Aus dem Institut für Experimentelle Pädiatrische Endokrinologie
der Medizinischen Fakultät Charité – Universitätsmedizin Berlin

DISSERTATION

Evaluation of a Rare Glucose-Dependent Insulinotropic Polypeptide Receptor Variant in a Patient with Diabetes

zur Erlangung des akademischen Grades
Doctor medicinae (Dr. med.)

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von

Simon Friedrich Jacobi
aus Frankfurt

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Abstract (deutsch)

Der nachfolgende Text entspricht dem übersetzten Abstrakt der folgenden Arbeit:

Jacobi SF, Khajavi N, Kleinau G, Teumer A, Scheerer P, Homuth G, Völzke H, Wiegand S, Kühnen P, Krude H, Gong M, Raile K, Biebermann H. Evaluation of a rare glucose-dependent insulinotropic polypeptide receptor variant in a patient with diabetes. *Diabetes Obes Metab*. 2019;21(5):1168-1176. doi:10.1111/dom.13634

„Einführung

Das glukoseabhängige insulinotrope Polypeptid (GIP) ist ein Inkretin-Hormon, welches in den K-Zellen des Magen-Darm-Traktes produziert wird. In den Zielorganen bindet GIP an seinen zugehörigen Rezeptor (GIPR). Durch Aktivierung von GIPR in den β-Zellen der Bauchspeicheldrüse wird die Insulinsekretion ins Blut verstärkt.

Genomweite Assoziationsstudien haben zuletzt die Assoziation des Einzelnukleotid-Polymorphismus (SNP) *rs1800437* im GIPR kodierenden Gen (*GIPR)* mit Adipositas und Insulinresistenz identifiziert. In der vorliegenden Studie haben wir untersucht, ob GIPR-Varianten in nach diesen Kriterien ausgesuchten Patientengruppen gehäuft auftreten und eine seltene Variante in *GIPR* funktionell charakterisiert.

Materialien und Methoden

Die kodierenden Regionen von *GIPR* wurden in 164 Kindern mit Adipositas und Insulinresistenz (Patientengruppe 1) und in 80 Kindern mit Diabetes unbekannter Genese (Patientengruppe 2) sequenziert. Zusätzlich wurde die 8320 Personen umfassende SHIP-Kohorte (Study of Health in Pomerania) auf die GIPR-Variante Arg217Leu untersucht.

Ausgewählte GIPR-Varianten wurden in COS-7-Zellen exprimiert und nach Stimulation mit GIP wurde die Produktion von cyclischem Adenosinmonophosphat (cAMP) gemessen. Die Expression des Rezeptors an der Zelloberfläche wurde durch ELISA (Enzyme-linked Immunosorbent Assay) bestimmt. Ein Homologie-Modell von GIPR mit Arg217Leu, Wildtyp und verschiedenen GIPR-Varianten wurde erstellt, um dreidimensionale Informationen über den Rezeptor zu erhalten.

Ergebnisse

Die missense Variante Arg217Leu (rs200485112) wurde heterozygot bei einem Kind asiatischer Abstammung aus der Patientengruppe 2 identifiziert. Bei der funktionellen

Charakterisierung zeigte Arg217Leu nach Stimulation mit GIP eine reduzierte Oberflächenexpression und eine verminderte Produktion von cAMP. Das Homologie-Modell der GIPR-Struktur unterstützt die gefundenen funktionellen Ergebnisse der Variante Arg217Leu.

In der SHIP-Kohorte lag Arg217Leu nicht vor. Die Häufigkeit der anderen SNP war in den untersuchten Patientengruppen und der durchschnittlichen Bevölkerung gleich.

Fazit

Die *In-vitro*-Funktionsstudien und die Modellierung der Proteinhomologie weisen auf eine Relevanz der GIPR-Variante Arg217Leu für die Rezeptorfunktion hin. In der betroffenen Familie zeigte die heterozygote Variante von Arg217Leu eine partielle Co-Segregation mit Diabetes. Auf Grund dieser Ergebnisse gehen wir davon aus, dass GIPR-Varianten bei gestörter Glukosehomöostase eine Rolle spielen und von klinischer Relevanz bei homozygoten Patienten sein können. Die genaue Rolle der GIP/GIPR-Achse und den Einfluss von Varianten in GIPR müssen weitere Studien klären.“

Abstract (englisch)

Der nachfolgende Text entspricht dem Abstrakt der folgenden Arbeit:

Jacobi SF, Khajavi N, Kleinau G, Teumer A, Scheerer P, Homuth G, Völzke H, Wiegand S, Kühnen P, Krude H, Gong M, Raile K, Biebermann H. Evaluation of a rare glucose-dependent insulinotropic polypeptide receptor variant in a patient with diabetes. *Diabetes Obes Metab*. 2019;21(5):1168-1176. doi:10.1111/dom.13634

“Aims

Glucose-dependent insulinotropic polypeptide (GIP) is an incretin hormone that augments insulin secretion in pancreatic β-cells via its receptor GIPR. Recent genome-wide association studies identified a single nucleotide variant (SNV) in *GIPR,* rs1800437, to be associated with obesity and insulin resistance. In the present study, we tested the hypothesis whether GIPR variants contribute to obesity and disturbed glucose homeostasis or diabetes in specific patient populations.

Materials and methods

Exon sequencing of *GIPR* was performed in 164 children with obesity and insulin resistance and in 80 children with pediatric-onset diabetes of unknown origin. The Study of Health in Pomerania (SHIP) cohort comprising 8320 adults were screened for the *GIPR* variant Arg217Leu. *GIPR* variants were expressed in COS-7 cells and cAMP production was measured upon stimulation with GIP. Cell surface expression was determined by ELISA. Protein homology modeling of the GIPR variants was performed to extract 3-dimensional information of the receptor.

Results

A heterozygous missense *GIPR* variant Arg217Leu (rs200485112) was identified in a patient of Asian ancestry. Functional characterization of Arg217Leu revealed reduced surface expression and signaling after GIP challenge. The homology model of the GIPR structure supports the observed functional relevance of Arg217Leu.

Conclusion

In vitro functional studies and protein homology modeling indicate a potential relevance for the *GIPR* variant Arg217Leu in receptor function. The heterozygous variant displayed partial co-segregation with diabetes. Based on these findings, we suggest that *GIPR* variants may play a role in disturbed glucose homeostasis and may be of clinical relevance in homozygous patients.”

Manteltext

INTRODUCTION

History and development of the incretins

In 1964, McIntyre and Elrick independently showed that after oral glucose challenge in humans, insulin concentrations in the blood were higher than after an intravenous injection of the same amount of glucose[1,2]. This observation was called “incretin-effect” and attributed to a substance, that enhances insulin secretion directly in the pancreas – independent of blood sugar values. The name “incretin” was introduced a few decades before by Zunz and La Barre[3,4] and even as early as in 1906, Moore and colleagues found that gut extracts contain a substance that lowered glucose in urine of patients with diabetes[5]. Subsequently, gastrointestinal peptide hormones were discovered and characterized: gastric inhibitory peptide (GIP) was first reported in 1970 by Brown et al.[6,7] and glucagon-like peptide 1 (GLP-1) approximately one decade later, in 1983 by Bell et al.[8–10]. In the following years, many actions of GIP and GLP-1 were discovered on not only pancreas and gastrointestinal tract, but also on bone, brain, adipose tissue[11]. Initially named gastric inhibitory peptide, GIP was renamed glucose-dependent insulinotropic polypeptide after its main biological action was clarified over the years. A brief overview of the history of incretins can be found e.g. in the reviews of Rehfeld or Creutzfeldt[12,13].

The promiscuous effects of GIP and GLP in different organs increased the search of the target receptors, which were discovered in the 1990s. GLP-1 receptor (GLP-1R) was first described and sequenced in 1992/1993[14–16]. Additionally, in 1992, Gutniak et al. showed for the first time that incretins, here GLP-1, can serve as antidiabetic agents[17]. In 1995 the sequence of glucose-dependent insulinotropic polypeptide receptor (GIPR) was reported by multiple groups[18–20]. Both incretin receptors belong to the class B of G-protein coupled receptors (GPCRs), that are described in detail below[21]. Incretin receptors are expressed amongst others in the endocrine pancreas, intestine, bone, brain, adipose tissue and many more, which explains the various effects of GIP (Figure 1) and GLP-1 action in humans. The reports on GIP/GIPR physiology and pathophysiology were controversial and until now it still remains undefined, whether GIPR agonism or antagonism is beneficial in metabolic diseases. However, GLP-1 and GLP-1R became an important drug target in the treatment of type-2 diabetes and obesity[22]. The first drugs developed for that pathway in the

beginning of the millennium were (1) exendin-4 (the synthetic form named exenatide), an incretin mimetic resembling GLP-1 and (2) sitagliptin, a dipeptidyl peptidase-4 (DPP-4) inhibitor stopping the degradation of GLP-1[23(p1)]. Both were successfully approved as antidiabetic drugs in humans and have been successfully used ever since.

