ORIGINAL ARTICLE

Genetic obesity: next-generation sequencing results of 1230 patients with obesity

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► Additional material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/jmedgenet-2018-105315).

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Received 6 February 2018
Revised 21 May 2018
Accepted 10 June 2018
Published Online First 3 July 2018

To cite: Kleinendorst L, Massink MPG, Cooiman MI, *et al*. *J Med Genet* 2018;**55**:578–586.

ABSTRACT

Background Obesity is a global and severe health problem. Due to genetic heterogeneity, the identification of genetic defects in patients with obesity can be time consuming and costly. Therefore, we developed a custom diagnostic targeted next-generation sequencing (NGS)-based analysis to simultaneously identify mutations in 52 obesity-related genes. The aim of this study was to assess the diagnostic yield of this approach in patients with suspected genetic obesity.

Methods DNA of 1230 patients with obesity (median BMI adults 43.6 kg/m^2; median body mass index-SD children +3.4 SD) was analysed in the genome diagnostics section of the Department of Genetics of the UMC Utrecht (The Netherlands) by targeted analysis of 52 obesity-related genes.

Results In 48 patients pathogenic mutations confirming the clinical diagnosis were detected. The majority of these were observed in the *MC4R* gene (18/48). In an additional 67 patients a probable pathogenic mutation was identified, necessitating further analysis to confirm the clinical relevance.

Conclusions NGS-based gene panel analysis in patients with obesity led to a definitive diagnosis of a genetic obesity disorder in 3.9% of obese probands, and a possible diagnosis in an additional 5.4% of obese probands. The highest yield was achieved in a selected paediatric subgroup, establishing a definitive diagnosis in 12 out of 164 children with severe early onset obesity (7.3%). These findings give a realistic insight in the diagnostic yield of genetic testing for patients with obesity and could help these patients to receive (future) personalised treatment.

INTRODUCTION

Obesity is a universal, severe health problem, with globally over 650 million adults with obesity and 124 million children and adolescents with obesity (aged 5–19 years) in 2016.[1] Because of their excessive accumulation of body fat, they are at risk for many health problems, such as cardiovascular disease, type 2 diabetes mellitus, depression and certain types of cancers (eg, breast cancer and colon cancer).[1] An adult is considered obese in case of a body mass index (BMI) >30 kg/m^2.[1] For children, BMI-SD scores (SDS) are used to define obesity (>2.3 SDS), representing the deviation from the BMI in gender and age-matched children. Obesity is caused by an imbalance between energy intake and expenditure. Environmental factors, for example, the easy accessibility of high caloric food, little physical activity or the use of obesogenic medication (eg, atypical antipsychotics or glucocorticoids),[2] can severely affect this energy balance. Therefore, obesity is regarded as a multifactorial disorder. On the other hand, meta-analysis of twin and family studies have shown that the heritability of BMI is around 46%–72%.[3]

A number of genetic factors have indeed been identified that cause obesity.[4] Nevertheless, these identified genes and chromosomal abnormalities have thus far only explained 7% of the heritability shown by twin studies.[5] This percentage, however, varies depending on the country or region where the genetic studies are performed. Reports from Pakistan and Guadeloupe show a much higher prevalence of rare monogenic forms of obesity (30% and 15%, respectively).[6 7] Different hypotheses have been suggested to explain the 'missing heritability' of human obesity, including CNVs, epigenetic events and rare highly penetrant variants.[8]

A genetic diagnosis is of great importance for patients since genetic counselling and (future) personalised therapy depending on the underlying gene defect can be offered.[9–11]

Additionally, a genetic diagnosis or insight in the genetic contribution to obesity might help to reduce the psychological burden of obesity, since the public distress and social stigma of being obese is a major problem for many patients with therapy-resistant obesity.[12]

Due to genetic heterogeneity, the identification of genetic defects in patients with obesity can be time consuming and costly. Therefore, we developed a next-generation sequencing (NGS) gene panel analysis for patients with suspected genetic obesity and offered it in our DNA diagnostics section. For the design of our gene panel (in 2012), we selected genes associated with an obesity phenotype from the OMIM catalogue, genes associated with obesity in Genome-Wide Association Studies, in obesity or diabetes pathways (Kyoto Encyclopaedia of Genes and Genomes pathway database) and several genes from known obesity CNVs. With this new test, 52 obesity-related genes are simultaneously analysed.

