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Original article

Rare gene variants and weight loss at 10 years after sleeve gastrectomy and gastric bypass - a randomized clinical trial

Petra Loid, M.D., Ph.D.[a,b,c,*], Sofia Grönroos, M.D., Ph.D.[d,e,f], Saija Hurme, M.Sc.[g], Paulina Salminen, M.D., Ph.D.[d,e,†], Outi Mäkitie, M.D., Ph.D.[a,b,c,h,†]

[a]*Folkhälsan Research Center, Genetics Research Program, Helsinki, Finland*
[b]*Pediatric Research Center, Children's Hospital, University of Helsinki and Helsinki University Hospital, Helsinki, Finland*
[c]*Research Program for Clinical and Molecular Metabolism, University of Helsinki, Helsinki, Finland*
[d]*Department of Surgery, University of Turku, Turku, Finland*
[e]*Division of Digestive Surgery and Urology, Turku University Hospital, Turku, Finland*
[f]*Department of Surgery, Satasairaala Central Hospital, Pori, Finland*
[g]*Department of Biostatistics, University of Turku and Turku University Hospital, Turku, Finland*
[h]*Department of Molecular Medicine and Surgery, Karolinska Institutet, and Clinical Genetics, Karolinska University Hospital, Stockholm, Sweden*

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Abstract

Background: Genetic background of severe obesity is inadequately understood. The effect of genetic factors on weight loss after metabolic bariatric surgery (MBS) has shown inconclusive results.
Objectives: To determine the prevalence of rare obesity-associated gene variants in a secondary analysis of a randomized clinical trial (RCT) comparing laparoscopic sleeve gastrectomy (LSG) and laparoscopic Roux-en-Y gastric bypass (LRYGB) for the treatment of severe obesity and examine their association with long-term weight loss at 10 years.
Setting: University Hospital, Finland.
Methods: Targeted sequencing panel was used to examine variants in 79 obesity-associated genes and 16p11.2 copy number variants. Weight loss was evaluated by percentage total weight loss (%TWL).
Results: Out of 240 patients, 113 patients [mean body mass index 48.4 kg/m^2, (6.8 standard deviation [SD]) kg/m^2 and median age 49 (range 26–64) years, LSG n = 60, LRYGB n = 53] were available for this post-hoc study. We identified 7 rare heterozygous likely/suspected pathogenic (LP/SP) variants in *SH2B1*, *PCSK1*, *DNMT3A*, *BDNF,* and *AFF4* in 6 patients (5.3%), 5 heterozygous variants of uncertain significance in *PLXNA4*, *PLXNA2*, *NRP1,* and *SEMA3D* in 5 patients (4.4%), heterozygous Bardet-Biedl syndrome variants in 3 patients (2.7%), and *PCKS1* risk allele p.Asn221Asp in 9 patients (8.0%). The patients with LP/SP variants had earlier age of obesity onset (P = .0089) and higher %TWL (P = .0446) compared with patients without LP/SP variants.
Conclusions: There were LP/SP pathogenic variants in 5% of the patients supporting the potential benefits of genetic testing to optimize targeted therapies in the future. Despite deleterious gene defects the long-term MBS outcome can be favorable. (Surg Obes Relat Dis 2024;■:1–9.)

Keywords: Metabolic bariatric surgery; Genetic obesity; Weight loss; Gene panel

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†Paulina Salminen and Outi Mäkitie are joint last authors.

*Correspondence: Petra Loid, M.D., Ph.D., Research Program for Clinical and Molecular Medicine, Faculty of Medicine, PO Box 63, 00014 University of Helsinki, Helsinki, Finland.
E-mail address: petra.loid@helsinki.fi (P. Loid).

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Obesity is a complex trait affected by genetic, epigenetic, and environmental factors. The genetic background and pathogenesis of severe obesity are incompletely understood. Rare gene variants and copy number variants have been shown to be involved in the development of severe early-onset obesity and monogenic forms of obesity have an estimated prevalence of 5%–10% in patients with severe obesity [1–5]. Data on the prevalence of monogenic obesity in adult bariatric surgery patients is limited. Identification of improved and optimally targeted strategies to treat severe obesity is of great importance. Advancements in the treatment of obesity require a deeper understanding of the underlying mechanisms. Recently, novel therapy has become available for patients with specific forms of genetic obesity. Setmelanotide, a melanocortin 4 receptor (MC4R) agonist, that functions by restoring the MC4R signaling, and thereby reducing hunger and increasing energy expenditure, has been approved for treatment of severe obesity with MC4R pathway impairment caused by biallelic variants in *LEPR*, *POMC,* or *PCSK1*, and in Bardet-Biedl syndrome [6–9]. Advances in targeted therapy for patients with specific genetic forms of obesity highlight the importance of identifying patients with genetic obesity who might benefit from various pharmacological and also combination therapies including metabolic bariatric surgery (MBS).

