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Sex-Specific Effects of GLP-1R Variants on BMI and Obesity Treatment Response

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Article

Keywords: Obesity, Incretin Mimetics, Sex, Gender, Bariatric Surgery, Genetics

Posted Date: November 4th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10686393/v1

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Additional Declarations: Competing interest reported. MB received honoraria as a consultant and speaker from Abbott, Amgen, AstraZeneca, Bayer, Boehringer Ingelheim, Daiichi–Sankyo, Lilly, MSD, Novo Nordisk, Novartis, Pfizer, and Sanofi; and reports chairing a Clinical Trial Data Safety Monitoring Board for Boehringer Ingelheim. All other authors declare no competing interests.

Abstract

Genetic variants may affect the body mass index (BMI) and the effectiveness of obesity treatments. However, it remains largely unclear whether these effects differ between men and women. Thus, this study investigates genetic variants in incretin-related genes (*GIP*, *GIP-R*, *GCG*, *GCG-R* and *GLP-1R*) using sex-stratified genetic and functional approaches.

First, sex-specific genome-wide association study data were analysed to identify variants with a sex-specific effect on BMI. Identified variants were then examined in an independent cohort of adults undergoing bariatric surgery and functionally assessed in a cellular model. Finally, all genes were screened for rare variants in children and adolescents with severe obesity.

Nine highly linked variants within *GLP-1R* showed stronger effects on BMI in men than in women. Among these, only rs1042044 (p.Leu260Phe) is located in the coding region. In a cohort of 807 bariatric surgery patients, rs1042044 showed a nominal association with achieving a weight loss of more than 10%, particularly in women. However, no differences in receptor internalisation were observed between wildtype and mutant GLP-1R *in vitro*. Additionally, 26 variants were identified across the genes in a paediatric cohort.

Future studies should incorporate sex-stratified analyses to uncover putative sex-dependent genetic modifiers of BMI and weight loss outcomes.

INTRODUCTION

Overweight and obesity remain a global health challenge. Worldwide, the prevalence of obesity has increased substantially over the last decades. According to current prevalence trends, it is projected that by the year 2030 approximately 50% of adults will be at least overweight. The proportion of men and women living with obesity is expected to reach 17% and 22%, respectively[1].

Historically, treatment options were limited to lifestyle interventions and bariatric surgery. The weight loss achieved through lifestyle interventions often proved to be modest or unsustainable, and even bariatric surgery may result in weight regain[2–4]. Recently, the development of incretin-based pharmacotherapies has marked the beginning of a new era in combating the global obesity epidemic.

Incretin hormones, including the glucose-dependent insulinotropic polypeptide (GIP) and the glucagon-like peptide 1 (GLP-1), are secreted from enteroendocrine cells in the small intestine in response to nutrient intake[5–7]. GIP and GLP-1 both stimulate insulin secretion in a glucose-dependent manner[7]. Over the past decade, incretin-based therapies have emerged as effective options for weight management[8]. Tirzepatide, the latest approved dual agonist targeting the receptors of GIP and GLP-1[7], has demonstrated remarkable efficacy with patients achieving on average a weight loss of up to 22.5% after 72 weeks of treatment[9–12]. More recently, a phase 2 clinical trial investigating retatrutide, a triple agonist targeting GIP, GLP-1 and glucagon (GCG) receptors, has even reached a greater efficacy. After 48 weeks, patients receiving retatrutide experienced an average weight loss of 24.2%[11,13].

Despite these therapeutic advances, substantial interindividual variability in treatment responses has been reported. Biological sex has emerged as a key determinant[13–15], with retatrutide showing a greater weight loss in women than men[13]. Additionally, genetic factors may play a critical role in modulating treatment efficacy[16–25]. Genetic variants may affect the effectiveness of incretin-based pharmacotherapies like tirzepatide by altering receptor expression, signal transduction or pharmacokinetics. Variants in incretin receptors, including *GIP-R* and *GLP-1R*, have been studied extensively. *GIP-R* variants may result in loss-of-function (LoF) or gain-of-function (GoF) effects on cyclic adenosine monophosphate (cAMP) production or β-arrestin recruitment, with carriers of LoF variants for both mechanisms exhibiting a lower BMI and a reduced body fat percentage[20]. For carriers of the *GLP-1R* variant rs6923761, it was implied that those homozygous for the A-allele experienced a greater weight loss following treatment with semaglutide[22]. Although another study was unable to confirm this, it reported that carriers of the A-allele of rs6923761 displayed an increased gastric emptying upon treatment with exenatide or liraglutide[16]. Recently, a genetic variant in *GLP-1R* was shown to influence the efficacy of incretin mimetics, with each effect allele (T-allele) of rs10305420 being associated with an additional weight loss of 0.76 kg[25]. Similarly, further variants, such as the *UCP2* variant rs660339, have been associated with post-surgery weight loss[24,26]. Of note, some of the reported associations should be interpreted with caution, as corrections for multiple testing were often lacking.

Despite previous studies suggesting that variants in *GIP-R* and *GLP-1R* may contribute to functional and phenotypic variability, it remains uncertain whether these effects on body mass index (BMI) and treatment response differ between men and women. Consequently, we aimed to identify and analyse genetic variants in genes related to the novel incretin-based obesity treatments (*GIP*, *GIP-R*, *GCG*, *GCG-R* and *GLP-1R*) in a sex-specific manner, employing both genetic and functional approaches.

RESULTS

Nine variants in GLP-1R exhibit a sex-specific effect on the BMI

Initially, we aimed to identify genome-wide significant variants for BMI that have a sex-specific effect and are located within the genes of interest or a 100 kb adjacent region. Therefore, data from a sex-stratified genome-wide association study (GWAS) for BMI[27] was extracted (see Table 6). The gene regions of *GIP*, *GIP-R*, *GCG*, *GCG-R* and *GLP-1R* encompassed a total of 99, 138, 73, 96 and 502 variants (regardless of p-value), respectively (see Table 1).

Table 1

Number of variants in gene and adjacent regions based on data of a GWAS for BMI variance[27]

Gene	Region	Women and men combined		Only women		Only men	
		Total variants	Significant variants	Total variants	Significant variants	Total variants	Significant variants
GIP	Gene region	99	5	99	0	99	0
	± 100 kb region	1,631	119	1,631	41	1,631	32
GIP-R	Gene region	138	12	138	8	138	11
	± 100 kb region	1,825	41	1,825	8	1,825	10
GCG	Gene region	73	0	73	0	73	0
	± 100 kb region	1,857	0	1,857	0	1,857	0
GCG-R	Gene region	96	0	96	0	96	0
	± 100 kb region	1,648	0	1,648	0	1,648	0
GLP-1R	Gene region	502	0	502	0	502	9
	± 100 kb region	2,138	0	2,138	0	2,138	0

Genetic variants were considered genome-wide significant, if the p-value was < $5*10^{-8}$. Kb: kilobase.

Table 6

Genomic positions applied for GWAS-based sex-specific analyses.

Gene	**Chr**	**Gene region**	**Gene ± 100 kb region**
GIP	17	47,035,916–47,045,958	49,935,916–47,145,958
GIP-R	19	46,171,479–46,186,980	46,071,479–46,286,980
GCG	2	162,999,392–163,008,757	162,899,392–163,108,757
GCG-R	17	79,762,026–79,771,884	79,662,026–79,871,884
GLP-1-R	6	39,016,557–39,059,079	38,916,557–39,159,079

Data of the respective genomic regions were extracted from the summary statistics of a sex-stratified GWAS for BMI variance[27]. The analysed regions refer to the GRCh37 genome version.

Notably, *GCG* and the gene encoding its respective receptor, *GCG-R*, did not carry any variants associated with BMI (GWAS-derived p-value ≤ $5*10^{-8}$, see Figs. 1C and 1D, see Table 1). In *GIP*, five variants showed a genome-wide significance for BMI when both sexes were analysed combined (see Figs. 1A, see Table 1). Sex-separated analyses revealed no variant associated with BMI in women or men only (see Table 1). *GIP-R* encompassed twelve variants associated with BMI when analysing both sexes, while eight variants were associated with BMI in women. Eleven variants in *GIP-R* were associated with BMI in men (see Fig. 1B, see Table 1). The gene region of *GLP-1R* contained nine variants with a genome-wide significance for BMI in men. No associated genetic variants were detected when analysing both sexes together and women only (see Fig. 1E). Additionally, the 100 kb adjacent regions of *GIP* and *GIP-R* encompassed numerous variants associated with BMI in all investigated groups (both sexes, women only, men only; see Figs. 1A and 1B, see Table 1).