Box 1 Inclusion criteria for the next-generation sequencing obesity gene panel

Patients should (apart from the obesity phenotype) have at least one of the criteria to be included in this study.
Principal inclusion criteria:
- Age of onset of obesity <5 years (prepubertal onset in adult subgroups)
- Family history of obesity (alarm symptom: single person with obesity in family)
- Hyperphagia
- Intellectual deficit/developmental delay
- Congenital malformations
- Visual impairment and/or deafness
- Abnormal growth parameters (head circumference and height)

Inclusion criteria for patients undergoing bariatric surgery:
- Extreme obesity (body mass index >50 kg/m^2)
- Repeat surgery after weight regain or insufficient weight loss

The gene panel includes genes involved in both syndromic and non-syndromic monogenic obesity. Genetic variants associated with polygenic forms of obesity and obesity-associated epigenetic variants have also been described in literature, but they are not the focus of this study.[13]

Monogenic syndromic obesity is defined as a genetic condition caused by a single gene defect in which the patient is obese, and has additional problems, like intellectual deficit, congenital malformations, dysmorphic features and/or organ dysfunction. Monogenic non-syndromic obesity is not accompanied by intellectual deficit in the majority of cases and is often caused by mutations in the leptin-melanocortin pathway, influencing energy expenditure and food intake.[13] Early onset of obesity, hyperphagia and a positive family history are often seen as warning signals for genetic non-syndromic obesity.[14]

METHODS

Patients

For this study, we reviewed the results of the diagnostic obesity gene panel analyses from December 2014 until April 2016. In this period, DNA samples of 1230 patients were analysed. Because of the diagnostic setting, the test was not performed on normal weight controls. The patients for which gene testing was requested, derived from 36 centres in The Netherlands and Dutch Caribbean, and two other European medical centres (from the UK and Finland). All patients/parents/guardians agreed to perform the diagnostic test and to the anonymous use of the test data. All patients were informed of their test result by the doctor who ordered the test or a genetic counsellor. Inclusion criteria to select eligible patients for the NGS obesity panel are listed in box 1. Patients who were already diagnosed with a genetic obesity disorder in the past were not included in this study.

Genetic consultations and phenotyping (figure 1) were routinely offered in five Dutch medical centres (more details are provided in the online supplementary appendix). We tried to obtain phenotypical information from the patients who were not referred for genetic consultations from the physicians who requested the test.

The median age of the total cohort was 33 years (range 0–79 years). The median age of the paediatric group was 9.5 years and of the adult group 43 years. Three hundred ninety-three patients were younger than 18 years when the test was performed; 837 patients were older than 18 years. The median BMI of the adult patients at the time of testing was 43.6 kg/m^2 (lowest 22,