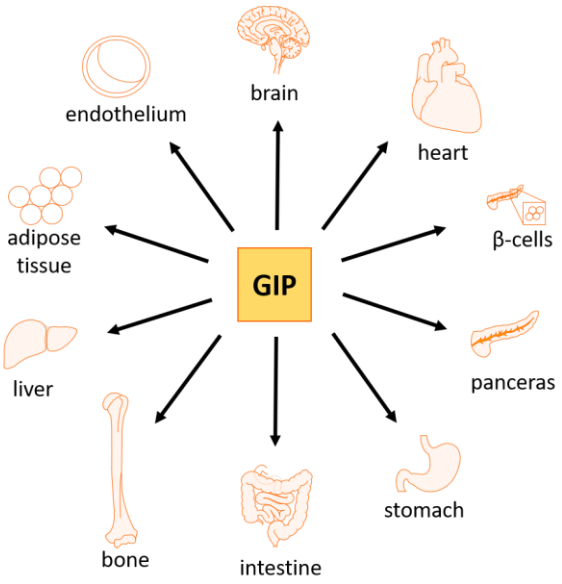
After years of mainly GLP-1/GLP-1R in the spotlight, the GIP/GIPR system again got more attention starting from 2010, when genome-wide associated studies (GWAS) showed associations of loci/single nucleotide polymorphisms in GIPR - and interestingly not GLP-1R - with obesity[24]. Subsequently, more studies focused on variants in incretin receptors and found associations of GIPR variants with obesity and disturbed insulin resistance[25,26]. Additionally, GIPR became a new drug target with the development of peptides being agonistic at both GLP-1R and GIPR[27]. This trend was reflected by the number of publications on PubMed (https://www.ncbi.nlm.nih.gov/pubmed) related to the GIP/GIPR field since 2009. For example, the search term “glucose-dependent insulinotropic polypeptide receptor” / “GIPR” achieved less than 10 results before the year 2009, but has increased considerably, which resulted in a new maximum of 34 results in the year 2019. Table 1 shows important landmarks of incretin research.

GIP

GIP is a polypeptide, consisting of 42 amino acids in its active form. Together with GLP-1, glucagon and secretin and various other peptides mainly originating from pancreas or intestine, it belongs to the secretin family of hormones. GIP is produced in K-cells of the duodenum and is released into the blood upon oral food intake, especially triggered by carbohydrates and fat[28,29]. The highest GIP concentrations measured in blood in healthy humans are reached 30-60 minutes after food intake[28]. In the human body, GIP is distributed via the bloodstream and acts as an endocrine hormone on multiple target organs (Figure 1). GIP in the bloodstream undergoes degradation and therefore inactivation within a few minutes, with an approximate half-life of 5 minutes[11]. This is mainly catalyzed via the enzyme that leads to the cleavage of the active form of GIP 1-42 to GIP 3-42. In GIP target organs, its cognate receptor, GIPR, is expressed[18–20]. The specific actions of GIP are displayed in Figure 1 and discussed in the subsequent paragraphs on ‘GIP/GIPR action in metabolism’ and ‘GIP/GIPR action in other organs’.

Table 1: Important discoveries in the incretin field in the 20th and 21st century

Year	Discovery	References
1906	Gut extracts contain a substance that lower glucose in urine of patients with diabetes	5
1929	Introduction of the term “incretin”	3,4
1964	Discovery of the “incretin effect”: after oral glucose challenge in humans, blood insulin concentrations were higher than after an intravenous injection of the same amount of glucose	1,2
1970	Gastric inhibitory peptide (GIP) reported	6,7
1983	Glucagon-like peptide-1 (GLP-1) reported	8–10
1992	Glucagon-like peptide-1 receptor (GLP-1R) was first described and sequenced	14–16
1995	Glucose-dependent insulinotropic polypeptide receptor (GIPR) was sequenced	18–20
from 2004	Exenatide (exendin-4), a GLP-1R agonist and sitagliptin, a dipeptidyl peptidase-4 (DPP-4) inhibitor, stopping the degradation of incretins, were the first drugs targeting incretin action	23
from 2010	Reappraisal of GIPR as a potential drug target	24,27



β-cells	- glucose-dependent insulin secretion ↑ - insulin biosynthesis ↑ - β-cell mass ↑ - β-cell survival ↑
panceras	Endocrine: - glucagon secretion ↑ Exocrine: - pancreatic amylase secretion ↑
adipose tissue	- fat accumulation / lipogenesis ↑ - interleukin-6 expression ↑ - lipolysis ↑ - lipoprotein lipase activity ↑
bone	- anabolic and proliferative actions ↑ - bone density ↑ - bone formation ↑
stomach	- gastric acid production ↓ - somatostatin secretion ↑

Figure 1: Actions of glucose-dependent insulinotropic polypeptide (GIP)

Target tissues of GIP (left) and actions of GIP in selected tissues (right) in humans. Data taken from McIntosh et al.[30].

GIPR

GIPR belongs to the G protein-coupled receptors (GPCR). This is a superfamily of proteins that are involved in numerous hormonal signaling processes in the body. Therefore, GPCRs are an important drug target and are targeted by approximately a third of all FDA-approved drugs[31]. Together with GLP-1R, the glucagon receptor and secretin receptor and others, GIPR belongs to the family-B (secretin-receptor family) GPCR. The common characteristic of this family are seven transmembrane domains, a large amino-terminal extracellular domain being essential for ligand binding and highly conserved parts of the transmembrane helices (TM) 1 and TM 7[21,30].

The gene encoding GIPR (*GIPR*) is located on chromosome 19q13.3, spans 12.5 kb and embraces 14 exons[18,19]. After transcription into a protein, it has 466 amino acids, an estimated molecular weight of approximately 59 kDa, and belongs to the glycoproteins.

The main signaling pathway after ligand binding for class B GPCR is $G\alpha_s$/adenylyl cyclase (AC) activation which, leads to Gs protein activation and subsequently the activation of AC and to the production of cyclic adenosine monophosphate (cAMP). The second messenger cAMP induces multiple subsequent signaling cascades. It (1) activates protein-kinase-A, which leads to an increased calcium influx and (2) potentiates membrane depolarization via closing of K+-channels, which also leads to

increased Ca^{2+} influx[30]. In pancreatic β-cells, increased Ca^{2+} accumulation leads to augmented insulin secretion[11].

GIP/GIPR action in the pancreas and adipose tissue

The main – and also name-giving – action of GIP is its positive effect on insulin secretion in the pancreatic β-cells (Figure 1). As a prerequisite, this effect needs at least mild hyperglycemia leading to insulin secretion. Consequently, incretin action does not lead to hypoglycemia, because insulin secretion is glucose-dependent and can only be augmented by GIP and GLP-1. Besides, GIP/GIPR agonism increases β-cell mass and improves β-cell survival[32]. Furthermore, GIP also triggers the secretion of glucagon secretion by pancreatic α-cells[33].

Additionally, GIP acts on adipose tissue and has lipogenic as well as lipolytic effects[30] Previous *in vitro* studies have shown that GIP directly increases lipoprotein lipase thereby inducing fat accumulation and increasing interleukin 6 (IL-6) expression in adipose tissue. A recent publication underlined these results, showing evidence to confirm that GIP is an obesity-promoting factor under high fat diet (HFD) conditions in mice[34]. Those findings go in line with the observation that deletion of GIPR signaling causes resistance to diet induced obesity as shown in GIPR knockout mice[35].

GIP/GIPR in diabetes and obesity

From early on, the GIP/GIPR system was studied mainly in patients with diabetes in order to determine the characteristics in pathophysiology and disease. In patients with type-2 diabetes, the incretin effect is reduced, partly due to defective pancreatic β-cell responsiveness to GIP. In obesity, fasting levels of GIP are normal. If there is impaired glucose tolerance, an increased GIP response to a glucose load is seen. There are, up to date, no clear causal relationships between elevated GIP, hyperinsulinemia and insulin resistance in type-2 diabetes mellitus[30].

GIP/GIPR action in other organs

The initial name of GIP, gastric inhibitory polypeptide, was given to it due to its ability to inhibit gastric acid production in an early, *ex vivo* experimental model. This effect was later on shown to be less relevant in physiological state[36]**.** GIP/GIPR, as well as GLP-1/GLP-1R, were investigated in multiple tissues and organs with suggested effects in bone, brain, gut and others (Figure 1). This is reviewed in detail by Nauck et al.[37].

