $P = 1.26 \times 10^{-6}$). For fasting insulin and HOMA-IR, no significant associations were observed ($P > 0.05$; Table 2). For the sensitivity analysis, we

Table 1 Characteristics of the study sample at different time points. Means (s.d.) are shown for normally distributed parameters. Anthropometric parameters are shown at different time points (at study entry, after 4 and 6 weeks of intervention). For HOMA-B, negative values were excluded.

Parameter	n (boys/girls)	Mean (s.d.) or median ^a (IQR)
Age (years)	310 (123/187)	14 (2)
Height (cm)	310 (123/187)	163.0 (10.9)
At study entry		
Weight (kg)	310 (123/187)	90.17 (22.93)
BMI-SDS	310 (123/187)	2.74 (0.55)
Waist circumference (cm)	297 (121/176)	109.30 (14.58)
Fasting glucose (mmol/l)	307 (121/186)	3.93 (0.45)
Fasting insulin (mU/l) ^a	306 (121/185)	10.00 (6.28)
HOMA-IR ^a	303 (119/184)	1.75 (1.23)
HOMA-B ^a	258 (92/166)	415.45 (416.06)
After 4 weeks of intervention		
Weight loss (kg)	297 (114/183)	8.26 (2.82)
BMI-SDS loss	297 (114/183)	0.36 (0.10)
Waist circumference loss (cm)	84 (36/48)	6.91 (4.60)
After 6 weeks of intervention		
Weight loss (kg)	187 (71/116)	10.94 (3.62)
BMI-SDS loss	187 (71/116)	0.47 (0.13)
Waist circumference loss (cm)	128 (55/73)	7.68 (4.81)

^aMedians (inter quartile ranges, IQR) are shown for the not normally distributed parameters (fasting insulin, HOMA-IR, and HOMA-B respectively).

excluded values below the 1% percentile and above the 99% percentile of the respective distribution and repeated the regression analyses. No major differences to the analyses in the full sample were observed. For HOMA-B, values below the 10% and above the 90% percentile were excluded (median (IQR): 413.86 (297.59), $n = 207$). Linear regression showed similar results (-0.209 , $P = 8.65 \times 10^{-6}$).

Diabetes-related traits according to BMI-SDS

To assess whether the genotype–glucose and the genotype–HOMA-B associations were modified by BMI-SDS, we performed an interaction analysis (Table 3).

The interaction term $\text{SNP} \times \text{BMI-SDS}$ (continuous) was not statistically significant for fasting glucose or HOMA-B ($P = 0.274$ and 0.068). In the analysis of BMI-SDS categories, according to sample size (Table 3A), the interaction term $\text{SNP} \times \text{BMI-SDS}$ category was statistically significant for fasting glucose ($P = 0.012$) and not significant for HOMA-B ($P = 0.083$). After exclusion of glucose values below the 1% and above the 99% percentile, the interaction term remained significant ($P = 0.014$) and of HOMA-B values below the 10% and above the 90% percentile, the interaction term became borderline significant ($P = 0.046$). In the analysis of dichotomized BMI-SDS categories, neither for fasting glucose nor for HOMA-B the interaction term was significant (Table 3B). The respective associations stratified by different BMI-SDS groups are shown in Table 3A and B. In subjects with a BMI-SDS < 2.5 , the association between rs10830963 and fasting glucose as

Table 2 Association between rs10830963 (*MTNR1B*) and fasting glucose, fasting insulin, HOMA-IR and HOMA-B as well as anthropometric traits. Genotype-specific means (s.d.) are shown for normally distributed parameters. β -estimates, s.e.m. and *P* values are given for the regression analysis. For regression analysis fasting insulin, HOMA-IR, and HOMA-B were log-transformed. Adjustments for age and sex were performed in all analyses. For HOMA-B, negative values were excluded.

