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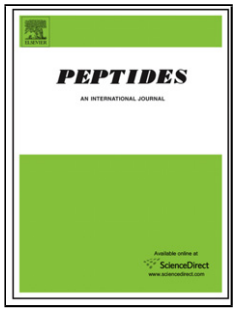

Predictive effect of GIPR SNP rs10423928 on glucose metabolism liver fat and adiposity in prediabetic and diabetic subjects

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Predictive effect of GIPR SNP rs10423928 on glucose metabolism liver fat and adiposity in prediabetic and diabetic subjects.

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Highlights

- A allele carriers of GIPR rs10423928 variant have lower glucose levels 2 hr after an oral glucose challenge.
- A allele contributes to a higher insulin sensitivity indicating an enhanced ß-cell response.
- GIPR rs10423928 variant is a promising target for therapeutic interventions involved in type 2 diabetes.

Introduction

Incretins mediate a major part of the food dependent secretion of insulin constituting the entero-insular axis [1-3]. Both, glucose-dependent insulinotropic peptide (GIP) and glucagon-like peptide-1 (GLP-1) exert their food-dependent insulin secretory effects by binding to their specific receptors on beta-cells, the GIP receptor (GIPR) [4, 5] and the GLP-1 receptor (GLP-1R) [6], respectively, triggering the exocytosis of insulin granules [7]. However, their function surpasses insulinotropic effects. Both hormones play critical roles in various biological processes in different tissues and organs that express their receptors [8]. In brief, GIP and GLP-1 together promote ß-cell proliferation and inhibit apoptosis. Interestingly GLP-1 inhibits the postprandial

glucagon response while GIP enhances it. GIP also facilitates fat deposition in adipose tissue, promotes bone formation and together with GLP-1 it may be involved in memory development and appetite control [2]. While the function of GLP-1 has been widely studied and is used pharmacologically in the treatment of T2DM [2], the physiological function of GIP remains unclear.

Consequences of disruption of GIPR signaling

Genetic deletion of GIPR in mice showed its critical role in the development of obesity, hepatic steatosis and insulin resistance [9-11]. Mice lacking GIPR and exposed to an obesogenic environment (ovariectomy, high-fat diet or sucrose-rich diet) do not exhibit body weight gain, liver steatosis nor an increase in visceral and subcutaneous fat mass compared to wild type (WT) mice. GIP signaling stimulates glucose uptake and free fatty acids (FFA) re-esterification in fat cells[12] and upregulates the lipoprotein lipase (LPL) gene [13]. All these factors contribute to the fat-accumulating effect of GIP. In fact, GIPR antagonists have been proposed as a strategy to prevent and even treat type 2 diabetes mellitus (T2DM) and adiposity [14]. An in vivo study suggested that deleting GIPR specifically in adipose tissue reduces IL-6 plasma levels which may be in part responsible for the protective effect against insulin resistance and hepatic steatosis of interrupting GIP-GIPR interactions [15].

In humans GIP seems to exert its adipogenic effect through the augmentation of blood flow in adipose tissue, glucose uptake and FFA re-esterification [16]. Furthermore, reducing ligand-receptor interaction by lowering the endogenous GIP secretion was shown to be effective for treating non-alcoholic fatty liver disease (NAFLD) [11] and

insulin resistance [17]. In support of this, genome-wide association studies (GWAS)-analysis have extended the knowledge associating GIPR variants and T2DM and co-morbidities [18].

GIPR Single Nucleotide Polymorphisms

The human GIPR gene, localized to chromosome 19, comprises 14 exons with a size of circa 14 kb [5]. Within the GIPR gene, 13 single nucleotide polymorphisms (SNP) have been described. SNP rs10423928 of GIPR is located within a noncoding region. It is well known, that intronic gene variants can affect gene splicing, transcription and translation, shifting gene expression [19]. This particular SNP rs10423928 consists of the exchange of thymine by an adenine base (T/A). The functional consequence of this base conversion was shown to be deeply involved in glucose and insulin response. The minor allele A has been repeatedly described as the risk allele in non-diabetic individuals due to its associations with increased 2-h glucose, fasting proinsulin levels, and lower ß-cell function [18, 20, 21]. However, the A allele was also linked to impaired glucose- and GIP-stimulated insulin secretion and a decrease in BMI, lean body mass, and waist circumference in T2DM and non-T2DM subjects [22]. On the other hand carriers of the A allele variant also showed better insulin sensitivity [23].