MBS is currently the most effective treatment for patients with severe obesity [10]. The clinical outcomes including weight loss after MBS differ significantly between individuals. Several factors may influence the effect of successful weight loss therapy such as age, sex, and preoperative body mass index (BMI). It has been suggested that genetic variants may contribute to the interindividual variability in response to all obesity treatments. Previous studies have shown inconclusive results regarding weight loss outcome after MBS [11–14].

The aim of our study was to determine the prevalence of rare obesity-associated gene variants in a secondary analysis of a randomized clinical trial (RCT) comparing laparoscopic sleeve gastrectomy (LSG) and laparoscopic Roux-en-Y gastric bypass (LRYGB) for the treatment of severe obesity and examine their association with long-term weight loss at 10 years after surgery.

Material and methods

Study subjects

The participants in this cohort are SLEEVEPASS RCT patients. This was a multicenter, RCT in Finland involving 240 patients with severe obesity randomized to undergo either LSG or LRYGB from April 2008 to June 2010. The trial was carried out at 3 centers in Finland (Turku, Vaasa, and Helsinki). The SLEEVEPASS study design, methods, and results have been described previously [15–17]. Briefly, the inclusion criteria were 18–60 years of age, BMI $\geq$40 or $\geq$35 with obesity-related comorbidity, and previous failed adequate conservative treatment. Exclusion criteria were BMI $>$60, serious psychiatric or eating disorder, active alcohol or substance misuse, active gastric ulcer, severe gastroesophageal reflux with large hiatal hernia, and previous MBS.

For the present secondary genetic analysis, the participants of the SLEEVEPASS study were contacted by telephone and/or mail and asked to participate in this post-hoc genetic study. Altogether 116 of the 240 SLEEVEPASS participants (48%) consented to participate. Three of the DNA samples were of poor quality; hence we had sequencing results from 113 participants. Additional new data on age of obesity onset and family history were collected by questionnaire combined with the data obtained during the trial follow-up, including age, baseline BMI, type 2 diabetes (T2DM), and weight loss defined as percentage total weight loss [%TWL = preoperative weight-follow up weight)/(preoperative weight) $\times$ 100]. Patient outcomes in the SLEEVEPASS study were assessed by a multidisciplinary team at 6 months, 1, 3, 5, 7, and 10 years with the final follow-up date of January 27, 2021, for the 10-year outcomes [16]. Blood samples were collected and DNA was extracted using standard procedures. This amendment to the initial SLEEVEPASS study protocol was approved by the Ethics Committee of Southwest Finland. Written informed consent was obtained from all participants. We applied the Finnish legislation on the protection of research and personal data in the study. All samples were pseudonymized and all the collected data and research results have been processed confidentially. The genetic data are not publicly available.

Genetic analyses

The targeted exome sequencing panel was developed by Rhythm Pharmaceuticals and included 79 obesity-associated genes and 16p11.2 chromosome region (Supplemental Table 1). The genes in the panel 1) are part of the leptin-melanocortin pathway, 2) interact with this pathway, 3) associate with the development of neurons involved in energy homeostasis or 4) are involved in syndromic forms of obesity. The sequencing was performed at CGC Genetics in Porto, Portugal. The variants detected in the exons and flanking intronic regions ($\pm$8 bp) of the genes in the panel were evaluated. Mean read depth (average number of times a specific base in the DNA is sequenced) for the analysis was 131-fold, and 98.3% of target nucleotides were covered with $>$10-fold read depth and 95.1% with $>$20-fold read depth.

The identified variants were classified according to American College of Medical Genetics (ACMG) guidelines [18]. In addition, we further classified some of the variants of uncertain significance (VUS) as “suspected pathogenic” or

"suspected benign". We distributed the study participants into 6 groups based on the classification of the detected genetic variants: 1) patients with no identified variants, 2) patients with likely pathogenic/suspected pathogenic (LP/SP) variants, 3) patients with VUS, 4) patients with suspected benign variants, 5) patients with heterozygous BBS variants, and 6) patients with *PCSK1* risk allele p.Asn221Asp.