Z-scores were exclusively calculated, if the respective gene of interest encompassed a genome-wide significant variant (GWAS-derived p- value ≤ $5*10^{-8}$) for BMI in women only and/or men only. These Z-scores were determined using harmonized data of BMI-associated variants in *GIP*, *GIP-R* and *GLP-1R* (see Figs. 1A, 1B, and 1E, see Table 1). This revealed nine variants located in *GLP-1R* associated with BMI, exhibiting a stronger effect on BMI in men than in women (see Fig. 2, see Supplementary Table 1). Of those, rs1042044 (p.Leu260Phe) was the only variant located within the coding region of *GLP-1R*. All other variants with a sex-specific effect on the BMI were located in intronic regions of *GLP-1R*.

The nine GLP-1R variants with sex-specific effects on BMI are in high linkage disequilibrium

Subsequently, we aimed to obtain further information on the nine *GLP-1R* located variants showing a sex-specific effect on BMI. Consequently, we assessed the linkage disequilibrium (LD) of those variants in the populations of Utah residents from North and West Europe (CEU) and British in England and Scotland (GBR) of the 1000 Genomes Project using the tool LDmatrix.

In the CEU population, all nine variants were in high LD ($r^2 > 0.8$, see Supplementary Fig. 1, see Supplementary Table 2) with the non-synonymous variant (NSV) rs1042044 (p.Leu260Phe) and the intronic variant rs2268644 being in perfect LD ($r^2 = 1.0$). Likewise, in the GBR population, all variants, except rs910165 and rs2254336 ($r^2 = 0.79$), exhibited a high LD ($r^2 > 0.8$; see Supplementary Fig. 2, see Supplementary Table 3). In both populations (CEU and GBR), the NSV rs1042044 exhibited a strong LD ($r^2 > 0.9$) with several variants, while both intronic variants, rs910163 and rs910162, were in perfect LD ($r^2 = 1.0$; see Supplementary Tables 2 and 3).

In-depth analyses of rs1042044 (p.Leu260Phe) in the Leipzig Obesity Biobank cohort comprising patients undergoing bariatric surgery

Based on our findings, we investigated the *GLP-1R* located NSV, rs1042044 (p.Leu260Phe), in greater detail to evaluate its potential clinical relevance. Therefore, we analysed a cohort of 807 severely obese patients who underwent bariatric surgery (either Roux-en-Y gastric bypass or sleeve gastrectomy; see Table 5). The mean postoperative body weight reduction experienced was 28.16%. First, we have performed a GWAS for total weight loss percentage (TWL%) in both sexes combined and sex-stratified with covariates age, surgery type and sex (for the combined analysis). Regardless of the groups analysed, no significant association of rs1042044 and other *GLP-1R* variants (rs910170 and rs10305420) was found (see Supplementary Table 4).

Table 5
Phenotypic characteristics of the investigated study groups.

Study group	Status		Sex (n)	Age in years	BMI in kg/m²
LOBB group	Individuals undergoing bariatric surgery		Female (542)	47.98 (11.62)	49.9 (9.16)
			Male (265)	48.89 (10.76)	50.17 (8.56)
Screening group	Children and adolescents with severe obesity		Female (48)	13.85 (3.26)	32.53 (6.88)
			Male (46)	13.13 (3.00)	31.40? (8.19)
			Both (94)	13.50 (3.14)	32.09 (7.52)
	Healthy, lean controls		Female (38)	24.48 (3.89)	17.54 (0.68)
			Male (58)	26.06 (3.96)	19.09 (0.95)
			Both (96)	25.43 (3.99)	18.48 (1.15)
Confirmation group	Family-based samples	Offspring with severe obesity	Female (424)	13.58 (3.19)	32.51 (6.14)
			Male (353)	13.29 (3.00)	31.73 (5.62)
			Both (777)	13.44 (3.11)	32.16 (5.91)
		Biological parents	Mother (777)	40.93 (5.44)	30.38 (7.06)
			Father (777)	44.25 (6.08)	30.42 (5.44)
	Independent larger study groups	Children and adolescents with severe obesity	Female (279)	14.56 (3.70)	33.45 (7.11)
			Male (210)	14.18 (3.80)	33.29 (6.39)
			Both (489)	14.40 (3.74)	33.38 (6.81)
		Healthy, lean controls	Female (240)	26.76 (6.62)	17.58 (0.97)
			Male (113)	24.97 (4.80)	18.79 (0.92)
			Both (353)	26.19 (6.15)	17.97 (1.11)

All data is presented as mean (standard deviation), if not otherwise stated. The mean and standard deviations were calculated with IBM's SPSS Statistics (version 29.0.0.0). Within the confirmation group, three children or adolescents with severe obesity were also part of the "offspring with severe obesity" as well as the "children and adolescents with severe obesity" study groups. kg: kilogram. LOBB: Leipzig Obesity Biobank, m²: squared meter. n: number of participants in respective study group. NA: not available for respective study group.

Next, we examined the allele frequencies and genotype distributions of rs1042044 in the 807 patients undergoing bariatric surgery. Here, a nominal association between rs1042044 genotype and responder status was observed at the 10% weight loss cut-off in the combined (women + men, p = 0.03, Chi-square test) and women only cohort (p = 0.004, Fisher-Freeman-Halton test; see Supplementary Tables 5 and 6). However, this association did not remain significant after correction for multiple testing. At all other weight loss cut-offs, no evidence for associations between genotype and responder status was observed (see Table 2, see Supplementary Tables 5 and 6).

Table 2
Allele frequencies and genotype distribution of rs1042044 in 807 patients who underwent bariatric surgery

TWL% cutoff	N (res: non-res)	MAF	Responder*			Non-responder*			Women % (res: non-res)	P-value
			AA	AC	CC	AA	AC	CC		
> 10%	788:19	A: 0.45	153 (0.19)	395 (0.50)	240 (0.31)	8 (0.42)	9 (0.47)	2 (0.11)	67%: 63%	0.03
> 15%	758:49	A: 0.44	148 (0.20)	377 (0.50)	233 (0.31)	13 (0.27)	27 (0.55)	9 (0.18)	68%: 55%	0.15
> 20%	685:122	A: 0.45	133 (0.19)	347 (0.51)	205 (0.30)	28 (0.23)	57 (0.48)	37 (0.30)	69%: 59%	0.61
> 40%	56:751	A: 0.39	10 (0.18)	24 (0.43)	22 (0.39)	151 (0.20)	380 (0.51)	220 (0.29)	75%: 68%	0.29
100 patients with highest TWL%	100:707	A: 0.40	15 (0.15)	49 (0.49)	36 (0.36)	146 (0.21)	355 (0.50)	206 (0.29)	72%: 66%	0.25

Here, both sexes are included. For sex-stratified analyses, please refer to Supplementary Tables 5 and 6. P-values correspond to either the Chi-Square or Fisher-Freeman-Halton test. *Here, the total number of samples with a respective genotype is presented. The corresponding allele frequency is shown in parenthesis. N = number of samples. MAF = minor allele frequency. TWL% = total weight loss percentage. Res = responder. Non-res = non-responder.

Notably, we additionally evaluated the allele frequencies and genotype distributions in the 100 individuals with the highest BMI in this cohort. These individuals (65% women) had on average a BMI of 66.61 kg/m², with the lowest BMI being 60.30 kg/m² and the highest being 97.30 kg/m². The MAF (A-allele) was 42%. Of the 100 individuals, 13 carried the A-allele homozygously, 58 were heterozygous carriers and 29 had a homozygous genotype for the C-allele (see Table 2).

No indications of an altered GLP-1R internalisation

To investigate the putative functional relevance of rs1042044 (p.Leu260Phe) located in *GLP-1R*, we assessed the ligand-induced GLP-1R internalisation using a BRET assay. Native and mutant (260Phe) GLP-1R internalisation was analysed in response to native GLP-1 (GLP-1(7-39amide) as well as semaglutide, an GLP-1 analogue approved for the treatment of obesity[8,28,29].