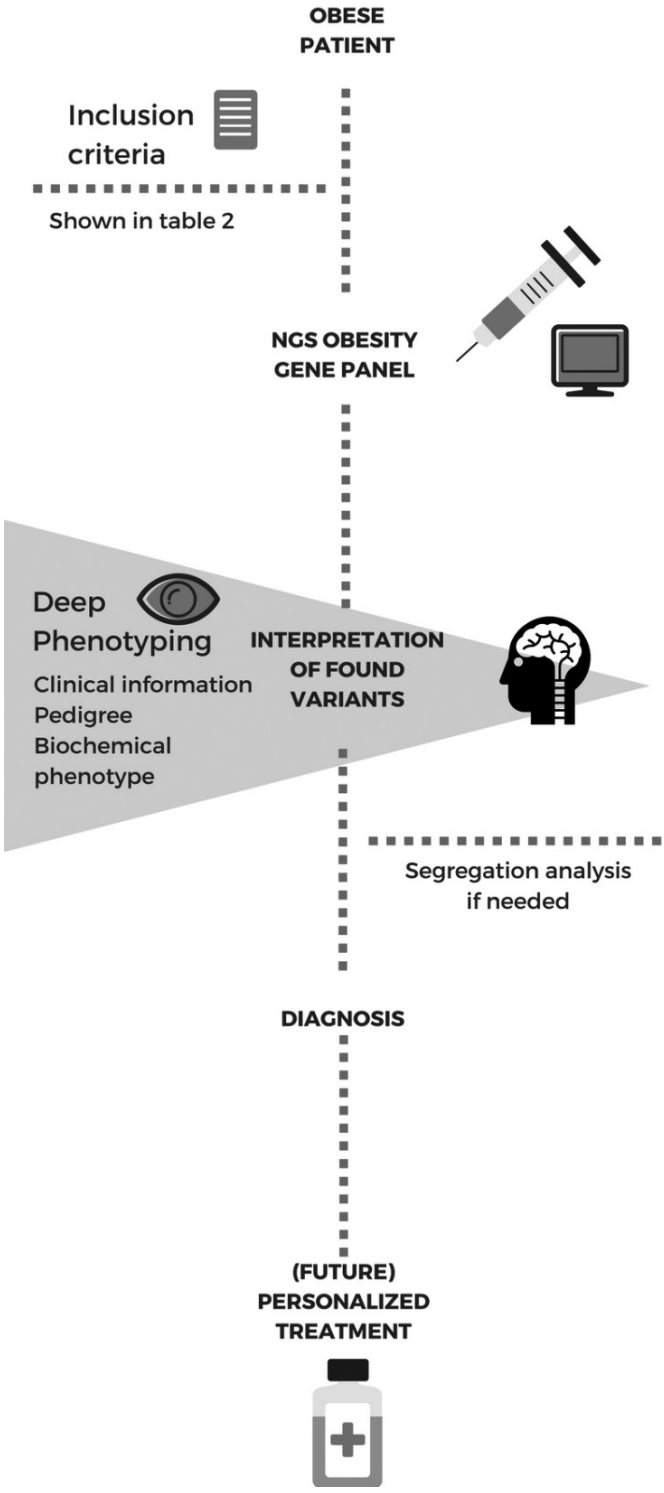

Figure 1 Diagnostic process. Patients with obesity who have one or more of the inclusion criteria can be tested with the next-generation sequencing (NGS) Obesity Gene Panel. We advised genetic counselling for all patients with abnormal results identified by the gene panel. Deep phenotyping (including pedigree information, biochemical tests and clinical dysmorphic evaluation) is needed to interpret the found variants. Sometimes, segregation analysis in the family is performed to interpret the significance of the found variant.

highest 91). The median BMI-SD of the children was +3.4 SD (lowest +1 SD, highest +9 SD). The few patients with a normal BMI were all obese or morbidly obese in the past, but lost weight before testing.

Patient subgroups

For analysis of the different patient groups (eg, children with early onset obesity or patients undergoing bariatric surgery), five subgroups were created in the Dutch medical centres where genetic consultations and phenotyping were routinely offered. Our largest patient subgroup is the bariatric surgery group of 659 patients. More details about the subgroups can be found in the online supplementary appendix.

Sequencing and bioinformatics analysis

Genomic DNA was isolated from peripheral blood samples at the ISO15189 accredited Genome Diagnostics section of the Department of Genetics, UMC Utrecht (The Netherlands). Subsequently, sequencing libraries were prepared from sheared genomic DNA. Each patient and thus each sequencing library received a unique barcode consisting of 10 nucleotides. This system allows for a cost-effective and time-effective approach for batches of ~50 patients simultaneously in a single enrichment procedure. The prepared libraries were pooled and target DNA capture was performed using a custom-designed Agilent SureSelectXT assay (elid#0561501).

The diagnostic genes included in the obesity gene panel are: *ALMS1, ARL6, BBS1, BBS2, BBS4, BBS5, BBS7, BBS9, BBS10, BBS12, BDNF, CCDC28B, CEP290, CRHR2, FLOT1, G6PC, GNAS, IRS1, IRS2, IRS4, KIDINS220, LEP, LEPR, LZTFL1, MAGEL2, MC3R, MC4R, MCHR1, MKKS, MKRN3, MKS1, MRAP2, NDN, NTRK2, PAX6, PCK1, PCSK1, PHF6, POMC, PRKAR1A, PTEN, SIM1, SNRPD2, SNRPN, SPG11, TBX3, THRB, TMEM67, TRIM32, TTC8, TUB,* and *WDPCP.* Sequencing was performed on a SOLiD 5500XL system (Life Technologies). We sequenced to an average depth of ~100X horizontal coverage to allow for optimal variant calling. Sanger sequencing of the fourth exon of *POMC* was performed to obtain >99% coverage for this gene.

Variant selection

Variant filtering and interpretation of clinical relevance

Filtering of variants was performed using the Cartagenia BENCHlab NGS module (V.3.1.2), with a validated 'classification tree'. The sequence data were compared with the dbSNP, GoNL (Genome of the Netherlands database), our in-house and Exome Variant databases (6500 exomes) to exclude common variants and select genes that contain non-synonymous variants, nonsense mutations, essential splice site mutations or coding frame-shift indels. Variants with (possible) clinical relevance were subsequently analysed in the Alamut mutation interpretation software program (V.2.6.0) using among others Polyphen2, SIFT, GERP and Grantham scores, and multiple splice-site prediction programs. The remaining (probable) pathogenic mutations were confirmed by Sanger sequencing. When the combined data were inconclusive, the variants were classified as variants of uncertain clinical significance (VUS).

Statistical analysis

Group comparisons were performed by means of the independent samples t-test. Statistical analyses were performed using SPSS software V.24.0.0.1. A Mann-Whitney U test was run to determine if there were differences in BMI in adults between those without a diagnosis and with a definite diagnosis, and in children between those with and without a definite diagnosis.

A permutation test was performed on the data of the Bardet-Biedl associated genes. We determined the population allele frequencies for a set of 27 curated pathogenic *BBS* gene mutations in our cohort. We determined the significance of this result by permutation testing on the obesity gene panel and ExAC NFE populations allele frequency data (details provided in the online supplementary appendix 1).

