CLINICAL STUDY

Association of variants in gastric inhibitory polypeptide receptor gene with impaired glucose homeostasis in obese children and adolescents from Berlin

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Abstract

Objective: In the past 20 years, obesity has become a major health problem due to associated diseases like type 2 diabetes mellitus. The gastric inhibitory polypeptide receptor (GIPR) modulates body weight and glucose homeostasis and, therefore, represents an interesting candidate gene for obesity and the comorbidity impaired glucose homeostasis. Recently, a *GIPR* variation was found to be associated with impaired insulin response in humans.

In this study, we screened the *GIPR* gene for mutations and examined the association between three single-nucleotide polymorphisms (SNPs; rs8111428, rs2302382, rs1800437) and childhood obesity, as well as impaired glucose homeostasis.

Methods: The coding region of the *GIPR* was screened for mutations by direct sequencing. We genotyped three known SNPs in 2280 healthy normal weight (1696) and obese (584) children and adolescents. Genotyping was performed using the SNaPshot protocol, the iPLEX, and matrix-assisted laser desorption/ionization time-of-flight spectrometry technique. Obesity was defined by a body mass index SDS above 2; homeostatic model assessment was calculated.

Results: No evidence for an association was found between the SNPs and the obesity phenotype. Significant association was found between the minor allele C of the SNP rs1800437 and elevated homeostasis model of insulin resistance values ($P=0.001$). No further sequence variations in the *GIPR* were found to be associated with childhood obesity.

Conclusion: Variations of the *GIPR* sequence are not associated with childhood obesity. This study points to a potential role for rs1800437 in glucose homeostasis. Further studies are necessary to confirm these results.

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Introduction

The gastric inhibitory polypeptide (GIP) is a 42-amino acid incretin released from endocrine K cells of the duodenum and the small intestine after oral glucose uptake (1, 2). Binding to its GIP receptor (GIPR), mainly expressed on the pancreatic β -cells (3, 4), it induces an activation of these cells with an increase in insulin release after meals. GIPR is a member of the G-protein-coupled receptor (GPCR) superfamily, and acts through the activation of adenyl cyclase (5).

Gipr knockout studies show that *GIPR*^{−/−} mice are resistant to diet-induced obesity (3, 6), and show improved insulin sensitivity in aged *GIPR*^{−/−} mice compared with wild-type mice (4). High-fat diet-induced obese mice, treated with a GIP antagonist, present a reduction in body weight, triglyceride, and cholesterol

levels and an improvement of insulin sensitivity (7). Furthermore, double knockout mice for GIPR and leptin are also resistant to diet-induced obesity (3). Recent studies showed, as well, that vaccination against GIP in mice has a preventive effect for the development of obesity (8). Along with a second incretin glucagon-like peptide 1 (GLP-1), the signaling pathway of GIP represents a target for pharmacological therapies for type II diabetes mellitus as shown in initial studies in mice (9). The role of *GIPR* single-nucleotide polymorphisms (SNPs) on the glucose and insulin homeostasis was recently shown by identifying a *GIPR* variant associated with lower 2-h insulin levels in non-diabetic patients (10).

Different association studies of SNPs in the *GIPR* have been reported so far albeit varying effects of these SNPs were found in association with obese phenotypes in adults (11, 12). Based on the assumption, that in

children the genetic impact on obesity and its comorbidities is higher, we investigated whether two intronic SNPs (rs8111428 and rs2302382), and the exonic rs1800437 (Glu354Gln) of *GIPR* are associated with severe obesity and elevated homeostasis model assessment values for insulin resistance (HOMA-IR) in children.

Sequencing of *GIPR* was performed to detect other possible variations within *GIPR* that might be associated with childhood obesity and impaired glucose homeostasis.