Dose-response fits indicated maximal ligand-induced internalisation (E_{max}) within a BRET ration range of 4.57 to 5.09 (see Fig. 3C, see Supplementary Table 7). Mixed-model analysis revealed significant main effects of time ($p < 0.0001$) and ligand ($p = 0.0018$), whereas the time x ligand interaction was not significant ($p = 0.761$), suggesting a general decline in internalisation over time similar across both ligands. Comparing the ligands, internalisation differed between native GLP-1 and semaglutide for both, the native ($p < 0.0001$) and the mutant GLP-1R ($p < 0.0001$). However, no significant differences in GLP-1R internalisation were observed between the native and mutant receptor for either agonist (native GLP-1 $p = 0.76$; semaglutide $p = 0.739$; see Figs. 3A and 3B).

Mutational analyses of the screening cohort revealed further variants in the genes of interest

In our previous study, we screened 761 German families, each comprising the biological parents and at least one offspring with severe obesity, for three tag-variants (rs8111428, rs2302382 and rs1800437) located in *GIP-R*. We detected an over-transmission of the A-allele of the intronic rs2302382[30]. Based on the previous publication and our preceding data on sex-specific *GLP-1R* variants, we aimed to identify rare variants in the genes of interest that may have a functional relevance. Therefore, we have performed a mutation screen of the coding regions of *GIP*, *GIP-R*, *GCG*, *GCG-R* and *GLP-1R* in 94 children and adolescents with severe obesity and 96 healthy, lean controls (see Table 5). Children and adolescents with severe obesity were selected based on their genotype at rs2302382, an intronic variant in *GIP-R*[30]. This strategy may have enriched the study group for additional variants of the *GIP-R* gene. The study group originated from obesity trios (affected offspring and their two biological parents), which largely contributed to the positive transmission disequilibrium test (TDT) observed in our previous study[30].

Six variants in GIP-R and one in its ligand, GIP

Within the coding region of *GIP-R*, six variants were detected (see Table 2). Five were NSVs and one was an SV. Of the NSVs, rs139215588 (p.Arg190Gln) and rs1800436 (p.Ala207Val) were each identified in one healthy, lean female, whereas rs776722055 (p.Arg429Trp) was detected exclusively in a male control individual (see Supplementary Tables 8 and 9). The variant rs1800437 (p.Glu354Gln) was detected in both study groups and both sexes (see Table 5, see Supplementary Tables 8 and 9). Yet, *in silico* analyses suggest that rs1800437 may be functionally deleterious, indicated by a strong evolutionary conservation at the affected nucleotide (see Supplementary Table 10). Thus, we have genotyped rs1800437 in a larger, independent group of

children and adolescents with severe obesity, that were not enriched for *GIP-R* variant rs2302382, and additional healthy, lean controls. The MAF of the C-allele was found to be 22.6% in children and adolescents with severe obesity, compared to 21.7% in the control group.

Within the coding region of *GIP*, a single non-synonymous variant (NSV) (rs2291725, p.Ser103Gly) was identified. This NSV was found at similar frequencies in both study groups (patients with obesity and healthy, lean controls) and across both sexes (women and men; see Table 5, see Supplementary Tables 8 and 9). *In silico* analyses indicate a benign effect (see Supplementary Tables 10).

The mutation screen of GCG and its receptor revealed 10 genetic variants in total

Similar to *GIP*, a single variant was detected within the coding regions of *GCG* (see Table 5, see Supplementary Tables 8 and 9). The synonymous variant (SV) rs5645 (p.Tyr5=) was determined in five carriers in each study group. Among children and adolescents with severe obesity, four carriers were female and one was male, while in the group of healthy, lean individuals, two carriers were women and three were men (see Supplementary Tables 8 and 9). *In silico* tools indicate a putative deleterious effect of rs5645 (see Supplementary Table 10).

In the coding region of *GCG-R*, a total of nine variants was identified, most of which were infrequent (MAF < 5%). Six of these were NSVs. Three NSVs were detected exclusively in children and adolescents with severe obesity (rs781413720, p.Val236Met; rs545914836, p.Ser438Leu and rs183571856, p.Gly440Ser; see Table 3). A rare NSV, rs1889736697 (p.Thr76Met; MAF < 2%), was predicted to be potentially pathogenic (see Supplementary Table 10) and was identified in three male children or adolescents with obesity and one female control individual (see Supplementary Tables 8 and 9). When stratified by sex, five NSVs in *GCG-R* were identified among women, including three rare NSVs found exclusively in women. Among men, three NSVs and three SVs were detected, including one NSV (rs138492107, p.Asp458His) found exclusively in a single male control individual (see Supplementary Tables 8 and 9).

Table 3
Identified variants within *GIP*, *GIP-R*, *GCG*, *GCG-R* and *GLP-1R*.

Gene	Variant	Genomic position (GRCh38)	Amino acid change	Allele 1	Allele 2	Individuals with obesity (n = 94)				Healthy, lean controls (n = 96)			
						11	12	22	MAF	11	12	22	MAF
GIP	rs2291725	chr17: 48961770	Ser103Gly	A	G	AA: 24	AG:45	GG:25	49%	AA: 29	AG:40	GG:27	49%
GIP-R[#]	rs191937467	chr19: 45674137	Ala150Thr	G	A	93	1	0	0.5%	96	0	0	0%
	rs139215588	chr19: 45674762	Arg190Gln	G	A	94	0	0	0%	95	1	0	0.5%
	rs1800436	chr19: 45674813	Ala207Val	C	T	94	0	0	0%	95	1	0	0.5%
	rs1800437	chr19: 45678134	Glu354Gln	G	C	GG:75	GC:19	CC: 0	10.1%	GG:50	GC:35	CC:11	30%
	rs137944672	chr19: 45678220	Ser321=	C	T	93	1	0	0.5%	96	0	0	0%
	rs776722055	chr19: 45681819	Arg429Trp	C	T	94	0	0	0%	95	1	0	0.5%
GCG	rs5645	chr2: 162149164	Tyr5=	C	T	89	5	0	3%	91	5	0	2.6%
GCG-R	rs1801483	chr17:81809839	Gly40Ser	G	A	91	3	0	1.6%	91	5	0	2.6%
	rs189736697	chr17:81810888	Thr76Met	C	T	91	3	0	1.6%	95	1	0	0.5%
	rs5386	chr17:81811293	Ala155=	C	G	77	15	2	10.4%	76	17	3	12.0%
	rs781413720	chr17:81811699	Val236Met	G	A	93	1	0	0.5%	96	0	0	0%
	rs368152053	chr17:81811803	Ile270=	C	T	93	1	0	0.5%	95	1	0	0.5%
	rs5384	chr17:81812864	Phe365=	C	T	63	26	5	19.2%	62	32	2	18.8%
	rs545914836	chr17:81813568	Ser438Leu	C	T	93	1	0	0.5%	96	0	0	0%
	rs183571856	chr17:81813573	Gly440Ser	G	A	93	1	0	0.5%	96	0	0	0%
	rs138492107	chr17:81813627	Asp458His	G	C	94	0	0	0%	95	1	0	0.5%
GLP-1R	rs10305420*	chr6: 39048860	Pro7Leu	C	T	CC:49	CT: 33	TT: 12	30%	CC:40	CT: 36	TT: 20	40%
	rs3765468	chr6: 39065817	Lys130=	G	A	86	7	1	5%	75	20	1	11.5%
	rs6918287	chr6: 39065826	Glu133=	A	G	92	2	0	1%	93	3	0	2%
	rs61754624	chr6: 39066295	Leu167=	C	T	94	0	0	0	95	1	0	0.5%
	rs6923761	chr6: 39066296	Gly168Ser	G	A	GG:50	GA:35	AA: 9	28%	GG:44	GA:36	AA: 16	35%
	rs2235868	chr6: 39072878	Arg176=	A	C	AA: 26	AC: 50	CC:18	46%	AA: 20	AC: 49	CC:27	46%
	rs1042044*	chr6: 39073726	Leu260Phe	A	C	AA: 19	AC: 53	CC:22	48%	AA: 17	AC: 52	CC:27	45%
	rs10305492	chr6: 39079018	Ala316Thr	G	A	92	2	0	1%	95	1	0	0.5%
	rs1126476	chr6: 39080715	Ile400=	A	C	AA: 30	AC: 50	CC:14	41%	AA: 22	AC: 46	CC:28	47%

Individuals with obesity have been selected based on their genotype of rs2302382, an intronic variant in *GIP-R* ([30]). All non-synonymous variants are marked in yellow. The sex-stratified data is presented in see Supplementary Tables 8 and 9. *These variants were additionally genotyped in 777 children and adolescents with severe obesity and both biological parents (see Tables 4 and 5). [#] As we screened a study group enriched for the

GIP-R rs2302382 variant, the group of individuals with obesity may not be truly representative.