RESULTS

Diagnostic yield

We established a definitive diagnosis of a genetic obesity disorder in 48 patients (3.9%), shown in tables 1 and 2, with the highest yield in a paediatric subgroup 12/164 (7.3%). A definitive diagnosis was established in 2.7% of the patients in the adult subgroup. Six of the 48 patients (12.5%) had pathogenic mutation that causes syndromic obesity. The majority of the identified mutations however, are linked to non-syndromic obesity. In 67 additional patients (5.4%), VUS were found that could possibly lead to a future diagnosis (see online supplementary table S1). Seventeen variants in comorbidity genes were identified (see online supplementary table S2). Eleven out of 52 genes in the panel harboured pathogenic mutations confirming the diagnosis; 44 genes showed (probable) pathogenic mutations or VUS.

BMI in patients with a genetic obesity disorder

The median BMI in adult patients with a definitive diagnosis was 41.8 kg/m^2 (range 34.2–72.7). Patients without a definitive or likely diagnosis had a median BMI of 43.7 kg/m^2 (range 22.4–91). Median BMI was not statistically significantly different between the two groups (details in the online supplementary appendix). The median BMI-SD in children with a definitive diagnosis was +3.84 (corrected for age and gender). In children without a definitive or likely diagnosis, the median BMI-SD was +3.4 (corrected for age and gender). This was also not a statistically significant difference (online supplementary appendix).

Carrier status

61 patients (5% of the total cohort) were identified as carriers of a heterozygous known pathogenic mutation that only leads to an obesity phenotype in an autosomal recessive mode of inheritance (*ALMS1, PCK1, SPG11, TUB, BBS* genes and modifiers). These findings were assessed as non-relevant for the development of the obesity phenotype, but patients were counselled about these results because the findings could impact the health of future generations or reproduction decisions. An additional 76 patients (6.2% of the total cohort) were carriers of a VUS in one of those genes. Most of them were carriers of a Bardet-Biedl syndrome (BBS)-related variant.

Bardet-Biedl syndrome

BBS is an autosomal recessive and genetically heterogeneous ciliopathy disorder characterised by obesity, intellectual deficit, retinitis pigmentosa, kidney dysfunction and polydactyly. Whether heterozygous carriers of *BBS* genes are predisposed to obesity or not was unclear at the onset of our study.[15 16]

We see a 1.7-fold higher population allele frequency for *BBS* mutation carriers in the obesity gene panel cohort compared with the ExAC's Non-Finnish European (NFE) population (see online supplementary table S3). Our permutation test showed that the permutation score was not statistically

Table 1 Confirmed diagnosis genetic obesity: autosomal recessive inherited conditions (homozygous or compound heterozygous)

Patient	Age (years)	Gender	Medical centre	Gene	Genotype	MCA/ID	Clinical information fitting with the clinical phenotype	Additional information/ coincidental phenotypic findings	BMI (kg/m2)	SD (children)	Family history of obesity (if available)
1	8	F	1	*BBS7*	**Compound heterozygous:** c.1037G>A(;)1657C>T; p.(Arg346Gln)(;)(Gln553*)	Yes	Postaxial polydactyly fingers and toes Intellectual deficit Hyperphagia onset obesity: 3 years		38.5	+6.1 SD	
2	3	F	2	*LEPR*	**Compound heterozygous:** c.1985T>C(;)2168C>T; p.(Leu662Ser)(;)(Ser723Phe)	No	Hyperphagia onset obesity: 2 months	AD: 34 weeks, birth weight of 2605 g (+1.9 SD for age)	34.5	+7.5 SD	No family history of obesity
3	1	F	2	*LEPR*	**Compound heterozygous:** c.2051A>C(;)2627C>A; p.(His684Pro)(;)(Pro876Gln)	Yes	Onset obesity: 3 months Birth weight: 3270 g (−0.4 SD for gestational age)	Cleidocranial dysplasia (*RUNX2* mutation identified)	34.6	+7.3 SD	No family history of obesity
4	17	M	2	*MC4R*	**Compound heterozygous:** c.446_450del(;)644T>G; p.(Phe149fs)(;)(Met215Arg)	No	Onset obesity: 1 year	Hypogonadism	43.8	–	No family history of obesity
5	18	M	2	*MC4R*	**Homozygous:** c.779C>A(;)779C>A; p.(Pro260Gln)(;)(Pro260Gln)	No	Onset obesity: 1 year	Autism	40.3	–	No childhood obesity in the parents, current BMI of father 32.9 kg/m^2
6	15	M	2	*SPG11*	**Compound heterozygous:** c.4534dup(;)5857?_6477+?del; p.(Asp1512fs)(;)?†	Yes	Spastic paraparesis IQ: 48 Onset obesity: 3 years		37.9	+3.7 SD	Mother and brother obese

Medical centre: 1=UMC Utrecht Clinical Genetics; 2=CGG Pediatrics.
*A singleasterisk is here used as a symbol for translation termination (stop) codon.
†The intragenic deletion in SPG11, comprising multiple protein coding exons, was identified after additional Sanger sequencing and MLPA copy number analysis at the DNA diagnostics department of the Radboud UMC, Nijmegen, The Netherlands. The NGS platform used at the time the patients in the present study were analysed is not suitable for reliable detection of genomic deletions>6 bp.

