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Research Article

Association analyses of GIP and GIPR polymorphisms with traits of the metabolic syndrome

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Glucose-dependent insulinotropic polypeptide (GIP) stimulates insulin release *via* interaction with its pancreatic receptor (GIP receptor (GIPR)). GIP also acts as vasoactive protein. To investigate whether variations in GIP and GIPR genes are associated with risk factors of the metabolic syndrome we sequenced gene regions and identified two coding SNPs (GIP Ser103Gly, GIPR Glu354Gln) and one splice site SNP (GIP rs2291726) in 47 subjects. Interestingly, *in silico* analyses revealed that splice site SNP rs2291726 results in a truncated protein and classified GIPR variant Glu354Gln as a functional amino acid change. Association analyses were performed in a case-cohort study of incident cardiovascular disease (CVD) nested in the EPIC-Potsdam cohort. No significant associations between incident CVD and GIP Ser103Gly and rs2291726 were found. For GIPR Glu354Gln, we obtained a nominal association of heterozygous minor allele carrier with CVD in a codominant model adjusted for BMI, sex, and age (OR: 0.67, CI: 0.50 – 0.91, $p = 0.01$) or additional covariates of CVD (OR: 0.72, CI: 0.52 – 0.97, $p = 0.03$). In conclusion, we identified a common splice site mutation (rs2291726) of the GIP gene which results in a truncated protein and provide preliminary evidence for an association of the heterozygous GIPR Glu354Gln genotype with CVD.

Keywords: Cardiovascular disease / Diabetes type 2 / GIP receptor / Gluocose-dependent insulinotropic peptide / Polymorphism

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1 Introduction

Glucose-dependent insulinotropic polypeptide (GIP) is a 42-amino acid gluco-incretin peptide hormone that is released postprandially from endocrine cells of the small intestine into the circulation. It is a potent mediator in the regulation of nutrient-dependent insulin release from the pancreas [1, 2]. This insulinotropic effect is responsible for approximately 50% of the insulin secretion after meal absorption. In type 2 diabetics, a reduced insulinotropic effect of GIP is described [3, 4]. Rask *et al.* [5] reported an association of impaired GIP responses with insulin resistance. In obese diabetic (ob/ob) mice Gault *et al.* [6] showed an association of GIP with obesity-related glucose intolerance. Because of these features GIP has been considered as a potential therapeutic target for type 2 diabetes. GIP has also been described to play a role in fat accumulation into adipose tissue [7]. Adipose tissue is a proinflammatory

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Abbreviations: CRP, C-reactive protein; **CVD,** cardiovascular disease; **EPIC,** European Investigation into Cancer and Nutrition Cohort; **GIP,** glucose-dependent insulinotropic polypeptide; **GIPR,** GIP receptor; **MI,** myocardial infarction

organ that is described to contribute to endothelial dysfunctions and cardiovascular disease (CVD) in obesity [8]. This implies a possible involvement of GIP in developing CVD.

GIP action requires binding to its specific receptor GIPR (GIP receptor), a class II G-coupled receptor which activates the adenylyl cyclase pathway [9]. GIPR is expressed in many tissues, including brain, adipose tissue, endothelium, intestine, pancreas, and bone [9 – 11]. Therefore, GIP also has multiple other features including control of fat metabolism and modulation of blood flow [12]. Tedde *et al.* [13] reported a contribution of altered GIP secretion in the pathogenesis of hyperinsulinemia in essential hypertension. GIPR knock out mice subjected to a high-fat diet were protected against obesity [14]. The inhibition of GIP signaling is considered to be a new target for antiobesity drugs [14, 15].