GIP/GIPR agonism and antagonism

Over the years of GIP/GIPR research in healthy state and disease, there were recurrent hints of GIPR antagonism being beneficial for metabolic disease. *Gipr* knockout mice showed no obese or insulin resistant phenotype, but were even resistant to diet-induced obesity[35]. In line, GIPR antagonism protects against obesity, insulin resistance, glucose intolerance and associated disturbances in high fat diet mice[38]. On top of that, vaccination against GIP in mice showed increased energy expenditure and confirmed protection against diet-induced obesity[39]. On the other hand, GIPR agonism was also suggested to be beneficial. GIPR agonism has been shown to have a positive impact on β-cell survival and glycemic control[40]. GIP/GIPR agonism is furthermore a part of modern, dual incretin agonists[41,42].

These new drugs seem to be beneficial in the treatment of both obesity and diabetes[27]. Taken together, both agonism and antagonism offer promising pathways for targeting GIPR in metabolic syndrome and its associated diseases[43].

Obesity in the population and in childhood

Obesity in the population in general is a worldwide problem with increasing prevalence over the last years[44]. In Germany, according to the *Statistisches Bundesamt*, more than 52% of the adult population has a body mass index > 25 kg/m² and is therefore classified overweight and 16% of the population is obese with a BMI > 30 kg/m²[45]. Nowadays, obesity is already a problem in early childhood and in adolescence with an increasing prevalence. In 2015 the overall prevalence for obesity in children worldwide was 5% with many countries even having a higher rate of increase in childhood obesity than in adult obesity[44]. Normal body weight is up to around two thirds genetically determined, with environmental factors and lifestyle then leading to the development of overweight and obesity. Only a small percentage of obesity in childhood is due to major gene defects, as for example monogenetic mutations (e.g in the pro-opiomelanocortin gene *POMC*[46,47]) or induced by drugs (steroid treatment). Independent of the cause of obesity, it is associated with a higher chance of obesity in adult life and with multiple comorbidities, such as metabolic syndrome with insulin resistance and type-2 diabetes in childhood and cardiovascular disease.

Insulin resistance and type-2 diabetes in the population and in childhood

Insulin resistance and ultimately type-2 diabetes are also an increasing health issue in the western society, often being a comorbidity of obesity. Nowadays, with an increasing

number of children being obese, more and more children also become insulin resistant or even have complete features of type-2 diabetes. Apart from that, there are intrinsic diabetes types: type-1 diabetes, caused from autoantibodies and maturity onset diabetes of the young (MODY) which is caused by genetic mutations in different genes and represents the cause of diabetes in approximately 1% of patients[48].

GIPR variations and genome wide association studies (GWAS)

Up to date, several single nucleotide polymorphisms and mutations in the coding region of *GIPR* are known. Some of these mutations were shown to have a functional impact, some appear in genome-wide association studies (GWAS) and others do not have effects on receptor function or on a phenotype in a population[27,30,49]. In 2010, Speliotes et al. showed in a GWAS a link between obesity and the *GIPR* SNP rs1800437 that leads to an exchange of glutamate to glutamine at amino acid position 354 of GIPR (Glu354Gln)[24]. Following up these results, there were more studies showing association of the minor allele of rs1800437 with increased insulin resistance measured by HOMA-IR in children[25] and impaired oral glucose-tolerance test[50]. Those results confirmed the relevance of genetic variations in *GIPR* for metabolic diseases.

However, up to date, the relevance of genetic aspects of GIPR is not completely clear[31]. There are no *GIPR* mutations, neither gain-of-function nor loss-of-function mutations known in humans that lead to clear phenotypes. Additionally, there are no studies investigating whether *GIPR* variants impact binding of GIP and/or GIPR agonists, which can be important for drug therapy.

Aim of our study

We investigated (1) if variants in *GIPR* in selected populations contribute to the obese phenotype and to impaired glucose homeostasis and (2) how these potential variants influence receptor function mechanistically on a molecular level.

MATERIALS AND METHODS

Principles of study population selection

One approach, in order to maximize the possibility of identifying a mutation in a study population, is the selection of the study population based on a phenotype that fits the function of the gene product (study population 1). Another option is to choose a study population with a phenotype or with symptoms that are so far unexplained, and then look at the genotype of this population (study population 2, Figure 2).

In children, the impact of genetic variations on metabolism is higher than in adults, due to less time of environmental factors and lifestyle being able to impact the phenotype.

Study population 1: Obesity and insulin resistance in children

Study population 1 was chosen according to the combination of phenotypes of two important studies: (1) Speliotes et al., who found an association of a *GIPR* variant with increased BMI and (2) Sauber et al., who found an association of a *GIPR* variant with increased insulin resistance measured by HOMAR-IR (Figure 2)[24,25].

In adults, a body-mass index (BMI) above 25 kg/m² is considered as overweight and a BMI above 30 kg/m² as obesity. In children, however, standard deviation from the mean BMI of an age adjusted population is used to ensure an adequate classification. This BMI standard deviation score (BMI-SDS) is given in standard deviation from the average BMI represented by the 50th percentile of the age adjusted group. In this case, 95 percent of all values are between -2.0 and +2.0 standard deviations. As a cut-off we chose a BMI-SDS of +2.0, which represents approximately the 98th percentile of a general healthy population (Figure 2, Table 2). The second chosen parameter was HOMA-IR, a calculated value of fasting insulin and fasting glucose levels, which is a measurement of insulin resistance and β-cell function (Allard et al. 2003). HOMA-IR is an easily accessible parameter, as it can be measured from one blood sample. That is often beneficial in pediatric patients, in which longer diagnostic procedures can be challenging. HOMA-IR is calculated with the following formula: $HOMA-IR = \frac{fasting\ insulin\left(\frac{\mu U}{ml}\right) * fasting\ glucose\left(\frac{mmol}{l}\right)}{22{,}5}$.

Additionally, we included some patients with a minimum of +1.77 BMI-SDS, which is on the ~95-96th percentile for BMI (Table 2). Study population 1 consisted of 164 children. Mean BMI was 32.67 ± 5.76, mean BMI-SDS was +2.75 ± 0.49, and mean HOMA-IR was 5.67 ± 3.39 (Table 2).

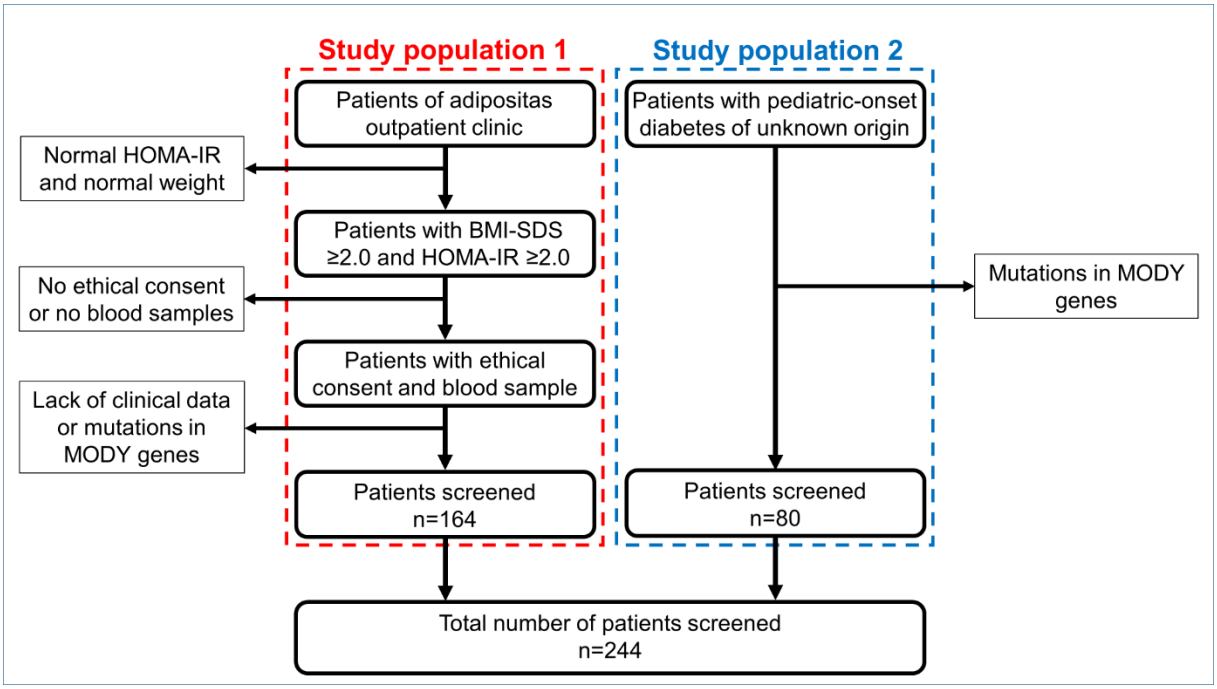


Figure 2: Study population selection criteria

Study population 1 (red) included 164 patients with overweight or obesity (BMI-SDS ≥+2.0) and increased insulin resistance by a HOMA-IR ≥2.0. Study population 2 (blue) consisted of 80 patients with pediatric-onset diabetes of unknown origin. Patients were antibody-negative and had no mutation in the MODY genes. 244 patients were screened in total for variants in *GIPR* exons. Figure and figure legend from Jacobi et al.[51] Reprinted and modified with permission.