Parameter	CC		CG		GG		β -Estimate (s.e.m.)	<i>P</i> value
	<i>n</i>	Mean (s.d.) or median ^a (IQR)	<i>n</i>	Mean (s.d.) or median ^a (IQR)	<i>n</i>	Mean (s.d.) or median ^a (IQR)		
At study entry								
Weight (kg)	162	87.90 (23.94)	121	91.60 (21.04)	27	97.45 (23.75)	0.601 (1.579)	0.704
BMI-SDS	162	2.71 (0.55)	121	2.73 (0.53)	27	2.91 (0.63)	0.028 (0.047)	0.544
Waist circumference (cm)	158	107.95 (15.19)	114	110.27 (13.53)	25	113.41 (14.69)	0.668 (1.151)	0.562
Fasting glucose (mmol/l)	160	3.84 (0.41)	120	3.97 (0.43)	27	4.28 (0.53)	0.205 (0.038)	1.17×10^{-7}
Fasting insulin (mU/l) ^a	161	10.10 (6.01)	118	9.65 (6.59)	27	12.00 (6.97)	0.007 (0.046)	0.878
HOMA-IR ^a	159	1.76 (1.20)	117	1.62 (1.23)	27	2.22 (1.34)	0.060 (0.052)	0.243
HOMA-B ^a	131	531.22 (556.10)	101	395.01 (370.36)	26	320.18 (124.38)	-0.353 (0.072)	1.58×10^{-6}
After intervention								
Weight loss (kg) after 4 weeks	154	8.11 (2.82)	116	8.52 (2.73)	27	8.01 (3.19)	-0.144 (0.212)	0.498
Weight loss (kg) after 6 weeks	98	10.61 (3.65)	74	11.28 (3.46)	15	11.39 (4.25)	0.277 (0.355)	0.436
BMI-SDS loss after 4 weeks	154	0.36 (0.11)	116	0.36 (0.10)	27	0.32 (0.10)	-0.013 (0.009)	0.163
BMI-SDS loss after 6 weeks	98	0.46 (0.13)	74	0.49 (0.14)	15	0.44 (0.12)	0.002 (0.015)	0.894
Waist circumference loss (cm) after 4 weeks	43	6.60 (3.64)	29	6.85 (5.29)	12	8.16 (5.97)	0.728 (0.728)	0.320
Waist circumference loss (cm) after 6 weeks	76	7.05 (4.51)	46	8.76 (5.33)	6	7.37 (2.97)	0.904 (0.731)	0.218

^aMedians (inter quartile ranges, IQR) are shown for not normally distributed parameters (fasting insulin, HOMA-IR and HOMA-B respectively).

well as HOMA-B was abolished ($P > 0.05$; Table 3A). However, compared to the association between fasting glucose and HOMA-B in the whole sample, in obese subjects (BMI-SDS: $2.5 \leq x < 3.0$) the β -estimates for fasting glucose and HOMA-B were moderately higher (glucose: 0.239 mmol/l , $P = 6.37 \times 10^{-5}$; HOMA-B: -0.377 , $P = 1.90 \times 10^{-3}$). In the very obese children and adolescents with a BMI-SDS ≥ 3.0 , the β -estimates for fasting glucose and HOMA-B were much higher (glucose: 0.299 mmol/l , $P = 6.97 \times 10^{-5}$; HOMA-B: -0.537 , $P = 2.39 \times 10^{-5}$) (Table 3A) compared with the whole study sample (Table 2). Analyzing the dichotomized BMI-SDS categories confirmed the results

that the effect size is bigger in the higher BMI-SDS category, whereas the association was significant for both the BMI-SDS groups (Table 3B). Furthermore, the SNP $\times$ gender interaction term was not significant (glucose: $P = 0.150$; HOMA-B: $P = 0.572$).

Anthropometric parameters (weight, BMI-SDS, waist circumference)

There was no significant association between rs10830963 and weight (0.601 kg , $P = 0.704$), BMI-SDS (0.028 , $P = 0.544$), or waist circumference

Table 3 Association between rs10830963 (*MTNR1B*) and fasting glucose and HOMA-B in different BMI-SDS categories. β -estimates, s.e.m. and *P* values are shown for the analyzed parameters in different BMI-SDS-categories. *P* values for the interaction term of SNP $\times$ BMI-SDS category are given. Adjustment for age and sex were performed in all analyses.

BMI-SDS categories	<i>n</i> (boys/girls)	β-estimate (S.E.M.)	<i>P</i> value	Interaction SNP × BMI-SDS category		
				β-estimate (S.E.M.)	<i>P</i> value	Global <i>P</i> value
A) According to sample size						
Fasting glucose (mmol/l)						
<2.5	108 (49/59)	0.035 (0.064)	0.589	—	—	0.012
2.5 ≤ × <3.0	103 (47/56)	0.239 (0.057)	6.37 × 10 ^{−5}	0.209 (0.093)	0.026	
≥3.0	96 (25/71)	0.299 (0.072)	6.97 × 10 ^{−5}	0.265 (0.091)	3.92 × 10 ^{−3}	
HOMA-B						
<2.5	85 (35/50)	−0.189 (0.133)	0.159	—	—	0.083
2.5 ≤ × <3.0	87 (35/52)	−0.377 (0.118)	1.90 × 10 ^{−3}	−0.144 (0.178)	0.419	
≥3.0	86 (22/64)	−0.537 (0.120)	2.39 × 10 ^{−5}	−0.380 (0.174)	0.030	
B) Dichotomized (</≥ mean)						
Fasting glucose (mmol/l)						
<2.74	168 (79/89)	0.141 (0.047)	2.93 × 10 ^{−3}	0.120 (0.074)	0.106	—
≥2.74	139 (42/97)	0.278 (0.060)	7.36 × 10 ^{−6}			
HOMA-B						
<2.74	140 (61/79)	−0.300 (0.096)	2.09 × 10 ^{−3}	−0.148 (0.139)	0.289	—
≥2.74	118 (31/87)	−0.428 (0.104)	7.52 × 10 ^{−5}			

(0.668 cm, $P=0.562$) at study entry respectively (Table 2). Furthermore, no significant result was observed for BMI-SDS reduction after 4 and 6 weeks of intervention (-0.013 , $P=0.163$; 0.002 , $P=0.894$) respectively. In addition, for weight and waist circumference loss no significant associations were observed (Table 2).