Table 2 Confirmed diagnosis genetic obesity: autosomal dominant inheritance

Patient	Age (years)	Gender	Medical centre	Gene	Genotype	MCA/ID	Clinical Information Fitting with the clinical phenotype	Additional information/ coincidental phenotypic findings	BMI (kg/m2)	BMI-SD	Family history of obesity
7	2	M	3	*GNAS*	**Heterozygous:** c.85C>T; p.(Gln29*)	Yes	Developmental delay Hyperphagia Onset obesity: 1.5 years		21.4	+2.9 SD	No family history of obesity, the *GNAS* mutation was identified in the mother.
8	6	F	1	*GNAS*	**Heterozygous:** c.1096G>A; p.(Ala366Thr)	Yes	Pseudohypoparathyroidism IQ 85		18.5	+2.4 SD	
9	50	F	4	*MC3R*	**Heterozygous:** c.31C>T; p.(Gln11*)	No	Hyperphagia Gastric band, followed by gastric bypass	Addison's disease	40	–	
10	40	M	4	*MC3R*	**Heterozygous:** c.149T>C; p.(Ile50Thr)	No	Sleeve gastrectomy, followed by single anastomosis duodenal-ilial bypass Onset obesity: 25 years		50.5	–	
11	52	F	4	*MC3R*	**Heterozygous:** c.446C>T; p.(Ala149Val)	No	Gastric bypass Onset obesity: 15 years	Special education	41.7	–	The *MC3R* mutation was identified in the brother with obesity.
12	50	M	4	*MC4R*	**Heterozygous:** c.20G>A; p.(Arg7His)	Yes	Gastric band, followed by gastric bypass	Spina bifida	44.7	–	
13	14	F	5	*MC4R*	**Heterozygous:** c.64A>T; p.(Arg22*)	No	Diabetes mellitus type 2 Onset obesity: 5 years		37.3	+38 SD	Mother obese, no segregation analysis performed.
14	15	F	2	*MC4R*	**Heterozygous:** c.105C>A; p.(Tyr35*)	No	Onset obesity: 9 years		36.8	+3 SD	The *MC4R* mutation was identified in the mother, but she has a normal weight.
15	2	F	2	*MC4R*	**Heterozygous:** c.105C>A; p.(Tyr35*)	No	Onset obesity: 1 year		20	+2.7 SD	The *MC4R* mutation was identified in the mother, but she has a normal weight.
16	14	F	6	*MC4R*	**Heterozygous:** c.105C>A; p.(Tyr35*)	No	Onset obesity: since birth		44.9	+4.4 SD	
17	48	F	7	*MC4R*	**Heterozygous:** c.105C>A; p.(Tyr35*)	No	Onset obesity: 1 year	Birth weight p3	41.5	–	The *MC4R* mutation was identified in the daughter with obesity, it was not identified in the normal weight father.
18	26	F	8	*MC4R*	**Heterozygous:** c.105C>A; p.(Tyr35*)	Yes	Onset obesity: 1 year	IQ 79 (VIQ 73) Autism 492 kb deletion 2q13	38.3	–	
19	36	M	4	*MC4R*	**Heterozygous:** c.105C>A; p.(Tyr35*)	No	Sleeve gastrectomy Onset obesity: 0–1 years		72.2	–	
20	61	V	4	*MC4R*	**Heterozygous:** c.105C>A; p.(Tyr35*)	No	Gastric bypass	B-cell lymphoma	40.9	–	
21	5	M	9	*MC4R*	**Heterozygous:** c.105C>A; p.(Tyr35*)	No	Early onset obesity		24.6	+5.3 SD	
22	7	F	10	*MC4R*	**Heterozygous:** c.240C>A; p.(Tyr80*)	No	Onset obesity: 5 years		28.6	+4 SD	
23	26	M	4	*MC4R*	**Heterozygous:** c.283G>A; p.(Val95Ile)	No	Gastric bypass Onset obesity: 12 years	Umbilical hernia	40.2	–	
24	5	M	11	*MC4R*	**Heterozygous:** c.493C>T; p.(Arg165Trp)	Unknown*	Unknown*		Unknown*	Unknown*	Unknown*.
25	7	M	10	*MC4R*	**Heterozygous:** c.719A>G; p.(Asn240Ser)	No	Onset obesity: 2.5 years		22.6	+2.9 SD	
26	41	M	4	*MC4R*	**Heterozygous:** c.757G>A; p.(Val253Ile)	No	Gastric bypass Onset obesity: 4 years		50	–	
27	5	M	11	*MC4R*	**Heterozygous:** c.913C>T; p.(Arg305Trp)	No	Hyperphagia Onset obesity: 3 years Length: 127.7 cm (+1.5 SD) Head circumference 56 cm (+2.5 SD)	SNP-array XXY syndrome	25.3	+5 SD	No family history of obesity, de novo mutation.
28	6	F	12	*MKRN3*	**Heterozygous:** c.482del; p.(Pro161fs) (paternal deletion)	Yes	Hyperphagia Precocious puberty	IQ 75 Synophris, almond-shaped eyes, tapering fingers	29.9	+4.5 SD	Both parents overweight.