Table 2: Study population 1 characteristics

Patient study population 1 comprises 164 children with a mean age of 13.02 years (range, 5–17 years). The mean HOMA-IR of the highest obtained value during all presentations at the outpatient clinic was 5.67 (range, 2.07–32.80). The mean BMI-SDS in study population 1 was +2.75 (range, +1.77–4.20). Table and table legend from Jacobi et al.[51] Reprinted and modified with permission.

n=164	Mean	Standard deviation	Median	Minimum	Maximum
Age [years]	13.02	2.34	13.18	5.68	17.76
HOMA-IR	5.67	3.39	5.00	2.07	32.80
BMI	32.67	5.76	31.99	23.30	56.90
BMI-SDS	+2.75	0.49	+2.73	+1.77	+4.20

HOMA-IR, Homeostasis Model Assessment for Insulin Resistance; BMI, Body Mass Index; BMI-SDS, Body Mass Index Standard Deviation Score.

Study population 2: Pediatric-onset diabetes of unknown origin

Study population 2 was chosen in order to have a broader approach and to increase the likelihood of finding relevant genetic variations. In study population 2, there were 80 children which had a phenotype of diabetes, without obesity being a criterium (Figure 2). The cause of diabetes was unknown in these patients. All patients underwent (1) testing for autoantibodies and (2) sequencing for known genes causing Maturity Onset Diabetes of the Young (MODY), which is a rare genetic cause of diabetes in children and adolescents. Neither autoantibodies, nor pathogenic mutations were found in study population 2.

Study of Health in Pomerania cohort (SHIP)

The analysis of the SHIP cohort was done in cooperation with A. Teumer, G. Homuth, and H. Völzke from the Department SHIP/Clinical-Epidemiological Research of the Institute for Community Medicine and the Department of Functional Genomics, Interfaculty Institute for Genetics at the University Medicine Greifswald. 8,230 individuals were screened for our *GIPR* variant of interest in SHIP, which is a population-based research project in West Pomerania, a region in northeast Germany[52]. The project consists of two independent cohorts (SHIP and SHIP-TREND), which were prospectively collected in order to assess the prevalence and incidence of common population-based diseases and their risk factors from 1997 until 2012. Details can be found in Jacobi et al.[51].

Variant screening and cloning of *GIPR* variants

All patients of both study populations and the family members of the index patient were screened for mutations in the coding region of *GIPR* including the exon/intron boundary using automated Sanger sequencing (ABI3710xl, Applied Biosystems, Foster City, CA, USA). The complete exons (n = 14 for *GIPR*) and ± 20 base pairs at the beginning and ending of the exons were sequenced, with the exception of exon 1, where we started sequencing 20 base pairs before the start-codon. Primers were designed with primer3web (http://primer3.ut.ee/) and tested with DNA samples for efficiency before used for screening[53]. To further investigate the function of the found *GIPR* variants, we used plasmids containing an expression vector encoding *GIPR.* We inserted the *GIPR* variant of interest rs200485112 Arg217Leu as well as other identified variants Ala207Val (rs1800436) and Glu354Gln (rs1800437) using Primer Extension as standard mutagenesis technique.

Cell culture and transfections

All functional assays were performed in COS-7 (CV-1 in Origin with SV40 genes) cells. The COS-7 cell line does not endogenously express incretin GPCRs and is therefore convenient for determining cell surface expression of GPCRs[54]. COS-7 cells originate from the kidney of the African green monkey and are CV-1 immortalized fibroblasts. The COS-7 cells are transformed with Simian Vacuolating Virus 40 (SV40) which is suitable for transfection with plasmids containing the SV40 promotor, like the used pcDps vectors of this study.

Cells for all experiments were grown in Dulbecco´s modified medium (DMEM/Biochrom, Berlin, Germany) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin in a humidified 5% CO_2 incubator at 37°C. For cell surface expression studies, cells were seeded in 48-well plates (3.8×10^5 cells/well) and for cAMP measurements, cells were seeded in 96-well plates (1.0×10^4 cells/well). Plasmids encoding the mutant or wild-type *GIPR* (*GIPR-WT*) were transfected into the cells. Transient transfection was performed 24 h after seeding in supplement-free DMEM medium using 45 ng plasmid DNA/well and 0.45 µl MetafecteneTM/well (Biontex, Martinsried, Germany) according to the manufacturer's protocol.

Cell surface expression

The cell surface expression was measured by an Enzyme-linked Immunosorbent Assay (ELISA) that detects cell surface proteins, which are N-terminally tagged with a peptide derived from the Influenza hemagglutinin glycoprotein (HA) (Figure 3). Cells were transfected with plasmids and metafectene and after 48 h, cell surface expression was analyzed. Cells were then washed, fixed with paraformaldehyde, and incubated with a biotin-labeled anti-HA antibody (Roche Applied Science, Mannheim, Germany). The bound anti-HA-biotin antibody was detected by using peroxidase-labeled streptavidin (BioLegend, London, UK). The peroxidase was used in a substrate/chromogen reaction as previously described[55]. The negative control was a C-terminally FLAG-tagged melanocortin-3 receptor (MC3R) not able to bind HA-antibodies. The positive control was an N-terminally HA-tagged MC3R. After first experiments, there was no detectable signal for GIPR constructs, however, the positive control showed expression. To rule out masking of the HA-tag due to the conformation of the N-terminal domain, we introduced a linker with eight additional glycine residues (GIPR-WT 8xGly), inserted directly after the HA tag in all examined GIPR variant

constructs. That increased flexibility and facilitated proper anti-HA-antibody binding. Subsequently, we were able to detect HA-tagged GIPR variants on the cell surface and measure cell surface expression. MC3R is highly expressed on the cell surface as shown by our group before and therefore served as positive control[56].

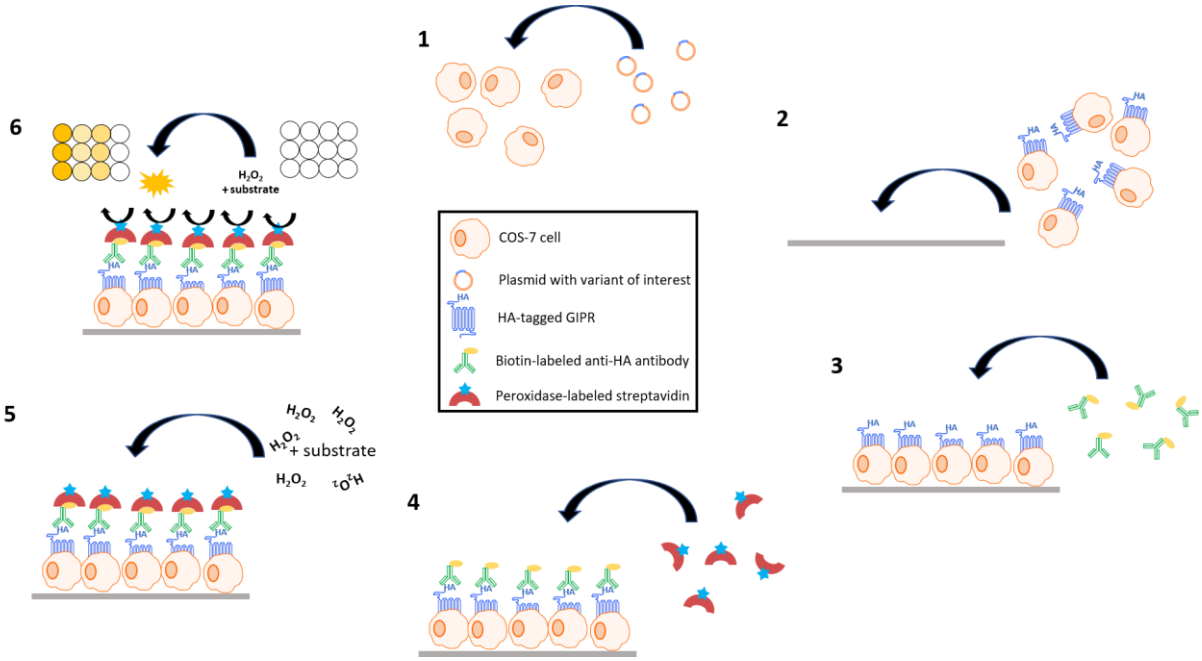


Figure 3: Measuring cell surface expression with ELISA

ELISA measuring HA-tags. Cells are transfected with the vectors containing the variants of interest (1). Cells express HA-tagged GIPR and are fixated (2). Biotin-labeled anti-HA-antibodies are added and bind to the HA-Tag of GIPR (3). Peroxidase-labeled Streptavidin is added and binds to biotin (4). After adding hydrogen peroxide, in a substrate chromogen-reaction the change of color is detected and cell surface expression quantified (5 and 6).