In order to investigate whether polymorphisms of the GIP and GIPR gene are associated with risk factors related to the metabolic syndrome we screened all exons including their flanking intron regions of both genes in 47 unrelated subjects. A coding SNP (rs2291725 G > A, Ser103Gly) and putative splice site SNP of GIP (rs2291726 A > G), and a coding SNP of GIPR (rs1800437 G > C, Glu 354Gln) were subsequently genotyped in two study samples from the large European Investigation into Cancer and Nutrition Cohort (EPIC)-Potsdam cohort. In a nested case-control study of incident type 2 diabetes ($N = 576$) no significant associations with disease risk were found [16]. Here, we describe the results of association analyses with disease risk and quantitative traits related to the metabolic syndrome in a nested case-cohort study of incident CVD ($N = 1042$) of the EPIC-Potsdam cohort.

2 Subjects and methods

2.1 EPIC-Potsdam

This population-based, prospective study comprises a total of 27 548 people from the area around Potsdam, Germany. Baseline examinations were conducted between 1994 and 1998 and included anthropometric and blood pressure measurements, blood sampling, a self-administered food-frequency questionnaire, and a personal interview on lifestyle habits and medical history [17]. Follow-up questionnaires were sent to the study participants every 2 – 3 years to obtain information on current medication and newly developed diseases, including diabetes and cardiovascular diseases [18]. Self-reported disease occurrence and medication or postbaseline changes in diet reported to be due to disease, or death certificates were requestioned and verified through contact with the patients attending physician. Informed consent was obtained from each study participant, and genotype assessments were approved by the local Ethics Committee.

2.1.1 Nested case-control study of incident type 2 diabetes (EPIC-Potsdam-T2DM)

During the first and second follow-up, 192 incident cases of type 2 diabetes were identified and confirmed by the primary care physicians [19]. A detailed description of the case verification process has been given previously [20]. Cases were then matched with two control subjects each by age and sex ($n = 384$). Gender distribution of the nested case-control study was 59% male and 41% female subjects with a mean age of 55.5 years (35 – 65 years).

2.1.2 Nested case – cohort study of incident CVD (EPIC-Potsdam-CVD)

The EPIC-Potsdam-CVD study has been designed as a case-cohort study. After exclusion of study subjects with a history of myocardial infarction (MI) or stroke at baseline and those without fully obtained follow-up data the source population comprised 26 490 subjects. Within a mean follow-up time of 6.0 (± 1.5) years, 156 participants were verified having suffered from an acute MI and 102 participants from an ischemic stroke. In two subjects, stroke occurred in association with a coronary event. Cases were verified from medical records and/or death certificates, applying criteria that were used in the WHO-MONICA-study [21]. Thus, incident MIs were defined according to ICD-10 I21.0-I21.9 and incident ischemic strokes were defined according to ICD-10 I63.0-I63.9. For the case-cohort design, a random sample of 851 subjects (subcohort) from the source population was selected including five subjects with incident MI and three subjects with incident ischemic stroke. Characteristics of the subcohort, including traits of the metabolic syndrome, are summarized in Supporting Table 1. Briefly, subjects were aged between 34 and 66 years at study entry (mean 50 ± 8.5 years), and gender distribution was 39.6% males and 60.4% females.

2.2 SNP identification and verification

Six exons of the GIP gene and 14 exons of the GIPR gene were analyzed for genetic variations by resequencing of dbSNPs in a group of 47 unrelated diabetics of the metabolic intervention cohort kiel (MICK). Sequencing was performed after "Touch Down" PCR using terminator cycle sequencing on an ABI 3700 capillary DNA sequencer (Applied Biosystems, Foster City, CA, USA).

2.3 *In silico* analyses of GIP rs2291725 and rs2291726 and GIPR rs1800437

Protein sequences of GIP and GIPR were obtained from NCBI. Domain structure and profile analyses were performed using ExPASy of the Swiss Institute of Bioinformatics (SIB). For predictions whether missense variations rs2291725 (Ser103Gly) of GIP and rs1800437 (Glu354Gln)

of GIPR could lead to consequences in protein function we used the PolyPhen tool [22] and the $SNPs_3D$ website (http://www.snps3d.org/).