Nine variants detected in GLP-1R

Nine variants were observed within the coding region of *GLP-1R*, including four NSVs and five SVs (see Table 2). Three NSVs were present in both study groups and across both sexes (see Supplementary Tables 8 and 9). The common NSV rs10305420 (p.Pro7Leu) was found in both groups. Another common NSV, rs1042044 (p.Leu260Phe) was previously identified in our sex-specific analyses and demonstrated a sex-specific effect on the BMI, with

stronger effects in men (see Fig. 2 and Supplementary Table 1). Yet, *in silico* analyses predominantly predicted benign effects (see Supplementary Table 10).

LD analysis of the identified *GLP-1R* variants, along with those associated with sex-specific BMI effects (see Fig. 2, see Supplementary Table 1), revealed a high LD between rs2235868 (p.Arg176=) and rs1126476 (p.Ille400=; CEU $r^2 = 0.96$, GBR $r^2 = 0.87$; see Supplementary Tables 11 and 12, see Supplementary Figs. 3 and 4). The strongest LD between a mutation screen derived variant and a variant demonstrating a sex-specific BMI effect was observed for rs2235868 (p.Arg176=) and the intronic (rs910165; CEU $r^2 = 0.94$, GBR $r^2 = 0.94$).

No Indications for a transmission disequilibrium of three GLP-1R variants

Subsequently, due to our findings in preceding analyses, we genotyped three variants located in *GLP-1R* in additional, independent family-based samples (777 families)[31]. Two variants based on our GWAS-based sex-specific analyses were selected, namely rs1042044 (p.Leu260Phe) and rs910170 (intronic variant; see Supplementary Table 1). The NSV rs1042044 was included as it represented the only coding variant with a sex-specific effect on BMI, whereas rs910170 was chosen due to its small p-value and strong LD with rs1042044 (see Supplementary Tables 1, 2, and 3). Additionally, rs10305420 was analysed. Genotype distributions for all three variants were consistent with the HWE in the confirmation group.

No evidence of transmission disequilibrium was observed for any of the risk alleles analysed. Furthermore, no sex-specific effects were detected (see Table 4).

Table 4
Results of the transmission disequilibrium tests for *GLP-1R* variants in families with an offspring with severe obesity

variant	Chromosomal position	Sex analysed*	N genotyped families	Risk allele	Transmitted (%)	Non-transmitted (%)	p-value
rs1042044	Chr 6: 39073726	Girls and boys	777	A	398 (51.89%)	369 (48.11%)	0.30
		Girls	424	A	217 (51.91%)	201 (48.09%)	0.43
		Boys	353	A	181 (51.86%)	168 (48.14%)	0.49
rs910170	Chr 6: 39064639	Girls and boys	777	A	413 (52.95%)	367 (47.05%)	0.10
		Girls	424	A	232 (53.83%)	199 (46.17%)	0.11
		Boys	353	A	181 (51.86%)	168 (48.14%)	0.49
rs10305420	Chr 6: 39048860	Girls and boys	777	T	363 (49.52%)	370 (50.48%)	0.80
		Girls	424	T	201 (49.88%)	202 (50.12%)	0.96
		Boys	353	T	162 (49.09%)	168 (50.91%)	0.74

The TDT included 777 families with an offspring with severe obesity and both biological parents previously analysed in a family-based GWAS[31]. The chromosomal position is based on GRCh38. *The sex indicated refers to the sex of the offspring with severe obesity.

DISCUSSION

Incretin mimetics, such as semaglutide or tirzepatide, have transformed clinical management of obesity by inducing substantial and sustained weight loss alongside marked metabolic improvements[8–12]. Previously, lifestyle interventions and bariatric surgery represented the primary options for weight reduction[2–4]. However, treatment responses across all available approaches show a marked heterogeneity, meaning some patients experience only marginal benefits, or none at all[25]. The underlying mechanisms for these variations remain incompletely understood. Growing evidence indicates that sex-specific effects and genetic variants in key incretin-related genes, such as *GIP*, *GIP-R* and *GLP-1R*, may contribute to this heterogeneity[13,16,18–20,22,25]. Yet, the extent to which such variants may exert sex-dependent effects on BMI and treatment outcomes was previously largely unknown.

In the present study, we analysed sex-specific effects of genetic variants in *GIP*, *GIP-R*, *GCG*, *GCG-R*, *GLP-1* and *GLP-1R*. Our analyses identified nine *GLP-1R* variants in high LD, one of which (rs1042044) is a non-synonymous variant, (p.Leu260Phe). While there have been reports of nominal associations of rs1042044 with the BMI and reduced HbA1c levels[32], other studies have failed to detect significant effects[33]. Additionally, a nominal association has been described between rs1042044 and the response to antipsychotic medications, with improved symptom severity reported following olanzapine treatment, among others[34]. However, it should be noted that the majority of these findings were not corrected for multiple testing, and as such, should be interpreted with caution.

We extended our investigation to a cohort of patients who had undergone bariatric surgery. A nominal association between rs1042044 genotype and achieving more than 10% total weight loss was observed in women only and the combined analyses (women + men). However, this association did not remain significant after correction for multiple testing. Previous studies have shown that post-surgical weight loss is strongly influenced by pre-operative body weight[35]. Therefore, it could be hypothesized that the observed association with weight loss is driven by the association of rs1042044 with BMI. However, rs1042044 was associated with BMI only in males, whereas nominal associations with post-operative weight loss was evident in females and in the combined cohort. Although these findings require validation, they may reflect sex-specific genetic effects, whereby the phenotypic impact of a genetic variant differs between males and females. Given these findings and due to the limited number of individuals achieving greater weight loss (e.g., 40%),

larger study groups are needed to validate potential effects. Still, our findings imply that sex-specific analyses may reveal additional genetic factors contributing to variability in postoperative outcomes.

A recent large-scale study including more than 10,000 individuals treated with *GLP-1R* agonists or undergoing bariatric surgery reported no significant association between individual genetic variants and weight loss after correction for multiple testing. Solely a higher polygenic risk score for BMI was associated with reduced weight loss following bariatric surgery[14]. However, this study did not perform sex-stratified analyses, which raises the question of whether sex-dependent effects, such as the trend observed in our study, may have gone undetected (e.g., [25]).

Yet, rs1042044 did not functionally impact the GLP-1R in our assay as no differences in receptor internalisation between the mutant (Phe260) receptor and the native GLP-1R were observed, regardless of the native GLP-1 or semaglutide was used. Several studies have functionally characterised *GLP-1R* variants, particularly rare missense variants, demonstrating various effects on receptor function, including significant effects on β-arrestin 2 recruitment, as well as LoF and GoF alterations in cAMP signalling[18], comparable to those observed for the melanocortin-4-receptor[36–39]. In addition, Koole et al. reported that rs1042044 (p.Leu260Phe) is associated with reduced GLP-1R cell surface expression, while ligand binding (native GLP-1 and exenatide) and downstream signalling remained largely persevered ([40]). Therefore, this variant may affect receptor trafficking or membrane localisation rather than agonist-induced receptor internalisation. Reduced GLP-1R surface expression has previously been reported to a be a risk factor for a higher BMI, while reduced β-arrestin 2 recruitment appeared to have beneficial metabolic effects[18]. Although rs1042044 did not affect GLP-1R internalisation in our assay, it may influence other aspects of GLP-1R function that were not assessed in this study. Furthermore, despite being the only coding variant within the identified LD block, rs1042044 may not be the causal variant. The observed association may instead reflect the combined effects of the nine variants in high linkage disequilibrium, or the influence of another causal variant in linkage disequilibrium. Consequently, further functional studies are required to identify the causal variant(s) and elucidate the underlying molecular mechanisms.