Receptor signaling measured by cAMP accumulation

cAMP accumulation was determined in wild-type GIPR and mutant GIPR transfected cells to measure Gs/adenylyl cyclase activation (Figure 4). Cells were exposed to human GIP in decadic concentrations from 1 pM to 1 µM for 40 min. Stimulation buffer contained 138 mM NaCl, 6 mM KCl, 1 mM $MgCl_2$, 5.5 mM glucose, 20 mM HEPES, 1 mM $CaCl_2$, and 1 mM 3-isobutyl-1-methylxanthine to inhibit cAMP degradation (IBMX, Sigma Aldrich, St. Louis, MO). As previously described in Fischer et al., cAMP accumulation was measured with AlphaScreen™ technology in a competitive assay (Perkin Elmer, Life Science, Zaventem, Belgium)[55].

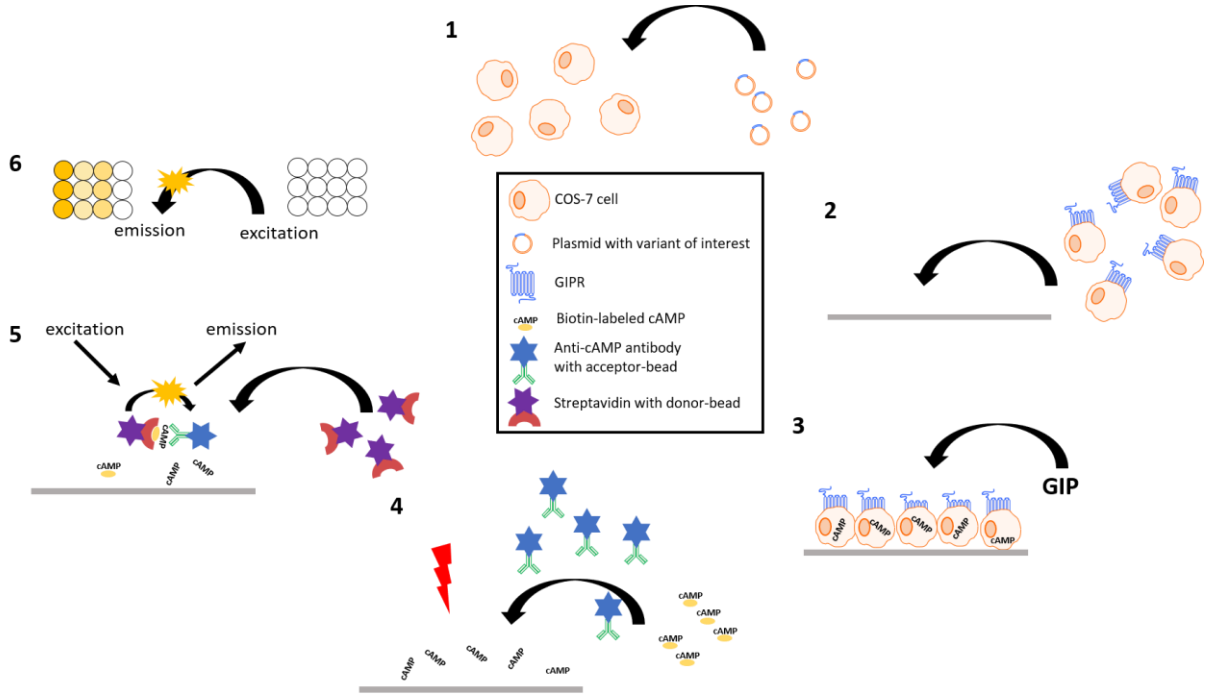


Figure 4: Alpha Screen Technology measuring intracellular cAMP production

COS-7 cells are transfected with the vectors containing the variants of interest (1). Cells express GIPR and are put in wells (2). Stimulation with GIP for 45 minutes in different concentrations is performed and cells accordingly produce endogenous cAMP (3). Cells are lysed and biotin-labeled cAMP and Anti-cAMP antibodies with acceptor-beads are added (4). Endogenous and biotinylated cAMP compete for binding with the acceptor-bead-lableled anti-cAMP antibody. Streptavidin with donor beads is added and binds to biotin-labeled cAMP (5). Using AlphaScreen™ Technology, light excitation is applied by 680nm wavelength and if donor and acceptor bead are in close proximity (when biotinylated cAMP is bound to the anti-cAMP-antibody), emission can be measured (6).

Structural modeling of the inactive GIPR conformation

All structural modeling was done in cooperation with G. Kleinau (Institut für Medizinische Physik und Biophysik, Group Protein X-ray Crystallography and Signal Transduction). The protein modeling of the human GIPR in an inactive state was created using the crystal structure of the transmembrane spanning domain of the glucagon receptor (GCGR). According to the sequence alignment of human GIPR and human GCGR the amino acids of the receptor-template were substituted and side chains and loops were adjusted. All model preparations were performed using Sybyl X2.0 software (Certara, NJ, US). More detailed information can be found in Jacobi et al.[51].

RESULTS

Study population 1: *GIPR* variants in obese and insulin-resistant children

In study population 1, we found the *GIPR v*ariants rs34125392, rs748182574, rs1800436, rs1800437, rs137944672, and rs12709891 (Table 3). No novel exonic *GIPR* variants were identified in our study population 1. Variant rs1800437, leading to, showed a similar minor allele frequency (MAF) of 0.168 in our study population 1 compared to reported global MAF 0.1611 (1000 Genomes Project[57]) and 0.2019 (Exome Aggregation Consortium[58]). For closer statistical separation and analysis of subgroups, the numbers of patients were not high enough. Rs1800437 (Glu354Gln) and rs1800436 (Ala207Val) were used for functional studies in subsequent experiments.

Study population 2: *GIPR* variants in patients with pediatric-onset diabetes of unknown origin

In study population 2, we identified the *GIPR* variant rs200485112, leading to the amino acid exchange Arg217Leu (Table 3). It was found heterozygous in one index patient, who is described in more detail below. To our knowledge, rs200485112 has never been detected in European cohorts, but is a known, however very rare variant in an East Asian population. In the East Asian (EAS) population, rs200485112 exhibits a MAF between 0.0198 (1000 Genomes Project) and 0.0118 (Exome Aggregation Consortium), whereas the minor allele of rs200485112 is not found in European (EUR), American (AMR), Africa (AFR), and South Asia (SAS). That leads to a global MAF of rs200485112 between 0.0009 (Exome Aggregation Consortium[58]) and 0.0040 (1000 Genomes Project[57]).

To date, Arg217Leu has not been further analyzed or functionally characterized in any studies. So far, there is no link or association known between the found variant Arg217Leu and obesity, insulin resistance, or diabetes.

Table 3: Minor allele frequencies (MAF) of *GIPR* variants in study populations

Minor allele frequencies (MAF) of study populations 1 and 2 for the found single nucleotide variants (SNV). rs200485112 (Arg217Leu), which was found heterozygous in the index patient in study population 2 (MAF = 0.0063), was not present in study population 1. rs1800437 was present in study population 1 (MAF = 0.1677) and in study population 2 (MAF = 0.2438). Table and table legend from Jacobi et al.[51]. Reprinted and modified with permission.

***GIPR* SNV** (reference ID)	rs34125392	rs748182574	rs1800436	**rs200485112**	rs1800437	rs137944672	rs12709891
Position of variant	UTR-5'	intron 4	exon 7	**exon 8**	exon 12	exon 12	UTR-3'
Nucleotide (Major/minor allele)	T/-	-/AGCACTTG GCCCACTGCGCAGT†	C/T	**G/T**	G/C	C/T	C/A
Amino acid exchanges			Ala207Val	**Arg217Leu**	Glu354Gln	silent mutation	
	Minor allele frequency (MAF)						
Study population 1 (n=164)	0.1951	0.0366	0.0061	**n. p.**	0.1677	0.0030	0.3232
Study population 2 (n=80)	0.2000	0.0188	n. p.	**0.0063**	0.2438	n. p.	0.3188
1000 Genomes Project	0.2428	n. a.	0.0012	**0.0040**	0.1611	0.0008	0.2602
Exome Aggregation Consortium	n. a.	n. a.	0.0032	**0.0009**	0.2019	0.0022	0.2970

n. p., minor allele not present in the study population; n. a.: not available; DIV, deletion insertion variant; UTR-3', Three prime untranslated region, UTR-5': Five prime untranslated region; † unknown ancestral allele.