Materials and methods

Study population

We examined 2280 children and adolescents aged 2.5–18 years from Berlin. They were either recruited from the obesity outpatient clinic of the 'Sozialpädiatrisches Zentrum' at the Otto-Heubner-Centrum for pediatrics, Charité Berlin ($n=657$) or from schools as part of the Berlin school children's cohort (BSCOC) conducted at the Institute of Experimental Pediatric Endocrinology at the Charité Berlin ($n=1623$) (13). No information on the degree of kinship was available for the girls of BSCOC. About 46% of all recruited children and adolescents had a migratory background with one or both parents not being German. Information on the ethnic background was used to assess whether ethnic differences occurred between the allele frequencies for the three analyzed SNPs. As these analyses provided no evidence for differences in genotypes by self-reported migration background (data not shown), we decided to analyze all data jointly. The purpose of recruiting children was to increase the genetic impact on obesity development, since the time of environmental impact on obesity is reduced compared with adults. To analyze the association between childhood obesity and the SNPs rs8111428, rs2302382, and rs1800437 in and nearby *GIPR* (Fig. 1), 1696 normal weight or lean and 584 obese children were genotyped. HOMA-IR values were available for a total of 357 of the obese children; among them, sequencing of all 14 exons of *GIPR* was performed for 87 children to detect further variations that might be associated with obesity.

Figure 1 Schematic presentation of the genomic DNA of the *GIPR* and relative localization of the SNPs analyzed in the association and sequencing study; exons represented as white boxes and numbered; the SNP which shows evidence for an association with HOMA-IR is highlighted.

All patients or their parents gave informed consent. Children with endocrine disorders or syndromal obesity were excluded from the study.

Anthropometric and laboratory measurements

We examined body weight and body height in 2280 children, clothed in underwear. Body weight was assessed in grams and height in centimeters. The body mass index (BMI)-SDS were calculated using the data from Kromeyer-Hauschild *et al.* (14). Obesity in childhood and adolescence was defined as BMI-SDS >2 , which corresponds to the 97.7th gender- and age-adjusted BMI percentile for children (15). This corresponds to the cut-off point proposed by the Childhood Group of the International Obesity Task Force (IOTF) as definition of obesity in children (16). In the analyses, dichotomized BMI-SDS was used as outcome variable. SDS values give a better feasibility of comparison of highly obese children and adolescences (17).

Fasting serum was used for insulin and glucose assessment using commercially available assays. The HOMA-IR was calculated as $\text{HOMA} = \text{insulin} \times \text{glucose} / 22.5$ (mmol/l) (18). The 95th HOMA-IR percentile as estimated by Allard *et al.* (18) was used to dichotomized HOMA-IR for the analyses (18).

Table 1 summarizes the anthropometric data of the association study population (total sample), and Table 2 summarizes the anthropometric data of the obese children explored in the sequencing study.

SNP analysis and sequencing of *GIPR*

DNA was extracted and purified from EDTA blood using a DNA-extracting procedure from Qiagen, or extracted and purified from chewed chewing gum as DNA source using the extracting procedure from Promega.

The *GIPR* genotype information was downloaded from the International HapMap Project (Build 36.2; www.hapmap.org) according to which, all three SNPs had a minor allele frequency larger than 5% in the CEU (Utah residents with Northern and Western European ancestry from the CEPH collection) population.

Subjects were either genotyped using the SNaPshot technique from Applied Biosystems (Darmstadt, Germany), or using the matrix-assisted laser desorption ionization time-of-flight spectrometry (MALDI-TOF) from Sequenom (Hamburg, Germany). The calling rate was 98.8% for rs8111428, 98.3% for rs2302382, and 97.4% for rs1800437. PCR was done for the 14 exons and the intron/exon boundaries, using primers assessed by Primer3 (19). *GIPR* was sequenced using an ABI 3130xl capillary DNA sequencer (Applied Biosystems). Except for rs1800437, no evidence for deviations from Hardy–Weinberg equilibrium (HWE) was detected (all $P \gg 0.10$). However, we observed evidence for deviations from HWE from rs1800437 in the obese

Table 1 Anthropometric variables of the association study population.

Group	No. of participants	Sex (M/F)	Mean age (years) $\pm$ s.d.	Mean BMI-SDS (kg/m ²) $\pm$ s.d.
Normal weight or lean children	1696	65/1631	12.31 $\pm$ 2.30	0.04 $\pm$ 0.95
Obese children	584	236/348	10.95 $\pm$ 3.56	2.80 $\pm$ 0.53

children ($P=0.03$) and in both HOMA-IR groups ($P=0.05$ and $P=0.005$). Most likely, these deviances also indicate the genetic association with HOMA-IR, which we observed.