Statistical analysis

Continuous variables were characterized using means and standard deviations (SDs) or medians and range of values for non-normally distributed variables, and in the case of categorical variables, frequencies and percentages were used. For normally distributed variables differences between groups were tested using an independent samples *t*-test; for non-normally distributed variables nonparametric Kruskal-Wallis –test was used.

A linear mixed model for repeated measurements was used to evaluate differences between genetic groups in % TWL. The model included genetic group, time after surgery, the interaction of patient group and time, operation type, baseline weight before surgery, T2DM, and study center. Because the interaction of genetic group and time was not statistically significant, the results of the model were reported using main effects of genetic group and time. The results of the model were reported using model-based mean estimates with 95% confidence intervals (95% CIs). Assumptions for model were checked with studentized residuals.

Two-sided tests were used and P values $<.05$ were considered statistically significant. Statistical analyses were carried out using the SAS system for Windows, Version 9.4 (SAS Institute Inc).

Results

Clinical characteristics

Our study included 113 MBS patients, 86 female (76%) and 27 male patients with a median baseline age of 49 years (range 26–64). Their mean baseline BMI was 48.8 kg/m^2 (+6.8 SD) and mean baseline weight was 139.4 kg (+24.4 SD). Out of the 113 patients, 60 (53%) underwent LSG and 53 (47%) underwent LRYGB. The patient baseline clinical characteristics are summarized in Table 1.

Genetic findings

Table 2 presents the identified genetic variants in the 113 participants. The gnomAD allele frequency (version 2.1.1) (http://gnomad.broadinstitute.org), Combined Annotation Dependent Depletion (CADD) score, the interpretation of the variants, and clinical information of the patients with the identified variants are presented in Supplemental Table 2 and summarized below.

Table 1
Characteristics of study subjects (n=113)

Female, n (%)	86 (76.1)
Age, median (range), yrs	49 (26–64)
Age of onset of obesity, median (range), yrs	13.0 (0–57.0)
BMI before surgery, mean (SD), kg/m^2	48.8 (6.8)
Weight before surgery, mean (SD), kg	139.4 (24.4)
%TWL at 10 yr after surgery, mean (SD)	22.5 (9.8)
Laparoscopic sleeve gastrectomy, n (%)	60 (53.1)
Laparoscopic Roux-en-Y gastric bypass, n (%)	53 (46.9)

BMI = body mass index; SD = standard deviation; %TWL = percentage total weight loss.

LP/SP variants

We detected 7 rare heterozygous LP/SP variants in *SH2B1*, *PCSK1*, *DNMT3A*, *BDNF,* and *AFF4* in 6 of the 113 patients (5.3%).

The novel heterozygous *SH2B1* frameshift variant c.1246del p.Glu416Argfs*127 found in patient 1 was classified as likely pathogenic. This female was also a carrier of the heterozygous *AFF4* missense variant c.1277A>G, p.Asp426Gly. The allele frequency of the *AFF4* missense variant was .009% in the Finnish population in gnomAD. CADD score was 23.7. The patient presented with hypertension, hypothyroidism, and Sjögren's syndrome, and the self-reported age of obesity onset was 10 years. She underwent LSG at 59 years of age with a preoperative BMI of 41.8 kg/m^2. At 10 years of follow-up her BMI was 32 kg/m^2 and %TWL 23.4%.

The novel heterozygous *PCSK1* deletion in patient 2 was confirmed by array comparative genomic hybridization (aCGH). The deletion comprised the whole gene and was classified likely pathogenic. This male patient presented with diabetes type 2 and hypercholesterolemia, and the age of obesity onset was 5 years. He underwent LRYGB at 39 years of age with a preoperative BMI of 56.4 kg/m^2. At 10 years of follow-up his BMI was 37.8 kg/m^2 and % TWL 33.0%.

The 2 heterozygous *PCSK1* missense variants p.Gly310Arg and p.Gly380Ser in patients 3 and 4 were predicted likely pathogenic. Both missense variants had high CADD scores and affect highly conserved residues (PhyloP 100-way scores 7.82) The *PCSK1* variant p.Gly310Arg had an allele frequency of .03% in Finnish population in gnomAD. The patient presented with hypertension and polyneuropathy, and obesity onset was already at .5 years of age. She underwent LSG at 29 years of age with a preoperative BMI of 43.5 kg/m^2. At 10 years of follow-up her BMI was 28.9 kg/m^2 and %TWL 33.6%. The female patient with the novel *PCKS1* variant p.Gly380Ser presented with obesity onset at 5 years of age. She underwent LRYGB at 34 years of age with a preoperative BMI of 62.3 kg/m^2. At 10 years of follow-up her BMI was 37.9 kg/m^2 and % TWL 39.1%.