GIPR variant Arg217Leu not present in the SHIP cohort

After finding rs200485112 in study population 2, we aimed to verify the absence of the minor allele in a German population, as stated by Exome Aggregation Consortium and 1000 Genomes Project. We analyzed the SHIP cohort, a large population-based study sample in Germany, for rs200485112. In 8,230 analyzed individuals, there was no minor allele of rs200485112 detectable. As a positive control, we also looked for the minor allele of rs1800437 (Glu354Gln), which was found at a frequency of 0.23 for the minor C allele (n = 8,229), verifying that otherwise there were *GIPR* variants present at a reasonable frequency.

Reduced cell surface expression of the GIPR variant Arg217Leu

In order to clarify the functional relevance, we investigated the cell surface expression and signaling effects of rs200485112 and other variants of interest in an overexpression cell culture model of COS-7 cells, which are established as a model for GPCR investigation[59–61]. *GIPR*-WT and the *GIPR* variants Ala207Val, Arg217Leu, and Glu354Gln were generated by site-directed mutagenesis. COS-7 were then transfected with the different plasmids and consecutively investigated for cell surface cell surface expression (Figure 5).

At first, the positive control N-HA-MC3R (black bar) was normally detected, whereas we were not able to observe cell surface expression of GIPR-WT (Figure 5A). After introducing eight glycine residues behind the ATG start codon (GIPR WT 8xGly) to prevent possible masking of the N-HA tag, which impedes binding of antibodies to the HA-tag, we were able to properly determine GIPR-WT. Following that, all mutant constructs contained the glycine (Gly) linker mentioned above. The introduction of Gly linker to proteins is a method that increases flexibility and simpler access of antibodies to the HA-tag [62].

Compared to the GIPR-WT, the surface expression of our variant of interest Arg217Leu (red bar) was reduced by about 50% (Figure 5A). For the Glu354Gln mutant (blue bar) we observed a 30% decrease compared to GIPR-WT. The cell surface expression of the Ala207Val variant (green bar) was not changed.

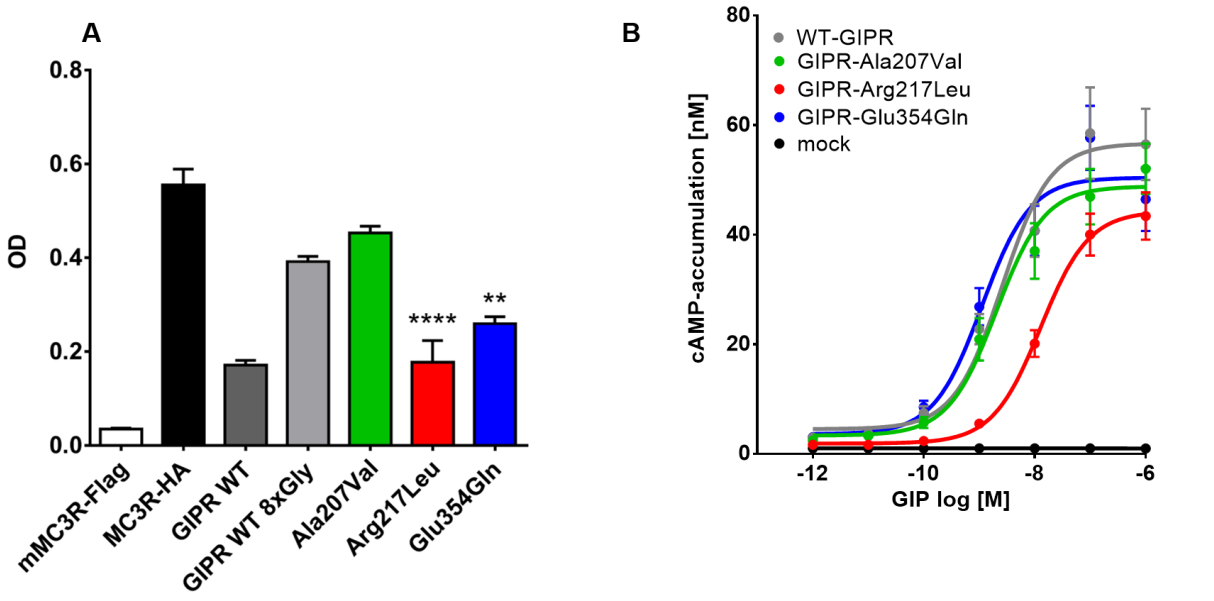


Figure 5: Functional characterization of the GIPR mutant Arg217Leu

A. COS-7 cells were transiently transfected with HA-tagged *GIPR*-WT, Ala207Val, Arg217Leu, and Glu354Gln. A C-terminally Flag-tagged MC3R and an N-terminally HA-tagged MC3R were used as negative and positive controls, respectively. To determine GIPR cell surface expression, eight additional glycine residues were inserted directly after the HA-tag (*GIPR*-WT 8×Gly). Cell surface ELISA was used for determining the N-terminal HA-tagged receptors. Cell surface receptor expression of the Arg217Leu and Glu354Gln variants measured as optical density (OD) significantly decreased relative to GIPR-WT 8×Gly. Data are expressed as mean ± SEM of four independent experiments performed in triplicate. One-way ANOVA was performed to compare the statistical significance between GIPR-WT 8×Gly and three variants, *** $P < 0.001$; **** $P < 0.0001$. **B.** For determining $G\alpha_s$ signaling, COS-7 cells were transiently transfected with GIPR-WT, Ala207Val, Arg217Leu, and Glu354Gln. Cells were stimulated with GIP (decadic concentration-response curves starting from 1 µM) and cAMP accumulation was measured with the AlphaScreen technology. Cells transfected with empty vector (mock) served as a negative control. In Arg217Leu variant-transfected cells, GIP potency shifted towards a higher ligand concentration relative to the GIPR-WT. Data are expressed as mean ± SEM of four independent experiments performed in triplicates. For statistical analysis, one-way ANOVA with the Kruskal-Wallis test for GIPR-WT was performed to test against all variants at all concentrations. There was no significant difference between Ala207Val and Glu354Gln compared to GIPR-WT. Arg217Leu was observed to differ from GIPT-WT at 10^{-12} M (* $P < 0.05$), at 10^{-11} M, 10^{-10} M and 10^{-8} M (** $P < 0.01$), at 10^{-9} M (**** $P < 0.0001$). Concentrations of 10^{-7} M and 10^{-6} M were not significant. Figure and figure legend from Jacobi et al.[51]. Reprinted and modified with permission.

The GIPR variant Arg217Leu reduces $G\alpha_s$ signaling

In a next step, the functional relevance of identified *GIPR* variants was investigated. COS-7 cells were transfected with GIPR-WT and other variants of interest. COS-7 cells were stimulated with increasing concentrations of GIP and the main signaling path of GIPR, $G\alpha_s$ signaling was investigated. GIPR variant Arg217Leu reduced the signaling

capacity and, in comparison to the GIPR-WT, GIP potency shifted towards a higher EC50 concentration (EC50 GIPR-WT: 2.5 × 10^{-9} M compared to GIPR variant Arg217Leu: 1.2 × 10^{-8} M) (Figure 5B). Both GIPR variants Glu354Gln or Ala207Val showed no significant change in GIP potency or efficacy. COS-7 cells transfected with an empty vector (mock) served as a negative control and did not result in an increase of intracellular cAMP after GIP challenge.

Amino acid Arg217 is of structural importance in wild-type GIPR

In cooperation with G. Kleinau, the alignment of the amino acid sequence of GIPR over class B GPCRs and a three-dimensional model of GIPR were created to further study GIPR variants regarding their relevance for the receptor structure. At position 217, the arginine residue is highly conserved among several class B GPCRs. In the three-dimensional structure, there is a likely interaction of the side chain of Arg217, located in the transmembrane helix (TMH) 3, with the backbone of the receptor's extracellular loop (ECL) 2 via hydrogen bonding. In the variant Arg217Leu, the substituted leucine at this particular position disturbs the hydrogen bonding and therefore the stabilizing contact between ECL2 and the TMH 3. The resulting instability could negatively impact the receptor function via changing structural features of the receptor. Details can be found in Jacobi et al.[51].

***GIPR* variant Arg217Leu index patient and family**

The rare *GIPR* variant Arg217Leu was found heterozygous in our index patient (Table 4). He is a 15-year-old Chinese male initially diagnosed with diabetes at the age of 15. Upon first evaluation, he was obese (BMI, 29.8 kg/m²; Z-score, +2.14) and presented with polyuria, polydipsia, and an elevated glycated hemoglobin HbA1c of 17.1%, which is a parameter for long-term glucose blood levels. Genetic investigation of the most frequent MODY genes (*GCK*, *HNF1A*, *HNF4A*, *HNF1B*, *ABCC8*, *KCNJ11*, *INS*) showed no pathogenic mutation[63]. To rule out type-1 diabetes, he was screened multiple times for autoantibodies, but always remained negative. Within the following months, the patient underwent treatment with insulin and managed to reduce his weight and the insulin dose was reduced, until stopping insulin therapy after a period of 18 months (BMI, 28.2 kg/m²; Z-score, +1.79). After finding the variant Arg217Leu, DNA of the family was screened for *GIPR* variant Arg217Leu. Both parents and two siblings (older brother and younger sister) were heterozygous for Arg217Leu. Other family members did not carry the minor allele (Table 4). In the screened family

members of the index patient, there was no coincidence of high HOMA-IR and the heterozygous Arg217Leu.