2.4 SNP genotyping

GIP rs2291725 (Ser103Gly), GIP rs2291726, and GIPR rs1800437 (Glu354Gln) were genotyped in the EPIC-Potsdam studies (T2DM and CVD). The genotyping technique was Taqman SNP allelic discrimination by means of an ABI 7900HT (Applied Biosystems). Assay details are available from the authors upon request.

2.5 Biochemical analyses

In EPIC-Potsdam-T2DM, total cholesterol, HDL-cholesterol, and triglycerides were measured with enzymatic colorimetric methods (Boehringer Mannheim, Mannheim, Germany). LDL-cholesterol was determined using Friedewald formula. In EPIC-Potsdam-CVD, LDL-cholesterol was directly quantified in fasting blood samples. Total cholesterol, HDL-cholesterol, LDL-cholesterol, C-reactive protein (CRP), and triglycerides were measured using standard methods with reagents from Horiba ABX (Shefford, UK). Intra-assay coefficients of variation (CVs) were 0.9, 1.2, 1.7, 2.6, and 3.4%, respectively. Interassay CVs were 4.7, 5.2, 5.2, 7.9, and 5.5%, respectively.

2.6 Details of covariate assessment

The presence of prevalent hypertension was defined on the basis of the mean values of second and third blood pressure readings during baseline examination as systolic blood pressure $\geq$ 140 mmHg and/or diastolic blood pressure $\geq$ 90 mmHg, and/or from self-reported disease-specific medication, or by verification from medical records. Definition of other prevalent diseases (diabetes, hyperlipidemia) was based on subjects' reports and/or disease-specific medication and/or verification from medical records. Sports activity was calculated from mean time spent on sporting activities during summer and winter seasons (h/wk) and categorized as "$<$2 h/wk" and "$\geq$2 h/wk". Smoking status was graded never/former/current smoker. Educational level was expressed as "no high school graduation", "high school graduation", and "university degree".

2.7 Statistical analyses

Allele and genotype frequencies were determined by gene counting. The study populations were tested as a whole and cases/controls individually for the distribution of genotypes according to the Hardy–Weinberg equilibrium with a χ^2 (1 degree of freedom) test. Associations between quantitative traits and SNPs were tested using ANOVA with adjustment for age and sex. We assumed that the alleles acted additively, hence a linear trend regression analysis of genotypes was used. Traits were transformed to approximate normality when necessary. We analyzed nine traits for EPIC-Potsdam-CVD subcohort subjects: BMI, WHR, waist circumference, total cholesterol, HDL-cholesterol, fasting triglycerides, and LDL-cholesterol, and systolic and diastolic blood pressure. Cox proportional hazard regression analysis was used to determine the relationship between SNPs and risk of CVD. Age was the underlying time variable in the counting process, with entry defined as the subjects' age at the time of recruitment and exit defined as age at the diagnosis of CVD or censoring. As suggested by Prentice [23], the Cox models were modified in order to account for the case-cohort design. Hazard rate ratios were calculated adjusting for age, sex, BMI, total cholesterol/HDL-cholesterol ratio, sports activity, smoking habits, comorbidities (prevalent diabetes and hypertension), and educational level. Statistics were computed with SAS software 9.1 (SAS Institute, Cary, NC).

3 Results and discussion

3.1 SNP verification in GIP and GIPR by resequencing

To investigate whether genetic variations in GIP and GIPR genes might influence risk factors of the metabolic syndrome we analyzed the exons including their flanking intron regions by resequencing both genes in 47 type 2 diabetic patients of the MICK cohort Kiel. For GIP nine SNPs, three exon, five intron, and one 3′ flanking SNP, were identified of which four were not listed in public databases (Table 1). GIPR resequencing revealed nine SNPs, one exon, and eight intron SNPs. Five of them were listed in public databases (Table 1). In both genes only one nonsynonymous coding SNP was detected, rs2291725 (A > G, Ser103Gly) in exon 4 of GIP and rs1800437 (G > C, Glu354Gln) in exon 12 of GIPR. A putative splice site SNP, rs2291726 in intron 3 of GIP was also concerned to be of potential interest. In NCBI dbSNP data the rs2291726 base change was defined as GA (allele frequencies = 0.5), in our resequencing analyses we found a A > G base change with a minor allele frequency $G = 0.28$, although, the minor allele of GIP Ser103Gly (A > G) was identified as major allele in 47 diabetic subjects (Table 1).