Continued

Table 2 Continued

Patient	Age (years)	Gender	Medical centre	Gene	Genotype	MCA/ID	Clinical Information Fitting with the clinical phenotype	Additional information/ coincidental phenotypic findings	BMI (kg/m2)	BMI-SD	Family history of obesity
29	23	M	13	*PCSK1*	**Heterozygous:** c.1A>G; p.(Met1?)	Unknown*	Unknown*		Unknown*	Unknown*	Unknown*.
30	33	F	4	*PCSK1*	**Heterozygous:** c.541T>C; p.(Tyr181His)	No	Gastric bypass Onset obesity: 7 years	ADHD	53.2	–	
31	11	F	12	*PCSK1*	**Heterozygous:** c.541T>C; p.(Tyr181His)	No	Hirsutism Precocious puberty Onset obesity: 2 years		30	+3.9 SD	The *PCSK1* mutation was identified in the mother with obesity.
32	13	F	5	*POMC*	**Heterozygous:** c.206del; p.(Pro69fs)	Yes	Onset obesity: 3 years	Special education Sacral dimple	43	+4.3 SD	The *POMC* mutation was identified in the mother with obesity.
33	54	F	4	*POMC*	**Heterozygous:** c.605_616delins18; p.(Gln202_Glu267delinsArgAlaGlnAlaAspLeu)	No	Hyperphagia Gastric band, followed by gastric bypass Onset obesity: 12 years		37.4	–	
34	25	M	4	*POMC*	**Heterozygous:** c.662A>G; p.(Tyr221Cys)	No	Gastric sleeve Onset obesity: 15 years		56.6	–	
35	47	F	7	*POMC*	**Heterozygous:** c.662A>G; p.(Tyr221Cys)	Yes		Clinical: Pendred syndrome (*SLC26A4*: neg.)	44.1	–	
36	14	M	5	POMC	**Heterozygous:** c.662A>G; p.(Tyr221Cys)	No	Onset obesity: 8 years		35.8	+3.8 SD	
37	60	M	4	*POMC*	**Heterozygous:** c.662A>G; p.(Tyr221Cys)	No	Onset obesity: 8 years	Myopia (−8 Dpt)	39.3	–	
38	6	F	1	*POMC*	**Heterozygous:** c.706C>G; p.(Arg236Gly)	No	Onset obesity: 3 years		26	+4 SD	The *POMC* mutation was identified in the mother with obesity.
39	54	F	4	*POMC*	**Heterozygous:** c.706C>G; p.(Arg236Gly)	No	Gastric bypass Onset obesity: 20 years	Crohn's disease	41.9	–	
40	52	F	4	*POMC*	**Heterozygous:** c.706C>G; p.(Arg236Gly)	No	Hyperphagia Gastric bypass Onset obesity: 20 years		54	–	
41	44	F	4	*POMC*	**Heterozygous:** c.706C>G; p.(Arg236Gly)	No	Sleeve gastrectomy Onset obesity: 5 years	Depression	70	–	
42	23	M	4	*POMC*	**Heterozygous:** c.706C>G; p.(Arg236Gly)	Yes	Onset obesity: 4 years	Special education Autism Macrocephaly (+2.4 SD)	57	–	The *POMC* mutation was not identified in the mother with obesity.
43	56	F	4	*POMC*	**Heterozygous:** c.706C>G; p.(Arg236Gly)	No	Gastric bypass Onset obesity: 25 years	Mitral prolapse Scoliosis	39.6	–	
44	10	M	14	*POMC*	**Heterozygous:** c.706C>G; p.(Arg236Gly)	No	Onset obesity: 4 years		28.2	+3.9SD	No family history of obesity.
45	33	F	4	*PTEN*	**Heterozygous:** c.202T>C; p.(Tyr68His)	No	Macrocephaly Onset obesity: 5 years		57	–	Obesity not reported Mother thyroid cancer, maternal grandmother and aunt breast cancer.
46	4	F	9	*PTEN*	**Heterozygous:** c.512A>G; p.(Gln171Arg)	No	Early onset obesity		22.6	+3.8 SD	
47	5	F	1	*SIM1*	**Heterozygous:** c.875_877del; p.(Thr292del)	No	IQ 71 Onset obesity: 2·5 years		20.8	+3 SD	
48	46	F	4	*SIM1*	**Heterozygous:** c.1532del;p.(Asn511fs)	No	Gastric band, followed by gastric bypass		38.6	–	