Table 4: Overview of family members of the index patient

The index patient (grey) with the heterozygous variant rs200485112 (Arg217Leu) is a 15-year old boy of Chinese descent. Table and table legend from Jacobi et al.[51] Modified and reprinted with permission.

	Clinical data			SNV
Relationship	**Diabetes**	**Year of birth**	**BMI – kg/m² (z-score)**	**rs200485112**
Father	Yes	1961	24.2 (+0.87)	G/**T (**Arg/Leu)
Mother	No	1963	24.9 (+1.19)	G/**T** (Arg/Leu)
Brother	No	1992	24.8 (+1.14)	G/**T** (Arg/Leu)
Index patient, male	Yes	1994	29.8 (+2.28)	G/**T** (Arg/Leu)
Sister†	No	1997	17.5 (-0.82)	G/G (Arg/Arg)
Brother†	No	1997	23.1 (+0.66)	G/G (Arg/Arg)
Sister	No	1998	16.0 (-1.00)	G/**T** (Arg/Leu)

† twins; major rs200485112 allele: G, minor allele: T.

New results of our study

(1) The rare *GIPR* variant rs200485112 leading to the amino acid changes Arg217Leu was identified in one of our patients. This variant has never been functionally characterized.

(2) Functional data showed a reduced cell surface expression for Arg217Leu and reduced $G\alpha_S$ signaling. That was supported by protein modeling of GIPR and introduction of Arg217Leu, which is at a highly conserved amino acid sequence important for receptor structure and function.

(3) We here found evidence that rare variants in the GIPR gene might predispose for disturbed glucose tolerance.

DISCUSSION

No new *GIPR* variants were detected in study population 1

In study population 1, which we selected based on both previously reported phenotypes that showed association with *GIPR* variants, namely obesity and increased HOMA-IR[24–26] no variants in addition to the already known ones were identified (Table 3). This could be due to various reasons, (1) possibly the study population was not big enough to find – potentially very rare – variants in *GIPR*; (2) due to the fact that even though Sauber et al. showed no association with obesity but only HOMA-IR, insulin resistance could be caused by obesity and therefore the chances to find variants are not increased by combining both phenotypes; (3) variants in the GIPR gene that are not located in exons, but in introns, influencing e.g. RNA splicing.

Activating or inactivating mutations in *GIPR*

In line with the arguments for both GIPR agonism or antagonism being beneficial for metabolic disease, it is difficult to define whether a variant in GIPR leads to decreased or increased GIPR activity. The *GIPR* variant Glu354Gln was identified as resulting in reduced GIPR activity and could therefore potentially lead to decreased glucose-dependent insulin secretion[25,26,49]. We would have expected a loss-of-function rather than a gain-of-function mutation in study population 1. In contrast, there are several studies suggesting, that inhibition of GIPR signaling could be beneficial in obesity prevention under e.g. high fat diet[35,38,39]. Accordingly, glucose homeostasis or obesity could be positively influenced by loss-of-function mutations. Gain-of-function mutations on the other hand are less frequent and could be masked in the case of GIPR. A gain-of-function phenotype in GIPR would potentially lead to concealed increased insulin secretion, because GIPR-dependent insulin secretion is only augmented dependent of glucose. Comparable to incretin mimetics used in drug therapy, no hypoglycemia as a key symptom would be present.

Since the discovery of the incretin effect, there have been efforts to target incretin receptors, especially as they have the pronounced benefit of not causing hypoglycemia (Table 1). In the case of GLP-1/GLP-1R, this was successful with the development of numerous compounds which made their way into clinical practice. With GIP/GIPR, so far there is no mono agonist or antagonist in clinical use. However, there are for some years now unimolecular compounds targeting both GIPR and GLP1R, so-called dual incretins[41,64]. Additionally, there has been the idea to combine also a agonistic part of

glucagon receptor in addition to both incretin receptors, so called triagonists[42,65]. All these compounds showed a promising effect on metabolic diseases, superior to the one seen for single receptor agonism, as e.g. in GLP-1R agonists[42]. When developing new incretin drugs, the numerous effects of GIP/GIPR in tissues have to be taken into consideration.

The GIPR variant Arg217Leu has a negative impact on receptor function

We described partial functional inactivation of the GIPR variant Arg271Leu. This is in contrast to the well-investigated GIPR variant Glu354Gln (rs1800437), which did not demonstrate an effect on GIP-induced cAMP accumulation in the COS-7 cell system used in this study, although cell surface expression was shown to be reduced[49]. To our knowledge, a structural integration of the amino acid exchange Glu354Gln in a three-dimensional model has not yet been done before. Investigating Glu354Gln on a protein level could help explain this discrepancy of strong association with the phenotype shown in GWAS[24–26], but mild functional impact *in vitro*[49,51]*.* In 2017, Zhang et al. published the resolved cryo-electron microscopy structure of the ligand-activated GLP-1R in 2017[66]. Due to the similarity of GLP-1R and GIPR, this first structure of a class B GPCR bound to its ligand could be used in the future to help modeling GIPR in more detail and increase the three-dimensional structural understanding, binding and signaling of the receptor.

Functional data and phenotype of the index patient and his family

The index family was of Chinese descent. Arg217Leu (rs200485112) is described to be rare in China with a minor allele frequency of 0.00179 in the large cohort of South Asian Genomes and Exomes (SAGE). That was due to one single individual, heterozygous for Arg217Leu[67]. No homozygous carrier of Arg217Leu has yet been described. This might be explained by the rarity or by an incompatibility of homozygous Arg271Leu with life. However, to date, that remains speculative, as even complete knockout of GIPR in mice is not lethal[35].

CONCLUSION AND OUTLOOK

For numerous years, the GLP-1/GLP-1R system was in the spotlight of incretin research, as it continues to serve as a target for effective drug therapy for metabolic diseases. GIP and GIPR have recently regained attention in drug research for obesity and type 2 diabetes[27]. Our study shows for the first time a potential functional association between the *GIPR* variant Arg217Leu and impaired GIPR signaling, displayed through functional data *in vitro* combined with protein homology modeling. G*IPR* variants might predispose to obesity and insulin resistance, but to which degree remains unclear to date. Our present study aimed to investigate study populations regarding their genetic variants in GIPR and study found variants regarding their potential impact on receptor function. Further studies are needed to place the results in a context:

(1) The investigation of larger study populations, comparable to the SHIP project, especially in the Asian population, could lead to a reliable prevalence of Arg217Leu in GIPR. If the total number of minor alleles of Arg217Leu is higher, association studies with BMI and HOMA-IR are feasible. In extended studies, potential homozygotic patients could be found and phenotyped. Interestingly, both parents in our index family were heterozygous carriers, but none of the five offspring was homozygous for Arg217Leu, which can be coincidence or a hint for a possible negative impact of homozygous Arg217Leu in e.g. development.

(2) The integration of all GIPR variants discovered so far into a three-dimensional receptor model could lead to a better understanding of the relevance of specific genetic variations. Combined with functional data from cell culture studies regarding signaling, surface expression, and ligand binding and phenotype characteristics of association studies, these models could help to increase the translation from judging a variation purely as an amino acid change in DNA up to clinical relevance. All data input could via constant reapproval and adjustment increase the integrity of the model.

(3) It was shown that GIPR and GLP-1R are able to form dimers, meaning two receptors – of two times the same protein or two different receptors – interact and thereby might lead to changed receptor functionality[68]. This effect called homo- and heterodimerization might play an important role in

(patho)physiology of GPCRs. Heterozygous variants in *GIPR* could be compensated via that mechanism by the *GIPR* wild-type allele and by GLP-1R. Additionally, a non-functional GIPR receptor could also exert a negative effect on other, per se functional, receptor proteins. These problems should be further examined, to better understand if genetic variants in different receptors – in our case the incretin receptors – could add up to show functional relevance.

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Eidesstattliche Erklärung

„Ich, Simon Friedrich Jacobi, versichere an Eides statt durch meine eigenhändige Unterschrift, dass ich die vorgelegte Dissertation mit dem Thema: „Evaluation of a Rare Glucose-Dependent Insulinotropic Polypeptide Receptor Variant in a Patient with Diabetes“ selbstständig und ohne nicht offengelegte Hilfe Dritter verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel genutzt habe.