In our mutation screen, which was conducted in an enriched study group of patients with obesity who contributed to a significant transmission disequilibrium test for a variant in GIP-R[30], we identified several variants across the five genes of interest. Some, particularly within *GIP-R* and *GLP-1R* have already been functionally characterised. For instance, the *GLP-1R* variant rs6923761 had been associated with a smaller reduction in HbA1c and a greater delay in gastric emptying following incretin therapy[16,17,22]. Yet, again caution is needed as most of these analyses did not correct for multiple testing.

Additionally, the *GIP-R* variant rs139215588 (p.Arg190Gln), which we identified exclusively in a healthy, lean female had been associated with a lower BMI and body weight[23]. Functional analyses further indicated an impaired G-protein coupling, reduced arrestin recruitment, defective internalisation and endoplasmic reticulum retention, classifying rs139215588 as a loss of function variant[20]. Interestingly, another *GLP-1R* variant rs10305420 has been associated with improved efficacy of incretin mimetics, with each risk allele contributing to an average additional weight loss of approximately 0.76 kg[25].

By contrast, the *GIP-R* variant rs1800437 (p.Glu354Gln) has been previously predicted to exhibit wildtype-like effects for both, cAMP production and β-arrestin 2 recruitment[20].

Overall, we identified several rare NSVs, including four in *GIP-R*, five in *GCG-R* and three in *GLP-1R* (see Table 5). All rare NSVs warrant further investigations in dedicated follow-up studies, even though some have already been functionally characterised (e.g., rs139215588[23]). This also applies to variants identified exclusively in healthy, lean individuals.

Generally, the majority of genetic studies on the genes of interest have not included sex-stratified analyses[14,16,25]. To our knowledge, we are the first to describe a sex-specific effect of rs1042044 and the respective variants located in *GLP-1R* in high LD. While these variants showed a stronger effect on the BMI in men, the clinical relevance of this findings remains to be elucidated. Nevertheless, our results emphasise the importance of conducting sex-specific analyses as the association with BMI and particularly the male-specific effect, would have been missed entirely if we had relied solely on sex-combined data. Collectively, these observations highlight the general need for larger, sex-stratified cohorts to uncover sex-specific genetic effects on BMI and weight loss outcomes following pharmacological and surgical interventions.

Taken together, our findings suggest that genetic variants, particularly within the *GLP-1R* gene, may contribute to BMI in a sex-specific manner. The nine variants in *GLP-1R* that are in high linkage disequilibrium represent candidate loci for future investigation, given their stronger association with BMI in men than in women. Whether the impact of genetic variants on weight loss following obesity therapies differs between men and women remains to be determined in larger cohorts. Future large-scale studies of individuals treated with semaglutide, tirzepatide, or undergoing bariatric surgery should therefore incorporate sex-stratified analyses and detailed genotype–phenotype assessments to identify potential sex-dependent genetic modifiers of weight loss outcomes.

METHODS

Study groups

Various study groups have been analysed (see Table 5). Analyses of a specific candidate variant (rs1042044, p.Leu260Phe) were performed in a cohort from the Leipzig Obesity Biobank (LOBB; https://www.helmholtz-munich.de/en/hi-mag/cohort/leipzig-obesity-bio-bank-lobb). The cohort included 807 human adipose tissue (AT) donors with longitudinal weight trajectory data. AT samples were obtained from individuals undergoing bariatric surgery, involving laparoscopic sleeve gastrectomy or laparoscopic Roux-en-Y gastric bypass, as previously described[41]. Approximately 500 mg of fresh visceral AT depots were immediately flash-frozen in liquid nitrogen and stored at – 80°C until further analysed. Body composition and obesity-related parameters were

assessed following established protocols[42]. Exclusion criteria comprised individuals under 18 years of age, with chronic substance or alcohol misuse, acute inflammatory diseases, end-stage malignant diseases, uncontrolled thyroid disorder and Cushing's disease as well as individuals that smoked within the last year leading up to surgery. The cohort consists of 542 female donors (body weight: 137.59 ± 27.74 kg; minimum measured body weight: 95.64 ± 22.74 kg; maximum measured body weight: 141.01 ± 29.27 kg; body weight one year after surgery: 100.27 ± 24.01 kg; see Table 5) and 265 male donors (body weight: 163.25 ± 8.56 kg; minimum measured body weight: 117.6 ± 26.13 kg; maximum measured body weight: 168.07 ± 31.59 kg; body weight one year after surgery: 122.52 ± 26.86 kg; see Table 5).

Sanger sequencing of the coding regions of *GIP*, *GIP-R*, *GCG*, *GCG-R* and *GLP-1R* was performed in a screening group of 94 children and adolescents with severe obesity (89% with BMI percentile > 95th) and 96 healthy, lean control individuals (BMI percentile ≤ 15th, see Table 5). Children and adolescents with severe obesity were selected based on their genotype at rs2302382, an intronic variant in *GIP-R*[30] and thus may be enriched for additional *GIP-R* variants. This group was derived from obesity trios (offspring with severe obesity and both biological parents) who predominantly contributed to a positive TDT in our previous study[30].

Two non-synonymous variants and one intronic *GLP-1R* variant were genotyped in additional, independent samples of a confirmation group. In total, 777 obesity trios (offspring with severe obesity and both biological parents) from a family-based genome-wide association study[31] were investigated (see Table 5). The variant rs1800437 (p.Glu354Gln) was additionally genotyped in study groups of 475 children and adolescents with severe obesity and 368 healthy, lean control individuals independent of the screening group (see Table 5).

All participants and in case of minors their parents gave written informed consent. The study was approved by the Ethics Committees of the University of Duisburg-Essen (approval number: 05-2954), University of Marburg (approval number: 153/97, 74/97, 78/97), and the University of Leipzig (approval number: 159-12-21052012 & 017-12ek) and was further performed in accordance with the *Declaration of Helsinki*[43], the Bioethics Convention (Oviedo) and EU Directive on Clinical Trials (Directive 2001/20/EC).

GWAS-based sex-specific analyses

To assess putative sex-specific effects of genetic variants within the genes of interest (*GIP*, *GIP-R*, *GCG*, *GCG-R* and *GLP-1R*) and an adjacent region of ± 100 kb, data of a sex-stratified GWAS for BMI variance[27] was extracted. Information regarding the chromosomal position (GRCh37, see Table 6), variant ID, alleles, minor allele frequency (MAF), standard error (SE), beta and p-values of the genetic variants were retrieved from the publicly available summary statistics data (downloaded from https://zenodo.org/records/1251813#.XCLJ7vZKhE4 on August 1, 2024)[27]. Effect alleles for men and women were checked for homogenous assignment, while palindromic variants with a MAF between 0.48 and 0.52 were excluded. The variants effect directions were subsequently harmonized to eliminate strand issues by converting the beta values of the tested variants for their major alleles. Next, the z-scores were calculated for variants with a genome-wide significance ($p < 5*10^{-8}$) within the investigated regions (see Eq. (1))[44].

$$z - score = \frac{beta_{female} - beta_{male}}{\sqrt{SE^2_{female} + SE^2_{male}}}$$

1

Genetic variants were classified to have a sex-specific effect if |z-scores| ≥ 3, as previously defined[44]. The analyses were performed in R (version 4.3.3). Graphs were generated using GraphPad's Prism (version 10.3.1).

Variant (rs1042044) look-up in the LOBB cohort comprising patients undergoing bariatric surgery

Genomic DNA was extracted from EDTA-anticoagulated whole blood using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. DNA samples were adjusted to a final concentration of 60 ng/µl in a volume of 15 µl and transferred into 96-well plates. Genotyping was performed by Life&Brain GmbH (Bonn, Germany) using the Infinium Global Screening Array-24 v3.0 (GSA) combined with the Multi Disease BeadChip (GSAMD-24v3-0-EA_20034606_A1), covering over 650,000 markers genome-wide. The genotyping data analyzed in this study were generated as part of a previously published project and are described in detail by [45].