Medical centre: 1=UMC Utrecht Clinical Genetics; 2=CGG Pediatrics; 3=Erasmus Clinical Genetics; 4=Vitalys Bariatric Surgery; 5=VUmc Louwesweg Pediatrics; 6=Jeroen Bosch Hospital; 7=CGG Internal Medicine; 8=VUmc Clinical Genetics; 9=UMCG Clinical Genetics; 10=Netherlands Antilles; 11=LUMC Clinical Genetics;12=Heideheuvel Pediatrics; 13=Franciscus Internal Medicine; 14=Franciscus Pediatrics.
*For some patients, the phenotype and BMI were not provided by the physician that requested the diagnostic test.
BMI, body mass index.

significant. Thus, the set of 27 curated pathogenic *BBS* mutations is not over-represented in the obesity gene panel cohort. This argues against a possible stronger predisposition to obesity for heterozygote *BBS* gene mutation carriers compared with the other genes on the panel. Furthermore, we were able to perform segregation analysis in the family in 12 out of 48 patients with BBS-associated mutations. The identified mutation cosegregated with obesity in only 6 out of 12 cases (see online supplementary table S4).

Illustrative cases and (future) personalised treatment

Melanocortin-4 receptor

Single pathogenic melanocortin-4 receptor (*MC4R*) mutations cause a hyperphagic phenotype resulting in obesity, which is milder than in patients with compound heterozygous or homozygous mutations.[17] In our cohort, pathogenic *MC4R* mutations were identified in 18 patients (1.5% of the total cohort), of which 16 patients were heterozygous for a *MC4R* mutation. The majority of these patients became obese before the age of 5 (see online supplementary table S5). Segregation analysis in families was performed in 9 of the 18 patients. Five out of nine patients showed cosegregation with the obesity phenotype. This result fits with the known variable penetrance.[18] Four of the heterozygous *MC4R* patients were treated with a gastric bypass. Although (long-term) response treatment studies are pending, there is evidence that patients with heterozygous *MC4R* mutations have good results after bariatric surgery.[19 20]

In 8 of the 18 patients with *MC4R* mutations, we identified the same pathogenic mutation c.105C>A; p.(Tyr35*). In all these patients, an additional c.110A>T; p.(Asp37Val) mutation was found in cis. The ExAC allele frequency of this mutation is 0.00004953%; only present in the European (non-Finnish) population. This result is highly suggestive that the c.105C>A p.(Tyr35*) mutation is a European founder mutation.

Leptin receptor

Leptin receptor (*LEPR*) deficiency can cause obesity with hyperphagia, delayed pubertal development and immune problems.[21] Patient 2 was diagnosed with a compound heterozygous leptin receptor deficiency. She was born at 33+6 weeks of gestation with a birth weight of 2605 g (+1.9 SD). The girl was severely hyperphagic since she was a few weeks old and became obese at the age of 2 months. At the age of 3 years, her BMI was 34.5 kg/m^2 (+7.5 SD). In the first 4 months after the diagnosis, her BMI lowered to 30 kg/m^2 (+6 SD). The identification of the *LEPR* mutations helped in the control of her weight due to supportive treatment. Treatment with setmelanotide, an MC4R agonist, might be a therapeutic option for patients with leptin receptor deficiency.[10]

Proopiomelanocortin

Homozygous and compound heterozygous proopiomelanocortin (*POMC*) mutations cause a combination of early onset obesity, ACTH deficiency, fair skin and red hair.[22] Individuals heterozygous for *POMC* mutations are only predisposed to the obesity phenotype.[23]

We identified 13 patients with a heterozygous *POMC* mutation. One of these was a girl aged 6 years with a BMI of 26 kg/m^2 (+4 SD). Besides hyperphagia, she had no physical or intellectual abnormalities. In this patient, the c.706C>G p.(Arg236Gly) mutation was identified, which was previously described in literature.[24] Segregation analysis showed the same mutation in her mother with obesity.

This *POMC* mutation was also identified in an adult patient. She suffered from obesity since the age of 5. At age 44, she had a BMI of 70 kg/m^2. Besides hyperphagia and depression, she had no other abnormalities. A sleeve gastrectomy was recently performed. Long-term follow-up results are needed to assess the success of the operation.

Treatment with setmelanotide, an MC4R agonist, is a therapeutic option for patients with homozygous or compound heterozygous *POMC* mutations.[10] Setmelanotide treatment might prove to be effective for heterozygous *POMC* patients as well.

DISCUSSION

Here, we present a large patient group for which diagnostic targeted NGS gene panel analysis of syndromic and non-syndromic obesity was performed (1230 affected individuals). A confirmed genetic diagnosis could be made in 48 of 1230 tested patients (3.9%), with the highest yield in a paediatric subgroup 12/164 (7.3%). In 67 additional patients, probable pathogenic mutations were found (5.4%). Further segregation analysis or functional studies are needed to prove the pathogenicity of these mutations. Our data again confirm that obesity is a heterogeneous condition, with diagnoses made on the basis of mutations in at least 11 different genes. Other studies using an NGS approach in genetic obesity showed variable results: a study in Norway had a diagnostic yield of 0.8%, only finding mutations in *MC4R*, whereas a study in Guadeloupean Afro-Caribbean children showed a yield of >15%.[7 25] From the 11 different genes in which mutations were found that lead to a definitive diagnosis in our cohort, *MC4R* mutations were the most frequent genetic cause of obesity. The results of our permutation analysis and segregation analysis argue against a possible stronger predisposition for obesity in heterozygote *BBS* gene mutation carriers than the general population.