Table 2
Genetic variants identified in our cohort of 113 bariatric patients

	Patients (n)	Gene	Variant	Variant effect	Zygosity
Likely/suspected pathogenic variants (LP/SP)	1	*SH2B1*	c.1246del	p.Glu416Argfs*127	HET
	1	*PCSK1*	c.(?_-1)_(*1_?)del	NA	HET
	1	*PCSK1*	c.928G>A	p.Gly310Arg	HET
	1	*PCSK1*	c.1138G>A	p.Gly380Ser	HET
	1	*DNMT3A*	c.1154C>T	p.Pro385Leu	HET
	1	*BDNF*	c.125C>T	p.Thr42Ile	HET
	1	*AFF4*	c.1277A>G	p.Asp426Gly	HET
Variants of uncertain significance (VUS)	1	*PLXNA4*	c.1996G>A	p.Val666Met	HET
	1	*PLXNA4*	c.5639C>T	p.Ala1880Val	HET
	1	*PLXNA2*	c.3277A>G	p.Thr1093Ala	HET
	1	*NRP1*	c.1403G>A	p.Arg468His	HET
	1	*SEMA3D*	c.1904A>C	p.Glu635Ala	HET
Suspected benign variants	1	*SEMA3D*	c.1892A>G	p.Asp631Gly	HET
	3	*SIM1*	c.383T>C	p.Ile128Thr	HET
	1	*POMC*	c.394C>G	p.Pro132Ala	HET
	1	*NRP2*	c.935A>C	p.His312Pro	HET
	1	*MC4R*	c.53G>A	p.Arg18His	HET
	1	*NCOA1*	c.3731A>G	p.Asn1244Ser	HET
	2	*TRPC5*	c.2463T>A	p.Ser821Arg	HET
Heterozygous Bardet-Biedl syndrome variants	1	*BBS4*	c.110G>A	p.Trp37*	HET
	1	*BBS7*	c.1611del	p.Gly538Glufs*4	HET
	1	*IFT74*	c.587+2del	NA	HET
	1	*BBS2*	c.823C>T	p.Arg275*	HET
PCSK1 risk allele	8	*PCSK1*	c.661A>G	p.Asn221Asp	HET
	1	*PCSK1*	c.661A>G	p.Asn221Asp	HOM

HET = heterozygous; HOM = homozygous; NA = not available.
Reference genome hg19.

The *DNMT3A* missense variant p.Pro385Leu in patient 5 was classified as suspected pathogenic. The variant was absent from the Finnish population in gnomAD and had an allele frequency of .003% in all populations in gnomAD. CADD score was 19.9. This variant was found in HGMD Professional and has previously been reported in a patient with Tatton-Brown-Rahman syndrome (TBRS) [19]. This female patient had onset of obesity at 5 years of age, and she suffered from hypertension and spinal stenosis. She underwent LSG at 42 years of age with a preoperative BMI of 42.5 kg/m^2. At 10 years of follow-up her BMI was 35.4 kg/m^2 and %TWL 16.8%.

The novel *BDNF* missense variant p.Thr42Ile in patient 6 was predicted suspected pathogenic. CADD score was 24.0 and PhyloP100way score 7.88. This female patient had obesity onset at 8 years of age. She underwent LRYGB at 47 years of age with a preoperative BMI 56.0 kg/m^2. At 10 years of follow-up her BMI was 33.2 kg/m^2 and % TWL 40.6%.

Other rare variants

Five rare heterozygous VUS in *PLXNA4*, *PLXNA2*, *NRP1,* and *SEMA3D* were identified in 5 patients (4.4%). The evidence of the association of class 3 semaphorins and their receptors with obesity is still limited and these variants were classified as uncertain significance.

We identified 7 suspected benign missense variants in 9 patients (8.0%); *POMC* variant p.Pro132Ala, *SIM1* variant p.Ile128Thr, *SEMA3D* variant p.Asp631Gly, *MC4R* variant p.Arg18His, *NCOA1* variant p.Asn1244Ser, *NRP2* variant p.His312Pro, and *TRPC5* variant p.Ser821Arg. These variants were classified as suspected benign based on allele frequency or benign predictions by in silico tools.