Quality control was conducted using PLINK v1.9 [46,47]. First, samples with more than 5% missing genotype data were excluded. Genetic variants with over 20% missingness across samples were initially filtered out, followed by a 2% cutoff, resulting in a final call rate of about 98%. Sex verification was conducted by examining the inbreeding coefficient (F values) on the X chromosome and individuals with F values inconsistent with their reported sex were excluded. Hardy-Weinberg equilibrium (HWE) testing was performed to identify and exclude variants deviating significantly from equilibrium (p-value < $1*10^{-6}$), first in controls and then in cases, to remove variants likely affected by genotyping errors or population stratification. Samples exhibiting heterozygosity rates exceeding three times the interquartile range above the median were excluded. Only autosomal variants with MAF ≥ 5% and minor allele count (MAC) ≥ 5 were retained. Identical twins (pihat = 1) were removed. Population stratification was evaluated through multidimensional scaling (MDS), comparing the samples to references populations from the 1000 Genomes Project (1000G Phase3 v5 Reference)[48]. Individuals of non-European ancestry were excluded to maintain population homogeneity. Data accuracy for genotype imputation was verified using the check-bim tool v4.3.0 (https://www.chg.ox.ac.uk/~wrayner/tools/), which detected strand inconsistencies, allele frequency deviations, palindromic variants, and other potential errors. Genotype imputation was performed using the Michigan Imputation Server 2[49] with Minimac4 v1.6.8, employing the 1000 Genomes Phase 3

reference panel v5 and Eagle v2.4 for phasing, focusing on European populations and array build hg19. Afterwards, invariant sites (also called monomorphic sites) were removed.

To define cohort subgroups, absolute weight loss was computed as maximum weight – weight one year after surgery. The total weight loss percentage (TWL%) was defined as absolute weight loss / maximum weight * 100. Cohort subsets were established based on individuals with more than 10, 15, 20 and 40 TWL%, as well as a group of 100 individuals with the highest TWL%. Allele frequencies were computed using vcftools (v0.1.16)[50] for the entire cohort, as well as separately for men and women.

A two-sided chi-squared test was performed using IBM's SPSS Statistics (version 29.0.0). If the expected frequency in at least one cell was less than 5, the Fisher-Freeman-Halton test was performed. $P < 0.05$ was considered to be nominally significant, while the Bonferroni-corrected threshold was $p < 0.0033$.

Cell Culture

HEK293T cells (female sex) were cultured in Dulbecco's Modified Eagle Medium (DMEM, Life Technologies) with 10% heat-inactivated fetal bovine serum (FBS, Life Technologies), 100 IU/mL of penicillin and 100 µg/mL of streptomycin solution (Pen-Strep, Sigma-Aldrich). All cells were maintained at 37°C in 5% CO_2.

Analyses of ligand-induced receptor internalization of native and mutant GLP-1R

Human GLP-1R-Rluc8 (hGLP-1R-Rluc8) was a gift from Professor Patrick Sexton (Monash University, Melbourne, Australia). EGFP-CAAX was a gift from Lei Lu (Addgene plasmid #86056).

Mutagenesis was performed using the Q5® Site-Directed Mutagenesis Kit according to the manufacturer's instructions. Mutagenic primers were designed using NEBaseChanger/NEB primer design software, and the plasmid template was amplified using the Q5 Hot Start High-Fidelity DNA Polymerase. PCR products were assessed on a 1% agarose gel, then treated with the KLD reaction mix, which phosphorylates and ligates the linearized PCR product while digesting methylated template plasmid with DpnI. The resulting plasmid was transformed into chemically competent *E.coli* and recovered in SOC medium before plating.

Bioluminescence resonance energy transfer (BRET) assays were performed in HEK293T cells, a widely and accepted cell model system for studying GPCR signaling and trafficking. To begin the assays, cells were seeded at 700k cells per well in 6-well plates. After 24h plasmids were overexpressed using Lipofectamine 2000 (Invitrogen) as per manufacturer's instructions. On the next day, cells were washed with phosphate buffered saline (PBS; Gibco) and resuspended in Fluorobrite (Gibco) supplemented with 5% HI-FBS and 1% L-glutamine, before being seeded at 100k cells per well into a poly-D-lysine (Sigma Aldrich) coated 96-well plate. The following day, baseline fluorescence and luminescence reading were recorded with a PHERAstar FSX plate reader, using Renilla luciferase (Rluc) substrate coelenterazine (Biomol). The luciferase substrate was added to Hanks Balanced Salt Solution (Sigma Aldrich) before being added to the cells. After recording baseline, agonists were applied and reading completed for a set number of cycles at an adjusted cycle time. A gain adjustment was performed to obtain the correct gain value before the initial baseline reading. Experiments were performed three times on three separate biological replicates. All ligand responses over time were normalized to the vehicle group and first two cycle readings. Dose response negative iAUC data is expressed as a log normalized means ± SEM, followed by three parameter non-linear regression. A linear mixed-effects model was fitted to evaluate the effects of time and ligend on the internalization levels, using time and ligand (WT + GLP-1(7–36)amide, WT + Semaglutide, Mutant + GLP-1(7–36)amide, Mutant + Semaglutide) as fixed effects and replicate as a random intercept. The statistical analyses were either performed with GraphPad's Prism (version 10.5.0) or IBM'S SPSS Statistics (version 29.0.0).

Mutation screen

The coding regions of *GIP* (chr17: 48,958,554–48,968,596; ENST00000357424.2; GRCh38), *GIP-R* (chr19: 45,668,221–45,683,722; ENST00000590918.6; GRCh38), *GCG* (chr2: 162,142,882–162,152,404; ENST000004118842.7; GRCh38), *GCG-R* (chr17:81,804,150–81,814,008; ENST00000400723.8; GRCh38) and *GLP-1-R* (chr6:39,048,781–39,091,303; ENST00000373256.5; GRCh38) were amplified in a semi-quantitative PCR using coding region-specific primers (see Suplementary Table 13). Amplicons were confirmed by a 2.5% agarose gel electrophoresis and subsequent unidirectional Sanger sequencing (performed by MicroSynth Seqlab GmbH, Göttingen, Germany). For exon 6 of *GIP-R*, an additional sequencing primer was used for Sanger sequencing to confirm the respective amplicon with high precision. For all other amplified fragments, the primers used in the PCR yielded sufficient sequencing quality to allow these fragments to be confirmed (see Supplementary Table 13). Evaluation of sequence quality and genotype assignment was performed independently by at least two scientists using the SeqMan Pro software (version 17). In case of discrepancies, consensus was reached, or the sample was re-sequenced. All detected variants were subsequently confirmed by bi-directional Sanger sequencing.

As our study cohort was intentionally enriched with children and adolescents with severe obesity who carry the *GIP-R* variant (rs1800437)[30], and who may therefore be carriers of further variants in the genes of interest, we did not assess deviations from HWE or perform statistical analyses for the variants detected in the mutation screen.

Genotyping

One non-synonymous (rs1042044, p.Leu260Phe) and one intronic variant (rs910170) located within *GLP-1R* showing a sex-specific effect and the *GLP-1R* variant rs10305420 (p.Pro7Leu) were subsequently genotyped in additional, independent family-based samples comprising offspring with severe obesity and both biological parents (see Table 5)[31]. Variant-specific TaqMan assays (ThermoFisher Scientific, assay ID: C_30514262_10 (rs10305420),

C___2491162_1_ (rs910170) and C___2491143_10 (rs1042044)) were performed on the QuantStudio3 (Applied Biosystems) based on the manufacturer's manual. Genotype assignment was performed and confirmed by at least two independent scientists. Samples with discrepancies and ambiguous genotypes were re-analysed.

The TDT[51] was applied for these three genetic variants genotyped. The TDT was calculated in R (version 4.3.3) using the McNemar's chi-squared test[52].

Additionally, the non-synonymous variant rs1800437 (p.Glu354Gln) was genotyped in independent study groups of children and adolescents with severe obesity, and in healthy lean individuals acting as controls, as we detected indications of a functional consequence (see Table 5, see Supplementary Table 10). Again, a variant-specific TaqMan assay (ThermoFisher Scientific, assay ID: C___2659022_50) was performed on the QuantStudio 3.

For all genotyped variants, the HWE was checked and showed no deviations.