Aim

Although evidence suggests beneficial and protective effects of reducing the GIP signal pathway, human studies of GIPR variants are missing. Therefore, the aim of this investigation was to examine the effect of SNP rs10423928 on several variables embedded in the T2DM dynamic network. We addressed the following questions: Are

A-allele carriers protected against insulin resistance, NAFLD and adiposity? Can current evidence be translated from healthy to prediabetic and T2DM subjects?

Materials and Methods

Participants and study design

In order to analyze the influence of GIPR rs10423928 A-allele on glucose metabolism, insulin sensitivity and fat accumulation, we collected cross-sectional and interventional data from four different dietary intervention studies.

For the cross-sectional analysis, baseline data of prediabetic subjects were obtained from two intervention trials, "Diabetes Nutrition Algorithms in PREDIABETIC (DiNA-P)" registered at clinicaltrials.gov as NCT 02609243 and the "Optimal Fibre Trial for Diabetes Prevention (OptiFiT)" as NCT 01681173. Complete details of DiNA-P and OptiFiT have been described elsewhere [24, 25]. Both studies were conducted in accordance with the Declaration of Helsinki and approved by the ethics committee of the University of Potsdam. All subjects provided written informed consent for their participation in the study. In total of 424 prediabetic subjects were included ($n_{DiNA\text{-}P}$ = 262, $n_{OptiFiT}$ = 162).

In addition, baseline data of 73 T2DM subjects were extracted from the DiNA-D (Diabetes Nutrition Algorithms in Patients With Overt Diabetes Mellitus) study [26] and LeguAN (Leguminosen – Anbau und Nutzung) study [27] (nDiNA-D = 42, nLeguAN = 31). Studies were registered at www.ClinicalTrials:gov as NCT02459496 and NCT02402985, respectively. Following the approval of the Ethics Committee of the Charité and of the University of Potsdam, conducted in accordance with the

Declaration of Helsinki.

Genotyping

Genomic DNA was isolated from buffy coat, whole blood or serum, depending on sample availability. Genotyping was performed by ViiA™ 7 System using TaqMan™ SNP Genotyping Assay rs10423928 ID C_30103605_10 (Applied Biosystems, Thermo Fisher Scientific).

Measurement of glucose metabolism and insulin sensitivity

2h glucose challenge (oGTT) was measured after a 12 h fast. Blood samples were taken before and at 30, 60, 90 and 120min to measure glucose, insulin and C-Peptide. Capillary blood glucose concentrations were measured immediately using the glucose oxidase method (Super-GL glucose analyzer; Dr Müller Gerätebau, Freital, Germany); venous serum blood samples were analyzed batch-wise after storage (Horiba ABX Pentra 400, Montpellier, France). Serum insulin and C-Peptide were measured using an ELISA technique (Mercodia, Uppsala, Sweden).

In order to study the ability to regulate blood glucose in T2DM subjects from DiNA-D study, the glycemic profile in response to the intake of a liquid formula mixed meal (BOOST® High Protein Complete Nutritional Drink Very Vanilla, Nestlé Nutrition., Vevey, Switzerland) was measured (Meal Tolerance Test (MTT)). Test meal was standardized to 360 mL (378 g). The standard test meal (360 mL) contained 365 kcal, 9 g fat, 50 g carbohydrates and 23 g protein. Serial sampling for analytes was performed 0, 30, 60, 90, and 120 min post meal. MTT performed in the LeguAN study is described elsewhere [3].

For the current analysis we included glucose values at baseline, 30, 60, 90 and 120 min after meal intake. For insulin and C-Peptide we included measurements at baseline 60 and 120 min after meal intake.

For the description of insulin sensitivity / resistance, common indices that are based on fasting parameters or describe the glycemic status under dynamic conditions were used. Fating and dynamic indices were calculated using oGTT or MTT data:

HOMA IR : $\frac{Ins_0 \frac{\mu U}{mL} \times Glc_0 \frac{mmol}{L}}{22.5}$ [28]

Stumvoll Index: $0.226 - \left(0.0032 \times BMI \frac{kg}{m^2}\right) - \left(0.0000645 \times Ins_{120} \frac{\mu U}{mL}\right) - (0.00375 \times Glc_{90} \frac{mmol}{L})$ [29]

Cederholm index : $\frac{(75000 + (Glc\_0 - Glc\_120) mmol/L \times 1.15 \times 180 \times 0.19 \times WEI)}{120\ x\ Glc_{0-120} \frac{mg}{dL} \times log\left(Ins_{0-120} \frac{\mu U}{mL}\right)}$ [30]

Measurements of adipose tissue accumulation

Calculation and analysis of total adipose tissue content (TAT), visceral adipose tissue (VAT) from MR images was performed by an automatic segmentation procedure based on fuzzy clustering and orthonormal snakes [31]. Intrahepatic lipids (IHL) were measured and quantified using a 1.5 T Eclipse multinuclear system (Philips Medical Systems, Cleveland, Ohio). Liver images were analyzed using an image segmentation software program in which liver contours were manually drawn for each slice as described previously [27].