3.2 SNP genotyping and association studies

For SNP genotyping we selected rs1800437 (Glu354Gln) in exon 12 of GIPR, rs2291725 (Gly103Ser) in exon 4 of GIP, and the putative splice site SNP rs2291726 in intron 3 of GIP. Genotyping was performed in two study samples of the EPIC-Potsdam cohort (EPIC-Potsdam-T2DM, EPIC-Potsdam-CVD). Compared to our resequencing results, GIP rs2291725 and rs2291726 allele frequencies were in

Table 1. Polymorphisms in GIP and GIPR genes detected by resequencing

Gene segment	SNP ID	Position	Allele	AA pos.	Freq. reseq.	dbFreq. (NCBI)	Freq. GT (Inz. diab.)
GIP							
Exon 4	rs2291725	50	A > G	S103G	*G* = 0.69 *A* = 0.30	*G* = 0.48 *A* = 0.52	*A* = 0.51
Exon 6	New	36 (3'UTR)	G > C		*G* = 0.76 *C* = 0.24		
Exon 6	New	37 (3'UTR)	G > T		*G* = 0.73 *T* = 0.27		
Intron 1	rs8078510	Ex1 + 17	C > T		*C* = 0.73 *T* = 0.27	*C* = 0.651 *T* = 0.349	
Intron 2	New	Ex2 + 46	G > A		*G* = 0.49 *A* = 0.51		
Intron 3	rs2291726	Exon4 - 73	G > A		*A* = 0,72 *G* = 0,28	*A* = 0.5 *G* = 0.5	*G* = 0.53
Intron 4	New	Ex5 - 85	C > G		*C* = 0.81 *G* = 0.24		
Intron 4	rs6504587	Ex5 - 42	G (refS = A)		*G* = 1	N.D.	
3′ Flanking	rs4793605	Ex6 + 49	G > A		*G* = 0.55 *A* = 0.45	*G* = 0.4 *A* = 0.6	
GIPR							
Exon 12	rs1800437	47	G > C	E354Q	*G* = 0.68 *C* = 0.32	*G* = 0.83 *C* = 0.17	*C* = 0.22
Intron 1	rs4803845	Ex1 + 48	C		*C* = 1	C/T N.D.	
Intron 1	rs2302382	Ex2 - 166	C > A		*C* = 0.79 *A* = 0.21	*C* = 0.9 *A* = 0.1	
Intron 2	New	Ex2 + 83	G > C		*G* = 0.99 *C* = 0.01		
Intron 7	New	Ex8 - 60	G > C		*G* = 0.99 *C* = 0.01		
Intron 7	rs4803846	Ex8 - 57	G > A		*G* = 0.8 *A* = 0.2		
Intron 7	New	Ex8 - 26	C > T		*C* = 0.99 *T* = 0.01		
Intron 7	rs11672660	E8 - 23	C > T		*C* = 0.7 *T* = 0.3	C/T N.D.	
Intron 8	New	E8 + 48	G > T		*G* = 0.7 *T* = 0.3		

Database frequency (dbFreq) is based on HapMap-CEU; Freq. GT = frequency of genotyping; Freq. reseq. = frequency of resequencing; Ex = exon; AA pos. = amino acid position; N.D. = not determined; S = serine, G = glycine, E = glutamate, Q = glutamine.

accordance with dbSNP entry A > G and G = A, respectively in EPIC-Potsdam study subjects (Table 1).