We identified 4 heterozygous pathogenic/likely pathogenic BBS variants (*BBS2*, *BBS4*, *BBS7,* and *IFT74*) in 3 patients (2.7%). One of these patients was a carrier of 2 heterozygous BBS variants, a novel likely pathogenic *BBS4* variant p.Trp37* and a previously reported pathogenic *BBS7* variant p.Gly538Glufs*4. This female patient had very early-onset obesity at 3 years of age but no other clinical features of BBS. The 2 other patients with the heterozygous likely pathogenic variants in *BBS2* and *IFT74* had adult-onset obesity and no characteristics of BBS.

The *PCKS1* variant p.Asn221Asp, which has been regarded as a risk allele for obesity, was detected in 9 patients (8.0%) (heterozygous in 8 patients and homozygous in one patient).

Association between genetic findings and clinical characteristics

We distributed the study patients into 6 groups based on the classification of the detected genetic variants in each

subject to evaluate whether clinical features or surgery outcomes differed according to genetic defects. The 6 genetic groups were: 1) no identified variants, 2) LP/SP variants, 3) VUS, 4) suspected benign variants, 5) heterozygous BBS variants, and 6) *PCSK1* risk allele p.Asn221Asp. In addition, we compared the patients with LP/SP variants with patients without LP/SP variants.

Age, BMI, and weight before surgery and age of obesity onset

Age, BMI, and weight before surgery and the self-reported age of obesity onset in the different genetic groups are shown in Table 3. The patients with LP/SP variants had an earlier age of obesity onset (median 5.0 years, range .5–10.0), compared with patients without LP/SP variants (median 15.0 years, range 0–57.0, $P = .0089$). There was no statistically significant difference in age, BMI, and weight at the time of surgery between the patients with LP/SP variants and patients without LP/SP variants (Table 4).

Weight loss after MBS

Descriptives of %TWL at .5, 1, 3, 5, 7, and 10 years in the different genetic groups are presented in Table 5. We used a linear mixed model to analyze %TWL in the LP/SP group compared with all other groups. The model included genetic group, time after surgery, the interaction of patient group and time, operation type, baseline weight before surgery, T2DM, and study center. Interaction of genetic group and time was not statistically significantly different between the groups (interaction of genetic group and time $P = .6707$). The patients with LP/SP variants had higher %TWL (mean estimate 31.3 (25.4–37.1) compared to patients without LP/SP variants (mean estimate 25.1) (23.7–26.5) ($P = .0446$).

Discussion

In our secondary analysis of a randomized MBS cohort, we identified LP/SP variants in 5% of the patients. The patients with LP/SP variants had higher %TWL compared to patients without LP/SP variants. The prevalence of LP/SP variants is in line with previous studies. Cooiman et al. studied 1014 bariatric patients and identified heterozygous pathogenic variants in *MC4R*, *POMC*, *PCSK1*, *SIM1,* and *PTEN* in 3% of the patients [14]. Campos et al. found heterozygous variants in leptin-melanocortin pathway genes in 9% of a cohort of 701 patients with a history of Roux-en-Y gastric bypass (RYBG) [11] and Li et al. detected rare gene variants in 8% of 131 patients who underwent sleeve gastrectomy [12]. In our cohort, we did not identify any pathogenic *MC4R* variants, which is the most frequent cause of monogenic nonsyndromic obesity. The prevalence of *MC4R* variants can vary with ethnicity and degree of population consanguinity. We have previously reported a prevalence

Table 3
Age, body mass index, and weight before surgery and age of obesity onset in the different genetic groups