Measurement of anthropometric and clinical parameters

Trained staff determined anthropometric variables with calibrated scales. Body mass index (BMI) was calculated as the weight in kilograms divided by the height in meters squared. Levels of glycated Hemoglobin A1c (HbA1c) were determined in fasting state

and were measured in serum/plasma by with ABX Pentra 400 (Horiba, Japan).

Statistical analysis

Hardy Weinberg equilibrium for GIPR genotype was calculated by Pearson's $\chi 2$ test to compare the genotype and allele frequencies within prediabetic and T2DM subjects using Microsoft® Excel ®, 2016.

Main statistical analysis was conducted using IBM SPSS for Windows (version 20.0, Chicago, USA). For comparison between homozygous carriers of major allele T (HMA) and A allele carriers (heterozygous T/A (HET) and homozygous for the minor allele A (HMI)) we used one-way analysis of variance (ANOVA) with genotype (HMA vs HET, HMI) as between-subject factor. Additionally, responses to oGTT and MTT were analyzed by repeated-measures ANOVA. ANOVA models for MTT analysis were adjusted for clinical study as possible confounder. P-value of less than 0.05 was considered significant.

Results

Genotype and allele frequencies of the prediabetic and T2DM cohorts are summarized below in Table 1

Glucose metabolism and insulin sensitivity in prediabetic subjects

Baseline data of 424 prediabetic subjects (263 females, 161 males) were analyzed. Within this cohort the major Allele T appeared 44% more often than allele A. HMI A frequency accounted for 5% (n=21) of the study population, while HET A/T to 46% (n=195).

Participants were on average 60 years old (range: 29 - 81 y), were mostly overweight to mildly obese (mean BMI= 31.6 ± 5.6 kg/m2) with a mean HbA1c level of 5.7 ± 0.4 %. Table 2 displays results of glucose metabolism and insulin sensitivity. Carriers of the A allele (HET, HMI)) showed significantly increased fasting glucose (p=0.015) and lower glucose levels 2 h (p = 0.021) after an oral glucose challenge compared to T/T homozygous individuals. ANOVA for repeated measurements showed no significant effect of rs10423928 variant for glucose, insulin nor C-Peptide. Interestingly, A-carriers showed significantly higher Cederholm Index value (p<0.001).

Glucose metabolism and insulin sensitivity in T2DM subjects

Baseline data of 73 diabetic subjects (35 females, 37 males) were investigated. Table 1 shows that 28% (n=3) of the T2DM subject carry the minor allele A, while allele T showed a frequency of 78% (n=70).

A total of 73 T2DM subjects aged between 46-76 years were genotyped and analyzed. Individuals had a mean HbA1c level of 6.7% and a mean BMI of 32.1 kg/m^2. We found no significant differences between rs10423928 variant carriers (HMA vs HET &HMI) regarding glucose metabolism and insulin sensitivity parameters analyzed as separate variables (Table 3). Similarly, after running ANOVA for repeated measurements no significant between-subjects effects for glucose, insulin or C-Peptide was found.

Total, visceral and liver fat accumulation in prediabetic and T2DM subjects

Content of body fat or liver fat measured by magnetic resonance imaging and spectroscopy did not differ between HAM and A-carriers, either in the prediabetic nor

in the T2DM cohort as shown in table 4.

Discussion

The current study provides interesting insights into the role of GIPR rs10423928 variant in glucose metabolism. The comparison of A allele carriers with non-carriers show that prediabetic A allele carriers have increased fasting glucose, but lower glucose levels 2 h after an oral glucose challenge and higher insulin sensitivity, indicating an improved ß-cell response compared to homozygous subjects for the T allele. The lack of effect in the T2DM cohort could be attributed to the medication for management of blood glucose and/or the smaller sample size. However, selection bias should be taken into consideration, since healthier subjects were omitted of the study and/or diabetics with very high glucose levels and insulin therapy were not included. Accordingly, a study showed that carriers of the A allele have better insulin sensitivity perhaps attributed to lower osteopontin (OPN), a cytokine, which is specifically abundant in adipose tissue of obese individuals. GIP/GIPR signaling stimulates OPN expression promoting adipose tissue inflammation and insulin resistance [23]. A recent rodent study demonstrated that the inhibition of GIPR signaling in adipose tissue reduced hepatic steatosis and enhanced insulin sensitivity in high-fat fed mice. This effect was also attributed in part to the reduction of a proinflammatory cytokine, interleukin-6 (IL-6) [15].