Statistical analysis

All statistical analyses were performed using the SPSS 17 program (Munich, Germany). All genotype distributions were explored for deviations from HWE using an exact test. The dichotomized BMI-SDS and the HOMA-IR data were also analyzed by an exact version of Pearson's χ^2 test for 2×3 tables. The significance level of each test was set to $\alpha=0.05$ (two-sided). We applied no correction for multiple testing, and all P values reported are two-sided.

Results

We analyzed the association of genetic variants in the *GIPR* with childhood obesity and dichotomized HOMA-IR. No evidence for an association could be found between obesity and the three analyzed SNPs rs8111428, rs2302382, and rs1800437. Though, we observed that the A-allele of the SNP rs2302382 was descriptively more often present in obese children than in normal weight or lean children (Table 3).

However, evidence for an association was found between the minor allele C of the SNP rs1800437 and increased dichotomized HOMA-IR values (odds ratio estimate_{CC versus CG&GG} = 5.48; exact 95% confidence interval 1.73...22.80; $P=0.001$; Table 4).

For sensitivity analyses, we performed stratified analyses by ethnicity and gender. We observed no large impact on the reported genetic association with obesity and dichotomized HOMA-IR (data not shown). Independent of genotype, however, we were able to replicate the known higher frequency of obesity in German children of Arabian and the Turkish ethnicity (20, 21) when compared with children of German ethnicity. This difference in obesity prevalence was also observable for HOMA-IR. For 357 of the children and adolescents, a HOMA-IR value was assessed with 47.1% of the children having a HOMA-IR value above the 95th

percentile according to Allard *et al.*; a total of 74.5% of the children with HOMA-IR values below the 95th percentile were of German origin, whereas only 59.2% of the children above the 95th percentile were of German origin. Nevertheless, the ethnical mixture of the population studied does not seem to bias the results as no significant differences between the allele frequencies could be detected for the three SNPs analyzed in the HOMA-IR study in children and adolescents of German and Turkish origin. Furthermore, all children, for whom HOMA-IR values were assessed, were obese, regardless of their ethnicity. Unfortunately, it was only possible to achieve meaningful evaluations for German and Turkish children and adolescents because the sample number was too low for the other ethnical groups. In the HOMA-IR study group, the German children and adolescents had a mean BMI-SDS of 2.82, and children and adolescents of Turkish origin had a mean BMI-SDS of 2.93. In addition, the children and adolescents analyzed in our study do not show any significant difference in terms of BMI between the two groups, HOMA-IR values below (mean BMI-SDS = 2.78) and above the 95th percentile (mean BMI-SDS = 2.95). Therefore, even if Turkish children are generally more prone to obesity, our study group was homogeneous in terms of BMI, and we finally decided to integrate all children and adolescents in the calculation, independently of their ethnical background. In terms of other factors, such as the socio-economical status, we could not make any reliable statements due to a lack of sufficient information.

In the sequencing study, a total of five SNPs, rs4803845, rs5390, rs12709891, rs11672660, and rs34783010 (Fig. 1) were detected among 87 children (Table 5). All variants were found in non-coding regions of the *GIPR* and were already known from the HapMap database. None of the variations showed evidence for an association with childhood obesity. Though, some evidence for a potential association of the A-allele with elevated HOMA-IR levels was provided for the SNP rs12709891 ($P=0.069$). Furthermore, we detected the variation Tyr240His in exon 8, which occurred in one normal weight child with normal HOMA-IR level but

Table 2 Anthropometric variables of the study groups for the sequencing study.

Group	No. of participants	Sex (M/F)	Mean age (years) $\pm$ s.d.	Mean BMI-SDS (kg/m ²) $\pm$ s.d.
HOMA-IR < 95th percentile	37	16/21	9.72 $\pm$ 2.92	2.22 $\pm$ 0.72
HOMA-IR $\geq$ 95th percentile	50	23/27	13.09 $\pm$ 2.29	2.78 $\pm$ 0.58

Table 3 Genotype and allele frequencies for normal weight or lean and obese children for the three analyzed single-nucleotide polymorphisms (SNPs).