	No identified variants, n = 84		LP/SP variants, n = 6		VUS, n = 5		Suspected benign variants, n = 6		Heterozygous BBS variants, n = 3		PCSK1 risk allele, n = 9	
Laparoscopic sleeve gastrectomy, n	47		3		3		2		1		4	
Laparoscopic Roux-en-Y gastric bypass, n	37		3		2		4		2		5	
Age, median (range), yrs	n = 84	47.7 (26.0–63.1)	n = 6	40.3 (29.0–59.3)	n = 5	46.9 (40.0–50.5)	n = 6	54.1 (45.8–61.2)	n = 3	44.0 (38.8–55.6)	n = 9	54.3 (46.7–63.7)
BMI before surgery, mean (SD), kg/m^2	n = 84	48.7 (6.7)	n = 6	50.4 (8.9)	n = 5	49.5 (9.0)	n = 6	46.6 (7.0)	n = 3	48.7 (6.5)	n = 9	49.4 (6.4)
Weight before surgery, (mean) kg	n = 84	139.5 (23.9)	n = 6	145.0 (35.8)	n = 5	139.7 (30.0)	n = 6	127.3 (21.7)	n = 3	142.7 (28.0)	n = 9	142.3 (23.2)
Age of obesity onset, median (range), yrs	n = 83	14.0 (0–57.0)	n = 6	5.0 (.5–10.0)	n = 5	15.0 (3.0–25.0)	n = 6	14.5 (5.5–22.0)	n = 3	28.0 (3.0–30.0)	n = 9	16.0 (6.0–55.0)

LP/SP = likely pathogenic/suspected pathogenic; VUS = variants of uncertain significance; BBS = Bardet-Biedl syndrome; BMI = body mass index; SD = standard deviation.

Table 4
Age, body mass index, and weight before surgery and age of obesity onset

	Patients with LP/SP variants, n = 6	Patients without LP/SP variants, n = 107	*P* value
Age, median (range), yrs	40.3 (29.0–59.3)	48.9 (26–63.7)	.0827*
BMI before surgery, mean (SD), kg/m^2	50.4 (8.9)	48.7 (6.7)	.5398†
Weight before surgery, (mean) kg	145.0 (35.8)	139.1 (23.9)	.5678†
Age of obesity onset, median (range), yrs	5.0 (.5–10.0)	15.0 (0–57.0)	.0089*

LP/SP = likely pathogenic/suspected pathogenic; BMI = body mass index; SD = standard deviation.
* Kruskal-Wallis test.
† Independent samples *t*-test.

of pathogenic *MC4R* variants of 2% in a cohort of Finnish patients with severe early-onset obesity [4]. The lack of *MC4R* variants in our bariatric cohort may be linked to the relatively small sample size and specific characteristics of the cohort, such as the higher prevalence of later-onset obesity.

We detected one female patient with a novel likely pathogenic frameshift *SH2B1* variant. *SH2B1* encodes for an adaptor protein that mediates the activation of receptor tyrosine kinases and cytokine receptors that have important roles in food intake, energy metabolism, and glucose homeostasis. Rare heterozygous variants in *SH2B1* have been associated with obesity [20–25]. Setmelanotide is being evaluated in a clinical trial (NCT05093634) for patients with rare variants in *SH2B1* and has shown good clinical response after 1 year treatment in a patient with a rare heterozygous missense variant in *SH2B1* [26]. Our patient was also a carrier of a rare missense variant in *AFF4*. This variant has been submitted to ClinVar as a VUS in a patient with CHOPS syndrome (OMIM#616368), a disorder characterized by cognitive impairment, coarse facies, heart defects, obesity, pulmonary involvement, short stature, and skeletal dysplasia [27,28]. The *AFF4* missense variant's pathogenicity and possible contribution to our patient's phenotype is unclear.

We detected 3 rare heterozygous variants in *PCSK1*, including one deletion and 2 missense variants. *PCSK1* encodes prohormone convertase 1/3 (PC1/3), whose activity is essential in the cleavage of peptide hormone precursors involved in appetite regulation, energy metabolism, and glucose homeostasis. PC1/3 deficiency is a rare autosomal recessive disorder with obesity and several endocrinopathies [29,30]. Setmelanotide has been shown to lead to weight loss in patients with pathogenic biallelic variants in *PCSK1* [8]. Recently, rare heterozygous *PCSK1* variants have been associated with obesity risk [31–38]. Variants in *PCSK1* can cause complete/partial loss of enzymatic activity or deficiencies in protein stability [33,36,39,40]. Folon et al. sequenced the coding exons of *PCSK1* in over 9000 subjects, analyzed the variants in vitro and clustered the variants into groups according to enzymatic activity, and assessed the effect of each cluster on obesity. Their results indicate that rare heterozygous null/loss of function variants in *PCSK1* contribute to obesity, whereas missense variants with neutral/partial deleterious effects on enzyme activity do not contribute to obesity risk [35]. Shah et al. performed in vitro functional characterization of missense variants in *PCSK1* using an enzyme activity assay and reported that the *PCSK1* variants p.Gly310Arg and p.Gly380Ser, the missense variants found in our study, showed loss-of-function activity [41]. The *PCSK1* deletion detected in our study comprised the whole gene and was predicted likely pathogenic. An ongoing phase 3 clinical trial (NCT05093634) is investigating setmelanotide in patients with rare heterozygous variants in *PCSK1*. Further studies