In silico analyses

Linkage disequilibrium (LD) structures were analysed using LDmatrix, which is publicly available via the LDlink website (https://ldlink.nih.gov/?tab=home). European-based populations of GBR as well as CEU extracted from the 1000 Genomes Project (GRCh38) were utilized. Two groups of variants were analysed: (1) variants that were identified in our GWAS-based sex-specific analysis that have a sex-specific effect on the BMI and (2) variants detected in the mutation screen located in *GLP-1R* together with variants of group (1). Genetic variants were considered to be in high LD if $r^2 > 0.8$.

To obtain hints at functional implications of the detected variants in the genes of interest, several *in silico* tools were applied. General implications on pathogenicity and conservation (phyloP score) were provided by MutationTaster2021[53]. Additionally, the Variant Effect Predictor (VEP)[54] was employed. VEP contains data of numerous *in silico* tools, such as the Sorting from Intolerant to Tolerant (SIFT)[55], AlphaMissense[56] and SpliceAI[57]. For non-synonymous variants, PolyPhen2[58] was used to predict further effects on the respective protein and its structure. The protein sequences required for PolyPhen2 were extracted from Ensembl.

Declarations

Competing Interests

MB received honoraria as a consultant and speaker from Abbott, Amgen, AstraZeneca, Bayer, Boehringer Ingelheim, Daiichi–Sankyo, Lilly, MSD, Novo Nordisk, Novartis, Pfizer, and Sanofi; and reports chairing a Clinical Trial Data Safety Monitoring Board for Boehringer Ingelheim. All other authors declare no competing interests.

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FUNDING

This study was funded by the Deutsche Forschungsgemeinschaft (DFG, HI 865/2 – 1, DFG Research Unit FOR2488, project number 209933838 - SFB 1052 (project B1)), the BMBF (01GS0820; PALGER 2017-33: 01DH19010), the Deutsches Zentrum für Diabetesforschung (DZD, Grant: 82DZD00601), and the Stiftung Universitätsmedizin Essen. We further acknowledge support by the Open Access Publication Fund of the University of Duisburg-Essen.

Author Contribution

AHi, TP, JH, YZ, ALV, SMM, CK and LSR have designed the study. LSR performed the GWAS-based sex-specific analyses. MB, JH and Ahi recruited patients and control participants. HH and MC generated SNP data for LOBB. TH, AHo, PK and MC performed genetic analyses based on LOBB cohort. AN, EK, CC and TDM conducted the functional in vitro analyses. JS, SK, SMM, NB, ALV, YZ and LSR performed the genetic analyses in the screening and confirmation cohort. TP and LSR performed the statistical analyses. JH, MB, TDM and AHi supervised the project. AN, AHo, TH, TO and LSR wrote the manuscript. All authors contributed to data analysis and interpretation. All authors reviewed the manuscript.

Acknowledgement

We thank all participants for their participation. Further, we like to thank Sieglinde Düerkop for her excellent technical support. We gratefully acknowledge Julius Honecker, Phil Kubitz and Emil Jørsboe for generating the SNP array data for the LOBB cohort.

Data Availability

The sequencing data generated or used in this study cannot be deposited in publicly accessible or external repositories with a controlled access due to restrictions arising from participant consent and applicable ethical and data protection requirements. However, the data are available to qualified researchers upon reasonable request and subject to the respective internal review and approval procedures described below.The Sanger sequencing data generated in this study are available upon reasonable request from Anke Hinney (anke.hinney@uni-due.de) or Luisa Rajcsanyi (luisa.rajcsanyi@uk-essen.de). Requests are subject to internal review and approval in accordance with the applicable ethical, consent, and data protection requirements.Access to the human RNA-seq data from the LOBB study is regulated by the LOBB Steering Committee. Data access is restricted to non-commercial research using non-identifiable data and requires an approved Data Use Agreement (DUA) outlining the permitted research purposes and safeguards for participant privacy. Requests will be acknowledged within 5–10 business days and, following review, will typically be granted or denied within 2–4 weeks, depending on the completeness of the application and the scope of the requested data. Access is restricted to non-commercial, non-identifiable research use and may be granted to named researchers or institutions following approval by the LOBB Steering Committee and execution of the DUA. Requests for access should be directed to Matthias Blüher (matthias.blueher@medizin.uni-leipzig.de) or Anne Hoffmann (anne.hoffmann@helmholtz-munich.de).In addition, publicly available datasets from previously published studies were analyzed in this study. Details on these datasets, including their respective sources and references, are provided in the Methods section and the corresponding publications.

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Figures

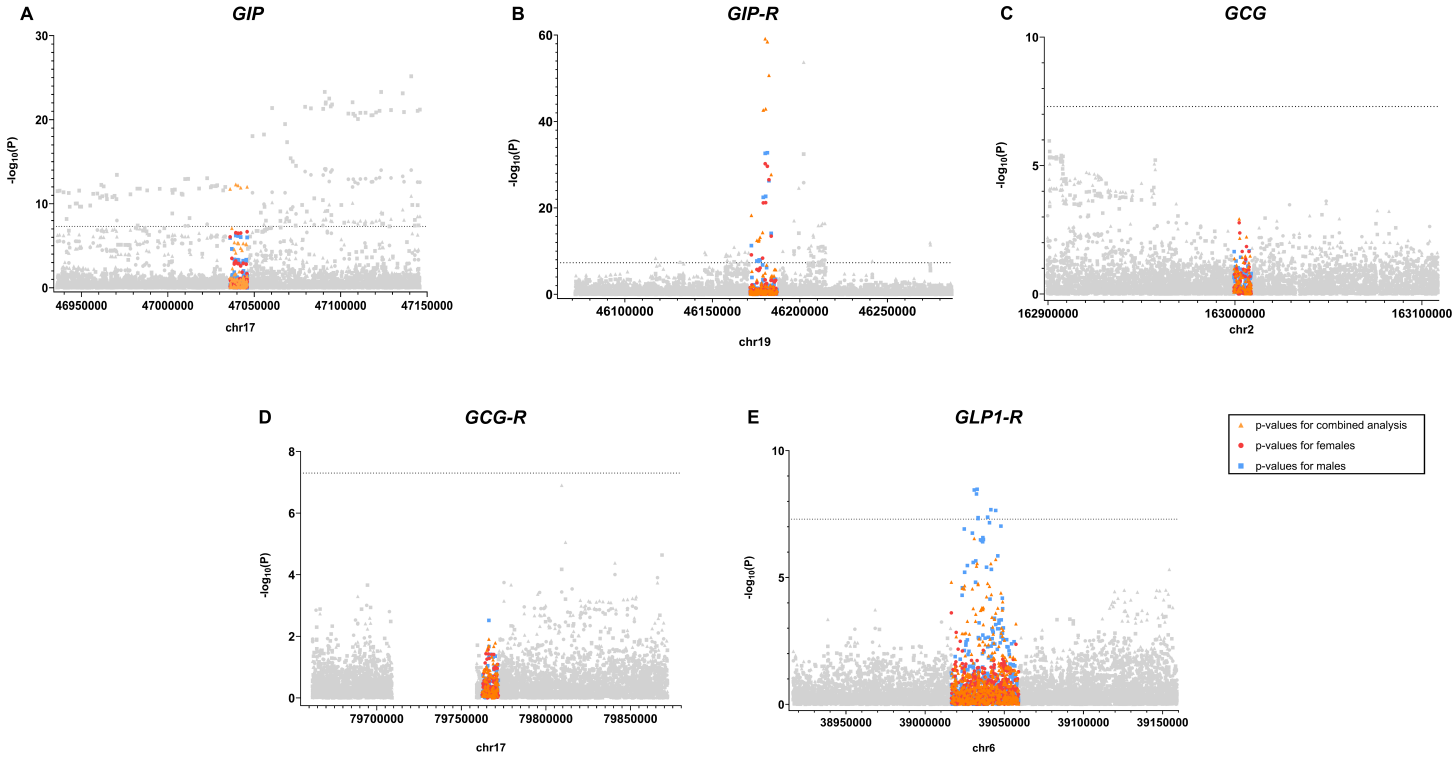


Figure 1

P-values of variants located in GIP, GIP-R, GCG, GCG-R and GLP-1R and adjacent regions. Regions of genetic variants located in GIP (A), GIP-R (B), GCG (C), GCG-R (D) and GLP-1R (E) and 100 kb adjacent regions were extracted from a sex-stratified BMI GWAS[27]. A large fragment within the analysed regions adjacent to GCG was not sufficiently covered by the GWAS dataset. Variants within the gene regions are shown in orange (men + women), red (women) and blue (males), while variants in the 100 kb adjacent regions are represented in light grey. Data regarding analyses with both sexes are plotted as a triangle, while women only and men only data are shown as dots or squared, respectively.