Discussion

This is the first independent study investigating the association of rs10830963 with traits related to glucose metabolism in overweight and obese children and adolescents. We observed a significant association with fasting glucose and HOMA-B, which was dependent on the obesity status, and no association with fasting insulin and HOMA-IR. However, no association could be detected between the SNP and weight, BMI-SDS, or waist circumference before and after the lifestyle intervention.

Compared to the effect size of 0.07 mmol/l (glucose) per minor allele in adults (6) and healthy children (8), the β -estimate observed in overweight and obese children and adolescents was higher (0.205 mmol/l), especially for the extremely obese (BMI-SDS ≥ 3.0) children (0.299 mmol/l). The interaction between SNP $\times$ BMI-SDS category supports the finding that the association might be related to obesity status.

One could argue that the lack of an association in children of the BMI-SDS-group < 2.5 is due to a lack of statistical power, because Kelliny *et al.* (8) observed a strong association between *MTNR1B* and fasting glucose in normal-weight children. Owing to the fact that in approach A (Table 3A) of BMI-SDS categorization, the sample size is the same in all the three groups, the finding might not be due to lack of statistical power. In the dichotomized BMI-SDS analysis, the association with fasting glucose in the lower BMI-SDS group (< 2.74) was significant. This difference might be due to the very small number of children homozygous for the minor allele.

Despite the very small effect in huge population-based studies, the early effect in life of the *MTNR1B* locus seen in our special cohort may have the potential for an early identification of obese children at high risk of diabetes. Given the fact that almost no investigation on diabetes in children has been made and that the early identification of children and adolescents at risk of type 2 diabetes might be a big challenge, the locus investigated in this study might be used together with some other loci for diagnostic purposes in the future.

Recent data indicate that melatonin, the ligand to *MTNR1B*, has an inhibitory effect on insulin secretion (7, 12–14). Therefore, our data replicate that children carrying the risk allele might be more sensitive to this inhibitory effect than noncarriers. Our results on the relationship between the *MTNR1B* SNP and increased fasting glucose as well as decreased HOMA-B are in

agreement with this hypothesis. Risk allele carriers were also shown to have increased mRNA levels of *MTNR1B* in β -cells (7), which may lead to a decreased insulin secretion resulting in elevated fasting glucose levels (16).

Our non-significant results for fasting insulin and HOMA-IR are in line with the results from recent publications (5, 6). In a recent study, the *MTNR1B* locus showed an association with lower insulinogenic index, but no association with insulin sensitivity (21).

In contrast to published data on an association between an *MTNR1B* variant (rs8192552) causing an amino acid exchange and obesity risk, BMI, and waist circumference, we found no association between rs10830963 and weight, BMI-SDS, and waist circumference or their changes during intervention (10). However, this finding might be due to the fact that rs10830963 is not in linkage disequilibrium with the polymorphism rs8192552 and also due to insufficient power of our ongoing weight-loss study.

Given the different effect size in adults and overweight and obese children and adolescents, young subjects as well as special groups may be particularly suitable for identifying new genetic variants related to glucose metabolism that has not yet been detected before in the adult population. This may also be of interest as other genes may be important before and after puberty. In a recently published genome-wide analysis by Scherag *et al.* (22), two new loci for obesity risk were detected in children and adolescents. The association between tankyrase, TRF1-interacting ankyrin-related ADP-ribose polymerase, and methionine sulfoxide reductase A (*TNKS/MSRA*) gene locus and obesity was limited to children and adolescents, whereas an association between this locus and waist circumference in adults was already shown (23).

The strength of our analyses is that we have included well-characterized overweight and obese children and adolescents participating in a prospective lifestyle intervention program and that we have data before and after weight loss. Thus, our analysis represents a clear approach to replicate the recent findings of Prokopenko *et al.* (6). Although the sample size of our study is rather limited, it seems to be sufficient, taking into account the effect size for fasting glucose and HOMA-B observed in our analysis. Owing to the small sample size our sub-analysis in the different BMI-SDS groups is risky and requires replication of the findings in a much larger study sample of overweight and obese children. The significant interaction term could be a false-positive finding. Furthermore, the analysis of a control group of normal-weight children would be interesting.

In conclusion, our data provide evidence that the rs10830963 polymorphism is associated with fasting glucose levels and HOMA-B in overweight and obese children and adolescents, especially in the higher BMI-SDS groups. The effect size for glucose levels per

minor allele seems to be higher than that reported for normal-weight children and adults. There was no association with anthropometric traits such as weight, BMI-SDS, or waist circumference.

Declaration of interest

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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