Table 5
Descriptives of percentage total weight loss at different time points after surgery

	No identified variants		LP/SP variants		VUS		Suspected benign variants		Heterozygous BBS variants		*PCSK1* risk allele	
% TWL at .5 yr	n = 82	25.3 (6.4)	n = 6	30.7 (2.7)	n = 5	23.9 (2.0)	n = 6	24.0 (6.2)	n = 3	23.7 (5.6)	n = 9	23.4 (5.2)
% TWL at 1 yr	n = 81	28.7 (8.0)	n = 5	35.6 (6.5)	n = 5	24.0 (4.7)	n = 6	28.7 (6.0)	n = 3	29.0 (5.8)	n = 9	24.8 (6.4)
% TWL at 3 yr	n = 77	27.2 (9.6)	n = 6	33.3 (9.4)	n = 5	19.1 (9.1)	n = 5	28.2 (3.1)	n = 3	29.8 (2.9)	n = 9	25.1 (8.7)
% TWL at 5 yr	n = 72	25.9 (10.2)	n = 6	33.7 (11.7)	n = 5	18.5 (9.7)	n = 5	26.8 (3.7)	n = 3	24.7 (1.7)	n = 9	22.8 (8.6)
% TWL at 7 yr	n = 68	24.5 (9.9)	n = 5	26.9 (8.5)	n = 5	15.6 (11.8)	n = 5	25.9 (4.8)	n = 3	20.9 (1.3)	n = 9	20.5 (4.5)
% TWL at 10 yr	n = 82	23.0 (10.0)	n = 6	31.1 (9.3)	n = 4	11.1 (7.8)	n = 6	21.0 (8.7)	n = 3	20.5 (4.3)	n = 9	19.0 (4.6)

LP/SP = likely pathogenic/suspected pathogenic; VUS = variants of uncertain significance; %TWL = percentage total weight loss; BBS = Bardet-Biedl syndrome.
Data is presented as mean (SD).

on heterozygous *PCSK1* variants are needed to determine their functional effect and clinical relevance in patients with obesity.

The suspected pathogenic *DNMT3A* missense variant p.Pro385Leu identified in our cohort has previously been reported in a patient with TBRS (OMIM #615879), a disorder characterized by overgrowth, mild to severe intellectual disability/developmental delay, obesity, and behavioral problems [19,42]. *DNMT3A* encodes for the DNA methyltransferase alpha3 enzyme, which plays an important role in epigenetic regulation. Human and mice studies have shown that loss of *DNMT3A* causes DNA hypomethylation and heterozygous *DNMT3A*-null mice develop severe obesity, show aberrant feeding behavior, display expanded fat depots, and disturbed adipose tissue formation and function [43,44]. The clinical significance of this variant should be evaluated in future studies.

We identified a novel suspected pathogenic *BDNF* missense variant p.Thr42Ile. *BDNF* is important for neuronal differentiation and proliferation and is highly expressed in the hypothalamus. It has been shown that *BDNF* is involved in regulating energy balance and acts as a downstream target of MC4R [45]. Several rare variants in *BDNF* have been reported in patients with severe obesity [46–50]. Their pathogenicity, and the pathogenicity of the *BDNF* variant in our patient, should be confirmed in functional studies.

We detected 5 heterozygous VUS in *PLXNA4*, *PLXNA2*, *NRP1,* and *SEMA3D*. Rare variants in the class 3 semaphorins (*SEMA3A-G*) and their receptors *NRP1*, *NRP2,* and *PLXNA1-4* have been associated with obesity. Van der Klaauw et al. identified 40 rare variants in these genes in a cohort of 573 patients with severe obesity and zebrafish and mice studies supported that *SEMA3A-G* signaling is involved in regulating the formation of neuronal melanocortin circuits in the hypothalamus [51].