Baseline characteristics of the matched case-control study (EPIC-T2DM) have been described elsewhere [20]. GIP rs2291726 and GIPR Glu354Gln were successfully genotyped in 190 cases of incident type 2 diabetes and 380 sex- and age-matched controls, GIP Gly103Ser in 188 cases and 378 controls. Genotype frequencies were found to be in Hardy-Weinberg equilibrium. Genotype distributions in diabetic and control subjects are shown in Table 2. Overall, there were no statistically significant differences in the distribution of genotypes comparing cases with control subjects and consequently no significant associations with type 2 diabetes of selected GIP and GIPR SNPs were obtained (data not shown). In agreement, previous studies found no associations between GIPR Glu354Gln polymorphism and type 2 diabetes in Japanese [24] and Caucasian [25].

For the two GIP SNPs 218 cases of CVD and 822 sex- and age-matched controls, for GIPR SNP 219 cases and 826 controls were genotyped. Genotype distributions in CVD cases and controls are given in Table 2. No significant association between CVD and GIP SNPs was found (Table 3). For GIPR Glu354Gln, we obtained a significant association of heterozygous minor allele carriers with CVD in the codominant model adjusted for BMI, sex, and age (OR: 0.67, CI: 0.50 – 0.91, $p = 0.01$). Comparable and significant OR was obtained when adjustment for additional covariates which are involved in CVD (OR: 0.72, CI: 0.52 – 0.97, $p = 0.03$) was performed. However, these associations were not found for the homozygous carrier of the minor allele. The association in the dominant model II (OR: 0.73, CI: 0.55 – 0.97, $p = 0.03$) resulted from the low frequency of homozygous carrier of the minor allele (Table 3). Additionally, we analyzed the associations between common traits

Table 2. GIP rs2291725 (Ser103Gly), GIP rs2291726, and GIPR rs1800437 (Glu354Gln) genotype frequencies, minor allele frequencies and test for deviation from Hardy – Weinberg equilibrium

EPIC-Potsdam-study		GIP Ser103Gly				HWE	GIP rs2291726				HWE	GIPR Glu354Gln				HWE
		AA	AG	GG	MAF	*P*	GG	GA	AA	MAF	*P*	GG	GC	CC	MAF	*P*
T2DM	Cases	41 (21.8)	106 (56.4)	41 (21.8)	0.500	0.10	47 (24.5)	109 (56.8)	36 (18.7)	0.471	0.05	114 (59.7)	65 (34.6)	12 (6.3)	0.233	0.51
	Controls	104 (27.4)	180 (47.5)	95 (25.1)	0.488	0.33	108 (28.2)	190 (49.6)	85 (22.2)	0.470	0.93	235 (61.2)	134 (34.9)	15 (3.9)	0.214	0.45
	P[a)]	0.13					0.27					0.45				
CVD	Cases	62 (28.4)	101 (46.3)	55 (25.2)	0.484	0.35	70 (32.1)	101 (46.3)	47 (21.6)	0.454	0.28	142 (64.8)	65 (29.7)	12 (5.5)	0.203	0.22
	Controls	218 (26.5)	424 (51.6)	180 (21.9)	0.477	0.39	241 (29.3)	426 (51.8)	155 (18.9)	0.448	0.13	458 (55.4)	326 (39.5)	42 (5.1)	0.248	0.10
	P[a)]	0.25					0.46					0.03				

Values in parenthesis are in percent, MAF, minor allele frequency; HWE, Hardy-Weinberg equilibrium.
a) Based on chi square test of independence.