Furthermore, GIPR antagonists studies also demonstrated the protective effect of GIP/GIPR signal interruption. Blockade of GIPR action has been newly extensively reviewed elsewhere [32] highlighting its effect against insulin resistance and obesity-

induced T2DM. Similarly, mice lacking GIPR exposed to an obesogenic environment (high-fat diet or sucrose-rich diet) did not show increased body weight, visceral, subcutaneous or liver fat content nor insulin resistance compared with wild type mice [10, 11].

Although it is not clear yet the functional consequence of GIPR rs10423928 base conversion, it may contribute to a diminished receptor signaling leading to improved insulin sensitivity.

In view of the direct impact of nutrition on endogenous GIP release, genetic variants in GIPR are excellent candidates of potential diet-gene interactions. Interestingly, dietary intake of carbohydrate and fat could potentially modulate the T2DM risk depending on the GIPR genotype similar to the GIPR knockout mice models. Homozygous for AA, non-diabetic subjects consuming high-fat, low-carbohydrate diets showed reduced risk. On the contrary, T/T genotype showed lower T2DM risk consuming a high-carbohydrate/ low-fat diet [33]. Taking this into consideration, macronutrients intake of study participants may have led to bias in the current analysis. Genotype-diet interactions could determine the susceptibility to impaired glucose metabolism and insulin sensitivity in response for instance to a high-fat diet. Definition of GIPR SNP rs10423928 -diet interaction effects is therefore essential in for better understanding of the impact of the variant. Therefore, cross-sectional or interventions evaluating dietary intake are needed.

The impact of GIPR SNP rs10423928 variant in glucose metabolism and insulin sensitivity is still a matter of speculation. Saxena et al., [18] showed a link between

GIPR A allele variant and T2DM. Another study in T2DM and non-diabetic subjects associated the A allele of GIPR rs10423928 with impaired glucose- and GIP-stimulated insulin secretion [22].

In conclusion, our results suggest an advantageous effect of GIPR SNP rs10423928 minor allele A in prediabetic subjects contributing to an improved ß-cell response. Understanding the role of GIPR rs10423928 might lead to individualized approaches to treat and prevent T2DM and co-morbidities.

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Disclosure Statement: The authors have nothing to disclose

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Tables, and figure legends

Table 1 Genotypic and allelic frequencies within the cohorts.

rs10423928	Genotype			Allele		p-value
	HMA	HET	HMI	AF	(%)	
Prediabetic (n)	208	195	21	T	72	**0.004**
(%)	49	46	5	A	28	
T2DM (n)	35	35	3	T	72	ns
(%)	48	48	4	A	28	

Table 2 Baseline Glucose metabolism and insulin sensitivity within the prediabetic cohort.

	HMA			HET / HMI			p-value
	n	Mean	± SD	n	Mean	± SD	
Weight [kg]	208	89.5	18.6	216	89.3	18.1	ns
BMI [kg/m²]	208	31.7	5.9	216	31.4	5.7	ns
Glucose Metabolism Parameters							
Hba1c [%]	197	5.7	0.4	201	5.6	0.4	ns
Fasting Glucose [mg/dL]	208	99.1	11.9	216	102.0	12.3	**0.015**
Glucose 30 [mg/dL]	206	177.2	25.8	213	179.1	29.2	ns
Glucose 60 [mg/dL]	208	196.5	35.8	216	192.2	40.3	ns
Glucose 90 [mg/dL]	206	176.9	36.3	211	171.8	41.9	ns
Glucose 120 [mg/dL]	208	150.0	28.3	216	143.5	29.6	**0.021**
Fasting insulin [mU/l]	168	10.0	6.6	171	9.4	5.2	ns
Insulin 60 [mU/l]	169	93.6	64.6	171	102.4	81.5	ns
Insulin 120 [mU/l]	169	94.6	72.1	171	99.3	109.3	ns
Fasting C-Peptid [µg/l]	154	1.8	1.1	168	1.6	0.8	ns
C-Peptid 60 [µg/l]	155	7.5	3.3	168	8.0	3.8	ns
C-Peptid 120 [µg/l]	155	8.6	4.0	168	8.7	4.1	ns
Insulin Sensitivity Indices							
HOMA IR	168	2.5	1.8	171	2.4	1.4	ns
Matsuda Index	105	6.6	3.8	142	6.0	3.9	ns
Disposition Index	105	934.6	932.8	142	974.9	1130.6	ns
Stumvoll Index	167	-0.6	0.2	166	-0.6	0.2	ns
Cederholm index	208	611.9	8.8	216	614.8	8.7	**<0.001**

Table 3 Baseline Glucose metabolism and insulin sensitivity within the T2DM cohort.