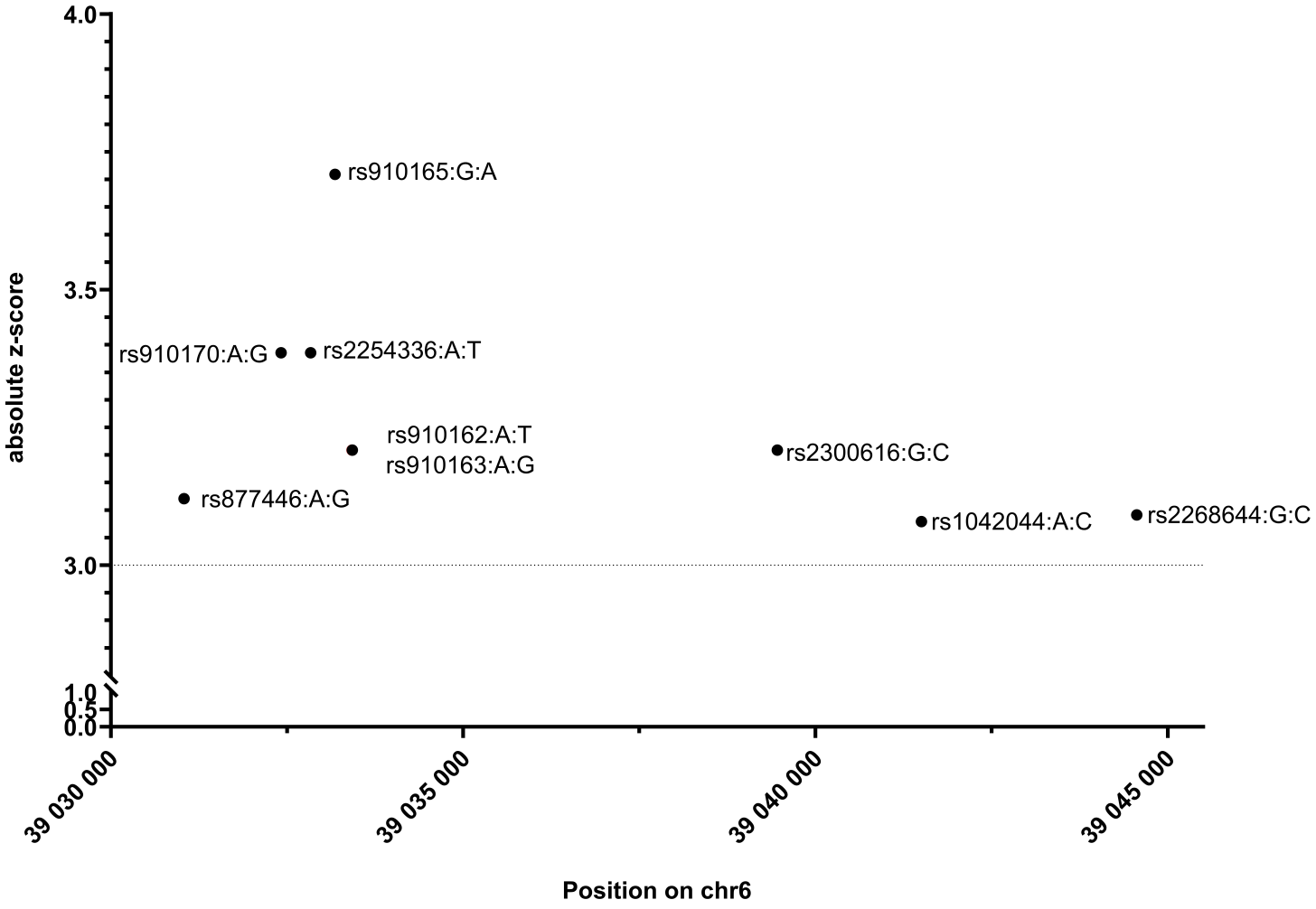


Figure 2

Z-Scores of genome-wide significant variants in GLP-1R. The absolute z-scores were plotted against the chromosomal position of each variant (GRCh37). All presented variants were exclusively significant for BMI in men[27]. For the respective p-values and effect sizes, please refer to Supplementary Table 1.

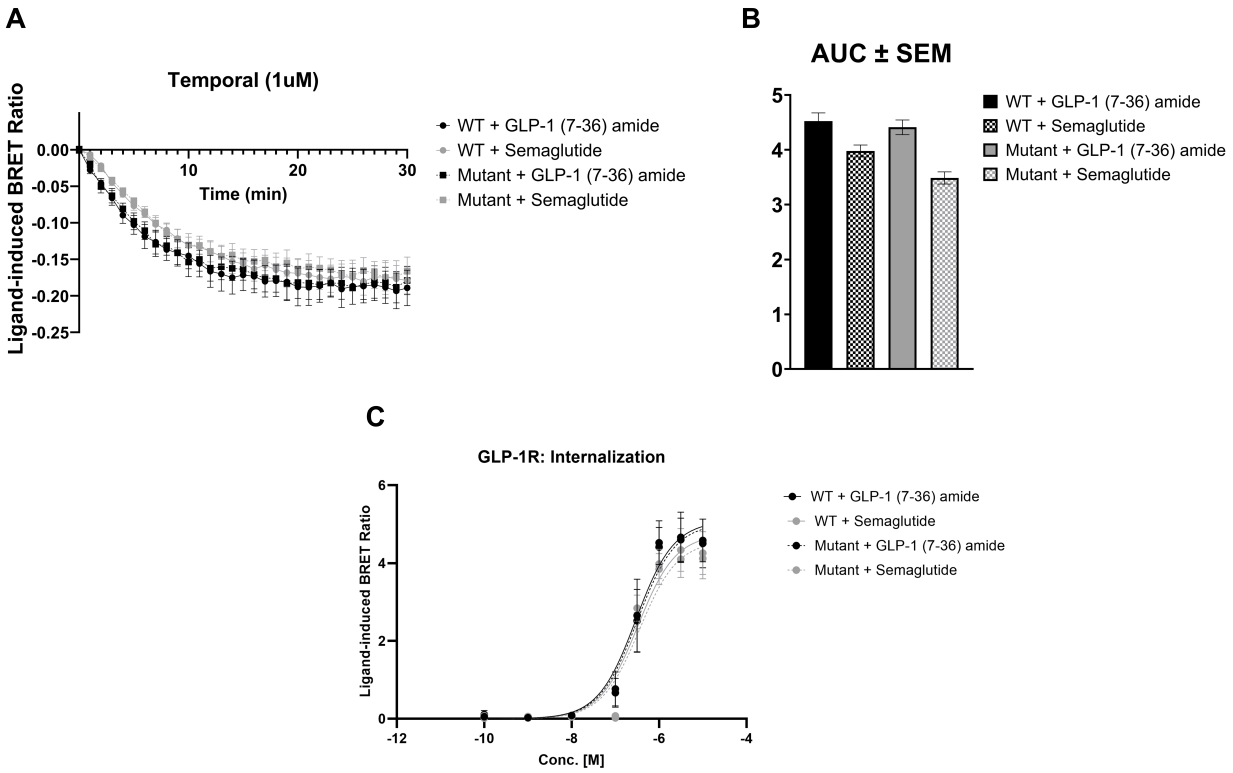


Figure 3

Temporal and dose-dependent effects on mutant and native GLP-1R internalisation. Time course of GLP-1R internalization upon stimulation with 1 μM of either GLP-1(7-36)amide or the GLP-1 analogue semaglutide (A & B) as measured by BRET. (C) Dose-response curves of ligand-induced GLP-1-R internalization for both agonists. The mutant GLP-1-R carried the Phe260 allele.

Supplementary Files

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