SNP	Group	Genotypes, <i>n</i> (frequency)			Frequency of allele		<i>P</i> value
rs8111428	Obese children	367 (0.630) AA	192 (0.330) AG	22 (0.038) GG	0.800 A	0.200 G	0.526
	Normal weight or lean children	1088 (0.650) AA	513 (0.228) AG	72 (0.032) GG	0.810 A	0.190 G	
rs2302382	Obese children	349 (0.602) CC	202 (0.348) AC	29 (0.050) AA	0.776 C	0.224 A	0.696
	Normal weight or lean children	1031 (0.620) CC	547 (0.329) AC	84 (0.037) AA	0.785 C	0.215 A	
rs1800437	Obese children	344 (0.616) GG	180 (0.323) CG	34 (0.061) CC	0.778 G	0.222 C	0.589
	Normal weight or lean children	984 (0.592) GG	571 (0.344) CG	107 (0.064) CC	0.764 G	0.236 C	

severely increased triglyceride levels. As the two sisters of this child were obese but do not carry the mutation, no further studies of this variant were performed to examine the association with obesity and impaired insulin sensitivity.

Discussion

So far, the role of genetic variations within the GIPR gene for different phenotypes like obesity and glucose metabolism remains controversial (12, 13), though physiological studies (3, 5, 9) clearly demonstrated the important role of the GIPR in weight regulation and glucose homeostasis.

We found evidence for a significant association between the minor allele of the exonic SNP rs1800437 of the *GIPR* and an elevated HOMA-IR value (above the 95th percentile) in these children and adolescents. The presence of the minor allele C induces an exchange from the amino acid glutamate to glutamine in transmembrane domain six (TM6; Fig. 2). Functional studies analyzing the impact of the amino acid exchange induced by rs1800437 on receptor function have not been published so far. However, naturally occurring mutations in other GPCRs, like the TSH receptor, indicate that the TM6 is highly susceptible for constitutive activation (22). Consistent with these data, one study reported an artificial activating mutation Thr340Pro in TM6 of the GIPR, which leads to an increase in basal cAMP compared with the wild-type receptor (23) (Fig. 2). Taking these observations together, we hypothesize that a constitutive activation of the GIPR by a variant in TM6 is conceivable.

As shown by several previous studies, *Gipr* knockout mice showed improved insulin sensitivity, and mice

treated with GIP antagonists had lower insulin levels (2, 4, 5). Therefore, loss of function of the GIPR would lead to a better insulin homeostasis capacity. An association between the SNP rs1800437 and a pathological HOMA-IR, which indicates an impaired glucose homeostasis prior to the manifestation of diabetes, may therefore be explained by gain of function effect implicated by the minor allele of this SNP on the GIPR.

To be sure, functional characterizations of the variant are necessary. Though the functional characterization of rs1800437 might be difficult to assess yet, since its effect on cAMP increase might be very small. Lessons can be learned from other GPCRs as for example, the melanocortin-4 receptor gene (*MC4R*) variant Val103Ile, which leads to a slight but significant and robustly replicated decreased body weight in individuals carrying the minor allele (103Ile) of this SNP (24, 25). Functional studies of Val103Ile, however, could only detect a marginal effect in response to inverse agonist challenge (26), whereas other functional studies failed to demonstrate a difference between cAMP levels in wild-type and in Val103Ile *MC4R* (25, 27). A possible explanation for this apparent discrepancy are very small individual effects at the functional level that are yet undetectable by the given study designs, which can nevertheless have an impact on the clinical phenotype if present for a longer period of time (28). In the case of inactivating variations within *GIPR*, a smaller effect on the phenotype expression might be accentuated by the complementary role of the incretin GLP-1 and its receptor (7, 29). GLP-1 and GIP seem to share a common pathway in the β -cell, which results in insulin release under feeding conditions. However, in the case of variations leading to constitutive activation of *GIPR*, the receptor effect of the activated GIPR is present in fasting state without ligand binding as both incretins are only secreted after nutrient intake.

Table 4 Allele frequency of the single-nucleotide polymorphisms (SNPs) rs8111428, rs2302382, and rs1800437 and the homeostasis model assessment values for insulin resistance (HOMA-IR) status.