	HMA			HET / HMI			p-value
	n	Mean	± SD	n	Mean	± SD	
Weight [kg]	35	91.3	14.3	38	95.7	18.3	ns
BMI [kg/m^2]	35	31.8	4.3	38	32.5	4.8	ns
Glucose Metabolism Parameters							
Hba1c [%]	35	6.8	1.1	38	6.6	0.7	ns
Fasting Glucose [mg/dl]	35	133.4	47.2	36	131.9	42.8	ns
Glucose 30 min [mg/dl]	34	176.4	54.0	33	169.6	50.0	ns
Glucose 60 [mg/dl]	35	193.1	62.2	35	181.7	53.2	ns
Glucose 90 [mg/dl]	35	168.6	65.6	35	171.7	62.7	ns
Glucose 120 [mg/dl]	34	181.2	63.4	33	180.3	63.0	ns
Fasting insulin [mU/l]	33	7.3	3.7	36	7.7	5.0	ns
Insulin 60 [mU/l]	33	66.7	47.1	36	62.7	46.6	ns
Insulin 120 [mU/l]	33	51.1	41.4	34	43.7	37.0	ns
Fasting C-Peptid [µg/l]	33	1.8	0.9	36	1.9	1.0	ns
C-Peptide 60 [µg/l]	33	3.9	2.3	36	3.6	2.4	ns
C-Peptide 120 [µg/l]	33	5.4	2.1	35	5.1	2.6	ns
Insulin Sensitivity Indices							
HOMA IR	33	46.6	34.0	36	44.3	30.4	ns
Stumvoll Index	32	-0.6	0.2	32	-0.6	0.2	ns
Cederholm index	35	615.8	8.6	35	614.2	8.9	ns

Table 4. Adipose tissue distribution and blood lipids within the prediabetic and T2DM cohort.

	HMA			HET / HMI			p-value
	n	Mean	± SD	n	Mean	± SD	
Prediabetic							
TAT [l]	106	20.2	7.6	153	20.7	7.1	ns
VAT [l]	107	4.9	2.1	153	5.2	2.2	ns
IHL [%]	107	10.2	13.6	151	9.3	7.8	ns
T2DM							
TAT [l]	33	21.0	6.0	35	22.0	5.6	ns
VAT [l]	33	6.3	2.3	35	5.8	2.8	ns
IHL [%]	33	14.2	9.2	34	12.9	10.2	ns

Tables, and figure legends

Table 1 Genotypic and allelic frequencies within the cohorts. A. Values are presented as frequencies and percentage. HMA: Homozygous major allele (T/T), HET: Heterozygous (A/T). HMI: Homozygous minor allele (A/A). AF: Allelic frequency. P value for HWE

Table 2 Baseline Glucose metabolism and insulin sensitivity within the prediabetic cohort. Values are presented as mean (±SD), p-values of one-way analysis of variance (ANOVA) with genotype as the between-subject factor. Difference significant at $p < 0.05$. HMA: Homozygous major allele (T/T), HET: Heterozygous (A/T), HMI: Homozygous minor allele (A/A). BMI =body mass index, HbA1c = glycated hemoglobin, HOMA IR = Homeostasis model assessment.

Table 3 Baseline Glucose metabolism and insulin sensitivity within the T2DM cohort. Values are presented as mean (±SD), p-values of one-way analysis of variance (ANOVA) with genotype as the between-subject factor. MTT analysis were adjusted for clinical study. Difference significant at $p < 0.05$. HMA: Homozygous major allele (T/T), HET: Heterozygous (A/T), HMI: Homozygous minor allele (A/A). BMI =body mass index, HbA1c = glycated hemoglobin, HOMA IR = Homeostasis model assessment.

Table 4. Baseline fat deposition and blood lipids within the prediabetic and T2DM cohort. Values are presented as mean (±SD), one-way analysis of variance (ANOVA) with genotype as the between-subject factor. Difference significant at $p < 0.05$. HMA: Homozygous major allele (T/T), HET: Heterozygous (A/T), HMI: Homozygous minor allele (A/A). TAT = total body fat, VAT = visceral adipose tissue, IHL = intra hepatic lipids,