Alle Stellen, die wörtlich oder dem Sinne nach auf Publikationen oder Vorträgen anderer Autoren/innen beruhen, sind als solche in korrekter Zitierung kenntlich gemacht. Die Abschnitte zu Methodik (insbesondere praktische Arbeiten, Laborbestimmungen, statistische Aufarbeitung) und Resultaten (insbesondere Abbildungen, Graphiken und Tabellen) werden von mir verantwortet.

Ich versichere ferner, dass ich die in Zusammenarbeit mit anderen Personen generierten Daten, Datenauswertungen und Schlussfolgerungen korrekt gekennzeichnet und meinen eigenen Beitrag sowie die Beiträge anderer Personen korrekt kenntlich gemacht habe (siehe Anteilserklärung). Texte oder Textteile, die gemeinsam mit anderen erstellt oder verwendet wurden, habe ich korrekt kenntlich gemacht.

Meine Anteile an etwaigen Publikationen zu dieser Dissertation entsprechen denen, die in der untenstehenden gemeinsamen Erklärung mit dem/der Erstbetreuer/in, angegeben sind. Für sämtliche im Rahmen der Dissertation entstandenen Publikationen wurden die Richtlinien des ICMJE (International Committee of Medical Journal Editors; www.icmje.og) zur Autorenschaft eingehalten. Ich erkläre ferner, dass ich mich zur Einhaltung der Satzung der Charité – Universitätsmedizin Berlin zur Sicherung Guter Wissenschaftlicher Praxis verpflichte.

Weiterhin versichere ich, dass ich diese Dissertation weder in gleicher noch in ähnlicher Form bereits an einer anderen Fakultät eingereicht habe.

Die Bedeutung dieser eidesstattlichen Versicherung und die strafrechtlichen Folgen einer unwahren eidesstattlichen Versicherung (§§156, 161 des Strafgesetzbuches) sind mir bekannt und bewusst.“

Datum Unterschrift

Ausführliche Anteilserklärung an der erfolgten Publikation

Publikation:

Jacobi, S.F., Khajavi, N., Kleinau, G., Teumer, A., Scheerer, P., Homuth, G., Völzke, H., Wiegand, S., Kühnen, P., Krude, H., Biebermann, H. (2019). Evaluation of a rare glucose-dependent insulinotropic polypeptide receptor variant in a patient with diabetes. Diabetes Obes Metab

Eigener Beitrag von Jacobi, SF zur Publikation im Einzelnen:

- Planung und Konzeption der Studie, Literaturrecherche zum Thema GIPR (zusammen mit Biebermann, H)
- Aussuchen der Study Population 1
- Datenauswertung für und Erstellen von Figure 1 und Supplementary Table 1
- Aussuchen der Study Population 2 (Hauptanteil, zusammen mit Raile, K und Biebermann, H)
- Datenauswertung für und Erstellen von Table 1 und Table 2
- Extraktion der DNA aus EDTA-Blut für Study Population 1 und 2
- Etablierung der PCR inklusive Design der Primer für *GIPR* zum Screening der Study Population 1 und 2
- Vorversuche der funktionellen Untersuchungen (Hauptanteil, zusammen mit Khajavi, N und Biebermann, H, Daten nicht in Paper)
- Klonierung der Funktionelle Assays und daraus Daten für Figure 2 A und B (zusammen mit Khajavi, N und Biebermann, H)
- Interpretation der Daten und Diskussion der Ergebnisse des Screenings, der funktionellen Daten (Hauptanteil, zusammen mit Biebermann, H)
- Interpretation der Daten aus der SHIP-Kohorte sowie der Ergebnisse des GIPR-Modelings (zusammen mit den Co-Autoren)
- Konzeption und Entwurf des Manuskripts (Hauptanteil, zusammen mit Biebermann, H)
- Schreiben des Manuskripts (Hauptanteil, zusammen mit Co-Autoren)
- Erstellen der Revisionen (Hauptanteil, zusammen mit Co-Autoren)

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Unterschrift, Datum und Stempel der erstbetreuenden Hochschullehrerin

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Unterschrift des Doktoranden

Auszug aus der Journal Summary List (ISI Web of Knowledge)

Journal Data Filtered By: **Selected JCR Year: 2017** Selected Editions: SCIE,SSCI
Selected Categories: **"ENDOCRINOLOGY and METABOLISM"**
Selected Category Scheme: WoS
Gesamtanzahl: 143 Journale

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
1	Cell Metabolism	29,834	20.565	0.101740
2	Nature Reviews Endocrinology	7,377	20.265	0.025190
3	Lancet Diabetes & Endocrinology	5,549	19.313	0.029810
4	ENDOCRINE REVIEWS	12,975	15.545	0.009920
5	DIABETES CARE	67,278	13.397	0.110140
6	JOURNAL OF PINEAL RESEARCH	9,079	11.613	0.008600
7	TRENDS IN ENDOCRINOLOGY AND METABOLISM	8,362	10.769	0.019900
8	Obesity Reviews	10,241	8.483	0.023840
9	THYROID	11,570	7.557	0.020730
10	DIABETES	53,645	7.273	0.074690
11	FRONTIERS IN NEUROENDOCRINOLOGY	3,924	6.875	0.006040
12	ANTIOXIDANTS & REDOX SIGNALING	19,324	6.530	0.032120
13	JOURNAL OF BONE AND MINERAL RESEARCH	26,804	6.314	0.036920
14	Molecular Metabolism	2,403	6.291	0.012250
15	JOURNAL OF CEREBRAL BLOOD FLOW AND METABOLISM	19,450	6.045	0.028280
16	DIABETOLOGIA	28,810	6.023	0.048370
17	FREE RADICAL BIOLOGY AND MEDICINE	40,089	6.020	0.043060
18	DIABETES OBESITY & METABOLISM	8,680	5.980	0.022060
19	METABOLISM-CLINICAL AND EXPERIMENTAL	13,117	5.963	0.016570
20	JOURNAL OF CLINICAL ENDOCRINOLOGY & METABOLISM	78,047	5.789	0.104690
21	ENDOCRINE-RELATED CANCER	7,114	5.331	0.012410
22	Cardiovascular Diabetology	4,796	5.235	0.011190
23	INTERNATIONAL JOURNAL OF OBESITY	22,185	5.151	0.032040

Druckexemplar der ausgewählten Publikation

Jacobi SF, Khajavi N, Kleinau G, Teumer A, Scheerer P, Homuth G, Völzke H, Wiegand S, Kühnen P, Krude H, Gong M, Raile K, Biebermann H. Evaluation of a rare glucose-dependent insulinotropic polypeptide receptor variant in a patient with diabetes. *Diabetes Obes Metab*. 2019;21(5):1168-1176.
https://doi.org/10.1111/dom.13634

Lebenslauf

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

Vollständige Publikationsliste

Jacobi SF, Khajavi N, Kleinau G, Teumer A, Scheerer P, Homuth G, Völzke H, Wiegand S, Kühnen P, Krude H, Gong M, Raile K, Biebermann H. Evaluation of a rare glucose-dependent insulinotropic polypeptide receptor variant in a patient with diabetes. *Diabetes Obes Metab.* February 2019. doi:10.1111/dom.13634 Impact Factor: 6.72

Schanze N, **Jacobi SF**, Rijntjes E, Mergler S, Del Olmo M, Hoefig CS, Khajavi N, Lehmphul I, Biebermann H, Mittag J, Köhrle J. 3-Iodothyronamine Decreases Expression of Genes Involved in Iodide Metabolism in Mouse Thyroids and Inhibits Iodide Uptake in PCCL3 Thyrocytes. *Thyroid.* 2017;27(1):11-22. doi:10.1089/thy.2016.0182 Impact Factor: 5.52

Mühlhaus J, Dinter J, Jyrch S, Teumer A, **Jacobi SF**, Homuth G, Kühnen P, Wiegand S, Grüters A, Völzke H, Raile K, Kleinau G, Krude H, Biebermann H. Investigation of Naturally Occurring Single-Nucleotide Variants in Human TAAR1. *Front Pharmacol.* 2017;8:807. doi:10.3389/fphar.2017.00807 Impact Factor: 3.68

Dinter J, Mühlhaus J, **Jacobi SF**, Wienchol CL, Cöster M, Meister J, Hoefig CS, Müller A, Köhrle J, Grüters A, Krude H, Mittag J, Schöneberg T, Kleinau G, Biebermann H. 3-iodothyronamine differentially modulates α-2A-adrenergic receptor-mediated signaling. *J Mol Endocrinol.* 2015;54(3):205-216. doi:10.1530/JME-15-0003 Impact Factor: 3.58

Hoefig CS, **Jacobi SF**, Warner A, Harder L, Schanze N, Vennström B, Mittag J. 3-Iodothyroacetic acid lacks thermoregulatory and cardiovascular effects in vivo. *Br J Pharmacol.* 2015;172(13):3426-3433. doi:10.1111/bph.13131 Impact Factor: 6.81

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