Table 3. Association of GIP and GIPR SNPs with CVD

	GIP Ser103Gly				GIP rs2291726				GIPR Glu354Gln			
	Model 1	*P*	Model 2	*P*	Model 1	*P*	Model 2	*P*	Model 1	*P*	Model 2	*P*
CVD I	1.0		1.0		1.0		1.0		1.0		1.0	
	0.90 (0.65 – 1.24)	0.52	0.84 (0.61 – 1.16)	0.30	0.84 (0.61 – 1.15)	0.27	0.83 (0.61 – 1.14)	0.26	0.67 (0.50 – 0.91)	0.01	0.72 (0.52 – 0.97)	0.03
	1.12	0.54	1.10	0.64	1.14	0.49	1.09	0.68	1.31	0.40	1.18	0.60
II	0.97	0.83	0.91	0.56	0.92	0.56	0.90	0.48	0.73	0.03	0.77	0.07
III	1.20	0.25	1.23	0.21	1.27	0.15	1.21	0.26	1.51	0.19	1.33	0.37

Cox proportional hazard ratios and 95% confidence intervals are given; model 1: adjusted for sex and age, model 2: adjusted for sex, age, BMI, physical exercise, smoking, educational level, cholesterol/HDL ratio, prevalent hypertension, and prevalent diabetes; IM, inheritance model, I: codominant, II: dominant, III: recessive.

of the metabolic syndrome and GIPR Glu354Gln (Table 4). ANCOVA analysis revealed significant associations for HDL-cholesterol levels (p-trend: 0.04). A replicative analysis in EPIC-Potsdam-T2DM did not show a significant trend of decreased HDL-cholesterol concentration associated with the minor allele of the polymorphism (data not shown).

3.3 Predictions of putative functional consequences of genotyped GIP and GIPR SNPs

Screening of domain and profile databases revealed that amino acid Ser103 of GIP is located in the propeptide (pro GIP) and not in the metabolic active hormone which comprises amino acids 52 – 93. The actually active alpha-helical motif spans the region from Ser(11) to Gln(29) [26].

The GIPR is composed of an important hormone receptor domain (amino acid residues 58 – 123) and the transmembrane domain 7tm-2 (amino acids 134 – 399) containing the variation Glu354Gln in transmembrane section six (amino acids 342 – 362). We used PolyPhen and SNPs3D to preestimate a putative effect of the coding SNPs rs2291725 (Ser103Gly) and rs1800437 (Glu354Gln) of GIP and GIPR on protein function. Both algorithms predicted the amino acid substitution Ser103Gly of GIP as tolerable with no functional impacts. Even so the GIPR missense mutation Glu354Gln describes a residue change from a strong to a weak α-helix former, PolyPhen and SNPs3D classified this variation as possibly damaging; however, no molecular effect is given. GIP SNP rs2291726 (related to our genotyping G > A, related to NCBI dbSNP G = A (Table 1)) located in intron 3 (exon four minus 73 bp) can be postulated as a putative splice site SNP (AG*A*T ⟷ AG*G*T). Nucleotide G introduced a putative alternative 3′ splice site 73 bp upstream of the constitutive exon 4. This putative splice site is in concordance with the 3′ splice consensus CAG-GT [27]. Translation of this putative transcript containing a 73 bp 5′-extension of exon 4 resulted in a truncation of six amino acid residues of the mature GIP peptide at the C-terminal part in combination with a frameshift introducing a stop codon after the first two amino acid residues in exon 4

Table 4. Association of GIPR Glu354Gln (G > C) with traits of the metabolic syndrome in EPIC-Potsdam CVD subcohort subjects