SNP	Group	Genotypes, <i>n</i> (frequency)			Frequency of allele		<i>P</i> value
rs8111428	HOMA-IR <95th percentile	116 (0.614) AA	66 (0.349) AG	7 (0.037) GG	0.788 A	0.212 G	0.923
	HOMA-IR $\geq$ 95th percentile	106 (0.639) AA	54 (0.325) AG	6 (0.036) GG	0.801 A	0.199 G	
rs2302382	HOMA-IR <95th percentile	111 (0.587) CC	71 (0.376) AC	7 (0.037) AA	0.775 C	0.225 A	0.396
	HOMA-IR $\geq$ 95th percentile	105 (0.633) CC	52 (0.313) AC	9 (0.054) AA	0.789 C	0.215 A	
rs1800437	HOMA-IR <95th percentile	106 (0.582) GG	72 (0.396) CG	4 (0.022) CC	0.780 G	0.220 C	0.001
	HOMA-IR $\geq$ 95th percentile	92 (0.594) GG	46 (0.297) CG	17 (0.110) CC	0.741 G	0.259 C	

Table 5 Variations found by sequencing *GIPR* in 87 children with known homeostasis model assessment values for insulin resistance (HOMA-IR) levels.

SNP	Group	Genotypes, n (frequency)			Frequency of allele		P value
rs4803845	HOMA-IR <95th percentile	37 (1.000) CC	0 (0.000) CT	0 (0.000) TT	1.000 C	0.000 T	0.198 ^a
	HOMA-IR ≥95th percentile	50 (1.000) CC	0 (0.000) CT	0 (0.000) TT	1.000 C	0.000 T	
rs5390	HOMA-IR <95th percentile	36 (0.973) CC	1 (0.270) CG	0 (0.000) GG	0.986 C	0.014 G	0.495
	HOMA-IR ≥95th percentile	49 (0.980) CC	1 (0.020) CG	0 (0.000) GG	0.990 C	0.010 G	
rs12709891	HOMA-IR <95th percentile	37 (1.000) CC	0 (0.000) CA	0 (0.000) AA	1.000 C	0.000 A	0.069
	HOMA-IR ≥95th percentile	45 (0.900) CC	5 (0.100) CA	0 (0.000) AA	0.950 C	0.050 A	
rs11672660	HOMA-IR <95th percentile	22 (0.595) CC	14 (0.378) CT	1 (0.027) TT	0.783 C	0.217 T	0.564
	HOMA-IR ≥95th percentile	32 (0.640) CC	18 (0.360) CT	0 (0.000) TT	0.820 C	0.180 T	
rs34783010	HOMA-IR <95th percentile	24 (0.649) GG	12 (0.324) GT	1 (0.027) TT	0.810 G	0.190 T	0.439
	HOMA-IR ≥95th percentile	36 (0.720) GG	14 (0.280) GT	0 (0.000) TT	0.860 G	0.140 T	

^aFor a binomial test.

Regarding their role for childhood and adolescence obesity, neither the three analyzed SNPs nor the five variants detected by sequencing provided evidence for a genetic association. Most likely, given the small genetic effect sizes reported for obesity so far (30), the power of our study has been too small to detect obesity effects related to these SNPs. So far, an association between variants in *GIPR* and obesity cannot be completely ruled out. Further investigation in much larger samples will be required to finally unravel the impact of *GIPR* as a gene for obesity.

Our findings concerning the role of *GIPR* as a candidate gene for insulin impairment are supported by the ‘Meta-Analyses of Glucose and Insulin-related traits Consortium’ (MAGIC) (10): very recently, within the scope of a meta-analysis of the MAGIC, Saxena *et al.* identified a variation in the *GIPR* (rs10423928) to be associated with increased 2-h glucose and an impaired insulin response on glucose challenge during oral glucose tolerance test. Additionally, this SNP is in strong linkage disequilibrium (LD) with the SNP rs1800437 showing association with elevated HOMA-IR levels in children. Both SNPs have an effect on the glucose homeostasis (10).

In sum, though this study could not provide evidence for a genetic association of all eight analyzed variants to

childhood and adolescence obesity, the role of *GIPR* as a candidate gene for insulin impairment is strengthened as we observed evidence for an association of the exonic SNP rs1800437 and the elevated HOMA-IR values in a relatively small sample of only 357 children and as this SNP is in strong LD with another *GIPR* variation showing an effect on insulin secretion.

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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Figure 2 Schematic structure of the *GIPR* with transmembrane domain 6 (TM6) in detail; activating variants highlighted: rs1800437 (Glu354Gln; E → Q) and the artificial variant Thr340Pro (T → P); relative localization of activating TSHR mutations in gray (according to http://www.ssfa-gphr.de/natural_ali.php) (31).

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