Some genetic causes of obesity such as CNVs (16p11.2 deletions), trinucleotide repeat expansion (fragile X-syndrome), uniparental disomies (UPD14) and methylation abnormalities (Prader-Willi syndrome) are not tested with the obesity gene panel. Because of the relatively high prevalence of 16p11.2 deletions as the cause of obesity and the variable phenotype of this syndrome, we would recommend to add SNP-array analysis to the diagnostic approach of a patient with suspected genetic obesity. This could result in a higher diagnostic yield than the definite molecular diagnosis of 3.9% that we present here with NGS gene panel testing. Since research in obesity genetics is rapidly progressing, recently identified obesity-associated genes, such as *CPE* were not included in this panel.[26] These genes can be added to the next version of our diagnostic obesity gene panel.

Six out of the 48 patients with a definitive diagnosis (12.5%) had a mutation that causes a syndromic form of obesity. The majority of the identified mutations however, are linked to non-syndromic monogenic forms of obesity. This may be caused by inclusion bias: patients with a syndromic form of obesity might already have a genetic diagnosis for their developmental disorders or congenital anomalies that presented at earlier age than the obesity. The diagnostic yield of genetic testing in obesity is low in unselected populations, but can be increased by targeting it to patients with specific phenotypes. From the patient's perspective, it can be an important test because of personalised treatment and future treatment options. Promising drug trials for *POMC* and *LEPR* deficiency are currently being performed.[10] An established diagnosis of genetic obesity might influence the choice for bariatric surgery as well. Short-term

effects of bariatric surgery in patients with monogenic obesity (due to *MC4R* heterozygous mutations) seem to be comparable to patients without a genetic diagnosis,[27 28] but there are only a few reports in literature about long-term effects. Two single case reports on long-term effects of bariatric surgery describe significant weight regain in the years after bariatric surgery in patients with homozygous mutations in *LEPR* and *MC4R*, respectively.[29 30] We are still awaiting the long-term follow-up results for the bariatric subgroup in our cohort.

A limitation of this study is that we compare the variants with the ExAC database, which does not exclude persons with obesity, so it is possible that rare pathogenic variants causing early onset obesity are present in ExAC resulting in an underestimation of our positive results. Moreover, the ExAC control group does not share the exact same geographic or ethnic characteristics with our Dutch cohort, possibly disregarding the occurrence of founder mutations in these populations.

Using our obesity gene panel, we have found more carrier statuses than definite diagnoses: 61 patients (5%) were carriers of a pathogenic mutation associated with recessive disease. However, to our opinion the importance of the diagnosis outweighs the downside of identifying carrier statuses, since finding the genetic cause of inherited obesity can have a significant clinical relevance. Genetic counselling can be provided (including information about risks for offspring to be affected with a severe recessive condition) and some patients are eligible for specific therapies. Single gene testing of the most common genetic causes would reduce the problem of finding unclear results or carrier statuses; however, the costs of multiple stand-alone Sanger sequencing tests are much higher than the costs of this multigene panel. Finally, it could also be possible that combinations of several VUS increase obesity risk (polygenic effect), but that was not the purpose of our study and thus not examined.

In conclusion, our NGS-based gene panel analysis in patients with obesity led to a definitive diagnosis of a genetic obesity disorder in 3.9% of the patients (48/1230). In 67 additional patients (5.4%), probable pathogenic mutations were found for which the causal role in the obesity phenotype has yet to be confirmed. The obesity gene panel showed the highest yield in a paediatric subgroup, establishing a definitive diagnosis in 12 out of 164 children with severe early onset obesity (7.3%).

The NGS-based gene panel analysis in patients with obesity is a useful tool for diagnosing genetic obesity and can have serious impact on the treatment of patients. Therefore, we recommend testing in selected patients with early onset severe obesity.

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Contributors LK, MM, GvH, BvdZ and MMvH contributed to the study design and acquisition and/or analysis of the data. All authors contributed to the data interpretation, provided critical revisions of the manuscript and approved the final version.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Disclaimer All authors, external and internal, had full access to all of the data (including statistical reports and tables) in the study and can take responsibility for the integrity of the data and the accuracy of the data analysis. We attest that we have obtained appropriate permissions. Required fees for use of copyright-protected materials: not applicable.

Competing interests None declared.

Patient consent Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement Extra clinical information might be available for some patients. Data that underlie the results reported in this article (after deidentification) can be requested if relevant. Proposals should be directed to m.vanhaelst@vumc.nl.

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