BBS is a rare autosomal recessive disorder characterized by retinal dystrophy, obesity, polydactyly, renal dysfunction, hypogonadism, and neurodevelopmental disorder [52,53]. There have been conflicting reports regarding the risk of obesity in heterozygous BBS carriers [47,54–57]. Previous studies have reported pathogenic/likely pathogenic BBS carrier status of 3%–7% in obesity cohorts, indicating a potential enrichment of rare BBS variants compared to the general population [56,57]. Kleinendorst et al. reported a 1.7-fold higher allele frequency of BBS carriers in their obesity cohort, but permutation and segregation analyses did not support a predisposition for obesity in heterozygous BBS carriers compared to the general population [47]. We found 4 rare heterozygous pathogenic/likely pathogenic BBS variants in 3 patients (2.7%). The possible contribution of BBS carrier status to the risk of obesity in our patients is unclear.

The common *PCSK1* p.Asn221Asp variant has been described as a polygenic risk variant for obesity and previous studies have shown that this variant causes a small decrease in PC1/3 enzyme activity and stability [33,38,39,58,59]. We detected this variant in 9 patients (8.0%) Roberts et al. reported a similar prevalence of this variant in a pediatric obesity cohort [56]. The allele frequency of the variant was 4.4% in our MBS cohort, which is only slightly higher than the allele frequency of 3.2% in the Finnish population in gnomAD.

When analyzing the effect of the identified rare gene variants on clinical course and MBS long-term outcome in our cohort, we observed that the patients with LP/SP variants had earlier onset of obesity, in accordance with previous studies [47]. Regarding weight loss outcomes after MBS, the data from previous studies are limited and have shown inconclusive results. Cooiman et al. found that weight loss 2 years after RYGB was not different for patients with *MC4R*, *POMC,* and *PCSK1* compared with patients without rare gene variants. Only patients receiving sleeve gastrectomy with pathogenic *MC4R* variants had less weight loss compared to those without variants [14]. Campos et al. reported that carriers of a heterozygous variant in the leptin-melanocortin pathway had lower weight loss and higher weight regain 15 years after RYGB [11]. Li et al. studied 131 bariatric patients who underwent sleeve gastrectomy and found that carriers of heterozygous variants in *LEP*, *LEPR*, *MC4R*, *MC3R*, *SIM1,* and *PCSK1* had less weight loss 6 years after surgery [12]. The variable results may depend on the characteristics of the bariatric cohort, type of surgery, examined genes, interpretation of the variants, and length of follow-up. In our study, the %TWL in subjects with LP/SP variants was even greater than in those without LP/SP variants. All the identified LP/SP variants in our study were heterozygous and further studies are needed to determine the clinical significance of heterozygous *PCSK1* variants. Patients with biallelic *PCSK1* variants may have different weight loss outcomes after MBS. Notably, the patient with the likely pathogenic *SH2B1* variant had similar %TWL compared to patients without any identified genetic variants. The number of study subjects with gene variants in our cohort is limited and larger studies are needed to further evaluate the effect of different gene variants on weight loss after MBS. On the other hand, it can be concluded that despite deleterious gene defects the long-term MBS outcome can be favorable.

In addition to rare gene variants, common genetic variants may also contribute to obesity and impact surgery outcomes, as shown in a recent study examining the role of genetic risk score on weight loss 5 years after surgery [60]. Future studies are needed to optimally incorporate various forms of genetic testing into MBS, in order to improve surgical and preoperative and postoperative interventions.

There were some limitations in our study. A major limitation is that we did not have family members available for genetic testing making the interpretation of the pathogenicity of the variants more difficult. Another limitation is the

lack of functional analyses to confirm the variants' effect on the protein and signaling pathway function. The limited number of genes in the targeted exome sequencing panel means that we may have missed potential rare variants in novel genes that were not included in the panel. However, the panel included the most common genes associated with monogenic and syndromic obesity and we also evaluated 16p11.2 copy number variants. Our study setting and the relatively small cohort size prevented us from making conclusions regarding the recommended type of surgery. Major strengths of the study are the randomized multicenter cohort and the long-term follow-up.

Conclusion

In conclusion, our results support the use of genetic testing in patients with severe obesity, particularly in patients with early-onset obesity. Advances in targeted therapy for patients with specific genetic forms of obesity highlight the significance of identifying patients with genetic obesity who might benefit from a more targeted treatment. Despite deleterious gene defects the long-term MBS outcome can be favorable.

Data availability

Restrictions apply to the availability of some data generated or analyzed during this study to preserve patient confidentiality. The corresponding author will on request detail the restrictions and any conditions under which access to some data may be provided.

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Disclosures

Paulina Salminen reports lecture fees and Outi Mäkitie reports consulting fees from Novo Nordisk.

Supplementary material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.1016/j.soard.2024.11.021.

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