	GG	GC	CC	*P*
Subjects (N)	460	330	42	
Age	49.9 (0.4)	49.8 (0.5)	48.5 (1.3)	0.6
Males (%)	39.8	39.7	31.0	0.5
				P trend
BMI (kg/m^2)[a)]	25.7 (0.16)	25.79 (0.19)	25.66 (0.52)	0.75
Waist (cm)[a)]	85.09 (0.42)	85.26 (0.49)	85.07 (1.39)	0.85
WHR[a)]	0.848 (0.003)	0.846 (0.003)	0.842 (0.009)	0.52
CRP (mg/l)[a)]	0.57 (0.50, 0.65)	0.54 (0.46, 0.63)	0.84 (0.54, 1.30)	0.56
Subjects (N)[c)]	448	315	41	
TC (mmol/l)[a)]	4.56 (4.49, 4.63)	4.46 (4.38, 4.54)	4.43 (4.21, 4.66)	0.07
HDL-C (mmol/l)[a)]	1.22 (1.20, 1.24)	1.17 (1.15, 1.20)	1.21 (1.14, 1.28)	0.04
Subjects (N)[c, d)]	122	85	6	
TG (mmol/l)[a)]	1.04 (0.96, 1.12)	1.09 (0.99, 1.20)	1.23 (0.86, 1.74)	0.29
LDL-C (mmol/l)[a)]	2.66 (2.54, 2.78)	2.48 (2.34, 2.62)	2.58 (2.09, 3.17)	0.09
Subjects (N)[e)]	396	277	38	
Diast BP (mmHg)[b)]	81.93(0.47)	82.91 (0.56)	83.93 (1.50)	0.09
Syst BP (mmHg)[b)]	125.79 (0.71)	125.93 (0.84)	128.39 (2.25)	0.45

a) Adjusted for age, and sex.
b) Adjusted for age, sex, and BMI.
c) Subjects taking lipid-lowering drugs were excluded.
d) Fasting blood samples only.
e) Subjects taking antihypertensive medication were excluded; CRP, C-reactive protein; TC, total cholesterol; TG, triglycerides; BP, blood pressure.

leading to a loss of exons 5 and 6 (Fig. 1). It would be an interesting approach to investigate if this putative GIP truncation exists and to what extent this truncation would affect insulin release or vasoactiv properties of GIP. Interestingly, two modified C-terminal truncated GIPs containing 16 amino acid residues showed low activity regarding insulin release *in vitro* and *in vivo* [28]. A synthetic truncated GIP with a similar bioactivity as the native GIP polypeptide was described by Manhart *et al.* [29].

4 Concluding remarks

Although numerous reports in the literature described GIP and its receptor as potent candidates that are linked to diabetes and obesity-related diseases our association studies in the EPIC Potsdam cohort revealed no effect of relevant GIP SNPs rs2291725 (Ser103Gly) and rs2291726 (putative splice site SNP) on risk prediction for diabetes type 2 and CVD. GIPR is described to modulate body weight and energy expenditure [30]. In relation to this, we found a nominal association between the heterozygous GIPR Glu354Gln genotype and CVD. Both Almind *et al.* [25] and Kubota *et al.* [24] found no significant functional consequences of GIPR Glu354Gln related to GIP binding and GIP-induced cAMP response in fibroblasts and ovary cells. However, Almind *et al.* [25] found an association between homozygous carrier of GIPR variant and reduced fasting and postoral glucose tolerance test serum C-peptide concentrations. Our *in silico* analysis classified the GIPR variant 354Gln as a putative damaging exchange. Therefore, future studies regarding the functional consequence of the GIPR variant in relation to CVD are of potential interest.

A

EKKEGHFSAL PSLPVGSHAK VSSPQPRGPR **YAEGTFISDY SIAMDKIHQQ DFVNWLLAQK** 60

GKKNDWKHNI TQREARALEL ASQANRKEEE AVEPQSSPAK NPSDEDLLRD LLIQELLACL 120

LDQTNLCRLR SR 132

B

EKKEGHFSAL PSLPVGSHAK VSSPQPRGPR **YAEGTFISDY SIAMDKIHQQ DFVNWLLAQK** 60

GKKNEY* 66

Figure 1. Amino acid sequence of proGIP carrying allele A (A) and proGIP containing allele G (B) of SNP rs2291726. The mature GIP peptide hormone comprising 42 amino acid residues is shown in bold. Allele G created a putative new splice site upstream of the regular splice site of exon 4 leading to a frameshift introducing a stop codon (*) after the two amino acids glutamate (E) and tyrosine (Y). This results in a truncated GIP peptide of 36 amino acids. Underlined amino acids are encoded by exon 4.

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